UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO

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AVALIAÇÃO DE POLIMORFISMOS DE ÚNICO NUCLEOTÍDEO (SNPS) ENVOLVIDOS COM A GRAVIDADE DA DOENÇA HEPÁTICA CRÔNICA CAUSADA PELO VÍRUS DA HEPATITE C (HCV)

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Avaliação de polimorfismos de único nucleotídeo (SNPs) envolvidos com a gravidade da doença hepática crônica causada pelo vírus da hepatite C (HCV)

Tese apresentada ao Programa de Pós-Graduação em Biotecnologia – RENORBIO, como parte dos requisitos exigidos para obtenção do título de Doutor em Biotecnologia, área de concentração Biotecnologia em Saúde

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(Martin Luther King)

RESUMO

O vírus da hepatite C (HCV) representa um problema de saúde mundial, acometendo mais de 170 milhões de pessoas em todo o mundo, o que corresponde a cerca de 3% da população mundial. Aproximadamente 70% dos indivíduos irão desenvolver a forma crônica da doença, 25% desenvolverão cirrose e cerca de 5% dos cirróticos desenvolverão carcinoma hepatocelular (HCC). O motivo pelo qual alguns indivíduos evoluem mais rapidamente para formas mais graves ainda é desconhecido, entretanto diversos estudos têm apontado a influência de fatores genéticos do hospedeiro envolvidos com a progressão da doença no fígado. Polimorfismos de único nucleotídeo (SNPs) são o tipo de variação genética mais comum em humanos, e podem influenciar os níveis séricos ou até mesmo a função de proteínas importantes. A pentraxina 3 (PTX3) é uma proteína de fase aguda capaz de se ligar a microrganismos e de regular o sistema complemento. Estudos têm demonstrado que a PTX3 pode influenciar positivamente a progressão de vários tipos de câncer. Além disso, alguns estudos têm demonstrado uma grande influência de uma região cromossômica (6q23) associada com a progressão da fibrose hepática na esquistossomose. O gene *IL22RA2* está localizado nesta região e poderia estar associado com a gravidade da fibrose no HCV, uma vez que este gene codifica um inibidor de uma importante citocina envolvida com a reparação de danos hepáticos, a interleucina-22 (IL-22). Portanto, o objetivo do presente trabalho foi associar a gravidade da doença hepática causada pelo HCV, com SNPs nos genes *PTX3* e *IL22RA2*, assim como identificar novos SNPs através da técnica de sequenciamento de exoma. Foram recrutados pacientes com hepatite C crônica, atendidos no serviço de Gastrohepatologia do Hospital Universitário Oswaldo Cruz/Instituto do Fígado de Pernambuco (Recife-PE, Brasil) entre agosto de 2010 e dezembro de 2014. A detecção dos SNPs foi realizada por PCR em tempo real através de sondas TaqMan®. Para o sequenciamento do exoma, foi utilizada a plataforma IonTorrent®. Um total de três estudos foram realizados, o primeiro estudo identificou dois polimorfismos (rs6570136 e rs2064501) no gene *IL22RA2*, associados com a gravidade da fibrose hepática, em um total de 532 pacientes. Foi observada uma maior frequência dos genótipos GG/GA do rs6570136 e TT/TC do rs2064501 no grupo de indivíduos com fibrose grave (p=0,007 OR 1,7 e p=0,004 OR 2,4). No segundo estudo, com um total de 524 pacientes, foi possível observar uma associação significativa do genótipo AA no gene *PTX3* (rs2305619) com o risco de HCC (p=0.024 OR 1,94). Por fim, foi realizado o sequenciamento do exoma de 9 casos com HCC e 10 controles cirróticos, onde foi possível identificar dois genes (*PRSS58* e *SOCS5*) possivelmente associados com o desenvolvimento de HCC. Portanto, através do presente estudo, foi demonstrado pela primeira vez a associação de SNPs no *IL22RA2, PTX3, PRSS58* e *SOCS5* com a progressão da doença hepática causada pelo HCV. Outros estudos são necessários para avaliar o uso desses SNPs como marcadores de progressão da hepatite C, bem como avaliar o possível uso dessas moléculas como alvos terapêuticos.

Palavras-chave: HCV. SNP. Polimorfismo. Fibrose. HCC.

ABSTRACT

The hepatitis C virus (HCV) represents a worldwide health problem, with over 170 million people infected all over the world, corresponding to almost 3% of the world's population. Approximately 70% of the individuals will develop the chronic form of the disease; 25% will develop cirrhosis and about 5% among the cirrhotic will develop hepatocellular carcinoma (HCC). The reason why some individuals evolve more rapidly to the severe forms is still unknown, however, several studies have pointed the influence of genetic factors of the host which are involved with the disease progression in the liver. Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation in humans, and they might alter serum levels or even the function of important proteins. Pentraxin 3 (PTX3) is an acute phase protein that is able to bind the surface of microorganisms and to regulate the complement system. Studies have demonstrated that PTX3 may influence positively the progression of various types of cancer. Additionally, some studies have demonstrated an important influence of a chromosomic region (6q23), associated with the progression of liver fibrosis in schistosomiasis. The *IL22RA2* gene is located in this region e may be associated with the severity of fibrosis in HCV, once this gene codifies an inhibitor of an important cytokine correlated with the repair of liver damage, the interleukin-22 (IL-22). Thus, the aim of the present study was to associate the severity of the liver disease caused by HCV with SNPs in the *PTX3* and *IL22RA2* genes, as well as to identify new SNPs through exome sequencing approach. Patients with chronic hepatitis C were recruited and attended at the service of Gastrohepatology of the Oswaldo Cruz University Hospital/Liver Institute of Pernambuco (Recife, Pernambuco, Brazil) between August 2010 and December 2014. The detection of SNPs was performed by real time PCR using TaqMan probes (Thermo Fisher Scientific). Regarding the exome sequencing, it was used the IonTorrent platform (Thermo Fisher Scientific). A total of three studies were performed, the first study identified two polymorphisms (rs6570136 and rs2064501) on the *IL22RA2* gene, associated with the severity of hepatic fibrosis, in a total of 532 patients. It was observed a higher frequency of genotypes GG/GA of rs6570136 and TT/TC of rs2064501 in the group of individuals with severe fibrosis (p=0,007 OR 1,7 and p=0,004 OR 2,4). On the second study, with a total of 524 patients, it was possible to notice a significant association of the genotype AA in the *PTX3* gene (rs2305619) with risk of HCC (p=0.024 OR 1,94). Finally, the exome sequencing was carried in 9 HCC cases and 10 cirrhotic controls, where it was possible to identify two genes (*PRSS58* and *SOCS5*), which are possibly associated with the development of HCC. Therefore, through this study we have demonstrated, for the first time, the association of SNPs in *IL22RA2*, *PTX3, PRSS58* and *SOCS5* with the progression of the hepatic disease caused by HCV. Other studies are needed in order to evaluate the use of these SNPs as progression markers of hepatitis C; as well as to evaluate the possible use of these molecules as therapeutic targets.

Keywords: HCV. SNP. Polymorphism. Fibrosis. HCC.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS

ALT – Alanina aminotransferase

apoA – Apolipoproteína A

apoB – Apolipoproteína B

apoC – Apolipoproteína C

apoE – Apolipoproteína E

AST – Aspartato aminotransferase

C1q – Componente 1q

CIS – Domínio SH2 induzida por citocina

CLDN1 – Claudina-1

cLDs – Gotículas lipídicas citoplasmáticas

ConA – Concanavalina A

DAAs – Antivirais de ação direta

DAMPs – Padrões moleculares associado ao dano

DGAT1 – Diacilglicerol aciltransferase-1

DNA – Ácido desoxirribonucleico

dsRNA – Ácido ribonucleico de fita dupla

EGF – Fator de crescimento epidérmico

EGFR – Receptor do fator de crescimento epidérmico

EGFR – Receptor do fator de crescimento epidérmico

HBV – Vírus da hepatite B

HCC – Carcinoma Hepatocelular

HCV – Vírus da Hepatite C

HLA – Antígeno leucocitário humano

HSCs – Células estreladas hepáticas

HVR-1 – Região hipervariável 1

IC – Intervalo de confiança

IFN – Interferon

IFNR – Receptor de interferon

IL – Interleucina

IL-10R – Receptor de interleucina-10

IL-22 – Interleucina 22

IL-22BP – Proteína de ligação a IL-22

ILCs – Células linfoides inatas

IPs – Inibidores de protease

IRF – Fator de regulação de interferon

IRF – Fator regulador de interferon

ISGs – Genes estimulados por interferon

JAK – Janus quinase

LDL – Lipoproteína de baixa densidade

LDLR – Receptor de lipoproteína de baixa densidade

LEPR – Receptor de leptina

LVPs – Lipoviropartículas

MAVS – Proteína de sinalização antiviral mitocondrial

MBL – Lectina ligadora de manose

NF-κB – Fator nuclear kappa B

NK – Natural Killer

OCLN – Ocludina

OMS – Organização Mundial de Saúde

PAMP – Padrões moleculares associado ao patógeno

PCR – Proteína C reativa

PEG-IFN-α – Interferon alfa peguilado

PKR – Proteína quinase R

PRSS58 – Serino protease 58

PTX3 – Pentraxina 3

RE – Retículo endoplasmático

RIG-I – Gene 1 induzível por ácido retinóico

RNA – Ácido ribonucleico

ROS – Espécies reativas ao oxigênio

RRP – Receptores de reconhecimento padrão

SAP – Amiloide sérico P

SNPs – Polimorfismos de único nucleotídeo

SOCS – Supressor de sinalização de citocina

SRB1 – Receptor scavenger B1

ssRNA – Ácido ribonucleico de fita simples

STAT – Transdutores de sinais e ativadores de transcrição

SVR – Resposta virológica sustentada

TGF-β – Fator de transformação de crescimento beta

TLR – Receptor semelhante ao Toll

TNF-α – Fator de necrose tumoral alfa

TRIF – Domínio TIR indutor de interferon beta

TYK – Tirosina quinase

VEGF – Fator de crescimento endotelial vascular

VLDL – Lipoproteína de muito baixa densidade

α-AML – α-actina de músculo liso

SUMÁRIO

1. INTRODUÇÃO

A hepatite C representa um dos principais problemas de saúde pública no mundo (LAVANCHY, 2009). Sua história natural, caracterizada pela escassez de sintomas durante os estágios precoces da doença, tornam o diagnóstico do vírus da hepatite C (HCV) um grande desafio para os profissionais de saúde. Devido aos sintomas serem inespecíficos, a doença pode evoluir por décadas sem diagnóstico, causando danos hepáticos cada vez mais graves no decorrer dos anos, dificultando assim o tratamento da doença. A infecção crônica pode levar à hepatite e a diferentes graus de fibrose. Nos casos mais graves, o indivíduo pode evoluir para cirrose e descompensação hepática, culminando com o desenvolvimento de carcinoma hepatocelular (HCC) (WESTBROOK;DUSHEIKO, 2014). As razões que levam alguns indivíduos a desenvolverem formas mais graves da doença ainda não foram completamente elucidadas, mas estudos têm demonstrado que fatores do hospedeiro, tais como sexo, idade, diabetes e fatores genéticos, desempenham grande importância no processo fisiopatológico da hepatite C (BATALLER;NORTH;BRENNER, 2003; ASSELAH *et al.*, 2009; WESTBROOK;DUSHEIKO, 2014).

Desde o sequenciamento completo do genoma em 2001 (VENTER *et al.*, 2001), grandes avanços têm sido feitos no que se refere a tecnologia de detecção de variantes genéticas, sobretudo no desenvolvimento de equipamentos de sequenciamento em larga escala, que permitem a descoberta de uma grande quantidade de polimorfismos associados a doenças (DAVEY *et al.*, 2011). Polimorfismos de único nucleotídeo (SNPs) são o tipo de variação genética mais comum em humanos, podendo influenciar os níveis séricos e a função de proteínas importantes. Estudos têm demonstrado que uma variedade de SNPs em genes envolvidos com vias inflamatórias, receptores de fatores de crescimento, moléculas envolvidas com a deposição de matrix extracelular, entre outros, influencia a progressão da fibrose e o desenvolvimento de HCC em pacientes com infecção crônica pelo HCV (BATALLER;NORTH;BRENNER, 2003; ROMERO-GOMEZ *et al.*, 2011; ESTRABAUD *et al.*, 2012). Esses fatores podem explicar, pelo menos em parte, as diferentes taxas de progressão observadas entre esses indivíduos.

A interleucina-22 (IL-22) é uma citocina que age em células epiteliais, hepatócitos e células pancreáticas, sugerindo uma importante função na reparação de danos teciduais locais, ou contribuindo com a inflamação fisiopatológica (WOLK *et al.*, 2010). A IL-22 também estimula a imunidade inata através da produção de peptídeos antimicrobianos, secreção de muco e produção de quimiocinas (RUTZ;EIDENSCHENK;OUYANG, 2013). Além disso, a

IL-22 possui um importante efeito hepatoprotetor, estimulando o reparo tecidual e reduzindo a fibrose em diversos modelos de lesão hepática (PAN *et al.*, 2014). A IL-22BP é considerada um inibidor solúvel da IL-22, impedindo sua ligação ao receptor celular e limitando seus efeitos em células-alvo. O gene que codifica a IL-22BP (*IL22RA2*), está localizado no lócus 6q23, que possui uma grande influência na fibrose hepática em indivíduos com esquistossomose (DESSEIN *et al.*, 1999). Até então, não existem informações relacionadas a influência de polimorfismos no gene da IL-22BP (*IL22RA2*) na doença hepática causada pelo HCV. Portanto, SNPs no *IL22RA2* poderiam influenciar também a progressão da fibrose hepática em indivíduos com HCV.

A pentraxina 3 (PTX3) é um receptor de reconhecimento padrão solúvel, produzido por fagócitos, células epiteliais, endoteliais e mesenquimais em sítios de inflamação (GARLANDA *et al.*, 2016). A PTX3 desempenha importantes funções na regulação da imunidade, sendo capaz de interagir com partículas microbianas e moléculas envolvidas em processos inflamatórios (INFORZATO *et al.*, 2013). Estudos recentes têm demonstrado um possível papel da PTX3 no desenvolvimento de alguns tipos de câncer (BONAVITA *et al.*, 2015; BOTTAZZI *et al.*, 2016; GARLANDA *et al.*, 2016). Além disso, níveis plasmáticos de PTX3 têm sido associados com a gravidade da fibrose hepática em pacientes com esteato-hepatite não alcoólica (NASH) (YONEDA *et al.*, 2008; BOGA *et al.*, 2015). Devido à escassez de estudos investigando o papel da PTX3 em infecções virais, não é conhecido se a PTX3 pode influenciar a progressão da doença hepática causada pelo HCV. Sendo assim, estudos investigando SNPs no gene *PTX3* são atraentes para determinar o papel desta molécula na patogênese do HCV.

Em pacientes com hepatite C crônica, o risco em desenvolver HCC aumenta de acordo com a progressão da fibrose. Uma vez alcançada o estágio de cirrose hepática, a incidência anual de HCC se torna extremamente elevada (1-7% por ano) (HOSHIDA *et al.*, 2014). Apesar dos protocolos clínicos atualmente utilizados recomendarem uma vigilância regular em busca de tumores, através de métodos de imagem, em pacientes com maior risco de HCC, dados apontam que apenas 12% dos pacientes com HCC são diagnosticados através dos programas de vigilância nos Estados Unidos (DAVILA *et al.*, 2011). Considerando uma limitação nas opções de tratamento, incluindo o transplante hepático, a prevenção do desenvolvimento de HCC em pacientes com fibrose avançada seria a melhor estratégia para aumentar a sobrevida desses indivíduos (HOSHIDA *et al.*, 2014).

Deste modo, o objetivo do presente estudo foi verificar a associação entre SNPs nos genes *PTX3* e *IL22RA2* com a gravidade da doença hepática causada pelo HCV, assim como

identificar novos SNPs através da técnica de sequenciamento do exoma. A identificação de marcadores genéticos relacionados a fibrose e susceptibilidade ao desenvolvimento de HCC, pode ser uma importante estratégia para selecionar indivíduos com maior predisposição ao desenvolvimento de formas graves, permitindo assim, um tratamento individualizado e precoce.

2. REVISÃO DE LITERATURA

2.1 Hepatite C

2.1.1 Epidemiologia

Desde sua descoberta em 1989 por Choo e colaboradores (CHOO *et al.*, 1989), o vírus da hepatite C (HCV) tem sido associado à principal causa de hepatite crônica, cirrose e carcinoma hepatocelular no mundo. Estima-se que a prevalência mundial da infecção pelo HCV seja entre 2-3%, equivalente a 130-170 milhões de pessoas, e que cerca de 3 a 4 milhões de pessoas são infectadas pelo vírus a cada ano. Entre os que possuem infecção crônica, 10-20% desenvolverão cirrose em 20 anos e 1-5% entre os cirróticos desenvolverão carcinoma hepatocelular (HCC) (LAVANCHY, 2009; LAVANCHY, 2011).

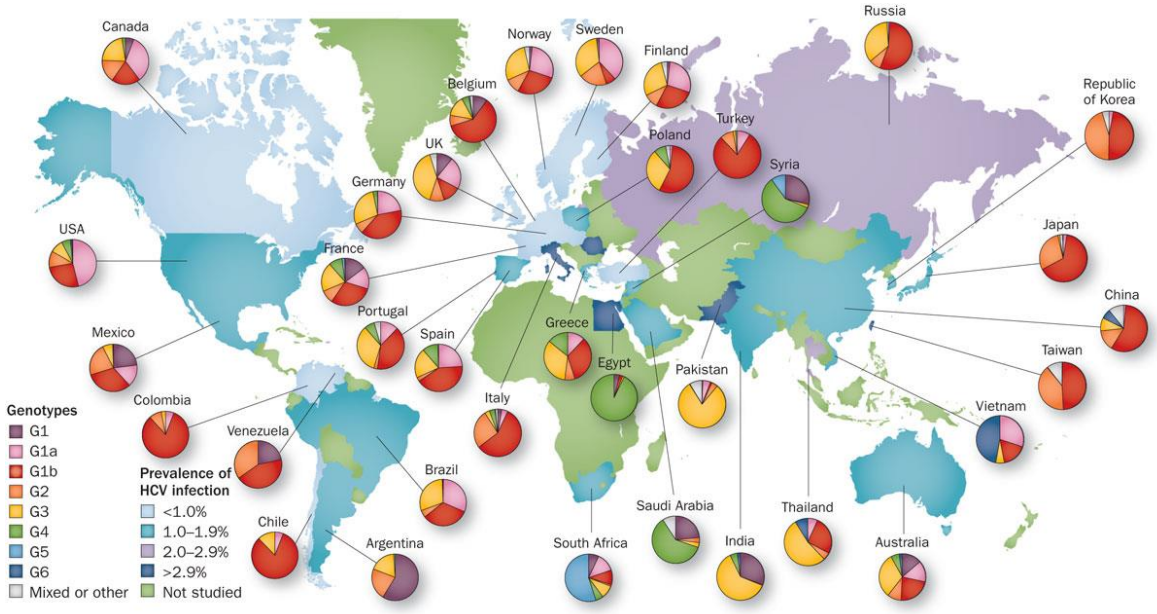
Apesar do HCV ser uma endemia mundial, existe um grande grau de variabilidade em sua distribuição (de <1% a >10% em diferentes países) (HAJARIZADEH;GREBELY;DORE, 2013). As maiores prevalências têm sido observadas na África e no Oriente Médio, com uma menor prevalência nas Américas, Austrália e na Europa Ocidental (LAVANCHY, 2011). Na África, a maior prevalência de infecção pelo HCV tem sido demonstrada no Egito e Camarões, com prevalências maiores que 10% (NERRIENET *et al.*, 2005; GUERRA *et al.*, 2012; HAJARIZADEH;GREBELY;DORE, 2013). Se tratando de números absolutos, a maioria das pessoas infectadas vive na Ásia Central/Sudeste e em regiões ocidentais do Pacífico, sendo a China o país com maior número de infectados (29,8 milhões), seguido da Índia (18,2 milhões), Egito (11,8 milhões), Paquistão (9,4 milhões) e Indonesia (9,4 milhões) (LAVANCHY, 2011).

Na América Latina, estimativas apontam uma prevalência de hepatite C entre 1% e 2,3% (KERSHENOBICH *et al.*, 2011). O Brasil é considerado um país de endemicidade intermediária, com prevalência entre 2,5% e 10%, segundo a Organização Mundial de Saúde (OMS) (TE;JENSEN, 2010). Entretanto, o último estudo de prevalência de base populacional realizado entre 2005 e 2009, em todas as 26 capitais e no Distrito Federal, apontou-se uma prevalência de 1,38% (IC 95% 1,12%-1,64%) na faixa etária entre 10 e 69 anos, colocando o Brasil como um país de baixa endemicidade (PEREIRA *et al.*, 2013).

No Brasil, a notificação compulsória de casos de hepatite C começou em 1996 e, em 1999, a taxa de detecção desse agravo no país era de 0,1 por 100.000 habitantes. A partir desse ano, a taxa aumentou, alcançando 5,0 em 2006 e mantendo-se estável, com oscilações entre 5,2 e 5,5, (5,4 em 2010) (BRASIL, 2012). No período de 1999 a 2011, foram notificados no Sistema de Informação de Agravos de Notificação (Sinan) 82.041 casos confirmados de hepatite C no Brasil, a maioria nas Regiões Sudeste (67,3%) e Sul (22,3%). Do total de casos, 49.291 (60,1%) dos infectados eram homens e 32.734 (39,9%) eram mulheres (BRASIL, 2012). Em uma publicação mais recente do Ministério da Saúde, um modelo matemático foi utilizado com o objetivo de estimar o número de casos possíveis e não diagnosticados no Brasil. A análise demonstrou que aproximadamente 1.450.000 pessoas possuíam HCV no ano de 2014 (BRASIL, 2015a). No período de 1999 a 2011 na região Nordeste foram notificados no Sinan 4.131 casos confirmados, representando 5% dos casos no Brasil. A maioria dos casos se concentrou nos estados da Bahia (38,3%), seguido pelo Ceará (13,2%) e Pernambuco (10,6%). Em relação a taxa de detecção, em 2010 todos os estados da Região Nordeste apresentaram taxas de detecção de hepatite C por 100.000 habitantes menores do que a média nacional (5,4 casos/100.000 habitantes). As maiores taxas foram observadas em Sergipe (2,4 casos/100.000 habitantes) e Bahia (1,9 casos/100.000 habitantes) (BRASIL, 2012).

A taxa de replicação do HCV é bastante elevada, de 10^{10} a 10^{12} vírions por dia, esta alta taxa de replicação juntamente com o erro da polimerase viral pode ser responsável pelo genoma do HCV sofrer mutações frequentemente, gerando seis principais genótipos e mais de 50 subtipos virais (SIMMONDS *et al.*, 1994; HOOFNAGLE, 2002). A distribuição dos genótipos varia de acordo com cada país ou região, sendo que os genótipos 1, 2 e 3 possuem uma ampla distribuição geográfica, enquanto que os genótipos 4, 5 e 6 estão normalmente confinados a regiões geográficas específicas (HAJARIZADEH;GREBELY;DORE, 2013). O genótipo 1 tem a maior distribuição no mundo, sendo mais comum na América do Norte (ALTER, 2007), no Oeste e Norte Europeu (ESTEBAN;SAULEDA;QUER, 2008; CORNBERG *et al.*, 2011), na América do Sul, Ásia e Austrália (KABA *et al.*, 1998; HAJARIZADEH;GREBELY;DORE, 2013). Os genótipos 2a e 2b são geralmente encontrados no Japão e norte da Itália, o genótipo 3 na Índia, o 4 é mais comum na África e Oriente Médio, enquanto os genótipos 5 e 6 são raros, porém encontrados em algumas regiões do sul da África e sudeste da Ásia (HOOFNAGLE, 2002). No Brasil, o padrão de distribuição se assemelha ao da Europa, onde os genótipos prevalentes são 1 e 3 (CAMPIOTTO *et al.*, 2005) (Figura 1). Em Pernambuco, o genótipo 1b é o mais prevalente (66,7%), seguido pelo 3a (25,4%), 1a (6,3%) e 2b (1,6%) (ALVARADO-MORA *et al.*, 2012).

Figura 1. Prevalência mundial estimada de infecção pelo HCV e distribuição de seus genótipos no mundo.



Fonte: (HAJARIZADEH;GREBELY;DORE, 2013)

2.1.2 Transmissão e Fatores de Risco

A transmissão da hepatite C ocorre principalmente por via parenteral, transmissões verticais e sexuais ocorrem em frequência muito baixa. Estudos apontam uma taxa de 5% para a transmissão vertical (NETWORK, 2005; PEMBREY;NEWELL;TOVO, 2005). A transmissão sexual ocorre principalmente em pessoas com múltiplos parceiros e com práticas sexuais desprotegidas. A co-infecção com HIV constitui relevante facilitador para a transmissão (SHEPARD;FINELLI;ALTER, 2005; SY;JAMAL, 2006).

No Brasil, a hepatite C teve como formas preferenciais de transmissão o uso de drogas injetáveis, hemodiálise, transfusão de sangue e uso de hemoderivados e outros procedimentos médicos invasivos, particularmente até o início dos anos 90, quando não havia exame que permitisse diagnosticar o portador do HCV (BRASIL, 2015a). Em 1992, foi desenvolvido o primeiro teste para detecção do anticorpo anti-HCV, proporcionando maior segurança em transfusões sanguíneas (LAUER;WALKER, 2001). De acordo com o Ministério da Saúde, constituem populações de risco para infecção pelo HCV (BRASIL, 2011):

- Pessoas que receberam transfusão de sangue e/ou hemoderivados antes de 1993;
- Usuários de drogas injetáveis, inaladas ou pipadas, que compartilham equipamentos contaminados como agulhas, seringas, canudos e cachimbos;
- Pessoas que compartilham equipamentos não esterilizados ao frequentar pedicures, manicures e podólogos;

- Pessoas submetidas a procedimentos para colocação de piercing e confecção de tatuagens;
- Pacientes que realizam procedimentos cirúrgicos, odontológicos, de hemodiálise e de acupuntura sem as adequadas normas de biossegurança.

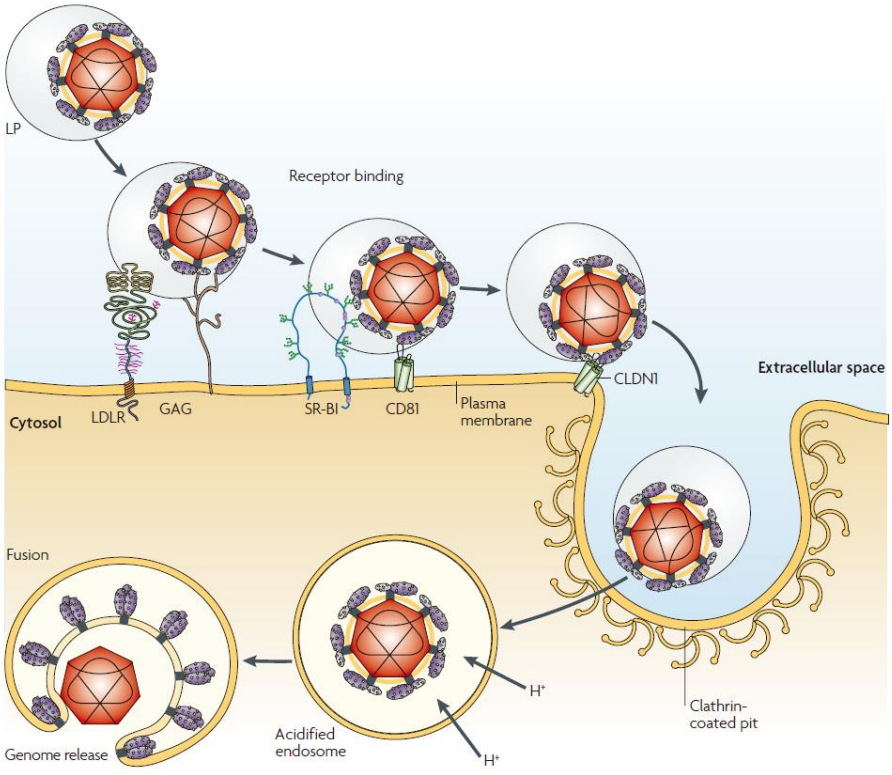
2.1.3 O vírus da hepatite C (HCV)

O HCV pertence ao gênero *Hepacivirus*, família Flaviviridae. Suas partículas virais medem cerca de 50-80 mm de diâmetro e são cobertas por heterodímeros glicoproteicos (E1 e E2) incorporados na bicamada lipídica cercando um nucleocapsídeo composto pela proteína core e de seu RNA de fita única (GASTAMINZA *et al.*, 2010). As partículas virais do HCV apresentam uma baixa densidade devido a sua associação com lipoproteínas de baixa densidade (LDL) e muito baixa densidade (VLDL) (THOMSSEN *et al.*, 1992; THOMSSEN;BONK;THIELE, 1993; ANDRE *et al.*, 2002). Devido a essa associação, apolipoproteínas como apoE, apoB, apoA1, apoC1, apoC2 e apoC3 podem também ser encontradas em partículas do HCV e desempenham fundamental importância no processo de invasão viral (NIELSEN *et al.*, 2006; CHANG *et al.*, 2007; MEUNIER *et al.*, 2008). Entretanto, as glicoproteínas E1 e E2 são os principais protagonistas no processo de entrada das partículas virais na célula hospedeira. Até então, estudos apontavam a E2 como uma proteína chave no processo de fusão entre o envelope viral e a membrana da célula hospedeira (KREY *et al.*, 2010), entretanto recentes estudos investigando a estrutura da glicoproteína E2 não confirmam esta hipótese, levando a especulação de que a glicoproteína E1 poderia ser a proteína de fusão, ou provavelmente atuar junto com E2 neste processo (KONG, L. *et al.*, 2013; DOUAM *et al.*, 2014; KHAN *et al.*, 2014).

Além de humanos, o HCV é capaz de infectar naturalmente apenas chimpanzés e musaranhos (*Tupaia belangeri*) (LINDENBACH;RICE, 2001; AMAKO *et al.*, 2010). Ao longo dos anos, modelos têm sido desenvolvidos para estudar os mecanismos de entrada, ligação e replicação do vírus na célula hospedeira, desde sistemas *in vitro* até modelos de camundongos geneticamente humanizados (ORTEGA‐PRIETO;DORNER, 2016). Esses estudos proporcionaram um grande avanço no conhecimento sobre o HCV nos últimos anos, entretanto algumas limitações ainda precisam ser superadas, uma vez que os modelos de camundongos modificados não apresentam as mesmas características da doença em humanos.

Durante uma infecção primária, as partículas do HCV são transportadas pela corrente sanguínea e entram em contato com os hepatócitos após atravessar o endotélio fenestrado dos sinusóides hepáticos. No espaço de Disse, os vírions têm contato direto com a superfície basolateral dos hepatócitos, permitindo a interação com fatores de ligação e receptores celulares (DUBUISSON;COSSET, 2014). Estudos recentes sugerem que esse contato inicial poderia ser mediado pela ApoE com o heparan-sulfato proteoglicana sindecano 1 ou sindecano 4 (SHI;JIANG;LUO, 2013; LEFÈVRE *et al.*, 2014) ou pelo receptor scavenger B1 (SRB1) (THI *et al.*, 2012; JIANG *et al.*, 2013), e não por glicoproteínas virais como pensava-se antes. Evidências sugerem que durante essa fase inicial, o receptor de LDL (LDLR) também desempenha um papel importante, devido a associação das partículas virais com lipoproteínas (AGNELLO *et al.*, 1999). Após esse contato inicial de baixa afinidade, ocorre a interação das glicoproteínas E1-E2 com os co-receptores SRB1 e CD81 (PILERI *et al.*, 1998; SCARSELLI *et al.*, 2002). Moléculas associadas a zônulas de oclusão, como a claudina-1 (CLDN1) e ocludina (OCLN), também têm sido associadas com a invasão do HCV nas células hospedeiras (EVANS *et al.*, 2007; PLOSS *et al.*, 2009). Fatores adicionais como o receptor do fator de crescimento epidérmico (EGFR) e o receptor de efrina tipo A2 possivelmente modulam a interação entre CD81 e CLDN1 (LUPBERGER *et al.*, 2011; SCHEEL;RICE, 2013) (Figura 2).

Figura 2. Mecanismos de entrada do HCV na célula hospedeira

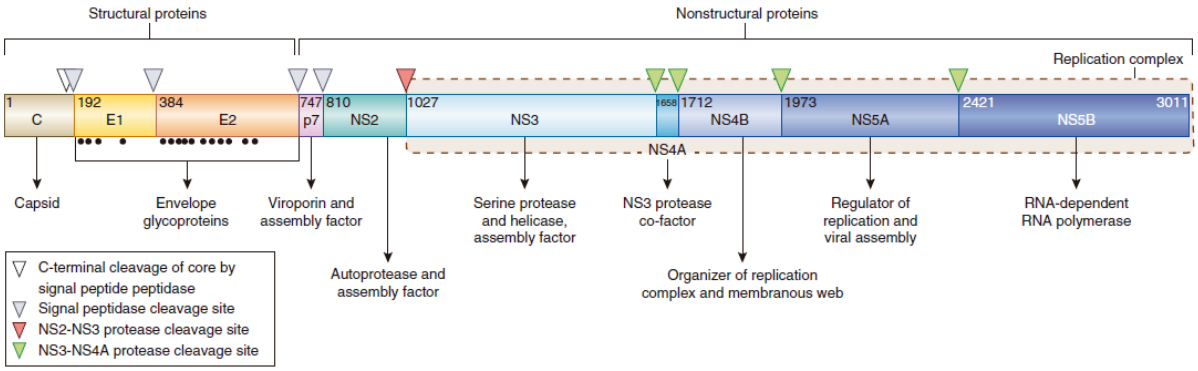


Fonte: (MORADPOUR;PENIN;RICE, 2007)

A absorção do vírion pela célula hospedeira ocorre através de endocitose mediada por clatrina (BLANCHARD *et al.*, 2006), e a fusão necessita de um compartimento de baixo pH, encontrada nos endossomos (TSCHERNE *et al.*, 2006; SCHEEL;RICE, 2013). Após o processo de fusão do vírus na célula, o genoma do HCV é liberado no citosol, onde ocorre o processo de tradução para a produção de proteínas virais (DUBUISSON;COSSET, 2014).

O HCV possui em seu genoma uma fita de RNA positiva simples contendo cerca de 9500 nucleotídeos, codificando uma poliproteína com aproximadamente 3000 aminoácidos. Essa poliproteína é clivada pós-tradução por enzimas virais e do hospedeiro, formando peptídeos estruturais e não-estruturais (NS). Os componentes estruturais incluem o nucleocapsídeo core (C) e duas proteínas pertencentes ao envelope viral, E1 e E2, enquanto a região não-estrutural codifica sete proteínas (p7, NS2, NS3, NS4A, NS4B, NS5A e NS5B) que exercem atividades distintas durante a replicação viral (GIANNINI;BRECHOT, 2003; LYRA;FAN;DI BISCEGLIE, 2004; MORADPOUR;PENIN, 2013) (Figura 3).

Figura 3. Representação esquemática do genoma do HCV com seus domínios proteicos.



Fonte: Figura adaptada de (SCHEEL;RICE, 2013)

A p7 é um polipeptídeo transmembrana que funciona como um canal iônico e exerce um papel fundamental na montagem e liberação de partículas infecciosas do HCV (GRIFFIN *et al.*, 2003; MADAN;BARTENSCHLAGER, 2015). A NS2 é uma protease que atua como uma proteína adaptadora, ela interage com outras proteínas estruturais e não estruturais e também coordena os estágios iniciais de montagem das partículas virais (POPESCU *et al.*, 2011; STAPLEFORD;LINDENBACH, 2011). A NS3 é uma proteína bifuncional, com um domínio de serino-protease na extremidade N-terminal e um domínio NTPase/RNA helicase na extremidade C-terminal. A NS3 possui importantes funções na replicação do RNA viral (KOLYKHALOV *et al.*, 2000; LAM;FRICK, 2006), na modulação da resposta imune inata antiviral (LI, K. *et al.*, 2005; MEYLAN *et al.*, 2005; LIN, W. *et al.*, 2006; LOO *et al.*, 2006),

assim como na montagem de partículas virais (MA *et al.*, 2008; PHAN *et al.*, 2009). A NS4A atua como um cofator para a NS3 através de um complexo transmembrana, esse complexo é essencial para a atividade de NS3 durante a replicação viral. A NS4A também é necessária para a fosforilação de NS5A e pode interagir diretamente com a NS5A (KIM *et al.*, 1996; ASHFAQ *et al.*, 2011). A NS4B induz alterações na membrana do RE que permitem a acomodação do complexo de replicação viral do HCV (EGGER *et al.*, 2002). A NS5A é uma fosfoproteína hidrofílica que desempenha importante função na replicação viral, na modulação de vias de sinalização celular e na via do interferon (ASHFAQ *et al.*, 2011). Por fim, a NS5B é uma polimerase dependente de RNA, responsável pela síntese do RNA genômico viral. Devido a seu papel central na replicação viral, esta enzima tem sido o principal alvo dos medicamentos antivirais (MORADPOUR;PENIN;RICE, 2007; ASHFAQ *et al.*, 2011).

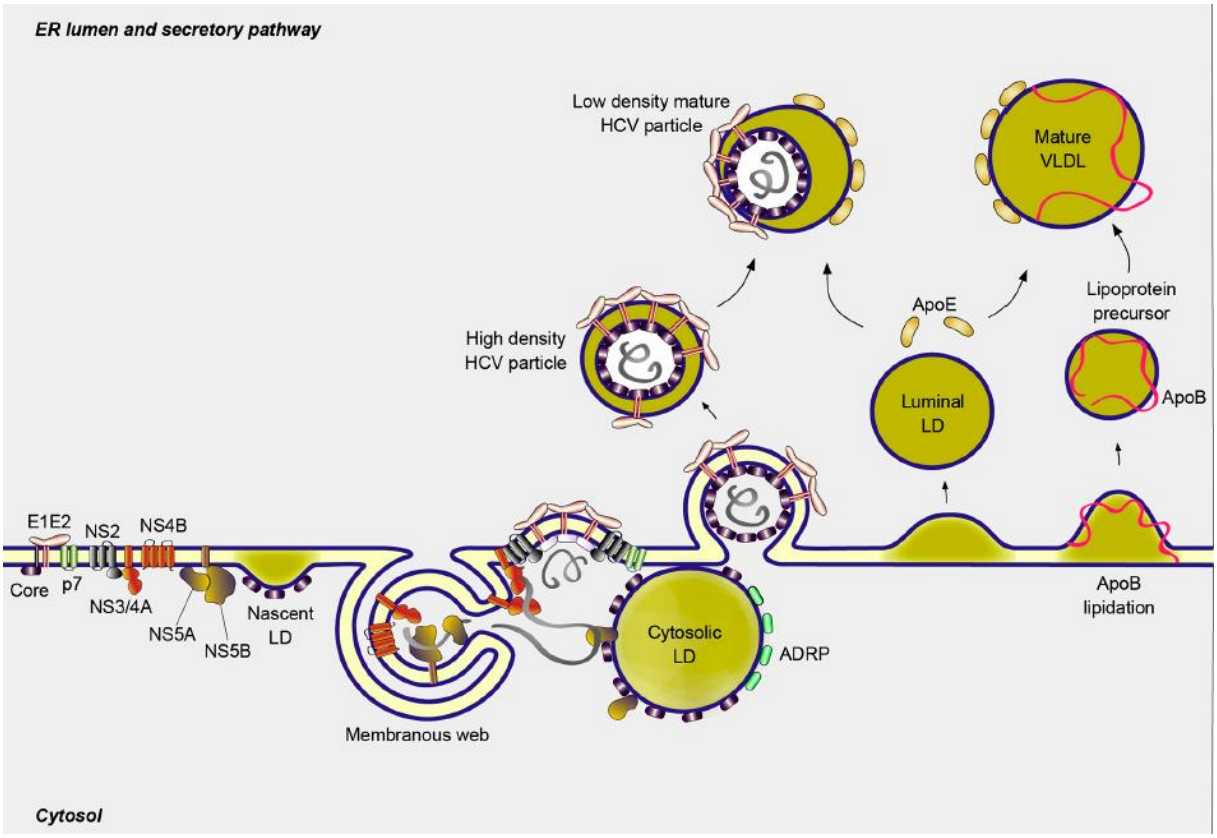
O HCV possui ainda uma região hipervariável (HVR-1) em seu genoma, localizada na seqüência que codifica a E2, envolvida na interação com o receptor SR-BI (receptor Scavenger classe B tipo I) que está relacionada à entrada de diversas lipoproteínas nas células (SCARSELLI *et al.*, 2002). Devido a sua alta variabilidade, essa região pode contribuir para a fuga da resposta imune do hospedeiro assim como para uma pior resposta ao tratamento (FARCI *et al.*, 2002; VON HAHN *et al.*, 2007).

A montagem e liberação das partículas virais do HCV é um processo regulado pela síntese lipídica da célula hospedeira (SCHEEL;RICE, 2013). A proteína core é deslocada da membrana do RE para gotículas lipídicas citoplasmáticas (cLDs) auxiliada pela diacilglicerol aciltransferase-1 (DGAT1) (MCLAUCHLAN *et al.*, 2002; BOULANT *et al.*, 2006; HERKER *et al.*, 2010). A formação do nucleocapsídeo envolve a interação da proteína core com a NS5A (MASAKI *et al.*, 2008). O processo de transporte do RNA viral para os sítios de montagem do nucleocapsídeo ainda é pouco conhecido, mas provavelmente é facilitado pela proximidade entre os sítios de montagem e replicação do RNA e também pela coordenação do processo de montagem exercido pela NS2 (JIRASKO *et al.*, 2010; POPESCU *et al.*, 2011; SCHEEL;RICE, 2013).

Durante os estágios finais de montagem, o nucleocapsídeo é então transferido para gotículas lipídicas precursoras do VLDL, que se fundem com apoE, apoB, apoA1, apoC1, apoC2 e apoC3 para formarem lipoviropartículas (LVPs), que são liberadas através do complexo de Golgi (CHANG *et al.*, 2007; GASTAMINZA *et al.*, 2008; COUNIHAN;RAWLINSON;LINDENBACH, 2011; COLLER *et al.*, 2012) (Figura 4). Estudos em culturas de células de hepatoma tem demonstrado que o HCV possui a capacidade de infectar células vizinhas diretamente (TIMPE *et al.*, 2008; RAMAKRISHNAIAH *et al.*,

2013), este mecanismo é corroborado por experimentos mostrando que hepatócitos infectados estão dispostos em aglomerados no fígado infectado (WIELAND *et al.*, 2014).

Figura 4. Representação esquemática das etapas finais do ciclo de vida do HCV



Fonte: (DUBUISSON;COSSET, 2014)

2.1.4 História natural

A hepatite C aguda normalmente está relacionada aos 6 primeiros meses de infecção e apresenta uma evolução subclínica envolvendo sintomas inespecíficos como anorexia, astenia, mal-estar, dor abdominal e icterícia em alguns casos. Apenas cerca de 15-30% dos casos apresentarão algum tipo de sintoma, que normalmente surge entre 5-12 semanas após infecção e dura entre 2-12 semanas (ORLAND;WRIGHT;COOPER, 2001). Devido ao quadro clínico ser semelhante a outras hepatites virais, o diagnóstico diferencial é somente possível através de testes sorológicos (HAJARIZADEH;GREBELY;DORE, 2013).

O curso da infecção é caracterizado pela detecção do HCV RNA no sangue entre 2-14 dias após infecção, aumento dos níveis séricos das transaminases hepáticas (aspartato aminotransferase – AST e alanina aminotransferase – ALT) entre 2-8 semanas e aumento gradual de anticorpos anti-HCV entre 20-150 dias após infecção (COX;NETSKI*; et al.*, 2005;

GLYNN *et al.*, 2005; PAGE-SHAFER *et al.*, 2008). Aproximadamente 25% dos indivíduos com infecção aguda conseguem eliminar o vírus espontaneamente, enquanto que 75% desenvolverão infecção crônica, caracterizado pela persistência dos níveis elevados de HCV RNA e transaminases por mais de 6 meses (MICALLEF;KALDOR;DORE, 2006). O motivo pelo qual alguns pacientes se curam espontaneamente ou desenvolvem a forma crônica ainda não é claro, mas provavelmente está relacionado a fatores do hospedeiro como sexo, idade, raça, desenvolvimento de icterícia, resposta imune eficiente, assim como fatores genéticos (HOOFNAGLE, 2002; ROMERO-GOMEZ *et al.*, 2011; ESTRABAUD *et al.*, 2012).

A mortalidade associada à hepatite C crônica é resultado, principalmente, do desenvolvimento de fibrose hepática e suas posteriores complicações, tais como insuficiência hepática, hipertensão portal e carcinoma hepatocelular (HCC). Aproximadamente 10-20% dos indivíduos com infecção crônica podem evoluir para cirrose em 20 anos (LAVANCHY, 2009). Uma vez que a cirrose é estabelecida, o risco anual para desenvolvimento de HCC é de 1-5% e de 3-6% para descompensação hepática (varizes esofágicas, hemorragia digestiva alta, ascite e encefalopatia) (WESTBROOK;DUSHEIKO, 2014) (Figura 5).

Figura 5. Curso clínico da hepatite C crônica.

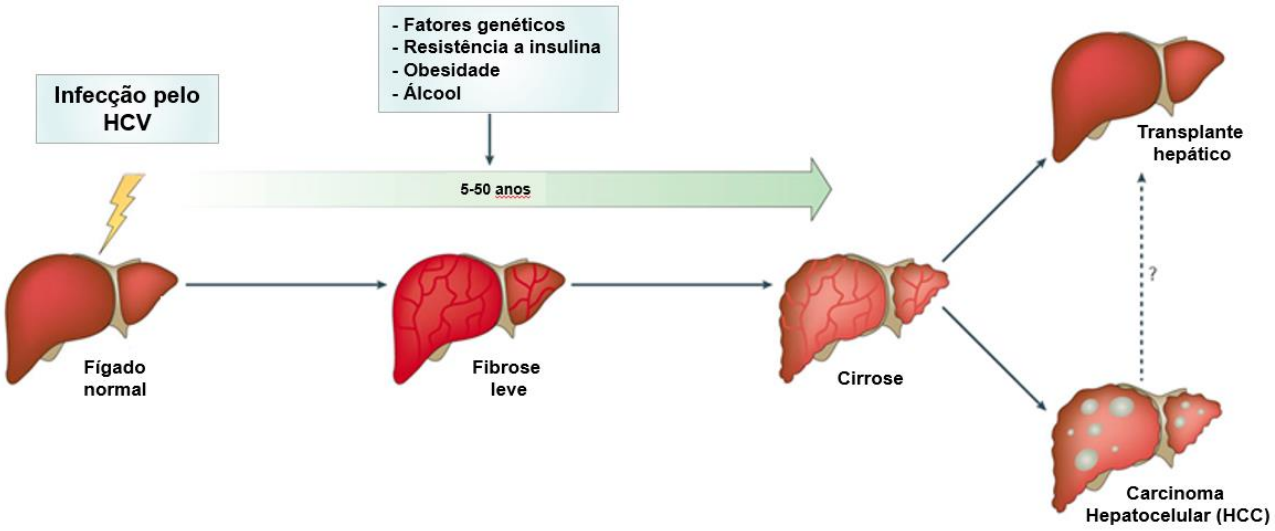


Figura adaptada de (PELLICORO *et al.*, 2014)

A fibrogênese é um processo complexo, acompanhado de necroinflamação e ativação de células estreladas hepáticas (HSCs), que no fígado resulta no acúmulo em excesso de proteínas da matriz extracelular, entre elas, o colágeno (FRIEDMAN, 2008). No fígado normal, as HSCs residem no espaço de Disse e são as principais fontes de vitamina A. Após lesão crônica, as HSCs são ativadas, passando a expressar características de miofibroblastos, com

geração e proliferação de α-actina de músculo liso (α-AML) e adquirindo propriedades pró-inflamatórias (MARRA, 1999; MANN;SMART, 2002).

Em pacientes com hepatite C crônica, o risco de HCC aumenta de acordo com a progressão da fibrose. O desenvolvimento de HCC é um processo de múltiplas etapas que pode levar entre 20-40 anos, envolvendo o estabelecimento da infecção crônica pelo HCV, inflamação hepática persistente, fibrose hepática avançada, surgimento de clones neoplásicos acompanhados por alterações genéticas/epigenéticas irreversíveis, e progressão de clones malignos em um microambiente cancerígeno (HOSHIDA *et al.*, 2014).

A progressão da fibrose determina o prognóstico da doença e a necessidade do tratamento, sendo a biópsia hepática o padrão ouro em hepatite crônica para determinar o estágio de fibrose (MARCELLIN, 2009; DHINGRA;WARD;THUNG, 2016). Para fornecer uma análise semi-quantitativa da fibrose e prognóstico clínico, escalas de pontuação (Knodell, Metavir, Ishak) têm sido utilizadas em diversos estudos (BEDOSSA;POYNARD, 1996; HOOFNAGLE, 2002; ASSELAH *et al.*, 2009; MARCELLIN, 2009). Existem vários fatores do hospedeiro envolvidos com a progressão da fibrose e desenvolvimento de HCC, como exemplo, idade avançada, sexo masculino, consumo excessivo de álcool, esteatose hepática, resistência à insulina, imunossupressão, diabetes, co-infecção com hepatite B e fatores genéticos do hospedeiro (POYNARD;BEDOSSA;OPOLON, 1997; HOOFNAGLE, 2002; MINOLA *et al.*, 2002; MOUCARI *et al.*, 2008; ESTRABAUD *et al.*, 2012; WESTBROOK;DUSHEIKO, 2014). Carga e genótipo viral não têm influenciado significativamente a taxa de progressão (MARCELLIN, 2009).

2.1.5 Tratamento da hepatite C crônica

Diferente das hepatites A e B, uma vacina para a hepatite C ainda não está disponível, e até então a única solução para os pacientes cronicamente infectados é o tratamento com medicamentos antivirais. O objetivo do tratamento antiviral é a eliminação completa do HCV, definida pelos níveis de HCV RNA indetectáveis por 12 ou mais semanas após o término do tratamento (resposta virológica sustentada ou SVR) (PANEL, 2015). A cura da infecção traz inúmeros benefícios para o paciente, incluindo uma diminuição na inflamação hepática, regressão da fibrose na maioria dos casos, e resolução da cirrose em metade dos indivíduos (POYNARD *et al.*, 2002). Além de melhorar a hipertensão portal, esplenomegalia e outras manifestações clínicas presentes nos indivíduos cirróticos. O alcance da SVR também reduz em 70% o risco de HCC e em 90% o risco de mortalidade associada a doença hepática e

transplante hepático (VELDT *et al.*, 2007; VAN DER MEER *et al.*, 2012; MORGAN *et al.*, 2013; PANEL, 2015).

Nos últimos anos ocorreu um enorme progresso no desenvolvimento de medicamentos antivirais para o tratamento da hepatite C. Antes de 2011, o esquema de tratamento padrão para o tratamento da hepatite C era o uso de interferon alfa peguilado (PEG-IFN-α) + ribavirina (RBV) por um período de 24 a 48 semanas, dependendo do genótipo viral (LIVER, 2011). Esse esquema terapêutico caracterizava-se por vários efeitos colaterais, longo período de tratamento e baixas taxas de SVR (aproximadamente 50% para indivíduos com genótipo 1 ou 4 e 80% para genótipos 2 ou 3) (GHANY *et al.*, 2009).

A partir de 2011, novas drogas denominadas antivirais de ação direta (DAAs) foram aprovadas para o tratamento da hepatite C. Essas drogas possuem a característica de atuar diretamente sobre proteínas virais, sendo classificadas em 3 grupos. O primeiro grupo são os inibidores de protease (IPs) (terminação *–previr*), que possuem como alvo a protease viral NS3/4A, o segundo grupo são os inibidores da polimerase NS5B (terminação *–buvir*), e o terceiro grupo os inibidores de NS5A (terminação *–asvir*) (ZOPF *et al.*, 2016).

Em 2011, duas novas drogas foram aprovadas para o tratamento de pacientes com genótipo 1, o boceprevir e o telaprevir. Essas drogas compõem a primeira geração dos DAAs e tem como alvo a protease viral NS3/4A. Ambos, boceprevir e telaprevir, são utilizados em esquema de terapia tripla, em combinação com o PEG-IFN-α e ribavirina. Com o esquema de terapia tripla, as taxas de SVR para indivíduos com genótipo 1 subiram para cerca de 65-75% (BACON *et al.*, 2011; JACOBSON *et al.*, 2011; POORDAD *et al.*, 2011; ZEUZEM *et al.*, 2011).

Em 2014, novos DAAs possibilitaram a redução da dose para uma vez ao dia e reduziram a duração do tratamento com PEG-IFN-α e ribavirina. O uso do simeprevir, daclatasvir ou sofosbuvir em combinação com PEG-IFN-α e ribavirina por 12-24 semanas aumentou para 60-100% a taxa de SVR, dependendo do esquema utilizado, do genótipo viral, da presença de mutações de resistência e da gravidade da doença hepática (POL *et al.*, 2012; FRIED *et al.*, 2013; KOWDLEY *et al.*, 2013; SULKOWSKI *et al.*, 2013; PAWLOTSKY;AGHEMO;BACK, 2015).

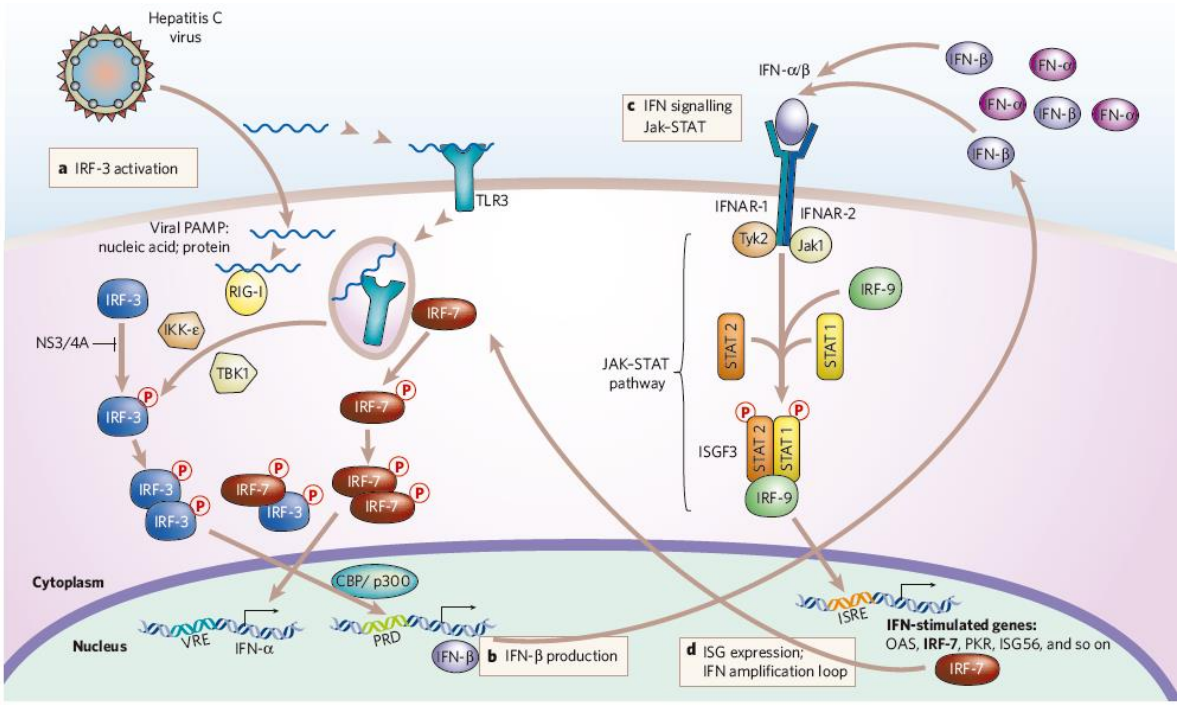
Em 2015, esquemas de tratamento livres de interferon (IFN-free) passaram a ser utilizados como principais armas no combate ao HCV. Esses esquemas apresentam uma taxa de SVR entre 92-100% para pacientes com genótipo 1, assim como maior tolerabilidade (WEBSTER;KLENERMAN;DUSHEIKO, 2015). Os novos medicamentos também permitem

que o tratamento de pacientes coinfectados com HIV seja realizado de forma análoga ao de monoinfectados pelo HCV (BRASIL, 2015b). Esses esquemas baseiam-se no uso de um IP ou um inibidor de NS5A mais um inibidor de NS5B, com ou sem ribavirina, ou o uso de um IP, um inibidor de NS5A e um inibidor de NS5B, com ou sem ribavirina, ou um IP mais um inibidor de NS5A, com ou sem ribavirina (WEBSTER;KLENERMAN;DUSHEIKO, 2015). Em meados de 2016 é esperado que novas drogas com efeito pangenotípico e com duração de 6-8 semanas estejam disponíveis para comercialização. Espera-se que essas drogas consigam aumentar as taxas de SVR em pacientes difíceis de tratar, como os indivíduos cirróticos com genótipo 3, que ainda apresentam taxas de SVR entre 86-90% (FLAMM *et al.*, 2014; ZOPF *et al.*, 2016).

2.1.6 Resposta imune na hepatite C

A resposta imune inata e adaptativa desempenham fundamental importância na infecção pelo HCV. O RNA viral durante as etapas de infecção ou replicação, que incluem sua forma em fita simples (ssRNA) ou fita dupla (dsRNA), atuam como padrões moleculares associado ao patógeno (PAMP), sendo reconhecido por receptores celulares de células hospedeiras e ativando cascatas de sinalização que induzem a produção de interferons tipo I e III (MEURS;BREIMAN, 2007; HEIM;THIMME, 2014) (Figura 6).

Figura 6. Processo de reconhecimento e ativação da imunidade na infecção por HCV.



Fonte: (GALE;FOY, 2005)

Os principais receptores de reconhecimento padrão (RRP) envolvidos no reconhecimento de PAMPs do HCV são o receptor semelhante ao Toll 3 (TLR3) e o receptor do gene 1 induzível por ácido retinóico (RIG-I). Após a ligação de PAMPs a esses receptores, vias de sinalização são ativadas, e convergem para a ativação de fatores de transcrição importantes como o fator nuclear kappa B (NF-κB) e fatores de regulação de interferon (IRF) 3 e 7, que atuarão na transcrição de genes relacionados ao interferon tipo I e III (LOO;GALE, 2011; LI *et al.*, 2012; HEIM;THIMME, 2014). O NF-kB também está envolvido na indução da expressão de quimiocinas e citocinas pró-inflamatórias que atuam na resposta imune contra o HCV (TAI *et al.*, 2000).

O genoma humano codifica 14 tipos de interferons tipo I, sendo um gene associado ao IFNβ e outros 13 associados a subtipos de IFNα (PESTKA, 2007). Por outro lado, os interferons tipo III (ou IFNλ) possuem 3 genes, IL29, IL28A e IL28B, que codificam os IFNλ1, IFNλ2 e IFNλ3, respectivamente (LI *et al.*, 2009). Uma vez que os interferons são produzidos, eles são secretados da célula e atuam de forma autócrina ou parácrina para promoverem a resposta antiviral. Os interferons tipo I se ligam aos receptores IFNAR1 e IFNAR2 (UZE *et al.*, 2007), enquanto os interferons tipo III se ligam aos receptores IL-10R2 e IL28RA (KOTENKO *et al.*, 2003; SHEPPARD *et al.*, 2003). A ligação dos interferons a seus respectivos receptores ativam a via Janus quinase/transdutores de sinais e ativadores de transcrição (JAK/STAT), através do recrutamento das quinases JAK1 e tirosina quinase 2 (TYK2), que levam a fosforilação e dimerização de STAT1 e STAT2. STAT1/STAT2 se associam com o fator regulador de interferon (IRF) 9 para formar o complexo ativo ISGF3, resultando na transcrição de vários genes estimulados por interferon (ISGs) (STARK;DARNELL, 2012; SCHOGGINS;RICE, 2013). Coletivamente, esses genes facilitam a eliminação dos vírus em células infectadas e protegem células vizinhas. Eles também agem recrutando células imunes efetoras para o sítio de infecção e promovem a resposta imune adaptativa (SCHOGGINS;RICE, 2013). Recentemente foi demonstrado que a proteína quinase R (PKR) também se liga ao dsRNA do HCV e ativa uma cascata de transdução de sinais que induz a produção de ISGs específicas e IFN-β, antes da ativação via RIG-I (KUMAR *et al.*, 1997; MCALLISTER;SAMUEL, 2009; ARNAUD *et al.*, 2011). Apesar de ainda não estar completamente elucidado os mecanismos de comunicação entre RIG-I e PKR, provavelmente essas duas moléculas atuam em conjunto para a ativação da resposta antiviral (HORNER;GALE JR, 2013).

A ativação da resposta mediada por interferon no fígado infectado pelo HCV aparentemente possui pouca eficácia na maioria dos pacientes, uma vez que cerca de 80% dos indivíduos infectados desenvolvem a forma crônica da doença. Esta alta taxa de cronicidade se deve ao fato do HCV ter desenvolvido mecanismos de evasão da resposta imune do hospedeiro, sendo a protease viral NS3/4A um componente importante na inibição da resposta imune (MORIKAWA *et al.*, 2011). A NS3/4A bloqueia a sinalização de RIG-I e TLR3 através da clivagem da proteína de sinalização antiviral mitocondrial (MAVS) e do adaptador contendo domínio TIR indutor de interferon-β (TRIF), respectivamente (LI, K. *et al.*, 2005; LI, X.-D. *et al.*, 2005; BARIL *et al.*, 2009). Esses componentes desempenham papel essencial na cascata de sinalização via RIG-I e TLR3, portanto sua clivagem pelo NS3/4A tem efeito direto na redução da produção de interferon pela célula infectada, assim como na resposta inflamatória, contribuindo para a persistência da infecção (HORNER;GALE JR, 2013). Além da NS3/4A, outros componentes virais também contribuem para a evasão viral da resposta imune, a proteína core interfere na via JAK/STAT através da ativação do inibidor SOCS-3, aumenta a degradação de STAT1 e inibe a ativação e translocação de STAT1 (BODE *et al.*, 2003; LIN *et al.*, 2005; LIN, R. *et al.*, 2006). A proteína E2 inibe a PKR e a função de células natural killer (NK) (CROTTA *et al.*, 2002; TSENG;KLIMPEL, 2002). A NS5A também desempenha importante função no bloqueio de proteínas antivirais estimuladas pelo interferon, além de bloquear a atividade da PKR (NOGUCHI *et al.*, 2001; GEISS *et al.*, 2003; ASHFAQ *et al.*, 2011).

A resposta antiviral mediada por interferon é de fundamental importância na supressão da replicação viral e modulação da resposta imune, e por isso o interferon tem sido utilizado por anos como droga de escolha para o tratamento da hepatite C. Entretanto grande parte dos pacientes tratados com PEG-IFN-α associado a ribavirina não conseguem eliminar o HCV ao término do tratamento, mantendo altos níveis de viremia (TAI;CHUNG, 2009). O genótipo viral explica, pelo menos em parte, por que alguns pacientes respondem ao tratamento e outros não. Pacientes infectados com genótipos 2 ou 3 apresentam uma maior taxa de SVR que pacientes infectados com genótipo 1 e 4 (PANG;PLANET;GLENN, 2009). O motivo pelo qual esses pacientes têm maior dificuldade em eliminar o HCV provavelmente está relacionado a maior eficácia que esses genótipos possuem em bloquear vias importantes na resposta imune antiviral (GALE *et al.*, 1997; TAYLOR *et al.*, 1999; NOGUCHI *et al.*, 2001). Além disso, polimorfismos no gene *IL28B* são os principais fatores do hospedeiro associados com a resposta a terapia antiviral e a resolução espontânea da infecção. Indivíduos com o genótipo favorável CC na posição rs12979860 possuem maiores chances em responder ao tratamento ou se curarem espontaneamente do que pacientes com genótipos CT/TT (HAYES *et al.*, 2012).

Portanto, uma combinação de fatores virais e genéticos do hospedeiro relacionados com a regulação da resposta imune e sinalização via interferon, irão contribuir para a resolução espontânea da infecção e resposta ao tratamento.

As vias de sinalização mediadas pelos RRP além de ativar um estado antiviral nas células através da produção de interferons, também modulam a resposta imune adaptativa durante a infecção pelo HCV. Anticorpos específicos contra o HCV são normalmente detectados cerca de 7-8 semanas após infecção, entretanto a maioria desses anticorpos não possui atividade antiviral (ASHFAQ *et al.*, 2011). Apenas uma porção dos anticorpos produzidos, conhecidos como anticorpos neutralizantes, são capazes de inibir a invasão viral, através da ligação a regiões hipervariáveis presentes nas glicoproteínas do envelope viral E1 e E2 (SABO *et al.*, 2011; THIMME;BINDER;BARTENSCHLAGER, 2012). Entretanto, esses anticorpos parecem não ter uma função importante na resolução da infecção pelo HCV, uma vez que mesmo indivíduos com hipogamaglobulinemia são capazes de eliminar a infecção após tratamento com interferon (CHRISTIE *et al.*, 1997; SEMMO *et al.*, 2006).

Vários estudos têm demonstrado que a eliminação do HCV está associada a uma resposta intensa e sustentada por células T CD4+ e CD8+ contra diferentes epítopos virais (DIEPOLDER *et al.*, 1995; MISSALE *et al.*, 1996; DIEPOLDER *et al.*, 1997; COOPER *et al.*, 1999; LECHNER *et al.*, 2000; THIMME *et al.*, 2001; DAY *et al.*, 2002; THIMME *et al.*, 2002; COX;MOSBRUGER*; et al.*, 2005; THIMME;BINDER;BARTENSCHLAGER, 2012). Após cerca de 6-8 semanas, linfócitos T vírus específicos passam a ser detectados e coincidem com o aumento das transaminases hepáticas, início dos sintomas e um leve declínio da carga viral (THIMME *et al.*, 2001; COX;MOSBRUGER*; et al.*, 2005; NEUMANN-HAEFELIN;THIMME, 2013). Os linfócitos T CD8+ são considerados as principais células efetoras na infecção pelo HCV, pois são capazes de controlar a replicação viral *in vivo* (SHOUKRY *et al.*, 2003) e alelos específicos do HLA de classe I têm sido associados com a resolução espontânea da infecção (THIMME;BINDER;BARTENSCHLAGER, 2012). Os linfócitos T CD8+ desempenham seus efeitos antivirais promovendo a morte de hepatócitos infectados e também através da secreção de citocinas como IFN-γ e TNF-α (GUIDOTTI;CHISARI, 2001; JO *et al.*, 2009) . Alguns estudos também têm demonstrado a presença de subpopulações de linfócitos T CD8+ secretoras de IL-17 envolvidas no controle da infecção pelo HCV (NORTHFIELD *et al.*, 2008; BILLERBECK *et al.*, 2010; GRAFMUELLER *et al.*, 2012).

Os linfócitos T CD4+ também desempenham importante função na resposta imune contra o HCV, reconhecendo antígenos ligados a moléculas de MHC de classe II na superfície

de células apresentadoras de antígeno (ASHFAQ *et al.*, 2011). Alelos relacionados ao HLA classe II também tem sido associados com a resolução espontânea da infecção pelo HCV (THIMME;BINDER;BARTENSCHLAGER, 2012; NEUMANN-HAEFELIN;THIMME, 2013). Portanto, esses achados demonstram que os linfócitos T CD8+ são as principais células efetoras na infecção pelo HCV, e que os linfócitos T CD4+ desempenham importante função na regulação da resposta imune adaptativa antiviral, entretanto essa resposta parece ser insuficiente para eliminação do HCV na maioria dos casos (THIMME;BINDER;BARTENSCHLAGER, 2012).

2.1.7 Imunopatogênese

Os mecanismos associados à lesão hepática na hepatite C ainda não são bem compreendidos, mas a resposta imune do hospedeiro parece exercer um papel importante na patogênese do HCV, uma vez que o HCV não possui efeito citopático nas células infectadas. A lesão hepática na hepatite C crônica é principalmente composta por infiltrado linfoide portal, necrose em ponte e focal e lesões lobulares degenerativas. O infiltrado linfoide é principalmente composto por linfócitos T CD4+ localizados dentro do espaço periportal, sendo predominante células do tipo Th1, e células T CD8+ localizadas nas regiões periportais e lobulares (BERTOLETTI *et al.*, 1997; FIORE *et al.*, 1997; PAWLOTSKY, 2004).

A fibrogênese é resultado de um mecanismo complexo envolvendo a resposta inflamatória mediada por citocinas e a ativação de HSCs. Na tentativa de eliminar o HCV, hepatócitos são lesados, gerando corpos apoptóticos que ativam HSCs quiescentes e células de Kupffer, que por sua vez contribuem para a liberação de citocinas pró-inflamatórias e mediadores fibrogênicos (CANBAY *et al.*, 2003; FRIEDMAN, 2008; LEE;FRIEDMAN, 2011). Evidências sugerem que padrões moleculares associados ao dano (DAMPs) liberados por hepatócitos lesados também podem contribuir com a fibrogênese (LUEDDE;KAPLOWITZ;SCHWABE, 2014; SZABO;PETRASEK, 2015). Este processo de cicatrização mediado pela deposição de tecido fibroso em lesões agudas possui um efeito benéfico pelas seguintes razões: 1) a fibrose proporciona estabilidade mecânica; 2) as células inflamatórias contribuem para a remoção de detritos celulares; e 3) sinais inflamatórios também exercem funções importantes na promoção da regeneração hepática (SEKI;SCHWABE, 2015). Entretanto, quando o dano local é persistente, mediado por uma resposta inflamatória crônica, o tecido normal é substituído por tecido fibroso, comprometendo a arquitetura e função hepática.

Vários fatores de crescimento, citocinas inflamatórias e quimiocinas, estão envolvidos no processo de ativação das HSCs e sua transformação em miofibroblastos, contribuindo para deposição de tecido fibroso. Citocinas são uma família de moléculas envolvidas na transmissão de sinais entre células. Elas incluem as quimiocinas, interferons, interleucinas, linfocinas e fatores de crescimento. Após a lesão hepática, vários tipos celulares produzem citocinas, incluindo células de Kupffer, hepatócitos, HSCs, células natural killer (NK), linfócitos e células dendríticas (HERNANDEZ-GEA;FRIEDMAN, 2011). O fator de transformação de crescimento β (TGF-β) é considerado uma das principais citocinas envolvidas no processo fibrogênico, junto com o fator de crescimento endotelial vascular (VEGF), elas contribuem para a ativação e proliferação das HSCs, assim como na angiogênese hepática (SEBASTIANI;GKOUVATSOS;PANTOPOULOS, 2014). O TGF-β também suprime a atividade das células NK, que são importantes na indução de apoptose das HSCs (JEONG *et al.*, 2011).

Estudos têm demonstrado que a produção de IL-1β por células de Kupffer, induz inflamação hepática através da via do inflamassoma, mediado por NLRP3, TLR7 e caspase-1. A ativação do inflamassoma em pacientes com HCV resulta em produção de IL-18, que por sua vez ativa células NKT (BURDETTE *et al.*, 2012; CHEN *et al.*, 2014; SERTI *et al.*, 2014; SZABO;PETRASEK, 2015). O fator de necrose tumoral alfa (TNF-α) é outra citocina pró-inflamatória, encontrada em níveis elevados em pacientes com HCV e fibrose avançada (AVRĂMESCU *et al.*, 2008; AROUCHA *et al.*, 2013). O TNF-α contribui para a apoptose de hepatócitos, ativação de células do sistema imune, e ativação de HSCs. Evidencias sugerem que uma quebra no balanço entre a produção de TNF-α e IL-10, uma interleucina anti-inflamatória, pode contribuir para a progressão da fibrose em pacientes com HCV (AROUCHA *et al.*, 2013). Vários estudos recentes em modelos animais e em humanos têm apontado o envolvimento de uma outra citocina, a IL-22, na redução da fibrose hepática causada por diferentes etiologias (COBLEIGH;ROBEK, 2013). Outros mediadores inflamatórios também têm sido associados com o processo de fibrogênese em hepatites virais, incluindo CCL2, CCL21, IL-8, IL-17, IL-20, CXCL9, CXCL10, CXCL11, CXCR1, entre outros (SEKI;SCHWABE, 2015).

O stress oxidativo também é um importante fator associado com a ativação de HSCs e miofibroblastos. Durante a resposta imune por vírus, neutrófilos e outras células produzem espécies reativas ao oxigênio (ROS) como um mecanismo de proteção (GIL *et al.*, 2004). O estresse oxidativo representa o desequilíbrio entre o aumento da produção de ROS e a baixa capacidade celular antioxidante, tendo a inflamação um importante papel no desencadeamento

desse processo (SAHNOUN;JAMOUSSI;ZEGHAL, 1996; YAMANE *et al.*, 2013). Vários estudos têm destacado o papel do stress oxidativo com a fisiopatologia da fibrose hepática (FARINATI *et al.*, 1995; DE MARIA *et al.*, 1996; GREENWEL *et al.*, 2000). Proteínas virais, NS3 e NS5A, atuam como importantes indutores de estresse oxidativo e inflamação. Evidências sugerem que a associação da proteína NS5A com o retículo endoplasmático estimula a produção de ROS através da liberação de cálcio presente no retículo (GONG *et al.*, 2001). Além do envolvimento do stress oxidativo com a deposição de tecido fibroso, estudos têm demonstrado sua relação com a supressão de mecanismos envolvidos no reparo de DNA, aumentando o risco de desenvolvimento de HCC em indivíduos com HCV (ZEKRI *et al.*, 2005; PAL *et al.*, 2010).

Sendo assim, é bastante evidente que a resposta imune montada pelo hospedeiro na tentativa de combater o HCV possui um papel importante na patogênese da hepatite C. Diferenças no padrão da resposta imune de cada indivíduo podem explicar, pelo menos em parte, diferentes taxas de progressão da fibrose e desenvolvimento de HCC em pacientes infectados com HCV.

2.1.8 Marcadores genéticos na hepatite C

Acredita-se que a diversidade genética do hospedeiro exerce função em cada etapa de diferentes resultados clínicos na infecção pelo HCV, como cura da infecção aguda, progressão da fibrose e resposta ao tratamento (ASSELAH *et al.*, 2009). Polimorfismos de único nucleotídeo (SNPs) são variações na sequência de DNA que afetam apenas uma base na sequência do genoma. São consideradas a forma mais comum de variação no genoma humano, ocorrendo em frequências >1%, podendo provocar mudanças estruturais ou quantitativas na proteína codificada (WANG *et al.*, 1998). Desde o sequenciamento completo do genoma humano em 2001 (VENTER *et al.*, 2001), grandes avanços têm sido feitos em relação às tecnologias de genotipagem, principalmente no custo e na descoberta em larga escala de novos SNPs (METZKER, 2010).

Vários estudos têm associado SNPs de genes envolvidos com a resposta imune e inflamatória com a progressão da fibrose e risco em desenvolver HCC. Polimorfismos nos genes da lectina ligadora de manose (*MBL2*) e alelos do antígeno leucocitário humano (HLA-II: DRB1*0405 e DQB1*0401), no qual exercem importante função na resposta imune do hospedeiro, têm sido associados à progressão da fibrose (AIKAWA *et al.*, 1996; ALVES PEDROSO *et al.*, 2008; HALLA *et al.*, 2010). Genes envolvidos com a expressão de citocinas,

como interleucina-12 (IL-12), interleucina-10 (IL-10), interleucina-6 (IL-6), interleucina-1 (IL-1) e fator de necrose tumoral-α (TNF-α) também têm sido investigados em pacientes com HCV (BATALLER;NORTH;BRENNER, 2003; JENG *et al.*, 2007; BOUZGARROU *et al.*, 2009; BOUZGARROU *et al.*, 2011; ROMERO-GOMEZ *et al.*, 2011; ESTRABAUD *et al.*, 2012; CORCHADO *et al.*, 2013; TARRAGÔ *et al.*, 2014; AROUCHA *et al.*, 2016). De modo geral, a expressão aumentada de citocinas pró-inflamatórias em pacientes com HCV parece estar associada à progressão mais rápida para formas graves de fibrose e desenvolvimento de HCC.

O sequenciamento do exoma é uma técnica que tem como objetivo sequenciar todos os genes codificadores em um genoma. O genoma humano é composto por 180.000 éxons, equivalente a cerca de 1% do genoma, ou aproximadamente 30 milhões de pares de base (NG *et al.*, 2009). A grande vantagem dessa técnica é o sequenciamento em larga escala com baixo custo, objetivando a identificação de variantes genéticas associadas a doenças raras. Através dela, novos genes associados ao desenvolvimento de vários de tipos de câncer têm sido identificados, incluindo HCC (WOO *et al.*, 2014; SCHULZE *et al.*, 2015; SOKOLENKO *et al.*, 2015; ZHANG *et al.*, 2016). Em relação aos estudos com HCC, a maioria deles inclui pacientes com HCC causada por diferentes etiologias e se restringem a análise de material extraído do tumor (HUANG *et al.*, 2012; CLEARY *et al.*, 2013; WOO *et al.*, 2014; SCHULZE *et al.*, 2015). Apenas um trabalho identificou novas mutações somáticas associadas ao HCC em pacientes infectados exclusivamente com HCV, neste estudo Ikeda *et al*. (2014) identificaram mutações no gene do receptor de leptina (*LEPR*) associadas com o desenvolvimento de HCC em amostras de tecido hepático. Neste mesmo estudo, foi demonstrado que camundongos knockout para o *LEPR* apresentaram maior inflamação hepática e maior susceptibilidade ao desenvolvimento de tumores, quando comparados a camundongos selvagem (IKEDA *et al.*, 2014).

Ainda é pouco conhecida, a forma pela qual o sistema imune do hospedeiro atua em cada indivíduo no combate à infecção pelo HCV, uma vez que a gravidade de danos ao fígado é diferente em cada paciente. Uma grande variedade de fatores, entre eles genéticos, determina o tipo de resposta imune utilizado pelo hospedeiro para combater o vírus, e consequentemente, o grau de lesão hepática que a infecção causará no paciente. Portanto, um melhor entendimento desses fatores se torna de fundamental importância para definir o melhor tratamento do indivíduo infectado, tornando o combate ao HCV mais eficaz.

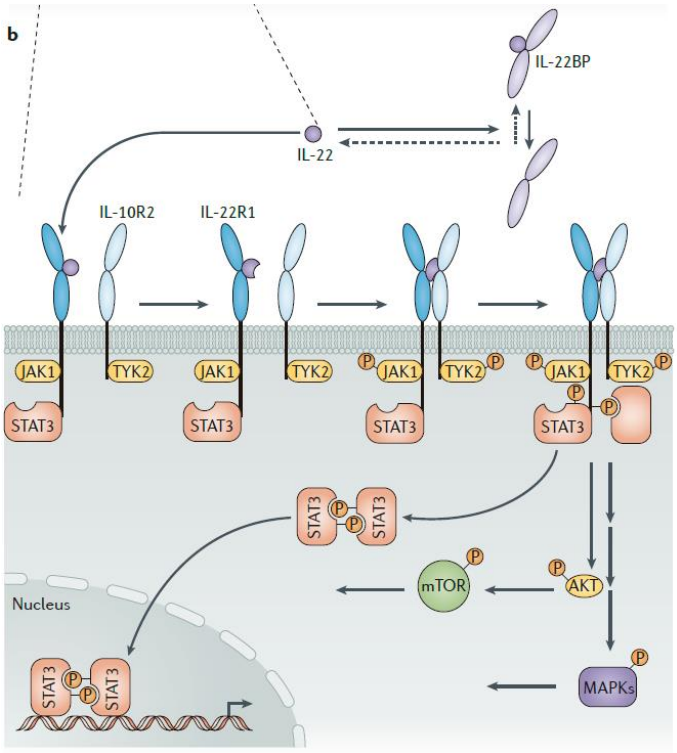
2.2 Interleucina-22 (IL-22)

A interleucina-22 (IL-22) é uma citocina pertencente à família da IL-10 (DUMOUTIER;LOUAHED;RENAULD, 2000). Seu gene (*IL22*) está localizado no cromossomo 12q15, codificando uma proteína de 146 aminoácidos (DUMOUTIER *et al.*, 2000; XIE *et al.*, 2000; SABAT;OUYANG;WOLK, 2014). Como outros membros da família da IL-10, a estrutura da IL-22 contém 6 α-hélices (de A a F) conectadas que se dobram em um pacote compacto (NAGEM *et al.*, 2002).

A IL-22 é produzida principalmente por células do sistema imune, incluindo linfócitos T CD4+ e T CD8+, linfócitos T γδ, células NK e células linfoides inatas (ILCs) (SABAT;OUYANG;WOLK, 2014). Entretanto, diferente da maioria das citocinas, a IL-22 não tem efeito sobre células hematopoiéticas, mas atua em células epiteliais, hepatócitos e células pancreáticas, sugerindo uma importante função na reparação de danos teciduais locais, ou contribuindo com a inflamação fisiopatológica (WOLK *et al.*, 2010).

O receptor da IL-22 é composto por duas subunidades transmembrana: o receptor IL-22-1 (IL-22R1) e o receptor IL-10-2 (IL-10R2) (RUTZ;EIDENSCHENK;OUYANG, 2013). A ligação da IL-22 ao complexo IL-22R1-IL-10R2 resulta na ativação da via JAK/STAT, levando a fosforilação desses receptores e das proteínas STAT (WOLK *et al.*, 2004) (Figura 7).

Figura 7. Interação da IL-22 com seus receptores e ativação da cascata de sinalização



Fonte: (SABAT;OUYANG;WOLK, 2014)

A sinalização dessas moléculas é capaz de regular a expressão de genes envolvidos em vários processos, como apoptose, ciclo celular e angiogênese (PAN *et al.*, 2014). Estudos tem demonstrado que a IL-22 possui um efeito benéfico na prevenção da doença inflamatória intestinal (LI *et al.*, 2014), danos hepáticos (RADAEVA *et al.*, 2004; ZENEWICZ *et al.*, 2008; KI *et al.*, 2010; KONG, X. *et al.*, 2013; PAN *et al.*, 2014) e colite ulcerativa (SUGIMOTO *et al.*, 2008). Por outro lado, a IL-22 tem sido associada a patogênese de algumas doenças como psoríase (HAO, 2014), artrite reumatoide (LEIPE *et al.*, 2011; DA ROCHA *et al.*, 2012), doença de Crohn (WOLK *et al.*, 2007) e hepatites virais (ZHAO *et al.*, 2014; WU *et al.*, 2015).

Além dos receptores transmembrana, a IL-22 também é capaz de se ligar a um receptor solúvel conhecido como proteína de ligação a IL-22 (IL-22BP ou IL-22RA2). Essa ligação é de alta afinidade (cerca de 1000 vezes maior que pelo IL-22R1) e previne que a IL-22 exerça seus efeitos celulares (DUMOUTIER;LOUAHED;RENAULD, 2000; GRUENBERG *et al.*, 2001; KOTENKO *et al.*, 2001; JONES;LOGSDON;WALTER, 2008; WOLK *et al.*, 2010). O gene que codifica a IL-22BP humana está localizado no cromossomo 6q23.3, entre os genes *IFNGR1* e *IL20RA*, e produz uma proteína de 210 aminoácidos, possuindo 34% de identidade com o domínio extracelular do IL-22R1 (DUMOUTIER;LOUAHED;RENAULD, 2000; KOTENKO *et al.*, 2001; XU *et al.*, 2001).

A expressão de IL-22BP tem sido demonstrada em diferentes tecidos, como placenta, mama, timo, baço, linfonodos, trato gastrointestinal, pulmões e pele (DUMOUTIER;LOUAHED;RENAULD, 2000; GRUENBERG *et al.*, 2001; XU *et al.*, 2001; WEISS *et al.*, 2004), apesar da principal fonte de IL-22BP ser constituída por células dendríticas imaturas (MARTIN *et al.*, 2014). Estudos anteriores têm demonstrado que a IL-22BP é inibida nos primeiros estágios de infecção (WEISS *et al.*, 2004; WOLK *et al.*, 2007; HUBER *et al.*, 2012), porém sua expressão é aumentada no fígado de camundongos em estágios tardios de infecção por *Toxoplasma gondii, Schistosoma mansoni and Mycobacterium avium* (WILSON *et al.*, 2010).

Vários estudos em camundongos demonstram que a IL-22 protege o fígado contra vários tipos de injúrias, promovendo a proliferação e sobrevivência dos hepatócitos (RADAEVA *et al.*, 2004; KI *et al.*, 2010; XING *et al.*, 2011; KONG *et al.*, 2012; KONG, X. *et al.*, 2013). Em modelos murinos de hepatite induzida por concanavalina A (ConA), CCl4 e ligante FAS, a superexpressão de IL-22 ou a administração de IL-22 recombinante protegeu o fígado de camundongos contra danos hepáticos graves, enquanto que a inibição de IL-22 com anticorpos anti-IL-22 ou através de camundongos knockout foi associado com uma maior gravidade de lesões hepáticas (PAN *et al.*, 2004; RADAEVA *et al.*, 2004; ZENEWICZ *et al.*, 2007; KONG

et al., 2012; LU *et al.*, 2015). A IL-22 foi também associada com a regeneração hepática e proteção em modelos de hepatectomia e de isquemia-reperfusão (REN;HU;COLLETTI, 2010; CHESTOVICH *et al.*, 2012).

Resultados contraditórios têm sido observados em estudos com hepatites virais. A IL-22 foi capaz de promover a proliferação de células tronco hepáticas, além de seus níveis intra-hepáticos terem sido inversamente correlacionados com estágios avançados de fibrose em pacientes com hepatite B crônica (FENG *et al.*, 2012; XIANG *et al.*, 2012). Entretanto, Zhao et al. (2014) observou uma relação positiva da expressão intra-hepática de IL-22 com estágios de fibrose em pacientes infectados com o vírus da hepatite B (HBV) (ZHAO *et al.*, 2014). Além disso, a neutralização de IL-22 reduziu o recrutamento de células hepáticas e consequentemente o dano hepático em modelos de camundongos transgênicos com HBV (ZHANG *et al.*, 2011; ZHAO *et al.*, 2014).

Pacientes com HCV apresentam níveis elevados de IL-22 (DAMBACHER *et al.*, 2008; PARK *et al.*, 2011; WU *et al.*, 2015), apesar da mesma não exercer nenhum efeito sobre a expressão de proteínas antivirais, nem inibir a replicação viral *in vitro* (DAMBACHER et al., 2008). Estudos investigando o papel da IL-22 na gravidade da doença hepática causada pelo HCV são escassos, apenas um estudo demonstrou uma associação positiva entre os níveis periféricos e intra-hepáticos de IL-22 com a gravidade da fibrose hepática (WU *et al.*, 2015).

Fica evidente através dos dados apresentados, que a IL-22 desempenha um efeito protetor na lesão hepática causada por diferentes agentes em modelos experimentais. Entretanto, o papel da IL-22 na progressão da lesão hepática causada pelo HCV ainda não é bem compreendido. Portanto, são necessários mais estudos para determinar o papel da IL-22/IL-22BP e suas variantes genéticas na progressão da doença hepática em pacientes com HCV.

2.3 Pentraxina 3 (PTX3)

A pentraxina 3 (PTX3) é um receptor de reconhecimento padrão solúvel, produzido por várias células mieloides (células dendríticas, monócitos, macrófagos, neutrófilos), epiteliais, endoteliais e mesenquimais (fibroblastos e adipócitos) (GARLANDA *et al.*, 2016). Seu gene (*PTX3*) está localizado no cromossomo 3, organizado em três exons e 2 introns, e produz uma glicoproteína multimérica de 381 aminoácidos, composta por uma região N-terminal única e um domínio C-terminal homólogo a outras pentraxinas, como a proteína C reativa (PCR) e o componente amiloide sérico P (SAP) (BREVIARIO *et al.*, 1992). Dois SNPs no *PTX3*

(rs1840680 e rs2305619) têm sido associados com alterações nos níveis de PTX3, o alelo A para ambos SNPs tem sido associado com níveis plasmáticos elevados de PTX3 (BARBATI *et al.*, 2012; DIAMOND *et al.*, 2012). Além disso, o alelo A tem sido associado com maior susceptibilidade a infecções bacterianas e fúngicas (OLESEN *et al.*, 2007; CHIARINI *et al.*, 2010; CUNHA *et al.*, 2014; WÓJTOWICZ *et al.*, 2015).

A PTX3 desempenha várias funções na imunidade inata e inflamação, sendo capaz de ativar e regular o sistema complemento (através de C1q, MBL e fator H), interagir com partículas microbianas e regular a inflamação (BOTTAZZI *et al.*, 1997; NAUTA *et al.*, 2003; INFORZATO *et al.*, 2013). A PTX3 atua como uma opsonina em infecções bacterianas e fúngicas, facilitando o reconhecimento e fagocitose, assim como é capaz de neutralizar partículas virais. Estudos apontam um papel protetor da PTX3 contra vários tipos de infecções fúngicas, bacterianas e virais, incluindo *Aspergillus fumigatus*, *Paracoccidoides brasiliensis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, influenza vírus (BOTTAZZI *et al.*, 2009). Embora estudos recentes têm demonstrado que a PTX3 é capaz de facilitar a invasão viral e replicação durante a fase aguda de infecção por alfavírus (vírus chikungunya e vírus do Rio Ross) (FOO *et al.*, 2015). Além disso, algumas evidências sugerem que a PTX3 pode estar envolvida no processo inflamatório hepático, uma vez que níveis plasmáticos elevados de PTX3 foram associados com a gravidade da fibrose hepática em pacientes com esteato-hepatite não alcoólica (NASH) (YONEDA *et al.*, 2008; BOGA *et al.*, 2015). Níveis intra-hepáticos de PTX3 também foram associados com uma maior inflamação hepática em indivíduos com lesão causada por paracetamol (CRAIG *et al.*, 2013).

Estudos também têm indicado um possível papel da PTX3 no desenvolvimento tumoral, uma vez que seus níveis têm sido associados com pior prognóstico em alguns tipos de câncer, como câncer gástrico, carcinoma pulmonar, carcinoma pancreático, liposarcoma, câncer de próstata e glioma (GERMANO *et al.*, 2010; DIAMANDIS *et al.*, 2011; KONDO *et al.*, 2013; LOCATELLI *et al.*, 2013; STALLONE *et al.*, 2014; CHOI *et al.*, 2015). Por outro lado, outros estudos têm demonstrado um papel oncosupressor da PTX3, uma vez que pacientes com carcinoma epidermóide esofágico apresentaram níveis de expressão diminuídos de PTX3 devido a uma hipermetilação na região promotora do gene (WANG *et al.*, 2011). Além disso, um estudo realizado em camundongos Ptx3 (-/-), observou uma maior susceptibilidade no desenvolvimento de carcinoma mesenquimal e epitelial, associado a um aumento na infiltração de macrófagos, na produção de citocinas pró-inflamatórias, na angiogênese, bem como um aumento na deposição dos componentes C3 e C5a do sistema complemento (BONAVITA *et al.*, 2015).

Ainda não está claro o papel da PTX3 no desenvolvimento de fibrose e câncer. Sem dúvida, a PTX3 possui um efeito importante na amplificação e regulação da resposta inflamatória, que por sua vez tem relação direta com o desenvolvimento de fibrose e câncer hepático, entretanto são necessários mais estudos com o objetivo de investigar o papel da PTX3 e suas variantes genéticas na susceptibilidade ao desenvolvimento de formas graves de HCV e outros agentes virais.

2.4 Supressor de sinalização de citocinas 5 (SOCS5)

A família de proteínas SOCS em mamíferos é constituída por 8 membros, que incluem a proteína contendo domínio SH2 induzida por citocina (CIS) e as proteínas SOCS1 a SOCS7, que se caracterizam por suas similaridades estruturais (PIESSEVAUX *et al.*, 2008). Essas moléculas desempenham importante papel na inibição da via JAK-STAT, que por sua vez está envolvida com a expressão de genes envolvidos com a imunidade, proliferação, diferenciação, apoptose e oncogênese (AARONSON;HORVATH, 2002; INAGAKI-OHARA *et al.*, 2013). A família SOCS está envolvida com o desenvolvimento de vários tipos de cânceres, estudos têm demonstrado que a diminuição na expressão de SOCS1 e SOCS3 está associada com o desenvolvimento de câncer de próstata, câncer de mama, carcinoma laríngeo, mieloma múltiplo, leucemia mieloide aguda, câncer pancreático e linfoma (ZHANG *et al.*, 2012; INAGAKI-OHARA *et al.*, 2013). Além disso, a diminuição na expressão de SOCS1 e SOCS3 está associada com a ativação de STAT1 e STAT3, respectivamente, e com o desenvolvimento de HCC (YOSHIDA *et al.*, 2004; NIWA *et al.*, 2005; OGATA *et al.*, 2006).

O SOCS5 é capaz de regular negativamente o receptor do fator de crescimento epidérmico (EGFR) e a sinalização da via JAK-STAT (KARIO *et al.*, 2005; NICHOLSON *et al.*, 2005; LINOSSI *et al.*, 2013). Estudos demonstram que a expressão de SOCS5 reduz a sinalização do fator de crescimento epidérmico (EGF), através da degradação do EGFR (KARIO *et al.*, 2005; NICHOLSON *et al.*, 2005). Além disso, o silenciamento epigenético da expressão de SOCS5 foi inversamente associado com a expressão de EGFR em hepatocarcinoma agressivo (CALVISI *et al.*, 2007). Em relação a via JAK/STAT, estudos mostram que SOCS5 é capaz de interagir diretamente com JAK1 a JAK4 e inibir a autofosforilação de JAK1 e JAK2 (LINOSSI *et al.*, 2013). Além disso, a inibição de SOCS5 está associada a um aumento na fosforilação de JAK1/2 e STAT1/3 (ZHUANG *et al.*, 2012).

As moléculas-alvo de SOCS5 (EGFR e JAK) desempenham importante papel no desenvolvimento de HCC, e também têm sido utilizadas como alvos terapêuticos no tratamento

de alguns tipos de câncer (PINES;KÖSTLER;YARDEN, 2010; PARDANANI *et al.*, 2011). Polimorfismos no gene *EGF*, associados com expressão aumentada de EGF, estão associados com o aumento da fibrose hepática e risco de desenvolvimento de HCC em pacientes com cirrose (TANABE *et al.*, 2008; DAYYEH *et al.*, 2011; CMET *et al.*, 2012; FALLETI *et al.*, 2012; SHEN *et al.*, 2015). Além disso, camundongos transgênicos com expressão hepática aumentada de EGF desenvolvem HCC rapidamente (TÖNJES *et al.*, 1995). Um outro estudo também demonstrou que a inibição do EGFR foi associada com uma redução da fibrogênese e prevenção de HCC (FUCHS *et al.*, 2014). A expressão aumentada da via JAK/STAT também está associada com a presença de HCC, quando comparados a tecidos normais (CALVISI *et al.*, 2006).

É bem estabelecida a relação de alguns membros da família SOCS com o desenvolvimento tumoral, principalmente SOCS1 e SOCS3, porém ainda não é conhecido o papel de SOCS5 no desenvolvimento de HCC e outros tipos de cânceres. Uma vez que esta molécula desempenha funções semelhantes com outros membros da família SOCS, regulando moléculas importantes associadas ao crescimento tumoral, SOCS5 é um alvo atraente para futuros estudos investigando sua relação com o câncer.

2.5 Serino protease 58 (PRSS58)

A PRSS58 (ou TRYX3) é membro da família das tripsinas. Seu gene (*PRSS58*) e vários outros genes relacionados ao tripsinogênio estão localizados no lócus do receptor de células T, no cromossomo 7 (ANTONACCI *et al.*, 2014). A função da PRSS58 ainda é desconhecida, entretanto outros membros da família, incluindo o PRSS1, PRSS2 e PRSS3 estão associados com pancreatite crônica, câncer de ovário, câncer colorretal e de próstata (WILLIAMS;GOTLEY;ANTALIS, 2001; WITT *et al.*, 2006; RADISKY, 2013; AZIZMOHAMMADI *et al.*, 2015; MA *et al.*, 2015). Estudos anteriores demonstraram que a ativação da tripsina pode bloquear ou aumentar a ativação de zimógenos, causando inflamação, danos celulares e moleculares a nível de DNA em células pancreáticas, aumentando assim o risco de câncer pancreático (CHEN *et al.*, 2013; YI *et al.*, 2015). Além disso, foi demonstrado em modelos de cultura, que a PRSS3 é capaz de promover a proliferação celular e invasão em câncer de ovário, sugerindo uma importante função da PRSS3 na metástase (MA *et al.*, 2015).

Apesar de ainda não existirem dados na literatura sobre a PRSS58, é possível especular uma possível associação com o desenvolvimento tumoral, uma vez que células tumorais

normalmente expressam proteases de forma atípica, desempenhando funções específicas que facilitam várias etapas da tumorigênese. Além disso, proteases são capazes de ativar quimiocinas, fatores de crescimento, receptores de fatores de crescimento, e outros receptores de sinalização, contribuindo com a sinalização de cascatas envolvidas na iniciação, proliferação e metástase tumoral (CUDIC;FIELDS, 2009; NAKANUMA *et al.*, 2010; MA *et al.*, 2015).

Portanto, ainda não existem dados na literatura sobre a função da PRSS58 e sua associação com doenças infecciosas ou câncer. Entretanto, a correlação de outros membros da família com o desenvolvimento tumoral pode sugerir uma possível associação da PRSS58 com o desenvolvimento de HCC ou outros tipos de câncer. Mais estudos são necessários para elucidar o papel da PRSS58 no desenvolvimento tumoral.

3. OBJETIVOS

Objetivo Geral

Investigar a associação de polimorfismos de único nucleotídeo (SNPs) com a gravidade da doença hepática causada pelo vírus da hepatite C (HCV).

Objetivos Específicos

- Verificar a associação de SNPs no gene *IL22RA2* com a gravidade da doença hepática em pacientes com HCV;
- Verificar a associação de SNPs no gene *PTX3* com a gravidade da doença hepática em pacientes com HCV;
- Associar os níveis plasmáticos de PTX3 com SNPs no gene *PTX3*;
- Associar os níveis plasmáticos de PTX3 com a gravidade da doença hepática em pacientes com HCV;
- Identificar novos SNPs associados com o desenvolvimento de carcinoma hepatocelular, por meio de sequenciamento do exoma, em pacientes com HCV;

4. REFERÊNCIAS

AARONSON, D. S.; HORVATH, C. M. A road map for those who don't know JAK-STAT. **Science,** v. 296, n. 5573, p. 1653-1655, 2002. ISSN 0036-8075.

AGNELLO, V. et al. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. **Proceedings of the National Academy of Sciences,** v. 96, n. 22, p. 12766-12771, 1999. ISSN 0027-8424.

AIKAWA, T. et al. HLA DRB1 and DQB1 alleles and haplotypes influencing the progression of hepatitis C. **Journal of medical virology,** v. 49, n. 4, p. 274-278, 1996. ISSN 1096-9071.

ALTER, M. J. Epidemiology of hepatitis C virus infection. **World Journal of gastroenterology,** v. 13, n. 17, p. 2436, 2007. ISSN 1007-9327.

ALVARADO-MORA, M. V. et al. Distribution and molecular characterization of hepatitis C virus (HCV) genotypes in patients with chronic infection from Pernambuco State, Brazil. **Virus research,** v. 169, n. 1, p. 8-12, 2012. ISSN 0168-1702.

ALVES PEDROSO, M. et al. Mannan-binding lectin MBL2 gene polymorphism in chronic hepatitis C: association with the severity of liver fibrosis and response to interferon therapy. **Clinical & Experimental Immunology,** v. 152, n. 2, p. 258-264, 2008. ISSN 1365-2249.

AMAKO, Y. et al. Pathogenesis of hepatitis C virus infection in Tupaia belangeri. **Journal of virology,** v. 84, n. 1, p. 303-311, 2010. ISSN 0022-538X.

ANDRE, P. et al. Characterization of low-and very-low-density hepatitis C virus RNA-containing particles. **Journal of virology,** v. 76, n. 14, p. 6919-6928, 2002. ISSN 0022-538X.

ANTONACCI, R. et al. Genomic characteristics of the T cell receptor (TRB) locus in the rabbit (Oryctolagus cuniculus) revealed by comparative and phylogenetic analyses. **Immunogenetics,** v. 66, n. 4, p. 255-266, 2014. ISSN 0093-7711.

ARNAUD, N. et al. Hepatitis C virus reveals a novel early control in acute immune response. **PLoS Pathog,** v. 7, n. 10, p. e1002289, 2011. ISSN 1553-7374.

AROUCHA, D. et al. High tumor necrosis factor-α/interleukin-10 ratio is associated with hepatocellular carcinoma in patients with chronic hepatitis C. **Cytokine,** v. 62, n. 3, p. 421-425, 2013. ISSN 1043-4666.

AROUCHA, D. C. et al. TNF-α and IL-10 polymorphisms increase the risk to hepatocellular carcinoma in HCV infected individuals. **Journal of medical virology**, 2016. ISSN 1096-9071.

ASHFAQ, U. A. et al. An overview of HCV molecular biology, replication and immune responses. **Virology journal,** v. 8, n. 1, p. 1, 2011. ISSN 1743-422X.

ASSELAH, T. et al. Gene expression and hepatitis C virus infection. **Gut,** v. 58, n. 6, p. 846-858, 2009. ISSN 1468-3288.

AVRĂMESCU, C. S. et al. Correlations among the serum levels of some interleukins and the histopathological aspects in chronic viral hepatitis C. **Rom J Morphol Embryol,** v. 49, n. 1, p. 57-62, 2008.

AZIZMOHAMMADI, S. et al. Clinical significance and expression of the PRSS3 and Wiskott-Aldrich syndrome protein family verprolin-homologous protein 1 for the early detection of epithelial ovarian cancer. **Tumour Biol**, Dec 10 2015. ISSN 1423-0380 (Electronic)

1010-4283 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26662304 >.

BACON, B. R. et al. Boceprevir for previously treated chronic HCV genotype 1 infection. **New England Journal of Medicine,** v. 364, n. 13, p. 1207-1217, 2011. ISSN 0028-4793.

BARBATI, E. et al. Influence of pentraxin 3 (PTX3) genetic variants on myocardial infarction risk and PTX3 plasma levels. **PloS one,** v. 7, n. 12, p. e53030, 2012. ISSN 1932-6203.

BARIL, M. et al. MAVS dimer is a crucial signaling component of innate immunity and the target of hepatitis C virus NS3/4A protease. **Journal of virology,** v. 83, n. 3, p. 1299-1311, 2009. ISSN 0022-538X.

BATALLER, R.; NORTH, K. E.; BRENNER, D. A. Genetic polymorphisms and the progression of liver fibrosis: a critical appraisal. **Hepatology,** v. 37, n. 3, p. 493-503, 2003. ISSN 1527-3350.

BEDOSSA, P.; POYNARD, T. An algorithm for the grading of activity in chronic hepatitis C. **Hepatology,** v. 24, n. 2, p. 289-293, 1996. ISSN 1527-3350.

BERTOLETTI, A. et al. Different cytokine profiles of intraphepatic T cells in chronic hepatitis B and hepatitis C virus infections. **Gastroenterology,** v. 112, n. 1, p. 193-199, 1997. ISSN 0016-5085.

BILLERBECK, E. et al. Analysis of CD161 expression on human CD8+ T cells defines a distinct functional subset with tissue-homing properties. **Proceedings of the National Academy of Sciences,** v. 107, n. 7, p. 3006-3011, 2010. ISSN 0027-8424.

BLANCHARD, E. et al. Hepatitis C virus entry depends on clathrin-mediated endocytosis. **Journal of virology,** v. 80, n. 14, p. 6964-6972, 2006. ISSN 0022-538X.

BODE, J. G. et al. IFN-α antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. **The FASEB Journal,** v. 17, n. 3, p. 488-490, 2003. ISSN 0892-6638.

BOGA, S. et al. Plasma Pentraxin 3 Differentiates Nonalcoholic Steatohepatitis (NASH) from Non-NASH. **Metabolic Syndrome and Related Disorders,** v. 13, n. 9, p. 393-399, 2015. ISSN 1540-4196.

BONAVITA, E. et al. PTX3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. **Cell,** v. 160, n. 4, p. 700-714, 2015. ISSN 0092-8674.

BOTTAZZI, B. et al. An integrated view of humoral innate immunity: pentraxins as a paradigm. **Annual review of immunology,** v. 28, p. 157-183, 2009. ISSN 0732-0582.

BOTTAZZI, B. et al. The pentraxins PTX3 and SAP in innate immunity, regulation of inflammation and tissue remodelling. **Journal of hepatology**, 2016. ISSN 0168-8278.

BOTTAZZI, B. et al. Multimer Formation and Ligand Recognition by the Long Pentraxin PTX3 similarities and differences with the short pentraxins c-reactive protein and serum amyloid p component. **Journal of Biological Chemistry,** v. 272, n. 52, p. 32817-32823, 1997. ISSN 0021-9258.

BOULANT, S. et al. Structural determinants that target the hepatitis C virus core protein to lipid droplets. **Journal of Biological Chemistry,** v. 281, n. 31, p. 22236-22247, 2006. ISSN 0021-9258.

BOUZGARROU, N. et al. Combined effect of pro-and anti-inflammatory cytokine gene polymorphisms on susceptibility to liver cirrhosis in Tunisian HCV-infected patients. **Hepatology international,** v. 5, n. 2, p. 681-687, 2011. ISSN 1936-0533.

BOUZGARROU, N. et al. Combined analysis of interferon-γ and interleukin-10 gene polymorphisms and chronic hepatitis C severity. **Human immunology,** v. 70, n. 4, p. 230-236, 2009. ISSN 0198-8859.

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de DST, Aids e Hepatites Virais. **Protocolo clínico e diretrizes terapêuticas para hepatite viral c e coinfecções**. Brasília: Ministério da Saúde, 2011

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de DST, Aids e Hepatites Virais. **Boletim epidemiológico: hepatites virais**. Brasília: Ministério da Saúde, 2012

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de DST, Aids e Hepatites Virais. **Boletim epidemiológico: hepatites virais**. Brasília: Ministério da Saúde, 2015a

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de DST, Aids e Hepatites Virais. **Protocolo Clínico e Diretrizes Terapêuticas para Hepatite C e Coinfecções**. Brasília: Ministério da Saúde, 2015b

BREVIARIO, F. et al. Interleukin-1-inducible genes in endothelial cells. Cloning of a new gene related to C-reactive protein and serum amyloid P component. **Journal of Biological Chemistry,** v. 267, n. 31, p. 22190-22197, 1992. ISSN 0021-9258.

BURDETTE, D. et al. Hepatitis C virus activates interleukin-1β via caspase-1-inflammasome complex. **Journal of General Virology,** v. 93, n. 2, p. 235-246, 2012. ISSN 1465-2099.

CALVISI, D. F. et al. Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. **Gastroenterology,** v. 130, n. 4, p. 1117-1128, 2006. ISSN 0016-5085.

CALVISI, D. F. et al. Mechanistic and prognostic significance of aberrant methylation in the molecular pathogenesis of human hepatocellular carcinoma. **The Journal of clinical investigation,** v. 117, n. 9, p. 2713-2722, 2007. ISSN 0021-9738.

CAMPIOTTO, S. et al. Geographic distribution of hepatitis C virus genotypes in Brazil. **Brazilian Journal of Medical and Biological Research,** v. 38, n. 1, p. 41-49, 2005. ISSN 0100-879X.

CANBAY, A. et al. Kupffer cell engulfment of apoptotic bodies stimulates death ligand and cytokine expression. **Hepatology,** v. 38, n. 5, p. 1188-1198, 2003. ISSN 1527-3350.

CHANG, K.-S. et al. Human apolipoprotein e is required for infectivity and production of hepatitis C virus in cell culture. **Journal of virology,** v. 81, n. 24, p. 13783-13793, 2007. ISSN 0022-538X.

CHEN, Q. et al. Trypsin-antitrypsin imbalance in immune escape and clonal proliferation of pancreatic cancer. **J Genet Syndr Gene Ther,** v. 4, p. 11-5, 2013.

CHEN, W. et al. HCV genomic RNA activates the NLRP3 inflammasome in human myeloid cells. **PloS one,** v. 9, n. 1, p. e84953, 2014. ISSN 1932-6203.

CHESTOVICH, P. J. et al. IL-22: implications for liver ischemia/reperfusion injury. **Transplantation,** v. 93, n. 5, p. 485, 2012.

CHIARINI, M. et al. PTX3 genetic variations affect the risk of Pseudomonas aeruginosa airway colonization in cystic fibrosis patients. **Genes and immunity,** v. 11, n. 8, p. 665-670, 2010. ISSN 1466-4879.

CHOI, B. et al. Pentraxin-3 silencing suppresses gastric cancer-related inflammation by inhibiting chemotactic migration of macrophages. **Anticancer research,** v. 35, n. 5, p. 2663-2668, 2015. ISSN 0250-7005.

CHOO, Q.-L. et al. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. **Science,** v. 244, n. 4902, p. 359-362, 1989. ISSN 0036-8075.

CHRISTIE, J. et al. Clinical outcome of hypogammaglobulinaemic patients following outbreak of acute hepatitis C: 2 year follow up. **Clinical & Experimental Immunology,** v. 110, n. 1, p. 4-8, 1997. ISSN 1365-2249.

CLEARY, S. P. et al. Identification of driver genes in hepatocellular carcinoma by exome sequencing. **Hepatology,** v. 58, n. 5, p. 1693-1702, 2013. ISSN 1527-3350.

CMET, S. et al. Carriage of the EGF rs4444903 A> G functional polymorphism associates with disease progression in chronic HBV infection. **Clinical & Experimental Immunology,** v. 167, n. 2, p. 296-302, 2012. ISSN 1365-2249.

COBLEIGH, M. A.; ROBEK, M. D. Protective and pathological properties of IL-22 in liver disease: implications for viral hepatitis. **The American journal of pathology,** v. 182, n. 1, p. 21-28, 2013. ISSN 0002-9440.

COLLER, K. E. et al. Molecular determinants and dynamics of hepatitis C virus secretion. **PLoS Pathog,** v. 8, n. 1, p. e1002466, 2012. ISSN 1553-7374.

COOPER, S. et al. Analysis of a successful immune response against hepatitis C virus. **Immunity,** v. 10, n. 4, p. 439-449, 1999. ISSN 1074-7613.

CORCHADO, S. et al. Influence of genetic polymorphisms of tumor necrosis factor alpha and interleukin 10 genes on the risk of liver cirrhosis in HIV-HCV coinfected patients. **PloS one,** v. 8, n. 6, p. e66619, 2013. ISSN 1932-6203.

CORNBERG, M. et al. A systematic review of hepatitis C virus epidemiology in Europe, Canada and Israel. **Liver International,** v. 31, n. s2, p. 30-60, 2011. ISSN 1478-3231.

COUNIHAN, N. A.; RAWLINSON, S. M.; LINDENBACH, B. D. Trafficking of hepatitis C virus core protein during virus particle assembly. **PLoS Pathog,** v. 7, n. 10, p. e1002302, 2011. ISSN 1553-7374.

COX, A. L. et al. Comprehensive analyses of CD8+ T cell responses during longitudinal study of acute human hepatitis C. **Hepatology,** v. 42, n. 1, p. 104-112, 2005. ISSN 1527-3350.

COX, A. L. et al. Prospective evaluation of community-acquired acute-phase hepatitis C virus infection. **Clinical Infectious Diseases,** v. 40, n. 7, p. 951-958, 2005. ISSN 1058-4838.

CRAIG, D. G. et al. Elevated levels of the long pentraxin 3 in paracetamol-induced human acute liver injury. **European journal of gastroenterology & hepatology,** v. 25, n. 3, p. 359-367, 2013. ISSN 0954-691X.

CROTTA, S. et al. Inhibition of natural killer cells through engagement of CD81 by the major hepatitis C virus envelope protein. **The Journal of experimental medicine,** v. 195, n. 1, p. 35-42, 2002. ISSN 0022-1007.

CUDIC, M.; FIELDS, G. B. Extracellular proteases as targets for drug development. **Current protein & peptide science,** v. 10, n. 4, p. 297, 2009.

CUNHA, C. et al. Genetic PTX3 deficiency and aspergillosis in stem-cell transplantation. **New England Journal of Medicine,** v. 370, n. 5, p. 421-432, 2014. ISSN 0028-4793.

DA ROCHA, L. F. et al. Increased serum interleukin 22 in patients with rheumatoid arthritis and correlation with disease activity. **The Journal of rheumatology,** v. 39, n. 7, p. 1320-1325, 2012. ISSN 0315-162X.

DAMBACHER, J. et al. The role of interleukin-22 in hepatitis C virus infection. **Cytokine,** v. 41, n. 3, p. 209-216, 2008. ISSN 1043-4666.

DAVEY, J. W. et al. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. **Nature Reviews Genetics,** v. 12, n. 7, p. 499-510, 2011. ISSN 1471-0056.

DAVILA, J. A. et al. Utilization of surveillance for hepatocellular carcinoma among hepatitis C virus–infected veterans in the United States. **Annals of internal medicine,** v. 154, n. 2, p. 85-93, 2011. ISSN 0003-4819.

DAY, C. L. et al. Broad specificity of virus-specific CD4+ T-helper-cell responses in resolved hepatitis C virus infection. **Journal of virology,** v. 76, n. 24, p. 12584-12595, 2002. ISSN 0022-538X.

DAYYEH, B. K. A. et al. A functional polymorphism in the epidermal growth factor gene is associated with risk for hepatocellular carcinoma. **Gastroenterology,** v. 141, n. 1, p. 141-149, 2011. ISSN 0016-5085.

DE MARIA, N. et al. Association between reactive oxygen species and disease activity in chronic hepatitis C. **Free Radical Biology and Medicine,** v. 21, n. 3, p. 291-295, 1996. ISSN 0891-5849.

DESSEIN, A. J. et al. Severe hepatic fibrosis in Schistosoma mansoni infection is controlled by a major locus that is closely linked to the interferon-γ receptor gene. **The American Journal of Human Genetics,** v. 65, n. 3, p. 709-721, 1999. ISSN 0002-9297.

DHINGRA, S.; WARD, S. C.; THUNG, S. N. Liver pathology of hepatitis C, beyond grading and staging of the disease. **World journal of gastroenterology,** v. 22, n. 4, p. 1357, 2016.

DIAMANDIS, E. P. et al. Pentraxin-3 is a novel biomarker of lung carcinoma. **Clinical Cancer Research,** v. 17, n. 8, p. 2395-2399, 2011. ISSN 1078-0432.

DIAMOND, J. M. et al. Variation in PTX3 is associated with primary graft dysfunction after lung transplantation. **American journal of respiratory and critical care medicine,** v. 186, n. 6, p. 546-552, 2012. ISSN 1535-4970.

DIEPOLDER, H. et al. Possible mechanism involving T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection. **The Lancet,** v. 346, n. 8981, p. 1006-1007, 1995. ISSN 0140-6736.

DIEPOLDER, H. M. et al. Immunodominant CD4+ T-cell epitope within nonstructural protein 3 in acute hepatitis C virus infection. **Journal of virology,** v. 71, n. 8, p. 6011-6019, 1997. ISSN 0022-538X.

DOUAM, F. et al. Critical interaction between E1 and E2 glycoproteins determines binding and fusion properties of hepatitis C virus during cell entry. **Hepatology,** v. 59, n. 3, p. 776-788, 2014. ISSN 1527-3350.

DUBUISSON, J.; COSSET, F.-L. Virology and cell biology of the hepatitis C virus life cycle–An update. **Journal of hepatology,** v. 61, n. 1, p. S3-S13, 2014. ISSN 0168-8278.

DUMOUTIER, L.; LOUAHED, J.; RENAULD, J. C. Cloning and characterization of IL-10-related T cell-derived inducible factor (IL-TIF), a novel cytokine structurally related to IL-10 and inducible by IL-9. **J Immunol,** v. 164, n. 4, p. 1814-9, Feb 15 2000. ISSN 0022-1767 (Print)

0022-1767 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10657629 >.

DUMOUTIER, L. et al. IL-TIF/IL-22: genomic organization and mapping of the human and mouse genes. **Genes Immun,** v. 1, n. 8, p. 488-94, Dec 2000. ISSN 1466-4879 (Print)

1466-4879 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11197690 >.

EGGER, D. et al. Expression of hepatitis C virus proteins induces distinct membrane alterations including a candidate viral replication complex. **Journal of virology,** v. 76, n. 12, p. 5974-5984, 2002. ISSN 0022-538X.

ESTEBAN, J. I.; SAULEDA, S.; QUER, J. The changing epidemiology of hepatitis C virus infection in Europe. **Journal of hepatology,** v. 48, n. 1, p. 148-162, 2008. ISSN 0168-8278.

ESTRABAUD, E. et al. Genomics and HCV infection: progression of fibrosis and treatment response. **Journal of hepatology,** v. 57, n. 5, p. 1110-1125, 2012. ISSN 0168-8278.

EVANS, M. J. et al. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. **Nature,** v. 446, n. 7137, p. 801-805, 2007. ISSN 0028-0836.

FALLETI, E. et al. Association between the epidermal growth factor rs4444903 G/G genotype and advanced fibrosis at a young age in chronic hepatitis C. **Cytokine,** v. 57, n. 1, p. 68-73, 2012. ISSN 1043-4666.

FARCI, P. et al. Early changes in hepatitis C viral quasispecies during interferon therapy predict the therapeutic outcome. **Proceedings of the National Academy of Sciences,** v. 99, n. 5, p. 3081-3086, 2002. ISSN 0027-8424.

FARINATI, F. et al. Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis. **Journal of hepatology,** v. 22, n. 4, p. 449-456, 1995. ISSN 0168-8278.

FENG, D. et al. Interleukin-22 promotes proliferation of liver stem/progenitor cells in mice and patients with chronic hepatitis B virus infection. **Gastroenterology,** v. 143, n. 1, p. 188-198. e7, 2012. ISSN 0016-5085.

FIORE, G. et al. In-situ immunophenotyping study of hepatic-infiltrating cytotoxic cells in chronic active hepatitis C. **European journal of gastroenterology & hepatology,** v. 9, n. 5, p. 491-496, 1997. ISSN 0954-691X.

FLAMM, S. L. et al. Ledipasvir/sofosbuvir with ribavirin for the treatment of HCV in patients with decompensated cirrhosis: preliminary results of a prospective, multicenter study. **Hepatology,** v. 60, n. Suppl 4, p. 321A, 2014.

FOO, S.-S. et al. Role of pentraxin 3 in shaping arthritogenic alphaviral disease: from enhanced viral replication to immunomodulation. **PLoS Pathog,** v. 11, n. 2, p. e1004649, 2015. ISSN 1553-7374.

FRIED, M. W. et al. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naïve genotype 1 hepatitis C: The randomized PILLAR study. **Hepatology,** v. 58, n. 6, p. 1918-1929, 2013. ISSN 1527-3350.

FRIEDMAN, S. L. Mechanisms of hepatic fibrogenesis. **Gastroenterology,** v. 134, n. 6, p. 1655-1669, 2008. ISSN 0016-5085.

FUCHS, B. C. et al. Epidermal growth factor receptor inhibition attenuates liver fibrosis and development of hepatocellular carcinoma. **Hepatology,** v. 59, n. 4, p. 1577-1590, 2014. ISSN 1527-3350.

GALE, M.; FOY, E. M. Evasion of intracellular host defence by hepatitis C virus. **Nature,** v. 436, n. 7053, p. 939-945, 2005. ISSN 0028-0836.

GALE, M. J. et al. Evidence that hepatitis C virus resistance to interferon is mediated through repression of the PKR protein kinase by the nonstructural 5A protein. **Virology,** v. 230, n. 2, p. 217-227, 1997. ISSN 0042-6822.

GARLANDA, C. et al. PTX3, a humoral pattern recognition molecule at the interface between microbe and matrix recognition. **Current opinion in immunology,** v. 38, p. 39-44, 2016. ISSN 0952-7915.

GASTAMINZA, P. et al. Cellular determinants of hepatitis C virus assembly, maturation, degradation, and secretion. **Journal of virology,** v. 82, n. 5, p. 2120-2129, 2008. ISSN 0022-538X.

GASTAMINZA, P. et al. Ultrastructural and biophysical characterization of hepatitis C virus particles produced in cell culture. **Journal of virology,** v. 84, n. 21, p. 10999-11009, 2010. ISSN 0022-538X.

GEISS, G. K. et al. Gene expression profiling of the cellular transcriptional network regulated by alpha/beta interferon and its partial attenuation by the hepatitis C virus nonstructural 5A protein. **Journal of virology,** v. 77, n. 11, p. 6367-6375, 2003. ISSN 0022-538X.

GERMANO, G. et al. Antitumor and anti-inflammatory effects of trabectedin on human myxoid liposarcoma cells. **Cancer research,** v. 70, n. 6, p. 2235-2244, 2010. ISSN 0008-5472.

GHANY, M. G. et al. Diagnosis, management, and treatment of hepatitis C: an update. **Hepatology,** v. 49, n. 4, p. 1335-1374, 2009. ISSN 1527-3350.

GIANNINI, C.; BRECHOT, C. Hepatitis C virus biology. **Cell Death & Differentiation,** v. 10, p. S27-S38, 2003. ISSN 1350-9047.

GIL, L. et al. Oxidative stress in adult dengue patients. **The American journal of tropical medicine and hygiene,** v. 71, n. 5, p. 652-657, 2004. ISSN 0002-9637.

GLYNN, S. A. et al. Dynamics of viremia in early hepatitis C virus infection. **Transfusion,** v. 45, n. 6, p. 994-1002, 2005. ISSN 1537-2995.

GONG, G. et al. Human hepatitis C virus NS5A protein alters intracellular calcium levels, induces oxidative stress, and activates STAT-3 and NF-κB. **Proceedings of the National Academy of Sciences,** v. 98, n. 17, p. 9599-9604, 2001. ISSN 0027-8424.

GRAFMUELLER, S. et al. Differential Antigen Specificity of Hepatitis C Virus–Specific Interleukin 17–and Interferon γ–Producing CD8+ T Cells During Chronic Infection. **Journal of Infectious Diseases,** v. 205, n. 7, p. 1142-1146, 2012. ISSN 0022-1899.

GREENWEL, P. et al. Hydrogen peroxide: A link between acetaldehyde-elicited α1 (i) collagen gene up-regulation and oxidative stress in mouse hepatic stellate cells. **Hepatology,** v. 31, n. 1, p. 109-116, 2000. ISSN 1527-3350.

GRIFFIN, S. D. et al. The p7 protein of hepatitis C virus forms an ion channel that is blocked by the antiviral drug, Amantadine. **FEBS letters,** v. 535, n. 1, p. 34-38, 2003. ISSN 0014-5793.

GRUENBERG, B. et al. A novel, soluble homologue of the human IL-10 receptor with preferential expression in placenta. **Genes and immunity,** v. 2, n. 6, p. 329-334, 2001. ISSN 1466-4879.

GUERRA, J. et al. HCV burden of infection in Egypt: results from a nationwide survey. **Journal of viral hepatitis,** v. 19, n. 8, p. 560-567, 2012. ISSN 1365-2893.

GUIDOTTI, L. G.; CHISARI, F. V. Noncytolytic control of viral infections by the innate and adaptive immune response. **Annual review of immunology,** v. 19, n. 1, p. 65-91, 2001. ISSN 0732-0582.

HAJARIZADEH, B.; GREBELY, J.; DORE, G. J. Epidemiology and natural history of HCV infection. **Nature Reviews Gastroenterology and Hepatology,** v. 10, n. 9, p. 553-562, 2013. ISSN 1759-5045.

HALLA, M. C. et al. Association of hepatitis C virus infection and liver fibrosis severity with the variants alleles of MBL2 gene in a Brazilian population. **Human immunology,** v. 71, n. 9, p. 883-887, 2010. ISSN 0198-8859.

HAO, J.-Q. Targeting interleukin-22 in psoriasis. **Inflammation,** v. 37, n. 1, p. 94-99, 2014. ISSN 0360-3997.

HAYES, C. N. et al. Genetics of IL28B and HCV—response to infection and treatment. **Nature Reviews Gastroenterology and Hepatology,** v. 9, n. 7, p. 406-417, 2012. ISSN 1759-5045.

HEIM, M. H.; THIMME, R. Innate and adaptive immune responses in HCV infections. **Journal of hepatology,** v. 61, n. 1, p. S14-S25, 2014. ISSN 0168-8278.

HERKER, E. et al. Efficient hepatitis C virus particle formation requires diacylglycerol acyltransferase-1. **Nature medicine,** v. 16, n. 11, p. 1295-1298, 2010. ISSN 1078-8956.

HERNANDEZ-GEA, V.; FRIEDMAN, S. L. Pathogenesis of liver fibrosis. **Annual review of pathology: mechanisms of disease,** v. 6, p. 425-456, 2011. ISSN 1553-4006.

HOOFNAGLE, J. H. Course and outcome of hepatitis C. **Hepatology,** v. 36, n. 5B, 2002. ISSN 1527-3350.

HORNER, S. M.; GALE JR, M. Regulation of hepatic innate immunity by hepatitis C virus. **Nature medicine,** v. 19, n. 7, p. 879-888, 2013. ISSN 1078-8956.

HOSHIDA, Y. et al. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. **Journal of hepatology,** v. 61, n. 1, p. S79-S90, 2014. ISSN 0168-8278.

HUANG, J. et al. Exome sequencing of hepatitis B virus-associated hepatocellular carcinoma. **Nature genetics,** v. 44, n. 10, p. 1117-1121, 2012. ISSN 1061-4036.

HUBER, S. et al. IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. **Nature,** v. 491, n. 7423, p. 259-263, 2012. ISSN 0028-0836.

IKEDA, A. et al. Leptin receptor somatic mutations are frequent in HCV-infected cirrhotic liver and associated with hepatocellular carcinoma. **Gastroenterology,** v. 146, n. 1, p. 222-232. e35, 2014. ISSN 0016-5085.

INAGAKI-OHARA, K. et al. SOCS, inflammation, and cancer. **Jak-Stat,** v. 2, n. 3, p. e24053, 2013. ISSN 2162-3996.

INFORZATO, A. et al. PTX3 as a paradigm for the interaction of pentraxins with the complement system. Seminars in immunology, 2013, Elsevier. p.79-85.

JACOBSON, I. M. et al. Telaprevir for previously untreated chronic hepatitis C virus infection. **New England Journal of Medicine,** v. 364, n. 25, p. 2405-2416, 2011. ISSN 0028-4793.

JENG, J.-S. et al. Tumor necrosis factor-α 308.2 polymorphism is associated with advanced hepatic fibrosis and higher risk for hepatocellular carcinoma. **Neoplasia,** v. 9, n. 11, p. 987-992, 2007. ISSN 1476-5586.

JEONG, W. I. et al. Suppression of innate immunity (natural killer cell/interferon-γ) in the advanced stages of liver fibrosis in mice. **Hepatology,** v. 53, n. 4, p. 1342-1351, 2011. ISSN 1527-3350.

JIANG, J. et al. Apolipoprotein E mediates attachment of clinical hepatitis C virus to hepatocytes by binding to cell surface heparan sulfate proteoglycan receptors. **PloS one,** v. 8, n. 7, p. e67982, 2013. ISSN 1932-6203.

JIRASKO, V. et al. Structural and functional studies of nonstructural protein 2 of the hepatitis C virus reveal its key role as organizer of virion assembly. **PLoS Pathog,** v. 6, n. 12, p. e1001233, 2010. ISSN 1553-7374.

JO, J. et al. Analysis of CD8+ T-cell–mediated inhibition of hepatitis C virus replication using a novel immunological model. **Gastroenterology,** v. 136, n. 4, p. 1391-1401, 2009. ISSN 0016-5085.

JONES, B. C.; LOGSDON, N. J.; WALTER, M. R. Structure of IL-22 bound to its high-affinity IL-22R1 chain. **Structure,** v. 16, n. 9, p. 1333-1344, 2008. ISSN 0969-2126.

KABA, S. et al. Molecular epidemiology of hepatitis C in Australia. **Journal of gastroenterology and hepatology,** v. 13, n. 9, p. 914-920, 1998. ISSN 1440-1746.

KARIO, E. et al. Suppressors of cytokine signaling 4 and 5 regulate epidermal growth factor receptor signaling. **Journal of Biological Chemistry,** v. 280, n. 8, p. 7038-7048, 2005. ISSN 0021-9258.

KERSHENOBICH, D. et al. Trends and projections of hepatitis C virus epidemiology in Latin America. **Liver International,** v. 31, n. s2, p. 18-29, 2011. ISSN 1478-3231.

KHAN, A. G. et al. Structure of the core ectodomain of the hepatitis C virus envelope glycoprotein 2. **Nature,** v. 509, n. 7500, p. 381-384, 2014. ISSN 0028-0836.

KI, S. H. et al. Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: role of signal transducer and activator of transcription 3. **Hepatology,** v. 52, n. 4, p. 1291-300, Oct 2010. ISSN 1527-3350 (Electronic)

0270-9139 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20842630 >.

KIM, J. et al. Crystal structure of the hepatitis C virus NS3 protease domain complexed with a synthetic NS4A cofactor peptide. **Cell,** v. 87, n. 2, p. 343-355, 1996. ISSN 0092-8674.

KOLYKHALOV, A. A. et al. Hepatitis C virus-encoded enzymatic activities and conserved RNA elements in the 3′ nontranslated region are essential for virus replication in vivo. **Journal of virology,** v. 74, n. 4, p. 2046-2051, 2000. ISSN 0022-538X.

KONDO, S. et al. Clinical impact of pentraxin family expression on prognosis of pancreatic carcinoma. **British journal of cancer,** v. 109, n. 3, p. 739-746, 2013. ISSN 0007-0920.

KONG, L. et al. Hepatitis C virus E2 envelope glycoprotein core structure. **Science,** v. 342, n. 6162, p. 1090-1094, 2013. ISSN 0036-8075.

KONG, X. et al. Hepatoprotective and anti-fibrotic functions of interleukin-22: therapeutic potential for the treatment of alcoholic liver disease. **J Gastroenterol Hepatol,** v. 28 Suppl 1, p. 56-60, Aug 2013. ISSN 1440-1746 (Electronic)

0815-9319 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23855297 >.

KONG, X. et al. Interleukin-22 induces hepatic stellate cell senescence and restricts liver fibrosis in mice. **Hepatology,** v. 56, n. 3, p. 1150-1159, 2012. ISSN 1527-3350.

KOTENKO, S. V. et al. IFN-λs mediate antiviral protection through a distinct class II cytokine receptor complex. **Nature immunology,** v. 4, n. 1, p. 69-77, 2003.

KOTENKO, S. V. et al. Identification, cloning, and characterization of a novel soluble receptor that binds IL-22 and neutralizes its activity. **The Journal of Immunology,** v. 166, n. 12, p. 7096-7103, 2001. ISSN 0022-1767.

KOWDLEY, K. V. et al. Sofosbuvir with pegylated interferon alfa-2a and ribavirin for treatment-naive patients with hepatitis C genotype-1 infection (ATOMIC): an open-label, randomised, multicentre phase 2 trial. **The Lancet,** v. 381, n. 9883, p. 2100-2107, 2013. ISSN 0140-6736.

KREY, T. et al. The disulfide bonds in glycoprotein E2 of hepatitis C virus reveal the tertiary organization of the molecule. **PLoS Pathog,** v. 6, n. 2, p. e1000762, 2010. ISSN 1553-7374.

KUMAR, A. et al. Deficient cytokine signaling in mouse embryo fibroblasts with a targeted deletion in the PKR gene: role of IRF-1 and NF-κB. **The EMBO journal,** v. 16, n. 2, p. 406-416, 1997. ISSN 0261-4189.

LAM, A. M.; FRICK, D. N. Hepatitis C virus subgenomic replicon requires an active NS3 RNA helicase. **Journal of virology,** v. 80, n. 1, p. 404-411, 2006. ISSN 0022-538X.

LAUER, G. M.; WALKER, B. D. Hepatitis C virus infection. **New England journal of medicine,** v. 345, n. 1, p. 41-52, 2001. ISSN 0028-4793.

LAVANCHY, D. The global burden of hepatitis C. **Liver International,** v. 29, n. s1, p. 74-81, 2009. ISSN 1478-3231.

LAVANCHY, D. Evolving epidemiology of hepatitis C virus. **Clinical Microbiology and Infection,** v. 17, n. 2, p. 107-115, 2011. ISSN 1469-0691.

LECHNER, F. et al. Analysis of successful immune responses in persons infected with hepatitis C virus. **The Journal of experimental medicine,** v. 191, n. 9, p. 1499-1512, 2000. ISSN 0022-1007.

LEE, U. E.; FRIEDMAN, S. L. Mechanisms of hepatic fibrogenesis. **Best practice & research Clinical gastroenterology,** v. 25, n. 2, p. 195-206, 2011. ISSN 1521-6918.

LEFÈVRE, M. et al. Syndecan 4 is involved in mediating HCV entry through interaction with lipoviral particle-associated apolipoprotein E. **PloS one,** v. 9, n. 4, p. e95550, 2014. ISSN 1932-6203.

LEIPE, J. et al. Interleukin 22 serum levels are associated with radiographic progression in rheumatoid arthritis. **Annals of the rheumatic diseases,** v. 70, n. 8, p. 1453-1457, 2011. ISSN 1468-2060.

LI, K. et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. **Proceedings of the National Academy of Sciences of the United States of America,** v. 102, n. 8, p. 2992-2997, 2005. ISSN 0027-8424.

LI, K. et al. Activation of chemokine and inflammatory cytokine response in hepatitis C virus–infected hepatocytes depends on toll-like receptor 3 sensing of hepatitis C virus double-stranded RNA intermediates. **Hepatology,** v. 55, n. 3, p. 666-675, 2012. ISSN 1527-3350.

LI, L.-J. et al. Role of interleukin-22 in inflammatory bowel disease. **World J Gastroenterol,** v. 20, n. 48, p. 18177-18188, 2014. ISSN 1007-9327.

LI, M. et al. Interferon-λs: the modulators of antivirus, antitumor, and immune responses. **Journal of leukocyte biology,** v. 86, n. 1, p. 23-32, 2009. ISSN 0741-5400.

LI, X.-D. et al. Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. **Proceedings of the National Academy of Sciences of the United States of America,** v. 102, n. 49, p. 17717-17722, 2005. ISSN 0027-8424.

LIN, R. et al. Dissociation of a MAVS/IPS-1/VISA/Cardif-IKKε molecular complex from the mitochondrial outer membrane by hepatitis C virus NS3-4A proteolytic cleavage. **Journal of virology,** v. 80, n. 12, p. 6072-6083, 2006. ISSN 0022-538X.

LIN, W. et al. Hepatitis C virus expression suppresses interferon signaling by degrading STAT1. **Gastroenterology,** v. 128, n. 4, p. 1034-1041, 2005. ISSN 0016-5085.

LIN, W. et al. Hepatitis C virus core protein blocks interferon signaling by interaction with the STAT1 SH2 domain. **Journal of virology,** v. 80, n. 18, p. 9226-9235, 2006. ISSN 0022-538X.

LINDENBACH, B. D.; RICE, C. Flaviviridae: the viruses and their replication. **Fields virology,** v. 1, p. 991-1041, 2001.

LINOSSI, E. M. et al. Suppressor of cytokine signaling (SOCS) 5 utilises distinct domains for regulation of JAK1 and interaction with the adaptor protein Shc-1. **PloS one,** v. 8, n. 8, p. e70536, 2013. ISSN 1932-6203.

LIVER, E. A. F. T. S. O. T. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. **Journal of hepatology,** v. 55, n. 2, p. 245-264, 2011. ISSN 0168-8278.

LOCATELLI, M. et al. The long pentraxin PTX3 as a correlate of cancer-related inflammation and prognosis of malignancy in gliomas. **Journal of neuroimmunology,** v. 260, n. 1, p. 99-106, 2013. ISSN 0165-5728.

LOO, Y.-M.; GALE, M. Immune signaling by RIG-I-like receptors. **Immunity,** v. 34, n. 5, p. 680-692, 2011. ISSN 1074-7613.

LOO, Y.-M. et al. Viral and therapeutic control of IFN-β promoter stimulator 1 during hepatitis C virus infection. **Proceedings of the National Academy of Sciences,** v. 103, n. 15, p. 6001-6006, 2006. ISSN 0027-8424.

LU, D.-H. et al. Interleukin-22 ameliorates liver fibrogenesis by attenuating hepatic stellate cell activation and downregulating the levels of inflammatory cytokines. **World journal of gastroenterology: WJG,** v. 21, n. 5, p. 1531, 2015.

LUEDDE, T.; KAPLOWITZ, N.; SCHWABE, R. F. Cell death and cell death responses in liver disease: mechanisms and clinical relevance. **Gastroenterology,** v. 147, n. 4, p. 765-783. e4, 2014. ISSN 0016-5085.

LUPBERGER, J. et al. EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. **Nature medicine,** v. 17, n. 5, p. 589-595, 2011. ISSN 1078-8956.

LYRA, A.; FAN, X.; DI BISCEGLIE, A. Molecular biology and clinical implication of hepatitis C virus. **Brazilian Journal of Medical and Biological Research,** v. 37, n. 5, p. 691-695, 2004. ISSN 0100-879X.

MA, R. et al. PRSS3 expression is associated with tumor progression and poor prognosis in epithelial ovarian cancer. **Gynecologic oncology,** v. 137, n. 3, p. 546-552, 2015. ISSN 0090-8258.

MA, Y. et al. NS3 helicase domains involved in infectious intracellular hepatitis C virus particle assembly. **Journal of virology,** v. 82, n. 15, p. 7624-7639, 2008. ISSN 0022-538X.

MADAN, V.; BARTENSCHLAGER, R. Structural and functional properties of the hepatitis C virus p7 viroporin. **Viruses,** v. 7, n. 8, p. 4461-4481, 2015.

MANN, D.; SMART, D. Transcriptional regulation of hepatic stellate cell activation. **Gut,** v. 50, n. 6, p. 891-896, 2002. ISSN 1468-3288.

MARCELLIN, P. Hepatitis B and hepatitis C in 2009. **Liver International,** v. 29, n. s1, p. 1-8, 2009. ISSN 1478-3231.

MARRA, F. Hepatic stellate cells and the regulation of liver inflammation. **Journal of hepatology,** v. 31, n. 6, p. 1106-1119, 1999. ISSN 0168-8278.

MARTIN, J. C. et al. Interleukin-22 binding protein (IL-22BP) is constitutively expressed by a subset of conventional dendritic cells and is strongly induced by retinoic acid. **Mucosal immunology,** v. 7, n. 1, p. 101-113, 2014. ISSN 1933-0219.

MASAKI, T. et al. Interaction of hepatitis C virus nonstructural protein 5A with core protein is critical for the production of infectious virus particles. **Journal of virology,** v. 82, n. 16, p. 7964-7976, 2008. ISSN 0022-538X.

MCALLISTER, C. S.; SAMUEL, C. E. The RNA-activated protein kinase enhances the induction of interferon-β and apoptosis mediated by cytoplasmic RNA sensors. **Journal of Biological Chemistry,** v. 284, n. 3, p. 1644-1651, 2009. ISSN 0021-9258.

MCLAUCHLAN, J. et al. Intramembrane proteolysis promotes trafficking of hepatitis C virus core protein to lipid droplets. **The EMBO journal,** v. 21, n. 15, p. 3980-3988, 2002. ISSN 0261-4189.

METZKER, M. L. Sequencing technologies—the next generation. **Nature reviews genetics,** v. 11, n. 1, p. 31-46, 2010. ISSN 1471-0056.

MEUNIER, J.-C. et al. Apolipoprotein c1 association with hepatitis C virus. **Journal of virology,** v. 82, n. 19, p. 9647-9656, 2008. ISSN 0022-538X.

MEURS, E. F.; BREIMAN, A. The interferon inducing pathways and the hepatitis C virus. **World journal of gastroenterology: WJG,** v. 13, n. 17, p. 2446-2454, 2007. ISSN 1007-9327.

MEYLAN, E. et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. **Nature,** v. 437, n. 7062, p. 1167-1172, 2005. ISSN 0028-0836.

MICALLEF, J.; KALDOR, J.; DORE, G. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. **Journal of viral hepatitis,** v. 13, n. 1, p. 34-41, 2006. ISSN 1365-2893.

MINOLA, E. et al. Age at infection affects the long-term outcome of transfusion-associated chronic hepatitis C. **Blood,** v. 99, n. 12, p. 4588-4591, 2002. ISSN 0006-4971.

MISSALE, G. et al. Different clinical behaviors of acute hepatitis C virus infection are associated with different vigor of the anti-viral cell-mediated immune response. **Journal of Clinical Investigation,** v. 98, n. 3, p. 706, 1996.

MORADPOUR, D.; PENIN, F. Hepatitis C virus proteins: from structure to function. In: (Ed.). **Hepatitis C Virus: From Molecular Virology to Antiviral Therapy**: Springer, 2013. p.113-142. ISBN 3642273394.

MORADPOUR, D.; PENIN, F.; RICE, C. M. Replication of hepatitis C virus. **Nature Reviews Microbiology,** v. 5, n. 6, p. 453-463, 2007. ISSN 1740-1526.

MORGAN, R. L. et al. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. **Annals of internal medicine,** v. 158, n. 5_Part_1, p. 329-337, 2013. ISSN 0003-4819.

MORIKAWA, K. et al. Nonstructural protein 3-4A: the Swiss army knife of hepatitis C virus. **Journal of viral hepatitis,** v. 18, n. 5, p. 305-315, 2011. ISSN 1365-2893.

MOUCARI, R. et al. Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. **Gastroenterology,** v. 134, n. 2, p. 416-423, 2008. ISSN 0016-5085.

NAGEM, R. A. et al. Crystal structure of recombinant human interleukin-22. **Structure,** v. 10, n. 8, p. 1051-62, Aug 2002. ISSN 0969-2126 (Print)

0969-2126 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12176383 >.

NAKANUMA, S.-I. et al. Tumor-derived trypsin enhances proliferation of intrahepatic cholangiocarcinoma cells by activating protease-activated receptor-2. **International journal of oncology,** v. 36, n. 4, p. 793-800, 2010. ISSN 1019-6439.

NAUTA, A. J. et al. Recognition and clearance of apoptotic cells: a role for complement and pentraxins. **Trends in immunology,** v. 24, n. 3, p. 148-154, 2003. ISSN 1471-4906.

NERRIENET, E. et al. Hepatitis C virus infection in cameroon: A cohort-effect. **Journal of medical virology,** v. 76, n. 2, p. 208-214, 2005. ISSN 1096-9071.

NETWORK, E. P. H. C. V. Three broad modalities in the natural history of vertically acquired hepatitis C virus infection. **Clinical Infectious Diseases,** v. 41, n. 1, p. 45-51, 2005. ISSN 1058-4838.

NEUMANN-HAEFELIN, C.; THIMME, R. Adaptive immune responses in hepatitis C virus infection. In: (Ed.). **Hepatitis C Virus: From Molecular Virology to Antiviral Therapy**: Springer, 2013. p.243-262. ISBN 3642273394.

NG, S. B. et al. Targeted capture and massively parallel sequencing of 12 human exomes. **Nature,** v. 461, n. 7261, p. 272-276, 2009. ISSN 0028-0836.

NICHOLSON, S. E. et al. Suppressor of cytokine signaling (SOCS)-5 is a potential negative regulator of epidermal growth factor signaling. **Proceedings of the National Academy of Sciences of the United States of America,** v. 102, n. 7, p. 2328-2333, 2005. ISSN 0027-8424.

NIELSEN, S. U. et al. Association between hepatitis C virus and very-low-density lipoprotein (VLDL)/LDL analyzed in iodixanol density gradients. **Journal of virology,** v. 80, n. 5, p. 2418-2428, 2006. ISSN 0022-538X.

NIWA, Y. et al. Methylation silencing of SOCS-3 promotes cell growth and migration by enhancing JAK/STAT and FAK signalings in human hepatocellular carcinoma. **Oncogene,** v. 24, n. 42, p. 6406-6417, 2005. ISSN 0950-9232.

NOGUCHI, T. et al. Effects of mutation in hepatitis C virus nonstructural protein 5A on interferon resistance mediated by inhibition of PKR kinase activity in mammalian cells. **Microbiology and immunology,** v. 45, n. 12, p. 829-840, 2001. ISSN 1348-0421.

NORTHFIELD, J. W. et al. CD161 expression on hepatitis C virus–specific CD8+ T cells suggests a distinct pathway of T cell differentiation. **Hepatology,** v. 47, n. 2, p. 396-406, 2008. ISSN 1527-3350.

OGATA, H. et al. Deletion of the SOCS3 Gene in Liver Parenchymal Cells Promotes Hepatitis–Induced Hepatocarcinogenesis. **Gastroenterology,** v. 131, n. 1, p. 179-193, 2006. ISSN 0016-5085.

OLESEN, R. et al. DC-SIGN (CD209), pentraxin 3 and vitamin D receptor gene variants associate with pulmonary tuberculosis risk in West Africans. **Genes and immunity,** v. 8, n. 6, p. 456-467, 2007. ISSN 1466-4879.

ORLAND, J. R.; WRIGHT, T. L.; COOPER, S. Acute hepatitis C. **Hepatology,** v. 33, n. 2, p. 321-327, 2001. ISSN 1527-3350.

ORTEGA-PRIETO, A.; DORNER, M. The expanding toolbox for hepatitis C virus research. **Journal of viral hepatitis**, 2016. ISSN 1365-2893.

PAGE-SHAFER, K. et al. Testing strategy to identify cases of acute hepatitis C virus (HCV) infection and to project HCV incidence rates. **Journal of clinical microbiology,** v. 46, n. 2, p. 499-506, 2008. ISSN 0095-1137.

PAL, S. et al. Hepatitis C virus induces oxidative stress, DNA damage and modulates the DNA repair enzyme NEIL1. **Journal of gastroenterology and hepatology,** v. 25, n. 3, p. 627-634, 2010. ISSN 1440-1746.

PAN, C. X. et al. Role of interleukin-22 in liver diseases. **Inflamm Res,** v. 63, n. 7, p. 519-25, Jul 2014. ISSN 1420-908X (Electronic)

1023-3830 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24623532 >.

PAN, H. et al. Hydrodynamic gene delivery of interleukin-22 protects the mouse liver from concanavalin A-, carbon tetrachloride-, and Fas ligand-induced injury via activation of STAT3. **Cell Mol Immunol,** v. 1, n. 1, p. 43-49, 2004.

PANEL, A. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. **Hepatology,** v. 62, p. 932-954, 2015.

PANG, P. S.; PLANET, P. J.; GLENN, J. S. The evolution of the major hepatitis C genotypes correlates with clinical response to interferon therapy. **PLoS One,** v. 4, n. 8, p. e6579, 2009. ISSN 1932-6203.

PARDANANI, A. et al. Safety and efficacy of TG101348, a selective JAK2 inhibitor, in myelofibrosis. **Journal of Clinical Oncology,** v. 29, n. 7, p. 789-796, 2011. ISSN 0732-183X.

PARK, O. et al. In vivo consequences of liver-specific interleukin-22 expression in mice: Implications for human liver disease progression. **Hepatology,** v. 54, n. 1, p. 252-261, 2011. ISSN 1527-3350.

PAWLOTSKY, J.-M. Pathophysiology of hepatitis C virus infection and related liver disease. **Trends in microbiology,** v. 12, n. 2, p. 96-102, 2004. ISSN 0966-842X.

PAWLOTSKY, J.-M.; AGHEMO, A.; BACK, D. EASL recommendations on treatment of hepatitis C 2015. **J hepatol,** v. 63, p. 199-236, 2015.

PELLICORO, A. et al. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. **Nature Reviews Immunology,** v. 14, n. 3, p. 181-194, 2014. ISSN 1474-1733.

PEMBREY, L.; NEWELL, M.-L.; TOVO, P.-A. The management of HCV infected pregnant women and their children European paediatric HCV network. **Journal of hepatology,** v. 43, n. 3, p. 515-525, 2005. ISSN 0168-8278.

PEREIRA, L. M. et al. Prevalence and risk factors of Hepatitis C virus infection in Brazil, 2005 through 2009: a cross-sectional study. **BMC infectious diseases,** v. 13, n. 1, p. 1, 2013. ISSN 1471-2334.

PESTKA, S. The interferons: 50 years after their discovery, there is much more to learn. **Journal of Biological Chemistry,** v. 282, n. 28, p. 20047-20051, 2007. ISSN 0021-9258.

PHAN, T. et al. Hepatitis C virus NS2 protein contributes to virus particle assembly via opposing epistatic interactions with the E1-E2 glycoprotein and NS3-NS4A enzyme complexes. **Journal of virology,** v. 83, n. 17, p. 8379-8395, 2009. ISSN 0022-538X.

PIESSEVAUX, J. et al. The many faces of the SOCS box. **Cytokine & growth factor reviews,** v. 19, n. 5, p. 371-381, 2008. ISSN 1359-6101.

PILERI, P. et al. Binding of hepatitis C virus to CD81. **Science,** v. 282, n. 5390, p. 938-941, 1998. ISSN 0036-8075.

PINES, G.; KÖSTLER, W. J.; YARDEN, Y. Oncogenic mutant forms of EGFR: lessons in signal transduction and targets for cancer therapy. **FEBS letters,** v. 584, n. 12, p. 2699-2706, 2010. ISSN 0014-5793.

PLOSS, A. et al. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. **Nature,** v. 457, n. 7231, p. 882-886, 2009. ISSN 0028-0836.

POL, S. et al. Daclatasvir for previously untreated chronic hepatitis C genotype-1 infection: a randomised, parallel-group, double-blind, placebo-controlled, dose-finding, phase 2a trial. **The Lancet infectious diseases,** v. 12, n. 9, p. 671-677, 2012. ISSN 1473-3099.

POORDAD, F. et al. Boceprevir for untreated chronic HCV genotype 1 infection. **New England Journal of Medicine,** v. 364, n. 13, p. 1195-1206, 2011. ISSN 0028-4793.

POPESCU, C.-I. et al. NS2 protein of hepatitis C virus interacts with structural and non-structural proteins towards virus assembly. **PLoS Pathog,** v. 7, n. 2, p. e1001278, 2011. ISSN 1553-7374.

POYNARD, T.; BEDOSSA, P.; OPOLON, P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. **The Lancet,** v. 349, n. 9055, p. 825-832, 1997. ISSN 0140-6736.

POYNARD, T. et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. **Gastroenterology,** v. 122, n. 5, p. 1303-1313, 2002. ISSN 0016-5085.

RADAEVA, S. et al. Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. **Hepatology,** v. 39, n. 5, p. 1332-1342, 2004. ISSN 1527-3350.

RADISKY, E. S. PRSS3/mesotrypsin in prostate cancer progression: implications for translational medicine. **Asian journal of andrology,** v. 15, n. 4, p. 439, 2013.

RAMAKRISHNAIAH, V. et al. Exosome-mediated transmission of hepatitis C virus between human hepatoma Huh7. 5 cells. **Proceedings of the National Academy of Sciences,** v. 110, n. 32, p. 13109-13113, 2013. ISSN 0027-8424.

REN, X.; HU, B.; COLLETTI, L. M. IL-22 is involved in liver regeneration after hepatectomy. **American Journal of Physiology-Gastrointestinal and Liver Physiology,** v. 298, n. 1, p. G74-G80, 2010. ISSN 0193-1857.

ROMERO-GOMEZ, M. et al. Genes and hepatitis C: susceptibility, fibrosis progression and response to treatment. **Liver international,** v. 31, n. 4, p. 443-460, 2011. ISSN 1478-3231.

RUTZ, S.; EIDENSCHENK, C.; OUYANG, W. IL-22, not simply a Th17 cytokine. **Immunol Rev,** v. 252, n. 1, p. 116-32, Mar 2013. ISSN 1600-065X (Electronic)

0105-2896 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23405899 >.

SABAT, R.; OUYANG, W.; WOLK, K. Therapeutic opportunities of the IL-22-IL-22R1 system. **Nat Rev Drug Discov,** v. 13, n. 1, p. 21-38, Jan 2014. ISSN 1474-1784 (Electronic)

1474-1776 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24378801 >.

SABO, M. C. et al. Neutralizing monoclonal antibodies against hepatitis C virus E2 protein bind discontinuous epitopes and inhibit infection at a postattachment step. **Journal of virology,** v. 85, n. 14, p. 7005-7019, 2011. ISSN 0022-538X.

SAHNOUN, Z.; JAMOUSSI, K.; ZEGHAL, K. [Free radicals and antioxidants: human physiology, pathology and therapeutic aspects]. **Therapie,** v. 52, n. 4, p. 251-270, 1996. ISSN 0040-5957.

SCARSELLI, E. et al. The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. **The EMBO journal,** v. 21, n. 19, p. 5017-5025, 2002. ISSN 0261-4189.

SCHEEL, T. K.; RICE, C. M. Understanding the hepatitis C virus life cycle paves the way for highly effective therapies. **Nature medicine,** v. 19, n. 7, p. 837-849, 2013. ISSN 1078-8956.

SCHOGGINS, J. W.; RICE, C. M. Innate immune responses to hepatitis C virus. In: (Ed.). **Hepatitis C Virus: From Molecular Virology to Antiviral Therapy**: Springer, 2013. p.219-242. ISBN 3642273394.

SCHULZE, K. et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. **Nature genetics,** v. 47, n. 5, p. 505-511, 2015. ISSN 1061-4036.

SEBASTIANI, G.; GKOUVATSOS, K.; PANTOPOULOS, K. Chronic hepatitis C and liver fibrosis. **World journal of gastroenterology: WJG,** v. 20, n. 32, p. 11033, 2014.

SEKI, E.; SCHWABE, R. F. Hepatic inflammation and fibrosis: functional links and key pathways. **Hepatology,** v. 61, n. 3, p. 1066-1079, 2015. ISSN 1527-3350.

SEMMO, N. et al. Maintenance of HCV-specific T-cell responses in antibody-deficient patients a decade after early therapy. **Blood,** v. 107, n. 11, p. 4570-4571, 2006. ISSN 0006-4971.

SERTI, E. et al. Monocytes activate natural killer cells via inflammasome-induced interleukin 18 in response to hepatitis C virus replication. **Gastroenterology,** v. 147, n. 1, p. 209-220. e3, 2014. ISSN 0016-5085.

SHEN, X. F. et al. Quantitative assessment of the effect of epidermal growth factor 61A/G polymorphism on the risk of hepatocellular carcinoma. **Oncology letters,** v. 10, n. 5, p. 3199-3205, 2015. ISSN 1792-1074.

SHEPARD, C. W.; FINELLI, L.; ALTER, M. J. Global epidemiology of hepatitis C virus infection. **The Lancet infectious diseases,** v. 5, n. 9, p. 558-567, 2005. ISSN 1473-3099.

SHEPPARD, P. et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. **Nature immunology,** v. 4, n. 1, p. 63-68, 2003.

SHI, Q.; JIANG, J.; LUO, G. Syndecan-1 serves as the major receptor for attachment of hepatitis C virus to the surfaces of hepatocytes. **Journal of virology,** v. 87, n. 12, p. 6866-6875, 2013. ISSN 0022-538X.

SHOUKRY, N. H. et al. Memory CD8+ T cells are required for protection from persistent hepatitis C virus infection. **The Journal of experimental medicine,** v. 197, n. 12, p. 1645-1655, 2003. ISSN 0022-1007.

SIMMONDS, P. et al. A proposed system for the nomenclature of hepatitis C viral genotypes. **Hepatology,** v. 19, n. 5, p. 1321-1324, 1994. ISSN 1527-3350.

SOKOLENKO, A. P. et al. Identification of novel hereditary cancer genes by whole exome sequencing. **Cancer letters,** v. 369, n. 2, p. 274-288, 2015. ISSN 0304-3835.

STALLONE, G. et al. Pentraxin 3: a novel biomarker for predicting progression from prostatic inflammation to prostate cancer. **Cancer research,** v. 74, n. 16, p. 4230-4238, 2014. ISSN 0008-5472.

STAPLEFORD, K. A.; LINDENBACH, B. D. Hepatitis C virus NS2 coordinates virus particle assembly through physical interactions with the E1-E2 glycoprotein and NS3-NS4A enzyme complexes. **Journal of virology,** v. 85, n. 4, p. 1706-1717, 2011. ISSN 0022-538X.

STARK, G. R.; DARNELL, J. E. The JAK-STAT pathway at twenty. **Immunity,** v. 36, n. 4, p. 503-514, 2012. ISSN 1074-7613.

SUGIMOTO, K. et al. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. **The Journal of clinical investigation,** v. 118, n. 2, p. 534-544, 2008. ISSN 0021-9738.

SULKOWSKI, M. S. et al. Faldaprevir combined with pegylated interferon alfa-2a and ribavirin in treatment-naïve patients with chronic genotype1 HCV: SILEN-C1 trial. **Hepatology,** v. 57, n. 6, p. 2143-2154, 2013. ISSN 1527-3350.

SY, T.; JAMAL, M. M. Epidemiology of hepatitis C virus (HCV) infection. **Int J Med Sci,** v. 3, n. 2, p. 41-6, 2006.

SZABO, G.; PETRASEK, J. Inflammasome activation and function in liver disease. **Nature Reviews Gastroenterology & Hepatology,** v. 12, n. 7, p. 387-400, 2015. ISSN 1759-5045.

TAI, A. W.; CHUNG, R. T. Treatment failure in hepatitis C: mechanisms of non-response. **Journal of hepatology,** v. 50, n. 2, p. 412-420, 2009. ISSN 0168-8278.

TAI, D. I. et al. Activation of nuclear factor κB in hepatitis C virus infection: implications for pathogenesis and hepatocarcinogenesis. **Hepatology,** v. 31, n. 3, p. 656-664, 2000. ISSN 1527-3350.

TANABE, K. K. et al. Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. **Jama,** v. 299, n. 1, p. 53-60, 2008. ISSN 0098-7484.

TARRAGÔ, A. M. et al. Combined impact of hepatitis C virus genotype 1 and interleukin-6 and tumor necrosis factor-α polymorphisms on serum levels of pro-inflammatory cytokines in Brazilian HCV-infected patients. **Human immunology,** v. 75, n. 11, p. 1075-1083, 2014. ISSN 0198-8859.

TAYLOR, D. R. et al. Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. **Science,** v. 285, n. 5424, p. 107-110, 1999. ISSN 0036-8075.

TE, H. S.; JENSEN, D. M. Epidemiology of hepatitis B and C viruses: a global overview. **Clinics in liver disease,** v. 14, n. 1, p. 1-21, 2010. ISSN 1089-3261.

THI, V. L. D. et al. Characterization of hepatitis C virus particle subpopulations reveals multiple usage of the scavenger receptor BI for entry steps. **Journal of Biological Chemistry,** v. 287, n. 37, p. 31242-31257, 2012. ISSN 0021-9258.

THIMME, R.; BINDER, M.; BARTENSCHLAGER, R. Failure of innate and adaptive immune responses in controlling hepatitis C virus infection. **FEMS microbiology reviews,** v. 36, n. 3, p. 663-683, 2012. ISSN 1574-6976.

THIMME, R. et al. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. **Proceedings of the National Academy of Sciences,** v. 99, n. 24, p. 15661-15668, 2002. ISSN 0027-8424.

THIMME, R. et al. Determinants of viral clearance and persistence during acute hepatitis C virus infection. **The Journal of experimental medicine,** v. 194, n. 10, p. 1395-1406, 2001. ISSN 0022-1007.

THOMSSEN, R. et al. Association of hepatitis C virus in human sera with β-lipoprotein. **Medical microbiology and immunology,** v. 181, n. 5, p. 293-300, 1992. ISSN 0300-8584.

THOMSSEN, R.; BONK, S.; THIELE, A. Density heterogeneities of hepatitis C virus in human sera due to the binding of β-lipoproteins and immunoglobulins. **Medical microbiology and immunology,** v. 182, n. 6, p. 329-334, 1993. ISSN 0300-8584.

TIMPE, J. M. et al. Hepatitis C virus cell-cell transmission in hepatoma cells in the presence of neutralizing antibodies. **Hepatology,** v. 47, n. 1, p. 17-24, 2008. ISSN 1527-3350.

TÖNJES, R. et al. Autocrine mitogen IgEGF cooperates with c-myc or with the Hcs locus during hepatocarcinogenesis in transgenic mice. **Oncogene,** v. 10, n. 4, p. 765-768, 1995. ISSN 0950-9232.

TSCHERNE, D. M. et al. Time-and temperature-dependent activation of hepatitis C virus for low-pH-triggered entry. **Journal of virology,** v. 80, n. 4, p. 1734-1741, 2006. ISSN 0022-538X.

TSENG, C.-T. K.; KLIMPEL, G. R. Binding of the hepatitis C virus envelope protein E2 to CD81 inhibits natural killer cell functions. **The Journal of experimental medicine,** v. 195, n. 1, p. 43-50, 2002. ISSN 0022-1007.

UZE, G. et al. The receptor of the type I interferon family. In: (Ed.). **Interferon: The 50th Anniversary**: Springer, 2007. p.71-95. ISBN 354071328X.

VAN DER MEER, A. J. et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. **Jama,** v. 308, n. 24, p. 2584-2593, 2012. ISSN 0098-7484.

VELDT, B. J. et al. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. **Annals of internal medicine,** v. 147, n. 10, p. 677-684, 2007. ISSN 0003-4819.

VENTER, J. C. et al. The sequence of the human genome. **science,** v. 291, n. 5507, p. 1304-1351, 2001. ISSN 0036-8075.

VON HAHN, T. et al. Hepatitis C virus continuously escapes from neutralizing antibody and T-cell responses during chronic infection in vivo. **Gastroenterology,** v. 132, n. 2, p. 667-678, 2007. ISSN 0016-5085.

WANG, D. G. et al. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. **Science,** v. 280, n. 5366, p. 1077-1082, 1998. ISSN 0036-8075.

WANG, J.-X. et al. Aberrant methylation of the 3q25 tumor suppressor gene PTX3 in human esophageal squamous cell carcinoma. **World J Gastroenterol,** v. 17, n. 37, p. 4225-4230, 2011.

WEBSTER, D. P.; KLENERMAN, P.; DUSHEIKO, G. M. Hepatitis C. **Lancet,** v. 385, n. 9973, p. 1124-35, Mar 21 2015. ISSN 1474-547X (Electronic)

0140-6736 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25687730 >.

WEISS, B. et al. Cloning of murine IL-22 receptor alpha 2 and comparison with its human counterpart. **Genes and immunity,** v. 5, n. 5, p. 330-336, 2004. ISSN 1466-4879.

WESTBROOK, R. H.; DUSHEIKO, G. Natural history of hepatitis C. **Journal of hepatology,** v. 61, n. 1, p. S58-S68, 2014. ISSN 0168-8278.

WIELAND, S. et al. Simultaneous detection of hepatitis C virus and interferon stimulated gene expression in infected human liver. **Hepatology,** v. 59, n. 6, p. 2121-2130, 2014. ISSN 1527-3350.

WILLIAMS, S. J.; GOTLEY, D. C.; ANTALIS, T. M. Human trypsinogen in colorectal cancer. **International journal of cancer,** v. 93, n. 1, p. 67-73, 2001. ISSN 1097-0215.

WILSON, M. S. et al. Redundant and pathogenic roles for IL-22 in mycobacterial, protozoan, and helminth infections. **The Journal of Immunology,** v. 184, n. 8, p. 4378-4390, 2010. ISSN 0022-1767.

WITT, H. et al. A degradation-sensitive anionic trypsinogen (PRSS2) variant protects against chronic pancreatitis. **Nature genetics,** v. 38, n. 6, p. 668-673, 2006.

WÓJTOWICZ, A. et al. PTX3 Polymorphisms and invasive mold infections after solid organ transplantation. **Clinical Infectious Diseases**, p. civ386, 2015. ISSN 1058-4838.

WOLK, K. et al. IL-22 increases the innate immunity of tissues. **Immunity,** v. 21, n. 2, p. 241-54, Aug 2004. ISSN 1074-7613 (Print)

1074-7613 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15308104 >.

WOLK, K. et al. IL-22 induces lipopolysaccharide-binding protein in hepatocytes: a potential systemic role of IL-22 in Crohn's disease. **The Journal of Immunology,** v. 178, n. 9, p. 5973-5981, 2007. ISSN 0022-1767.

WOLK, K. et al. Biology of interleukin-22. **Semin Immunopathol,** v. 32, n. 1, p. 17-31, Mar 2010. ISSN 1863-2300 (Electronic)

1863-2297 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20127093 >.

WOO, H. G. et al. Profiling of exome mutations associated with progression of HBV-related hepatocellular carcinoma. **PloS one,** v. 9, n. 12, p. e115152, 2014. ISSN 1932-6203.

WU, L.-Y. et al. Up-regulation of interleukin-22 mediates liver fibrosis via activating hepatic stellate cells in patients with hepatitis C. **Clinical Immunology,** v. 158, n. 1, p. 77-87, 2015. ISSN 1521-6616.

XIANG, X. et al. IL-22 and non-ELR-CXC chemokine expression in chronic hepatitis B virus-infected liver. **Immunology and cell biology,** v. 90, n. 6, p. 611-619, 2012. ISSN 0818-9641.

XIE, M. H. et al. Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. **J Biol Chem,** v. 275, n. 40, p. 31335-9, Oct 6 2000. ISSN 0021-9258 (Print)

0021-9258 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10875937 >.

XING, W.-W. et al. Interleukin-22 protects against acute alcohol-induced hepatotoxicity in mice. **Bioscience, biotechnology, and biochemistry,** v. 75, n. 7, p. 1290-1294, 2011. ISSN 1347-6947.

XU, W. et al. A soluble class II cytokine receptor, IL-22RA2, is a naturally occurring IL-22 antagonist. **Proceedings of the National Academy of Sciences,** v. 98, n. 17, p. 9511-9516, 2001. ISSN 0027-8424.

YAMANE, D. et al. Liver injury and disease pathogenesis in chronic hepatitis C. In: (Ed.). **Hepatitis C Virus: From Molecular Virology to Antiviral Therapy**: Springer, 2013. p.263-288. ISBN 3642273394.

YI, Q. et al. PRSS1 mutations and the proteinase/antiproteinase imbalance in the pathogenesis of pancreatic cancer. **Tumor Biology**, p. 1-6, 2015. ISSN 1010-4283.

YONEDA, M. et al. Plasma Pentraxin3 is a novel marker for nonalcoholic steatohepatitis (NASH). **BMC gastroenterology,** v. 8, n. 1, p. 1, 2008. ISSN 1471-230X.

YOSHIDA, T. et al. SOCS1 is a suppressor of liver fibrosis and hepatitis-induced carcinogenesis. **The Journal of experimental medicine,** v. 199, n. 12, p. 1701-1707, 2004. ISSN 0022-1007.

ZEKRI, A. et al. Mismatch repair genes (hMLH1, hPMS1, hPMS2, GTBP/hMSH6, hMSH2) in the pathogenesis of hepatocellular carcinoma. **World journal of gastroenterology: WJG,** v. 11, n. 20, p. 3020-3026, 2005. ISSN 1007-9327.

ZENEWICZ, L. A. et al. Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. **Immunity,** v. 27, n. 4, p. 647-659, 2007. ISSN 1074-7613.

ZENEWICZ, L. A. et al. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. **Immunity,** v. 29, n. 6, p. 947-957, 2008. ISSN 1074-7613.

ZEUZEM, S. et al. Telaprevir for retreatment of HCV infection. **New England Journal of Medicine,** v. 364, n. 25, p. 2417-2428, 2011. ISSN 0028-4793.

ZHANG, J. et al. Role of SOCS1 in tumor progression and therapeutic application. **International Journal of Cancer,** v. 130, n. 9, p. 1971-1980, 2012. ISSN 1097-0215.

ZHANG, L. et al. Whole-exome sequencing identifies a somatic missense mutation of NBN in clear cell sarcoma of the salivary gland. **Oncology Reports**, 2016. ISSN 1021-335X.

ZHANG, Y. et al. A proinflammatory role for interleukin-22 in the immune response to hepatitis B virus. **Gastroenterology,** v. 141, n. 5, p. 1897-1906, 2011. ISSN 0016-5085.

ZHAO, J. et al. Pathological functions of interleukin-22 in chronic liver inflammation and fibrosis with hepatitis B virus infection by promoting T helper 17 cell recruitment. **Hepatology,** v. 59, n. 4, p. 1331-1342, 2014. ISSN 1527-3350.

ZHUANG, G. et al. Tumour-secreted miR-9 promotes endothelial cell migration and angiogenesis by activating the JAK-STAT pathway. **The EMBO journal,** v. 31, n. 17, p. 3513-3523, 2012. ISSN 0261-4189.

ZOPF, S. et al. Advances in hepatitis C therapy: What is the current state-what come's next? **World journal of hepatology,** v. 8, n. 3, p. 139, 2016.

5. RESULTADOS

5.1 Artigo 1

Título: Genetic variation in PTX3 and plasma levels associated with hepatocellular carcinoma in patients with HCV.

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Genetic variation in *PTX3* and plasma levels associated with hepatocellular carcinoma in patients with HCV

Running title: Association of PTX3 variants with HCV

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Abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; PTX, pentraxin; HIV, human immunodeficiency virus; OR, odds ratio; CI, confidence interval; BMI, body mass index; SNPs, single nucleotide polymorphisms; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor-alpha; CRP, C-reactive protein; IL-6, interleukin-6; MIF, macrophage migration inhibitory factor.

Abstract

Hepatitis C virus (HCV) is the main cause of chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC) worldwide. The risk to develop HCC increases with the severity of liver inflammation and fibrosis. Long pentraxin 3 (PTX3) is a soluble pattern-recognition receptor produced by phagocytes and nonimmune cells at sites of inflammation or injury. The aim of the present study was to determine the association of PTX3 polymorphisms and its plasma levels with HCC occurrence among patients with HCV. Samples from 524 patients with chronic hepatitis C were evaluated in this study. Two polymorphisms (rs1840680 and rs2305619) in the PTX3 gene were determined by real-time PCR. PTX3 plasma levels were measured by Enzyme-linked Immunosorbent Assay (ELISA). Our data presented a significant association between PTX3 polymorphisms and HCC occurrence in univariate and multivariate analysis (p=0.024). Patients with HCC had higher PTX3 plasma levels compared to individuals with mild or severe fibrosis (p<0.0001 and p=0.002, respectively). In addition, PTX3 rs2305619 polymorphism and plasma levels were correlated with Child-Pugh scores B and C in HCC individuals. PTX3 seems to be a risk factor for HCC occurrence in chronic hepatitis C. This is the first study that evaluates PTX3 in the context of hepatitis C.

Keywords: fibrosis, HCC, HCV, hepatitis C, polymorphism, PTX3.

Introduction

It is estimated that 3% of the world's population is infected with hepatitis C virus (HCV), representing 170 million people. In these patients, the risk of hepatocellular carcinoma (HCC) gradually increases as liver fibrosis progresses. Once cirrhosis is established, the annual incidence of HCC is extremely high (1–7% per year), although HCC rarely develops in less fibrotic livers [1,2].

The risk to develop HCC increases with the severity of liver inflammation and fibrosis. The hepatic inflammation caused by HCV involves various mechanisms that include the regulatory immune response of the host, mediated by cytokines that play important roles against viral infections and viral polypeptides, which interact with cells involved in innate and adaptive immunity [3-6].

Long pentraxin 3 (PTX3) is a soluble pattern-recognition receptor produced by phagocytes and nonimmune cells at sites of inflammation or injury. PTX3 plays multiple roles in innate immunity and inflammation, being able to activate and regulate complement system (via C1q and factor H), interact with microbial moieties (outer membrane protein A of *K. pneumonia* and viral haemoagglutinin) and regulate inflammation [7-9].

PTX3 gene, located in human chromosome 3, is organized in three exons and two introns. Two single nucleotide polymorphisms (SNPs) in *PTX3* (rs1840680 and rs2305619) have demonstrated functional significance. The A allele of both SNPs has been associated with higher PTX3 plasma levels [10,11]. In addition, the A allele of the rs1840680 and rs2305619 have been associated with susceptibility to *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa* infections [12,13]. However, studies investigating the association of *PTX3* in the context of viral infections in humans are lacking.

Studies have indicated a possible role of PTX3 in cancer development, since then its serum and expression levels have been associated with worse prognostic in some types of cancer, including gastric cancer [14], lung carcinoma [15], pancreatic carcinoma [16], liposarcoma [17], prostate cancer [18] and glioma [19]. The mechanisms by which PTX3 promotes cancer development are still unknown, but it may be associated with inflammation, indeed further studies are necessary to elucidate this relationship.

The regular surveillance of HCC is essential to detect early tumors and to achieve cure with the treatment. In addition, the identification of molecular biomarkers may open new paths

toward the discovery of therapeutic targets [20]. Thus, the aim of the present study was to evaluate the association of two *PTX3* polymorphisms (rs1840680 and rs2305619) and PTX3 plasma levels with HCC occurrence in patients with chronic HCV.

Patients and Methods

Patients

A total of 524 patients from the Gastrohepatology Service of the Oswaldo Cruz University Hospital/Liver Institute of Pernambuco (Recife, Northeastern Brazil) were selected from August 2010 to November 2014. Patients were included in this study if they had persistent anti-HCV antibodies and HCV-RNA positivity. Presence of hepatitis A, hepatitis B, and human immunodeficiency virus (HIV) antibodies were considered as exclusion criteria. Written informed consent was obtained from all patients and a profile with clinical, biochemical and HCV genotype information was generated by means of a questionnaire.

The present study was approved by the Ethical Committee in Research of the University of Pernambuco under the protocol 47/2010 - CAAE: 0041.0.106.000-10 and was conducted in accordance with the provisions of the declaration of Helsinki and Good Clinical Practice guidelines.

Study groups

Liver biopsies were evaluated by a single expert pathologist and assessed according to the METAVIR scoring system, in which fibrosis is scored as F1 (portal fibrosis), F2 (portal fibrosis with few septa), F3 (septal fibrosis) and F4 (cirrhosis) [21]. Patients with METAVIR score F0 or F1 were classified as mild fibrosis group, while those with F3 or F4 were classified as severe fibrosis group. The presence of HCC was considered as an exclusion criteria in these two groups. Once F2 is considered an intermediate phenotype between mild and severe fibrosis, we decided not to include this group in this study to ensure more homogeneous phenotype.

Hepatocellular carcinoma caused by HCV was diagnosed by ultrasound, computerized tomography, magnetic resonance imaging, arteriography and tumor biopsy according to the AASLD criteria [22]. Patients with HCC were further characterized by HCC size and number

of nodules. Child-Pugh score was determined according Pugh et al. (1973) to access patients' prognosis. Individuals were classified in three groups of increasing severity (A, B and C) [23].

DNA Extraction and Genotyping

Genomic DNA was extracted from whole blood by using QIAamp DNA Blood Kit (QIAGEN Inc, Chatsworth, CA) following the manufacturer's instructions. The extracted DNA was stored at -20ºC until further analysis.

A 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) performed PTX3 rs1840680 (Assay: C__12069244_10) and rs2305619 (Assay: C__22275654_10) genotyping with TaqMan Genotyping Assays in 96-well plates.

Determination of PTX3 plasma levels

PTX3 plasma levels were determined in 234 patients (Mild group: 85; Severe group: 90 and HCC group: 59). None of the patients had received previous treatment with interferon. Blood samples were collected in EDTA-containing tubes and centrifuged at 1500g for 10 min. Plasma was separated and stored at -80ºC until analysis. PTX3 levels were measured in duplicate using a commercially available enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN). Absorption was read at 450 nm with wavelength correction set to 540 nm using an ELISA plate reader (Bio-Rad, Hercules, CA, USA).

Statistical analysis

The statistical analysis of the data was performed using SPSS statistical software package version 22.0 (SPSS, Inc., Chicago, IL). Categorical variables were compared using the χ^2 test or Fisher's exact test when appropriate. Kolmogorov-Smirnov test was used to check for normal distribution of continuous variables. Two-group comparisons were performed using Student's t-test or Mann-Whitney U-test for parametrically or nonparametrically distributed data. For comparisons of more than two groups, ANOVA or Kruskal-Wallis was performed for parametrically or nonparametrically distributed data. Multivariate logistic regression using the stepwise backward approach was performed to identify predictors of HCC occurrence. The

results are presented using odds ratio (OR) with 95% confidence interval (CI). The differences were considered statistically significant when $p < 0.05$.

Results

Patients characteristics

Among the 524 HCV-infected patients, 210 cases were classified as mild fibrosis, 218 as severe fibrosis and 96 had HCC. The baseline characteristics of the population are provided in Table 1. Patients with HCC were significantly older than those with severe or mild fibrosis (mean age 63.4±8.8, 57.5±9.01 and 52.8±11.6, respectively; $p<0.0001$). Male sex and diabetes were also more prevalent in the HCC group (69.8% and 36.0%, respectively; $p<0.0001$). However, the body mass index (BMI) was found higher in patients with severe fibrosis when compared to those with HCC or mild fibrosis (26.7±4.6, 25.6±4.1 and 25.6±4.2, respectively; $p=0.027$).

Regarding biochemical characteristics, total bilirubin, AST (aspartate aminotransferase), GGT (gamma, glutamyl transpeptidase), ALP (alkaline phosphatase) and AFP (alpha-fetoprotein) were all significantly higher in the HCC group ($p<0.0001$). Platelets counting was higher in patients with mild fibrosis, and ALT was associated with severe fibrosis ($p<0.0001$). Differences in the HCV genotype among the groups were not significant (Table 1).

Association of PTX3 plasma levels with genetic variants and HCV outcome

Figure 1 shows the association of PTX3 plasma levels with the rs1840680 and rs2305619 genotypes. The A/A genotype presented the highest values of PTX3 plasma levels when compared to G/A and G/G genotypes for both rs1840680 and rs2305619. The mean PTX3 plasma levels for the rs1840680 A/A, A/G and G/G genotypes were 2.80 ng/mL, 1.62 ng/mL and 1.55 ng/mL, respectively (AA vs. AG, $p=0.008$; AA vs. GG, $p=0.003$) (Figure 1A). Regarding rs2305619, the mean levels for A/A, A/G and G/G genotypes were 2.34 ng/mL, 1.66 ng/mL and 1.50 ng/mL, respectively (AA vs. AG, $p=0.02$; AA vs. GG, $p=0.03$) (Figure 1B).

We assessed the PTX3 plasma levels according to fibrosis severity and HCC occurrence. Patients with HCC presented higher PTX3 plasma levels than those with severe or mild fibrosis (Mild: 0.97 ng/mL; Severe: 1.84 ng/mL; HCC: 2.90 ng/mL; HCC vs. Mild, $p<0.0001$; HCC vs. Severe, $p=0.002$) (Figure 2).

PTX3 polymorphisms and HCC occurrence

All groups assessed in this study were in Hardy-Weinberg equilibrium. Table 2 summarizes genotypic frequencies of *PTX3* rs1840680 and rs2305619 variants according to fibrosis severity and HCC occurrence. When comparing HCC patients to those with mild or severe fibrosis, carriage of the *PTX3* A/A genotype, for both rs1840680 and rs2305619, was significantly associated with HCC occurrence. For the rs1840680, the frequency of the A/A genotype in the HCC, severe and mild group was 25.0%, 15.1% and 14.8%, respectively ($p<0.05$). The rs2305619 A/A genotype had a frequency of 33.3%, 20.6% and 24.3 for the HCC, severe and mild group, respectively (Severe fibrosis vs. HCC, $p<0.05$).

Multivariate logistic regression model for HCC predictors

Multivariate logistic regression analysis was performed to verify if the *PTX3* A/A genotype could be an independent predictor for HCC occurrence in a multivariate model. Possible confounding variables that were significantly associated with fibrosis severity and HCC in the univariate model were included in the multivariate model. After adjustment, the *PTX3* rs2305619 A/A genotype remained independently associated with HCC occurrence (Severe fibrosis vs HCC, OR 1.94, 95% CI 1.09 – 3.43, $p=0.024$). Age, male sex and diabetes were also associated with HCC after stepwise regression (Table 3).

PTX3 and HCC characteristics

We classified the 96 patients with HCC according to their *PTX3* rs1840680 and rs2305619 genotypes and analyzed their tumor characteristics (Table 4). Patients carrying the A/A genotype for both polymorphisms had higher levels of plasmatic PTX3 ($p=0.0002$ for rs 1840680 and $p=0.009$ for rs 2305619). The frequency of the rs2305619 A/A genotype was higher in patients with Child-Pugh score B and C ($p=0.032$) (Table 4). This association was also observed regarding to PTX3 plasma levels and Child-Pugh score, which patients with Child-Pugh B and C had higher PTX3 plasma levels than those with score A (Mean: 3.45 ng/mL vs. 2.14 ng/mL, $p=0.03$) (Data not shown). Differences in AFP levels, HCC diameter and number of nodules were not significant (Table 4).

Discussion

The present study provides the first evidence of the association between PTX3 and HCC occurrence in patients with chronic HCV. We observed that *PTX3* (rs2305619) A/A genotype and PTX3 plasma levels were associated with HCC in individuals chronically infected by HCV. In a multivariate analysis adjusting potential confounding factors, patients carrying the A/A genotype had almost two times more chance to have HCC.

How the *PTX3* (rs2305619) A/A genotype drives HCC occurrence is unclear. We have demonstrated that this genotype was significantly associated with high PTX3 plasma levels, corroborating previous studies in patients with acute myocardial infarction and in individuals after lung transplantation [10,11]. Although PTX3 plays host-protective roles in some circumstances, many studies have shown detrimental effects of PTX3 in certain experimental settings such as post-ischemic renal injury, intestinal ischemia and reperfusion and ventilation-induced lung injury models [24-27]. Regarding cancer, literature suggests PTX3 has an important role as an inflammatory mediator involving tumor occurrence [14-19]. Thus, HCV infected patients with A/A genotype may activate more pro-inflammatory mediators than those with G/A or G/G genotypes, and consequently increase the risk to develop HCC.

In line with our findings concerning *PTX3* polymorphism, individuals with HCC had higher PTX3 plasma levels than those with mild or severe fibrosis without HCC. Studies showed that PTX3 levels were associated with different types of cancer such as, gastric cancer [14], lung carcinoma [15], pancreatic carcinoma [16], liposarcoma [17] and prostate cancer [18].

The mechanisms through which PTX3 could be associated with HCC are still unknown, but they probably involve inflammation. PTX3 is an acute phase protein which is able to bind to several microbes, including fungi, bacteria and viruses (influenza virus type A, human and murine cytomegalovirus and murine hepatitis virus) [27]. In addition, PTX3 actives the Complement system via the classical and the lectin pathways and it can regulate all pathways by interacting with Complement inhibitors [9]. Previous studies have demonstrated that PTX3 facilitates neutrophil infiltration and stimulates production of pro-inflammatory cytokines, such as TNF-α, IL-1b, CCL2 and CXCL1 in a model of intestinal ischemia and reperfusion [24,27,28].

Hepatocarcinogenesis is considered a multistep process involving subsequent mutations of genes that control proliferation and/or apoptosis in the hepatocytes subjected to continuous

inflammatory and regenerative stimuli, starting from the initial phases of chronic hepatitis to liver cirrhosis [29]. Accordingly, a recent report demonstrated that TNF-α stimulates PTX3 expression via the NFkB pathway, and that PTX3 acts as a tumor-promoting factor by facilitating gastric cancer cell migration and recruitment of macrophages [14]. Moreover, it is also known that PTX3 has an important role in promoting fibrocyte differentiation at sites of fibrosis in vitro and in human fibrotic lung tissue [30].

We found a significant association between the PTX3 rs2305619 A/A genotype, as also high PTX3 levels, and Child-Pugh scores B and C in individuals who had HCC. Previous studies have demonstrated a positive correlation between PTX3 and more advanced stages of pancreatic carcinoma [16], bone metastatic breast cancer [31] and glioma [19]. These findings suggest PTX3 has a role in tumor progression in some types of cancer.

There are some limitations within our study. The association of *PTX3* polymorphism with HCC occurrence needs to be confirmed in a replication cohort. Additionally, due to the design of the present study, we cannot conclude if PTX3 levels are the cause or consequence of HCC occurrence. A further prospective study would be necessary to elucidate this relationship.

In summary, our results support the idea that high levels of PTX3 have a role in HCC development. Additionally, the frequency of A/A genotype, associated with high PTX3 levels, was significantly higher in HCC patients. The release of PTX3 in HCV infection could increase the inflammation process causing liver damage and increasing HCC risk. Nevertheless, more studies are necessary to elucidate the mechanisms concerning the role of PTX3 in HCC development and HCV infection.

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No conflicts of interest exist.

References

1. Lavanchy D. The global burden of hepatitis C. *Liver Int* 2009;29 (Suppl. 1):74-81.
2. Hoshida Y, Fuchs BC, Bardeesy N, Baumert TF, Chung RT. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. *J Hepatol* 2014;61 (1 Suppl):S79-90.
3. Wang YY, Lo GH, Lai KH, Cheng JS, Lin CK, Hsu PI. Increased serum concentrations of tumor necrosis factor-alpha are associated with disease progression and malnutrition in hepatocellular carcinoma. *J Chin Med Assoc* 2003;66:593–8.
4. Mondelli MU, Barnaba V. Viral and host immune regulatory mechanisms in hepatitis C virus infection. *Eur J Gastroenterol Hepatol* 2006;18:327–31
5. Farinati F, Cardin R, Bortolami M, *et al*. Hepatitis C virus: from oxygen free radicals to hepatocellular carcinoma. *J Viral Hepat*. 2007;14:821-9.
6. Aroucha DC, do Carmo RF, Moura P, *et al*. High tumor necrosis factor-α/interleukin-10 ratio is associated with hepatocellular carcinoma in patients with chronic hepatitis C. *Cytokine*. 2013;62:421-5.
7. Bottazzi B, Vouret-Craviari V, Bastone A, *et al*. Multimer formation and ligand recognition by the long pentraxin PTX3.Similarities and differences with the short pentraxins C-reactive proteinand serum amyloid P component. *Journal of Biological Chemisty* 1997;272:32817–23.
8. Nauta AJ, Daha MR, van Kooten C, Roos A. Recognition and clearance of apop-totic cells: a role for complement and pentraxins. *Trends in Immunology* 2003;24:148–54.
9. Inforzato A, Doni A, Barajon I, *et al*. PTX3 as a paradigm for the interaction of pentraxins with the complement system. *Semin Immunol* 2013;25:79-85.
10. Diamond JM, Meyer NJ, Feng R, *et al*. Variation in PTX3 is associated with primary graft dysfunction after lung transplantation. *Am J Respir Crit Care Med* 2012;186:546-52.
11. Barbati E, Specchia C, Villella M, *et al*. Influence of pentraxin 3 (PTX3) genetic variants on myocardial infarction risk and PTX3 plasma levels. *PLoS One* 2012;7:e53030.
12. Olesen R, Wejse C, Velez DR, *et al*. DC-SIGN (CD209), pentraxin 3 and vitamin D receptor gene variants associate with pulmonary tuberculosis risk in West Africans. *Genes Immun* 2007;8:456-67.

13. Chiarini M, Sabelli C, Melotti P, *et al*. PTX3 genetic variations affect the risk of Pseudomonas aeruginosa airway colonization in cystic fibrosis patients. *Genes Immun* 2010;11:665-70.
14. Choi B, Lee EJ, Park YS, *et al*. Pentraxin-3 Silencing Suppresses Gastric Cancer-related Inflammation by Inhibiting Chemotactic Migration of Macrophages. *Anticancer Res* 2015;35:2663-8.
15. Diamandis EP, Goodglick L, Planque C, Thornquist MD. Pentraxin-3 is a novel biomarker of lung carcinoma. *Clin Cancer Res* 2011;17: 2395-9.
16. Kondo S, Ueno H, Hosoi H, *et al*. Clinical impact of pentraxin family expression on prognosis of pancreatic carcinoma. *Br J Cancer* 2013;109:739-46.
17. Germano G, Frapolli R, Simone M, *et al.* Antitumor and anti-inflammatory effects of trabectedin on human myxoid liposarcoma cells. *Cancer Res* 2010;70:2235-44.
18. Stallone G, Cormio L, Netti GS, *et al.* Pentraxin 3: a novel biomarker for predicting progression from prostatic inflammation to prostate cancer. *Cancer Res* 2014;74:4230-8.
19. Locatelli M, Ferrero S, Martinelli Boneschi F, *et al.* The long pentraxin PTX3 as a correlate of cancer-related inflammation and prognosis of malignancy in gliomas. *J Neuroimmunol* 2013;260: 99-106.
20. Hoshida Y, Fuchs BC, Tanabe KK. Genomic risk of hepatitis C-related hepatocellular carcinoma. *J Hepatol*. 2012 Mar;56:729-30.
21. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996;24:289-293.
22. Bruix J, Sherman M. American association for the study of liver diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011;53:1020–2.
23. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973;60:646–9.
24. Souza DG, Soares AC, Pinho V, *et al*. Increased mortality and inflammation in tumor necrosis factor-stimulatedgene-14 transgenic mice after ischemia and reperfusion injury. *Am J Pathol* 2002;160:1755–65.
25. Chen J, Matzuk MM, Zhou XJ, Lu CY. Endothelial pentraxin 3 con-tributes to murine ischemic acute kidney injury. *Kidney Int* 2012;82:1195–207.
26. Real JM, Spilborghs GM, Morato-Marques M, *et al*. Pentraxin 3 accelerates lung injury in high tidal volume ventilation in mice. *Mol Immunol* 2012;51:82–90.

27. Daigo K, Mantovani A, Bottazzi B. The yin-yang of long pentraxin PTX3 in inflammation and immunity. *Immunol Lett* 2014;161:38-43
28. Souza DG, Amaral FA, Fagundes CT, *et al*. The long pentraxin PTX3 is crucial for tissue inflammation after intestinal ischemia and reperfusion in mice. *Am J Pathol* 2009;174:1309–18.
29. Tan A, Yeh SH, Liu CJ, Cheung C, Chen PJ. Viral hepatocarcinogenesis: from infection to cancer. *Liver Int* 2008;28:175-88.
30. Pilling D, Cox N, Vakil V, Verbeek JS, Gomer RH. The long pentraxin PTX3 promotes fibrocyte differentiation. *PLoS One*. 2015;10:e0119709.
31. Choi B, Lee EJ, Song DH, *et al.* Elevated Pentraxin 3 in bone metastatic breast cancer is correlated with osteolytic function. *Oncotarget* 2014;5:481-92.

Tables

Table 1: Baseline characteristics of chronic HCV patients according to severity of liver fibrosis and HCC

Variable	Mild Fibrosis (n=210)	Severe Fibrosis (n=218)	HCC (n=96)	*P*
Clinical				
Age (years)	52.8±11.6	57.5±9.01	63.4±8.8	**<0.0001**
Male sex	96 (45.7)	94 (43.1)	67 (69.8)	**<0.0001**
BMI (kg/m^2)	25.6±4.2	26.7±4.6	25.6±4.1	**0.027**
Diabetes	25 (12.0)	55 (25.2)	31 (36.0)	**<0.0001**
Biochemical				
Total Bilirubin (mg/dL)	0.60 (0.20-6.30)	0.90 (0.30-3.30)	1.27 (0.36-7.22)	**<0.0001**
AST (U/L)	43.00 (7.00-149.00)	77.00 (12.00-346.00)	93.00 (16.00-235.00)	**<0.0001**
ALT (U/L)	53.00 (12.00-561.00)	88.00 (7.00-578.00)	76.00 (18.00-491.00)	**<0.0001**
GGT (U/L)	56.00 (11.00-474.00)	118.00 (14.00-883.00)	160.00 (26.00-1629.00)	**<0.0001**
ALP (U/L)	70.00 (12.00-410.00)	93.00 (23.00-332.00)	138.00 (36.00-630.00)	**<0.0001**
Platelets	$2.07x10^5$ ($1.93x10^4$-$3.94.10^5$)	$1.36x10^5$ ($4.1x10^4$-$3.25x10^5$)	$1.06x10^5$ ($3.00x10^4$-$3.24x10^5$)	**<0.0001**
AFP (ng/mL)	2.71 (0.00-92.9)	7.19 (1.30-112.0)	37.5 (1.97-20302.00)	**<0.0001**
HCV genotype 1	141 (69.5)	141 (67.1)	53 (63.9)	0.647

Abbreviations: BMI, body mass index; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma, glutamyl transpeptidase; ALP, alkaline phosphatase; AFP, alpha-fetoprotein

Values are presented as mean ± standard deviation, median (range) or n (%).

Significant associations are in bold.

Table 2. Genotyping distribution of *PTX3* rs1840680 and rs2305619 according to fibrosis severity and HCC

	Genotype	Mild Fibrosis (n=210)	Severe Fibrosis (n=218)	HCC (n=96)	*P*[1]	*P*[2]
rs1840680	G/G	78 (37.1)	76 (34.9)	30 (31.3)	AA vs	AA vs
	A/G	101 (48.1)	109 (50.0)	42 (43.8)	AG+GG	AG+GG
	A/A	31 (14.8)	33 (15.1)	24 (25.0)	**0.024**	**0.029**
rs2305619	G/G	51 (24.3)	54 (24.8)	20 (20.8)	AA vs	AA vs
	A/G	108 (51.4)	119 (54.6)	44 (45.8)	AG+GG	AG+GG
	A/A	51 (24.3)	45 (20.6)	32 (33.3)	0.066	**0.013**

[1]Mild fibrosis vs HCC ; [2]Severe fibrosis vs HCC. Significant associations are in bold.

Table 3. Stepwise multivariate logistic regression for predictors of HCC occurrence

	Mild vs HCC		Severe vs HCC	
	OR (95% CI)	***P***	**OR (95% CI)**	***P***
Age	1.13 (1.09 – 1.17)	**<0.0001**	1.09 (1.05 – 1.12)	**<0.0001**
Male sex	4.42 (2.47 – 7.87)	**<0.0001**	4.20 (2.41 – 7.30)	**<0.0001**
Diabetes	2.99 (1.56 – 5.74)	**0.001**	1.39 (0.78 – 2.45)	0.263
rs2305619 (A/A)	1.48 (0.81 – 2.68)	0.197	1.94 (1.09 – 3.43)	**0.024**

Significant associations are in bold.

Table 4. Hepatocellular carcinoma characteristics in 96 patients classified according to their *PTX3* genotypes groups

	***PTX3* Genotypes rs1840680**			***PTX3* Genotypes rs2305619**		
Variable	**A/A (n=24)**	**G/A+G/G (n=72)**	***P***	**A/A (n=31)**	**G/A+G/G (n=65)**	***P***
PTX3 levels (ng/mL)	4.66±3.04	2.21±1.38	**0.0002**	3.63±2.13	2.45±2.18	**0.009**
AFP levels (ng/mL)	34.7	39.75	0.86	75.50	33.63	0.334
	(2.56-10685.00)	(1.97-60500.00)		(2.56-10685.00)	(1.97-60500.00)	
Child-Pugh						
A	14 (58.3%)	43 (59.7%)	0.711	14 (45.2%)	43 (66.2%)	**0.032**
B	9 (37.5%)	28 (38.9%)		15 (48.4%)	22 (33.8%)	
C	1 (4.2%)	1 (1.4%)		2 (6.5%)	0	
HCC diameter (cm)	3.58±2.17	4.67±2.76	0.086	4.33±2.76	4.44±2.63	0.841
Number of nodules						
Single	9 (27.5%)	25 (34.7%)	0.805	9 (29.0%)	25 (38.5%)	0.494
Multiple	15 (62.5%)	47 (65.3%)		22 (71.0%)	40 (61.5%)	

Values are presented as mean ± standard deviation, median (range) or n (%).

Significant associations are in bold.

Figure legends

Figure 1. Distribution of PTX3 plasma levels according to the rs1840680 (A) and rs2305619 (B) genotypes. Results are shown as Mean±S.D. Mann-Whitney U test was used for comparisons between genotypes.

Figure 2. PTX3 plasma levels according to groups of fibrosis severity and HCC occurrence. Results are shown as Mean±S.D. Mann-Whitney U test was used for comparisons between groups.

Figure 1

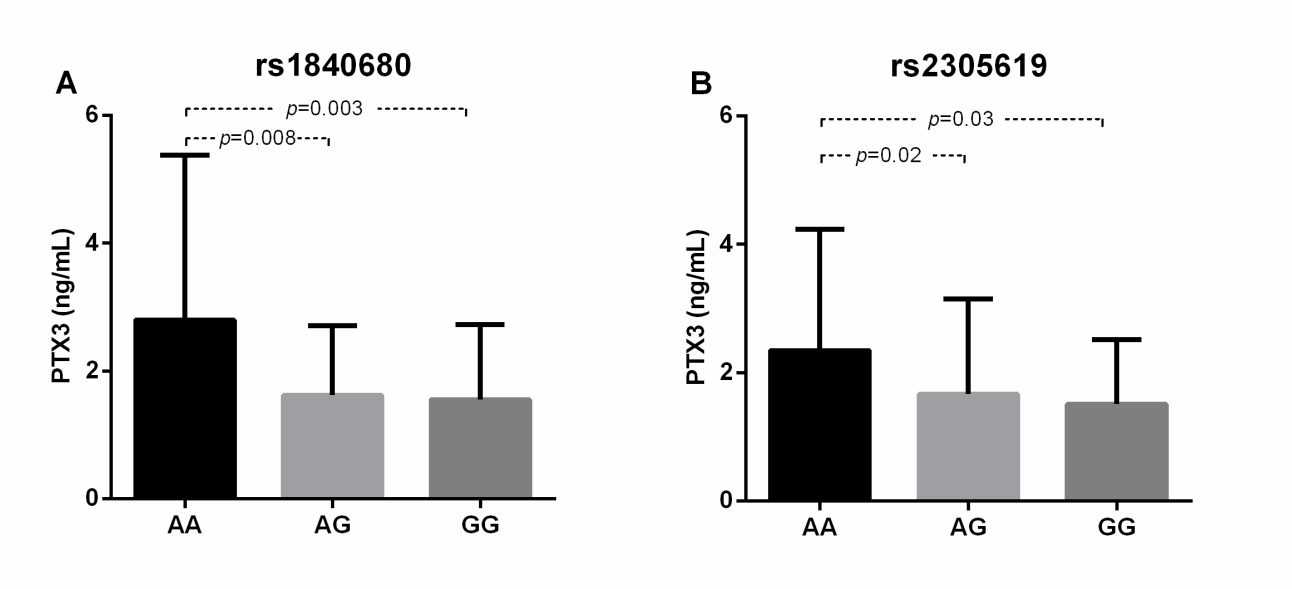
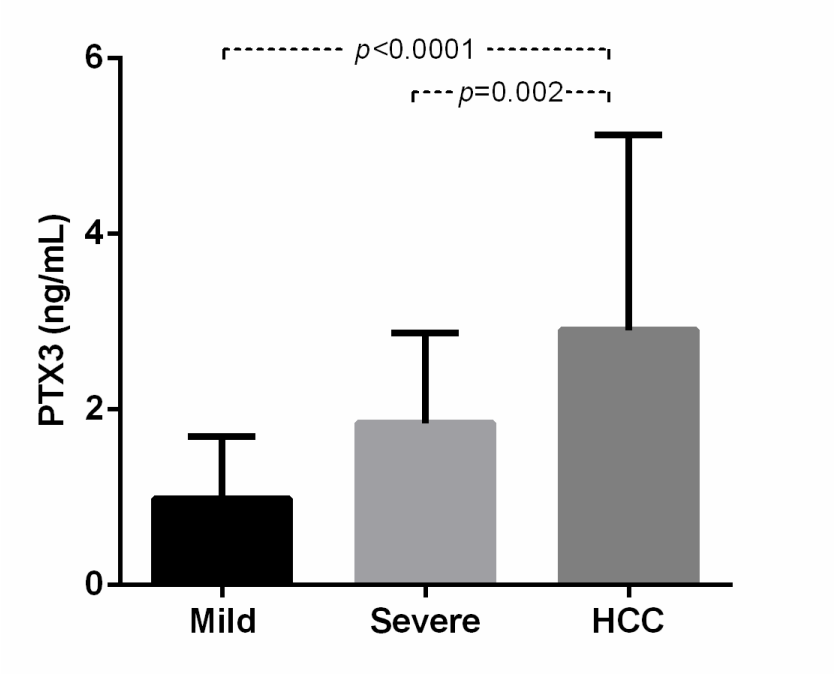


Figure 2



5.2 Artigo 2

Título: IL-22 and IL-22 Binding Protein (IL-22BP) Regulate Fibrosis and Cirrhosis in Hepatitis C Virus and Schistosome Infections

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Interleukin-22 and IL-22 binding protein (IL-22BP) regulate fibrosis and cirrhosis in hepatitis C virus and schistosome infections

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[3] Abbreviations: CentF, central hepatic fibrosis; CTGF, connective tissue growth factor; HBV, hepatitis B virus; HCV, hepatitis C virus; HF, hepatic fibrosis; IL, interleukin; IL-22BP, IL-22 binding protein; ILCs, innate lymphoid cells; NetF, network fibrosis; LD, linkage disequilibrium; LPS, lipopolysaccharide; mRNA, messenger RNA; OR, odds ratio; PBMCs, peripheral blood mononuclear cells; PH, portal hypertension; SNPs, single-nucleotide polymorphisms; Th, T helper.

Conflict of interest

Prof. Dessein consults for, owns stock in, and holds intellectual property rights with GENEPRED Biotechnologies. Dr. Bourliere consults for, advises, is on the speakers' bureau of, and received grants from Janssen, MSD, and Bristol-Myers Squibb. He consults for, advises, and received grants from Gilead. He consults for and advises AbbVie and Roche.

Summary

Interleukin (IL)-22 acts on epithelia, hepatocytes, and pancreatic cells and stimulates innate immunity, tissue protection, and repair. IL-22 may also cause inflammation and abnormal cell proliferation. The binding of IL-22 to its receptor is competed by IL-22 binding protein (IL-22BP), which may limit the deleterious effects of IL-22. The role of IL-22 and IL-22BP in chronic liver diseases is unknown. We addressed this question in individuals chronically infected with schistosomes or hepatitis C virus (HCV). We first demonstrate that schistosome eggs stimulate production of IL-22 transcripts and inhibit accumulation of IL22-BP transcripts in schistosome-infected mice, and that schistosome eggs selectively stimulate production of IL-22 in cultures of blood leukocytes from individuals chronically infected with *Schistosoma japonicum*. High IL-22 levels in cultures correlated with protection against hepatic fibrosis and portal hypertension. To test further the implication of IL-22/IL-22BP in hepatic disease, we analyzed common genetic variants of IL22RA2, which encodes IL-22BP, and found that the genotypes, AA, GG of rs6570136 (*P*=0.003; odds ratio [OR]=2), and CC, TT of rs2064501 (*P*=0.01; OR=2), were associated with severe fibrosis in Chinese infected with *S. japonicum*. We confirmed this result in Sudanese (rs6570136 GG [*P*=0.0007; OR=8.2], rs2064501 TT [*P*=0.02; OR=3.1]), and Brazilians (rs6570136 GG [*P*=0.003; OR=26], rs2064501 TC, TT (*P*=0.03; OR=11]) infected with *S. mansoni*. The aggravating genotypes were associated with high IL22RA2 transcripts levels. Furthermore, these same variants were also associated with HCV-induced fibrosis and cirrhosis (rs6570136 GG, GA [*P*=0.007; OR=1.7], rs2064501 TT, TC (*P*=0.004; OR=2.4]). Conclusions: These results provide strong evidence that IL-22 protects against and IL-22BP aggravates liver fibrosis and cirrhosis in humans with chronic liver infections. Thus, pharmacological modulation of IL-22 BP may be an effective strategy to limit cirrhosis.

Introduction

Interleukin (IL)-22 is produced by a variety of hematopoietic cells, such as innate lymphoid cells (ILCs), lymphoid tissue-inducers,[1] T-helper (Th) cells,[2] and γ/δ T cells.[3] However, unlike most cytokines, IL-22 does not act on hematopoietic cells, but affects epithelia, hepatocytes, and pancreatic cells,[4,5] suggesting an important role for this cytokine at epithelial barriers of the intestine, skin, and lungs and in the liver and pancreas. Indeed, IL-22 stimulates innate immunity by promoting production of antimicrobial peptides,[2,6,7] secretion of mucus, and release of chemokines[8-10] and by enhancing cell mobility.[6,7,11] IL-22 also protects tissues from damage and mediates tissue repair.[5,12,13] Deregulated IL-22 responses may cause pathological inflammation,[12] abnormal cell proliferation,[14] and enhanced chemokine production as occurs in experimental models of psoriaris,[8-10] *Toxoplasma gondii*–induced ileitis,[15] and arthritis.[16] Furthermore, release of both IL-17A and IL-22 in the same inflammatory sites may aggravate pathology given that IL-17A enhances the proinflammatory effects of IL- 22.[9,16,17] Few studies have evaluated the role of IL22/IL-22 binding protein (IL-22BP) in disease, and with the exception of skin psoriasis, it is not known whether this cytokine protects against or aggravates various human diseases. Here, we evaluated whether IL-22 and its inhibitor, IL-22BP,[18,19] influence liver disease in humans infected with hepatitis C virus (HCV) or schistosomes. Hepatic fibrosis (HF) and cirrhosis develop during chronic liver inflammation caused by HCV, hepatitis B virus (HBV), schistosomes, steatosis, and alcohol. Fibrosis is an excessive deposition of extracellular matrix proteins in healing lesions. HF and cirrhosis cause varices, ascites, liver failure, and death in millions of patients. IL-22 protects against acute hepatitis[20] and stimulates tissue regeneration[21] in experimental models of liver disease, but aggravates inflammation in a mouse model of HBV infection.[17] Furthermore, in a murine model of schistosomiasis, IL-22 was not detected during infection.[22] Intestinal ILC produce IL-22 in inflammatory conditions and when damage occurs to the intestinal barrier; therefore, we hypothesized that IL-22 is produced when schistosome eggs perforate the intestine. The consequences of these egg-induced intestinal lesions on the human immune response during schistosomiasis deserve further investigations given that most studies have focused on splenomegaly and on periportal fibrosis. Here, we present evidence that IL-22/IL-22BP play significant roles in HF and cirrhosis in patients with chronic schistosome and HCV infections.

Materials and Methods

Study Population and Evaluation of Hepatic Fibrosis.

The human protocols were approved by the research ethics committees of the University of Gezira and the National Cancer Institute (Gezira, Sudan), the Hunan Institute of Parasitic Diseases (Yueyang, China), the University do Triangulo Mineiro (Uberaba, Brazil), Fiocruz (Salvador) and University Estadual de Pernambucco (Recife). The protocols were also approved by French INSERM ethics committees and by the CNIL.

All study samples are described in Table 1. Subjects infected with schistosomes were obtained from populations exposed to infection with *S. japonicum* (China) or *S. mansoni* (Brazil, Sudan) as described previously.[23,24] Individuals infected with HCV (genotypes 1, 2 or 3) were recruited among patients attending the outpatient clinic at the Instituto do Fígado in Recife and at the Hospital das clinicas in Salvador (Brazil).

Evaluation of Hepatic Fibrosis.

Fibrosis in individuals infected with schistosomes was evaluated with ultrasound (US) that was carried out with a portable ultrasound machine and a 3.5MHz convex probe (LOGIQ Book XP 2410786, Jiangsu, China), based on the standardized procedures of the World Health Organization (WHO).[25] Hepatic fibrosis in individuals infected with *S. mansoni* or *S. Japonicum* manifests as central fibrosis (CentF) around the central vein, and in *S. japonicum* infections, a network fibrosis pattern (NetF) in the parenchyma is also present. WHO guidelines graded CentF as A, B, C, CL, D, E, or F and NetF as GN if the diameter of the network mesh was less than 12mm (resembling fish scales) or GW if it is more than 12mm (resembling a tortoise shell). CentF A pattern is a normal liver struc A, diffuse echogenic foci or “starry sky” is graded CentF B, an uninterrupted thickness of the venous wall distinguished grade CL from grade C, a patchy pattern was graded as D, E, and as F if around portal veins.

WHO grading was modified for *S. japonicum* infections as described in Supporting Materials.

Fibrosis stages in HCV infections were determined on liver biopsies with the Metavir scale. All covariates (alcohol consumption, schistosome infections, mode of contamination,

virus genotypes) were assessed either by interview or by genotyping. None of the HCV-infected individuals had active HBV or HIV infections.

Samples for immunological analysis (Fig. 2) were obtained from the *S. japonicum* Chinese cohort, comprising 66 individuals with HF and 18 controls living in the same region. Controls had not been exposed to infection with *S. japonicum* and were negative when tested by ELISA using (IgG) schistosome Ag. None of the cases and controls had active HBV infections and all cases had been treated with Praziquantel fewer than 10 times over the last 15 years. The mean age of cases and controls did not differ significantly and all individuals were >30 and <66 years old.

Cell Cultures and Cytokine Titration.

PBMCs were separated from heparin-treated venous blood by Ficoll-Hypaque gradient sedimentation (400g for 30min at 18ºC). PBMCs were washed and placed in cultures as described[24] and stimulated with 500 eggs/1ml well prepared as described.[24] Supernatants were collected at 72h and 144h and stored at 280ºC. IL-22 and IL-17A concentrations were determined in the supernatant by ELISA (DuoSet kit, R&D, detection limits, 16 pg/ml).

Cellular Staining and Cytometry Analysis.

PBMCs from Chinese patients were stained ex vivo with FITC-conjugated anti-CD4 and PE-Cy7-conjugated anti-CD3 (BD biosciences) antibodies. The labeled cells were treated for 5 hrs with 100 ng/ml phorbol-12-myristate-13-acetate (PMA) and 1 μg/ml ionomycin in the presence of monensin (Golgistop, BD biosciences). Cells were then fixed and permeabilized with BD Cytofix/Cytoperm according to the manufacturer's instructions and incubated with Alexa647-conjugated anti-IL-17 (eBiosciences) and PE-conjugated anti-IL-22 (R&D) antibodies. Isotype control antibodies were obtained from the corresponding manufacturers. Compensation settings were determined with Comp Beads (BD biosciences). Data were collected on a FACS Calibur cytometer with Cellquest software (BD biosciences) and analyzed with DIVA software (BD biosciences). Cells in the lymphocyte region were gated based on FSC and SSC proprieties and $CD3^+CD4^+$ cells or $CD3^-CD4^-$ cells in the lymphocyte gate were analyzed for the expression of IL-17 and IL-22.

RNA Extraction and Quantitative RT-PCR.

Liver, spleen, Peyer patches and intestine biopsies were conserved in RNAlater (Ambion, Life Technologies) at -80ºC. Tissue (20-30mg of tissue) homogenization was carried out with the Precellys-24 device (Bertin Technologies, Ozymee), with ceramic beads (1.4mm in diameter, CK14), in 400 µl of lysis buffer RLT plus (Qiagen) supplemented with 4µl β-mercapto-ethanol. RNA was recovered with the RNeasy plus following the manufacturer's instructions (Qiagen). RNA integrity was assessed with a 2100 Bioanalyzer (Agilent).

Total RNA (1µg), with a RIN > 7, was reverse-transcribed with the high Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Real-time quantitative PCR, from 20ng of cDNA, was performed with the ABI 7900HT Fast Real Time PCR System and TaqMan Universal PCR Master Mix (Applied Biosystems, Life Technologies). The TaqMan mouse gene expression assays: *Il22* (Mm00444241_m1), *Il22ra2* (Mm00617572_m1), *Il22ra1* (Mm00663697_m1), *Hprt1* (Mm00446968_m1) were from Applied Biosystems. Gene expression values were normalized to the value of the housekeeping gene *Hprt1* (hypoxanthine phosphoribosyltransferase).

Genotyping.

DNA extraction and genotyping were performed as in Dessein et al.[23] Genotyped tag SNPs (Supporting Fig.2A) were selected from the 1000 Genomes Project and from the Hapmap database release #24. Genotyped SNPs had an R-square higher than 0.8 and minor allele frequency higher than 0.10. Primers are described in Supporting Table 2.

Statistical Analysis.

Group comparisons performed on data from Fig. 1A-C were carried out with nonparametric Mann-Whitney tests with SPSS software. Linear regression analysis was performed to test for correlations between IL-22 concentrations, hepatic fibrosis and portal hypertension. CentF was divided into binary classes. NetF was divided in the same manner. Linear regression was performed with these binary variables[23] and with age, sex and exposure. Multivariate logistic regression (SPSS statistical software) was used to investigate possible associations of genotypes with advanced fibrosis or cirrhosis as described previously.[23]

Results

IL22, IL22RA2, IL22RA1 transcripts in mice infected with *Schistosoma mansoni*: Schistosome eggs stimulate the production of IL-22 in the intestine.

We measured *Il22*, *Il22ra2*, and *Il22ra1* messenger RNA (mRNA) levels in the intestine, liver, and spleen of mice infected with *Schistosoma mansoni* to test whether schistosomes induce an IL-22/IL-22BP response (Fig. 1A-D). *Il22* mRNA levels were low before infection (Fig. 1A); they started to increase in the colon and in Peyer patches, at 4 weeks when egg laying had just begun, and peaked at 6 weeks (Fig. 1B). *Il22* mRNA was not detectable in liver or spleen. *Il22ra2* mRNA levels were high in the intestine and spleen (but not in the liver) before infection (Fig. 1A) and were 5- to 10-fold lower after 11 weeks of infection (Fig. 1C). Thus, schistosome eggs promote production of *Il22* mRNA and inhibit that of *Il22ra2*. *Il22ra1* mRNA levels remained stable in the intestine and liver, and were high in the spleen after infection (Fig. 1D), indicating that these tissues can respond to IL-22.

Production of IL-22 by blood mononuclear cells from individuals with chronic schistosome infection: IL-22 is produced by at least two different cell populations.

We evaluated the amount of IL-22 produced by peripheral blood mononuclear cells (PBMCs) from 66 Chinese fishermen infected with *S. japonicum* and 18 nonexposed controls (described in Materials and Methods). IL-22 was detectable in resting cultures of patient PBMCs at 72 and 144 hours of cell culture, and its production was stimulated by schistosome eggs ($P<10^{-3}$; (Fig. 2A). IL-22 was detectable in cultures of control PBMCs only at 144 hours of culture, and its production was unaffected by eggs. IL-17A was detectable in cultures of PBMCs at 144 hours of culture, and its production was unaffected by eggs (data not shown). Flow cytometry analysis of patient PBMCs showed that both $CD3^+CD4^+$ and $CD3^-CD4^-$ cell populations produced IL-22, and neither cell population produced IL-17A (Supporting Fig. 1).[1,26,27]

IL-22 produced by PBMCs from individuals with chronic schistosomiasis correlates with protection against HF and portal hypertension.

HF in individuals infected with *S. mansoni* or *S. japonicum* manifests as central fibrosis (CentF) around the central vein, and in *S. japonicum* infections, a network fibrosis (NetF) pattern in the parenchyma is also present. In this study, CentF was graded as light (+/-), advanced (+), severe (++), and very severe (+++). Severe and very severe fibrosis were associated with portal blood hypertension and splenomegaly. We found that IL-22 concentrations in resting (*P*=0.005) and in egg-stimulated (*P*=0.04) cultures of PBMCs were inversely correlated with CentF (Fig. 2B) in PBMC donors infected with *S. japonicum*. Moreover, multilinear regression analysis showed that both severe and very severe CentF (*P*=0.01) and NetF (P=0.004) were independently associated with low IL-22 concentrations (Fig. 2C). Furthermore, patient portal vein diameter (an indicator of portal hypertension [PH]) was negatively correlated (*P*=0.02) with IL-22 concentrations (Fig. 2D).

Polymorphisms in *IL22RA2* are associated with severe HF in Chinese fishermen exposed to *S.japonicum*; the aggravating genotypes are associated with an increase of IL-22RA2 transcripts.

We determined whether polymorphisms in *IL22RA2* affect the risk of severe HF to test further the hypothesis that high IL-22 production protects against schistosome-induced HF. *IL22RA2* is located on Chr.6q23, which controls HF.[28] Mutations in connective tissue growth factor (CTGF) account for part of the control exerted by this locus.[23] *IL22RA2*, the gene encoding IL-22BP, is located in the same region and may also be involved in the control. To test this hypothesis, we genotyped single-nucleotide polymorphisms (SNPs) representative of the six major SNP correlation bins (TagSNP, r^2=0.8) in *IL22RA2* (Supporting Fig. 2A; Supporting Table 1) in 327 Chinese fishermen (Table 1) with long exposure to *S. japonicum* infections. For this analysis, we have used a binary fibrosis phenotype that included, as cases, subjects with severe CentF or severe NetF and subjects with advanced CentF if they also had advanced or severe NetF. Control groups included individuals who exhibited no NetF and light CentF or less (see Materials and Methods). The genotypes AA, GG of SNP rs6570136 (*P*=0.003; odds ratio [OR]=2), and CC, TT of SNP rs7774663 (*P*=0.004; OR=2.1), both in bin I, and the genotypes CC, TT of SNP rs2064501 (*P*=0.01; OR=2), in bin VI were significantly

associated with HF (Table 2; Fig. 3A,B). Multivariate analysis could not separate the effects of these SNPs owing to the high linkage disequilibrium (LD) between these SNPs (Fig. 3C).

Thus, rs6560136 and/or rs2064501, or other variants highly correlated with either of these SNPs, modulate susceptibility to HF in fishermen. We built a map of all SNPs correlated with either rs6560136 or rs2064501 in a region extending 5 Mb from the 30 and 50 end of *IL22RA2* to rule out the possibility that the casual variants may lie outside of *IL22RA2*. Supporting Fig 2B-D shows the various common SNPs (minor allele frequency >5%) in this region and their correlation with the SNPs of interest (y-axis). None of these SNPs outside *IL22RA2* were strongly correlated with rs6560136 or rs2064501 and could account for the association. Then, the causal SNPs must lie in *IL22RA2*.

Next, we evaluated whether rs6560136 and rs2064501 were associated with modulation of *IL22RA2* transcripts. We performed this analysis in healing skin tissue that highly expresses *IL22RA2*. We found a significant association of the genotypes GG, AA of rs6570136 (*P*=0.005) and a suggestive association of the genotype TT of rs2064501 (*P*=0.065) with the highest *IL22RA2* mRNA levels (Fig. 3D,E). This evaluation shows that the genotypes of rs6560136 that are associated with aggravation of fibrosis (Fig. 3A) are also associated with enhancement of *IL22RA2* transcripts (Fig. 3D); they also suggest that the genotype TT of rs2064501, which is also associated with HF (Fig. 3B), also enhances *IL-22RA2* transcripts (Fig. 3E).

Validation/extension of the associations observed in Chinese subjects to Sudanese and Brazilians infected with *S. mansoni*.

We sought to confirm the association between HF and SNPs in *IL22RA2* in Sudanese and Brazilian populations infected with *S. mansoni*. We genotyped 201 Sudanese subjects (described in Table 1) and found that SNPs rs6570136 GG (*P*=0.03; OR=2.1), rs11154915 TT (*P*=0.04; OR=9.4), and rs2064501 CC, TT (*P*=0.03; OR=2.1) were associated with severe HF (Table 3). The best multivariate model included SNPs rs6570136 GG (*P*=0.0007; OR=8.2) and rs2064501 TT (*P*=0.02; OR=3.1).

We then genotyped 186 Brazilians (Brazil [1]; described in Table 1) and found that SNPs rs6570136 GG (*P*=0.001; OR=4.8), rs7774663 TT (*P*=0.02; OR=2.5), and rs779054 TT (*P*=0.02; OR=2.4) were associated with aggravation of HF (Table 3; Fig. 4A). No significant association was found between rs2064501 TT and HF in univariate analysis. Nevertheless, the

best multivariate model included SNPs rs6570136 GG (*P*=0.003; OR=26) and rs2064501 TT, TC (*P*=0.03; OR=11). Thus, both rs6570136 and rs2064501 independently contribute to the association with severe HF, with rs6570146 GG and rs2064501 TT corresponding to the aggravating genotypes. In conclusion, variants of *IL22RA2* that are associated with a high abundance of *IL22RA2* transcripts are also associated with severe hepatic disease in three genetically different populations.

The same genetic variants of *IL22RA2* are associated with susceptibility to fibrosis and cirrhosis in individuals infected with HCV.

We evaluated whether these same polymorphisms affected fibrosis and cirrhosis in HCV-infected patients (n=532, cohort Brazil [2]; Table 1) with different grades of HF (from F0=no fibrosis to F3=advanced fibrosis and F4=cirrhosis). We found that the genotypes GG and AG of SNP rs6570136 (*P*=0.04; OR=1.6) and TT, CT of genotype rs2064501 (*P*=0.02, OR=2) and the genotype AA of the SNP rs202563 (*P*=0.07; OR=1.8) were associated with advanced fibrosis or cirrhosis (F3 and F4; n=210) and were less prevalent in individuals with mild or no fibrosis (F0+F1+F2; n=322; Table 4). Multivariate analysis demonstrated that rs6570136 GG, AG (*P*=0.007; OR=1.7) and rs2064501 TT, CT (*P*=0.004; OR=2.4) were independently associated with severe fibrosis (F3+F4). The data obtained in comparing F3+F4 with F0+F1 are shown on Fig. 4B. These associations in HCV induced HF in Brazilians are similar to the associations we have observed in schistosome-induced HF in Brazilians (Fig. 4A). The strong LD between the two associated SNPs (Fig. 4C) explains why the strength of the association is high when both SNPs are analyzed simultaneously.

We confirmed these results in an independent cohort (Brazil [3]; Table 1) of 149 (F0+F1 and F3+F4) patients infected with HCV, which showed that SNP rs6570136 GG (0.04) and rs2064501 TT (0.05) were independently associated with severe fibrosis (F3+F4).

Discussion

We evaluated production of IL-22 in a mouse model of schistosome infection to examine the role of IL-22/IL-22 BP in HF. In a previous study, IL-22 was not detected in the liver and spleen of schistosome infected mice.[22] Although we confirmed this result, we also showed that the abundance of IL22 transcripts is high in Peyer patches when schistosome eggs

reach the mouse intestine. IL-22 is thought to protect epithelia against damage and mediates tissue repair.[5,12,13] lL-22 also stimulates production of antimicrobial peptides that limit the invasion of microbes across epithelial lesions.[2,6,7,11] Schistosome eggs perforate the intestinal wall; therefore, epithelial lesions caused by eggs, which allow the entry of bacteria and toxic bacterial products, should induce a strong IL-22 response. Our failure to detect lL-22 in mouse liver is probably owing to a lack of sensitivity, because cells producing IL-22 in the liver are outnumbered by hepatocytes that do not produce it. We also found that the abundance of *IL-22RA2* transcripts in the intestine was reduced after the arrival of eggs. Thus, the balance between IL-22/IL-22BP is tipped in favor of IL-22 when eggs lodge in the liver and perforate the intestine. This indicates that the biological activity of IL-22 is high when hepatic and intestinal diseases begin to develop. A previous study found no evidence to suggest that IL-22 is involved in HF in schistosome-infected mice.[22] However, there are many differences between schistosomiasis in mice and humans. Liver disease in mice is evaluated within a few weeks of infection, whereas we examined hepatic disease in humans after more than 10 years of infection. Furthermore, the parasite load in mice is hundreds of times higher than in infected humans. For these reasons, we evaluated IL-22 in chronically infected individuals who have been living in an endemic region for their whole life. IL-22 was produced by PBMCs obtained from these individuals; IL-22 production was stimulated by schistosome eggs. Eggs had no effect on IL-22 production by PBMCs from local individuals who had no exposure to schistosomes. IL-17A production in these cultures was low and was not stimulated by eggs.[9,16,17] Our findings suggest that synergy between IL-22 and IL-17 does not occur in humans with long chronic infections, although we cannot fully exclude the possibility that IL-17 may be produced locally in particular tissues and not in the blood. Our data indicate that IL-22 in PBMC cultures was produced by at least two cell types: $CD3^{+}CD4^{+}$ and $CD3^{-}CD4^{-}$. This suggests that IL-22 is probably produced by Th22 cells[26,27] and natural killer–like innate lymphoid cells.[1] However, more work is required to characterize IL-22-producing cells in schistosome-infected patients. IL-22 was not produced by Th17 because little IL-17 was produced in cultures and IL-22-producing cells were IL-17 negative. There may be a high abundance of ILCs in the intestine because inflammation of the intestine strongly stimulates the multiplication of ILCs producing IL-22, as also indicated by our data in mice. We found that IL-22 production in cultures was positively correlated with protection against HF. Indeed, certain fishermen presented with no disease or only mild disease, although they have been exposed daily to infection for more than 20 years. This was owing to the effective control of HF and not inherent protection against infection. Portal vein diameter of the studied fishermen was inversely correlated with IL-22

levels. This measure is used to detect PH. Thus, high IL-22 levels are associated with protection against the most severe stages of HF.

To investigate further the link between IL-22/IL-22BP and HF, we performed a genetic analysis of *IL22RA2* to search for genetic variants associated with HF. We first studied *IL22RA2* because it is located in the 6q23 locus that exerts major control on HF in schistosome-infected populations.[28] Mutations in the CTGF gene, which is present at this same locus, contribute to this major genetic effect.[23] Nevertheless, CTGF cannot account for the entire effect of the locus. We obtained convincing associations between HF and two polymorphisms in *IL22RA2*. The observation that heterozygous individuals are better protected from HF than homozygous individuals of either genotype has been found in other infectious diseases. We believe that the strong LD between rs657136 and rs2064501 explains this effect. Our study in Sudan and China shows that the aggravating homozygous genotypes are rs657136 GG and rs2064501 TT. The protective homozygous genotype of one SNP is almost always associated with the aggravating homozygous genotype of the other SNP owing to the strong LD between these SNPs. This association between homozygous genotypes with opposite effects neutralizes the effects of the protective genotypes and gives rise to a genotype that is associated with disease aggravation. The association of variants in *IL22RA2* with HF directly implicates IL-22BP in HF. Homozygous genotypes, which are associated with susceptibility to severe HF, are also associated with high levels of *IL22RA2* transcripts, strongly suggesting that IL-22BP aggravates HF. This finding is consistent with the association we observed between IL-22 and protection against both HF and PH.

The underlying mechanisms that lead to HF in schistosome and HCV infections are similar in many aspects. However, in HCV infections, liver fibrosis, and viral hepatotoxicity are associated with a vigorous multiplication of hepatocytes, which is not observed in schistosome infections. IL-22 promotes liver cell regeneration by increasing cell proliferation and hepatocyte migration.[29] However, such proliferation contributes to the regeneration nodules that greatly augment loss of liver architecture and organization and impairment of liver function in cirrhosis. Hepatocyte proliferation may be aggravated by IL-22, which stimulates tissue regeneration and inhibits apoptosis[10,30]; furthermore, uncontrolled IL-22 activity may promote development of hepatocarcinoma.[14] For these reasons, we investigated these same polymorphisms in the context of HCV-induced cirrhosis. We showed that genetic variants of *IL22RA2* that are associated with susceptibility to severe HF in schistosomiasis are also

associated with HCV-induced cirrhosis, indicating that IL-22/IL-22BP exerts hepatoprotective effects in both HCV and schistosome infections.

Several biological effects may account for the protective action of IL-22 against schistosome-induced HF. IL-22 may stimulate production of anti-inflammatory molecules,[31,32] promote liver repair by limiting apoptosis, or stimulating mitosis, cell migration,[20,31] and progenitor cell growth.[33] IL-22 may also promote stellate cell senescence.[34] Regulation of IL-22 by IL-22BP may also limit entry of profibrogenic bacterial products, such as lipopolysaccharide (LPS), by stimulating antibacterial innate immunity,[4,35] reducing intestinal inflammation,[36,37] and promoting healing of the intestinal epithelium,[12,38] which is perforated by thousands of schistosome eggs. LPS is known to stimulate hepatic fibrogenesis through direct effects on hepatic stellate cells, which express the Toll-like receptor 4 receptor. Finally, IL-22 may stimulate the liver to produce LPS-binding protein.[39] Intestinal damage does not typically occur during HCV infections; therefore, the protective effects of IL-22 are likely to result from a direct protective action on the liver[10,40] and probably involve tissue repair and regeneration[21] and stellate cell senescence,[34] which was shown to be crucial for limiting HF. Hepatic tissue repair is probably more critical in HCV infections than in schistosome infections because HCV is very cytotoxic for hepatocytes, whereas schistosomes are not because eggs are trapped in the liver sinusoids and toxic substances are prevented from diffusing by sequestration in the granuloma.

In conclusion, we show that IL-22 is associated with protection against liver fibrosis in human schistosomiasis, and mutations that promote IL-22BP expression, the physiological inhibitor of IL-22, aggravate fibrosis and cirrhosis in both schistosome and HCV infections. These results strongly suggest that IL-22 protects against HF and cirrhosis. This is also the first direct evidence that IL-22BP plays a significant regulatory role in human inflammatory diseases. IL-22 and IL-22BP do not act on hematopoietic cells; therefore, pharmacological intervention against these molecules should have fewer side effects than treatments that target classical cytokines such as tumor necrosis factor. Thus, IL-22 and IL-22BP may be good therapeutic targets[3] in the prevention and treatment of fibrosis and cirrhosis.

References

1. Walker JA, Barlow JL, McKenzie AN. Innate lymphoid cells—how did we miss them? Nat Rev Immunol 2013;13:75-87.

2. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med 2006;203:2271-2279.

3. Sabat R, Ouyang W, Wolk K. Therapeutic opportunities of the IL-22-IL-22R1 system. Nat Rev Drug Discov 2014;13:21-38.

4. Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 increases the innate immunity of tissues. Immunity 2004;21:241-254.

5. Rutz S, Eidenschenk C, Ouyang W. IL-22, not simply a Th17 cytokine. Immunol Rev 2013;252:116-132.

6. Wolk K, Witte E, Wallace E, Docke WD, Kunz S, Asadullah K, et al. IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. Eur J Immunol 2006;36:1309-1323.

7. Boniface K, Bernard FX, Garcia M, Gurney AL, Lecron JC, Morel F. IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. J Immunol 2005;174:3695-3702.

8. Wolk K, Haugen HS, Xu W, Witte E, Waggie K, Anderson M, et al. IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not. J Mol Med (Berl) 2009;87:523-536.

9. Ma HL, Liang S, Li J, Napierata L, Brown T, Benoit S, et al. IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation. J Clin Invest 2008;118:597-607.

10. Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, Ouyang W. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature 2007;445:648-651.

11. Sa SM, Valdez PA, Wu J, Jung K, Zhong F, Hall L, et al. The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. J Immunol 2007;178:2229-2240.

12. Sonnenberg GF, Fouser LA, Artis D. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. Nat Immunol 2011;12:383-390.

13. Pan CX, Tang J, Wang XY, Wu FR, Ge JF, Chen FH. Role of Interleukin-22 in liver diseases. Inflamm Res 2014;63:519-525.

14. Jiang R, Tan Z, Deng L, Chen Y, Xia Y, Gao Y, et al. Interleukin-22 promotes human hepatocellular carcinoma by activation of STAT3. HEPATOLOGY 2011;54:900-909.

15. Munoz M, Heimesaat MM, Danker K, Struck D, Lohmann U, Plickert R, et al. Interleukin (IL)-23 mediates Toxoplasma gondii induced immunopathology in the gut via matrixmetalloproteinase-2 and IL-22 but independent of IL-17. J Exp Med 2009;206:3047-3059.

16. Geboes L, Dumoutier L, Kelchtermans H, Schurgers E, Mitera T, Renauld JC, Matthys P. Proinflammatory role of the Th17 cytokine interleukin-22 in collagen-induced arthritis in C57BL/6 mice. Arthritis Rheum 2009;60:390-395.

17. Zhao J, Zhang Z, Luan Y, Zou Z, Sun Y, Li Y, et al. Pathological functions of interleukin-22 in chronic liver inflammation and fibrosis with hepatitis B virus infection by promoting T helper 17 cell recruitment. HEPATOLOGY 2014;59:1331-1342.

18. Dumoutier L, Lejeune D, Colau D, Renauld JC. Cloning and characterization of IL-22 binding protein, a natural antagonist of IL-10-related T cell-derived inducible factor/IL-22. J Immunol 2001;166:7090-7095.

19. Xu W, Presnell SR, Parrish-Novak J, Kindsvogel W, Jaspers S, Chen Z, et al. A soluble class II cytokine receptor, IL-22RA2, is a naturally occurring IL-22 antagonist. Proc Natl Acad Sci USA 2001;98:9511-9516.

20. Radaeva S, Sun R, Pan HN, Hong F, Gao B. Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. HEPATOLOGY 2004;39:1332-1342.

21. Ren X, Hu B, Colletti LM. IL-22 is involved in liver regeneration after hepatectomy. Am J Physiol Gastrointest Liver Physiol 2010;298:G74-G80.

22. Wilson MS, Feng CG, Barber DL, Yarovinsky F, Cheever AW, Sher A, et al. Redundant and pathogenic roles for IL-22 in mycobacterial, protozoan, and helminth infections. J Immunol 2010;184:4378-4390.

23. Dessein A, Chevillard C, Arnaud V, Hou X, Hamdoun AA, Dessein H, et al. Variants of CTGF are associated with hepatic fibrosis in Chinese, Sudanese, and Brazilians infected with schistosomes. J Exp Med 2009;206:2321-2328.

24. Arnaud V, Li J, Wang Y, Fu X, Mengzhi S, Luo X, et al. Regulatory role of interleukin-10 and interferon-gamma in severe hepatic central and peripheral fibrosis in humans infected with Schistosoma japonicum. J Infect Dis 2008;198:418-426.

25. Richter J, Domingues AL, Barata CH, Prata AR, Lambertucci JR. Report of the second satellite symposium on ultrasound in schistosomiasis. Mem Inst Oswaldo Cruz 2001;96(Suppl):151-156.

26. Eyerich S, Eyerich K, Pennino D, Carbone T, Nasorri F, Pallotta S, et al. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. J Clin Invest 2009;119:3573-3585.

27. Nograles KE, Zaba LC, Shemer A, Fuentes-Duculan J, Cardinale I, Kikuchi T, et al. IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. J Allergy Clin Immunol 2009;123:1244-1252.e2.

28. Dessein AJ, Hillaire D, Elwali NE, Marquet S, Mohamed-Ali Q, Mirghani A, et al. Severe hepatic fibrosis in Schistosoma mansoni infection is controlled by a major locus that is closely linked to the interferon-gamma receptor gene. Am J Hum Genet 1999;65:709-721.

29. Brand S, Dambacher J, Beigel F, Zitzmann K, Heeg MH, Weiss TS, et al. IL-22-mediated liver cell regeneration is abrogated by SOCS-1/3 overexpression in vitro. Am J Physiol Gastrointest Liver Physiol 2007;292:G1019-G1028.

30. Zhang W, Chen Y, Wei H, Zheng C, Sun R, Zhang J, Tian Z. Antiapoptotic activity of autocrine interleukin-22 and therapeutic effects of interleukin-22-small interfering RNA on human lung cancer xenografts. Clin Cancer Res 2008;14:6432-6439.

31. Park O, Wang H, Weng H, Feigenbaum L, Li H, Yin S, et al. In vivo consequences of liver-specific interleukin-22 expression in mice: implications for human liver disease progression. HEPATOLOGY 2011;54:252-261.

32. Ki SH, Park O, Zheng M, Morales-Ibanez O, Kolls JK, Bataller R, Gao B. Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: role of signal transducer and activator of transcription 3. HEPATOLOGY 2010;52:1291-1300.

33. Feng D, Kong X, Weng H, Park O, Wang H, Dooley S, et al. Interleukin-22 promotes proliferation of liver stem/progenitor cells in mice and patients with chronic hepatitis B virus infection. Gastroenterology 2012;143:188-198.e7.

34. Kong X, Feng D, Wang H, Hong F, Bertola A, Wang FS, Gao B. Interleukin-22 induces hepatic stellate cell senescence and restricts liver fibrosis in mice. HEPATOLOGY 2012;56:1150-1159.

35. Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med 2008;14:282-289.

36. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. Immunity 2008;29:947-957.

37. Sugimoto K, Ogawa A, Mizoguchi E, Shimomura Y, Andoh A, Bhan AK, et al. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. J Clin Invest 2008;118:534-544.

38. Huber S, Gagliani N, Zenewicz LA, Huber FJ, Bosurgi L, Hu B, et al. IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. Nature 2012;491:259-263.

39. Wolk K, Witte E, Hoffmann U, Doecke WD, Endesfelder S, Asadullah K, et al. IL-22 induces lipopolysaccharide-binding protein in hepatocytes: a potential systemic role of IL-22 in Crohn's disease. J Immunol 2007;178:5973-5981.

40. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Karow M, Flavell RA. Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. Immunity 2007;27:647-659.

Supporting Materials and Methods

Evaluating Hepatic Fibrosis in *S.japonicum*-infected subjects

WHO grading was modified for *S. japonicum* infections as described (39) because 80% of Chinese fishermen had grade CL (CentF) and >40% of fishermen had grade GN (NetF). These large groups were inappropriate for further studies, so CL and GN were divided into three subgroups: light (CL^L/GN^L), advanced (CL^M/GN^M), and severe (CL^H/GN^H). CentF C^L was observed only in the left lobe of the liver, whereas CL^M and CL^H were observed in both liver lobes. Fibrosis in the right lobe of the liver extending to the second order branches was classified as CL^M, and thickness that extended further down the second order branches was classified as CL^H. For NetF, a network mesh < 2mm thick was classified as light GN (GN^L), a network mesh between 2 to 4 mm thick was classified as medium GN (GN^M), and a network mesh > 4mm thick was classified as heavy GN (GN^H). PV diameter (in mm) corresponded to the internal (inner to inner) diameter of the portal vein at the entry point of the portal vein into the liver. PV diameter was adjusted according to the average height of the healthy population from the same region. Only severe CentF CL^H and very severe CentF (D, E, F) and severe NetF (GN^H,GW) were associated with portal hypertension. In Fig. 2.b, very light (B, C), light (CL^L) advanced (CL^M) and severe (CL^H) and very severe CentF (D,E,F) are coded CentF +/-, +, ++, and +++, respectively. The genetic analysis uses binary phenotypes that included as cases, the Chinese fishermen with severe or very severe CentF or severe NetF and subjects with advanced CentF if they also had advanced or severe NetF. The inclusion of advanced CentF fishermen was necessary since the numbers of severe HF cases were too small (<100 individuals). Control groups included individuals who exhibited no NetF and light CentF or less. The HF cases in *S. mansoni* infections included D, E, and F grades. All other grades were considered as controls.

Figure legends

Figure 1

Schistosome eggs stimulate the production of *IL22* mRNA and impair that of *IL22RA2* in infected mice (A-D).

(A) Abundance of *Il22*, *Il22ra2*, and *Il22ra1* mRNA in colon, intestine, Peyer's patches, liver, and spleen of noninfected CBA/J mice **(B-D)** and mice infected with 30 *S. mansoni* cercariae. Transcript levels were evaluated at 4, 6, or 11 weeks post infection. One representative experiment of two (5-6 mice per group) is shown. Data are the geometric mean **(A)** or the log (geometric mean) **(B-D)** of the fold change between infected and non-infected animals. Comparisons were performed by non-parametric analysis (*P<0.05).

Figure 2

IL-22 response in humans infected with schistosomes. IL-22 concentrations correlate with protection against HF and portal blood hypertension.

(A) Concentration of IL-22 produced in egg-stimulated cultures of PBMCs from 66 Chinese fishermen and 18 controls. A total of 106 PBMCs were cultured for 72 or 144 hours with or without 500 schistosome eggs. **(B)** IL-22 concentrations in resting (P=0.005) and egg-stimulated (P=0.04) cultures at 144 hours are inversely correlated with CentF severity. Light CentF (CL^L) is (+/-) (n=41), advanced CentF (CL^M) is (+) (n=18), severe CentF (CL^H) is (++) (n=7), and very severe CentF D+E+F is (+++) (n=3). **(C)** Multivariate analysis shows that CentF and NetF are independently correlated with IL-22 levels in egg-stimulated cultures (P=0.009 and P=0.004, respectively). CentF was divided into CL^L, CL^M (n=51), and CL^H, D+E+F (n=7); NetF was divided into not detectable (n=35) and detectable (n=23). **(D)** PH (portal vein [PV] diameter) is inversely correlated with IL-22 produced in resting cultures at 144 hours (P=0.02). IL-22 concentrations were divided into classes 11-100 (n=26), 101-300 (n=16), 301-900 (n=16), and >900 ng/mL (n=8). Bars show standard error of the mean.

Figure 3

***IL22RA2* genotypes that are associated with severe fibrosis in Chinese fishermen are associated with high *IL22RA2* mRNA levels in healing skin.**

(A and B) The genotypes AA and GG of SNP rs6570136 and the genotypes TT and CC of SNP rs2064501 are associated with susceptibility to severe HF. The sample of Chinese fisherman is described in Supporting Table 1, and data are also shown in Table 1. Data are represented for men and women separately ($P<0.001$) and for fishermen who were exposed for more >20 years (longer exposure is not significantly associated with HF). **(C)** SNPs rs6570136 and rs2064501 are in strong linkage disequilibrium. **(D)** The genotypes AA and GG of SNP rs6570136 are associated ($P=0.005$) with the highest *IL22RA2* mRNA levels in healing skin of 34 individuals. **(E)** Suggestive association of the genotype TT of SNP rs2064501 ($P=0.065$) with the highest *IL22RA2* mRNA levels in the skin biopsies. The abundance of mRNA is expressed as arbitrary units (6 standard error of the mean).

Figure 4

The same alleles that are associated with susceptibility to severe HF in Brazilians infected with schistosomes are also associated with a high risk of fibrosis and cirrhosis in Brazilians infected with HCV.

(A) The genotype GG of SNPs rs6570136 GG ($P=0.003$) and the genotype TT, TC of SNP rs2064501 ($P=0.03$) are independently associated with HF in Brazilians (Brazil [1]) infected with *S. mansoni*. **(B)** SNPs rs6570136 ($P=0.007$) and rs2064501 ($P=0.004$) are also independently associated with HF and cirrhosis in Brazilians (Brazil [2]) infected with HCV. The analysis compares F0+F1 with F3+F4, whereas data in Table 4 compare F0, F1, and F2 with F3 and F4. The SNP that shows the strongest association with HF is shown on the x-axis. **(C)** Distribution of rs6570136 and rs2064501 genotypes among Brazilians reveals strong LD between these two SNPs. The same genotype distribution was observed with schistosome and with HCV-infected Brazilians inhabiting the same region of Brazil.

Supporting Figure 1

Flow cytometry analysis of IL-22 producing cells in the blood of control and exposed individuals.

Data are from one representative experiment out of 20. Cell labeling was performed as indicated in Material and Methods.

Supporting Figure 2

Description of the SNP bins in *IL22RA2* (a); lack of high correlation (r^2<0.6) between SNP 6570136 or 2064501 and other SNPs in a 1Mb region surrounding IL22RA2 (b-d).

SNPs shown in **(a)** have a minor allele frequency >10% in the Chinese population; the analysis extended 10 Mb from the 3' and 5' end of the gene. Plot in **(b)** shows correlation values for all SNPs of the 10 Mb region surrounding *IL22RA2*, with SNPs 6570136 (closed symbols) and 2064501 (open symbols) whereas plots shown in **(c)** and **(d)** show data for each SNP respectively in the 1 MB region. Relevant genes located in the region are indicated (*CCN2* encodes CTGF). There is no SNP outside *IL22RA2* that correlates significantly (r^2>0.6) with SNP rs6570136 and rs2064501 that could account for the observed association with HF. Data were collected from the Hapmap and 1000 Genomes databases.

Table legends

Table 1.

Description of the study samples.

Exposure to infection was evaluated with "years of fishing" (F. yrs) in Chinese fishermen and with age in Sudanese and Brazilians infected with *S. mansoni*. Duration of infection could not be accurately evaluated in HCV-infected individuals. The analysis presented in Table 4 (Cohort Brazil [2]) compares F0, F1, and F2 with F3 and F4. The analysis presented in Fig. 4B,C (Cohort Brazil [2]) compares F0 and F1 with F3 and F4.

Table 2.

***IL22RA2* genotypes associated with advanced fibrosis in Chinese fishermen infected with *S. japonicum*.**

Cases and controls are described in Material and Methods and in Table 1. Significant covariates in the logistic regression analysis were sex ($P=10^{-3}$) and exposure ($P<10^{-3}$). Bins represent correlation groups ($r^2>0.8$), and the aggravating genotype is shown. Abbreviation: CI, confidence interval of OR.

Table 3.

***IL22RA2* genotypes associated with advanced fibrosis in Sudanese and Brazilian farmers infected with *S. mansoni*.**

The two principal schistosome strains that cause HF are *S. japonicum* in Asia and *S. mansoni* in Africa and South America. We investigated whether allelic variants of *IL-22RA2* also affect susceptibility to severe HF in an *S. mansoni*–endemic region in Sudan and Brazil. *IL22RA2* polymorphisms that were associated with HF in Chinese fishermen were genotyped in both samples. The phenotypes of cases and controls and the cohort size are described in Material and Methods and in Supporting Table 1. We first tested for associations between the SNPs and HF phenotypes separately (upper part of the table) and then tested the SNPs simultaneously (lower part). The aggravating genotype is shown. Age $P=0.025$ was a covariate in the Sudanese multivariate analysis. Abbreviation: CI, confidence interval of OR.

Table 4.

***IL22RA2* SNP rs6570136 and SNP rs2064501 are also associated with fibrosis and cirrhosis in humans infected with HCV.**

Co-variable: Age (45 yrs., $P = 0.001$), Alcohol ($P = 0.01$). Severe (F3, F4) and mild (F0, F1, F2) fibrosis phenotypes and the cohort size are described in supporting Material and Methods and in Table 1. We first tested for associations between the SNPs and HF phenotypes separately (upper part of the table) and then tested the SNPs simultaneously by logistic regression analysis (lower part). Significant covariates ($P < 0.01$) were age (45 yrs., <46 yrs.) and alcohol intake. The aggravating genotypes are shown.

Supporting Table 1.

Primers used in the analysis of *IL22RA2*.

Table 1.

	Pathogen	N Total (Cases/Controls)	Age (Mean) Case/Controls Exposure (Mean) Case/Controls	Women % Case/% Controls	Severe Fibrosis	Mild or No Fibrosis
Chinese	*S. japonicum*	327 (122/205)	Age: 44.1 yrs/52.2 yrs F. yrs: 25.5 yrs/34.8 yrs	13.1/26.0	CL^H, D, E, F with GN^H CL^M with GN^M or GN^H	A, B, C, CL^L with GN0
Sudan	*S. mansoni*	217 (68/149)	Age = exposure 47.1 yrs/50.7 yrs	17.2/28.7	CL, D, E, F with gall bladder wall thickening	A, B, C no gall bladde thickening
Brazil (1)	*S.mansoni*	186 (45/141)	Age: 46.6 yrs/40 yrs	47.8/62.9	CL, D, E, F	A, B, C
Brazil (2)	HCV	532 (210/322) 364 (210/154)	F3-F4/F0-F1/F0: Age: 57 yrs/52.1 yrs/53.7 yrs	57.0/53.8/52.9	F3+F4 (cirrhosis)	F0+F1+F2 or F0+F1
Brazil (3)	HCV	149 (53/96)	F3-F4/F0-F1/F0: Age: 57 yrs/52.1 yrs/53.7 yrs	39/46.2	F3+F4 (cirrhosis)	F0+F1+F2

Table 2

			Chinese Fishermen					
Analysis	**SNP**	**Bins**	**Genotype**	**Controls**	**Cases**	***P* Value**	**OR**	**95% CI**
Univariate	6570136	I	AA, GG	45	62	0.003	2.07	1.5-5.4
	7774663	I	CC, TT	44	61	0.004	2.1	1.4-5.2
	7749054	II	GG, TT	46.2	54.8	0.12	1.45	0.9-2.3
	202563	III	AA	39.7	48.8	0.10	1.47	0.9-2.4
	276466	IV	AA	69.2	78.7	0.07	1.65	0.9-2.9
	11154915	V	TT, TC	25.6	34.6	0.10	1.5	0.9-2.6
	2064501	VI	CC, TT	53.6	66.4	0.01	2	1.2-3.3

Table 3

			Sudanese Farmers						Brazilian Farmers					
Analysis	SNP (rs)	Bins	Genotype	Controls	Cases	*P* Value	OR	95% CI	Genotype	Controls	Cases	*P* value	OR	95% CI
Univariate	6570136	I	AA, GG	59.7	75.4	0.03	2.1	1.1-3.9	GG	13	35.6	0.001	4.8	2.1-11
	7774663	I	CC, TT	47	67.7	0.006	2.4	1.3-4.4	TT	25.7	44.2	0.02	2.5	1.1-5.2
	7749054	II	TT	34.2	47.7	0.07	1.7	0.95-3.1	TT	47.2	67.4	0.02	2.4	1.2-4.9
	202563	III	GG	22.8	35.5	0.06	1.9	0.98-3.5	GG, AG	74.2	86	0.13	2.1	0.8-5.5
	276466	IV				>0.3			AA	26.7	41.9	0.07	2	0.9-4.2
	11154915	V	TT	0.7	6.3	0.04	9.4	1.1-85	TT, TC	75.4	87	0.1	2.1	0.8-5.6
	2064501	VI	CC, TT	65.1	80	0.03	2.1	1.1-4.3	CC, CT	56.7	66.7	0.05	2.1	1.0-4.6
Multivariate	6570136		GG			0.0007	8.2	2.4-28.0	GG			0.003	26	3.1-222
	2064501		TT			0.02	3.1	1.2-8.1	TT, TC			0.03	11	1.2-102

Table 4

			HCV					
Analysis	SNP	Bins	Genotype	Controls	Cases	p	OR	95% CI
Univariate	6570136	I	GG,AG	65.2	72.2	0.04	1.6	1.0-2.2
	202563	III	AA	18.7	26	0.07	1.8	1.1-2.9
	2064501	VI	TT,CT	83.5	90.9	0.02	2	2.4
Multivariate	6570136	I	GG,AG			0.007	1.7	1.2-2.6
	2064501	VI	TT,CT			0.004	2.4	1.3-4.3

Supporting Table 1

Bin	SNP	Position	Taqman Assay #	SNP sequence	MAF (HapMap) CEU	YRI	CHB
I	rs9376263	137489626	C__2523610_10	AGTAAA[C/T]AAATA	0.41 (T)	0.13 (C)	0.34 (C)
	rs6570136	137494622		TGGGAC[A/G]CCATGT	0.42 (A)	0.25 (G)	0.34 (G)
	rs6570137	137498645		CCCAGC[C/T]CTGCCT	0.38 (C)	0.13 (T)	0.37 (T)
	rs6570138	137501914		ACCCAC[G/T]CTACAT	0.41 (T)	0.25 (G)	0.34 (G)
	rs6570139	137502056		CTAAAA[A/G]AGTACA	0.41 (A)	0.12 (G)	0.31 (G)
	rs6907167	137503761		TTGTGA[G/T]TGATAG	0.41 (T)	0.25 (G)	0.33 (T)
	rs9402876	137509025		GAGTGA[C/T]TCATAA	0.41 (C)	0.09 (T)	0.32 (T)
	rs9402877	137509075		AACTAG[A/T]TCCTTG	0.41 (A)	0.13 (T)	0.34 (T)
	rs9402878	137509292		TGGAAA[G/T]AATTGA	0.37 (G)	0.29 (T)	0.33 (T)
	rs7774663	137510893	C__30217943_10	AAAAAA[C/T]CCTGGA	0.36 (C)	0.33 (T)	0.38 (T)
II	rs13217897	137471327		GGCAAT[A/G]CATGCA	0.18 (A)	0.26 (A)	0.44 (A)
	rs11154913	137474838		CAAGGC[A/G]TAATAT	0.17 (G)	0.27 (G)	0.44 (G)
	rs12664889	137481612		GCAGAG[A/C]CTGCCA	0.18 (A)	0.30 (G)	0.46 (G)
	rs13197049	137491211		TTTCTA[A/T]TCGGAA	0.18 (T)	0.26 (T)	0.44 (T)
	rs7749054	137500786	C__32241951_10	CCCTCT[G/T]CCTGGA	0.19 (G)	0.26 (G)	0.43 (G)
III	rs202563	137461492	C__3010272_10	TAAATT[A/G]TTCCAC	0.49 (G)	0.42 (A)	0.26 (G)
	rs156751	137463294		TCCACC[C/T]TTCTCC	0.49 (T)	0.19 (T)	0.26 (T)
IV	rs85462	137463154		AAACCT[C/G]GAAAGT	0.21 (G)	0.08 (G)	0.16 (G)
	rs276467	137464218		CTCCACT[A/G]ATAAGG	0.20 (A)	0.07 (A)	0.16 (A)
	rs276466	137466614	C__3010277_10	GAATGG[A/G]TAAACA	0.21 (G)	0.07 (G)	0.014 (G)
V	rs7750867	137470186		CCTTCC[C/T]GCATA	0.16 (T)	0.09 (T)	0.07 (T)
	rs9389475	137478484		TATCTC[C/T]AGCAAT	0.17 (T)	0.07 (T)	0.06 (T)
	rs11154914	137480411		GGTTCA[A/G]GGTTT	0.17 (G)	0.07 (G)	0.07 (G)
	rs11154915	137482982	C__9800072_30	CACTCC[C/T]GGGTTT	0.16 (T)	0.07 (T)	0.05 (T)
	rs1040622	137483258		AACTAG[C/T]GGGGCC	0.16 (C)	0.08 (C)	0.07 (C)
	rs10457018	137484893		CCAGAC[A/G]TAAGTG	0.16 (A)	0.08 (A)	0.07 (A)
	rs10457019	137484979		GGTCTC[A/G]GGAGAG	0.17 (A)	0.07 (A)	0.07 (A)
	rs13441747	137488608		AGGTGA[C/G]GTCTCG	0.17 (C)	0.09 (C)	0.07 (C)
	rs9385786	137497052		GTGTTT[C/T]GATTTT	0.16 (T)	0.07 (T)	0.06 (T)
	rs9402875	137498018		ATGAGC[A/C]GCCACC	0.18 (C)	0.08 (C)	0.06 (C)
	rs9385787	137500399		TGGAAG[C/G]CATTAA	0.17 (C)	0.08 (C)	0.07 (C)
	rs9373180	137503455		TCTATT[A/G]GTTCAG	0.17 (G)	0.04 (G)	0.07 (G)
	rs9385789	137505172		TGAGTG[A/T]TATAAG	0.17 (A)	0.08 (A)	0.07 (A)
VI	rs202567	137470844		GCTCCT[A/G]AATAAA	0.48 (G)	0.06 (A)	0.22 (A)
	rs7774349	137475858		ACAGAT[C/T]GCGAGA	0.48 (G)	0.06 (T)	0.21 (T)
	rs2064501	137477823	C__11693858_10	TTTATA[C/T]AATCTT	0.48 (T)	0.06 ©	0.23 (C)

Figure 1

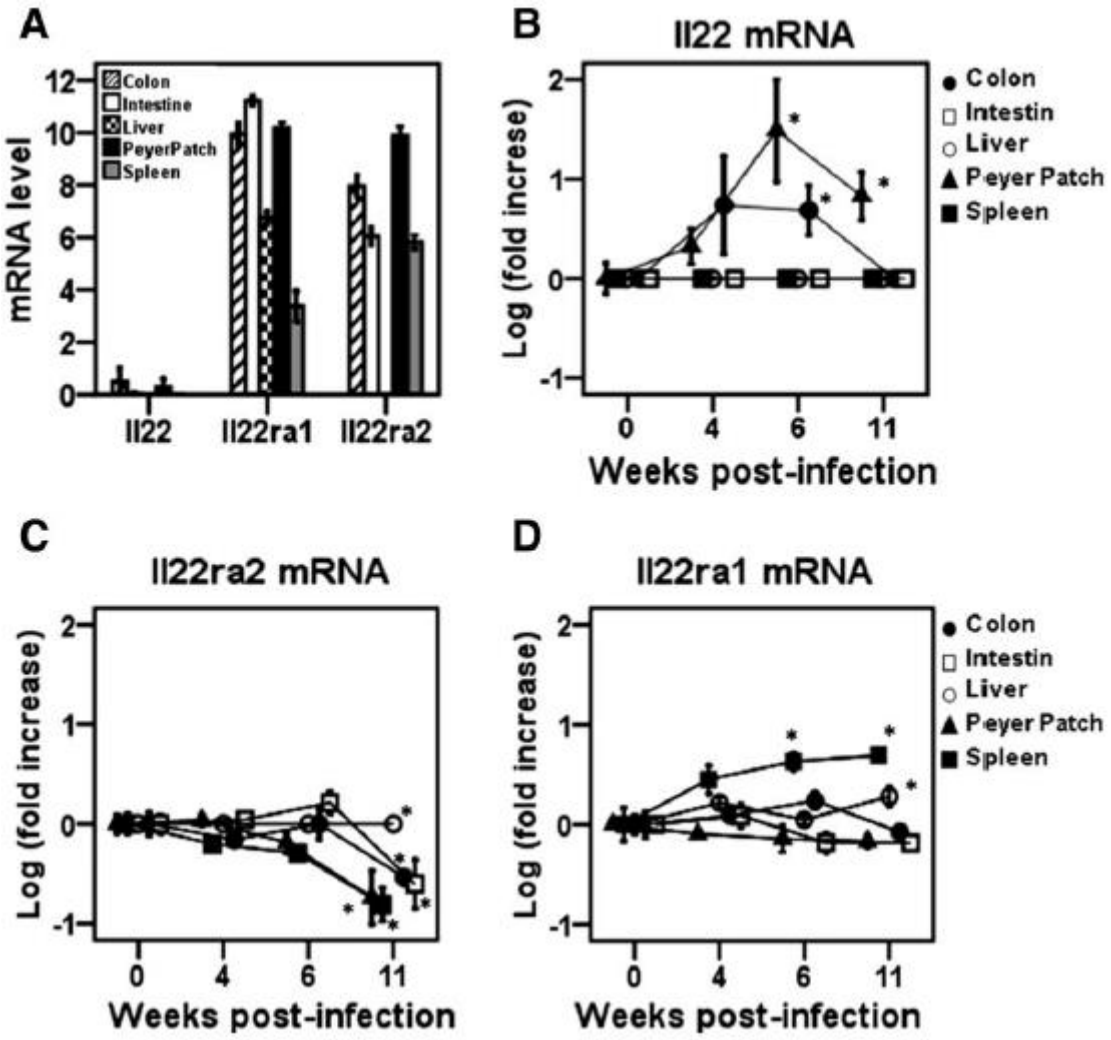

A
mRNA level
Colon
Intestine
Liver
PeyerPatch
Spleen
Il22
Il22ra1
Il22ra2
B
Il22 mRNA
Log (fold increse)
Colon
Intestin
Liver
Peyer Patch
Spleen
Weeks post-infection
C
Il22ra2 mRNA
Log (fold increase)
Weeks post-infection
D
Il22ra1 mRNA
Log (fold increase)
Colon
Intestin
Liver
Peyer Patch
Spleen
Weeks post-infection

Figure 2

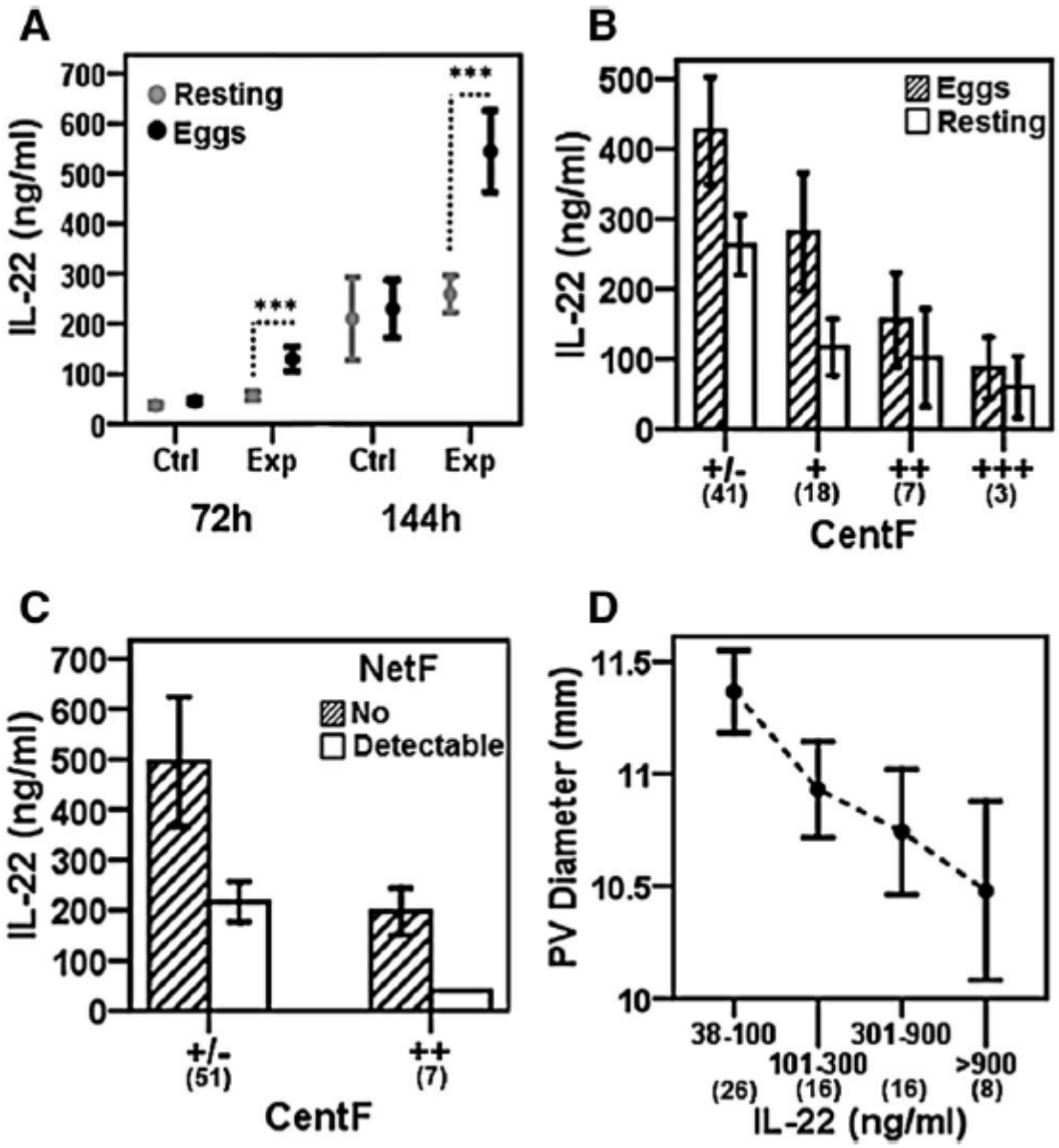
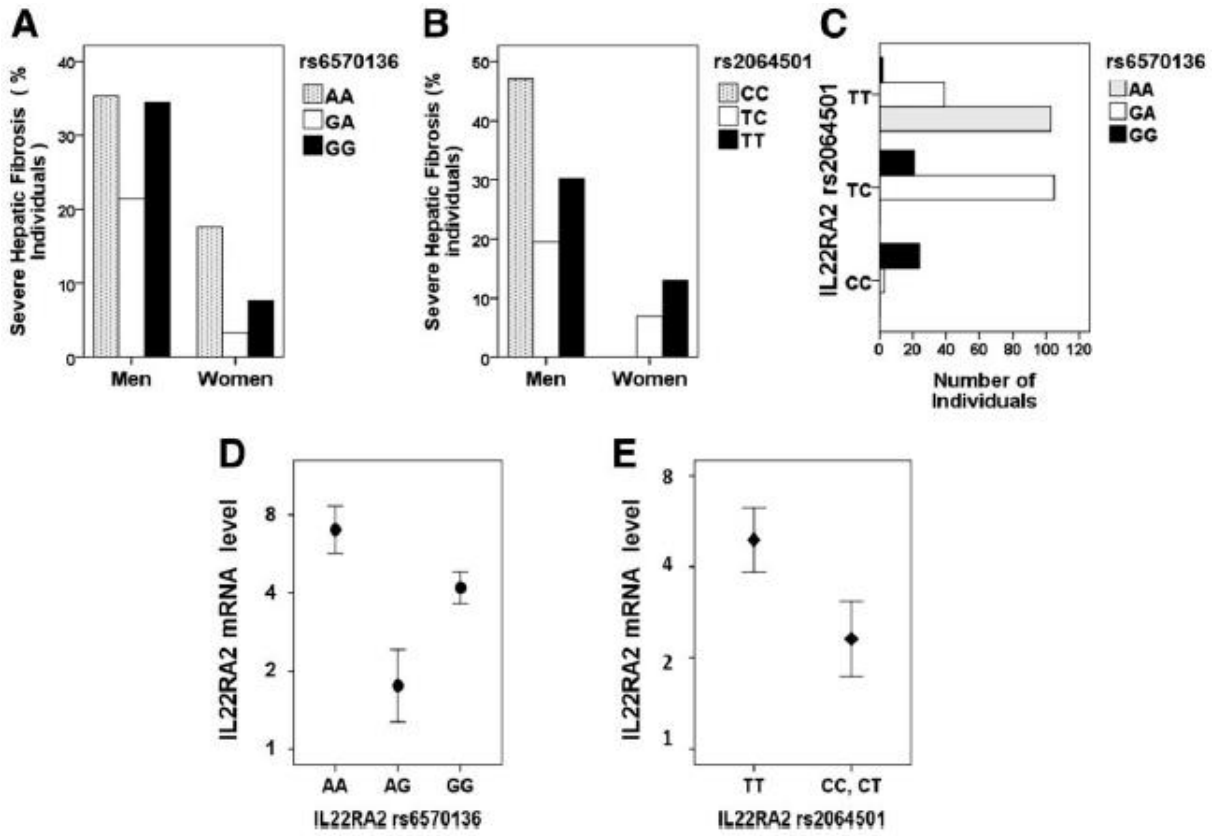

A
IL-22 (ng/ml)
Resting
Eggs
***
***
Ctrl
Exp
Ctrl
Exp
72h
144h
B
IL-22 (ng/ml)
Eggs
Resting
+/-
(41)
+
(18)
++
(7)
+++
(3)
CentF
C
IL-22 (ng/ml)
NetF
No
Detectable
+/-
(51)
++
(7)
CentF
D
PV Diameter (mm)
38-100
(26)
101-300
(16)
301-900
(16)
>900
(8)
IL-22 (ng/ml)

Figure 3


A
Severe Hepatic Fibrosis (% Individuals)
rs6570136
AA
GA
GG
Men
Women
B
Severe Hepatic Fibrosis (% individuals)
rs2064501
CC
TC
TT
Men
Women
C
IL22RA2 rs2064501
TT
TC
CC
rs6570136
AA
GA
GG
Number of Individuals
D
IL22RA2 mRNA level
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AG
GG
IL22RA2 rs6570136
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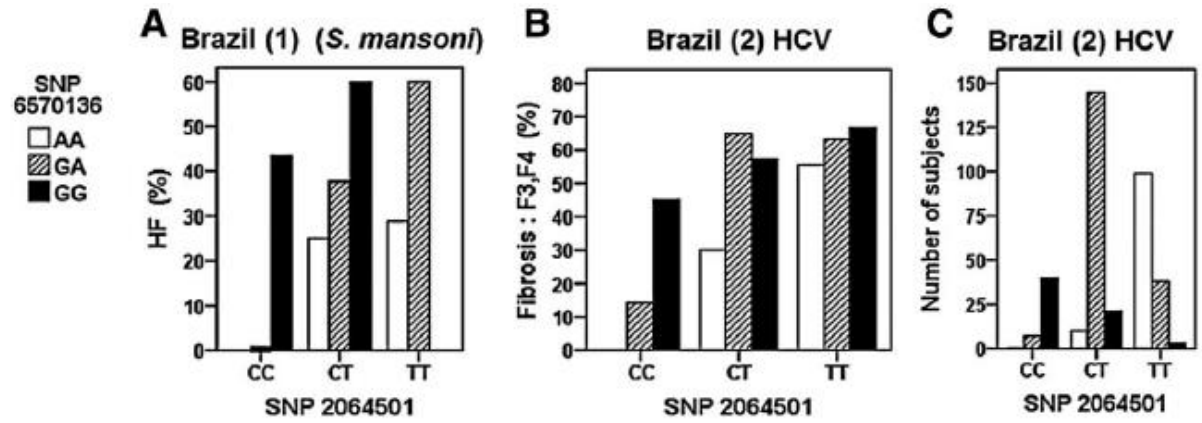
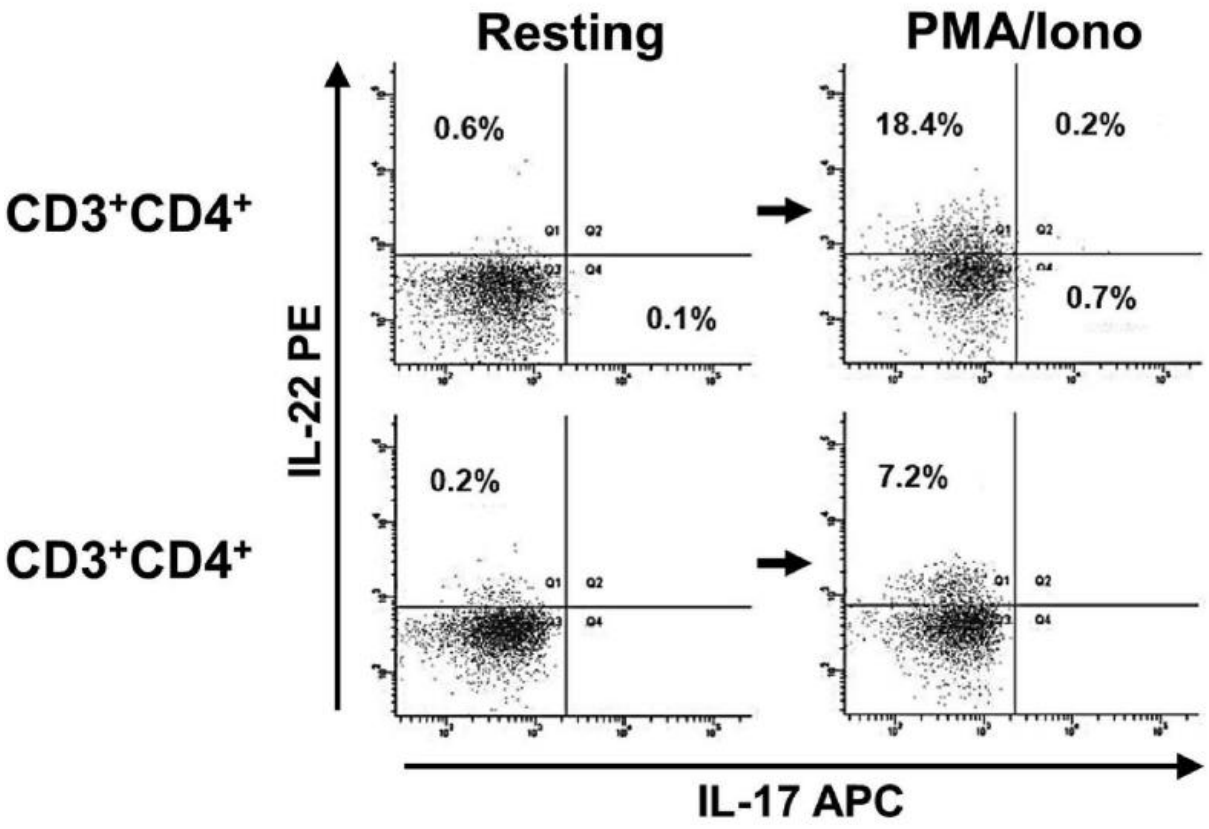
IL22RA2 mRNA level
TT
CC, CT
IL22RA2 rs2064501

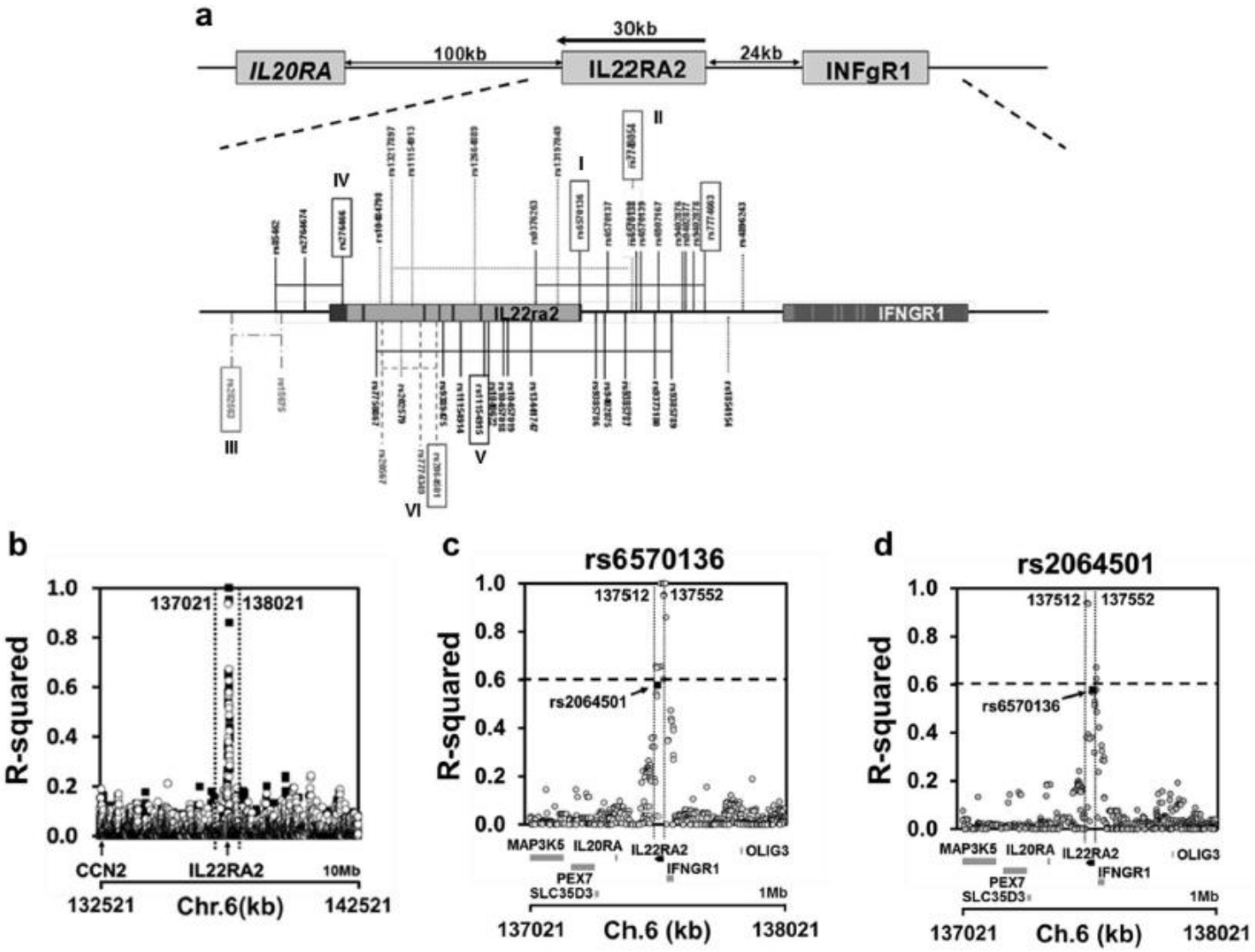
Figure 4


A
Brazil (1) (S. mansoni)
SNP 6570136
AA
GA
GG
HF (%)
0
10
20
30
40
50
60
CC
CT
TT
SNP 2064501
B
Brazil (2) HCV
Fibrosis : F3,F4 (%)
0
10
20
30
40
50
60
70
80
CC
CT
TT
SNP 2064501
C
Brazil (2) HCV
Number of subjects
0
25
50
75
100
125
150
CC
CT
TT
SNP 2064501

Supporting Figure 1



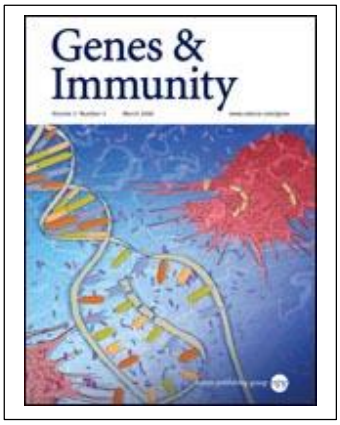
Supporting Figure 2



5.3 Artigo 3

Título: Exome sequencing identifies mutations associated with HCC development in HCV infected individuals.

A ser submetido na revista Genes and Immunity (JCR: 2,91 / Qualis CAPES: B1)



Short Communication

Exome sequencing identifies mutations associated with HCC development in HCV infected individuals

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Abstract

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide and hepatitis C virus (HCV) infection is the main risk factor. Given the limited treatment options available, prevention of HCC development in patients with advanced liver fibrosis may be the most effective strategy to impact patient survival. We report the exome sequencing analysis of 19 HCV infected individuals, of whom 9 had advanced stages of HCC and 10 had liver cirrhosis at older age. The exome sequencing was performed using the ion proton sequencing system. A total of 2,981 variants in 2,276 genes were found in all 9 HCC cases. After filtering against variants found in the cirrhotic group, there were 3 variants in 2 genes (*PRSS58* and *SOCS5*) present exclusively in all HCC cases. Sanger sequencing confirmed the findings. Our results suggest that variants in *PRSS58* and *SOCS5* may be associated with HCC development in chronic HCV infected individuals.

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide and one of the leading causes of death in patients with cirrhosis [1]. In patients with chronic HCV infection, the risk of HCC gradually increases as liver fibrosis progresses. Once cirrhosis is established, HCC develops at an annual rate of about 1-7%, although HCC rarely develops in less fibrotic livers [2-4].

In addition to viral and environmental behavioral factors, host genetic diversity is believed to influence the natural history of HCV infection, including HCC occurrence. Recently, the advance of highthroughput sequencing technology, called next generation sequencing (NGS), have enabled the discovery of genetic variations in a whole-genome scale. Through the use of NGS technology, studies have investigated tumor-associated mutations in samples from patients with HCC caused by different etiologies [5-10].

Whole exome sequencing (WES) can efficiently detect mutations in protein- coding exons, which are much more easily interpretable than mutations or variants in non-coding regions. In the present study, we sequenced the exome of 19 HCV infected individuals: 9 diagnosed with HCC and a control group with 10 cirrhotic individuals. Patients characteristics are summarized in Table 1.

On average, 15.9 gigabases of sequence were generated per sample, 98% of which successfully aligned against the human reference genome (*hg19*). A total of 116,769 variants in 18,054 genes were found in at least one control individual, 57,873 were single nucleotide polymorphisms (SNPs) in exonic regions. Among them, 2,679 were not previously reported in single nucleotide polymorphism database (dbSNP) (http://www.ncbi.nlm.nih.gov/SNP/). Moreover, 1,425 indels were also detected in these patients. Regarding the HCC samples, a total of 111,206 variants in 17,876 genes were found in at least one individual, including 54,784 SNPs in exonic regions (2,416 absent in dbSNP) and 1,499 indels.

Considering that the HCC development may be associated with a recessive allele, we investigated the homozygous variants present in more than one HCC case simultaneously. A total of 2,981 variants in 2,276 genes were found in all 9 HCC cases, 4,712 variants (3,308 genes) in 8 cases, 8,495 variants (5,111 genes) in 6 cases, and so on as the number of cases decreases more variants in common are found (Figure 1A). To deal with the large amount of variants identified, we filtered the variants by selecting homozygous variants present in HCC cases but absent in at least 90% of controls. Using this approach, none variants were obtained

when considering all HCC cases, however 8 cases presented 2 variants in the *CFAP43* gene (Figure 2A).

Regarding the heterozygous model, 674 variants in 356 genes were found in 9 HCC samples (Figure 1B). However, when only the variants absent in at least 90% of controls were considered, 3 variants in 2 genes (*PRSS58* and *SOCS5*) were found in all cases (Figure 2B).

To access the sequencing quality of the selected variants in the *CFAP43*, *PRSS58* and *SOCS5* genes, a second filter was applied using IGV software (Table 2). Variants with poor alignment quality and coverage less than 20X were excluded. After analysis, the variants in *CFAP43* were excluded due the low coverage and alignment quality. Thus, the variants in the *PRSS58* (protease, serine 58) and in *SOCS5* (suppressor of cytokine signaling 5) may be associated with the risk to HCC development. Due the high probability of false positives in high-throughput sequencing methods, the selected variants were validated by Sanger sequencing.

The variant found in *SOCS5* is a synonymous SNP (A→G change) located in a exonic region of the *SOCS5* gene, located at chromosome 2. Members of the suppressor of cytokine signaling (SOCS) family play key roles in the negative regulation of cytokine signal transduction, mainly through inhibition of Janus-activated kinase-STAT (JAK/STAT) signaling pathway [11,12]. The interaction networks for SOCS5 were generated by STRING (http://string-db.org/) [13] (Supplementary figure 1).

SOCS5 is able to regulate the epidermal growth factor receptor (EGFR) and JAK signaling, both implicated in the development of many types of cancer and validated for the treatment of human cancer [14-21]. Studies have demonstrated that exogenous expression of SOCS5 reduces EGF signaling, by promoting EGF-R degradation in a ligand independent manner [22,23]. Additionally, epigenetic silencing of SOCS5 expression has been shown to correlate inversely with EGF-R expression in aggressive hepatocarcinoma [24]. Several evidences suggest an important role of EGF pathway in both cirrhosis and HCC. Polymorphisms in the *EGF* gene that leads to increased EGF expression is associated with increased fibrosis and cirrhosis progression and elevated risk of developing HCC in patients with cirrhosis [25-29]. In addition, transgenic mice with liver-specific overexpression of EGF rapidly develop HCC [30]. Finally, EGFR inhibition is associated with reduction of fibrogenesis and prevention of HCC [31].

SOCS5 can interact directly with JAK1-4 and selectively inhibits autophosphorylation of JAK1 and JAK2 [16]. Furthermore, down-regulation of SOCS5 expression by tumor-derived miR-9 results in enhanced JAK1/2 and STAT1/3 phosphorylation in endothelial cells [32]. The JAK-STAT pathway mediates important biological mechanisms, including inflammation, cell proliferation and antiviral activity [33,34]. In HCC, the JAK/STAT signalling pathway is up-regulated in tumour tissues compared to adjacent normal tissues [35]. Thus, these findings highlight the potential role of SOCS5 as a tumor supressor, however the molecular mechanisms by which it acts in these pathways have not been well-defined and require further studies.

In the present study, we found a correlation between a synonymous polymorphism in *SOCS5* and HCC occurrence. We cannot explain why a synonymous polymorphism may be related with a pathological condition, such as HCC, however this SNP may be in linkage disequilibrium with the real causative SNP associated with HCC development. Supplementary table 1 shows the SNPs in linkage disequilibrium with the selected variant in the European population based in data from the 1000 genomes project (http://www.broadinstitute.org/mammals/haploreg/haploreg.php) [36]. Therefore, *SOCS5* is a potential candidate gene for further studies investigating its correlation with HCC occurrence and other types of cancer.

The PRSS58 encodes a member of the trypsin family of serine proteases. This gene and several related trypsinogen genes are localized to the T cell receptor beta locus on chromosome 7 [37]. Both SNPs found in *PRSS58* are in complete linkage disequilibrium in a intronic region and involves a substitution from G to A and C to T, respectively. The role of PRSS58 is still unknown and data in literature are lacking, but other family members such as PRSS1, 2 and 3 are strongly associated with chronic pancreatitis, as also with ovarian, colorectal and prostate cancer [38-43]. Usually, proteases are abnormally expressed in tumor cells, performing specific functions to facilitate various steps of tumorigenesis. Additionally, proteases activate chemokines, growth factors, growth factor receptors, and other signaling receptors, contributing to the signaling cascades involved in tumor initiation, proliferation, and metastasis [40,44,45]. Thus, *PRSS58* is a potential candidate to genetic marker associated with HCC, however further studies are necessary to confirm this association in a larger cohort and to better characterize the role of PRSS58 in cancer occurrence.

In summary, we identified 3 variants in 2 genes (PRSS58 and SOCS5) associated with HCC development in HCV infected individuals using whole-exome sequencing. Data about the

role of these genes in HCC development are lacking, therefore further studies are necessary to confirm these data in larger cohorts.

Methods

Patients

A total of 9 patients with HCC and 10 patients with liver cirrhosis from the Gastrohepatology Service of the Oswaldo Cruz University Hospital/Liver Institute of Pernambuco (Recife, Northeastern Brazil) were included in the present study. The inclusion criteria for the HCC group were the follow: individuals with HCV in advanced stages of HCC according to the BCLC staging (B or C) and/or individuals with multiple nodules and/or individuals diagnosed with HCC in less than 4 years, counting from the last negative result for HCC. For the control group with liver cirrhosis, the following inclusion criteria were applied: HCV individuals with liver cirrhosis confirmed by histopathology and advanced age. The HCC group had a mean age of 58.3 years old, while the control group had a mean age of 68.4 years old.

Liver biopsies were evaluated by a single expert pathologist and assessed according to the METAVIR scoring system [46]. Hepatocellular carcinoma caused by HCV was diagnosed by ultrasound, computerized tomography, magnetic resonance imaging, arteriography and tumor biopsy according to the AASLD criteria [47].

The present study was approved by the Ethical Committee in Research of the University of Pernambuco under the protocol 47/2010 - CAAE: 0041.0.106.000-10 and was conducted in accordance with the provisions of the declaration of Helsinki and Good Clinical Practice guidelines.

DNA extraction and quality control

Genomic DNA was extracted from whole blood by using QIAamp DNA Blood Kit (QIAGEN Inc, Chatsworth, CA) following the manufacturer's instructions. The extracted DNA was stored at -20ºC until further analysis. The DNA quality was verified by agarose electrophoresis at 2%. The DNA concentration was measured by using the Qubit dsDNA BR Assay Kit (Invitrogen) and Qubit 2.0 Fluorometer (Invitrogen).

Exome sequencing

The library preparation was performed using Ion AmpliSeq Exome RDY Library Kit (Thermo Fisher Scientific). Library was made with 100ng of DNA by sample and 294,000 pairs of probes distributed in 12 wells, covering 97% of coding regions. Samples were barcoded with the Ion Xpress Barcode Adapter (Thermo Fisher Scientific) in order to pool two exomes per chip. The Gene Amp PCR System (Life Technologies) was used for library amplification, probes digestion and barcoding.

The library purification and quantitation was done using the AMPure XP-PCR Purification kit (Life Technologies) and Ion Library Quantitation kit (Life Technologies), respectively. After library quantitation by Real Time PCR system (Life Technologies), the library was diluted to 100pM.

Library fragments were clonally amplified with emulsion PCR using the Ion PI Hi-Q OT2 200 kit v2 and the Ion OneTouch 2 System (Thermo Fisher Scientific), and the positive-ion sphere particles enriched in the Ion OneTouch ES machine (Thermo Fisher Scientific). The percentage of covered ISPs was then quantified by the Qubit Fluorometer 2.0 and Quality Control Sphere Ion kit (Thermo Fisher Scientific). Finally, the positive spheres were loaded in Ion Xpress Chip and sequenced in the Ion Proton System (Thermo Fisher Scientific). All procedures were carried out according to the manufacturer's instructions.

Exome mapping and variant calling

The data generated from were processed with the Torrent Suite Software 4.1 (Thermo Fisher Scientific). Reads were mapped against the human reference genome hg19 using Torrent Mapping Alignment Program version 4.0.6 (Thermo Fisher Scientific). Variant calling was performed by running Torrent Variant Caller plugin version 4.0, using the optimized parameters for exome-sequencing recommended for AmpliSeq sequencing (Thermo Fisher Scientific).

Variants selection

The VCF (Variant Caller File) files generated by the Torrent Suite Software were analyzed by the VarAFT software and the sequences were annotated using Annovar, dbSNP and UMD Predictor. Initially, two local data bases were created with the homozygous and heterozygous variants present in at least 1 control sample. Later, the homozygous and

heterozygous variants present in the maximum number of HCC cases but absent in at least 90% of cirrhotic individuals were selected. The selected variants were then analyzed according to the lecture deep and alignment quality with the Integrative Genome Viewer (IGV) software. The flowchart of the selection criteria is summarized in Supplementary figure 2.

Confirmation of variants with Sanger sequencing

Sanger sequencing of PCR amplicons from genomic DNA was used to confirm the presence and identity of variants in the candidate genes identified via exome sequencing. Primers were designed with Primer 3 v0.4.0 and specificity verified through Primer Blast from NCBI website. Resulting PCR products were purified using Agencourt AMPure XP Reagent Kit (Beckman Coulter, Inc) and subjected to a new cycling reaction prepared with BigDye Terminator kit v3.1 (Thermo Fisher Scientific). Sequencing reactions were analyzed in an ABI 3730xl genetic analyzer (Thermo Fisher Scientific).

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Declaration of Interest

No conflicts of interest exist.

References

1. Singal, A.G. & El-Serag, H.B. Hepatocellular carcinoma from epidemiology to prevention: translating knowledge into practice. *Clinical Gastroenterology and Hepatology* **13**, 2140-2151 (2015).
2. Hoshida, Y., Fuchs, B.C., Bardeesy, N., Baumert, T.F. & Chung, R.T. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. *Journal of hepatology* **61**, S79-S90 (2014).
3. Yang, J.D. & Roberts, L.R. Hepatocellular carcinoma: a global view. *Nature Reviews Gastroenterology and Hepatology* **7**, 448-458 (2010).
4. Yoshida, H. *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. *Annals of internal medicine* **131**, 174-181 (1999).
5. Schulze, K. *et al.* Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nature genetics* **47**, 505-511 (2015).
6. Woo, H.G. *et al.* Profiling of exome mutations associated with progression of HBV-related hepatocellular carcinoma. *PloS one* **9**, e115152 (2014).
7. Nakagawa, H. & Shibata, T. Comprehensive genome sequencing of the liver cancer genome. *Cancer letters* **340**, 234-240 (2013).
8. Kan, Z. *et al.* Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma. *Genome research* **23**, 1422-1433 (2013).
9. Cleary, S.P. *et al.* Identification of driver genes in hepatocellular carcinoma by exome sequencing. *Hepatology* **58**, 1693-1702 (2013).
10. Totoki, Y. *et al.* High-resolution characterization of a hepatocellular carcinoma genome. *Nature genetics* **43**, 464-469 (2011).
11. Cooney, R.N. Suppressors of cytokine signaling (SOCS): inhibitors of the JAK/STAT pathway. *Shock* **17**, 83-90 (2002).
12. Croker, B.A., Kiu, H. & Nicholson, S.E. SOCS regulation of the JAK/STAT signalling pathway. in *Seminars in cell & developmental biology* Vol. 19 414-422 (Elsevier, 2008).
13. Jensen, L.J. *et al.* STRING 8—a global view on proteins and their functional interactions in 630 organisms. *Nucleic acids research* **37**, D412-D416 (2009).

14. Gan, H.K., Burgess, A.W., Clayton, A.H. & Scott, A.M. Targeting of a conformationally exposed, tumor-specific epitope of EGFR as a strategy for cancer therapy. *Cancer research* **72**, 2924-2930 (2012).
15. Levine, R.L. *et al.* Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer cell* **7**, 387-397 (2005).
16. Linossi, E.M. *et al.* Suppressor of cytokine signaling (SOCS) 5 utilises distinct domains for regulation of JAK1 and interaction with the adaptor protein Shc-1. *PloS one* **8**, e70536 (2013).
17. Mullighan, C.G. The molecular genetic makeup of acute lymphoblastic leukemia. *ASH Education Program Book* **2012**, 389-396 (2012).
18. Pardanani, A. *et al.* Safety and efficacy of TG101348, a selective JAK2 inhibitor, in myelofibrosis. *Journal of Clinical Oncology* **29**, 789-796 (2011).
19. Pines, G., Köstler, W.J. & Yarden, Y. Oncogenic mutant forms of EGFR: lessons in signal transduction and targets for cancer therapy. *FEBS letters* **584**, 2699-2706 (2010).
20. Verstovsek, S. *et al.* Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. *New England Journal of Medicine* **363**, 1117-1127 (2010).
21. Zhang, J. *et al.* The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature* **481**, 157-163 (2012).
22. Kario, E. *et al.* Suppressors of cytokine signaling 4 and 5 regulate epidermal growth factor receptor signaling. *Journal of Biological Chemistry* **280**, 7038-7048 (2005).
23. Nicholson, S.E. *et al.* Suppressor of cytokine signaling (SOCS)-5 is a potential negative regulator of epidermal growth factor signaling. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 2328-2333 (2005).
24. Calvisi, D.F. *et al.* Mechanistic and prognostic significance of aberrant methylation in the molecular pathogenesis of human hepatocellular carcinoma. *The Journal of clinical investigation* **117**, 2713-2722 (2007).
25. Cmet, S. *et al.* Carriage of the EGF rs4444903 A> G functional polymorphism associates with disease progression in chronic HBV infection. *Clinical & Experimental Immunology* **167**, 296-302 (2012).
26. Dayyeh, B.K.A. *et al.* A functional polymorphism in the epidermal growth factor gene is associated with risk for hepatocellular carcinoma. *Gastroenterology* **141**, 141-149 (2011).

27. Falleti, E. *et al.* Association between the epidermal growth factor rs4444903 G/G genotype and advanced fibrosis at a young age in chronic hepatitis C. *Cytokine* **57**, 68-73 (2012).
28. Shen, X.F. *et al.* Quantitative assessment of the effect of epidermal growth factor 61A/G polymorphism on the risk of hepatocellular carcinoma. *Oncology letters* **10**, 3199-3205 (2015).
29. Tanabe, K.K. *et al.* Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. *Jama* **299**, 53-60 (2008).
30. Tönjes, R. *et al.* Autocrine mitogen IgEGF cooperates with c-myc or with the Hcs locus during hepatocarcinogenesis in transgenic mice. *Oncogene* **10**, 765-768 (1995).
31. Fuchs, B.C. *et al.* Epidermal growth factor receptor inhibition attenuates liver fibrosis and development of hepatocellular carcinoma. *Hepatology* **59**, 1577-1590 (2014).
32. Zhuang, G. *et al.* Tumour-secreted miR-9 promotes endothelial cell migration and angiogenesis by activating the JAK-STAT pathway. *The EMBO journal* **31**, 3513-3523 (2012).
33. O'Shea, J.J. & Plenge, R. JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. *Immunity* **36**, 542-550 (2012).
34. Stark, G.R. & Darnell, J.E. The JAK-STAT pathway at twenty. *Immunity* **36**, 503-514 (2012).
35. Calvisi, D.F. *et al.* Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. *Gastroenterology* **130**, 1117-1128 (2006).
36. Ward, L.D. & Kellis, M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic acids research* **40**, D930-D934 (2012).
37. NCBI. National Center for Biotechnology Information Search database. Vol. 2016.
38. Yi, Q. *et al.* PRSS1 mutations and the proteinase/antiproteinase imbalance in the pathogenesis of pancreatic cancer. *Tumor Biology*, 1-6 (2015).
39. Azizmohammadi, S. *et al.* Clinical significance and expression of the PRSS3 and Wiskott–Aldrich syndrome protein family verprolin-homologous protein 1 for the early detection of epithelial ovarian cancer. *Tumor Biology*, 1-5 (2015).
40. Ma, R. *et al.* PRSS3 expression is associated with tumor progression and poor prognosis in epithelial ovarian cancer. *Gynecologic oncology* **137**, 546-552 (2015).
41. Radisky, E.S. PRSS3/mesotrypsin in prostate cancer progression: implications for translational medicine. *Asian journal of andrology* **15**, 439 (2013).

42. Williams, S.J., Gotley, D.C. & Antalis, T.M. Human trypsinogen in colorectal cancer. *International journal of cancer* **93**, 67-73 (2001).
43. Witt, H. *et al.* A degradation-sensitive anionic trypsinogen (PRSS2) variant protects against chronic pancreatitis. *Nature genetics* **38**, 668-673 (2006).
44. Nakanuma, S.-I. *et al.* Tumor-derived trypsin enhances proliferation of intrahepatic cholangiocarcinoma cells by activating protease-activated receptor-2. *International journal of oncology* **36**, 793-800 (2010).
45. Cudic, M. & Fields, G.B. Extracellular proteases as targets for drug development. *Current protein & peptide science* **10**, 297 (2009).
46. Bedossa, P. & Poynard, T. An algorithm for the grading of activity in chronic hepatitis C. *Hepatology* **24**, 289-293 (1996).
47. Bruix, J. & Sherman, M. Management of hepatocellular carcinoma: an update. *Hepatology* **53**, 1020-1022 (2011).

Tables

Table 1: Clinical aspects of the sequenced individuals

Diagnosis	Sex	Age	AFP	Nodules	Tumor size (cm)	BCLC	Child-Pugh	Time to HCC diagnosis (years)
HCC	M	49	44.0	Multiple	5.1	B	B	1
HCC	F	70	6.60	Unique	1.1	A	B	3
HCC	F	55	23.50	Multiple	3.1	A	B	2
HCC	M	66	13.0	Multiple	4.0	A	A	8
HCC	M	49	16.0	Multiple	8.1	C	B	5
HCC	F	73	1672.0	Multiple	4.1	B	C	---
HCC	M	54	37.5	Multiple	2.8	A	A	2
HCC	F	60	4.5	Unique	1.6	A	A	1
HCC	M	49	681.0	Multiple	8.6	B	B	---
Cirrhosis	F	65	---	---				
Cirrhosis	M	69	15.8	---				
Cirrhosis	M	68	5.7	---				
Cirrhosis	F	71	16.9	---				
Cirrhosis	M	64	33.6	---				
Cirrhosis	F	71	8.8	---				
Cirrhosis	M	63	2.5	---				
Cirrhosis	M	74	4.3	---				
Cirrhosis	F	73	18.1	---				
Cirrhosis	F	66	---	---				

Table 2. Criteria used for variants selection.

Filter 1: Variants selection	**Selected genes by filter 1**	**Selected genes by filter 1 and 2***
"Homozygous variants without MAF filter" "Absent in at least 9/10 (90%) controls" "Variants present in 8/9 (88%) of cases"	CFAP43	------
"Heterozygous variants without MAF filter" "Absent in at least 9/10 (90%) controls" "Variants present in 9/9 (100%) of cases"	PRSS58 SOCS5	PRSS58 SOCS5

*Filter 2: IGV alignment and sequence coverage.

Figure legends

Figure 1. Number of variants and genes found in HCC cases. A) Number of variants and genes in homozygosis regarding to the number of HCC cases. B) Number of variants and genes in heterozygosis regarding to the number of HCC cases.

Figure 2. Number of variants and genes found in HCC cases and absent in 90% of controls. A) Number of variants and genes in homozygosis regarding to the number of HCC cases. B) Number of variants and genes in heterozygosis regarding to the number of HCC cases.

Figure 1

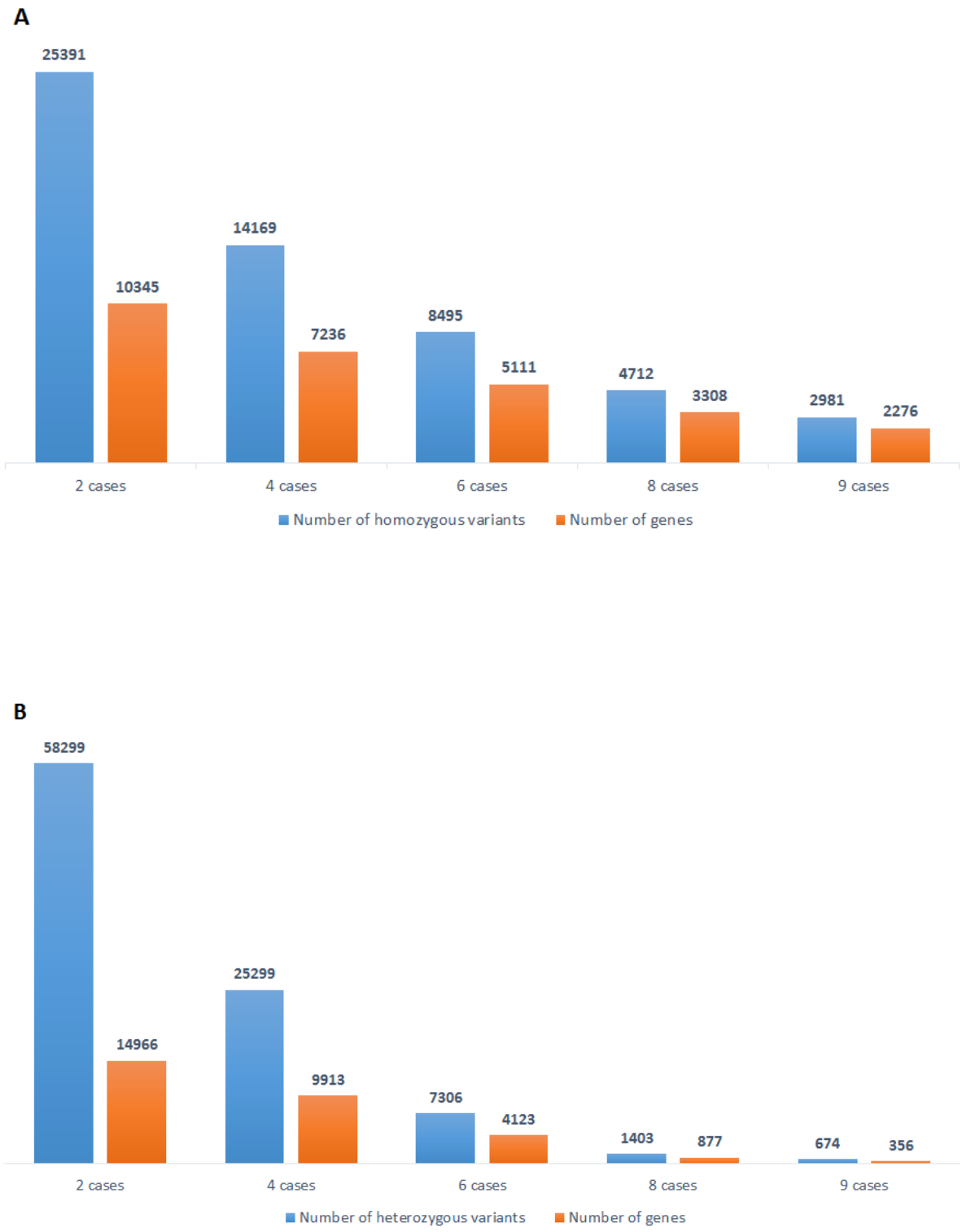
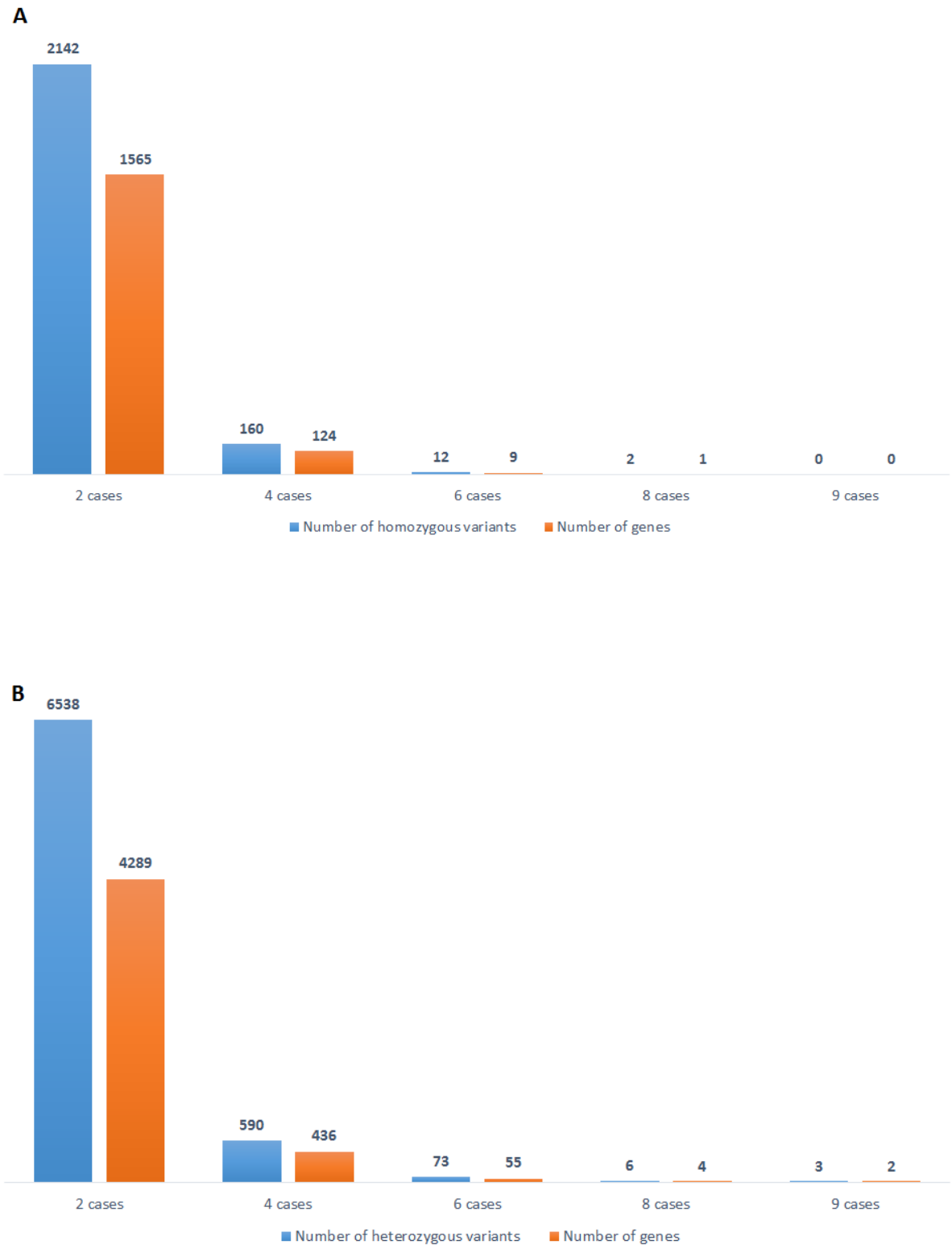

A
25391
10345
14169
7236
8495
5111
4712
3308
2981
2276
2 cases
4 cases
6 cases
8 cases
9 cases
Number of homozygous variants
Number of genes
B
58299
14966
25299
9913
7306
4123
1403
877
674
356
2 cases
4 cases
6 cases
8 cases
9 cases
Number of heterozygous variants
Number of genes

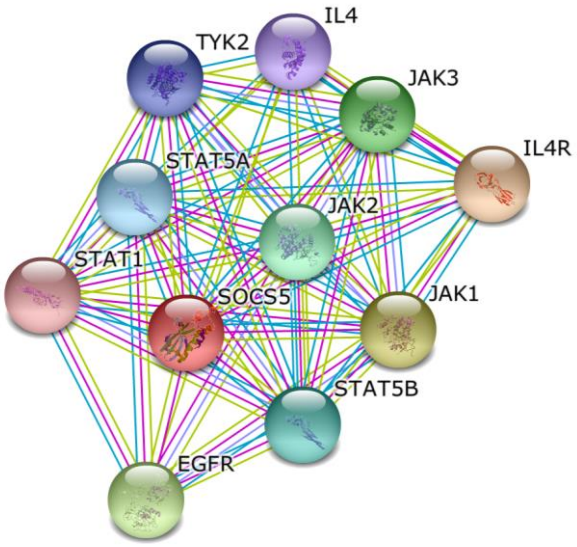
Figure 2


A
2142
1565
160
124
12
9
2
1
0
0
2 cases
4 cases
6 cases
8 cases
9 cases
Number of homozygous variants
Number of genes
B
6538
4289
590
436
73
55
6
4
3
2
2 cases
4 cases
6 cases
8 cases
9 cases
Number of heterozygous variants
Number of genes

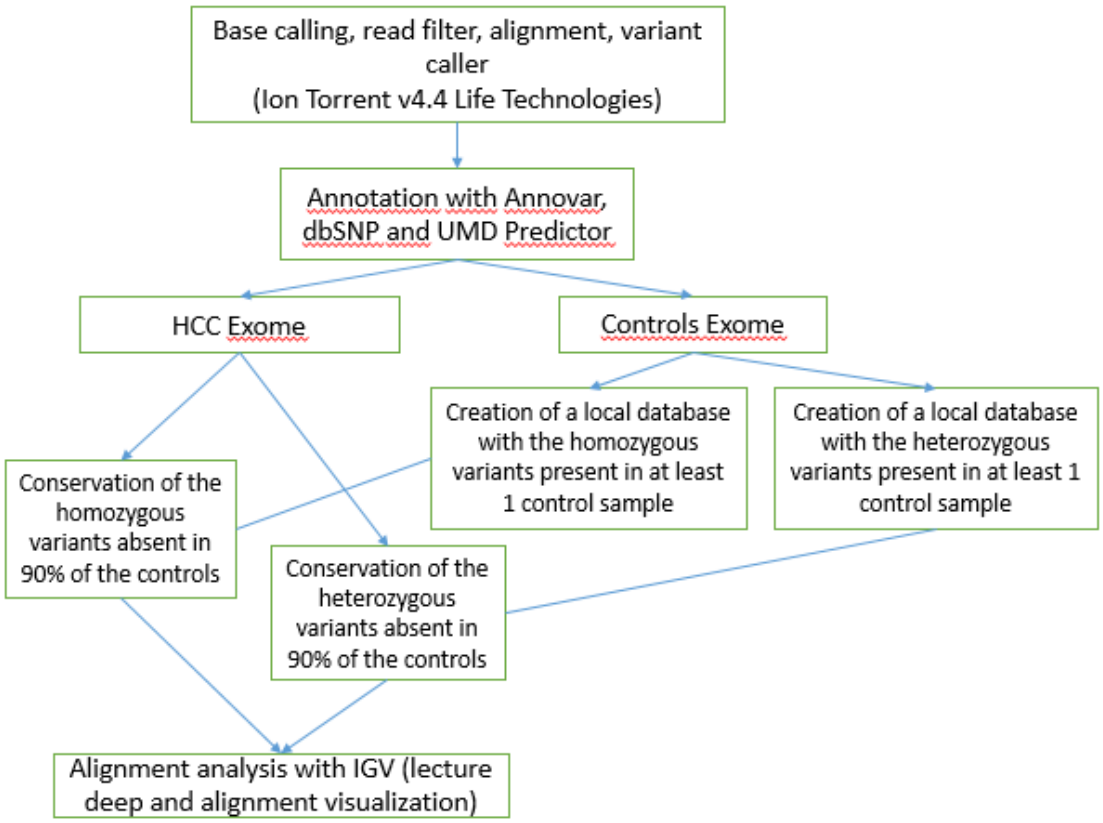
Supplementary material

Supplementary table 1: List of SNPs in linkage disequilibrium with the rs6738426 and r^2>0.8

chr	LD (r^2)	LD (D')	variant	Ref	Alt	AFR freq	AMR freq	ASN freq	EUR freq	GENCODE genes	dbSNP func annot
2	0.84	0.96	----	G	A	0.20	0.42	0.12	0.32	4.8kb 5' of SOCS5	
2	0.83	0.96	----	C	A	0.09	0.40	0.07	0.33	360bp 5' of SOCS5	
2	0.83	0.96	----	C	T	0.13	0.40	0.07	0.33	301bp 5' of SOCS5	
2	0.85	0.96	----	T	C	0.14	0.40	0.08	0.33	SOCS5	intronic
2	0.8	0.9	----	T	G	0.14	0.41	0.08	0.34	SOCS5	intronic
2	0.8	0.9	----	G	C	0.29	0.42	0.09	0.34	SOCS5	intronic
2	0.81	0.91	----	A	G	0.10	0.41	0.08	0.34	SOCS5	intronic
2	0.81	0.91	----	A	C	0.10	0.41	0.08	0.34	SOCS5	intronic
2	0.86	0.96	----	C	T	0.15	0.41	0.07	0.33	SOCS5	intronic
2	0.8	0.91	----	T	C	0.14	0.41	0.08	0.34	SOCS5	intronic
2	0.98	0.99	----	C	G	0.14	0.41	0.08	0.35	SOCS5	intronic
2	0.88	0.98	----	C	T	0.13	0.40	0.07	0.33	SOCS5	intronic
2	0.93	0.99	----	A	G	0.10	0.41	0.08	0.36	SOCS5	intronic
2	0.9	0.99	----	G	A	0.14	0.40	0.08	0.33	SOCS5	intronic
2	0.85	0.93	----	G	T	0.28	0.41	0.08	0.34	SOCS5	intronic
2	0.85	0.93	----	T	C	0.08	0.40	0.07	0.34	SOCS5	intronic
2	0.9	0.99	----	A	C	0.24	0.41	0.09	0.33	SOCS5	intronic
2	0.89	0.99	----	T	A	0.41	0.44	0.08	0.37	SOCS5	intronic
2	0.98	0.99	----	G	C	0.13	0.41	0.09	0.35	SOCS5	intronic
2	0.99	1	----	A	G	0.14	0.41	0.09	0.35	SOCS5	intronic
2	0.94	1	----	A	G	0.14	0.41	0.09	0.36	SOCS5	intronic
2	1	1	----	A	G	0.13	0.41	0.09	0.35	SOCS5	synonymous
2	0.9	0.99	----	G	A	0.13	0.40	0.08	0.33	SOCS5	3'-UTR
2	0.93	0.99	----	T	G	0.13	0.41	0.08	0.36	SOCS5	3'-UTR
2	0.89	0.96	----	A	G	0.13	0.41	0.07	0.36	2.7kb 5' of RP11-333I13.1	



Supplementary figure 1. Network interactions of SOCS5 generated by STRING (http://string-db.org/)

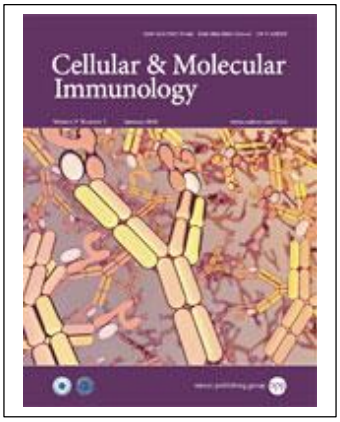


Supplementary figure 2. Workflow for exome analysis. After variants annotation, two local databases with homozygous and heterozygous variants present in at least 1 control sample were created, these databases were used to filter variants present in the HCC cases. Homozygous and heterozygous variants present in HCC cases but absent in 90% of the controls were conserved to further alignment analysis.

5.4 Artigo 4

Título: Role of Interleukin-22 in liver fibrosis

A ser submetido na revista Cellular & Molecular Immunology (JCR: 4,11 / Qualis CAPES: A1)



Review Article

Role of Interleukin-22 in liver fibrosis

Short title: IL-22 in fibrosis

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Abstract

Liver fibrosis is the result of an exacerbated wound-healing response associated with chronic liver injury. Advanced liver fibrosis results in cirrhosis, liver failure, and portal hypertension and frequently requires liver transplantation. The host immune response has an important role driving fibrosis deposition by activating hepatic stellate cells (HSCs). Interleukin-22 (IL-22) is a cytokine that plays a key role in promoting antimicrobial immunity and tissue repair at barrier surfaces. Data from literature suggest that IL-22 has a protective role in the liver by reducing fibrosis in some pathological conditions, however the results are contradictory. This review highlights current knowledge of IL-22' role in liver fibrosis, as well as its therapeutic potential for the treatment of liver fibrosis.

Introduction

Liver fibrosis is the result of an exacerbated wound-healing response associated with chronic liver injury. It is caused by the accumulation of extracellular matrix and scar formation[1]. Subsequent to liver injury, hepatic stellate cells (HSCs) transdifferentiate into myofibroblast-like cells, acquiring contractile, proinflammatory, and fibrogenic properties[1,2]. Advanced liver fibrosis results in cirrhosis, characterized by distortion of the liver parenchyma associated with septae and nodule formation, altered blood flow, and risk of liver failure[3]. A variety of etiologies, such as hepatitis B and C, chronic alcohol abuse, non-alcoholic steatohepatitis (NASH), cholestasis, and autoimmune hepatitis, ultimately progress to liver cirrhosis. Although the removal of causative agents of liver fibrosis regress liver tissue scarring, it is difficult to treat advanced cirrhosis[4,5].

Despite remarkable advances in understanding the fibrotic process, the exact molecular mechanisms of the disease are still poorly understood. However, it is well known that the immune system plays an important role in liver fibrosis. Several immune components (neutrophils, monocytes/macrophages and lymphocytes) are involved in the mechanisms of hepatic fibrosis by controlling hepatic myofibroblast (MFB) accumulation[3].

Interleukin-22 (IL-22) is a member of the IL-10 cytokine family (which also includes IL-10, IL-19, IL-20, IL-24, IL-26, IL-28α, IL-28β, and IL-29) produced by a variety of adaptive and innate immune system cells, such as: αβ T cells, γδ T cells, NKT cells, and innate lymphoid cells (ILCs)[6,7]. Unlike most cytokines, IL-22 does not directly regulate the function of immune cells. Preferably, IL-22 targets cells at outer-body barriers, such as the skin and tissues of the digestive and respiratory systems, as well as cells from pancreas, liver, kidney and joints[8]. Although it can have a profound effect in regenerating epithelial tissues following injury - largely by promoting survival by inducing proliferation and inhibiting apoptosis of epithelial

cells - this same function has also been implicated in pathological states such as malignancy and psoriasis. In addition, a role for IL-22 has been described in host defense within barrier tissues such as the intestine, oral mucosa, skin, and lungs[6].

Numerous studies suggest that IL-22 has a strong protective effect against hepatocyte damage in a variety of liver injury models. In a murine model of T cell-mediated hepatitis induced by concanavalin A, neutralization of IL-22 worsened liver damage, whereas a pretreatment with IL-22 prevented damage[9]. Furthermore, IL-22 treatment reduced liver injury, fatty liver and hepatic oxidative stress in mouse models of acute and chronic alcohol-induced liver damage[10]. High levels of IL-22 are found in several liver injury models induced by pathogens; however, the roles of IL-22 in the pathogenesis of infection-related liver injury have been inconclusive[11]. In this review, we summarize the current state of knowledge about IL-22' role in liver fibrosis, as well as its therapeutic potential for the treatment of liver fibrosis.

Interleukin-22 (IL-22)

IL-22 is a member of the IL-10 cytokine family[12]. The human *IL22* gene is located at chromosome 12q15, about 52- and 99-kbp upstream from the *IL26* and *IFNG* locus, respectively[13]. *IL22* share a similar size and organization when compared with other members of the IL-10 family. Its genomic structure presents 5 introns and 6 exons, including a short non-coding exon of 22 nucleotides starting 24 nucleotides downstream a putative TATA box[13]. Human *IL22* gene encodes a protein which has 179 amino acids in length. After the removal of the predicted 33-amino-acid signal peptide, the cytokine is secreted as a protein which is 146 amino acids long[8,14]. Like other IL-10 family members, the IL-22 structure contains six α-helices (referred to as helices A to F) and connecting loops that fold in a compact bundle[15].

IL-22 exerts its biological activities via a transmembrane receptor complex composed by two subunits: IL-22 receptor 1 (IL-22R1) and IL-10 receptor 2 (IL-10R2), which is mainly expressed on intestinal and respiratory epithelial cells, keratinocytes, and hepatocytes, but not on cells of hematopoietic origin[16]. The human gene encoding IL-22R1 (*IL22RA1*) is located at chromosome 1p36.11, near the IL28RA locus, whereas the gene encoding IL-10R2 (*IL10RB*) is located on chromosome 21q22.11, near the IFNARA, IFNARB, and IFNGRB loci[7]. Studies have demonstrated a high affinity between IL22 and IL-22R1 but no affinity between IL-22 and IL-10R2. However, a strong affinity of IL-10R2's subunit for IL-22-IL-22R1's complex was observed[17-20]. These data suggest that the IL-22–IL-22R interaction is a multistep process, in which IL-22 first binds to its high-affinity receptor subunit, IL-22R1, then it induces a conformational change in the IL-22 protein that confers enough affinity to allow the protein to bind secondarily to the IL-10R2 subunit. IL-10R2 then stabilizes the association of IL-22 with IL-22R1[8].

Ligation between IL-22 and IL-22R1-IL-10R2 complex results in the activation of JAK/STAT pathway, leading to the phosphorylation of these receptors and STAT proteins. Phosphorylation of STAT3 at the Tyr705 residue is considered the main event observed in the IL-22 signaling pathway, although phosphorylation of STAT1 and STAT5 have also been observed[21]. The signaling of these molecules enables the regulation of the expression of genes involved in many processes, such as apoptosis, cell-cycle progression, and angiogenesis[22]. Besides the STAT signaling, the activation of mitogen-activated protein kinase (MAPK) and p38 pathways have been observed after IL-22 activation[23].

IL-22 is produced by various immunity cells, including CD4+ and CD8+ T cells, γδ T cells, natural killer (NK) cells and innate lymphoid cells (ILCs)[8]. However, unlike most cytokines, IL-22 does not act on hematopoietic cells, but affects epithelia, hepatocytes, and pancreatic cells, suggesting an important role in repairing local tissue damage, or contributing

to pathophysiologic inflammation[7]. Studies have shown that IL-22 might have a beneficial effect in preventing the inflammatory bowel disease[24], liver injury[9-11,22,25] and ulcerative colitis[26]. In contrast, IL-22 has been associated with the pathogenesis of some diseases, including psoriasis[27], rheumatoid arthritis[28,29], Crohn's disease[30] and viral hepatitis[31,32]. Thus, the context in which IL-22 is expressed may influence its role in the immune response's modulation.

IL-22 binding protein

Besides cell surface receptors, IL-22 can also bind to a secreted, single-chain receptor known as IL-22 binding protein (IL-22BP or IL-22RA2), which binds to this cytokine with strong affinity (up to 1,000-fold higher than IL-22R1) and prevents its cellular effects[12,18,30,33,34]. The gene encoding human IL-22BP is located at chromosome 6q23.3, between the *IFNGR1* and *IL20RA* genes, and encodes a 210-aa protein with 34% amino acid identity to the extracellular domain of IL-22R1[12,34,35].

The expression of IL-22BP has been demonstrated in different tissues such as placenta, breast, thymus, spleen, lymph nodes, gastrointestinal tract, lungs and skin[12,33,35,36]. However, the main source of IL-22BP is constituted by immature dendritic cells[37]. Interestingly, previous studies have demonstrated that IL-22BP is down-regulated during the early stages of infection[30,36,38]; however, IL-22BP was up-regulated in the liver of mice at later stages after infection with *Toxoplasma gondii*, *Schistosoma mansoni* and *Mycobacterium avium*[39]. These data indicate a regulatory role of IL-22BP controlling IL-22's effects.

IL-22 and liver fibrosis

Hepatocytes are the main target of IL-22 in the liver. Previous reports have demonstrated that IL-22 induces production of acute-phase proteins such as serum amyloid A, α1-antichymotrypsin, haptoglobin and lipopolysaccharide (LPS)-binding protein[21,30,40-42]. IL-22 also induces expression of proteins associated with tissue repair such as anti-apoptotic proteins (BCL-2, BCL-X_L, myeloid cell leukaemia sequence 1 [MCL1]), mitogenic proteins (such as retinoblastoma-like protein 2, cyclin D1, p21 and cyclin-dependent kinase 4 [CDK4])[9,43].

Several studies have demonstrated that IL-22 protects the liver against many types of injuries by promoting hepatocyte proliferation and survival[9-11,44,45] In a murine model of T cell-mediated hepatitis induced by concanavalin A (Con A), neutralization of IL-22 reduced STAT3 activation and worsened liver injury, whereas pretreatment with IL-22 prevented the damage[9]. Additionally, the overexpression of IL-22 by hydrodynamic gene delivery protected mice against liver injury, necrosis and apoptosis in experimental models of hepatic injury induced by Con A, CCl4 and FAS ligand (FASL)[46]. Furthermore, transgenic mice with an overexpression of IL-22 were protected from the liver injury induced by ConA injection[43], while IL-22 deficient mice were highly susceptible to such injury[47]. In addition, a study demonstrated that IL-22 promotes liver cell regeneration by increasing hepatic cell proliferation and hepatocyte migration in vitro through the activation of AKT and STAT3 signaling[48]. Finally, evidences suggest that the treatment with recombinant IL-22 reduces liver fibrosis by targeting HSCs. These experiments were performed in murine models of CCl4-induced liver fibrosis, and were confirmed by the lower hepatic fibrosis pathological scores[44,49].

IL-22 was also able to enhance liver regeneration in mice after 70% of partial hepatectomy, whereas its blockage with anti-IL-22 antibody decreased hepatocyte

proliferation[50]. The hepatoprotective role of IL-22 was also observed in a model of liver ischaemia–reperfusion injury, where mice which were treated with recombinant IL-22 had significant reduction in AST serum levels; local inflammation and leukocyte sequestration[51].

On the other hand, evidence also supports that IL-22 plays a pathological role in exacerbating chronic liver inflammation and fibrosis in HBV-infected patients and HBV transgenic mice[32,42], as well as in patients with hepatitis C[31]. In addition, IL-22 did not show any protective effects against liver lesions in mice infected by *Schistosoma mansoni* or *Mycobacterium avium*[39].

Taken together, these findings suggest that although IL-22 plays an important hepatoprotective role in the most liver disease models, it seems that under some circumstances such as infectious diseases, IL-22 may have a detrimental effect in the liver.

Hepatitis B

Hepatitis B is an infectious disease that may lead to chronic severe liver disease. Chronic HBV carriers have an increased risk of developing liver cirrhosis, hepatic decompensation, and hepatocellular carcinoma[52]. Worldwide it is estimated that 350 million individuals are chronically infected with HBV, and 15–40% of them will develop serious sequelae during their lifetime[53]. HBV itself is not cytopathic, however interactions between the virus and the host immune system will determine the natural history of infected individuals[54].

Hepatic IL-22 is up-regulated in patients with chronic HBV[32,42,43,55]. Although IL-22 has been demonstrated to have no antiviral activity against HBV replication[42], hepatic expression of IL-22 was increased in patients with HBV and correlated with the grade of inflammation and proliferation of liver progenitor cells (LPCs)[56].

Furthermore, IL-22 has been demonstrated to play a key role in promoting tissue repair by inducing the production of anti-apoptotic, mitogenic and antioxidant molecules in damaged hepatocytes via STAT3[9,43]. The protective role of IL-22 in preventing liver damage is supported by a study which investigates the intra-hepatic expression of IL-22 in HBV infected patients. In this study, IL-22 was inversely correlated with liver inflammation and fibrosis stages, determined by liver histology, whereas the intra-hepatic expression of IL-17 showed a positive correlation with fibrosis progression. Additionally, the expression of IL-22 was significantly higher in patients with alanine aminotransferase (ALT) levels ≤ twice the upper limit of normal, while IL-17 expression was higher in patients with ALT levels > twice the upper limit of normal[55].

On the other hand, other studies have shown evidences that IL-22 plays a detrimental role in exacerbating chronic liver injury. IL-22 was positively correlated with the grade of liver inflammation and fibrosis by recruiting hepatic Th17 cells[32]. IL-22 was also associated with serum levels of aspartate aminotransferase (AST) in cirrhotic patients with HBV and HCV[43]. Additionally, IL-22 neutralization ameliorated liver damage by reducing chemokine expression on hepatocytes, and subsequently preventing hepatic recruitment of inflammatory cells in a HBV transgenic mice model[32,42].

Despite differences in the course of HBV infection between mouse and humans that may explain, at least in part, the contradictory finding results, chronic HBV is a complex disease that progresses through different phases from immune tolerance, immune activity, and immune inactivity[57]. It is possible that IL-22 exerts a dual role in HBV infection; in early stages IL-22 may exert a protective role by promoting liver repair. However, since HBV is not eliminated and the chronic infection is maintained for several years, IL-22 may contribute to liver damage. Further studies are needed using a cohort of HBV-infected individuals, with a well-defined immunological status, to elucidate the effect of IL-22 in the pathogenesis of liver fibrosis.

Hepatitis C

It is estimated that 3% of the world's population is infected with hepatitis C virus (HCV), representing 170 million people[58]. Liver inflammation in chronic HCV infection is controlled by several mechanisms, including host regulatory immune responses and viral polypeptides interaction with cells of the host innate and adaptive immunities[59]. It is believed that an effective immune response will clear HCV infection in 20%–40% of cases. However, the failure of an adequate immune response will tolerate continuous viral replication, with chronic recruitment of inflammatory cellular infiltration to the liver[60]. An important factor in the pathogenesis of HCV chronic infection is the liver damage sustained by the chronic inflammation and tissue fibrosis, which could lead to cirrhosis and hepatocellular carcinoma[61].

Hepatic IL-22 expression is up-regulated in patients with HCV[31,43,62], although IL-22 does not regulate the gene expression of the antiviral proteins 2',5'-OAS and MxA in hepatic cells nor inhibit HCV replication *in vitro*[62]. Furthermore, evidences show that single nucleotide polymorphisms (SNPs) in *IL22* gene may influence treatment response and viral clearance in HCV infected individuals[63].

Recent studies addressing the role of IL-22 in HCV fibrosis progression presented contradictory conclusions. Methodological differences may explain the contradictory findings in these studies. Sertorio et al (2015) demonstrated that the carriage of a genetic variant in *IL22RA2* - the physiological inhibitor of IL-22 - increased the risk to develop severe fibrosis, this result was replicated in four different cohorts (2 cohorts with HCV and 2 with schistosomiasis). Although the expression of IL-22 was not evaluated in HCV individuals, this study observed a protective effect of IL-22 in fibrosis progression in individuals with schistosomiasis [64]. On the other hand, Wu et al. (2015) found that peripheral and intrahepatic IL-22 were positively associated with liver fibrosis severity in HCV infected individuals [31]; although IL-22 positive cells were clearly accumulated in the liver tissue of individuals with

more fibrosis than individuals with less fibrosis, IL-22 may be increased due to a protective mechanisms trying to control liver damage. Finally, a recent study demonstrated that IL-22 was associated with fibrosis progression in individuals with HCV after orthotopic liver transplantation. IL-22 also promoted HSCs proliferation and activation as well as inhibited apoptosis *in vitro* [65]. This study supports the pathological role of IL-22 in liver fibrosis caused by HCV, however it cannot be directly compared with the other previously published studies, since the clinical course of HCV infection after liver transplant is considerably different due to the patients' immunological status and high viremia.

Though there are different conclusions, these studies raised an important role of IL-22 in the fibrosis severity of HCV infection. Further studies are necessary to elucidate the role of IL-22/IL-22BP as well as their genetic variants in the progression of liver fibrosis in patients with HCV.

Schistosomiasis

Schistosomiasis (or bilharzia) is an infectious disease that affects more than 230 million people worldwide[66]. It is caused by trematode parasites of the genus *Schistosoma*, of which three major species—*Schistosoma mansoni, S. japonicum, and S. haematobium*—cause severe disease in humans[67].

The main pathogenesis of schistosomiasis is caused by eggs deposition in the liver, which results in periportal fibrosis and may extend to end-stage liver disease, such as cirrhosis, portal hypertension, splenomegaly, ascites and eventually lead to death[68]. It remains unknown why certain patients develop severe liver complications and others do not, despite living in a similar endemic environment.

Studies investigating the association between IL-22 and liver fibrosis in schistosomiasis infection are scarce. IL-22 concentrations were inversely correlated with severe fibrosis in PBMC cultures from donors infected with *S. japonicum*. In addition, polymorphisms in *IL22RA2* (rs6560136 and rs2064501) were significantly correlated with the severity of liver fibrosis in patients infected with schistosomiasis. The functional analysis of these variants revealed an association with high expression of IL-22BP in healing skin tissue[64]. Therefore, the authors concluded that IL-22 may protect against liver fibrosis in human schistosomiasis as well as suggested in HCV infection. On the other hand, Wilson et al. (2010) failed to demonstrate association between IL-22 and fibrosis severity in il-22$^{-/-}$ mice infected with *S. mansoni*[39].

IL-22 appears to play a significant role in the pathogenesis of schistosomiasis infection in humans, although significant results were not found in mice. Differences between schistosomiasis infection in mice and humans may explain the contradictory results. Thus, further studies are necessary to confirm the role of IL-22 in human schistosomiasis infection.

Alcoholic liver disease

Excessive alcohol consumption is a major cause of chronic liver disease worldwide and encompasses a broad spectrum of liver injuries, including fatty liver, alcoholic hepatitis, cirrhosis, and hepatocellular carcinoma[69,70]. The exact pathogenesis mechanisms of alcoholic liver injury are still not clear, but may involve direct hepatotoxicity of ethanol and its metabolites; oxidative stress generated by ethanol metabolism; activation of innate immunity; elevation of pro-inflammatory cytokines and chemokines and others[11]. Interleukin-6 (IL-6) seem to have an hepatoprotective role in alcoholic liver disease through activation of STAT3, however, its clinical application is limited due to many potential side effects[71,72]. Since, IL-22

is able to activate similar pathways, such as STAT3, its role in alcoholic liver disease has been addressed.

In a murine model of chronic-binge ethanol feeding, treatment with recombinant IL-22 reduced liver injury and steatosis via STAT3. Moreover, IL-22 increased the expression of antioxidant, antiapoptotic and antimicrobial genes[10]. Accordingly, IL-22 was demonstrated to play a protective effect mediated by the attenuation of oxidative stress, decreasing hepatic TNF-alpha expression and hepatocyte apoptosis inhibition, in a model of acute alcohol-induced liver injury[73]

These findings suggest that a treatment with IL-22 could be a potential therapeutic option to alcoholic liver disease, due to its hepatoprotective role and possibly few side effects.

Nonalcoholic fatty liver disease (NAFLD)

Nonalcoholic fatty liver disease (NAFLD) is one of the most common forms of chronic liver disease worldwide and it is strongly associated with obesity and type 2 diabetes[74]. NAFLD refers to a wide spectrum of liver disorders ranging from simple fatty liver (steatosis) to nonalcoholic steatohepatitis (NASH) with increased risk of developing progressive fibrosis, cirrhosis, and liver cancer[75]. The mechanisms involved in the progression from steatosis to more severe liver injury remains unknown, but may involve oxidative stress, mitochondrial dysfunction, fatty acids lipotoxicity, innate immunity and inflammatory cytokines[76].

Beyond its role in modulating inflammation and host defense, IL-22 seems to regulate lipid metabolism and steatosis in the liver. In a murine model of hepatic steatosis induced by high fat diet (HFD), the administration of recombinant IL-22 reduced the expression of lipogenesis-related genes, as well as triglyceride, cholesterol, AST and ALT levels[77]. Additionally, it was suggested a protective activity of IL-22 when there was an absence of IL-

17. Rolla et al. (2016) observed that IL-17$^{-/-}$ mice are protected from NASH development and are characterized by an extensive liver infiltration of Th22 lymphocytes[78]. These data support IL-22 as a potential candidate for treatment of NASH, however, further studies are necessary to confirm the beneficial role of IL-22 in humans.

Therapeutic potential of IL-22

Liver fibrosis is a significant health problem, with a worldwide mortality of around 1.5 million deaths per year, attributed to cirrhosis and primary liver cancer [79]. Despite an improved understanding of the pathogenetic mechanisms of liver fibrosis, effective antifibrotic drugs are lacking and only preventive measures are addressed to resolve the underlying causative factors of liver degeneration (i.e., antiviral therapies for HCV and HBV or strategies to reduce metabolic syndrome)[80]. The development of therapies for delaying or regressing the liver fibrosis are extremely important mainly for those patients for whom a specific treatment is not available or ineffective, reducing the complications of severe liver disease and delaying the need of liver transplantation.

The use of IL-22 as a therapeutic agent for liver diseases is promising, since IL-22 treatment has been shown to ameliorate liver damage in several models of liver injury[9,10,45,49,73]. *In vitro* treatment with recombinant IL-22 or overexpression of IL-22 promoted liver regeneration or hepatocyte proliferation[9,43,48]. Furthermore, IL-22 treatment attenuated HSC activation and down-regulated the levels of inflammatory cytokines, thereby reducing the severity of liver fibrosis[49]. Finally, IL-22R1 expression is restricted to epithelial cells (e.g. hepatocytes and LPCs)[7,56], besides, studies investigating liver-specific IL-22 transgenic mice observed no obvious adverse phenotypes[43], thus IL-22 treatment may have fewer side effects.

The increase of IL-22–IL-22R1's activity could be achieved by supporting the generation and/or stability of IL-22-producing cells, increasing IL-22 production by these cells or by direct IL-22 application[8]. On the other hand, in some situations where IL-22 activity has a detrimental effect, the inhibition of IL-22 production or its neutralization could be an alternative approach. Therefore, IL-22 appears to be a promising therapeutic target for the treatment of liver fibrosis although further investigations are necessary for the implementation of these strategies considering the source of liver damage.

Conclusions

Considerable progress has been achieved in recent years regarding the role of IL-22 in the pathogenesis of liver fibrosis. It is evident that IL-22 plays an important role in the pathogenesis of liver fibrosis. Its final effect in promoting or protecting the liver will be determined by the type of liver disease and liver injury models. However, it is worthy to note that IL-22 presented a protective role in most of the studies, except in some reports which the source of liver injury was mediated by an infectious agent. In the course of an infectious disease, IL-22 will interact with many others cytokines that may amplify or inhibit its response (the protection of IL-22 was null in the presence of IL-17 [78]). Thus, it is possible that IL-22 plays dual role in controlling liver fibrosis, depending on the intrahepatic microenvironment (Figure 1). Further studies are necessary, especially to investigate the role of IL-22/IL-22BP genetic polymorphisms in the severity of liver fibrosis.

Conflicts of interest

No conflicts of interest exist.

References

1. Bataller, R. & Brenner, D.A. Liver fibrosis. *Journal of Clinical Investigation* **115**, 209-218 (2005).
2. Marra, F. Hepatic stellate cells and the regulation of liver inflammation. *J Hepatol* **31**, 1120-30 (1999).
3. Friedman, S.L. Mechanisms of hepatic fibrogenesis. *Gastroenterology* **134**, 1655-69 (2008).
4. Brenner, D.A. Reversibility of liver fibrosis. *Gastroenterol Hepatol (N Y)* **9**, 737-9 (2013).
5. Seki, E. & Brenner, D.A. Recent advancement of molecular mechanisms of liver fibrosis. *J Hepatobiliary Pancreat Sci* **22**, 512-8 (2015).
6. Dudakov, J.A., Hanash, A.M. & van den Brink, M.R. Interleukin-22: immunobiology and pathology. *Annu Rev Immunol* **33**, 747-85 (2015).
7. Wolk, K., Witte, E., Witte, K., Warszawska, K. & Sabat, R. Biology of interleukin-22. *Semin Immunopathol* **32**, 17-31 (2010).
8. Sabat, R., Ouyang, W. & Wolk, K. Therapeutic opportunities of the IL-22-IL-22R1 system. *Nat Rev Drug Discov* **13**, 21-38 (2014).
9. Radaeva, S., Sun, R., Pan, H.n., Hong, F. & Gao, B. Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. *Hepatology* **39**, 1332-1342 (2004).
10. Ki, S.H. *et al.* Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: role of signal transducer and activator of transcription 3. *Hepatology* **52**, 1291-300 (2010).

11. Kong, X., Feng, D., Mathews, S. & Gao, B. Hepatoprotective and anti-fibrotic functions of interleukin-22: therapeutic potential for the treatment of alcoholic liver disease. *J Gastroenterol Hepatol* **28 Suppl 1**, 56-60 (2013).

12. Dumoutier, L., Louahed, J. & Renauld, J.C. Cloning and characterization of IL-10-related T cell-derived inducible factor (IL-TIF), a novel cytokine structurally related to IL-10 and inducible by IL-9. *J Immunol* **164**, 1814-9 (2000).

13. Dumoutier, L., Van Roost, E., Ameye, G., Michaux, L. & Renauld, J.C. IL-TIF/IL-22: genomic organization and mapping of the human and mouse genes. *Genes Immun* **1**, 488-94 (2000).

14. Xie, M.H. *et al.* Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. *J Biol Chem* **275**, 31335-9 (2000).

15. Nagem, R.A. *et al.* Crystal structure of recombinant human interleukin-22. *Structure* **10**, 1051-62 (2002).

16. Rutz, S., Eidenschenk, C. & Ouyang, W. IL-22, not simply a Th17 cytokine. *Immunol Rev* **252**, 116-32 (2013).

17. Bleicher, L. *et al.* Crystal structure of the IL-22/IL-22R1 complex and its implications for the IL-22 signaling mechanism. *FEBS letters* **582**, 2985-2992 (2008).

18. Jones, B.C., Logsdon, N.J. & Walter, M.R. Structure of IL-22 bound to its high-affinity IL-22R1 chain. *Structure* **16**, 1333-1344 (2008).

19. Logsdon, N.J. *et al.* The IL-10R2 binding hot spot on IL-22 is located on the N-terminal helix and is dependent on N-linked glycosylation. *Journal of molecular biology* **342**, 503-514 (2004).

20. Logsdon, N.J., Jones, B.C., Josephson, K., Cook, J. & Walter, M.R. Comparison of interleukin-22 and interleukin-10 soluble receptor complexes. *Journal of interferon & cytokine research* **22**, 1099-1112 (2002).

21. Wolk, K. *et al.* IL-22 increases the innate immunity of tissues. *Immunity* **21**, 241-54 (2004).

22. Pan, C.X. *et al.* Role of interleukin-22 in liver diseases. *Inflamm Res* **63**, 519-25 (2014).

23. Lejeune, D. *et al.* Interleukin-22 (IL-22) activates the JAK/STAT, ERK, JNK, and p38 MAP kinase pathways in a rat hepatoma cell line Pathways that are shared with and distinct from IL-10. *Journal of Biological Chemistry* **277**, 33676-33682 (2002).

24. Li, L.-J., Gong, C., Zhao, M.-H. & Feng, B.-S. Role of interleukin-22 in inflammatory bowel disease. *World J Gastroenterol* **20**, 18177-18188 (2014).

25. Zenewicz, L.A. *et al.* Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* **29**, 947-957 (2008).

26. Sugimoto, K. *et al.* IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *The Journal of clinical investigation* **118**, 534-544 (2008).

27. Hao, J.-Q. Targeting interleukin-22 in psoriasis. *Inflammation* **37**, 94-99 (2014).

28. da ROCHA, L.F. *et al.* Increased serum interleukin 22 in patients with rheumatoid arthritis and correlation with disease activity. *The Journal of rheumatology* **39**, 1320-1325 (2012).

29. Leipe, J. *et al.* Interleukin 22 serum levels are associated with radiographic progression in rheumatoid arthritis. *Annals of the rheumatic diseases* **70**, 1453-1457 (2011).

30. Wolk, K. *et al.* IL-22 induces lipopolysaccharide-binding protein in hepatocytes: a potential systemic role of IL-22 in Crohn's disease. *The Journal of Immunology* **178**, 5973-5981 (2007).

31. Wu, L.-Y. *et al.* Up-regulation of interleukin-22 mediates liver fibrosis via activating hepatic stellate cells in patients with hepatitis C. *Clinical Immunology* **158**, 77-87 (2015).

32. Zhao, J. *et al.* Pathological functions of interleukin-22 in chronic liver inflammation and fibrosis with hepatitis B virus infection by promoting T helper 17 cell recruitment. *Hepatology* **59**, 1331-1342 (2014).

33. Gruenberg, B. *et al.* A novel, soluble homologue of the human IL-10 receptor with preferential expression in placenta. *Genes and immunity* **2**, 329-334 (2001).

34. Kotenko, S.V. *et al.* Identification, cloning, and characterization of a novel soluble receptor that binds IL-22 and neutralizes its activity. *The Journal of Immunology* **166**, 7096-7103 (2001).

35. Xu, W. *et al.* A soluble class II cytokine receptor, IL-22RA2, is a naturally occurring IL-22 antagonist. *Proceedings of the National Academy of Sciences* **98**, 9511-9516 (2001).

36. Weiss, B. *et al.* Cloning of murine IL-22 receptor alpha 2 and comparison with its human counterpart. *Genes and immunity* **5**, 330-336 (2004).

37. Martin, J.C. *et al.* Interleukin-22 binding protein (IL-22BP) is constitutively expressed by a subset of conventional dendritic cells and is strongly induced by retinoic acid. *Mucosal immunology* **7**, 101-113 (2014).

38. Huber, S. *et al.* IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. *Nature* **491**, 259-263 (2012).

39. Wilson, M.S. *et al.* Redundant and pathogenic roles for IL-22 in mycobacterial, protozoan, and helminth infections. *The Journal of Immunology* **184**, 4378-4390 (2010).

40. Dumoutier, L., Van Roost, E., Colau, D. & Renauld, J.-C. Human interleukin-10-related T cell-derived inducible factor: molecular cloning and functional characterization as an hepatocyte-stimulating factor. *Proceedings of the National Academy of Sciences* **97**, 10144-10149 (2000).

41. Liang, S.C. *et al.* IL-22 induces an acute-phase response. *The Journal of Immunology* **185**, 5531-5538 (2010).

42. Zhang, Y. *et al.* A proinflammatory role for interleukin-22 in the immune response to hepatitis B virus. *Gastroenterology* **141**, 1897-1906 (2011).

43. Park, O. *et al.* In vivo consequences of liver-specific interleukin-22 expression in mice: Implications for human liver disease progression. *Hepatology* **54**, 252-261 (2011).

44. Kong, X. *et al.* Interleukin-22 induces hepatic stellate cell senescence and restricts liver fibrosis in mice. *Hepatology* **56**, 1150-1159 (2012).

45. Xing, W.-w. *et al.* Hepatoprotective effects of IL-22 on fulminant hepatic failure induced by d-galactosamine and lipopolysaccharide in mice. *Cytokine* **56**, 174-179 (2011).

46. Pan, H., Hong, F., Radaeva, S. & Gao, B. Hydrodynamic gene delivery of interleukin-22 protects the mouse liver from concanavalin A-, carbon tetrachloride-, and Fas ligand-induced injury via activation of STAT3. *Cell Mol Immunol* **1**, 43-49 (2004).

47. Zenewicz, L.A. *et al.* Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. *Immunity* **27**, 647-659 (2007).

48. Brand, S. *et al.* IL-22-mediated liver cell regeneration is abrogated by SOCS-1/3 overexpression in vitro. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **292**, G1019-G1028 (2007).

49. Lu, D.-H. *et al.* Interleukin-22 ameliorates liver fibrogenesis by attenuating hepatic stellate cell activation and downregulating the levels of inflammatory cytokines. *World journal of gastroenterology: WJG* **21**, 1531 (2015).

50. Ren, X., Hu, B. & Colletti, L.M. IL-22 is involved in liver regeneration after hepatectomy. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **298**, G74-G80 (2010).

51. Chestovich, P.J. *et al.* IL-22: implications for liver ischemia/reperfusion injury. *Transplantation* **93**, 485 (2012).

52. Beasley, R.P. Hepatitis B virus. The major etiology of hepatocellular carcinoma. *Cancer* **61**, 1942-1956 (1988).

53. Lavanchy, D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *Journal of viral hepatitis* **11**, 97-107 (2004).

54. Trépo, C., Chan, H.L. & Lok, A. Hepatitis B virus infection. *The Lancet* **384**, 2053-2063 (2014).

55. Xiang, X. *et al.* IL-22 and non-ELR-CXC chemokine expression in chronic hepatitis B virus-infected liver. *Immunology and cell biology* **90**, 611-619 (2012).

56. Feng, D. *et al.* Interleukin-22 promotes proliferation of liver stem/progenitor cells in mice and patients with chronic hepatitis B virus infection. *Gastroenterology* **143**, 188-198. e7 (2012).

57. Gao, W., Fan, Y.-C., Zhang, J.-Y. & Zheng, M.-H. Emerging Role of Interleukin 22 in Hepatitis B Virus Infection: a Double-edged Sword. *Journal of Clinical and Translational Hepatology* **1**, 103 (2013).

58. Lavanchy, D. The global burden of hepatitis C. *Liver International* **29**, 74-81 (2009).

59. Mondelli, M.U. & Barnaba, V. Viral and host immune regulatory mechanisms in hepatitis C virus infection. *European journal of gastroenterology & hepatology* **18**, 327-331 (2006).

60. Zimmermann, M. *et al.* Hepatitis C virus core protein impairs in vitro priming of specific T cell responses by dendritic cells and hepatocytes. *Journal of hepatology* **48**, 51-60 (2008).

61. Fahey, S., Dempsey, E. & Long, A. The role of chemokines in acute and chronic hepatitis C infection. *Cellular & molecular immunology* **11**, 25-40 (2014).

62. Dambacher, J. *et al.* The role of interleukin-22 in hepatitis C virus infection. *Cytokine* **41**, 209-216 (2008).

63. Hennig, B.J. *et al.* Influence of IL-10RA and IL-22 polymorphisms on outcome of hepatitis C virus infection. *Liver International* **27**, 1134-1143 (2007).

64. Sertorio, M. *et al.* IL-22 and IL-22 binding protein (IL-22BP) regulate fibrosis and cirrhosis in hepatitis C virus and schistosome infections. *Hepatology* **61**, 1321-1331 (2015).

65. Gao, Y. *et al.* Pathological Roles of Interleukin-22 in the Development of Recurrent Hepatitis C after Liver Transplantation. *PloS one* **11**, e0154419 (2016).

66. Colley, D.G., Bustinduy, A.L., Secor, W.E. & King, C.H. Human schistosomiasis. *The Lancet* **383**, 2253-2264 (2014).

67. Weerakoon, K.G., Gobert, G.N., Cai, P. & McManus, D.P. Advances in the Diagnosis of Human Schistosomiasis. *Clinical microbiology reviews* **28**, 939-967 (2015).

68. Andrade, Z.d.A. Schistosomiasis and liver fibrosis. *Parasite immunology* **31**, 656-663 (2009).

69. Gao, B. & Bataller, R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology* **141**, 1572-1585 (2011).

70. O'Shea, R.S., Dasarathy, S. & McCullough, A.J. Alcoholic liver disease. *Hepatology* **51**, 307-328 (2010).

71. Hong, F. *et al.* Interleukin 6 alleviates hepatic steatosis and ischemia/reperfusion injury in mice with fatty liver disease. *Hepatology* **40**, 933-941 (2004).

72. Kammüller, M.E. Recombinant human interleukin-6: safety issues of a pleiotropic growth factor. *Toxicology* **105**, 91-107 (1995).

73. Xing, W.-W. *et al.* Interleukin-22 protects against acute alcohol-induced hepatotoxicity in mice. *Bioscience, biotechnology, and biochemistry* **75**, 1290-1294 (2011).

74. Ahmed, A., Wong, R.J. & Harrison, S.A. Nonalcoholic fatty liver disease review: Diagnosis, treatment, and outcomes. *Clinical Gastroenterology and Hepatology* **13**, 2062-2070 (2015).

75. Marchesini, G. *et al.* Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* **37**, 917-923 (2003).

76. MA, X. & LI, Z. Pathogenesis of nonalcoholic steatohepatitis (NASH). *Chinese journal of digestive diseases* **7**, 7-11 (2006).

77. Yang, L. *et al.* Amelioration of high fat diet induced liver lipogenesis and hepatic steatosis by interleukin-22. *Journal of Hepatology* **53**, 339-347 (2010).

78. Rolla, S. *et al.* The balance between IL-17 and IL-22 produced by liver-infiltrating T-helper cells critically controls NASH development in mice. *Clinical Science* **130**, 193-203 (2016).

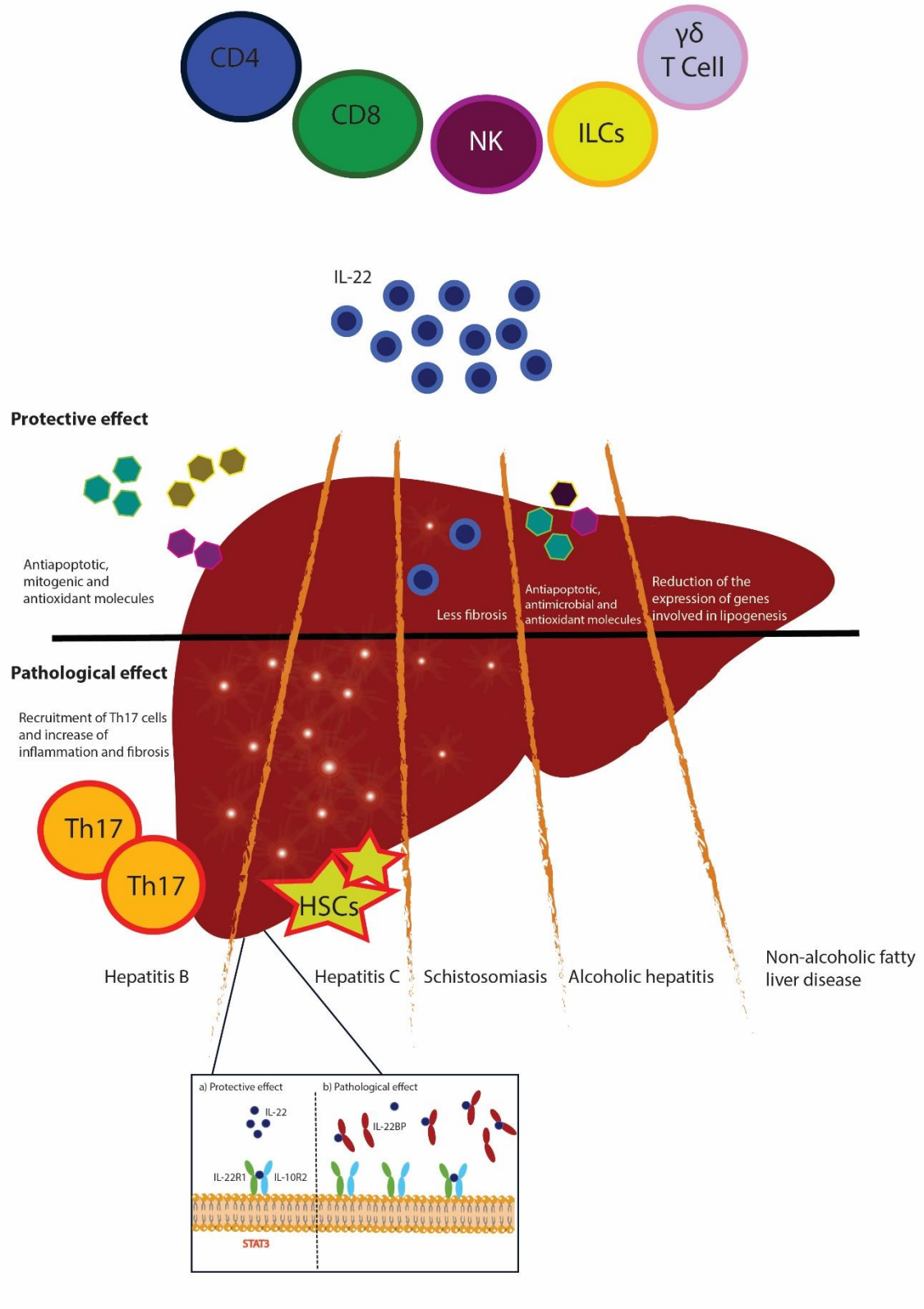
79. Poynard, T. *et al.* Prevalence of liver fibrosis and risk factors in a general population using non-invasive biomarkers (FibroTest). *BMC gastroenterology* **10**, 1 (2010).

80. Fagone, P. *et al.* Emerging therapeutic targets for the treatment of hepatic fibrosis. *Drug discovery today* (2015).

Figure legends

Figure 1. Protective and pathological effects of IL-22 under different environments. IL-22 may be expressed by different cells (CD4, CD8, NK, ILCs and γδ T lymphocytes). Its protective effect is mainly associated with the expression of antiapoptotic, mitogenic, antimicrobial and antioxidant molecules. Moreover, a reduction of the expression of genes involved in lipogenesis have been observed in NAFLD. On the other hand, IL-22 may also increase inflammation and fibrosis by recruiting Th17 cells in viral hepatitis. The balance between protective and pathological effects of IL-22 may be associated with variations in IL-22BP levels and other cytokines, such as IL-17, that in turn may be expressed differently in different diseases and disease stages.

Figure 1


CD4
CD8
NK
ILCs
γδ T Cell
IL-22
Protective effect
Antiapoptotic, mitogenic and antioxidant molecules
Less fibrosis
Antiapoptotic, antimicrobial and antioxidant molecules
Reduction of the expression of genes involved in lipogenesis
Pathological effect
Recruitment of Th17 cells and increase of inflammation and fibrosis
Th17
Th17
HSCs
Hepatitis B
Hepatitis C
Schistosomiasis
Alcoholic hepatitis
Non-alcoholic fatty liver disease
a) Protective effect
b) Pathological effect
IL-22
IL-22BP
IL-22R1
IL-10R2
STAT3

6. CONCLUSÕES

- O presente trabalho identificou SNPs em 4 genes (*IL22RA2, PTX3, PRSS58, SOCS5)*, associados com a progressão da doença hepática em pacientes com hepatite C crônica;
- Pacientes com HCV que possuem genótipos GG/GA e TT/TC no rs6570136 e rs2064501, respectivamente, possuem maiores chances em desenvolver formas graves de fibrose. Essas variantes, presentes no gene *IL22RA2*, foram associadas com níveis de expressão aumentados de IL-22BP, um inibidor solúvel da IL-22. É possível que o efeito protetor da IL-22 no tecido hepático seja inibido em indivíduos que possuem essas variantes;
- As variantes rs6570136 e rs2064501 do gene *IL22RA2* poderiam ser utilizados como marcadores genéticos de progressão da fibrose, permitindo que indivíduos com maior risco sejam identificados precocemente e possuam tratamento individualizado;
- A presença do genótipo AA no rs2305619 do gene *PTX3* foi associada com um risco de desenvolvimento de HCC quase duas vezes maior em pacientes com hepatite C. Esse genótipo foi associado a níveis plasmáticos aumentados de PTX3. Níveis elevados de PTX3 poderiam aumentar o risco de HCC através do aumento do processo inflamatório no fígado de pacientes infectados.
- São necessários mais estudos em outras populações avaliando o papel do PTX3 no desenvolvimento de HCC, para que ele possa então ser considerado um marcador de predisposição ao HCC;
- Variantes nos genes *PRSS58* e *SOCS5* foram encontrados exclusivamente em nove pacientes com HCC e ausentes em dez pacientes cirróticos sem HCC. Variantes nesses genes poderiam estar associados com uma maior susceptibilidade no desenvolvimento de HCC;

7. PERSPECTIVAS

- O presente estudo foi o primeiro na literatura a associar variantes nos genes *IL22RA2, PTX3, PRSS58 e SOCS5* com a progressão da doença hepática em pacientes com hepatite C crônica. Sendo assim, este estudo abre novo caminhos para a avaliação do papel dessas moléculas na progressão da fibrose hepática e desenvolvimento de HCC;
- Pretende-se ampliar este estudo em outras populações para a confirmação de nossos achados;
- Nossos achados abrem caminhos para o estudo de outras variantes nesses genes, que também poderiam estar associados com a gravidade da doença hepática em pacientes com HCV;
- Estudos em modelos experimentais de fibrose hepática e HCC em camundongos *knockouts* seria uma abordagem interessante para a compreensão do papel dessas moléculas na fisiopatologia da doença hepática;
- O estudo genético da *IL22RA2, PTX3, PRSS58 e SOCS5* também poderia ser estendido para outras doenças no fígado, como hepatite B, NASH, hepatite alcoólica, etc.
- O desenvolvimento de fármacos que atuem na modulação da IL-22BP, poderia ser uma estratégia eficiente no tratamento da fibrose hepática;
- O PRSS58 e SOCS5 são potenciais candidatos para futuros estudos avaliando a influência dessas moléculas no desenvolvimento de HCC;

APÊNDICE A

Questionário utilizado durante entrevista dos pacientes

Investigação sobre a relação de gravidade da doença hepática com polimorfismos genéticos			
Data (coleta): ___ ___ / ___ ___ / ___ ___ ___ ___		Prontuário HUOC:	IFP:
Nome completo:			
Estado atual de tratamento: 1. SEM TRATAMENTO 2. EM TRATAMENTO 3.TRATADO (Follow-Up)			
Idade (momento da biópsia) ___ ___		Qual a data do seu nascimento.	___ ___ / ___ ___ / ___ ___ ___ ___
Sexo: 1. Masc. 2. Fem. :		Cor de pele: 1. Branca 2. Parda 3. Negro 4.Indio 5. Outro :	
Cidade/Estado (mora):		Cidade/Estado (origem):	
Telefone convencional para contato:		Celular:	
Antecedentes: 1. Hemotransfusão Ano:_____ 3. Medicação por seringa de vidro 5.Cirurgias 2. Drogas Injetáveis 4. Tatuagens			
Consumo de álcool (antes de descobrir a doença): 1. Sim 2. Não Homem Mulher ( ) Maior que 40 g/dia ( ) Maior que 20 g/dia ( ) Menor que 40 g/dia ( ) Menor que 20 g/dia			
Tabagismo: 1. Nunca fumou 2. Ex-fumante 3. Fumou até 1 ano antes da pesquisa 4. Fumante			
Investigação clínica.			
Além da Hepatite C o (a) senhor (a) tem ou teve alguma outra doença. 1. Sim 2. Não 9. Não sabe			
Se sim, qual a outra doença que o (a) senhor (a) tem ou teve. 1. Hipertensão 2. Diabetes 3. Esquistossomose 4. Outra. Especifique _____			
Exame físico			
Peso.____ ____, ____ ____ ____ (quilo, gramas)		51. Altura. _____, _____ _____ (metro, centímetro)	
Índice de BM [peso/ (altura/m2)]			
Circunferência abdominal + quadril (cm):		P.A:	
Exames Laboratoriais			
Glicemia: TG: HDL: Albumina: Bilirrubina: CT: LDL: Insulina:			
Síndrome Metabólica (PA; Glic.; Cintura abdm; TG; HDL): 1. Sim 2.Não			
AST: ALT: Alfa-fetoproteína: gGT: FA: Plaquetas:			
Genótipo viral: **Carga viral:**			
Histopatológico			
Grau de fibrose (METAVIR) 1. F1 2. F2 3. F3 4. F4 5.HCC Data:____/____/_____		1. F1 2. F2 3. F3 4. F4 5.HCC Data:____/____/_____	
Grau de inflamação 1. A0 2. A1 3. A2 4. A3 5.A4		1. A0 2. A1 3. A2 4. A3 5.A4	
Grau de esteatose 1. Leve 2. Moderada 3. Intensa 4. Esteatohepatite (NASH)		1. Leve 2. Moderada 3. Intensa 4. Esteatohepatite (NASH)	
Resposta ao tratamento			
Week 4:	POS	NEG	
Week 12:	POS	NEG	
Week 24:	POS	NEG	
Week 48:	POS	NEG	
6 meses pós-tto:	POS	NEG	

ANEXO A

Artigo 1

JOURNAL OF VIRAL HEPATITIS JVH

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Genetic variation in *PTX3* and plasma levels associated with hepatocellular carcinoma in patients with HCV

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SUMMARY. Hepatitis C virus (HCV) is the main cause of chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC) worldwide. The risk to develop HCC increases with the severity of liver inflammation and fibrosis. Long pentraxin 3 (PTX3) is a soluble pattern-recognition receptor produced by phagocytes and nonimmune cells at sites of inflammation or injury. The aim of the present study was to determine the association of PTX3 polymorphisms and its plasma levels with HCC occurrence among patients with HCV. Samples from 524 patients with chronic hepatitis C were evaluated in this study. Two polymorphisms (rs1840680 and rs2305619) in the PTX3 gene were determined by real-time PCR. PTX3 plasma levels were measured by Enzyme-linked Immunosorbent Assay (ELISA). Our data show a significant association between PTX3 polymorphisms and HCC occurrence in univariate and multivariate analysis ($P = 0.024$). Patients with HCC had higher PTX3 plasma levels compared to individuals with mild or severe fibrosis ($P < 0.0001$ and $P = 0.002$, respectively). In addition, PTX3 rs2305619 polymorphism and plasma levels were correlated with Child-Pugh scores B and C in HCC individuals. PTX3 seems to be a risk factor for HCC occurrence in chronic hepatitis C. This is the first study that evaluates PTX3 in the context of hepatitis C.

Keywords: fibrosis, hepatitis C, hepatitis C virus, hepatocellular carcinoma, polymorphism, pentraxin 3.

INTRODUCTION

It is estimated that 3% of the world's population is infected with hepatitis C virus (HCV), representing 170 million people. In these patients, the risk of hepatocellular carcinoma (HCC) gradually increases as liver fibrosis progresses. Once cirrhosis is established, the annual incidence of HCC is extremely high (1–7% per year), although HCC rarely develops in less fibrotic livers [1,2].

The risk to develop HCC increases with the severity of liver inflammation and fibrosis. The hepatic inflammation caused by HCV involves various mechanisms that include the regulatory immune response of the host, mediated by cytokines that play important roles against viral infections and viral polypeptides, which interact with cells involved in innate and adaptive immunity [3–6].

Long pentraxin 3 (PTX3) is a soluble pattern-recognition receptor produced by phagocytes and nonimmune cells at sites of inflammation or injury. PTX3 plays multiple roles in innate immunity and inflammation, being able to activate and regulate the complement system (via C1q and factor H), interact with microbial moieties (outer membrane protein A of *K. pneumonia* and viral haemagglutinin) and regulate inflammation [7–9].

PTX3 gene, located on human chromosome 3, is organized in three exons and two introns. Two single nucleotide polymorphisms (SNPs) in *PTX3* (rs1840680 and rs2305619) have demonstrated functional significance. The A allele of both SNPs has been associated with higher PTX3 plasma levels [10,11]. In addition, the A allele of the rs1840680 and rs2305619 have been associated with susceptibility to *Mycobacterium tuberculosis* and *Pseudomonas*

Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IL-1β, interleukin-1 beta; IL-6, interleukin-6; MIF, macrophage migration inhibitory factor; OR, odds ratio; PTX, pentraxin; SNPs, single nucleotide polymorphisms; TNF-α, tumour necrosis factor-alpha.

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ANEXO B

Artigo 2

HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

IL-22 and IL-22 Binding Protein (IL-22BP) Regulate Fibrosis and Cirrhosis in Hepatitis C Virus and Schistosome Infections

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Interleukin (IL)-22 acts on epithelia, hepatocytes, and pancreatic cells and stimulates innate immunity, tissue protection, and repair. IL-22 may also cause inflammation and abnormal cell proliferation. The binding of IL-22 to its receptor is competed by IL-22 binding protein (IL-22BP), which may limit the deleterious effects of IL-22. The role of IL-22 and IL-22BP in chronic liver diseases is unknown. We addressed this question in individuals chronically infected with schistosomes or hepatitis C virus (HCV). We first demonstrate that schistosome eggs stimulate production of IL-22 transcripts and inhibit accumulation of IL22-BP transcripts in schistosome-infected mice, and that schistosome eggs selectively stimulate production of IL-22 in cultures of blood leukocytes from individuals chronically infected with *Schistosoma japonicum*. High IL-22 levels in cultures correlated with protection against hepatic fibrosis and portal hypertension. To test further the implication of IL-22/IL-22BP in hepatic disease, we analyzed common genetic variants of IL22RA2, which encodes IL-22BP, and found that the genotypes, AA, GG of rs6570136 ($P = 0.003$; odds ratio [OR] = 2), and CC, TT of rs2064501 ($P = 0.01$; OR = 2), were associated with severe fibrosis in Chinese infected with *S. japonicum*. We confirmed this result in Sudanese (rs6570136 GG [$P = 0.0007$; OR = 8.2], rs2064501 TT [$P = 0.02$; OR = 3.1]), and Brazilians (rs6570136 GG [$P = 0.003$; OR = 26], rs2064501 TC, TT ($P = 0.03$; OR = 11]) infected with *S. mansoni*. The aggravating genotypes were associated with high IL22RA2 transcripts levels. Furthermore, these same variants were also associated with HCV-induced fibrosis and cirrhosis (rs6570136 GG, GA [$P = 0.007$; OR = 1.7], rs2064501 TT, TC ($P = 0.004$; OR = 2.4]). *Conclusions*: These results provide strong evidence that IL-22 protects against and IL-22BP aggravates liver fibrosis and cirrhosis in humans with chronic liver infections. Thus, pharmacological modulation of IL-22 BP may be an effective strategy to limit cirrhosis. (HEPATOLOGY 2015;61:1321-1331)

Abbreviations: CentF, central hepatic fibrosis; CTGF, connective tissue growth factor; HBV, hepatitis B virus; HCV, hepatitis C virus; HF, hepatic fibrosis; IL, interleukin; IL-22BP, IL-22 binding protein; ILCs, innate lymphoid cells; NetF, network fibrosis; LD, linkage disequilibrium; LPS, lipopolysaccharide; mRNA, messenger RNA; OR, odds ratio; PBMCs, peripheral blood mononuclear cells; PH, portal hypertension; SNPs, single-nucleotide polymorphisms; Th, T helper.

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**Drs. Abdelwahed, Hamdoun, and Abdelmaboud contributed to this work, but did not provide a signed author agreement.*

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Additional Supporting Information may be found at http://onlinelibrary.wiley.com/doi/10.1002/hep.27629/suppinfo.

ANEXO C

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título da Pesquisa: “Avaliação de polimorfismos genéticos na doença hepática crônica causada pelo vírus da hepatite C”

Nome do (a) Pesquisador (a): Rodrigo Feliciano do Carmo

1. Natureza da pesquisa: o sr. (sra.) está sendo convidado (a) a participar desta pesquisa que tem como finalidade investigar a relação de polimorfismos genéticos com a gravidade da doença hepática em pacientes com hepatite C.
2. Justificativa: Ainda é desconhecido o porquê de alguns pacientes com hepatite C desenvolverem formas graves da doença de forma mais rápida que outros pacientes. O presente estudo ajudará a uma melhor compreensão de fatores genéticos agravantes, podendo fornecer subsídios para um melhor manejo dos pacientes infectados pelo vírus da hepatite C.
3. Objetivos: O objetivo do presente estudo é avaliar a presença de mutações genéticas relacionadas com a gravidade da doença hepática em pacientes com hepatite C.
4. Procedimentos metodológicos: O estudo genético para a determinação das mutações associadas com a gravidade da doença será realizado através da análise do DNA, obtida através do sangue de pacientes com formas graves e leves da doença causada pelo vírus da hepatite C. Após obtenção das amostras serão comparados os dois grupos em busca da presença de fatores envolvidos com a gravidade da doença hepática.
5. Participantes da pesquisa: Serão recrutados 250 pacientes com hepatite C para o desenvolvimento deste estudo.
6. Envolvimento na pesquisa: ao participar deste estudo a sra (sr) permitirá que o (a) pesquisador (a) retire uma amostra de 10 ml de sangue da sua veia, por profissionais durante o seu atendimento. Também será obtida uma pequena parte do fragmento do fígado (aprox. 0,5 cm) que é retirado para o exame histopatológico de rotina requisitado pelo médico para orientar o tratamento do paciente. Este procedimento é obrigatório para que o paciente seja avaliado quanto sua necessidade de tratamento, dessa forma não será realizada uma biopsia especificamente para esse estudo. Algumas perguntas serão feitas ao senhor (a), relacionadas ao seu tratamento, através de um questionário. A sra (sr.) tem liberdade de se recusar a participar e ainda se recusar a continuar participando em qualquer fase da pesquisa, sem qualquer prejuízo para a sra (sr.). Sempre que quiser poderá pedir mais informações sobre a pesquisa através do telefone do (a) pesquisador (a) do projeto e, se necessário através do telefone do Comitê de Ética em Pesquisa.
7. Sobre as entrevistas: o pesquisador fará algumas perguntas relacionadas ao seu tratamento através de um questionário previamente elaborado para o estudo.
8. Riscos e desconforto: a participação nesta pesquisa não infringe as normas legais e éticas. A realização da biópsia oferece riscos inerentes ao procedimento, e será realizado por um médico habilitado. A região da punção poderá ficar dolorida durante as primeiras 24 horas após o procedimento. Também será retirada uma única coleta de 10 ml de sangue por punção venosa, por profissionais habilitados e treinados para tal, entretanto um risco de maior gravidade seria a não coleta por dificuldade em puncionar a veia e esse fato pode representar um pequeno risco de ocasionar uma pequena mancha roxa no local da punção, mesmo que esse acontecimento seja muito raro de acontecer. Caso você venha a sentir algo dentro desses padrões, comunicar imediatamente ao pesquisador para que sejam tomadas as devidas providências através do profissional de enfermagem presente no local. Os procedimentos adotados nesta pesquisa obedecem aos Critérios da Ética em Pesquisa com Seres Humanos conforme Resolução no. 466/2012 do Conselho Nacional de Saúde. Nenhum dos procedimentos usados oferece riscos à sua dignidade.

9. Confidencialidade: todas as informações coletadas neste estudo são estritamente confidenciais. Somente o (a) pesquisador (a) e seu (sua) orientador (a) (e/ou equipe de pesquisa) terão conhecimento de sua identidade e nos comprometemos a mantê-la em sigilo ao publicar os resultados dessa pesquisa.
10. Benefícios: ao participar desta pesquisa a sra (sr.) não terá nenhum benefício direto. Entretanto, esperamos que este estudo traga informações importantes sobre a influência de mudanças genéticas na gravidade da doença hepática causada pelo vírus da hepatite C, de forma que o conhecimento que será construído a partir desta pesquisa possa fornecer subsídios para o desenho de estratégias terapêuticas empregando as moléculas estudadas e fornecer subsídios para estabelecer o melhor manejo clínico dos pacientes com hepatite C, onde o pesquisador se compromete a divulgar os resultados obtidos, respeitando-se o sigilo das informações coletadas, conforme previsto no item anterior.
11. Pagamento: a sra (sr.) não terá nenhum tipo de despesa para participar desta pesquisa, bem como nada será pago por sua participação.

Após estes esclarecimentos, solicitamos o seu consentimento de forma livre para participar desta pesquisa. Portanto preencha, por favor, os itens que se seguem: Confiro que recebi cópia deste termo de consentimento, e autorizo a execução do trabalho de pesquisa e a divulgação dos dados obtidos neste estudo.

Obs: Não assine esse termo se ainda tiver dúvida a respeito.

Consentimento Livre e Esclarecido

Tendo em vista os itens acima apresentados, eu, de forma livre e esclarecida, manifesto meu consentimento em participar da pesquisa

_____
Nome do Participante da Pesquisa

_____
Assinatura do Participante da Pesquisa

_____
Assinatura do Pesquisador

_____
Assinatura do Orientador

Pesquisador: Rodrigo Feliciano do Carmo. Contato: (87) 2101-6862
Coordenador do Comitê de Ética em Pesquisa: Professor Alexandre H. Reis
Vice-Coordenadora: Márcia Bento Moreira
Telefone do Comitê: 87 21016896
E-mail cedep@univasf.edu.br

ANEXO D

Folha de aprovação do CEP

UNIVERSIDADE FEDERAL DO VALE DO SÃO FRANCISCO - UNIVASF
COMITÊ DE ÉTICA E DEONTOLOGIA EM ESTUDOS E PESQUISAS – CEDEP
COMISSÃO DE ÉTICA E PESQUISA - CEP

Prezado pesquisador,

É com satisfação que informamos formalmente ao Vº. Sr. que o projeto "**Avaliação de polimorfismos genéticos na doença hepática crônica causada pelo vírus da hepatite C**" foi aprovado pelo Comitê de Ética e Deontologia em Estudos e Pesquisas – (CEDEP) em reunião realizada no dia 19 de setembro de 2013. A partir de agora, portanto, o vosso projeto pode dar início à fase prática ou experimental. Informamos ainda que no prazo máximo de 1 (um) ano a contar dessa data deverá ser enviado a esse Comitê um relatório sucinto sobre o andamento da presente pesquisa. Informamos que para efeito de publicação, o presente projeto encontra-se registrado sob o nº 0005/190913 CEDEP/UNIVASF.

Pesquisador responsável: **Rodrigo Feliciano do Carmo**

Data da entrada: **21/08/2013**

Petrolina-PE, 23 de outubro de 2013.

(Alexandre H. Reis)
Coordenador CEDEP/UNIVASF

Av. José de Sá Maniçoba, s/n – Centro – Petrolina - PE
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