



Review article

Toll like receptor 4 and hepatocellular carcinoma; A systematic review

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ABSTRACT

Introduction: Toll like receptor 4 (TLR4) is an extracellular pathogen recognition receptor (PRR) which recognizes a wide range of pathogens and damage associated molecular patterns (PAMPs and DAMPs). It can activate intracellular signaling and consequently transcription factors which participate in transcription from either immune related or malignancy genes. Thus, it has been hypothesized that TLR4 may be a cause of hepatocellular carcinoma (HCC). This article has reviewed the roles of TLR4 in the pathogenesis of HCC.

Method: “TLR4”, “hepatocellular carcinoma”, “liver tumor” and “liver cancer” were used as key words for searching in Scopus, Google Scholar and MEDLINE scientific databases.

Results: Most of the investigations documented the roles of TLR4 in induction of HCC via several mechanisms including increased number of T regulatory lymphocytes and liver resident follicular helper like cells, increased production of pro-inflammatory and malignancy related molecules including cytokines, NANOG, Caspase-1, Ephrin-A1, NO and BCL6. TLR4 participates in the proliferation of the cells and also production of the molecules in both chronic infectious and non-infectious inflammatory diseases.

Discussion: TLR4 is an innate immunity receptor which plays a pathogenic role during chronic inflammation and can induce HCC in human.

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1. Introduction

Hepatocellular carcinoma (HCC) is a prevalent malignancy worldwide [1]. It is more prevalent in Asia, and less common in Europe,

America and Middle East countries [2]. Several factors and mechanisms are responsible for inducing HCC in human. It has been reported that liver cirrhosis, primary biliary cirrhosis, hepatitis viral infections, co-infection of hepatitis viruses and human immunodeficiency virus (HIV), nonalcoholic fatty liver disease, silent chronic liver disease, alcoholic liver disease, nonalcoholic steatohepatitis, hypersensitivities including autoimmune hepatitis, toxins such as aflatoxin B1 and hereditary hemochromatosis are the most important risk factors for HCC [3,4]. HCC has been classified to 8 categories based on the histomorphologic features including well vascularized tumors with wide trabeculae (>3 cells), small cell changes, prominent acinar pattern, absence of Kupffer cells, mitotic activity, cytologic atypia, vascular invasion and the loss of the reticulin network [5]. HCC surveillance is variable and significantly dependent on early detection, curative therapy administration, and existence of cirrhosis [6]. It has been hypothesized that the viral factors and host immune system can be considered as the main risk factors for induction of HCC in the population [7]. It appears that pathogen recognition receptors (PRRs), as the molecules involved in the pathogen/damage associated molecular patterns (PAMPs/DAMPs) recognition, can be considered as candidates to induce or stimulate HCC. The main mechanisms used by the PRRs in the pathogenesis of HCC have been evaluated previously. Among PRRs, toll like receptors (TLRs) are the important PRRs which have been evaluated regarding their roles in the pathogenesis of HCC. TLRs can recognize PAMPs/DAMPs and induction of related intracellular signaling. There are 10 TLRs in/on the human immune and non-immune cells which use myeloid differentiation primary response (MYD88) alone (TLR1, TLR2, TLR5, TLR6, TLR7, TLR8 and TLR9), TIR-domain-containing adapter-inducing interferon- β (TRIF) alone (TLR3) and both TRIF and MYD88 (TLR4), as adaptor proteins [8,9]. Accordingly, it appears that TLR4 has a wider range of functions than other TLRs [8], which corroborates its role as an important molecule involved in the pathogenesis of HCC. The aim of this article is to review the main mechanisms used by TLR4 in the induction of HCC.

2. TLR4 recognizes several ligands and induces both TRIF and MYD88 dependent pathways

TLR4 is a known highly conserved member of PRRs and has been recognized as age-related macular degeneration 10 (ARMD10) and cluster of differentiation 282 (CD284) [10]. Its gene location is far from other TLRs and is located on 9q33.1 [11,12]. TLR4 consists of three main domains including extracellular leucine-rich repeats (LRRs), hydrophobic intramembrane domain, and intracytoplasmic toll-IL-1 receptor (TIR) domain. Its extracellular LRRs domain recognizes several ligands including Lipopolysaccharide (LPS), free fatty acids, microbial lipids, monophosphoryl lipid A (MPLA), hyaluronan, high-mobility group box-1, allergenic nickel and heat shock protein 60 and 70 [13,14]. Interaction between TLR4 and their ligands leads to activation of two distinct pathways which are dependent on the location of TLR4/ligands interactions [15]. TLR4/ligands interactions on the cell membrane lead to activation of MYD88 dependent pathway. Accordingly, TIR domain-containing adaptor protein (TIRAP), which is also known as MyD88 adapter-like (Mal), interacts with TIR domain of TLR and consequently activates MYD88 molecule [15]. Activated MYD88 mediates phosphorylation of Interleukin-1 receptor associated kinase (IRAK)-4 and IRAK-1 [16]. Activation of IRAK-1 leads to phosphorylation and activation of TNF receptor associated factor (TRAF)-6 molecule [17]. Activated TRAF-6 is a key stage of TLRs intracellular signaling pathways which can activate important transcription factors including nuclear factor- κ B (NF- κ B), activator protein 1 (AP-1) and interferon regulatory factor 3 (IRF3) [17]. The transcription factors translocate to the nucleus and, hence, transcripts from pro-inflammatory cytokines as well as type interferons [15]. TLR4/ligands interaction in the endosomes leads to recruitment of TRIF-related adaptor molecule (TRAM) which contains TIR domain and consequently activation of TRIF molecule [18]. TRIF, as an

adaptor protein, mediates phosphorylation of I κ K, as inhibitor of NF- κ B, and consequently translocation of the transcription factor to the nucleus [18]. TRIF also mediates activation of mitogen-activated protein kinase (MAPK) pathway which leads to activation of AP-1 [18]. TRAF3 is another target of TRIF which mediates activation of IRF5 transcription factor [18]. Intracellular signaling pathways of anti-inflammatory cytokine receptors, including Janus kinase (JAK)/Signal transducer and activator of transcription 3 (STAT3) pathway are the main inhibitory pathways for TLR4 signaling. Fig. 1 shows the intrasignaling pathways of TLR4.

3. Hepatocellular carcinoma

HCC is the sixth most common cancer worldwide and is associated with increasing mortality [19,20]. It is the most serious chronic hepatitis B/C complication [21]. Multiple risk factors can influence HCC and, hence, HCC has a complex pathogenesis [21]. Based on the HCC heterogeneity, developing effective therapies against this dangerous cancer is challenging. Despite the advances in our knowledge regarding the etiology and mechanisms responsible for development of HCC, the molecular pathogenesis of this lethal cancer is yet to be completely understood. HCC has clinical and histopathological heterogeneity and its cellular differentiation has a wide range from very well to poorly differentiated tumors. Additionally, HCC has various morphologic features such as cirrhotomimetic, clear cell, myxoid, sarcomatoid, fibrolamellar, neutrophilic-rich, lymphocyte-rich, cirrhotic, biphenotypic (a combination of hepatocellular and cholangiocarcinoma) and steatohepatic HCCs [22]. Although there is a wide histological diversity of HCC, there is limited information regarding the molecular or genetic mechanisms for development of HCC. Therefore, it is worthy to expand our knowledge regarding the main risk factors and the mechanisms which lead to development of HCC.

Several agents have been introduced as risk factors for development of HCC which can be categorized into three classes including viral, liver and host factors [23,24]. Viral factors consist of the hepatitis B virus (HBV) and hepatitis C virus (HCV) related molecules which may participate in induction of HCC. These risk factors include high HBV-DNA/HCV-RNA copy numbers, HBV/HCV mutants, hepatitis B virus e antigen (HBeAg), HBV/HCV genotype and chronic raised liver enzymes [24–26]. Liver factors include advanced liver cirrhosis and fibrosis, impaired liver function and active viral/non-viral hepatitis [24,27,28]. Interestingly, host and environmental factors including gender, diabetes mellitus, obesity, some genetic variations, older age, cirrhosis, family history of HCC, opium addiction, smoking, alcohol and immune status are the most prevalent risk factors for HCC. The roles played by immune system and its related molecules in the pathogenicity of HCC have yet to be clearly elucidated. Accordingly, the roles of TLR4 as a plausible risk factor for development of HCC and participation in its pathogenesis are discussed in this review article.

4. Methods

Medical Literature Analysis and Retrieval System Online (MEDLINE), Google Scholar and Scopus, as the main three databases, were searched regarding “TLR4, liver tumor, liver cancer and hepatocellular carcinoma” as key words. The research studies which evaluated the TLR4 expression and functions in other liver diseases rather than HCC and the publications in a language other than English have been excluded from the current review article as well. The articles published from 1990 to 2017 have been enrolled in this project. Accordingly, after initial searching, 90 papers were included in the investigation and after excluding the papers according to the exclusion criteria, 63 papers were presented in the current review article.

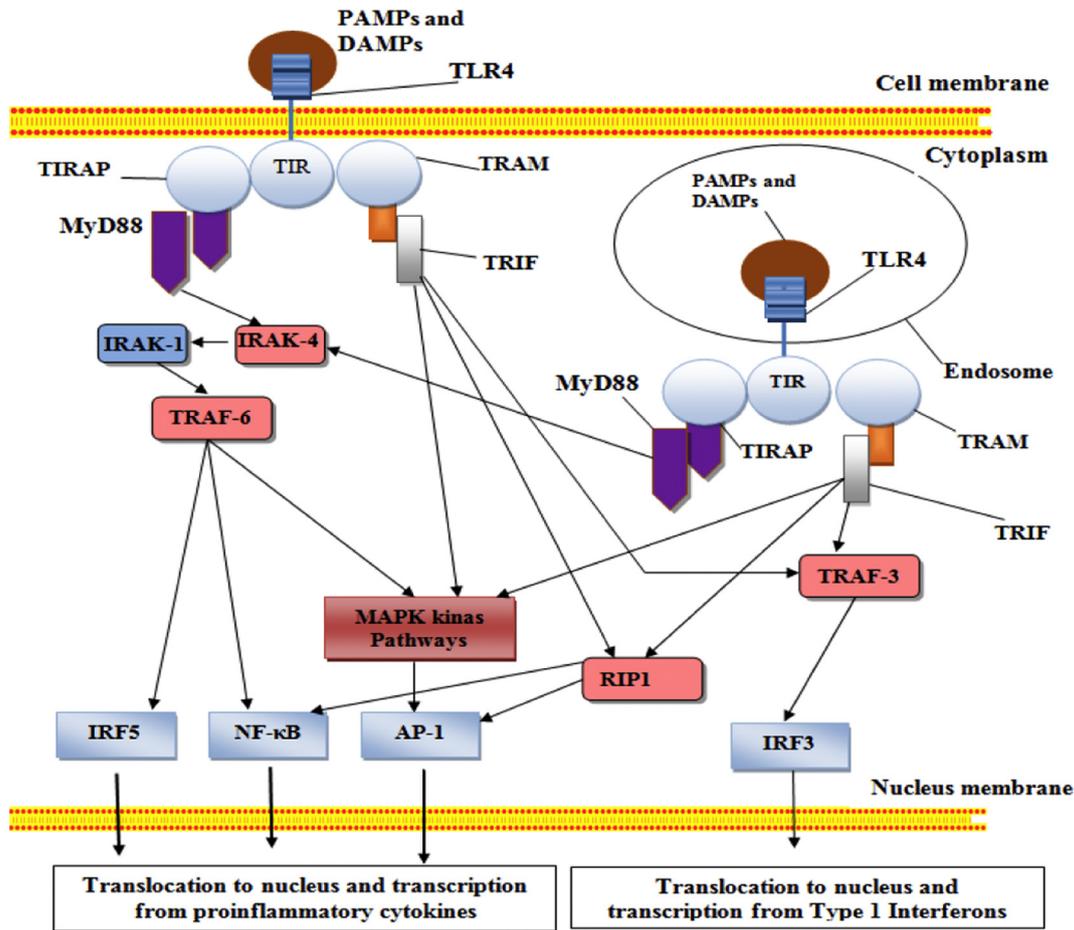


Fig. 1. TLR4 signaling pathways. TLR4 interacts with its ligands on both cytoplasm and endosome membranes and activates MYD88 and TRIF dependent pathways. The pathways lead to activation of IRF3, IRF5, AP-1 and NF- κ B, as pro-inflammatory transcription factors. The transcription factors are the important factors for up-regulation of the molecules involved in the induction of appropriate immune responses to cancers and in some cases the molecules involved in the cancer development and metastasis.

5. TLR4 and hepatocellular carcinoma

Due to the key roles of TLR4 in recognition of PAMPs and DAMPs along with using both MYD88 and TRIF dependent pathways and also based on the critical roles of NF- κ B, a transcription factor which is activated in the TLR4 intracellular pathway, in the induction of malignancies [29], it has been hypothesized that TLR4 may significantly participate in the pathogenesis of HCC. The role of TLR4 in the pathogenesis of HCC has been evaluated by Lin et al., who reported that HCC cells functionally expressed TLR4 [30]. Additionally, increased expression of TLR4 on the HCC cells proved the hypothesis [31]. The strengths of this study was to evaluate TLR4 expression in both mRNA and protein levels. It appears that TLR4 can use some mechanisms to induce survival of HCC cells. Moreover, TLR4 positive HCC cells have stem-like properties including migration, chemotherapy resistance and tumor invasion [32].

TLR4 uses several mechanisms to induce HCC in the patients with high risk factors. For example, Li et al., reported that increased expression of TLR4 on the T regulatory lymphocytes was associated with increased expression of the chemokine (C-X-C motif) ligand 10 (CXCL10) and its receptor, C-X-C motif receptor 3 (CXCR3), on T regulatory lymphocytes [33]. The investigators also revealed that increased TLR4 expression was associated with increased recruitment of T regulatory lymphocytes to the liver of HCC patients, probably via CXCR3/CXCL10 interaction, and consequently increased the chance of tumor recurrence [33]. T regulatory lymphocytes are the main survival factors for cancers which inhibit immune responses to tumors [34]. It appears that TLR4 induces HCC progression via increased numbers of T regulatory

lymphocytes in the HCC tissue [35]. However, it seems that Lin et al., had to neutralize CXCL10 by blocking antibodies to confirm the hypothesis and this is the weakness of the study. Recent data demonstrated that TLR4 induces histone mediated chemokine production via activation of NF- κ B [36]. Suppression of TLR4-NF- κ B signaling leads to impaired histone related chemokine production which finally results in inhibition of HCC cell migration and metastasis [36]. Studies have been performed on both TLR4-/- and normal mice, which is their strength property. TLR4 appears to induce tumor associated molecules and chemotactic factor for cells involved in HCC development in MYD88 dependent manner which activates NF- κ B as transcription factor [15]. Chen and colleagues also reported the roles of TLR4 in the development of HCC and reported that TLR4 induces development of tissue-resident T follicular helper like cells, which play key roles in development of HCC [36]. It seems that TLR4 develops tissue-resident T follicular helper like cells via up-regulation of T cell survival related molecules as well. As mentioned previously, heat shock proteins (HSPs) are considered as TLR4 ligands [13]. Previous investigations showed that extracellular HSP70-peptide complexes are the ligands for TLR4 and promote HCC cells proliferation in C-Jun N-terminal kinases 1/2 (JNK1/2) and MAPK pathways [37–39]. Other studies also reported the same data and revealed that LPS/TLR4 interaction leads to development of HCC in TLR4/JNK/MAPK dependent signaling pathways [40–42]. Inhibition of HCC development by suppression of TLR4-MyD88-TGF- β -Activated Kinase-1 (TAK1)-mediated NF- κ B and MAPK pathway proved these findings [43]. Dong et al., also reported that TLR4 is able to promote HCC cells invasion in MAPK4/JNK dependent pathway [44]. Hsiao also showed that LPS/TLR4 interaction results in increased

proliferation of HepG2 cells, a HCC cell line, and also nitric oxide synthase (NOS) expression, as well as NO production in the cell line in Akt and MAPK dependent pathways [45]. Based on the fact that MAPK pathway activation leads to phosphorylation and activation of activator protein 1 (AP-1), another transcription factor down-stream of TLR4-MYD88 dependent signaling pathways [46], hence, it appears that TLR4 uses AP-1, in addition to NF- κ B, to induce expression of HCC related molecules [47,48]. TLR4 signaling also induces other intracellular signaling pathways to promote HCC cell proliferation and multidrug resistance through a cyclooxygenase-2 (COX-2)/prostaglandin E2 (PGE2)/signal transducer and activator of transcription 3 (STAT3) positive feedback loop [30,49]. Miura and colleagues also reported that macrophage related TLR4 participates in development of steatohepatitis-related HCC by production of IL-6 and TNF- α in response to LPS in mice [50]. The Janus kinase (JAK)/STAT pathways are the signaling pathways which are used by cytokines to induce intracellular signaling pathways [51].

On the other hand, interactions of the cytokine by their receptors activate JAK/STAT signaling pathway [51], which is a positive factor for up-regulation of TLR4 and its signaling pathways in a positive feedback loop. Accordingly, TLR4/ligands interactions lead to up-regulation of pro-inflammatory cytokines such as IL-6 and TNF- α , and interaction of the pro-inflammatory cytokines with their receptors results in up-regulation of TLR4 and its signaling molecules. So, it seems that the ligands which activate TLR4 signaling can be considered as the inducers of HCC. B-cell lymphoma 6 (BCL6) up-regulation is another mechanism used by TLR4 to develop programmed death 1 (PD-1) B cells, which interact with programmed death-ligand 1 (PD-L1) on the T cells and result in T-cell dysfunction and consequently HCC progression [52]. Nanog is a key transcription factor which is involved in the self-renewal and maintenance of stem cells [53]. Based on the fact that it is expressed in the stem cells, it has been considered as a stem cell marker [53]. It has been demonstrated that NANOG plays key roles in cancers development and drug resistance including HCC [54]. Investigators revealed that TLR4 up-regulates NANOG [54,55], which may be considered as a main mechanism used by TLR4 in induction of HCC.

Interestingly, Wang and colleagues revealed that activation of TLR4 signaling pathways leads to upregulation of HMGB1 in a positive feedback manner [56]. As mentioned previously, alcohol is a risk factor for HCC, and investigators identified interaction between alcohol and TLR4 as a possible risk factor for induction of HCC [55,57,58]. It appears that alcohol/TLR4 related HCC may happen through up-regulation of some HCC related molecules such as CXCL1, TLR4 and its intracellular signaling molecules [59–61].

The significant role of TLR4 in the pathogenesis of HCC was also demonstrated via *in vitro* and *in vivo* experiments by Liu and colleagues which revealed that TLR4 enhances invasion and migration of HCC cells [32]. The investigators also demonstrated that TLR4/LPS interaction can lead to HCC cell proliferation and also resistance to chemotherapy [30]. Liu et al., also demonstrated that TLR4 expressions were significantly increased in HCC tissues with microvascular invasion which is a main mechanism for development of chemotherapy resistance and also tumor growth [32].

Interestingly, investigations on HCC patients showed that elevated expression of TLR4 in HCC tissues was significantly associated with either early recurrence or poor survival [32,62]. Furthermore, administration of drugs which inhibit TLR4 signaling such as tumor suppressor serine/threonine-protein kinase 4 (STK4) [63], cisplatin and doxorubicin [45], protein tyrosine phosphatase receptor type O (PTPRO) [64], Emodin (1,3,8-trihydroxy-6-methylanthraquinone) [65] and TAK-242 (Resatorvid) [66] were also associated with protection against HCC.

TLR4 also induces metastasis of HCC. Accordingly, investigations showed that interactions between S100 calcium-binding protein A8 (S100A8)/high-mobility group box 1 (HMGB1), as internal DAMPs, and TLR4 lead to up-regulation of Ephrin-A1 (an important molecule for metastasis of cancer cells) and activation of Caspase-1, respectively

[67,68]. Caspase-1 activates pro-inflammatory cytokines and in combination with Ephrin-1 increases the chance of HCC progression and metastasis [68].

However, most studies which are listed here have been performed on the HCC cell lines and also animal models, hence, their results need to be verified in human models.

6. TLR4 and its roles in viral hepatitis induced HCC

In addition to mentioned investigations, it seems that the roles of TLR4 in the HCC related viral hepatitis can improve our knowledge regarding the roles of TLR4 in HCC pathogenesis, because it has been documented that HBV/HCV infections are the main risk factor for development of HCC. Accordingly, it has been hypothesized that HBV/HCV antigens may be considered as inducers of TLR4 functions and up-regulation.

LPS, as activator of TLR4 signaling, can be derived from either internal or external sources including intestinal microbiota [69,70]. Interactions between hyaluronan derived from malignant cells with TLR4 on neutrophils results in promotion of cancer cells motility in a TLR4/phosphoinositide 3-kinase (PI3K) dependent activation loop [71]. Investigations revealed that expression of TLR4 has a positive relation with deterioration of chronic hepatitis B liver injuries [72,73]. Thus, it seems that internal and external LPS, HBV/HCV antigens, alcohol, and internal DAMPs such as S100A8 and HMGB1 are the main TLR4 agonists and risk factors for HCC progression in susceptible patients. Based on the fact that some of the ligands are produced during chronic inflammation, such as chronic HBV/HCV infection, hence, it may be hypothesized that TLR4 induces immune surveillance in acute and HCC development in chronic inflammation. Interestingly, the effects of chronic inflammation on the induction of HCC have been demonstrated by previous studies [74,75].

Accordingly, a study by Wang et al., revealed that TLR4/HBxAg interaction leads to activation of extracellular signal-regulated kinases 1/2 (ERK1/2) signaling and consequently deterioration of HBV-related HCC [76]. Lu and colleagues reported that TLR4 is expressed on the liver dendritic cells (DCs) higher in HBV-related HCC than chronic HBV infected patients [77]. Thus, it appears that chronic HBV infection can be associated with production of HBxAg and consequently interaction of HBxAg with TLR4 on the liver DCs which is an important mechanism for induction of HCC.

HCV also up-regulates NANOG via interaction with TLR4 and consequently HCC development [78,79]. Moreover, TLR4 mediates synergism effects between alcohol and HCV, two important risk factors for HCC, involving NANOG [80]. Imran et al., demonstrated that nonstructural 5A (NS5A), a HCV protein, down-regulates the expressions of NKG2D on natural killer (NK) cells via interaction with TLR4. Down-regulation of NKG2D leads to decreased functions of NK cells and, hence, increased the chance of HCC progression [81]. Ectopic expression and activation of TLR4 can also participate in the pathogenesis of alcohol/HCV-associated HCC models [82]. As mentioned in previously, TLR4 is expressed by immune cells in normal conditions, but its expression can be induced on the hepatocytes and epithelial parenchyma cells ectopically during alcohol consumption and also HCV infection. Based on the study performed by Tsukamoto et al., ectopic expression of TLR4 is associated with increased risk of HCC in alcohol consumption and HCV infected animal models [82].

7. TLR4 genetic variations and HCC

It seems that up-regulation of TLR4 is associated with increased chances of HCC progression from inflamed liver. In addition to the roles played by TLR4 ligands in increased expression of TLR4 on the cell surface of immune cells and hepatocytes, it seems that genetic factors are also major factors to alter expression of TLR4 in susceptible patients. Zhang and colleagues reported that the rs11536889 single

nucleotide polymorphism (SNP) in TLR4 gene is associated with HCC risk in chronic hepatitis B infected patients [83]. On the other hand, the SNP has been more prevalent in the chronic hepatitis B infected patients with HCC than the patients without HCC [83]. Jiang et al., showed rs1057317 SNP in TLR4 gene is associated with increased expression of TLR4 and significantly increased the risk of HCC in a Chinese population [84]. Association between TLR4 adenosine/cytosine (A/C) (rs4986790, rs4986791) haplotype with HCC in a population from Saudi Arabia has also been demonstrated by Al-Qahtani and colleagues [85]. Another study on Spanish population demonstrated that TLR4 rs2148356 T allele can be associated with a reduced HCC risk [86]. The associations between decreased risk of HCC and rs10759930, rs2737190, rs10116253, rs1927914, rs12377632 and rs1927911 polymorphisms have been demonstrated in a Chinese population [87]. Interestingly, TLR4 polymorphisms are not only associated with HCC, but a meta-analysis review article by Zhang et al., also demonstrated that two SNPs within TLR4 gene including rs4986790 and rs4986791 are associated with increased risk of malignancy in other cancers [88]. Thus, it seems that genetic variations are risk factors for development of cancers including HCC.

8. TLR4 roles against HCC

Contrary to the aforementioned investigations, some investigators reported TLR4 may play protective roles against HCC. Zhang et al., revealed that hypoxia-inducible factor-1 α (HIF- α) silencing is associated with activation of TLR4-MYD88 dependent signaling pathway leading to regression of HCCs [89]. Another investigation revealed that synergistic effects of TLR4 by other innate immunity receptors including LRR and PYD domains-containing protein 3 (NACHT), LRR and pyrin domain

(PYD) domains-containing protein 3 (NALP3) or nucleotide-binding oligomerization domain-containing protein 1 (NOD1) can lead to dendritic cells (DCs) activation and consequently suppression of HCC [90]. Additionally, TLR4 deficiency is associated with decreased expression of X-ray repair cross complementing (XRCC)5, XRCC6 and Ku70, as DNA repair proteins, and it leads to failure in DNA repair, and may promote HCC development [91]. Earl and colleagues showed that TLR4 silencing leads to increase in liver tumor recurrence in a murine model [92].

Despite overwhelming evidence regarding the positive roles of TLR4 in progression of HCC, the controversy with the limited papers regarding the negative roles of the molecules in HCC progression may be defined as follow: 1. Previous investigations identified that the concentrations of TLR ligands are important factors to determine outcome of TLR functions [12]. Thus, it may be hypothesized that the concentrations of TLR4 ligands may be considered as critical factors for determination of TLR4 activation. 2. Types and structures of ligands and also the period of interaction (acute or chronic) may be considered as other factors to determine effects of TLR4/ligands interaction [12]. Thus, it seems that microenvironment of HCC cells are critical factors to induce TLR4 to play a role as tumor-induction factor. It has been demonstrated that normal tissue stroma acts as a barrier against tumor formation; however, in tumorigenic condition, the microenvironment changes in such a way to develop cancers [93,94]. A typical HCC microenvironment is composed of several immune and non-immune cells including immune and inflammatory cells, fibroblasts, pericytes, myofibroblasts, endothelial cells, adipose cells, and the elements [94]. The main immune cells infiltrated to the HCC are tumor-associated macrophages [95]. The macrophages induce anti-tumor immune responses via tumor cell phagocytosis and presentation of the antigens

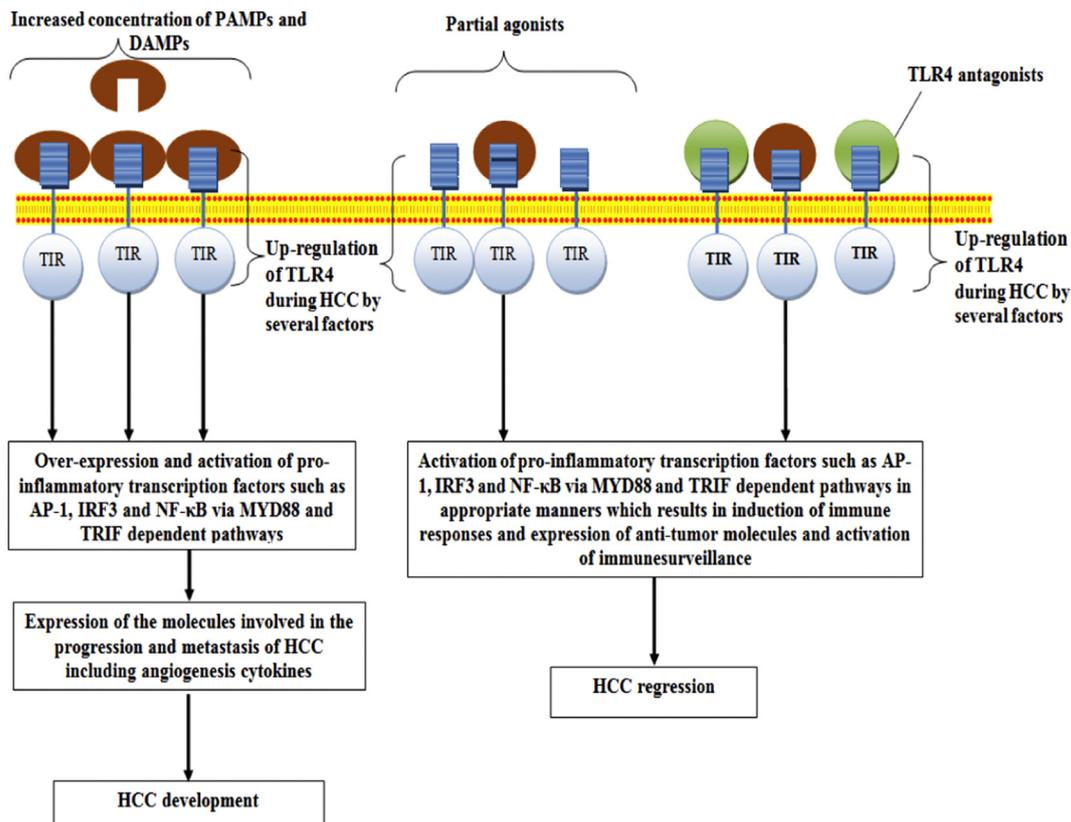


Fig. 2. Up-regulation of TLR4 happened during HCC by several factors which are described in Fig. 3. Hyperactivation of TLR4 by DAMPs and PAMPs is associated with over-expression and activation of pro-inflammatory transcription factors in a considerable manner which is associated with over-expression of the molecules involved in the HCC and development. Using TLR4 antagonists and partial agonists may be associated with involvement of lower expressed TLR4, hence, intracellular signaling has been induced by the limited TIRs which interact with partial agonists. TLR4 antagonists also block the up-regulated TLR4 and decreased the interaction of TLR4 with its ligands. Both strategies, using antagonists and partial agonists lead to under controlled activation of TLR4 which results in induction of appropriate immune responses and consequently HCC regression.

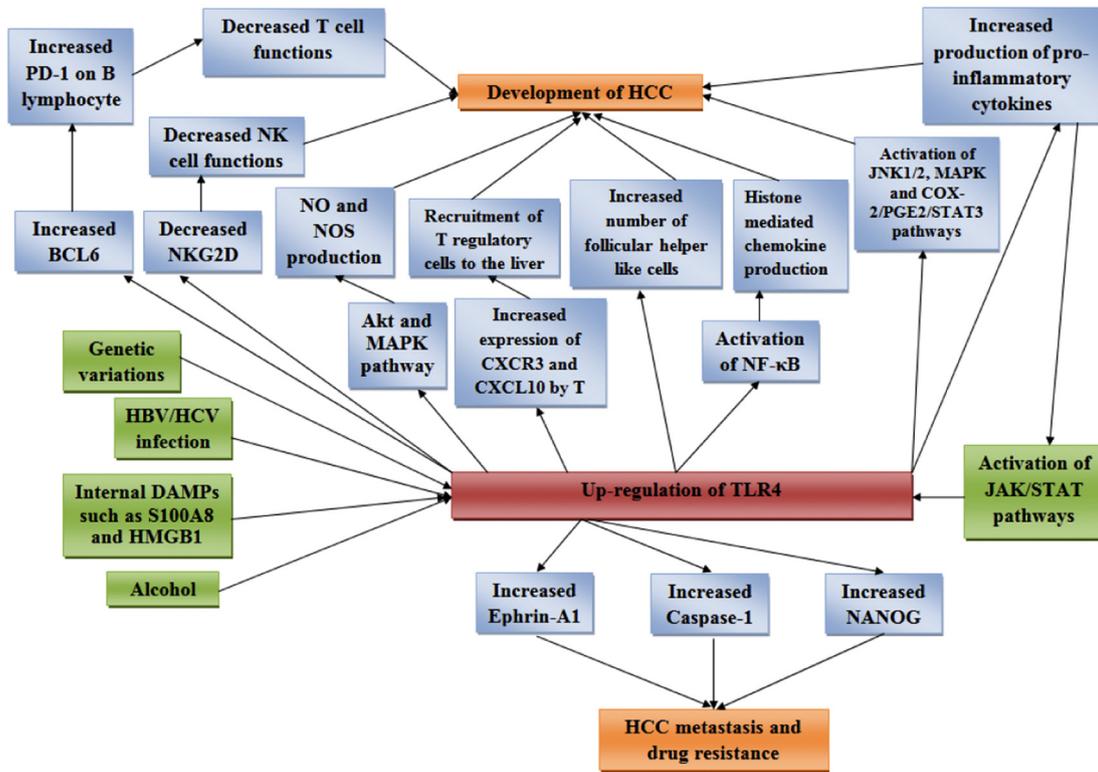


Fig. 3. The mechanisms used by TLR4 in development, metastasis and drug resistance of HCC. Genetic variations, chronic HBV/HCV infection, alcohol, internal DAMPs and chronic inflammation, via activation of JAK/STAT pathways, lead to up-regulation of TLR4. Increased expressions of TLR4 by resident immune cells and also hepatocytes result in increased Ephrin-A1, Caspase-1 and NANOG, which are the main inducers of HCC metastasis and drug resistance. Increased and decreased expressions of BCL6 and NKG2D, respectively, lead to impaired immunosurveillance against HCC development. No, NOS and pro-inflammatory cytokine production are also risk factors for development of HCC. Increased numbers of T regulatory and follicular T helper cells, via up-regulation of chemokines, are other effects of up-regulated TLR4 during HCC, which are associated with development of HCC.

to effector T lymphocytes at the site of HCC [96]. However, in the micro-environment of HCC, the functions of the macrophages are changed to alternative mode, which leads to tissue repairing and tumor development [97]. Based on the results presented in the current review article, it is hypothesized that altered expression and functions of TLR4, following interaction with HCC microenvironment, may be the main causes of the altered macrophage functions.

9. Conclusion

As mentioned in the aforementioned sentences, TLR4 can be considered as either anti- or pro-tumorigenic factor. In chronic inflammation, TLR4 uses several mechanisms to induce cell survival and HCC induction. Several TLR4 ligands up-regulate TLR4 and increase its functions, which leads to HCC progression. Due to the information presented in this review article, up-regulation and altered functions of TLR4 are a result of chronic inflammation and induction of HCC. Collectively, TLR4 activation is necessary to induce appropriate immune responses against HCC but TLR4 hyperactivation is not desirable. Thus it may be hypothesized that using TLR4 antagonists and partial agonists can be considered as a good strategy to induce TLR4 functions to elucidate appropriate immune responses against HCC (Fig. 2). Moreover, it may be possible to hypothesize that inhibition of TLR4-MyD88-TAK1-mediated NF-κB and MAPK pathways could be considered as a new approach for discovery of new drugs. Fig. 3 describes the main risk factor for up-regulation of TLR4 and also the mechanisms used by TLR4 for development, metastasis and drug resistance of HCC.

Conflict of interest

There are no conflicts of interest to declare.

Abbreviations

- AP-1 activator protein 1
- ARMD10 age-related macular degeneration 10
- BCL6 B-cell lymphoma 6
- CD cluster of differentiation
- COX-2 cyclooxygenase-2
- CXCL C-X-C motif ligand
- CXCR C-X-C motif receptor
- DAMP damage associated molecular patterns
- DCs dendritic cells
- ERK extracellular signal-regulated kinases
- HBV hepatitis B virus
- HBeAg hepatitis B virus e antigen
- HBxAg hepatitis B x antigen
- HCC hepatocellular carcinoma
- HCV Hepatitis C virus
- HIF-α hypoxia-inducible factor-1α
- HMGB1 high-mobility group box 1
- HSP heat shock protein
- IL interleukin
- IRF3 interferon regulatory factor 3
- JNK C-Jun N-terminal kinases
- LPS lipopolysaccharide
- LRRs leucine-rich repeats
- MAPK mitogen-activated protein kinase.
- MPLA monophosphoryl lipid A
- MYD88 myeloid differentiation primary response
- NF-κB nuclear factor-kappaB
- NALP3 NACHT, LRR and PYD domains-containing protein 3
- NK cells natural killer cells

NOD1	nucleotide-binding oligomerization domain-containing protein 1
NOS	nitric oxide synthase
NS5A	nonstructural 5A
PAMP	pathogen associated molecular patterns
PBMC	peripheral blood mononuclear cell
PD-1	programmed death 1
PD-L1	programmed death-ligand 1
PGE2	prostaglandin E2
PI3K	phosphoinositide 3-kinase
PRR	pathogen recognition receptors
PTPRO	protein tyrosine phosphatase receptor type O
PYD	pyrin domain
TAK1	TGF- β -Activated Kinase-1
TIR	toll/interleukin-1 receptor
TLR	toll like receptor
TNF	tumor necrosis factor
TRIF	TRIF-domain-containing adapter-inducing interferon- β
S100A8	S100 calcium-binding protein A8
SNP	Single nucleotide polymorphism
STAT3	Signal transducer and activator of transcription 3
STK4	serine/threonine-protein kinase 4
XRCC5	X-ray repair cross complementing

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