Potential Involvement of the ILC2 - Regulatory T-Cell-Amphiregulin Axis in Liver Cancer Development: Novel Concepts for Immunotherapy of Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver tumor and its incidence is increasing. Since this type of cancer is largely resistant to systemic therapies, there is urgent need to identify cellular and molecular pathways involved in the pathogenesis of HCC. Recent evidence implicates inflammation-induced, immune cell-derived amphiregulin (AREG) about interactions between immune cells of the innate and adaptive immune system by AREG in HCC development. We postulate an immunological network with pro-tumorigenic activity comprising AREG, type 2 innate lymphoid cells and regulatory T cells that might constitute a promising target for novel cancer immunotherapies.

Keywords: Hepatocellular carcinoma; AREG; IL-33; ILC2; Tregs

Abbreviations: HCC: Hepatocellular Carcinoma; NASH: Non-Alcoholic Steatohepatitis; AREG: Amphiregulin; EGFR: Epidermal Growth Factor Receptor; Tregs: Regulatory T Cells; ILC2s: Type 2 Innate Lymphoid Cells; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; HSCs: Hepatic Stellate Cells; ECM: Extracellular Matrix; KLRG1: Killer-Cell Lectin Like Receptor G1; Foxp3: Forkhead Box Protein P3

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and is among the most lethal and prevalent cancers in humans [1,2]. The majority of HCC cases develops in the setting of hepatic cirrhosis as a result of chronic liver inflammation predominantly caused by viral infection, alcohol, and nonalcoholic steatohepatitis (NASH), the progressive form of nonalcoholic fatty liver disease [3]. Chronic liver injury induces repeated cycles of hepatic inflammation, necrosis, and compensatory regeneration causing fibrogenesis, a reversible, organ integrity-maintaining response characterized by excessive wound-healing and extracellular matrix deposition. Progression of fibrosis towards non-reversible cirrhosis is a result of hepatocyte necrosis, replacement of functional liver parenchyma by scar tissue and regenerative nodules, as well as hepatocyte dysplasia accompanied by loss of liver function and a high risk to develop HCC. So far, there are very limited therapeutic options and no effective targeted therapy exists for patients with advanced HCC. The multikinase inhibitor Sorafenib is the only systemically active drug approved for the treatment of advanced HCC but it improves survival of HCC patients by only few months [4]. Therefore, identifying cellular and molecular effectors involved in the pathogenesis of HCC is essential for developing novel immunotherapies to treat HCC and hence to improve patient prognosis.

The AREG/EGFR Axis in HCC

The epidermal growth factor amphiregulin (AREG) mediates mitogenic signals to various cell types and its expression was found to be up-regulated in human and rat cirrhotic livers [5]. AREG conveys anti-apoptotic signals in hepatocarcinoma cells thereby enhancing their survival and chemoresistance [6]. In patients with HCC, elevated AREG levels were associated with poor prognosis [7] and there are multiple studies that have correlated AREG expression with tumorogenesis and tumor progression in breast, lung, ovarian, and gastric cancer [8], suggesting AREG as a potential target for cancer therapy. Indeed, administration of an AREG neutralizing antibody inhibited growth of lung [9] and ovarian tumors in mice and strongly enhanced chemotherapy efficacy [10]. AREG binds to the epidermal growth factor receptor (EGFR), which belongs to a family of tyrosine kinases involved in the development and growth of various types of cancer including HCC [11,12]. Upon ligand binding, EGFR induces signal transduction cascades crucial for processes in tumorogenesis such as cell proliferation and angiogenesis as well as resistance to apoptosis and invasive behavior. Similar to AREG itself, EGFR is frequently overexpressed in a variety of tumors and serves as a negative prognostic factor for HCC [13,14]. Upon ligand binding, EGFR induces signal transduction cascades crucial for processes in tumorogenesis such as cell proliferation and angiogenesis as well as resistance to apoptosis and invasive behavior. Similar to AREG itself, EGFR is frequently overexpressed in a variety of tumors and serves as a negative prognostic factor for HCC [13,14]. Thus, several studies have addressed inhibition of EGFR activity as therapeutic strategy in HCC treatment and are reviewed elsewhere [15]. However, targeting the AREG/EGFR axis in cancer therapy bear the risk of severe side effects as AREG is involved in many physiological processes such as mammary gland development and lung morphogenesis and is essential for tissue repair and integrity following damage as demonstrated in liver regeneration [5,16]. Thus, identifying tumor-associated, inflammation-induced modulators of the AREG/EGFR axis may help to establish more selective cancer immunotherapies.

IL-33, ILC2s and AREG

There is increasing body of evidence that the AREG/EGFR axis plays an important role in immunological networks that are critical for
mediating immunity and tolerance. During inflammation, AREG is expressed by different activated immune cells such as Th2 cells, macrophages, dendritic cells, mast cells, and basophils, but also regulatory T cells (Tregs) and type 2 innate lymphoid cells (ILC2) [17]. Numerous mediators stimulate AREG expression in immune cells including prostaglandin E2, transforming growth factor-β, and the cytokine IL-33. Cytokines released during carcinogenesis are thought to be essential mediators of interactions between immune cells in the inflammatory tumor environment. In this context, IL-33 has been implicated as a modulator of tumorigenesis in various cancers [18]. In an immunological network comprising IL-33 and ILC2 in liver disease, one might speculate that hyperactive ILC2s concur to progression of inflammation, fibrosis, and thus, might increase the risk to develop HCC.

So far, there is no direct link between hepatic ILC2s and HCC but one might speculate that hyperactive ILC2s concur to progression of liver fibrosis towards cirrhosis with the possibility to develop HCC. In general, ILC2s have been considered as a population with predominant pro-tumorigenic activity and the IL-33/ILC2/IL-13 circuit was shown to be critically involved in promoting cholangiocyte hyperplasia in murine cholangiocarcinoma [26] and in activating myeloid-derived suppressor cells, which inhibit anti-cancer immunity in breast cancer [27]. Interestingly, IL-33-activated ILC2s also express AREG early after tissue damage. In murine models of colitis [28] and infection-induced inflammation, ILC2s express AREG was shown in murine models of liver injury, fibrosis [21], and thus, might increase the risk to develop HCC. HSCs are another liver-resident cell population that is activated during chronic inflammation. The capability of activated HSCs to express AREG was shown in murine models of liver fibrosis [37] and NASH, as well as in NASH patients [39]. AREG induced proliferation, survival and ECM production of HSCs [39] thereby stimulating their pro-fibrogenic activity that may result in development of cirrhosis with progression to HCC. Unlike Kupffer cells that do not express ST2 [40], HSCs express the IL-33 receptor and become activated by IL-33 as shown in murine bile-duct ligation-induced hepatic fibrosis [41], pointing to another mechanism by which IL-33 drives chronic inflammation and tissue remodeling in the liver.

**AREG and Tregs**

Tregs play an important role in tumorigenesis and constitute a tumor escape mechanism, for example by inhibiting tumor-infiltrating CD8+ T cells with anti-tumor activity as shown in various types of cancer including HCC [42]. One recently described mechanism by which hepatocarcinoma cells improve immunosuppressive intratumoral Treg function is via release of AREG [43]. During inflammation, activated Tregs up-regulate expression of EGFR and AREG/EGFR signaling was found to be crucial for effective Treg function in lung and gastric cancer [44]. In HBV infection, EGFR+ Tregs responded to increased AREG expression by potent inhibition of anti-viral CD8+ T cells resulting in immune tolerance and persistent HBV infection [45]. As chronic HBV infection is the major cause for HCC, this indicates that infection-induced AREG might concur to HCC development by activation of intrahepatic Tregs and maintenance of Treg function in the chronically inflamed liver.

The function of Tregs is critically linked with sustained expression of the transcription factor forkhead box protein P3 (Foxp3) and AREG/EGFR signaling was found to stabilize Foxp3 expression in Tregs thereby preserving Treg function during inflammation [44]. In addition to the direct regulation of Tregs by AREG, it was shown that a functionally distinct Treg subset expresses AREG upon activation [46], suggesting an autocrine feedback loop to further maintain Treg function independently of exogenous AREG. Tregs are also poised to respond to IL-33 through expansion of a Treg subset expressing the IL-33 receptor ST2 as well as AREG.

**AREG, Macrophages and HSCs**

Macrophages can adopt a pro-tumorigenic phenotype in the tumor microenvironment thereby suppressing anti-tumor immune responses and promoting tumor cell invasion and persistent growth [34]. Recent data indicate that AREG participates in both recruitment of tumor-associated macrophages (TAMs) by inducing macrophage-attracting chemokines [35] and suppressive function of TAMs in carcinogenesis [36]. Liver-resident macrophages, namely Kupffer cells, were shown to up-regulate AREG expression during murine hepatic fibrosis that in turn triggers ECM production by HSCs [37]. Moreover, in HBV-infected livers, Kupffer cells also expressed AREG and promoted viral persistence by promoting Treg-mediated inhibition of anti-viral CD8+ T-cell responses [38], indicating that Kupffer cell-derived AREG supports chronic liver inflammation and thus, might increase the risk to develop HCC.

**References**

Figure 1: The ILC2/Treg/AREG axis in HCC development. In the chronically inflamed liver, IL-33 is released by necrotic hepatocytes and leads to activation of tissue-resident type 2 innate lymphoid cells (ILC2s) and hepatic stellate cells (HSCs) and recruitment of regulatory T cells (Tregs). All cell populations start to express amphiregulin (AREG), which promotes regenerative processes following liver tissue damage. Activated Tregs increase expression of the epidermal growth factor receptor (EGFR) and AREG/EGFR signaling further sustains Treg function in the inflammatory environment. Chronic inflammation-induced constitutive activation of AREG-expressing ILC2s and Tregs might trigger excessive wound healing and particularly Tregs might contribute to the development of hepatocellular carcinoma (HCC) by favoring an immune tolerant milieu and by inhibiting anti-tumor activity of cytotoxic CD8+ T cells (CTLs). KC: Kupffer Cells, iMΦ: Inflammatory Macrophages, TAM: Tumor-Associated Macrophages.

IL-33/ST2 signaling also ensures Treg function and adaption to the inflammatory environment as demonstrated in intestinal inflammation [47]. Interestingly, IL-33-activated ST2+ Tregs infiltrate the inflamed liver during immune-mediated hepatitis [24,48] and murine cytomegalovirus (MCMV)-induced liver damage [49]. IL-33−/− mice showed reduced infiltration of ST2+ Tregs in the liver and developed more severe immune-mediated hepatitis [48]. Similar results were shown in MCMV-infected ST2−/− mice where ST2 deficiency aggravated liver pathology due to impaired hepatic accumulation of Tregs [49], indicating immunosuppressive function of ST2+ Tregs in liver disease pathogenesis. In the setting of chronic inflammation, it is conceivable that inflammation-triggered IL-33 and AREG expression leads to constitutively active Tregs with high suppressive function providing the basis of an immunotolerant milieu that allows and supports the development of HCC.

Conclusion

Accumulating evidence indicates that dysregulation of the dialog between the immune system and the AREG/EGFR signaling pathway contributes to pathology in the context of chronic liver disease and HCC. We here postulate a so far not described immunological network that might concur to HCC development and therefore might be a target for novel immunotherapeutic strategies in cancer treatment. In this network, IL-33 is an important link between liver inflammation-driven tissue damage and local immune response since it activates tissue-resident ILC2s and HSCs and recruits Tregs, all of them expressing the pro-tumorigenic growth factor AREG and other immune mediators that favor carcinogenesis. AREG in conjunction with IL-33 might further ensure effective intratumoral Treg function in the inflammatory tumor environment and might induce recruitment of TAMs resulting in immunosuppression of anti-tumor immunity (Figure 1). However, despite the present data, a clear link between the ILC2/Treg/AREG circuit and cancer has not been identified in humans until now. Thus, further research is needed to characterize the contribution of this immunological network to carcinogenesis and to define its impact in the development of HCC.

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