Cytokines Related to Hepatocellular Carcinoma

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Abstract

Irrespective of the different etiologies, unresolved chronic inflammation is a common denominator and a feature present in more than 90% of patients with HCC. Local activation of cell populations upon sensing pathogens and/or tissue damage in the liver may trigger a tightly regulated and coordinated multi-step process, followed by immune cell infiltration, and subsequent engagement in tissue repair as the ultimate goal. It is in this fine orchestration of events that the release of a wide array of soluble factors, such as cytokines, takes place. In this regard, cytokines have been investigated as potential biomarkers to predict different stages of HCC, and to further understand mechanisms of HCC formation. In the presence of HCC-promoting risk factors, the initial inflammatory response in the liver is unresolved, and as a result, the unbalanced expression of cytokines promotes a persistent healing response. This response may lead to sequential development of fibrosis, cirrhosis, and eventually HCC by enhancing hepatocyte proliferation and regeneration which can lead to mutagenesis and set the stage for HCC development. Once HCC is established, cytokines released by the tumor, neighboring non-tumor cells, or immune cells can act on the malignant lesion to promote tumor survival by multiple mechanisms. Since cytokines are present throughout the different stages of HCC progression, their evaluation may provide insightful information on HCC detection and management. The ability to detect cytokines in sera and/or plasma could potentially serve as biomarkers to increase early HCC detection rates which would improve disease outcome as well as be used as prognostic factors in response to therapies

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Introduction:

Liver cancer is a leading cause of cancer-related death worldwide with approximately 800,000 deaths per year, with hepatocellular carcinoma (HCC) representing the great majority of primary liver cancers. Epidemiological data have shown marked differences in HCC incidence among different ethnic-racial groups, genders, and across geographic regions of the globe, partially dictated by different risk factors. Among the main risk factors are infection with the hepatitis B virus (HBV) or hepatitis C virus (HCV) and alcohol use. ***(Rico et al., 2021)***

Irrespective of the different etiologies, unresolved chronic inflammation is a common denominator and a feature present in more than 90% of patients with HCC. Local activation of cell populations upon sensing pathogens and/or tissue damage in the liver may trigger a tightly regulated and coordinated multi-step process, followed by immune cell infiltration, and subsequent engagement in tissue repair as the ultimate goal. It is in this fine orchestration of events that the release of a wide array of soluble factors, such as cytokines, takes place. ***(El–Serag, 2007)***

In this regard, cytokines have been investigated as potential biomarkers to predict different stages of HCC, and to further understand mechanisms of HCC formation. In the presence of HCC-promoting risk factors, the initial inflammatory response in the liver is unresolved, and as a result, the unbalanced expression of cytokines promotes a persistent healing response. ***(Debes et al., 2018)***

This response may lead to sequential development of fibrosis, cirrhosis, and eventually HCC by enhancing hepatocyte proliferation and regeneration which can lead to mutagenesis and set the stage for HCC development. Once HCC is established, cytokines released by the tumor, neighboring non-tumor cells, or immune cells can act on the malignant lesion to promote tumor survival by multiple mechanisms. ***(Fu et al., 2019)***

In addition, these cytokines can act on the tumor microenvironment to induce immune escape and metastasis. Interestingly, as the treatment of advanced HCC has evolved from no reasonable therapy to tyrosine kinase inhibitors that significantly prolong survival to immune therapy, cytokines can act as markers of response to therapy. ***(Perera, 2020)***

Since cytokines are present throughout the different stages of HCC progression, their evaluation may provide insightful information on HCC detection and management. The ability to detect cytokines in sera and/or plasma could potentially serve as biomarkers to increase early HCC detection rates which would improve disease outcome as well as be used as prognostic factors in response to therapies. ***(Parikh et al., 2020)***

It is important to highlight, however, that certain cytokines—although involved in a common carcinogenic program, such as angiogenesis—might more accurately depict a given stage in HCC progression than others, and that cytokines with prognostic potential in one stage may not be relevant in another. ***(Rico et al., 2021)***

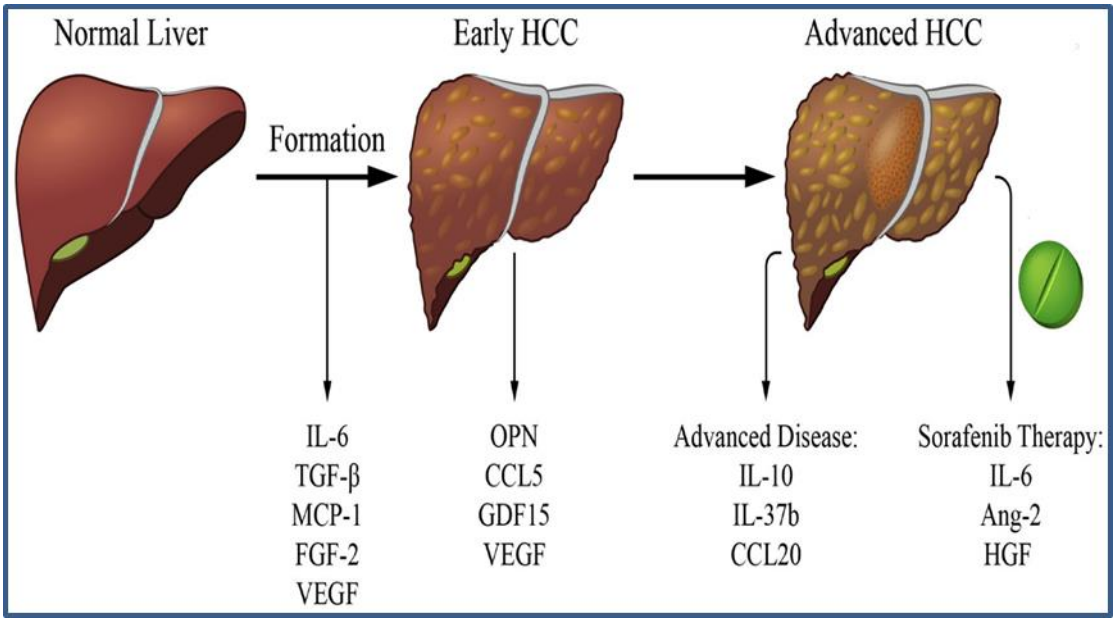

Figure (1): Cytokines of clinical relevance in the different stages of liver cancer. ***(Rico et al., 2021)***

Cytokines Related to HCC Formation:

Due to its physiologic role and anatomic location the liver is exposed to chronic infections and environmental insults resulting in an unresolved inflammation state that may lead to HCC. It is in this setting that the presence of pro-inflammatory cytokines in peritumoral tissues contributes to tumor formation as well as progression. Most of these cytokines participate in carcinogenesis by inducing cell survival and proliferation, epithelial mesenchymal transition (EMT), and angiogenesis. ***(Debes et al., 2018)***

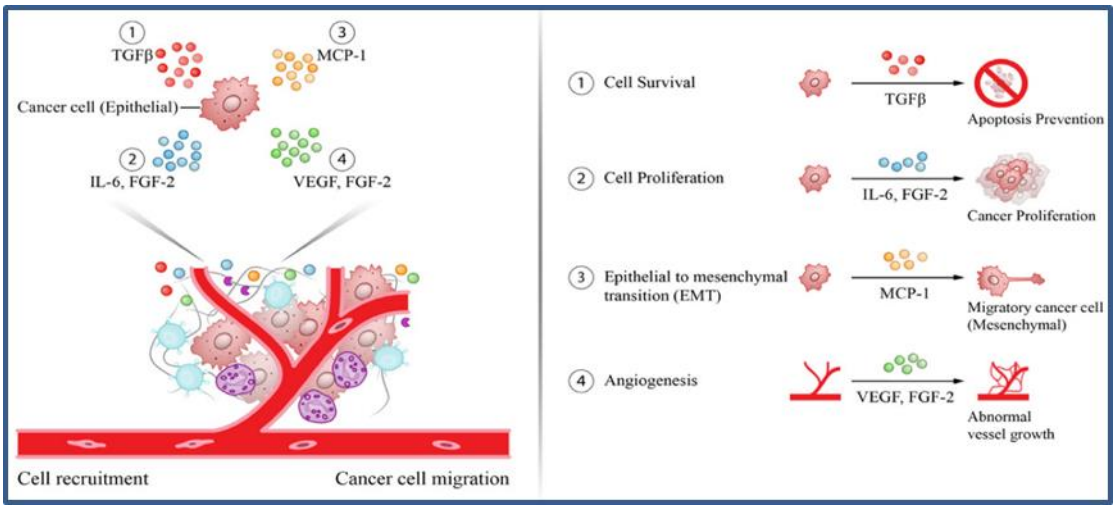

Figure (2): Pro-carcinogenic cytokines in the tumor microenvironment involved in HCC formation. ***(Rico et al., 2021)***

Interleukin-6 (IL-6):

One of the cytokines most frequently examined in many studies with respect to the early stages of cancer formation is IL-6. This pro-inflammatory cytokine has a critical role in host defense and in the orchestration of inflammation leading to cancer. In HCC, the constant exposure to triggering insults in the liver (i.e., during chronic viral hepatitis infection, or alcohol use) leads to a chronic inflammatory state that eventually promotes cancer formation. In human studies, increased levels of serum IL-6 in HCC patients -compared to chronic hepatitis and cirrhosis patients- have been consistently shown. ***(Shakiba, 2018)***

Furthermore, among HCC patients, IL-6 levels have been found to be increased in advanced vs. early stages of HCC supporting the conception of IL-6 as an important cytokine in hepatocarcinogenesis. Moreover, it has been shown that elevated serum IL-6 levels in HCC patients who undergo hepatectomy are associated with lower overall survival and experience early HCC recurrence. ***(Lai et al., 2019)***

In vivo experiments performed in a diethylnitrosamine (DEN) HCC mouse model with hepatocyte-specific knockout of the IL-6 receptor gp130 have demonstrated a reduced number of liver tumor nodules and macrophages compared to their control counterparts, supporting a role for IL-6 in HCC formation. ***(Hatting et al., 2015)***

Moreover, Kupffer cells, the macrophages of the liver, can act as source of IL-6 upon stimulation with the microbial product lipopolysaccharide, which supports pre-malignant hepatocyte proliferation under DEN-induced carcinogenesis. Interestingly, estrogen-mediated inhibition of IL-6 production by activated Kupffer cells reduced chemical hepatocarcinogenesis in DEN-HCC mice and has been proposed as a mechanism behind sex disparities in HCC. ***(Naugler et al., 2007)***

Similarly, IL-6 blockade in multidrug resistance 2 knockout mice showed decreased liver carcinogenesis. This effect likely occurred due to a decrease in hepatocytes harboring genomic instability, instated by a genotoxic environment, which reinforces a role of IL-6 in promoting survival of pre-malignant hepatocytes. ***(Rico et al., 2021)***

Furthermore, mice models have shown the immune-suppression role of IL-6 by inducing PD-L1 expression on tumor-associated macrophages, which are associated with immune-evasion. Lastly, studies in mouse HCC models have demonstrated that isolated HCC progenitor cells can give rise to cancer when there is ongoing liver damage, and that these cells promote their own growth and progress towards malignancy via autocrine IL-6 signaling. ***(He et al., 2013)***

Transforming Growth Factor Beta (TGF-β):

The cytokine TGF-β regulates many inflammatory processes, which generally lead to inhibition of cellular processes, such as proliferation, differentiation, and survival. Since the TGF-β receptors (TGF-βR) are broadly expressed, TGF-β can act on virtually all cells. The TGF-βR heterodimer

consists of 2 chains which upon triggering, activates SMAD-dependent signal transduction cascades to induce gene expression of the target genes. ***(Debes et al., 2018)***

During carcinogenesis, malignant cells can often blunt their suppressive TGF-β signaling by altering the expression of its receptors, but also hijack the signaling cascade to inactivate growth-inhibitory functions. In HCC, mutations have been described in the TGFBRII poly(A) region of the gene, which were found to encode for non-active receptors. ***(Cheng et al., 2019)***

Moreover, HCC cell lines with metastatic potential have been described to downregulate TGF-βR2. Interestingly, reduced TGF-βR2 expression in HCC tissues was found to correlate with larger tumor size and various metastatic features, such as poor differentiation, portal vein invasion and intrahepatic metastasis. ***(Cheng et al., 2019)***

In addition, mutations in SMAD2 and SMAD4 genes have been observed in HCC which can result in cell cycle progression via disruption of cyclin inhibitors, such as p15INK4b and p21CIP1. Furthermore, methylation of the cyclin inhibitors p16INK4a and p15INK4b is an event found in early stages of HCC as well as in cirrhotic patients, although at a smaller rate, suggesting that these epigenetic modifications play a role in certain aspects of hepatocarcinogenesis. ***(Rico et al., 2021)***

Interestingly, non-canonical SMAD-independent signal transduction via TAK1—also known as mitogen-activated protein kinase 7—can activate p38 and JNK kinases, which are known to participate in HCC. Upon JNK activation, a non-canonical SMAD3 isoform (pSmad3L) becomes active, resulting in silencing of signals of cell cycle arrest and augmented cell proliferation. ***(Matsuzaki et al., 2007)***

In contrast, JNK inhibition has been shown to reduce HCC tumors. Interestingly, immunostaining of oncogenic JNK signaling molecules in livers of chronic HBV patients was found to be increased during progression from cirrhosis to HCC. Similar results were found in HCV-induced HCC livers as fibrotic and necro-inflammatory grades progressed. ***(Matsuzaki et al., 2007)***

Moreover, TGF-β signaling has been shown to induced surface tumor associated markers (i.e., CD133 and CD90) in liver progenitor cells which coffered them tumor intrinsic cell properties such as, increased self-renewal potential and greater chemoresistance potential. A proposed mechanism for the increased chemoresistance potential was recently proposed where TGF-β induced the expression drug-efflux transporters via the induction of the xenobiotic nuclear receptor, PXR. ***(Debes et al., 2018)***

<u>Monocyte Chemoattractant Protein 1 (MCP-1):</u>

Produced by parenchymal and non-parenchymal liver cells upon tissue injury, MCP-1 acts as a potent chemoattractant of immune cells by interacting with the CC chemokine receptor 2

(CCR2). In HCC mouse models increase in MCP-1 expression plays a pivotal role in the recruitment of monocyte-derived macrophages. ***(Kapanadze et al., 2013)***

In the tumor microenvironment, these cells can support dysplastic lesions by promoting angiogenesis and cancer cell proliferation by the release of metalloproteinases (MMPs) and cytokines such as IL-6 and TGF-ß. In addition, these macrophages also suppress effective anti-tumor immune responses by limiting antigen presentation and inducing immunotolerance in favor of the tumor. ***(Shih et al., 2015)***

Further illustrating the relevance of MCP-1 in relation to macrophages, it was shown that CCR2 antagonists inhibit HCC growth. This outcome was accompanied by a reduction of recruited pro-tumorigenic monocytes and an increase of anti-tumor cytotoxic CD8 T cells. In line with this, human HCC livers with increased MCP-1 expression show a higher number of macrophages and reduced CD8 T cell numbers in the tumor. ***(Kapanadze et al., 2013)***

On the other hand, laboratory assays have shown MCP-1 to promote migration and invasion in hepatoma-lines (i.e., Huh7 and Hep3B) by downstream activation of activating protein-1 (AP-1) which in turn induces the onco-microRNA miR-21 promoting cancer cell migration and invasion. ***(Shih et al., 2015)***

MCP-1-stimulated HCC cell lines also showed an EMT phenotype which encompassed morphological changes with increased expression of stem markers (i.e., N-cadherin, vimentin) and enhanced metastatic potential when transplanted into nude mice. Interestingly, human data on MCP-1 have shown an increase in the number of MCP-1—expressing endothelial progenitor cells—associated with advanced HCC stages and have been hypothesized to promote neo-vascularization by promoting angiogenesis via release of pro-angiogenic cytokines. ***(Shih et al., 2015)***

<u>Vascular Endothelial Growth Factor (VEGF):</u>

The role of VEGF as an angiogenic and tumorigenesis factor has been known for almost three decades and has been extensively reviewed elsewhere. Under normal liver homeostasis, VEGF is predominantly expressed by hepatic stellate cells and myofibroblast at low levels. ***(Fernandez et al., 2009)***

In contrast, during HCC formation and progression, VEGF expression by these cells in human livers is increased. Oxidative stress, hypoxia, and nutrient deprivation are hallmarks of tumor formation and have been shown to stimulate VEGF expression. Interestingly, malignant hepatocytes in human HCC tumors have been shown to expressed higher cytoplasmatic VEGF levels than non-malignant hepatocytes located in cirrhotic areas. ***(Rico et al., 2021)***

As an angiogenic factor, VEGF induces new vessel formation, which can act as new ports for the recruitment of inflammatory cells, inducing further inflammation. In addition, new vessels may

act as exit windows for tumor cells to gain access to the circulation to metastasize. Interestingly, the lack of well-defined vessel architecture can offer sub-optimal oxygen and nutrient supply, which may select for more aggressive forms of tumors, while increasing hepatocyte damage and hypoxia. ***(Fernandez et al., 2009)***

All of these factors play a critical role in hepatocarcinogenesis. As a liver nodule transitions to a tumor, the so-called "portal triad" becomes less frequent and "unpaired arteries" become the norm. It is in this setting that VEGF promotes HCC neovascularization. ***(Fernandez et al., 2009)***

Fibroblast Growth Factor 2 (FGF-2):

FGF-2 has been shown to be expressed in human tumors since the late 80s and early in vitro work on hepatoma cell lines demonstrated that almost all cells express FGF-2 at the mRNA level. Importantly, exogenous FGF-2 can induce cell proliferation rendering this cytokine an attractive target in HCC therapy. FGF-2 neutralization with monoclonal antibodies in HCC xenograft mouse models has demonstrated reduced tumor growth. ***(Debes et al., 2018)***

FGF-2's mode of action is not limited to cell proliferation but has also been indirectly linked to tumor angiogenesis. Interestingly, T-cadherin expression is often observed in intra-tumoral capillary endothelial cells in HCC tissues, but not in liver normal tissues. Moreover, serum FGF-2 levels are increased during progression of chronic liver disease and correlate with large tumors (>5 cm), with the presence of venous invasion and with advanced TNM stage, suggesting a role for FGF-2 in HCC angiogenesis progression. ***(Cheng et al., 2019)***

Cytokines Linked to Early Detection of HCC:

Early detection of HCC remains the best tool in HCC management as curative treatment at this stage achieves the highest survival rates of patients. However, ultrasound surveillance for HCC detection—the standard approach for patients at risk—estimates a pooled 45% sensitivity for early HCC detection. An attractive option to replace ultrasound, is the use of blood biomarkers as they are easily quantifiable and interpretable through standardized assays. ***(Tzartzeva et al., 2018)***

Osteopontin (OPN):

OPN has been examined as an early HCC marker by many research groups. OPN is highly expressed at sites of inflammation and tissue remodeling and can be produced by Kupffer cells, hepatic stellate cells, and hepatocytes. This cytokine mediates a wide array of biological functions in the immune and vascular system and has been studied extensively in numerous cancers. ***(Zhao et al., 2018)***

Increased serum and plasma levels of OPN in individuals with HCC compared to those with liver cirrhosis or chronic liver disease controls have been reported in several studies. Despite promising results for HCC vs. non-HCC, the specific diagnostic efficacy of OPN in detecting early-stage HCC from non-HCC patients varies considerably depending on the study. ***(Zhao et al., 2018)***

CC Chemokine Ligand 5 (CCL5):

CCL5 is a chemoattractant of memory T cells and other immune cell types, which has been shown to be critical in controlling chronic viral infections. CCL5 has also been shown to be associated with liver inflammation in the setting of chronic HCV and HBV as well. ***(Chen et al., 2020)***

Growth Differentiation Factor 15 (GDF15):

A divergent member of the TGF-β superfamily, GDF15, is rarely detected under homeostatic conditions, except in human placenta where it is abundant. Increased levels of this marker are observed in pathological conditions such as inflammation, ischemia, and some forms of cancer. ***(Liu et al., 2015)***

In the context of HCC, comparison of serum GDF15 levels in a Chinese cohort of 223 HCC cases, predominantly due to viral hepatitis, showed elevated levels in sera of HCC patients as compared to HBV/HCV controls. Importantly, although serum GDF15 levels were increased in HCC patients compared to chronic HBV and HCV, no statistical differences were found between HCC and cirrhotic patients. ***(Liu et al., 2015)***

Vascular Endothelial Growth Factor (VEGF):

Besides its role as a potent angiogenic factor for vascular endothelial cells during HCC formation, as described above, VEGF has also been studied as a potential biomarker for HCC detection. ***(Kaseb et al., 2009)***

Cytokines Related to Advanced HCC:

The definition of advanced disease in HCC could be evaluated by a variety of factors. Of these, the BCLC staging is endorsed by the major liver disease societies and has been well validated. The BCLC staging system denotes stage C as advanced stage and stage D as terminal stage. Multiple cytokines and stimulatory molecules are associated with the risk for advanced disease in patients with HCC. ***(Rico et al., 2021)***

Interleukin-10 (IL-10):

IL-10 is a potent anti-inflammatory cytokine. Produced by most activated immune cells, including monocytes and macrophages, IL-10 acts by reducing the production of inflammatory mediators, inhibiting antigen presentation, and suppressing numerous other immune parameters. ***(Debes et al., 2018)***

Its role in viral infections is well documented, but its role in HCC is less clearly understood. A meta-analysis showed that IL-10 levels in HCC patients are increased compared to cirrhotic patients and healthy controls, but not to patients with viral hepatitis, thereby adding complexity to the interpretation of IL-10 data for HCC. ***(Shakiba, 2018)***

Interleukin-37b (IL-37b):

IL-37b is the largest of the five different isoforms of IL-37. This cytokine is secreted by monocytes, macrophages, and epithelial cells, and suppresses proinflammatory cytokine production and block EMT via downregulation of IL-6/STAT3 signaling. Moreover, in vivo experiments with recombinant IL-37b in mice showed lower tumor volume than in untreated controls. In many studies conducted in HBV-related HCC patients, IL-37b serum levels had an inverse correlation to the prognosis of advanced HCC. ***(Liu et al., 2016)***

These findings in HCC as well as the attenuated production and expression of IL-37b in metastatic cancers suggest an involvement for IL-37b in the signaling pathways that modulate metastasis, suggesting a potential role in histopathologic prognostication. ***(Cheng et al., 2019)***

CC Chemokine Ligand 20 (CCL20):

CCL20 (also known macrophage inflammatory protein-3 alpha) interacts with CC chemokine receptor 6 (CCR6), resulting in chemoattraction of immune cells to inflammation sites. CCL20 has been shown to display a variety of roles in overall inflammation, rheumatoid arthritis, and several cancers. In vitro and in vivo assays have highlighted a role for the CCL20-CCR6 axis in inducing HCC proliferation, growth, and invasion. ***(Guo et al., 2019)***

Several studies on subjects with HCC, found that tumor-infiltrating regulatory T cells could be selectively recruited to the tumor through the CCR6-CCL20 axis. The expression of CCL20 in the tumor was positively correlated with the number of tumor-infiltrating regulatory T cells. Importantly, the increased numbers of tumor-infiltrating regulatory T cells predicted poorer prognosis in HCC patients. ***(Guo et al., 2019)***

Cytokines Related to HCC Systemic Therapy Response:

Most patients present at advanced HCC stages where treatment options are restricted to recently approved immune-checkpoint inhibitors or kinase inhibitors, such as sorafenib, regorafenib, and lenvatinib among others, all which block tumor growth and angiogenesis pathways. Thus, evaluation of cytokines associated with these carcinogenic processes may help identify prognostic factors in response to therapy. ***(Debes et al., 2018)***

In recent years, immune therapy has become a key player in the systematic treatment of HCC with several combinations approved for first- and second-line treatment. Moreover, the success of bevacizumab, a VEGF antibody, in combination with atezolizumab, a PD-L1 inhibitor, for the treatment of advanced HCC highlights the potential role of these immune players in the treatment of HCC. ***(Rico et al., 2021)***

Interleukin-6 (IL-6):

Although promising, further studies, which are currently being conducted, are needed to solidify the role of IL-6 in response to therapy in HCC. Interestingly, recent studies in cellular models have described decreased resistance to sorafenib by inhibiting IL-6-related pathways. ***(Li et al., 2020)***

Angiopoietin-2 (ANG-2):

ANG-2 is almost exclusively produced by epithelial cells and acts as a key regulator in vessel maturation supporting the activities of other endothelial-acting cytokines. Many studies on patients with progressive HCC disease showed increased ANG-2 levels at the start of therapy, compared to those with non-progressive disease. ANG-2 levels, however, only increased in patients with progressive disease during follow-up. ***(Adachi et al., 2019)***

Hepatocyte Growth Factor (HGF):

HGF can have either promoting or a suppressive role in the development of HCC. Higher pretreatment plasma HGF levels are an independent prognostic factor for lower overall survival. Interestingly, lower HGF levels at the start of therapy tended to yield greater benefit from sorafenib in overall survival and time to progression. ***(Cheng et al., 2019)***

Vascular Endothelial Growth Factor (VEGF):

As a key cytokine driving angiogenesis, multityrosine kinase inhibitors such as Sorafenib target VEGF signaling. Similar to the ANG-2, higher VEGF pretreatment levels were associated with lower survival in many studies. In addition, elevated VEGF levels at baseline correlated with reduced overall survival and progression free survival. ***(Debes et al., 2018)***

Cytokines Associated with Response to Immune Checkpoint Inhibitor Therapy:

In recent years, immune checkpoint inhibitors (ICI) have expanded the treatment options for HCC. These agents target the co-inhibitory cell signals via the programmed death ligand/receptor (PD-L1/PD-1) and/or cytotoxic T-lymphocyte associated antigen-4 (CTLA-4). Despite the promise shown by these agents in clinical trials, the response rates in clinical practice may be less than 40%, hence the need for predictors of response to ICI treatment. ***(Kim, 2018)***

Nonetheless, further research and confirmation is needed for those markers to be considered in clinical practice. Pretreatment levels of PD-1/PD-L1 are well observed to predict response to ICI therapy, as well as the risk of acute cellular rejection when used in liver transplant recipients. ***(Munker, 2018)***

Beyond PD-1/PD-L1, the use of other peripheral biomarkers in the prediction of response to ICI is somewhat limited, but there have been a few biomarkers of interest with early assessment, including OPN, T-cell immunoglobulin and mucin domain-containing-3 (TIM-3), V-domain

immunoglobulin suppressor of T-cell activation (VISTA), and C-C motif chemokine 5 (CCL5/RANTES). ***(Ji et al., 2020)***

Interleukin-20:

The interleukin (IL)-20 is a subfamily part of the IL-10 family of cytokines that helps the liver respond to damage and disease, they participate in the control of tissue homeostasis, and in the immunological responses developed in this organ. The best-studied member of the family in inflammatory balance of the liver is the IL-22 cytokine, which on the one hand may have a protective role in fibrosis progression but on the other may induce liver tissue susceptibility in hepatocellular carcinoma development. ***(Caparros, 2018)***

Other members of the family might also carry out this dual function, as some of them share IL receptor subunits and signal through common intracellular pathways. Investigators are starting to consider the potential for targeting IL-20 subfamily members in liver disease. The recently explored role of miRNA in the transcriptional regulation of IL-22 and IL-24 opens the door to promising new approaches for controlling the local immune response and limiting organ injury. The IL-20RA cytokine receptor has also been classified as being under miRNA control in non-alcoholic steatohepatitis. ***(Keller, 2016)***

Molecular features:

The IL-20 subfamily of cytokines represents one of the three subfamily groups comprising the IL-10 family of cytokines. This family also includes the IL-10 cytokine itself and the type III IFN group (with IL-28A, IL-28B, and IL-29 members), categorized according to their biological function. They all work together to maintain epithelial tissue homeostasis and integrity, enhancing innate epithelial immunity, and regulating the healing process after infection or inflammatory events. ***(Rutz, 2014)***

The IL-20 subfamily includes IL-19, IL-20, IL-22, IL-24, and IL-26, which all have the common function of communicating leukocytes and epithelial cells in different tissues such as the liver. They play an important role in controlling tissue regeneration following injury, promoting survival as well as inhibiting apoptosis of epithelial cells. ***(Rutz, 2014)***

The IL-20 subfamily of cytokines is encoded by genes located in different clusters, which all share genomic organization, primary and secondary structures, and receptor complexes. IL-19, -20, and -24 genes are confined in chromosome 1q32, close to IL-10 gene location, while IL-22 and IL-26 are enclosed in chromosome 12q16. ***(Caparros, 2018)***

Regarding their regulation, different transcription factors have been reported for IL-19 (PE1 and AML-1), IL-20 (NF-κB), and IL-24 (Jak1, Stat3, Stat6, Spcs3, and AP-1), though only putative regulators have been suggested for IL-22 and IL-26 transcriptional control. ***(Keller, 2016)***

Signalling events by IL-20 subfamily members result in receptor dimerization; Janus kinase (Jak) 1, Jak2, and Tyrosine kinase 2 phosphorylation; and final signal transducer and activator of transcription (STAT)1 STAT3, and STAT5 activation. The best-studied cytokine signalling pathways are those regulated by IL-10 and IL-22, with the mitogen-activated protein kinases' final recruitment for anti-apoptotic and mitogenic gene expression in target cells. ***(Rutz, 2014)***

Cellular sources of the IL-20 subfamily of cytokines include monocytes, macrophages, dendritic cells (DCs), B-cells, T helper (Th) 2 and Th17 cells, cytotoxic CD8+ T cells, natural-killer (NK) cells, innate lymphoid cell (ILC) 3, fibroblasts, NKT cells, and γδ T cells to epithelial cells. They regulate new cytokine secretion according to their cellular targets which are immune system cells, such as monocytes, DCs, neutrophils or T cells, and also hepatocytes, acinar cells, fibroblasts, epithelial cells, keratinocytes, or adipocytes. ***(Keller, 2016)***

In the liver, the IL-20 cytokine subfamily has a key role in inflammatory pathological processes. The best-studied member of the family in liver homeostasis is the IL-22 cytokine. In a mouse model, it has been shown to lessen metabolic syndrome—a condition related to chronic low-level inflammation—by inducing the activation and expression of lipogenesis-related genes and helping with the triglyceride and cholesterol metabolism. ***(Wang et al., 2014)***

IL-22 also prevents apoptosis of hepatic stellate cells and attenuates liver fibrosis in mice and rat models and reported as a predictive severity marker in advanced stages of liver cirrhosis. Due to its hepatoprotective and anti-fibrotic properties, different studies have proposed IL-22 as a plausible candidate in the treatment of alcoholic liver disease (ALD). ***(Rutz, 2014)***

Commensal bacteria, such as Lactobacillus, have been shown to induce the production of IL-22 by gut ILCs. Rising IL-22 levels provoke the recruitment of regulatory DCs into the liver, constricting the hepatic inflammatory response, and favouring a tolerant tissue microenvironment. ***(Keller, 2016)***

It is still unknown whether the protective role attributed to IL-22 is unique or shared with other IL-20 family members, but IL-19, IL-20, and IL-24 may also function as protective cytokines during liver inflammation, as they all share signalling pathways through the IL-20R2 receptor subunit, which is greatly induced during LPS liver challenge. ***(Caparros, 2018)***

IL-20 and IL-24 can also signal through heterodimeric receptor formation with IL-22RA1, which could entail some redundancy in their action. Both cytokines participate in lipid metabolism regulation, although they have failed to improve metabolic disorder in obese mice. Also, IL-19, IL-22, and IL-24 participate in wound healing, an event present not only in the skin but also in liver disease as a first step for fibrogenesis, cirrhosis, and liver failure. ***(Keller, 2016)***

<u>IL-20 Cytokine Family in different hepatic disorders:</u>

IL-20 Cytokine Family in Viral Hepatitis:

Hepatitis B virus (HBV) and HCV are the most frequent virus types that induce chronic or acute and chronic forms of hepatitis, respectively. In patients, there is an increase in the number of cells producing IL-22 in both HBV and HCV. Furthermore, there is an increase in the number of liver progenitor cells in infected mice, a finding that has been corroborated in patients with chronic HBV. IL-22 production in HBV-infected patients seems to be under Notch pathway control. ***(Caparros, 2018)***

Experiments performed in a mouse model in vivo with Notch signalling inhibitors show that IL-22 was clearly diminished in the liver. It has also been reported to promote the modulation of the immune response during viral infection by downregulating the S100 family of viral proteins. Nevertheless, a dual role in protection and inflammation induction has been proposed for IL-22 in chronic HBV infection, either promoting cell proliferation and improving fibrosis, or inducing chemokine production and neutrophil recruitment. ***(Wei et al., 2016)***

More recently, different controversial roles have emerged for IL-22 in viral infection. For example, IL-22 has been associated with liver fibrosis severity in patients infected with HCV, with a possible pathological role residing in the accumulation of IL-22 in fibrotic areas due to its role in ameliorating liver tissue damage. In this sense, Sertorio et al. found that IL-22 functions as a protective factor, while IL-22 binding protein, a natural protein antagonist for IL-22, contributes to worsening liver fibrosis in chronic HCV infection. ***(Sertorio et al., 2015)***

On the other hand, many authors attribute a pathogenic role to IL-22 because of its participation in promoting Th17 recruitment in chronic liver inflammation and fibrosis in HBV infection, other authors postulate that IL-22 works as a proinflammatory cytokine in response to HBV, and they raise the possibility that the inflammatory tissue milieu represents different scenes for different functions of IL-22. More studies are needed to shed light on the IL-22 function in liver fibrosis, but this cytokine may be considered a therapeutic opportunity for future clinical management of liver disease. ***(Sertorio et al., 2015)***

Another IL-20 family member implicated in HCV infection is IL-26. This cytokine is overexpressed in serum when HCV infection occurs, and it is detected in liver lesions in chronic infection, particularly in patients with severe liver inflammation. ***(Wei et al., 2016)***

As previously indicated, IL-26 boosts NK cell response to HCV challenge through the upregulation of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) expression in the membrane surface of NK cells. Therefore, IL-26 has been proposed as a marker of inflammation in chronic HCV infection. ***(Sertorio et al., 2015)***

IL-20 Cytokine Family in ALD:

Alcoholic liver disease comprises different categories of liver alterations, from liver steatosis to cirrhosis or cancer. Alcohol consumption induces hepatotoxicity through intermediaries of ethanol metabolism, or by generating different mediators of the inflammatory response, like products of the oxidative stress response or proinflammatory cytokines secreted by liver Kupffer cells, such as TNF-α. ***(Szabo et al., 2011)***

Serum concentrations of IL-20 Cytokine Family have been proposed as correlates of liver damage progression in ALD. Moreover, high-IL-20 levels seem to be a good prognostic marker. Many authors also show an improvement in hepatocyte function and survival from ethanol-induced cell death when exposed to IL-20. Moreover, in a murine binge-drinking model, alcoholic liver injury is improved after treatment with IL-20 recombinant protein. ***(Caparros, 2018)***

IL-20 Cytokine Family in Non-ALD:

Non-alcoholic fatty liver disease (NAFLD) comprises a range of pathologies, from steatosis to non-alcoholic steatohepatitis (NASH). The mechanisms underlying this aggravation remain undefined, but a title role has been given to the innate immunity, inflammatory cytokines, and commensal microbiota. ***(Zai et al., 2021)***

The protective role of the intestinal mucus coat has been analyzed in a murine model of fatty liver disease [high-fat diet (HFD)]. The absence of mucin-2 (muc2) in muc2-deficient HFD-fed mice causes the activation of the mucosal immune system resulting in higher plasmatic and intestinal levels of IL-22, with a role regulating lipid metabolism and inflammation in the liver and adipose tissue. ***(Zai et al., 2021)***

Increased IL-20 levels are present in visceral adipose tissue of NASH-diagnosed patients, and they are considered a target for miR-26a. This would induce a reduction in the expression of IL-20 cytokine. IL-20RA is located in pericellular areas of fibrotic zones and that the expression of IL-20RB is also upregulated in NASH patients. ***(Caparros, 2018)***

This proinflammatory cytokine signals through STAT3 transcription factor, also activating the downstream IL-22 or IL-10 signalling pathway. STAT3 has been directly implicated in the progression of hepatic insulin resistance and its polymorphisms linked to NAFLD advancement, so it could be a potential objective for modulating cytokine response during NAFLD development. ***(Zai et al., 2021)***

IL-20 Cytokine Family in Hepatocellular Carcinoma:

Hepatocellular carcinoma (HCC) is the most frequent liver cancer variant, with incidence increasing every year. IL-22 is a well-established hepatoprotective cytokine; it promotes liver healing and tissue repair, preventing cellular apoptosis. It has a dual role in the control of liver

disease, with new evidence showing that it participates in controlling viral and alcohol-induced HCC. ***(Saalim et al., 2016)***

Nonetheless, IL-22 can also promote tumor growth both in vitro and ex vivo, and its levels are increased in patients with hepatocellular carcinoma. These data suggest that IL-22 might enhance liver tissue susceptibility to HCC development. Thus, although these results have been obtained from the animal model, clinicians must consider the risk of developing liver cancer when making therapeutic decisions for patients with hepatic disease in the future. ***(Niess, 2019)***

Interleukin-24 has been shown to inhibit HCC cells metastasis. This cytokine has been recently described to be regulated by the onco-microRNA miT-203a-3p.1. This microRNA controls IL-24 expression, and its inhibition can reverse HCC cell proliferation and metastasis. ***(Saalim et al., 2016)***

In addition, IL-24 acts synergistically with Notch pathway inhibitors in reducing tumor cell invasion and migration of HepG2 liver cancer cell line. In combination, the Notch pathway, miRNA miT-203a-3p.1, and IL-24 can be now considered possible therapeutic objectives in HCC management. ***(Wei et al., 2016)***

An in vitro anti-tumor effect has also been described for IL-26 due to the fact that NK cell-specific cytotoxicity against the hepatocellular carcinoma line HuH7.5 is highly upregulated in the presence of IL-26. The induction mechanisms for this upregulation depend on the overexpression of the TRAIL receptor in NK cells, which contributes to their cytotoxic activity. ***(Caparros, 2018)***

Future directions:

The IL-20 subfamily are key targets for the treatment of liver illness because of their protective in minimizing tissue damage and inflammation.:

(a) IL-22 has shown both pathological and hepatoprotective roles, so IL-22-IL-22R1 therapeutic possibilities seem to depend on the presence of different inflammatory conditions, which may either require the activation of this pathway or justify its inhibition.

(b) IL-19, IL-20, and IL-24 have a key role in the wound healing process of fibrogenesis in the liver, and IL-22 in the angiogenesis and neo-vascularization events.

(c) IL-26 can be considered a promising scope in infectious diseases management, given the antimicrobial and antiviral function already reported for this cytokine. ***(Caparros, 2018).***

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