

Review

Signal Transducer and Activator of Transcription 4 in Liver Diseases

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Abstract

STAT4 is a member of the signal transducer and activator of transcription (STAT) family of molecules that localizes to the cytoplasm. STAT4 regulates various genes expression as a transcription factor after it is phosphorylated, dimerizes and translocates to the nucleus. STAT4 activation is detected virtually in the liver of several mouse models of liver injury, as well as the human liver of chronic liver diseases. STAT4 gene polymorphism has been shown to be associated with the antiviral response in chronic hepatitis C and drug-induced liver injury (DILI), primary biliary cirrhosis (PBC), HCV-associated liver fibrosis and in hepatocellular carcinoma (HCC). However, the roles of STAT4 in the pathogenesis of liver diseases are still not understood entirely. This review summarizes the recent advances on the functional roles of STAT4 and its related cytokines in liver diseases, especially in regulating hepatic anti-viral responses, inflammation, proliferation, apoptosis and tumorigenesis. Targeting STAT4 signaling pathway might be a promising strategy in developing therapeutic approaches for treating hepatitis in order to prevent further injury like cirrhosis and liver cancer.

Key words: STAT4, cytokines, liver injury, chronic hepatitis, liver fibrosis, hepatocellular carcinoma.

Introduction

The Janus Kinase-Signal Transducers and Activators of Transcription (JAK-STAT) pathways have already been clearly shown to play critical roles in the immune, neuronal, hematopoietic and hepatic systems. Among these complex pathways, STAT4 is an important transcription factor that is crucial for the differentiation of Th1 cells in promoting cellular-mediate immune response (1). Generally, it has been accepted that STAT4 expression is restricted to myeloid cells (especially in activated monocytes, macrophages, and dendritic cells (2)), thymus, and testes. In contrast, the expression of STAT4 in resting human T cells is very low. Diverse cytokines can activate STAT4, including interleukin (IL)-12 (3-5), interferon (IFN)- α/β (6-8), IL-2 (9) and IL-17 (10) pro-

duced by T cell, natural killer (NK) cell and several other types of immune cells. In addition, growth arrest and DNA-damage-inducible protein GADD45- β/γ can also activate STAT4 via MKK6/p38 pathway (11). After activation, STAT4 enters the nucleus, binds to the STAT4 element and activates its target genes. Among these target genes, interferon γ (IFN- γ) is the most characterized one. Although it has been extensively investigated in immune system (12-19) and cancer (20), the roles of STAT4 in the pathogenesis of liver diseases are largely unknown. This review summarizes the recent advances on the functions of STAT4 and its related cytokines involved in liver injury, hepatitis, fibrosis and cancer (Figures 1-3).

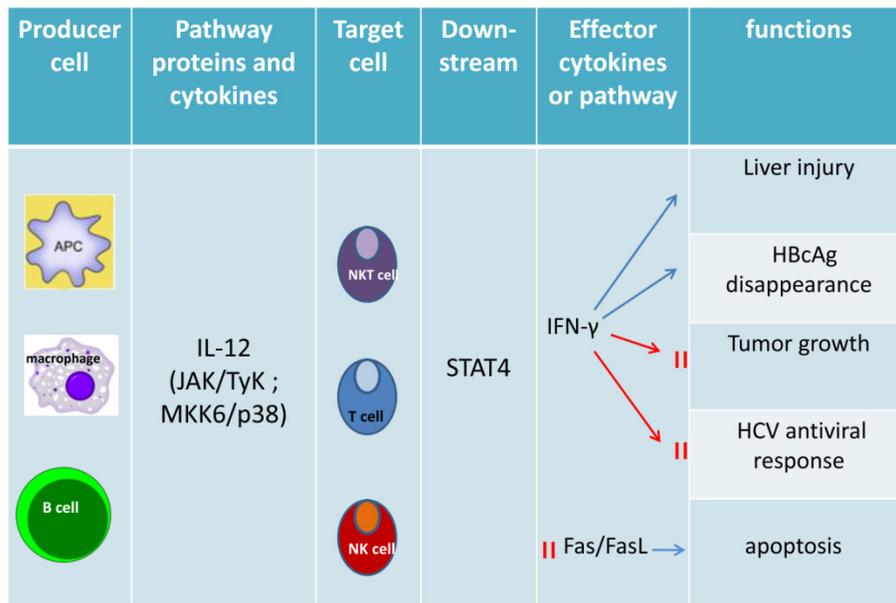


Figure 1. Functions of IL-12-dependent STAT4. IL-12, synthesized predominantly by dendritic cells, macrophages and human B-lymphoblastoid cells, as the major upstream cytokine of STAT4, that can activate STAT4 via stimulating target cells (NK cell, NKT cell and T cell) to release IFN- γ . IFN- γ plays a crucial role on cell proliferation, NK cell cytotoxicity, Th1 differentiation, liver fibrosis, liver tumor, viral replication and anti-viral therapy response as well as inhibiting Fas/FasL mediated apoptosis.

STAT4 Upstream gene	STAT4 Target cell	STAT4 Target gene	function
IL-12	T cell NK cell NKT cell	IFN- γ	Th1 cell immunity; necrotic hepatic cell death
		IRF-1	
		ERM	Th1 cell immunity
		IL-12R	Positive feedback loop
		AP-1	Active IFN- γ promoter
		IL-10	Histone acetylation
IFN- α		IRF-1	
IFN- β	Mast cell	MCP-1 SOCS-3	Vascular smooth muscle cell proliferation
IL-2	NK cell	No clear	Cytotoxicity probably
IL-17	U937 cell line	No clear	No clear

Figure 2. The mainly upstream genes and target genes of STAT4. IL-12, as a major upstream cytokine, stimulate T cell, NK cell and NKT cell to release target cytokines or activated some receptors, transcription factors or proteins by activated STAT4 to regulate Th1 immune response, autophagic-associated necrotic hepatic cell death, histone acetylation or positive feedback loop. IFN- α and IFN- β both play a crucial role on Th1 cell immunity and cell proliferation by activated STAT4 via different target genes respectively. IL-2 and IL-17 also can be upstream genes that activate STAT4.

Activation of STAT4 and its downstream target genes

STAT4 is predominately activated by IL-12, which mainly regulates tissue inflammation, fibrogenesis and antiviral defense (21). In CD4⁺ Th1 cells, the binding of IL-12 and its receptor, results in phosphorylation of tyrosine 693 and serine 721 of STAT4

(22). Then phosphorylated STAT4 translocates into nucleus, binds to specific DNA sequences, leading to the production of inflammatory cytokines, like IFN- γ (22). IFN- γ combined with other inflammatory cytokines may induce liver injury associated with Th1 cellular immunity and autophagy-related necrotic hepatocyte death (23). IFN- α can also activate STAT4, resulting in tyrosine and serine phosphorylation,

DNA binding of STAT4, and consequently transcription of its target genes (6). In mast cells, IFN- β may activate STAT4 to induce monocyte chemoattractant protein 1 (MCP-1) expression (7). IL-2 can also induce tyrosine phosphorylation of STAT4 in NK and NKT cells (9). It has also been reported that IL-17 can induce STAT4 activation in human leukemic monocyte lymphoma cell line U937 (10).

The development of ChIP (chromatin immunoprecipitation)-on-Chip technology greatly expanded our understanding on the genomic occupancies of STAT4. Good SR *et al* explored that STAT4 target genes could be clustered into several subsets by functions, including Th1 phenotype-related cluster (*Furin, Ifng* and *Il18r1, Myd88, Gadd45g*), TCR signaling-related cluster (*LCE1B, Lcp2*), other receptor (*IL-12ra*), and cytokine cluster (*IL-10, IL-21, IL-24* and *IL-17f*) (24). *Ifng* is one of the main target genes for STAT4, which plays a key role in the IL-12-induced differentiation of T cells into the Th1 pathway. Activator Protein 1 (AP-1) is an important target gene, regulated by STAT4. STAT4 can bind with c-Jun, and the phosphorylated c-Jun-STAT4 complex efficiently interacts with the AP-1-relevant promoter sequence. AP-1 binds to the promoter sequence to elicit IFN- γ promoter activation depending on the presence of STAT4 (25). The Ets transcription factor ERM is also one of STAT4 downstream target genes and ERM is Th1-specific which is induced by IL-12 through the STAT4-dependent pathway (26). IFN regulatory factor-1 (IRF-1) has been well known to be regulated by IFN- α . More interestingly, STAT4 also participates in the regulation of IRF-1 in two different mechanisms. First, STAT4 is required for the IL-12-dependent transactivation of IRF1. Second, STAT4 can directly bind to the IRF-1 promoter (27). In addition, suppressor of cytokine signaling 3 (SOCS-3) and monocyte chemoattractant protein-1 (MCP-1) contribute to the human vascular smooth muscle cells (VSMC) proliferation by STAT4-dependent pathway (28). An interesting paper reported STAT4-binding element in the fourth intron of the IL-10 gene. This intronic STAT4 motif is a target for cytokine-induced histone acetylation, which indicates that STAT4 may play a role in regulating histone acetylation via binding downstream gene IL-10 (29).

STAT4 and its related cytokines in liver injury

IL-12 and liver injury

IL-12 is the major cytokine that can activate STAT4, synthesized predominantly by dendritic cells, macrophages and human B-lymphoblastoid cells (30). It comprises of two subunits, including the light chain

p35 (IL-12A) and the heavy chain p40 (IL-12B). There are other heterodimeric interleukins, such as IL-23, IL-27, and IL-35, whose subunits consist of either or both the IL-12 p35 or p40 chains (31). The IL-12 receptor belongs to the hematopoietin receptor superfamily and it is composed of two chains that are termed as IL-12R β 1 and IL-12R β 2. Ligand binding results in heterodimer formation and activation of the receptor associated with JAK kinases, Jak2 and Tyk2 (32). IL-12 activates the JAK2 and Tyk2, leading to phosphorylation of STAT4 on tyrosine 693 (6). Moreover, IL-12 can also activate the p38/MKK6 signaling pathway that in turn phosphorylates STAT4 on serine 721 (22). Numerous studies have suggested that IL-12 acts as a pro-inflammatory cytokine which induces liver injury and also inhibits liver tumor growth by activating NK and NKT cells to produce IFN- γ (33). IL-12-mediated increased IFN- γ production, cellular proliferation, enhanced NK cell cytotoxicity, and Th1 cell differentiation, were all impaired in STAT4 knockout mice (34, 35). IL-12 therapy enhances type 1 immunity and induces IFN- γ expression. IL-12 could also strongly synergize with other cytokines like IL-18 to enhance both cytolytic activity and IFN- γ production by T, NKT, and NK cells, thereby promoting liver inflammation (36, 37). Overexpression of IL-12 in the liver by hydrodynamic injection of IL-12 cDNA resulted in liver injury (38). However, prolonged IL-12 stimulation of NK cells specifically decreased the level of activated STAT4 protein and induced apoptosis with increasing ROS production in human NK cells (39). Deletion of IL-12 suppressed liver inflammation in dominant negative TGF- β receptor transgenic mice (40), but in concanavalin A (ConA)-induced hepatitis, the role of IL-12 remains as a controversial issue (41, 42). Our recent study showed that disruption of IL-12a or IL-12b augmented Con A-induced hepatocellular damage (43). Other studies have focused on the antiviral role of IL-12. Some data showed that IL-12 treatment results in the disappearance of cytoplasmic hepatitis B core antigen (44). In contrast, other results reveal that IL-12 induces a strong immunosuppressive reaction in the liver of chronic woodchuck hepatitis virus (WHV) carriers that counteracts the antiviral effect of the treatment (45). Moreover, clinical data showed that low serum IL-12p40 levels are associated with a non-virological response in chronic hepatitis C patients whose IL28B polymorphism is TG or GG genotypes (46).

IFN- α/β and liver injury

Type-I IFN α/β , mainly produced by macrophages and fibroblasts, has antiviral, anti-proliferative and immune regulatory activities (47). Type-I IFN receptor (IFNAR) is a common cell surface receptor

complex, composed of subunits IFNAR1 and IFNAR2. The binding of IFN α/β to its cell surface receptor leads to the phosphorylation of the transcription factor STAT1, STAT4 and activates IFN target genes (48). Previous work has suggested that type-I IFN signaling is required in the innate immunity-mediated ischemia-reperfusion induces liver transplant damage (49). Disruption of type-I IFN signaling decreased serum alanine aminotransferase (ALT) levels, improved histological architecture, and increased 14-day survival in a clinically relevant murine model of extended hepatic cold followed by orthotopic liver transplantation (OLT). The physiological role of type I IFN in controlling IFN- γ production remains to be fully evaluated.

STAT4 and liver injury in mouse models

IFN- γ serves as an important target gene of STAT4 and it has been well documented in liver inflammatory response, liver injury and inhibition of virus replication. Moreover, the activation of STAT4 was also detected in several models of liver injury including Con A-induced T cell hepatitis (1, 50) and hepatic ischemia/reperfusion injury (51, 52). The role of STAT4 in the pathogenesis of animal liver injury models is complicated and summarized below.

In ConA-induced mouse model, peak STAT4 activation in liver was detected at 9 hours (50). Recently, Li *et al.* reported that suppressive oligodeoxynucleotides (ODNs) inhibit the development of Con A-induced hepatitis through down-regulation of the STAT1/4 and T-bet pathways, suggesting that STAT4 may be involved in the development of liver injury (53). Our studies further found after Con A administration, STAT4 was observed being activated in multiple types of liver immune cells (T cells, NK T cells, macrophages and Kupffer cells). Disruption of IL-12a, IL-12b, or STAT4 significantly augmented ConA-induced liver injury even though the reduced production of Th1 and Th2 cytokines. Higher FasL expression and increased cytotoxicity against hepatocytes in hepatic NKT cells from ConA-treated STAT4^{-/-} mice than those from wild-type mice can explain this phenomenon. In summary, our results show that despite of the up-regulation of pro-inflammatory cytokines, STAT4 protects mice against acute T-cell hepatitis, which is mediated by direct or indirect down-regulation of FasL expression on NKT cells (43).

The function of STAT4 in ischemia/reperfusion (I/R) model remains to be controversial. Shen XD *et al.* reported that STAT4-deficient mice were resistant to hepatic ischemia/reperfusion injury, suggesting that disruption of STAT4 may be protective in I/R induced liver injury progression (52). However, another

study showed that there was no significant difference in the severity of liver injury between STAT4-deficient and wild-type mice after liver ischemia/reperfusion injury (51).

STAT4 and its related cytokines in human liver injury

IL-12 and viral hepatitis

IL12B 3'UTR gene polymorphisms may be associated with HBV susceptibility (54). Serum low IL-12p40 level is relative to non-virological responses in chronic hepatitis C patients who take the treatment with pegylated interferon and ribavirin (46). Modulation of regulatory T-cell activity in combination with IL-12 induces a strong immunosuppressive reaction in the liver of chronic WHV carriers which may indicates that IL-12 involves in counteracting the antiviral effect (45). Recently, *in vivo* and *in vitro* experiments have shown that IFN- α -induced regulation of STAT1 and STAT4 phosphorylation, which plays a role in increasing cytotoxicity and decreasing IFN- γ production in HCV infection (55). Another study revealed that type I IFNs can promote IFN- γ production by activating STAT4, but can also inhibit production of IL-12. So the efficacy of IFN- α in treatment of hepatitis C, may depend in part on the balance of IFN- γ -inducing and IL-12-suppressing effect (56).

STAT4 and viral hepatitis

The NK cells from chronic hepatitis C patients treated with IFN- α have higher level of phosphorylated-STAT4 and phosphorylated-STAT1 than those from healthy subjects. It suggested that both STAT4 and STAT1 activation in NK cells may play a crucial role in the antiviral response in chronic hepatitis C (57). There is evidence suggesting that STAT4 variants (rs7574865) might contribute to chronic hepatitis B disease progression, and the variants seemed to be population specific in Tibetans in China (58).

STAT4 and drug induced liver injury (DILI)

The mechanisms of drug-induced liver injury remain unclear. An analysis of 285 DILI patients indicated a trend association between STAT4 variants (rs7574865) and hepatocellular injury (59). Another study which included over 800 patients and over 3000 control subjects showed there is an association between a single-nucleotide polymorphisms (SNP) in the STAT4 gene and a common type of DILI (hepatocellular injury) (60).

STAT4 and its related cytokines in liver fibrosis

Liver fibrosis is an accompanying response and common characteristic of chronic liver disease. The

pathologic features of hepatic fibrosis include the accumulation of extracellular matrix (ECM) proteins and the activation of hepatic stellate cells (HSCs). After liver injury and inflammation, HSCs become activated, express alpha-smooth muscle actin (α -SMA), and produce large amounts of collagen (61-63). The JAK-STAT signaling pathway has been proposed to play a central role in liver inflammation and fibrosis development. Kong X et al reported that IL-22 induces HSC senescence through the activation of STAT3, SOCS3, and p53 pathways, thereby inhibiting liver fibrosis (64). It has been well studied that IL-12-mediated activation of STAT4 plays an important role in regulating tissue inflammation, fibrogenesis and antiviral defense (1, 51, 65-67). Cheng et al. reported granuloma size was significantly larger and granuloma fibrosis remarkably intensified in anti-IL-12-treated mice after schistosoma infection. These results suggest that IL-12 may play an impeditive role in granuloma formation as well as in liver fibrosis (68). However, the roles of STAT4 in HSC senescence or activation and also in the pathogenesis of liver fibrosis still remain largely unknown.

STAT4 and its related cytokines in human liver fibrosis

Primary biliary cirrhosis

Using the advent of genome-wide association studies, *STAT4*, *IL12A*, and *IL12RB2* have been identified to be the risk loci that is associated with development of primary biliary cirrhosis (69).

Liver fibrosis

A clinical study genotyped 160 transplant patients with HCV recurrence for *STAT4* (rs7574865) and determined their fibrotic stage based on pathologic analysis. This result indicates *STAT4*-T-allele is associated with development of advanced fibrosis (70).

STAT4 and its related cytokines in liver tumor

IL-12 and liver tumor

Being the *STAT4* upstream cytokine, IL-12 is well known to play an important role in the regulation of innate and adaptive immune responses (71). IL-12 can induce prolonged IFN- γ production, NK cell activation and inhibition of liver metastasis of CT-26 colon cancer cells. Upon IL-12 administration, *STAT4* activation in NK cells enhanced and IFN- γ production increased (72). It has been shown that IL-12 therapy can induce regression of primary tumors, inhibit the distant metastasis, and prolong the survival of tu-

mor-bearing mice (73-76). Intratumoral gene transfer of murine IL-12 enhances lymphocytic infiltration into the tumor and significantly reduces the number of microvessels and inhibits the growth of HCC (77). A recent study indicates that *IL12A* rs568408 may contribute to the risk of HCC and modify HCC risk associated with HBV infection in Chinese patients (78). IL-12-mediated anti-tumor effects depend on the production of IFN- γ *in vivo* in several animal models (74, 79-82). IL-12 can inhibit tumor angiogenesis mainly through IFN- γ -dependent production of the chemokine IP-10 (83). Combined GM-CSF and IL-12 gene therapy induces various types of effectors and high levels of IFN- γ , thereby suppresses the growth of orthotopic liver tumors (84).

IFN- α/β and liver tumor

It is shown that endogenous type I IFN prevents the growth of primary carcinogen-induced and transplantable tumors but does not act directly on tumor cells. Hematopoietic cells, in particular NK cells, are critical IFN- α/β targets involving in the anti-tumor responses (85, 86). The combination therapy of IFN- α and sorafenib synergistically suppresses HCC growth and apoptosis (87).

STAT4 and human liver tumor

The studies about *STAT4* in liver tumor development are very few. Recent studies have shown that *STAT4* deficiency may contribute to impaired IFN- γ production in lymphoma patients after autologous peripheral blood stem cell transplantation (PBSCT). In fact, *STAT4* deficiency in these patients when acquired may also promote lymphoma development (88). Chemotherapy-induced *STAT4* deficiency could be due to the reduced levels of *STAT4* mRNA and the protein stability (20).

A genome-wide association study (GWAS) was conducted in 2,514 Chinese patients with chronic HBV carriers (1,161 HCC cases and 1,353 controls) followed by a 2-stage validation among 6 independent populations of chronic HBV carriers (4,319 cases and 4,966 controls). The analysis showed that HCC risk was significantly associated with loci rs7574865 at *STAT4*. The risk allele G of these loci was strongly associated with lower levels of *STAT4* mRNA in HCC tumor tissues (89). These findings indicate that *STAT4* is associated with HCC development, although more consolidated studies are still needed.

Conclusions

In summary, *in vivo* and *in vitro* studies from recent decades have suggested that *STAT4* and its related cytokines exhibit complex biological functions in regulating hepatic anti-viral responses, inflammation,

proliferation, apoptosis and tumorigenesis. These findings can help with the characterization of pathophysiology and treatments of liver diseases that may be associated with STAT4 and its cytokines. However,

there is still a long way to explore and elucidate STAT4 as a therapeutic approach in treating liver disease (Figure 3).

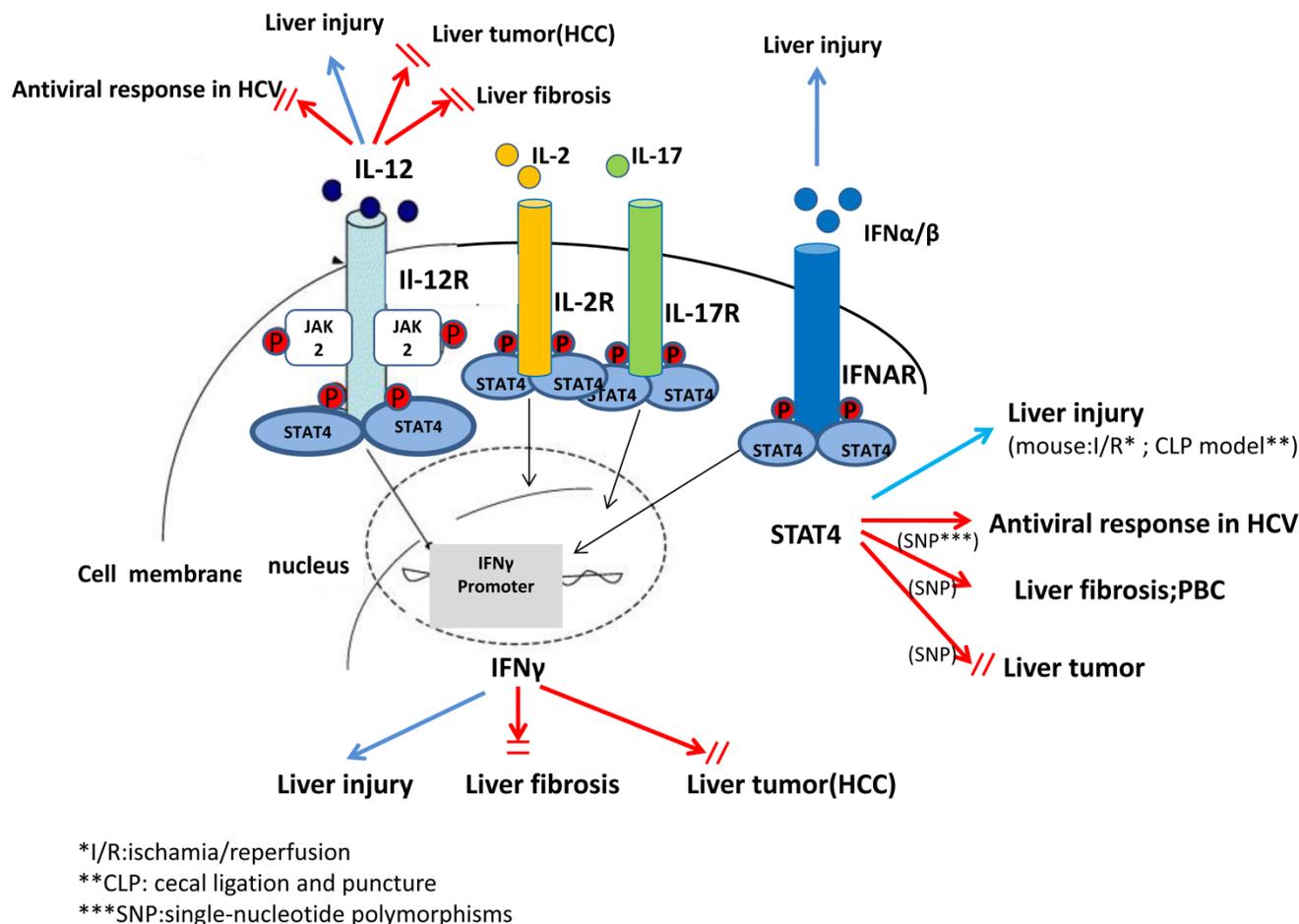


Figure 3. A complex role of IL-12, IFN- γ and STAT4 in pathogenesis of liver injury, liver fibrosis and liver tumor. In summary, studies from animal models and human in the recent decades have suggested that STAT4 and related cytokines, such as IL-12, IFN- α/β exhibit complex biological functions, especially in regulating antiviral responses in HCV, liver injury, liver fibrosis and tumorigenesis.

Acknowledgments

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Competing Interests

The authors have declared that no competing interest exists.

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