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**Innate Immunity and hepatocarcinoma: Can Toll Like Receptors open the door to oncogenesis?**

Lopes JA *et al*. Innate Immunity and hepatocarcinoma: Can Toll Like Receptors open the door to oncogenesis?

**Table 1: Table of original studies**

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| **Study** | **Year** | **Type of study** | **Methods** | **Limitations** | **Conclusions** |
| [Chew V](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chew%20V%5BAuthor%5D&cauthor=true&cauthor_uid=23197495), *et al1* | 2012 Dec | Experimental | Natural killer cell activation and cytotoxicity were assessed in vitro after treatment with the TLR3 ligand poly(I:C). The effect of TLR in a spontaneous liver tumor mouse model and a transplanted tumor mouse model were determined by Immunohistochemistry and PCR. | The effect of poly(I:C) on tumor growth was only analyzed in a transplanted, nonorthotopic model of HCC. The effect of poly(I:C) on human NK cells was assessed only with cells from healthy donors. Not all HCC cell lines undergo apoptosis after TLR3 triggering and the reason is not known. | TLR3 is an important modulator of HCC progression and is a potential target for novel immunotherapy. |
| [Mohamed FE](http://www.ncbi.nlm.nih.gov/pubmed/?term=Mohamed%20FE%5BAuthor%5D&cauthor=true&cauthor_uid=24990399), *et al2* | 2015 Mar | Experimental | Tissue microarrays containing liver samples from patients with cirrhosis, viral hepatitis and HCC were examined for expression of TLR7 and TLR9. Proliferation of human HCC cell lines was studied following stimulation of TLR7 and TLR9 using agonists (imiquimod and CpG-ODN respectively) and inhibition with a specific antagonist (IRS-954) or chloroquine. The effect of these interventions was confirmed in a xenograft model and diethylnitrosamine (DEN)/nitrosomorpholine (NMOR)-induced model of HCC. | Before translation to the clinical arena, it is important to further characterize the exact mechanisms through which  TLR7 and TLR9 exert their actions and determine what  effects their inhibition may have on the immune system . | Inhibiting TLR7 and TLR9 with IRS-954 or chloroquine could potentially be used as a novel therapeutic approach for preventing HCC development and/or progression in susceptible patients. |
| [Dapito DH](http://www.ncbi.nlm.nih.gov/pubmed/?term=Dapito%20DH%5BAuthor%5D&cauthor=true&cauthor_uid=22516259), *et al3* | 2012 Apr | Experimental | TLR2-deficient mice, TLR4-deficient mice, TNFR1-/IL-1R1-double deficient and C57Bl/6 mice were used. HCC was induced by intraperitoneal injection of DEN. Gut-sterilization was done using a combination of ampicillin (1 g/l), neomycin (1 g/l), metronidazole (1 g/l) and vancomycin (500 mg/l) in drinking water. Samples from patients with features of alcoholic hepatitis were used. Liver biopsies were obtained from mice and from cadaveric donners or resection of liver metastases. | Clinically feasible methods of targeting the intestinal microbiota or TLR4 need to be established. The quadruple combination of antibiotics employed is not suitable for long-term treatment due to known side effects in patients with advanced liver disease. | Gut sterilization restricted to late stages of hepatocarcinogenesis reduced HCC, suggesting that the intestinal microbiota and TLR4 represent therapeutic targets for HCC prevention in advanced liver disease. |
| [Eiró N](http://www.ncbi.nlm.nih.gov/pubmed/?term=Eir%C3%B3%20N%5BAuthor%5D&cauthor=true&cauthor_uid=23742263), *et al7* | 2014 Jul; | Experimental | The expression levels of TLR3, TLR4 and TLR9 were analyzed from 30 patients with HCC and correlated with various clinicopathological findings and with overall survival. | In the scoring system, after immunostaining analysis, when setting of the threshold for positive staining and the determination  of the intensity different observers can set different thresholds and intensity levels. | An association between TLR3, TLR4 and TLR9 expression and tumor aggressiveness and poor prognosis in HCC has been observed |
| [Liu S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20S%5BAuthor%5D&cauthor=true&cauthor_uid=12065483), *et al11* | 2002 Jul; | Experimental | Cultures of primary mouse hepatocytes were incubated with LPS to assess its effects on the global gene expression, hepatic transcription factors, and mitogen-activated protein (MAP) kinase activation | Using Hepatocytes’ cell lines loses the capacity to observe the importance of a direct response to LPS by hepatocytes | NF-kappa B activation was reduced in TLR4-mutant or -null hepatocytes compared to control hepatocytes. |
| [Matsumura T](http://www.ncbi.nlm.nih.gov/pubmed/?term=Matsumura%20T%5BAuthor%5D&cauthor=true&cauthor_uid=11054280), *et al12* | 2000 Oct; | Experimental | PCR analysis of mice’s hepatocytes and an Murine hepatoma cell line Hepa 1-6. | Murine hepatoma cell line Hepa 1-6  may have reached  an overquantitative level after stimulation. | LPS and proin-flammatory cytokines differentially regulate gene expression of TLR2 and TLR4 in murine hepatocytes, which may lead to pathologic and host defense reactions in the liver. |
| [Thobe BM](http://www.ncbi.nlm.nih.gov/pubmed/?term=Thobe%20BM%5BAuthor%5D&cauthor=true&cauthor_uid=17117477), *et al13* | 2007 Mar | Experimental | Wester blotting and cytokine analisis in a cell culture. Evaluation of Kupfer cells response after a trauma-hemorrage procedure. | Does not explain if the increase in MAPK-activity is due to TLRs’ overexpression. | Kupffer cell TLR signaling employs different MAPK pathways in eliciting cytokine and chemokine responses following trauma-hemorrhage. |
| [Knolle P](http://www.ncbi.nlm.nih.gov/pubmed/?term=Knolle%20P%5BAuthor%5D&cauthor=true&cauthor_uid=7790711), *et al14* | 1995 Feb; | Experimental | Human Kupffer cells were isolated by collagenase perfusion followed by centrifugal elutriation and analyzed for cytokine secretion after 3 days in culture. | Only interleukine 10 and 6 were analysed. | The important role for interleukin-10 in the regulation of the local immune response in the liver sinusoid after Kupffer cells exposure to to lipopolysaccharide |
| [Edwards AD](http://www.ncbi.nlm.nih.gov/pubmed/?term=Edwards%20AD%5BAuthor%5D&cauthor=true&cauthor_uid=12672047), *et al15* | 2003 Apr | Experimental | Splenocyte reparations were enriched for D11c+ and for Ly6C+ cells using magnetic selection. Four populations were routinely isolated and TLR’s mRNA was amplified by PCR. | To analyze the functional significance of TLR mRNA expression in DCs subsets it was only used ligands for TLR7 and TLR9 | mRNA for most TLRs is expressed at similar levels by murine splenic DC sub-types. TLR expression between plasmacytoid and non-plasmacytoid DC is not conserved between species. |
| [Sawaki J](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sawaki%20J%5BAuthor%5D&cauthor=true&cauthor_uid=17289654), *et al16* | 2007 Mar | Experimental | Total RNA was extracted, and mRNA for TLR1, 2, 3, 4, 5, 6, 7, 9 and b-actin was determined by reverse transcription–PCR. Nuclear localization of NF-kB was determined and cytokines and chemokines were measured by a commercially available kit. | It was not evaluated  precise roles of NK cell responses in vivo. | Upon microbial infection, macrophages produce IL-12 that renders NK cells highly responsive to TLR agonists to produce IFN-gamma and chemokines, which might in turn recruit and fully activate macrophages |
| [Meyer-Bahlburg A](http://www.ncbi.nlm.nih.gov/pubmed/?term=Meyer-Bahlburg%20A%5BAuthor%5D&cauthor=true&cauthor_uid=18039950), *et al17* | 2007 Dec | Experimental | It was compared the TLR response profile of germinal center after immunization versus naive mature B cell subsets, using real time PCR, ELISA and Western Blotting to evaluate MyD88 pathway. | TLRs’ role in B-cells immune response was only accessed in splenic B cells from MyD88 WT, heterozygote (Het), or  KO, being studied only the MyD88-dependent pathway. | B cell-intrinsic TLR signals are not required for antibody production or maintenance |
| [Paik YH](http://www.ncbi.nlm.nih.gov/pubmed/?term=Paik%20YH%5BAuthor%5D&cauthor=true&cauthor_uid=12717385), *et al18* | 2003 May | Experimental | LPS-associated signalling molecules in culture-activated HSCs and HSCs isolated from patients with hepatitis C virus-induced cirrhosis was evaluated by NF-kappaB-dependent luciferase reporter gene assays, electrophoretic mobility shift assays and in vitro kinase assays. | It does not fully explain why only full activated HSCs respond to LPS. It was not evaluated the activation of TLR4 downstream molecules like MyD88. | Human activated HSCs utilize components of TLR4 signal transduction cascade to stimulate NF-kappaB and JNK and up-regulate chemokines and adhesion molecules |
| [Wu J](http://www.ncbi.nlm.nih.gov/pubmed/?term=Wu%20J%5BAuthor%5D&cauthor=true&cauthor_uid=19922426), *et al19* | 2010 Mar; | Experimental | Isolated Kupffer cell and liver sinusoidal endothelial cells from wild-type C57BL/6 mice and examined their responses to TLR1 to TLR9 agonists. Characterization of cell surface protein expression was done by flow cytometry and quantification of mRNA was done by reverse transcription–polymerase chain reaction. | The in vitro assay does not explore the organ-specific regulation of immune responses. For the identification of TLR-induced antiviral cytokine(s) only TLR3 and TLR4 were used. | Non-parenchymal cells display a restricted TLR-mediated activation profile when compared with 'classical' antigen-presenting cells which may, at least in part, explain their tolerogenic function in the liver. |
| [Huang Y](http://www.ncbi.nlm.nih.gov/pubmed/?term=Huang%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=22815694), *et al20* | 2012 Jul | Experimental | TLR expression in BLE-7402 cells was assayed by RT-PCR, real-time PCR and flow cytometry (FCM). To investigate the function of TLR2 in hepatocarcinoma growth, BLE-7402 cells were transfected with recombinant plasmids expressing one TLR2 siRNA | Only the effect on tumour volume is evaluated after tumour implantation in nude mice. | TLR2 knockdown inhibit proliferation of cultured hepatocarcinoma cells and decrease the secretion of cytokines. |
| [Kim S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kim%20S%5BAuthor%5D&cauthor=true&cauthor_uid=19122641), *et al21* | 2009 Jan | Experimental | LLC cells were implanted in mice. Metastasis enhancing factors were identified on a QSTAR XL qQTOF mass spectrometer. Gene and protein expression were monitored by Q-PCR and immunoblot analysis. Tumors were analyzed by immunohistochemistry and indirect immunofluorescence. | It does not explain if the interaction between versican and TLR2 is direct or depends on a versican’s ligand. | By activating TLR2:TLR6 complexes and inducing TNF-alpha secretion by myeloid cells, versican strongly enhances Lewis Lung Carcinoma metastatic growth. |
| [Lin H](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lin%20H%5BAuthor%5D&cauthor=true&cauthor_uid=22859216), *et al23* | 2013 Jan | Experimental | A DEN injection was done in TLR2-/- and WT mice. Than they were sham-treated or treated with interferon-gamma. TUNEL, heterochromatin and SA b-gal staining were performed. | The mechanism by which  TLR2 signaling participates in the regulation of cellular  senescence to maintain growth arrest and promote programmed  cell death remains inconclusive. | Loss of immune networks may play a  role in the failure of initiating and maintaining cellular  senescence and autophagy flux in the TLR2-mutant liver  tissue. |
| [Lin H](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lin%20H%5BAuthor%5D&cauthor=true&cauthor_uid=24098333), *et al24* | 2013 Oct | Experimental | WT mice were pre-treated with anti-TLR2 antibody and a subset of TLR2-/- mice were pre-treatment with NAC (antioxidant) or physiological saline. Both were submitted to DEN. Histology was submitted to western blotting, ROS assay, immunohistochemistry and immunofluorescence. | It does not report any results about the effects on non-parenchymal cells like Kuppfer cells. It does not reveal interactions that regulate the signal from TLR2 activation to suppression of oxidant and ER stressors in HCC. | A TLR2 activity defends against hepatocarcinogenesis through diminishing the accumulation of ROS and alleviating ER stress and unfold protein response. |
| [Li S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Li%20S%5BAuthor%5D&cauthor=true&cauthor_uid=25600646), *et al25* | 2015 Mar | Experimental | WT and Tlr2–/– mice were used. Flow cytometry, Histopathological analysis and Immunofluorescence, Western blot and ELISA were performed. MDSC induction  in vitro and functional T cell suppression assay and knockdown of IL-18 and caspase-8 in hepatocytes with Quantitative PCR were also done. | The exact role of IL-18 in  MDSC generation is still unknown.  It does not reveal the levels of TLR2 that determine the possible use of IL-18 as a therapeutic target. | TLR2 deficiency accelerates IL18-mediated immunosuppression during liver carcinogenesis, providing new insights into immune control that may assist the design of effective immunotherapies to treat HCC. |
| [Soares JB](http://www.ncbi.nlm.nih.gov/pubmed/?term=Soares%20JB%5BAuthor%5D&cauthor=true&cauthor_uid=22330637), *et al26* | 2012 Oct | Analytic – cross sectional | It was used samples from patients with hepatitis, cirrhosis and hepatocarcinoma. mRNA isolation and quantification of TLR2, TLR4, NF-kB, TNF-α and COX-2 were performed. Immunohistochemical evaluation of TLR2 and TLR4 was also done. | Most patients included in the reference group have evidence  of NAFLD and it was demonstrated that NAFLD is associated with increased hepatic TLR2 and TLR4-mRNA expression. the hepatitis,  cirrhosis and hepatocarcinoma groups included both patients with HBV infection or HCV infection. Included only patients with virus-induced chronic hepatitis. The method used for quantification of protein expression was semi-quantitative. | Increased expression of TLR2 and TLR4 in hepatitis and cirrhosis and maintained expression in hepatocarcinoma. Up-regulation of TLR2, TLR4 and their pro-inflammatory mediators is associated with virus-induced hepatic IFC sequence. |
| [Dolado I](http://www.ncbi.nlm.nih.gov/pubmed/?term=Dolado%20I%5BAuthor%5D&cauthor=true&cauthor_uid=17292829), *et al30* | 2007 Feb | Experimental | WT and p38a-/- were used. Growth in soft agar was evaluated. Intracellular ROS levels were determined, immunoblot Analysis was performed. To induce p38 MAPK activation, cells were treated with H2O2, sorbitol and cisplatin. | The tumorigenesis enhanced by ROS is not evaluated on hepatocarcinoma. | Oxidative stress sensing plays a key role in the inhibition of tumor initiation by p38alpha. |
| [Kang TW](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kang%20TW%5BAuthor%5D&cauthor=true&cauthor_uid=22080947), *et al31* | 2011 Nov | Experimental | For transposon-mediated intra-hepatic gene transfer mice received a transposon- to transposase encoding vector (30  mg total DNA). DNA was administered by hydrodynamic tail vein injection. Immunohistochemical analyses  were performed. | It was not investigated if factors secreted from pre-malignant senescent hepatocytes also contribute to the oncogenic transformation of neighbouring cells. | Indicates that senescence surveillance represents an important extrinsic component of the senescence anti-tumour barrier, and illustrates how the cellular senescence program is involved in tumour immune surveillance. |
| [Ogata M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ogata%20M%5BAuthor%5D&cauthor=true&cauthor_uid=17030611), *et al33* | 2006 Dec | Experimental | Electron microscopic analysis was performed using neuroblastoma SK-N-SH cells exposed to ER stressors. GFP-LC3 fluorescence was used to monitor autophagy in cells transiently transfected with an expression vector for GFP-LC3. Then was performed an Amino acid uptake assay and autophagosome formation was evaluated. | A signalling pathway other than the IRE1-JNK pathway  may also play important roles in the activation of autophagy  signalling after ER stress. The detailed signalling pathway for  activation of the autophagy induced by ER stress is still unknown. | Disturbance of autophagy rendered cells vulnerable to ER stress, suggesting that autophagy plays important roles in cell survival after ER stress. |
| [Pikarsky E](http://www.ncbi.nlm.nih.gov/pubmed/?term=Pikarsky%20E%5BAuthor%5D&cauthor=true&cauthor_uid=15329734), *et al35* | 2004 Sep | Experimental | The possibility that NF-kβ activation is  involved in Mdr2-knockout hepatocarcinogenesis was investigated by RelA (p65) immunostaining. Hystological analysis was performed. To study the relationship between the TNFα-producing cells and NF-kβ activation in the hepatocytes, liver sections were stained for both TNFα and p65. | It does not explain how the inflammatory process in Mdr2-knockout mice is maintained in the double mutants as it is independent of hepatocyte NF-kβ activity. | NF-kappaB is essential for promoting inflammation-associated cancer, and is therefore a potential target for cancer prevention in chronic inflammatory diseases. |
| [Gong W](http://www.ncbi.nlm.nih.gov/pubmed/?term=Gong%20W%5BAuthor%5D&cauthor=true&cauthor_uid=23836405), *et al36* | 2013 Sep | Experimental | BALB/c mice were used and inoculated with H22 hepatocarcinoma cells into the hind thigh muscle. They were treated with TLR2/ 4 ligands, HSP70 and HMGB1. The main tumor nodules were measured and satellite tumor nodes counted. To downregulate HMGB1, RAGE or Beclin-1 in tumor cells, cells were transduced with *short interfering RNA.* | It does not explain the mechanisms responsible by the Nf-kβ’s phosphorylation in the first 30 minutes. It was observed only one of the pathways responsible for the involvement of HMGB1/RAGE in the NF-κB signaling. | Activation of NF-κB was indispensable for the effect of HSP70. HSP70 induced a positive feedback loop involving Beclin-1/HMGB1 production, causing re-phosphorylation of NF-κB. |
| [Shi W](http://www.ncbi.nlm.nih.gov/pubmed/?term=Shi%20W%5BAuthor%5D&cauthor=true&cauthor_uid=24964964), *et al37* | 2014 Oct | Experimental | Human hepatocellular carcinoma cell lines were used. Into the cell lines were transfected small-interfeering-RNAs and at 48 h after transfection, the TLR2-siRNA-transfected group, scramble control group, and blank  Group were treated with recombinant-HMGB1. Evaluation included real time PCR, Western blot, MTT assay, Transwell assay and Flow cytometry assay. | It does not explore the signaling pathway that regulates NF-kβ through TLR2 inhibition or stimulation with recombinant-HMGB1. | TLR2-siRNA could  effectively inhibit the growth, migration, invasion, and expression  of NF-κB/P65, and HMGB1 promoted HCC progression  via TLR2 |
| [Wu FH](http://www.ncbi.nlm.nih.gov/pubmed/?term=Wu%20FH%5BAuthor%5D&cauthor=true&cauthor_uid=22115967), *et al38* | 2012 Apr | Experimental | It was used mice and HCC cell lines. Eukaryotic expression vectors psTLR2 and psTLR4 were created. An adhesion assay, a tumor cell proliferation assay, a flow cytometric analysis, an apoptosis analysis, an analysis of gene expression by RT-PCR and a western blot analysis were performed. | More than one signaling pathways activated by HSPA1A might be required for the survival of tumor cells. The effect of eHSPA1A was only evaluated in one cell line. Injection of HSPA1A suppressed  tumor growth in early stage of tumor development, but promoted tumor growth in later stage | Extracellular HSPA1A functions as endogenous ligand for TLR2 and TLR4 to facilitate tumor growth. |
| [Yoneda K](http://www.ncbi.nlm.nih.gov/pubmed/?term=Yoneda%20K%5BAuthor%5D&cauthor=true&cauthor_uid=18949355), *et al40* | 2008 Nov | Experimental | HCC cell lines and 74 HCC samples were used. Poly I:C, cycloheximide and actinomycin were included in the study. Profiling analysis of TLRs recognized by viral components, Flow cytometric analysis, Immunohistochemical staining, Detection of TLR3 by immunofluorescence, Detection of cell viability and apoptosis assays, Detection of apoptosis-related proteins by immunoblotting, NF-kB activity assays and measurement of IFN-ß were also performed. | Further evaluation of the possible roles and the type of regulation associated with TLR3 needs to be undertaken. | Intracellular TLR3 signalling is involved in cell death, while in contrast, the cell surface TLR3 signalling is responsible for activation of NF-κB. |
| [Zorde-Khvalevsky E](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zorde-Khvalevsky%20E%5BAuthor%5D&cauthor=true&cauthor_uid=19441101), *et al41* | 2009 Jul | Experimental | It was used TLR3-WT mice and TLR3-/- mice. Partial hepatectomy was done followed by Immuonhistochemistry Stainings, Plasma Aminotransferase Activity Assay, Measurements of Serum Cytokine Levels, Semi-quantitative Reverse-Transcription Polymerase Chain Reaction, Western Blotting, Caspase-8 Immunopurification and injection with poly(IC) or saline solution. | It is not explained what happens to the levels of ALT in mice’s serum before the 10-hour time point following 70% PHx. Cytokine evaluation only includes IL-6 and IL-22. | TLR3 plays an inhibitory role in the priming of liver regeneration, thus reinforcing the role of the innate immune system in balancing tissue regeneration. |
| [Khvalevsky E](http://www.ncbi.nlm.nih.gov/pubmed/?term=Khvalevsky%20E%5BAuthor%5D&cauthor=true&cauthor_uid=17243100), *et al43* | 2007 Apr | Experimental | Various cell lines and Plasmids pTLR7, pTLR8, and pTLR9, carrying the respective human TLR gene, were used. Transfection Assays, RNA Quantification, Immuno-Staining and Flow Cytometry, were performed. | The role of TLR3 signaling in normal hepatocytes requires further investigation in vivo. It is not specified the degree of NF-kβ activation obtained from the overexpression of TLR3 nor the degree of this overexpression that is needed. | Preferential induction of the apoptotic pathway over the cytokine induction pathway by TLR3 signaling in hepatocellular carcinoma cells with potential implications for therapeutic strategies. |
| [Chen L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chen%20L%5BAuthor%5D&cauthor=true&cauthor_uid=22552584), *et al44* | 2012 Jul | Experimental | The human HCC cell line HepG2.2.15 was used. After treating HepG2.2.15 with BM-06 or poly(I:C), NF-κB activity was checked by dual luciferase reporter gene kit. Then it was performed a Nuclear and cytoplasmic extraction, Western blot analysis, a Cell proliferation assay, Cell invasion assays and Flow-cytometry was used to determine the apoptotic rate. | The role of TLR3 in the antiviral defense against HBV was not analyzed according to differences in the type of viruses, the type of cells that are infected, the viral load, its model of infec­tion (endoplasmic versus cytoplasmic), and stage of infection. | BM-06 inhibited the proliferation, invasion and secretion of HBV, and induced apoptosis in HepG2.2.15 cells. In addition, the antitumor effects of BM-06 were superior to poly(I:C). |
| [Guo Z](http://www.ncbi.nlm.nih.gov/pubmed/?term=Guo%20Z%5BAuthor%5D&cauthor=true&cauthor_uid=22075935), *et al45* | 2012 Feb | Experimental | Cell cultures were used and submitted to BM-06 and poly(I:C) treatment. RNA isolation and one-step quantitative real-time PCR were performed. Analysis included Detection of TLR3 by immunocytochemistry, Luciferase reporter assays, Endothelial cell tube formation assay, rat aortic ring assay, Annexin V/PI for cell apoptotic analysis and Cell migration assays. | It does not evaluate the molecular mechanisms after TLR3 stimulation that lead to modulation of endothelial tube-forming activity of HUVECs and vascular sprouting or enhanced apoptosis. | TLR3 agonists not only affect tumor microenvironment by suppressing angiogenesis but also directly induce tumor cell apoptosis and inhibit tumor cell migration. |
| [Bergé M](http://www.ncbi.nlm.nih.gov/pubmed/?term=Berg%C3%A9%20M%5BAuthor%5D&cauthor=true&cauthor_uid=20971743), *et al46* | 2010 Dec | Experimental | It was injected transgenic mice developing hepatocellular carcinoma (HCC) with either control siRNAs or siRNA targeting neuropilin-1. The study used antibodies (goat anti-TLR3 and rabbit anti-tubulin antibody), Western Blotting, and Immunofluorescence Analysis. Real-Time RT-PCR, ELISA, MTT Assay and three-Dimensional Collagen Assay were also performed. | It is not known why INF-γ does not inhibit cells’ functions in the in vitro study despite the high levels in HCC. In vivo evaluation was not performed. | Synthetic siRNAs inhibit target-independently HCC growth and angiogenesis through the activation of the innate interferon response and by directly inhibiting endothelial cell function. |
| [Xu YY](http://www.ncbi.nlm.nih.gov/pubmed/?term=Xu%20YY%5BAuthor%5D&cauthor=true&cauthor_uid=23970360), *et al47* | 2013 Oct | Experimental | Thirty rats were used, all 30 were fed with 2‑acetylaminofluorene to establish the HCC model. Two animal groups were treated, respectively, with the drug candidate (BM‑06) and poly(I:C). It was performed a Hematoxylin and eosin (H&E) staining, an Immunohistochemical staining, a Western blot analysis | It does not explore the pathway though which BM‑06 and poly(I:C) are capable of inducing cell death. It is not evaluated TLR3’s downstream molecules to explain the signalling pathway responsible for these results. | Treatment with BM-06, showed a decrease in tumor growth and cell proliferation, and an increase in apoptosis compared with that in a phosphate‑buffered saline control group. |
| [Wang L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Wang%20L%5BAuthor%5D&cauthor=true&cauthor_uid=23828139), *et al48* | 2013 Aug | Experimental | Fifty-three HCC and ten normal liver specimens were analyzed by immunohistochemistry, and three cell lines were used for in vitro studies. Lipopolysaccharide was used to activate TLR4 signaling. Cell survival, proliferation and invasion were examined | Only a specific amount of LPS has shown to have an effect on the mRNA expression of IL-6, EGFR  and HB-EGF. Opposing to HL-7702 cell line, PLC/PRF/5, with a moderate level of TLR4 expression, was not affected by inhibiting p38. | Indicate that TLR4 signaling in cancer cells promotes cell survival and proliferation in HCC. |
| [Liu WT](http://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20WT%5BAuthor%5D&cauthor=true&cauthor_uid=25511737), *et al49* | 2015 Mar | Experimental | Two HCC cell lines and a splenic vein metastasis of the nude mouse model were used. A total of 88 clinical samples from HCC patients were used. A fluorescence activated cell sorting system and flow cytometry analysis were performed. Nude mouse splenic vein metastasis assay, Immunohistochemistry analysis, Real-time quantitative PCR, Western blot analysis, Immunofluorescence and Cell apoptosis assay were also done. | More pathological specimens should be enrolled to verify the tendencies of association between TLR4 expression and malignant characteristics of HCC found in this study. A particular signaling pathway involved in the relationship between TLR4 expression and stem cell features remains elusive. | There is a relationship between TLR4 expression and CSC's features, TLR4 may act as a CSC marker, prompting tumor invasion and migration, which contributes to the poor prognosis of HCC. |
| [Li H](http://www.ncbi.nlm.nih.gov/pubmed/?term=Li%20H%5BAuthor%5D&cauthor=true&cauthor_uid=25053598), *et al51* | 2014 Oct | Experimental | A HCC cell line was used where a Scratch assay was performed. Invasion assay, Western blot analysis, Quantitative real-time reverse transcription PCR and siRNA knockdown of TLR4 gene expression were also done. | It does not reveal the time needed for induction of epithelial-mesenchymal transition after LPS stimulus. Does not explore influence of LPS on TLR2. | TLR4/JNK/MAPK signaling is required for LPS-induced EMT, tumor cell invasion and metastasis, which provide molecular insights for LPS-related pathogenesis and a basis for developing new strategies against metastasis in HCC. |
| [Jing YY](http://www.ncbi.nlm.nih.gov/pubmed/?term=Jing%20YY%5BAuthor%5D&cauthor=true&cauthor_uid=22938142), *et al52* | 2012 Aug | Experimental | Four HCC cell lines and a splenic vein metastasis of the nude mouse model were used and stable TLR4-expressed and knocked-down cell lines were generated. 106 clinical samples from HCC patients were also used. Quantitative real-time polymerase chain reaction, Western-blot analysis, Immunofluorescence, FACS Analysis and IHC analysis were performed. | HCC development is a multifactorial  and complicated process, which has a close association with various risk factors. Many gene alterations and  cytokines also could induce EMT. HCC cells with low expression  or even a lack of TLR4 are not susceptible to LPS, they might perform EMT induced by other TLR4-independent mechanisms. | TLR4 signaling is required for LPS-induced EMT, tumor cell invasion and metastasis, which provide molecular insights for LPS-related pathogenesis and a basis for developing new strategies against metastasis in HCC. |
| [Xu D](http://www.ncbi.nlm.nih.gov/pubmed/?term=Xu%20D%5BAuthor%5D&cauthor=true&cauthor_uid=25034527), *et al*.53 | 2014 Oct | Experimental | HCC and adjacent tissues were obtained from 84 patients. HCC cell lines were used and a PLV-PTPRO-GFP plasmid was constructed. Real-time polymerase chain reaction, Immunofluorescence, Western blot analysis and Cell proliferation assay were performed. | It does not specify the doses of NF-κB specific inhibitor needed to result in a decreasing of PTPRO’s levels  in HuH7 cells stimulated with LPS. | The effect of PTPRO on TLR4 signaling is dependent on NF-κB pathway, suggesting an interesting PTPRO/TLR4/NF-κB signaling feedback loop in HCC carcinogenesis and progression. |
| [Wang Y](http://www.ncbi.nlm.nih.gov/pubmed/?term=Wang%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=25475726), *et al54* | 2015 Jan | Experimental | It was used LPS-induced human hepatocellular carcinoma cell lines. Cell viability was assessed using the MTT assay. Double staining for Annexin V-FITC and propidium iodide was performed. Inflammatory mediators were evaluated through a specific ELISA kit. Immunoprecipitation and Western blot analysis were also used. | Only one type of cell line is used to observe the effect of CXC-195. It does not reveal the level (high or low) of TLR4 expression. It does not explore the influense of LPS in TLR2. | Treatment with CXC195 could attenuate the TLR4-mediated proliferation and inflammatory response in LPS-induced HepG2 cells |
| [Yu LX](http://www.ncbi.nlm.nih.gov/pubmed/?term=Yu%20LX%5BAuthor%5D&cauthor=true&cauthor_uid=20803560), *et al55* | 2010 Oct | Experimental | Rats and mice were used, including TLR4-deficient mice. Immunohistochemical analysis and Bone Marrow Transplantation were performed. | It does not explore the effect of modulating gut flora. It does not evaluate the effect of different LPS’ levels | Sustained LPS accumulation represents a pathological mediator of inflammation-associated hepatocellular carcinoma (HCC) and manipulation of the gut flora to prevent pathogenic bacterial translocation. |
| [Lin Y](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lin%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=22617167), *et al57* | 2012 Sep | Experimental | It was used wild-type and TLR4-deficient mice. A Flow cytometry analysis and Isolation and Culture of CD4+ cells were performed. | TLR4 knockout showed decreased liver injury induced by Con A, contrarily to what was expected. It is needed to determine whether the regimen with antiendotoxin effects will prove beneficial in preventing or delaying Tcell–mediated hepatitis and hepatitis-induced HCC. | Gut-derived LPS and TLR4 play important positive roles in Con A-induced hepatitis and modulation of the gut microbiotia may represent a new avenue for therapeutic intervention |
| [Chen CL](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chen%20CL%5BAuthor%5D&cauthor=true&cauthor_uid=23921128), *et al59* | 2013 Jul | Experimental | It used HCV Tg mouse models and patients with HCC functional cDNA. Then, functional cDNA screening for oncogenes was performed. In vitro and in vivo oncogenic activities were evaluated. It was also done a Liver TIC engraftment via splenic injection  . | The degree of attenuation of  Tlr4 expression in TICs by Nanog, implying a feedback loop is not shown. Besides this, the underlying mechanisms are not known. | TLR4/NANOG oncogenic pathway is linked to suppression of cytostatic TGF-β signaling and could potentially serve as a therapeutic target for HCV-related HCC. |
| [French SW](http://www.ncbi.nlm.nih.gov/pubmed/?term=French%20SW%5BAuthor%5D&cauthor=true&cauthor_uid=23773849), *et al62* | 2013 Aug | Experimental | Liver biopsies from patients diagnosed with alcoholic hepatitis, with or without cirrhosis were selected. Double Immunohistochemistry was performed. | The antibody stain was only against TLR4. | The Mallory-Denk-bodies forming cells expressed two additional progenitor cell markers. These markers were CD49f and TLR4. |
| [Machida K](http://www.ncbi.nlm.nih.gov/pubmed/?term=Machida%20K%5BAuthor%5D&cauthor=true&cauthor_uid=25427905), *et al*.63 | 2014 Nov | Experimental | An immunostaining of liver tumor sections from alcohol-fed Ns5a mice was performed along with TLR4 silencing with lentiviral short-hairpin RNA | LPS- independent mechanisms of TLR4 activation in TICs remain to be elucidated.  The oncogenic role of TLR4 is explored only around the synergism alcohol-HCV. | TLR4-dependent mechanisms of  TIC generation actually contribute to or at least promote the initiation of HCC |
| [Yan W](http://www.ncbi.nlm.nih.gov/pubmed/?term=Yan%20W%5BAuthor%5D&cauthor=true&cauthor_uid=22234969), *et al64* | 2012 Jun | Experimental | Human HCC liver samples and mice were used. Stable HMGB1-Expressing Cells and HMGB1 Knockdown Cells were established. Immunoblotting Analysis, RNA Interference by Short Interfering RNA, Enzyme-Linked Immunosorbent Assay, Confocal Microscopy exam, Caspase-1 Colorimetric Assay, Cell Migration and Invasion Assays and metastatic potencial exam were all performed. | Mechanisms by which caspase-1 affects tumor cancer progression remain incompletely understood. | In hypoxic HCC cells, HMGB1 activates TLR4- and RAGE-signalling pathways to induce caspase-1 activation which, in turn, promote cancer invasion and metastasis. |
| [Xu N](http://www.ncbi.nlm.nih.gov/pubmed/?term=Xu%20N%5BAuthor%5D&cauthor=true&cauthor_uid=18215354), *et al66* | 2008 Feb | Analytic – cross sectional | 52 patients were studied. The protein and mRNA levels of TLR7 and TLR9 were evaluated using real-time PCR, Western blot analysis, and flow cytometry. We also detected the serum viral load of HBV in the patients and analyzed the correlation between HBV-DNA copies and the TLR expression. | The statistical analysis indicated no difference in the TLR9 levels  among the HCC and LC groups. If the sample size was enlarged, the results may be different. The expression of TLR7 was not different among the groups of patients, suggesting that TLR7 has no correlation with HCC. | There are downregulations of TLR7 expression and TLR9 mRNA in PBMC of HBV-infected patients, but an increased TLR9 expression at the protein level. |
| [Tanaka J](http://www.ncbi.nlm.nih.gov/pubmed/?term=Tanaka%20J%5BAuthor%5D&cauthor=true&cauthor_uid=20811701), *et al67* | 2010 Oct | Experimental | HCC cell lines and 42 HCC tissues were used. The type C CpG oligonucleotide was used as TLR9 ligand. Flow cytometric analysis, Immunohistochemical staining, Cell proliferation assay, Immunoblotting, NF-κB activity assays and expression analysis of IRF-7, RNA extraction and oligonucleotide microarray and Microarray data analysis were all performed. | Despite being present both intracellular or extracellualar TLR9´s intracellular function is not observed with TLR9 ligands and its function is not known. | Functional cell surface expression of TLR9 in human HCC may play an important role in tumorigenesis and cancer progression. |
| [Liu Y](http://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=25681553), *et al68* | 2015 Feb | Experimental | C57BL6 mice were injected with Hepa1-6 cancer cells. TLR9 and HMGB1 were inhibited using shRNA or direct antagonists. HuH7 and Hepa1-6 cancer cells were investigated in vitro to determine how the interaction of HMGB1 and mtDNA activates TLR9 signaling pathways. | The contribution of TLR9 to cancer pathophysiology remains incompletely understood. The regulation of TLR9 signaling and the  physiological ligands which may induce TLR9 mediated tumor growth remain poorly characterized. | Reveals a novel mechanism by which the interactions of HMGB1 and mtDNA activate TLR9 signaling during hypoxia to induce tumor growth. |
| [Zhang Y](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhang%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=25224571), *et al69* | 2014 Dec | Experimental | It was used HCC cell lines to where was transfected CpG oligodeoxynucleotide and poly(I:C). Proliferation analyses, Detection of apoptosis with an Apoptosis Detection Kit, Quantitative real-time PCR analysis, Western blot analysis and Fluorescence microscopy were also performed. | The precise molecular interactions that likely occur between CpG ODNs and poly(I:C) to block poly(I:C) entry, remain to be established. Poly(I:C) may be influenced by many molecules in the microenvironment. | When combining poly(I:C) and CpG ODN for cancer therapy, these agents should be used in an alternating rather than simultaneous manner to avoid the blocking effect of phosphorothioate-modified TLR9 ligands. |
| [Zhang Y](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhang%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=24452201), *et al70* | 2014 Apr | Experimental | Human hepatoma cell lines were used. Cells were transfected with CpG ODNs or small interfering RNAs targeting TLR9. Reverse transcriptase polymerase chain reaction assay, Proliferation measurements, Cell cycle analysis, Detection of apoptosis, Quantitative real-time PCR analysis, Western blot analysis were all performed. An in vivo study was also done. | Apoptosis induced by ODN M362 Ctrl and ODN M362 occurred independently of TLR9 stimulation. TLR9- and MyD88-independent mechanisms in ODN-stimulated immune cells, including B lymphocytes and neutrophils may exist. | Phosphorothioate-modified TLR9 agonist ODN M362, and its control, elicit antitumor activity in HCC cells and may serve as a novel therapeutic target for HCC therapy. |
| [Bubici C](http://www.ncbi.nlm.nih.gov/pubmed/?term=Bubici%20C%5BAuthor%5D&cauthor=true&cauthor_uid=15611622), *et al73* | 2004 Dec | Perspective |  |  | Induction of FHC and Mn-SOD represents an additional, indirect means by which NF-kappaB controls proapoptotic JNK signaling. |
| [Liu X](http://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20X%5BAuthor%5D&cauthor=true&cauthor_uid=19287992), *et al74* | 2009 Apr | Experimental | Cell cultures were used. Immunocytochemistry stain for TLR9, a Cell proliferation assay, reverse transcriptase PCR for TLR9 and real-time reverse transcriptase PCR for DNMT-1 and Bcl-2, NF-kβ activation measurement and Cellular apoptosis analysis were all performed. | L-02 cells were used to allow in vitro studies but cells may behave differently *in vivo. Future in vivo* models are needed. | Identified a possible novel mechanism that indicates how CpG DNA of HBV DNA may contribute to the malignant transformation of benign liver cells. |
| [Nischalke HD](http://www.ncbi.nlm.nih.gov/pubmed/?term=Nischalke%20HD%5BAuthor%5D&cauthor=true&cauthor_uid=21500195), *et al75* | 2012 Mar | Analytic – cross sectional | A total of 197 patients with HCV-associated HCC, 192 HCV-infected patients without HCC and 347 healthy controls were included. HCV antibodies were detected for diagnosis. Determination of TLR2-196 to -174 del/ins polymorphism was performed by LightCycler real-time PCR. In vitro induction of TLR2 expression and interleukin-8 was performed. | Analysis of the functional role of TLR2 -196 to -174 del/ins alleles with respect to TLR2 expression was based on in vitro stimulation studies but it is not known if an in vivo analysis would have the same results. | TLR2 -196 to -174 del allele to affect HCV viral loads and to increase the risk for HCC in HCV genotype1-infected patients. |
| [Junjie X](http://www.ncbi.nlm.nih.gov/pubmed/?term=Junjie%20X%5BAuthor%5D&cauthor=true&cauthor_uid=22309608), *et al76* | 2012 Feb | Single center-based case-control | SNaPshot method was used to genotype sequence variants of TLR2 and TLR9 in 211 patients with HCC and 232 subjects as controls. | Despite the SNP rs3804099 and rs3804100 were out of  HWE (P= 0.01 - 0.02), they were retained in the analyses. | TLR2 rs3804099 C/T and rs3804100 C/T polymorphisms were closely associated with HCC. In addition, the haplotypes composed of these two TLR2 synonymous SNPs have stronger effects on the susceptibility of HCC. |
| [Jiang ZC](http://www.ncbi.nlm.nih.gov/pubmed/?term=Jiang%20ZC%5BAuthor%5D&cauthor=true&cauthor_uid=25179842), *et al78* | 2014 Dec | Single center-based case-control study | 426 HCC subjects and 438 cancer-free control subjects were used. SNP genotyping was performed. A Vector was constructed and luciferase reporter assays were done. TLR4 mRNA levels were evaluated and Western blotting was done. | The hypothesis that the overexpression of TLR4 induced by the rs1057317 polymorphism miRNA-disrupting function may influence the development of hepatocellular carcinoma is possible but still not proved. More studies in this area are needed. | The risk of hepatocellular carcinoma was associated with a functional variant at miR-34a binding site in toll-like receptor 4 gene. miR-34a/TLR4 axis may play an important role in the development of hepatocellular carcinoma. |
| [Minmin S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Minmin%20S%5BAuthor%5D&cauthor=true&cauthor_uid=21559380), *et al79* | 2011 Apr | Analytic – Case-control | A systematic genetic analysis of sequence variants of TLR4 by evaluating ten single-nucleotide polymorphisms was performed from 216 hepatocellular carcinoma cases and 228 controls. | The contribution of the SNPs in TLR4 to HCC is modest. More studies are needed to validate this finding in independent populations and to understand the mechanism by which TLR4 sequence variants affect the pathological role of TLR4 in the signaling pathways that control carcinogenesis. | The risk of hepatocellular carcinoma was associated with TLR4 sequence variation. TLR4 single nucleotide polymorphisms may play an important protective role in the development of hepatocellular carcinoma. |
| [Kawamoto T](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kawamoto%20T%5BAuthor%5D&cauthor=true&cauthor_uid=18299127), *et al81* | 2008 Apr | Experimental | Mouse cells were used together with plasmids containing TLRs. Cels were submited to LPS and TAK-242. Nitrite and TNF-α were measured. Reporter gene assay for ligand-dependent signaling by TLRs, Reporter gene assay for ligand-independent signaling by  TLR4, CD4-TLR or adaptors and Western blot analysis were performed. | Human studies are needed as the interacting affinity of TAK-242 with TLR4 may be affected by a subtle difference in the amino acid sequences of TIR between humans and mice. | TAK-242 selectively suppresses TLR4-signaling mediated by the intracellular domain. |
| [Matsunaga N](http://www.ncbi.nlm.nih.gov/pubmed/?term=Matsunaga%20N%5BAuthor%5D&cauthor=true&cauthor_uid=20881006), *et al82* | 2011 Jan | Experimental | 293 cells of human embryonic kidney and murine resident peritoneal macrophages were used. They were subited to TAK-242 and LPS. Vectors for FLAG-TLR4 and FLAG-TLR2 were cloned. Measurement of Nitrite and Cytokine Concentrations in Culture Supernatants, Radiolabeling of the Cells, Immunoprecipitation, Western Blot Analysis and Autoradiography, Reporter Gene Assay and In Vitro Interleukin-1 Receptor-Associated Kinase-1 Kinase Assay were all performed. | To fully understand the physical basis  whereby TAK-242 disturbs signaling complex formation and intracellular signal transduction, a crystal structure analysis of the TLR4-TAK-242 complex is needed. | TAK-242 binds selectively to TLR4 and subsequently disrupts the interaction of TLR4 with adaptor molecules, thereby inhibiting TLR4 signal transduction and its downstream signaling events. |
| [Xu YY](http://www.ncbi.nlm.nih.gov/pubmed/?term=Xu%20YY%5BAuthor%5D&cauthor=true&cauthor_uid=24195809), *et al83* | 2013 Nov | Experimental | Four dsRNAs were designed and synthesized. The expression of proteins was compared. The migration, proliferation and apoptosis of HepG2.2.15 cells were evaluated in presence of BM-06, sorafenib alone or in combination of both. The similar treatments were also applied in an SD rat primary HCC model. | Since synthetic siRNAs must be transfected into the target cells through a vector, such as Lipofectamine™ 2000 reagent, they always exhibit cytotoxicity, which may limit their use in clinic. | dsRNA alone was capable of inhibiting the proliferation of HepG2.2.15 cells and tumor growth of orthotopic HCC SD rats, but the effect of combination of dsRNA with sorafenib was more prominent. |
| [Behm B](http://www.ncbi.nlm.nih.gov/pubmed/?term=Behm%20B%5BAuthor%5D&cauthor=true&cauthor_uid=25524262), *et al84* | 2014 Dec | Experimental | Rabbits were randomised to receive RFA, CpG B, their combination or no therapy, further tested by rechallenging a separate group with intravenously injected VX2 tumour cells after 120 days. Animals were assessed for survival, tumour size and spread, and tumour and immune related histological markers after 120 days. Peripheral blood mononuclear cells were tested for tumour-specific T cell activation and cytotoxicity. Immune modulatory cytokines were measured in serum. | Lack of antibody reagents for the VX2-tumour model in rabbits. It was not possible to elucidate indepth histopathological changes. | The combination of TLR9 stimulation with RFA resulted in a potentiated antitumour T cell response and cytotoxicity in the VX2 tumour model. Only this combination prevented subsequent tumour spread and resulted in a significantly improved survival. |