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Oncogenic Signal and Tumor Microenvironment in Hepatocellular Carcinoma

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Abstract
During tumor development, several immunosuppressive molecules are released from cancer cells and contribute to the establishment of immunosuppressive tumor environment. In tumor tissues, cytokines, chemokines, growth factors, and metabolites are present and could counter the effects of immune checkpoint inhibitors. From this point of view, monotherapy of anti-PD-1/PD-L1 antibody might not be enough to exert a sufficient antitumor effect; additional blockade of immunosuppressive molecules in tumor microenvironment could enhance the antitumor effect of anti-PD-1/PD-L1 antibody. Importantly, the production of immunosuppressive molecules in cancer cells is attributed to the activation of cellular signaling through genetic and epigenetic alterations and environmental stimulation, such as inflammation and hypoxia. In this review, we focus on the establishment of immunosuppressive microenvironment of hepatocellular carcinoma in the context of activation of oncogenic signals, and discuss how the immunosuppressive condition could be overcome using tyrosine kinase inhibitors.
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Introduction

During tumor development, genetic and epigenetic alterations take place that lead to the transformation of hepatocytes [1–5]. These events induce several tumor-associated antigens (TAAs), and a variety of nonsynonymous passenger mutations that could be a source of neoantigen and a target of antitumor immune response.

On the other hand, at the early stage of tumor development, cancer cells are under surveillance of the immune system and could be eliminated by $CD8^+$ cytotoxic T cells that target TAAs and neoantigens. The extent of TAA-specific $CD8^+$ T cell responses to hepatocellular carcinoma (HCC) is more prominent in patients with an early stage of tumor compared to those in the late stage, and are correlated with survival of the patients [6]. On the contrary, immunosuppressive environment against cancer cells could develop during the progression of tumor, which is attributed to a selective pressure of the immune system on cancer cells. Consequently, less immunogenic tumor cells could survive and expand, which is a phenomenon called immune editing [7] (Fig. 1). During this process, several cytokines, chemokines, growth factors, and metabolites are released from immune cells as well as cancer cells, and contribute to the establishment of an immunosuppressive condition in tumor [8, 9]. Importantly,

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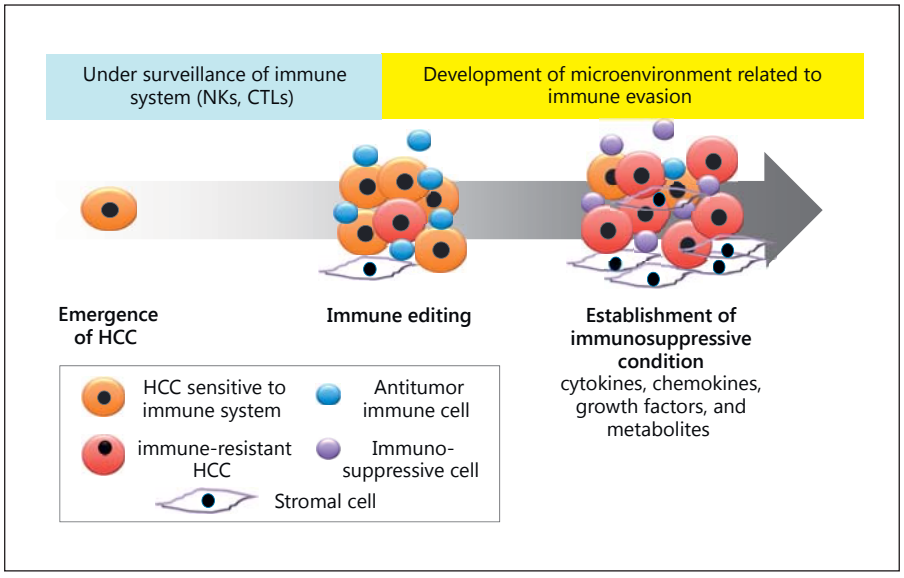

Fig. 1. Development of immunosuppressive environment in cancer. At the early stage of tumor, cancer cells are under surveillance of natural killer cells (NKs) and cytotoxic T cells (CTLs). During this process, less immunogenic cancer cells are selected (immune editing). Immunosuppressive cytokines, chemokines, growth factors, and metabolites are released from immune cells and cancer cells, and play a role for the development of immunosuppressive microenvironment in tumor at the advanced stage. HCC, hepatocellular carcinoma.

cellular signals activated through genetic and epigenetic alterations could induce the production of immunosuppressive molecules in HCC tissues [8]. In this review, we focus on the establishment of immunosuppressive microenvironment in the context of activation of oncogenic signaling.

Activation of Oncogenic Signaling and Immunosuppressive Molecules

In melanoma cells, it is reported that constitutive activation of mitogen-activated protein kinase (MAPK) by gain-of-function BRAF mutation ($BRAF^{V600}$) induces the increase in interleukin (IL)-6, IL-10, and vascular endothelial growth factor (VEGF), and results in the recruitment of myeloid-derived suppressor cells (MDSCs) and regulatory T (Treg) cells in tumor tissues [10]. It also reduces the expression of major histocompatibility complex class I molecule, and suppresses the $CD8^+$ T cells and natural killer (NK) cells in tumor microenvironment [11–13]. Reportedly, MAPK inhibition increased the number of antigen-specific $CD8^+$ T cells in tumor, and more importantly, combination of MAPK/extracellular signal-regulated kinase (MEK) inhibition with anti-programmed death-ligand 1 (PD-L1) antibody induced a synergistic effect for tumor regression compared to monotherapy of MEK inhibitor or anti-PD-L1 antibody [13, 14]. These observations indicate that activation of the oncogenic pathway in cancer cells could play an important role for the establishment of immunosuppressive tumor microenvironment.

Induction of Growth Factors

So far, overexpression of VEGF and basic fibroblast growth factor are reported in HCC tissues compared to noncancerous livers [15]. VEGF is known to induce MDSC accumulation, inhibit maturation of dendritic cells (DCs), and induce Treg cells [16]. VEGF also exerts immunosuppressive function through the expression of immune checkpoint molecules on $CD8^+$ T cells, such as PD-1, T-cell immunoglobulin and mucin domain 3 (TIM-3) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) [16]. Expression of VGEF is reportedly controlled by microRNA (miR)-146a as well as hypoxia-inducible factor-1α (HIF-1α), and downregulation of miR-146a through DNA methylation takes place in HCC cells [17]. High expression of transforming growth factor-β (TGF-β), which is known as an immune modulator, could also be a predictor of poor prognosis in HCC patients [18]; transcriptional expression of TGF-β is induced by the activation of β-catenin, which is one of the commonly mutated genes in HCC [19]. Expression of TGF-β leads to the induction of Treg cells, inhibits DCs and NK cell activity [20], and induces expression of TIM-3 on tumor-associated macrophages (TAMs) [21]. Hypoxia in tumor cells also induces HIF-1α and results in the increase of several immune modulators, such as VEGF, platelet-derived growth factor (PDGF), lactic acid, and adenosine [22].

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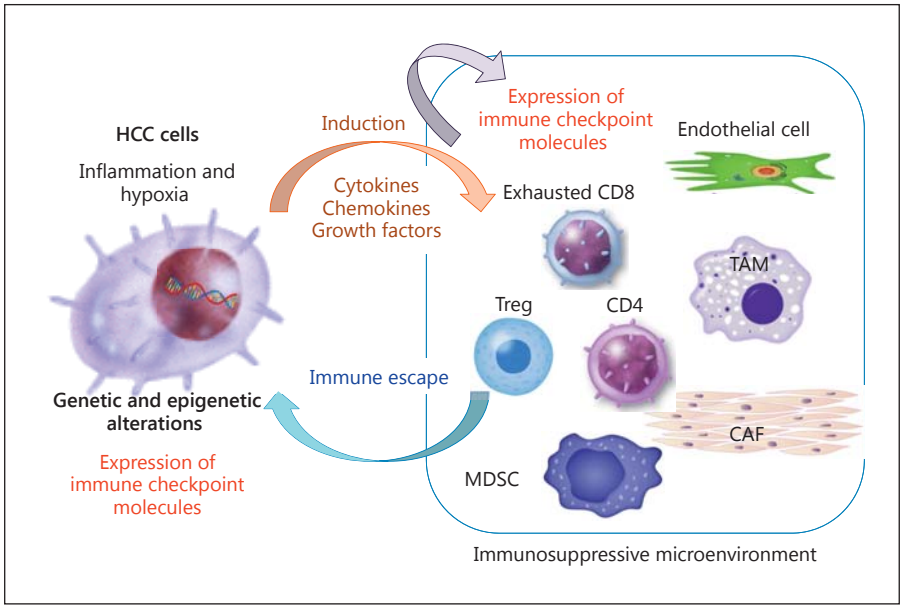

Fig. 2. Role of activation of oncogenic signal in cancer for development of immunosuppressive microenvironment. Oncogenic signal in hepatocellular carcinoma (HCC) cells, which is activated thorough genetic and epigenetic alterations and environmental factors, could induce the components of tumor microenvironment, such as regulatory T (Treg), tumor-associated macrophages (TAM), myeloid-derived suppressor cells (MDSC), and cancer-associated fibroblast (CAF).

Cytokines, Chemokines, and Metabolites

HCC cells also produce several cytokines, chemokines, and metabolites as immune modulators to the surrounding environment. Amphiregulin, the ligand of endothelial growth factor receptor (EGFR), could be induced in Treg cells as well as tumor cells [23]. Immunoregulatory enzyme, indoleamine 2,3-dioxygenase (IDO), is overexpressed in HCC cell lines and human HCC tissues, which is an independent prognostic factor for HCC patients [24]. IDO is upregulated by proinflammatory cytokines, such as interferon-γ, and inhibits T-cell activation and promotes expansion of Treg cells [25, 26]. Similarly, lactic acid, which is generated through glycolysis in tumor cells, stimulates the expression of VEGF and M2-like polarization of TAMs [27]. Activation of NF-κB could also increase the expression of immunosuppressive cytokines, such as IL-2, IL-6, and IL-8 in cancer cells; intratumoral CC chemokine ligand (CCL)-20 prompts the migration of Treg cells through its receptor CCR6 and plays a role for the establishment of immunosuppressive condition in HCC [28].

Conclusion

The blockade of PD-1-PD-L1 axis is a promising approach for the control of advanced HCC; the phase I/II trials of nivolumab (CheckMate 040) showed an objective response rate of 20% and disease control was observed in 64% of HCC patients [29]. However, several immune modulators are expressed and released from cancer cells through the activation of cellular signaling, which is a consequence of genetic and epigenetic alterations and stimulation of environmental factors such as inflammation and hypoxia (Fig. 2). Therefore, it is conceivable that combination with inhibitors of oncogenic signaling improves the antitumor effect of anti-PD-1 and PD-L1 antibody. For example, IL-6 and PD-L1 blockade combination reportedly inhibits HCC development in mouse model [30]. Inhibition of C-X-C receptor type 4 in tumor microenvironment facilitates anti-PD-L1 immunotherapy of mice HCC [31]. Recently, it was also reported that PD-1 expression promotes tumor growth, where PD-1 binds the downstream effectors of mammalian target of rapamycin (mTOR) and promotes their phosphorylation; the combination of mTOR inhibition with anti-PD-1 antibody results in a synergic tumor regression [32]. Based on these findings, clinical trials of immunomodulation of tumor microenvironment through intervention of oncogenic signaling are ongoing [33–39]. These trials include immune checkpoint inhibition combined with tyrosine kinase inhibitors [40, 41] and other types of immune checkpoint inhibitors such as CTLA-4 antibody as well as immune checkpoint therapy combined with locoregional therapy such as resection [42–44], ablation [45, 46], transarterial chemoembolization [47, 48], and hepatic arterial infusion chemotherapy [49, 50].

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Disclosure Statement

The authors have no conflicts of interest to disclose.

Author Contributions

Naoshi Nishida drafted the manuscript and wrote the final version. Masatoshi Kudo approved the final version of the manuscript.

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