Original Article

Accumulating evidence: TLR signaling facilitates the development of hepatocellular carcinoma

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Abstract: Chronic inflammatory diseases are well-known major risk factors for hepatocellular carcinoma (HCC). Inflammatory signals are also involved in tumorigenesis, and the role of the TLR signaling in hepatocarcinogenesis is just beginning to emerge. Here, we review the links between TLR and HCC, and we explore the mechanisms by which TLR signaling facilitates the promotion and initiation of HCC.

Keywords: Hepatocellular carcinoma, inflammation, Toll-like receptor, MyD88, chronic inflammatory disease

Introduction

Hepatocellular carcinoma (HCC) is the most common type of liver cancer. HCC is the fifth most frequently diagnosed cancer and is the second highest cause of cancer mortality [1]. The main risk factors for HCC are alcoholism, hepatitis B, hepatitis C, aflatoxin, cirrhosis of the liver, nonalcoholic steatohepatitis, hemochromatosis, Wilson’s disease, type 2 diabetes, and hemophilia [2-6]. The risk factors for HCC display regional variability: in China, for example, hepatitis B is endemic and is the predominant cause of HCC [7], while cirrhosis of the liver is the major cause of HCC in the United States [8].

Inflammation and HCC

Inflammation is a protective response that involves immune cells and immune molecules, and inflammatory responses are classified as either acute or chronic. Chronic inflammation leads to a progressive shift in the types of cells present at the site of inflammation, and can lead to tissue damage by the continuous secretion of proinflammatory cytokines [9]. It is well known that chronic inflammatory diseases, such as chronic viral hepatitis (with either the hepatitis B virus or the hepatitis C virus) and liver cirrhosis, are some of the most important risk factors for HCC [8, 9]. Inflammatory cells, cytokines, and other proinflammatory molecules found within tumors have a fundamental and intricate relationship to tumor growth, progression, and immune evasion through suppression of anti-tumor immune responses. Functional polymorphisms of cytokine genes have been reported to be associated with cancer susceptibility and severity [10]. Recently, the role of chronic inflammation in the development and progression of cancer has sparked intense scientific interest.

TLR structure

Toll-like receptors (TLRs) are a family of pattern recognition receptors that play a key role in innate and adaptive immunity, and the deregulation or dysfunction of TLRs is implicated in inflammatory disease and cancer [11, 12]. The TLRs recognize molecular patterns that are broadly shared by exogenous pathogens, pathogen-associated molecular patterns (PAMP) [13, 14]. TLR receptors are highly evolutionarily conserved germline-encoded type I transmembrane proteins containing an extracellular domain, a transmembrane region, and an intracellular tail. The extracellular domain contains leucine-rich repeats (LRR) of differing size and abundance. Within each LRR repeat, the conserved residues provide a rigid structural
framework, whereas variable residues are available for interaction with ligands. The Toll/interleukin-1 receptor (TIR) homology domain is another important domain in the cytoplasmic region, and plays a key role in cytoplasmic signal transduction following ligand stimulation of TLR [13].

**TLR signaling in inflammation**

When TLR recognizes PAMP, a series of intracellular signal cascades activate, inducing and activating downstream target genes. Individual TLRs interact with specific adapter proteins to transduce the intracellular signal [15]. TLR signaling can be divided into two distinct signaling pathways: a MyD88-dependent pathway and a TRIF-dependent pathway, as shown in Figure 1. Among the TLRs, only TLR3 and TLR4, which are activated by dsRNA and LPS, respectively, can transduce the signal via the TRIF-dependent pathway [16].

With the exception of TLR3, all TLRs signal via the MyD88-dependent pathway after dimerization of the TLR receptor, subsequently activating the downstream NF-κB and MAPK pathways [17]. TLR cytoplasmic domain binds the adaptor protein MyD88, forming functional signaling complexes, then recruits IRAK1, IRAK2, and IRAK4. These kinases phosphorylate and activate TRAF6, TAK1, and IKK-β, which phosphorylates iκB, activating transcription and consequent induction of inflammatory cytokines [16, 18].

**TLRs regulate HCC-associated inflammation in Kupffer cells and hepatocytes**

To better understand liver tumorigenesis, the role of TLRs in liver disease is summarized (Table 1). Kupffer cells (Browicz-Kupffer cells and stellate macrophages) are specialized macrophages that line the walls of the sinusoids in the liver. Ethanol-induced liver injury is common in chronic alcoholics, which can activate Kupffer cells [19]. In the liver, TLRs are predominantly expressed on the Kupffer cells, whereas only a handful of TLRs are expressed on hepatocytes, the liver parenchymal cells; hepatocytes are less sensitive to TLR ligands than Kupffer cells [20]. Activation of Kupffer cells by TLRs induces the production of proinflammatory cytokines and hepatomitogens, which can promote the formation of HCC [21, 22]. Exposure of Kupffer cells to diethylnitrosamine (DEN) promoted production of IL-6 in a manner that was dependent on the Toll-like receptor adaptor protein 88 (MyD88) [22]. MyD88 has a critical role in mediating innate immune signaling by TLR family members, and has been associated with both pro- and anti-tumorigenic responses in HCC [23, 24].

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**Figure 1.** Role of Toll-like receptors (TLRs) signaling in hepatocarcinogenesis. TLR signaling can be divided into two distinct signaling pathways, the MyD88-dependent and TRIF-dependent pathway. Elevated MyD88 expression may facilitate HCC metastasis by promoting an EMT phenotype and enhancing tumour-initiating capabilities via activation of the PI3K/Akt pathway. NF-κB regulated proinflammatory factors (IL-6, IL-1β, and TNF-α) contribute to hepatocarcinogenesis, while the antiinflammatory regulatory factors (CD11b, SOCS1, and MyD88) inhibit the development of HCC via repressing TLR/MyD88-triggered signals.
The links between TLR and HCC

Recent studies have shown that TLR4 and gut microbiota have been reported to be associated with HCC promotion, and are associated with the main features of HCC promotion, including up-regulation of epiregulin, secretion of proinflammatory cytokines, cell proliferation, and reduction in apoptosis [25, 26]. Evidence indicates that TLR4 is associated with inflammation and carcinogenesis in chronically injured liver tissue. Genetic TLR4 inactivation significantly reduced the development of HCC, revealing that the promotion of HCC by TLR4 involves a similar mechanism as that observed in intestinal tumorigenesis promoted by TLR4 and MyD88 [25]. Clinical and epidemiological data show that hepatitis C virus-induced tumorigenesis can be promoted via TLR4 overexpression under conditions of long-term alcohol consumption [21]. Yoon Seok Roh et al. discovered that a mouse harboring a hepatocyte-specific knockout of TAK1 (TAK1DHEP) spontaneously develops HCC [27]. Knocking out MyD88, TLR4, or TLR9 signaling in TAK1DHEP mice hindered the development of HCC [11, 28].

Regulatory factors can inhibit HCC development via TLR/MyD88-triggered signals

It is well known that MyD88 signals downstream from the TLRs, activating pathways that drive the promotion of HCC. Enhanced expression of MyD88 in HCC cells can induce epithelial-mesenchymal transition (EMT) and cancer stem cell-like features, as well as contribute to HCC metastasis [29]. Additionally, MyD88 binds to the regulatory subunit of PI3K and p85, activating the PI3K/AKT/GSK3B/SNAIL pathway to induce EMT and tumor metastasis [29, 30]. The characteristics of elevated MyD88 induced EMT in HCC cells indicated in Figure 1. However, MyD88s, a MyD88 spliceosome, can be detected under continuous stimulation with proinflammatory cytokines or inflammation-promoting bacterial products. MyD88s can serve as a negative regulator of TLR/MyD88-triggered signals, repressing the development of HCC [31, 32]. In addition, suppressor of cytokine signaling 1 (SOCS1) also acts as a negative regulator of TLR/MyD88-triggered signals. SOCS1 binds and degrades the adapter protein Mal, which is involved in TLR2/MyD88 and TLR4/MyD88-triggered signals [33] (Figure 1), indicating that SOCS1 can also inhibit hepatocarcinogenesis by repressing TLR/MyD88 signaling. Integrins are also involved in TLR/MyD88 signaling, inflammation, and hepatocarcinogenesis. TLR-triggered, active CD11b integrin engages in crosstalk with the MyD88 and TRIF pathways, which feedback to inhibit TLR signaling by activating the tyrosine kinases Src and Syk [34].

Conclusion

A large number of studies indicate that TLRs signaling play significant roles in hepatocarcinogenesis as well as inflammation. TLRs and MyD88 appear to have multiple roles in hepatocarcinogenesis, and further elucidation of these complex roles will contribute to better understand of the mechanisms that regulate TLR-triggered HCC promotion and liver tumorigenesis. Furthermore, targeting these proteins may provide novel therapeutic options for preventing the progression of liver disease from chronic liver disease to HCC.

Acknowledgements

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Table 1. Characteristics of TLRs in liver-related disease

<table>
<thead>
<tr>
<th>Toll-like Receptor</th>
<th>Cellular Localization</th>
<th>Adaptor Protein</th>
<th>Live-related Disease</th>
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<tbody>
<tr>
<td>TLR2</td>
<td>Kupffer Cells</td>
<td>MyD88</td>
<td>HCV [35, 36]</td>
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<td></td>
<td>Hepatocytes</td>
<td>TIRAP</td>
<td>HBV [37]</td>
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<td>TLR3</td>
<td>Kupffer Cells</td>
<td>TRIF</td>
<td>HBV [38, 39]</td>
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<td></td>
<td>Hepatocytes</td>
<td></td>
<td>HCC [40]</td>
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<tr>
<td>TLR4</td>
<td>Kupffer Cells</td>
<td>MyD88</td>
<td>HCC [41]</td>
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<tr>
<td></td>
<td>Hepatocytes</td>
<td>TIRAP</td>
<td>HCV [42, 43]</td>
</tr>
<tr>
<td></td>
<td>HSs</td>
<td>TRIF</td>
<td>HBV [37, 39]</td>
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<tr>
<td></td>
<td>LSECs</td>
<td>TRAM</td>
<td>ALD [44]</td>
</tr>
<tr>
<td>TLR5</td>
<td>Hepatocytes</td>
<td>MyD88</td>
<td></td>
</tr>
<tr>
<td>TLR9</td>
<td>Kupffer cells</td>
<td>MyD88</td>
<td>HCV [45]</td>
</tr>
<tr>
<td></td>
<td>HSs</td>
<td></td>
<td>HBV [46, 47]</td>
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</tbody>
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HCV: hepatitis C virus; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; ALD: alcohol induced liver disease; LSECs: liver sinusoidal endothelial cells; HSs: hepatic stellate cells.
The links between TLR and HCC

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