Role and mechanism of programmed death-ligand 1 in hypoxia-induced liver cancer immune escape (Review)

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Abstract. Immune escape plays a vital role in the development of liver cancer. The interaction between programmed death-ligand 1 (PD-L1) and programmed cell death-1 is a key mediator of cancer immune escape, which leads to the suppression of anticancer immunity and promotion of tumor progression. Hypoxia is a common phenomenon in the tumor microenvironment. Under hypoxic conditions, suppressive immune cells, such as regulatory T cells, myeloid-derived suppressor cells and M2 macrophages, are frequently recruited to tumor tissues to form the immunosuppressive microenvironment in liver cancer. These cells secrete cancer-promoting inflammatory cytokines, which activate the STAT3 and NF-κB signaling pathways. Recent studies have shown that STAT3 is associated with NF-κB and that these transcription factors are often co-activated to regulate tumor proliferation, survival, angiogenesis and invasion. The activation of STAT3 and NF-κB signaling pathways can directly and indirectly induce PD-L1 expression. Therefore, further understanding of the association between hypoxia and PD-L1 may help in the future treatment of liver cancer. The present review summarizes the recent progresses on PD-L1-mediated regulation and facilitation of liver cancer cell immune escape in response to hypoxia.

Contents

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1. Introduction

Hepatocellular carcinoma (HCC) is one of the most malignant tumors that pose a severe threat to human health. The latest cancer statistics showed that the number of new liver cancer cases and liver cancer-associated deaths worldwide in 2018 was 841,000 and 782,000, respectively (1). Liver cancer ranked sixth in terms of new cancer cases and fourth in terms of cancer-associated deaths worldwide in 2018 (1). The available evidence indicates that immune escape of liver cancer cells plays a vital role in the development of this malignancy (2,3) and impairs the effectiveness of antitumor treatment (4). Therefore, effective blockage of the occurrence of immune escape has become the focus of attention in the prevention and treatment of HCC.

It is now known that the activation or inhibition of immune cells in the body is regulated by positive and negative signals (5,6). Among them, the interaction between programmed cell death-1 (PD-1, also termed CD279) and programmed death-ligand 1 (PD-L1, also termed CD274 and B7-H1) is the primary negative immune regulatory signal, which inhibits the antitumor immune activity of effector cells and mediates tumor immune escape (7-10). Furthermore, immune checkpoint blockers have recently emerged as a mainstream strategy for the treatment of multiple solid tumors, including liver cancer (11-14).

Hypoxia, a common phenomenon in the tumor microenvironment, induces the expression of PD-L1 to promote immune escape (15-17). A number of immune cells with immunosuppressive activities, including tumor-associated regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), are recruited to the tumor tissue to form an immunosuppressive microenvironment (18-20). Moreover, under hypoxic

conditions, the expression of PD-L1 is rapidly upregulated in these immunosuppressive cells in a hypoxia-inducible factor 1α (HIF-1α)-dependent manner (21,22). In this regard, the comprehensive analysis of the role and mechanism of PD-L1 in hypoxia-induced immune escape is essential for the improved treatment of liver cancer. The present review summarizes the recent findings regarding the regulation of PD-L1-mediated hypoxia-induced immune escape in HCC cells and discusses the underlying mechanisms.

2. PD-L1/PD-1 interaction in the immune escape of liver cancer

The compromised immune status of the body is associated with the occurrence of liver cancer. When the immune function is weakened or suppressed, the incidence of liver cancer will increase significantly. Normally, once liver cancer cells are formed in the body, the immune system can inhibit or kill these cells in a variety of ways (23-25). However, despite the immune surveillance and scavenger receptors, it remains challenging to curb the occurrence and development of liver cancer (26). The main reason is that liver cancer cells may escape from the immune system attack through various mechanisms. The PD-L1/PD-1 pathway, which promotes cancer cell survival and proliferation, is a key mediator of the immune escape of HCC cells (27-29).

Previous studies have shown that T cell-mediated cellular immunity plays a pivotal role in the recognition and killing of tumor cells (30,31). T cells recognize major histocompatibility complexes that bear antigens derived from the surface of cancer cells, which allows subsequent tumor recognition and targeted killing (32). Recently, it has been demonstrated that various mechanisms play a role in increasing the expression of PD-L1 in tumor cells and in the tumor microenvironment. PD-L1 is a transmembrane glycoprotein composed of 290 amino acids, which belongs to the B7 family of immune-regulatory ligands. The binding of PD-L1 to its PD-1 receptor suppresses T-cell migration, proliferation and secretion of cytotoxic mediators, and restricts tumor cell killing, leading to the occurrence of tumor cell immune escape (33). In the healthy immune system, the PD-L1/PD-1 pathway plays a critical role in maintaining the balance between protective immunity and immune tolerance. However, aberrant activation of the PD-L1/PD-1 signaling pathway in the tumor microenvironment is associated with the development of liver cancer. A multivariate analysis showed that PD-L1 expression is an independent predictor of postoperative recurrence of HCC (7,34).

Accumulating evidence has revealed that the elevated level of PD-L1 in the tumor microenvironment constrains antitumor immunity via the inhibition of antitumor effector cell function and enhancement of the inhibitory activity of immunosuppressive cells (12,16,35,36). Cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells are the main local antitumor immune effector cells. Activated CTLs are marked by granzyme B, which is the primary molecular mediator of apoptosis (37). It has been shown that the activation of the PD-1/PD-L1 signaling pathway restrains CTL function by inducing apoptosis, anergy and exhaustion, and promoting the secretion of immunosuppressive factors, leading to the immune escape of tumor cells (38). Hepatoma tumor-infiltrating CTLs express

PD-1 molecules, which bind to PD-L1 that are expressed on the surface of tumor cells, resulting in the depletion and apoptosis of CTLs (39).

There are a large number of active immunosuppressive cells in the tumor microenvironment, including Tregs, MDSCs and TAMs (40). These immune cells form a complex multi-cell population, which is an important part of the tumor microenvironment. Indeed, various molecular interactions between immune and cancer cells are considered a crucial step in the direct or indirect induction of the occurrence and development of tumors. These immunosuppressive cells also express a large number of PD-L1 molecules, which induce apoptosis in CTLs by binding to PD-1 (41). Tregs, characterized by the expression of CD4, CD25 and Forkhead box protein P3 (FOXP3), are the most characteristic immunosuppressive cells. The inhibition of the immune response by Tregs is also mediated by cell contact or the secretion of inhibitory cytokines, such as interleukin (IL)-10 and transforming growth factor-β (TGF-β) (42). A previous study found that PD-L1 promoted Treg differentiation by converting CD4+CD25+FOXP3- T cells to CD4+CD25+FOXP3+ Tregs. Furthermore, higher expression levels of PD-L1 on hepatic dendritic cells were associated with an increased Treg cell induction (43). Specific blocking of PD-L1 by small interfering (si)RNA or monoclonal antibodies decreased the production of CD4+CD25+FOXP3+ Tregs and induced Treg apoptosis (44). In a pig xenograft model, PD-L1 was found to enhance Treg function and stimulate IL-10 production, thereby further promoting the immune inhibitory function (45). Clinical data also showed that PD-L1 effectively stimulated the secretion of IL-10 in patients with liver cancer, thereby further enhancing the immunosuppressive effect of Tregs (46). Collectively, these studies have shown that the PD-L1/PD-1 pathway inhibits the antitumor function of CTLs, enhances the immunosuppressive activity of Tregs, and promotes the secretion of immunosuppressive factors by transmitting inhibitory signals, leading to the occurrence of tumor immune escape.

3. Hypoxia-induced recruitment of immunosuppressive cells and regulation of PD-L1 expression

Hypoxia is a common phenomenon in the tumor microenvironment (47-49). Previous studies have revealed that tumor hypoxia alters the composition and activity of tumor-associated immune cells, and that numerous immune cells with immunosuppressive activities are recruited to tumor tissues to form the immunosuppressive microenvironment (18,50,51). Under hypoxic conditions, tumor cells and macrophages secrete a variety of cytokines and chemokines, including C-C motif chemokine (CCL)22, CCL28 and IL-10, which results in the recruitment of CD4+CD25+FOXP3+ Tregs from peripheral blood to inhibit T cell-mediated antitumor responses (18,52,53). Hypoxia also promotes the recruitment of MDSCs (19). MDSCs are a group of undifferentiated, immunosuppressive, bone marrow-derived heterogeneous cell populations, which have a strong immunosuppressive function (54). MDSCs expressing arginase-1, which mediate the depletion of L-arginine, impede T cell proliferation, and are associated with the downregulation of T cell receptor (TCR) subunit CD3ζ, resulting in decreased TCR response (55-57). The occurrence of a tumor in the liver
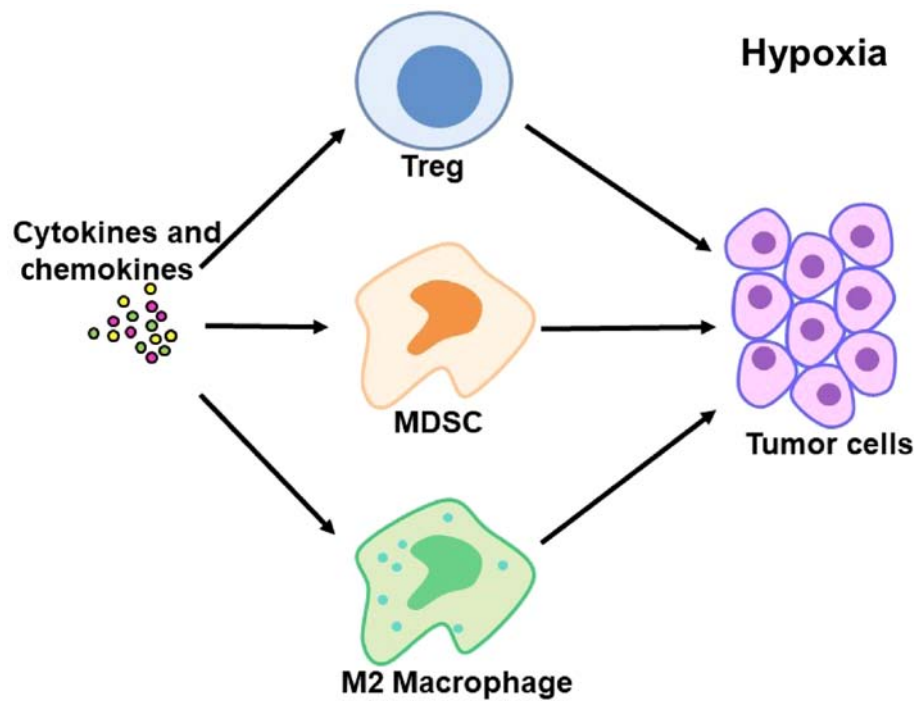
Figure 1. Hypoxia induces the recruitment of immunosuppressive cells to the tumor tissue to promote the immune escape of hepatoma cells. Under hypoxic conditions, Tregs, MDSCs and M2 macrophages are recruited to tumor tissues to form an immunosuppressive microenvironment. These cells exhibit potent immunosuppressive activity and foster the occurrence of tumor immune escape. Treg, regulatory T cell; MDSC, myeloid-derived suppressor cell.

results in increased levels of MDSCs at the tumor site, and the activation of the MyD88-NF-κB pathway stimulates the secretion of IL-10 to inhibit the expression of IL-12 in dendritic cells and the activation of T cells (58). MDSCs also induce NK cell inactivation through TGF-β and the NK receptor p30 on the cell surface (59). Furthermore, a previous study indicated that MDSCs inhibit immune response and promote the development of liver cancer by inducing the generation of CD4+CD25+FOXP3+ Tregs (60). In addition, the tumor hypoxia microenvironment directly induces macrophage M2 polarization, angiogenesis, and tumor growth and metastasis (20). M2 type macrophages inhibit the antitumor immune response by producing TGF-β and IL-10, and their numbers in the tumor microenvironment are negatively correlated with the prognosis of liver cancer patients (61,62). M2 microphages also secrete a range of specific chemokines, including CCL17, CCL22 and CCL24, which recruit regulatory T cells to tumor sites (62). As a result, Tregs, MDSCs and M2 macrophages have potent immunosuppressive activities and together promote the occurrence of tumor immune escape (Fig. 1).

Under hypoxic conditions, HIF-1α is a crucial transcription factor that mediates the effect of hypoxia on the adaptive regulation of tumor cells and the tumor microenvironment (63-65). Under normoxic conditions, HIF-1α is hydroxylated by prolyl hydroxylase (PHD) and ubiquitinated/degraded by the von Hippel-Lindau E3 ubiquitin ligase complex. Under hypoxic conditions, PHD activity is inhibited, and HIF-1α ubiquitination and degradation are decreased, thereby stabilizing HIF-1α (66). Previous studies have shown that HIF-1α is associated with PD-L1 expression (15,22). Under hypoxic conditions, tumor cells, myeloid suppressor cells, macrophages and dendritic cells all undergo rapid upregulation of PD-L1 in a HIF-1α-dependent manner. Chromatin immunoprecipitation and luciferase reporter assays showed that HIF-1α induced the expression of PD-L1 by directly binding to the hypoxia response element region of the PD-L1 promoter. Furthermore, the inhibition of PD-L1 expression significantly decreased the secretion of IL-6 and IL-10 by MDSC, leading to the activation of T cells (22). Another *in vitro* study also revealed that hypoxia stimulated the expression of PD-L1 in a variety of human and murine tumor cells through HIF-1α (15). These studies demonstrate that hypoxia induces PD-L1 expression by activating the HIF-1α cascade.

4. Involvement of STAT3 and NF-κB in the regulation of PD-L1 expression in liver cancer

Accumulating evidence has indicated that the essential mechanism underlying tumor immune escape is associated with the presence of a large number of cytokines and growth factors with immunosuppressive activities in the tumor microenvironment, such as IL-6, vascular endothelial growth factor, TGF-β, IL-10, IL-13, macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (67-69). These cytokines stimulate immune inhibitory cells, including Tregs, TAMs and MDSCs, and mediate the expression of a series of genes by activating various signaling pathways. Among them, the STAT3 and NF-κB pathways are essential hubs linking these cytokines to cellular responses (70-73).

STAT3 is a member of the STAT family of transcription factors. When cytokines in the tumor microenvironment bind to their receptors, the Janus kinase and/or proto-oncogene tyrosine-protein kinase Src will be activated and able to
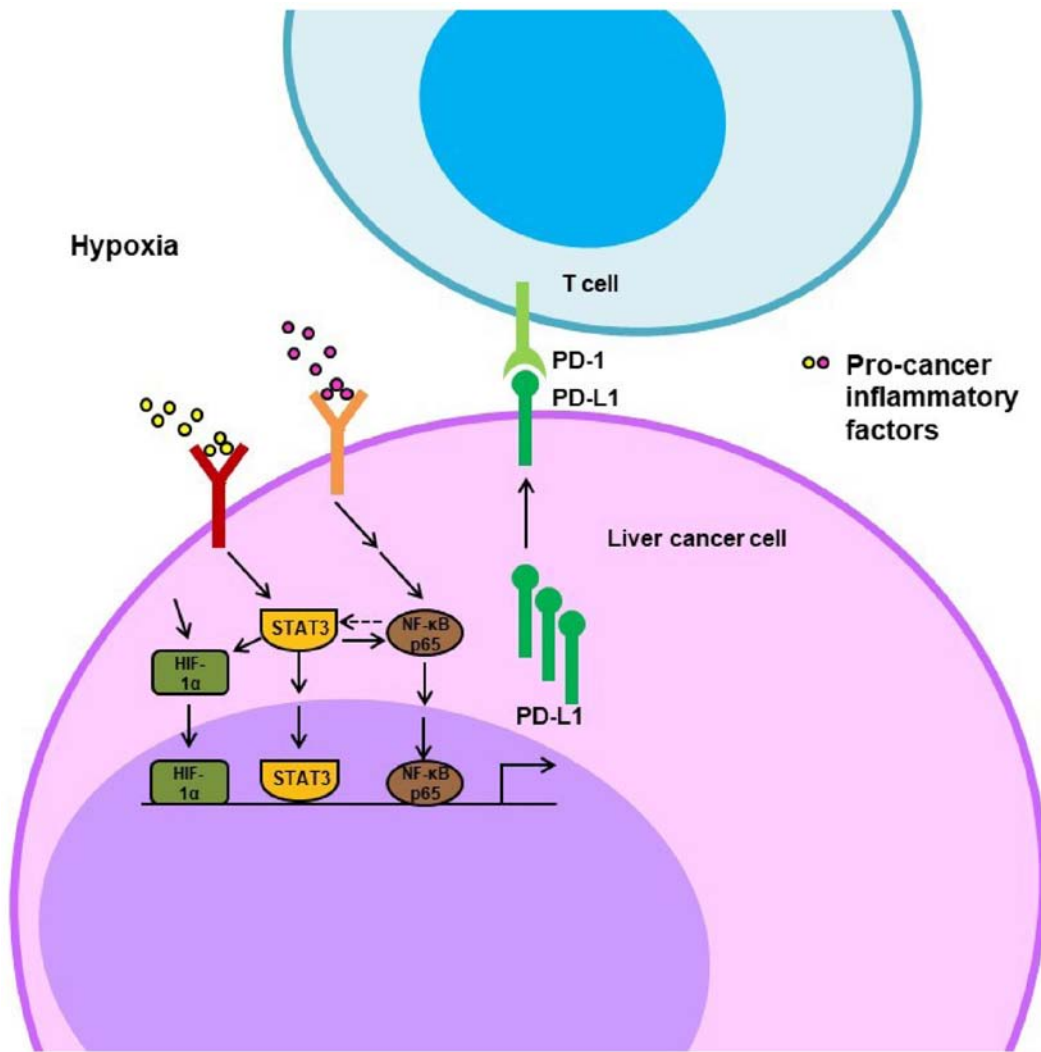
Figure 2. Schematic representation of hypoxia-induced activation of STAT3, NF-κB and HIF-1α pathways resulting in increased PD-L1 expression. Under hypoxic conditions, the expression of PD-L1 is upregulated in a HIF-1α-dependent manner. Furthermore, immunosuppressive cells secrete inflammatory cytokines to activate the STAT3 and NF-κB signaling pathways, which are often coactivated to induce the expression of PD-L1 directly, by binding to and stimulating its promoter, or indirectly, by increasing the expression level of HIF-1α. HIF-1α, hypoxia inducible factor-1α; PD-L1, programmed death-ligand 1; PD-1, programmed cell death-1.

phosphorylate STAT3. Following dimerization and nuclear translocation, STAT3 will initiate the transcription of downstream genes. A previous study found that STAT3 activation in tumor cells induces the secretion of IL-6 and IL-10 cytokines, which results in Treg proliferation. Moreover, STAT3 is also activated in Tregs and further stimulates the expression of FOXP3, TGF-β and IL-10, which inhibits CTLs and promotes the formation of an immunosuppressive environment (70,74,75).

In addition, STAT3 and NF-κB are often coactivated in tumor cells and play a vital role in the regulation of the expression of cancer-promoting inflammatory genes (76). The coordination between STAT3 and NF-κB is mainly manifested in the following aspects: i) Multiple inflammatory factors, especially IL-6, induced by NF-κB are essential activators of STAT3; ii) STAT3 directly interacts with the NF-κB family member transcription factor p65 (RelA), leading to its acetylation and inhibition of nuclear export, and constitutive activation of NF-κB; iii) STAT3 and NF-κB co-regulate the expression of a number of oncogenes and inflammatory

genes; and iv) the inflammatory factors induced by NF-κB and STAT3 form a positive feedback loop to further activate NF-κB and STAT3 (77,78).

Notably, it has been shown that the expression of HIF-1α is regulated by both NF-κB and STAT3. Under hypoxic conditions, STAT3 is activated by phosphorylation, which not only blocks HIF-1α degradation but also increases the synthesis of HIF-1α (79). In human breast cancer MCF-7 cells, the depletion of STAT3 by siRNA inhibited $CoCl_2$-induced HIF-1α nuclear accumulation (80). The NF-κB signaling pathway is also activated under hypoxic conditions (81). Gel shift assay and chromatin immunoprecipitation experiments confirmed that the NF-κB subunits p50 and RelA bind to the promoter of HIF-1α and activate its transcription (82). Since HIF-1α transcriptionally induces PD-L1, these studies indicate that the activation of the STAT3 and NF-κB pathways may indirectly stimulate PD-L1 expression under hypoxic conditions.

Furthermore, several studies have shown that the STAT3 and NF-κB signaling pathways are also involved in the direct regulation of PD-L1 at the transcriptional level (83-86). It has

been demonstrated that the co-culture of liver cancer cells (BEL-7402 and SMMC-7721) with macrophages resulted in increased PD-L1 mRNA and protein levels and that blocking either the NF-κB or the STAT3 signaling pathway inhibited this co-culture effect on PD-L1 expression (83). Another study showed that EB virus latent membrane protein 1 (LMP1) induced the expression of PD-L1 by the activation of NF-κB or STAT3; the inhibition of one of these pathways notably decreased LMP1-stimulated PD-L1 expression (84). Chromatin immunoprecipitation and reporter assays revealed direct binding of STAT-3 and NF-κB to the PD-L1 promoter, triggering PD-L1 transcription (85,86). These studies indicate that the STAT3/NF-κB pathways directly and indirectly regulate PD-L1 expression in the hypoxic microenvironment (Fig. 2).

5. Relevance for clinical practice

Immunotherapy is emerging as an appealing and attractive strategy for the treatment of HCC. Novel immune checkpoint inhibitors have revolutionized pharmacological treatment options for cancer with remarkable clinical outcomes in a number of human malignancies, including advanced HCC. It has been shown that the inhibition of PD-L1 improves overall survival rates in patients with HCC (87). Moreover, since HIF-1α plays a vital role in regulating immune escape in the hypoxic tumor microenvironment, a HIF-1α inhibitor is being investigated for the treatment of HCC (88-91). Several inhibitors of STAT3 and/or NF-κB are undergoing clinical trials for HCC (92,93). In addition, due to the upregulation of PD-L1 by STAT3, NF-κB and HIF-1α, a combination of a PD-L1 antibody with small molecule inhibitors of STAT3, NF-κB or HIF-1α could be a more effective therapeutic strategy in advanced liver cancer.

6. Conclusions

Immune escape is a key cause of tumor development. Enhancing antitumor immunity of the body, as the core treatment strategy, is being extensively studied in cancer care and research. In the tumor hypoxic microenvironment, PD-L1 overexpression is a crucial factor contributing to liver cancer immune escape and is associated with the activation of the STAT3/NF-κB pathway and HIF-1α. Therefore, the inhibition of STAT3 and NF-κB pathways or HIF-1α should decrease PD-L1 expression and reverse immune escape. Agents blocking STAT3, NF-κB or HIF-1α have great potential for cancer immunotherapy, particularly in patients developing resistance to PD-L1 and PD1 inhibitors.

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Authors' contributions

SJ contributed to the conception of the study. QW wrote the manuscript with support from SJ, TH and ZW. All authors have read and approved the final version of the manuscript.

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Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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