Oncogenic Pathways and Immune Microenvironment

Subjects: Oncology

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Oncogenic signals affect the expression of several immune-related molecules, including immune regulatory receptors, ligands, growth factors and other humoral factors, which affect diverse stromal cells as well as cancer cells. The cellular components of tumors and their states are major players in the regulation of the tumor immune microenvironment.

Keywords: cancer ; hepatocellular carcinoma ; immune evasion ; immunotherapy ; immune checkpoint inhibitors ; oncogenic signaling pathway ; molecular targeted agents ; genome ; epigenome ; tumor immune microenvironment

1. Introduction

Hepatocellular carcinoma (HCC) is highly refractory and is the third leading cause of cancer-related deaths worldwide. Recent advancements in molecular targeted agents (MTAs) for HCC have dramatically improved the prognosis for patients with this disease. Following the approval of sorafenib as the first MTA for advanced HCC, lenvatinib has also been applied as a first-line systemic chemotherapeutic for HCC, while regorafenib, cabozantinib and ramucirumab have been approved as second-line agents. Because MTAs primarily target molecules involved in oncogenic signaling pathways that play an important role in the development of cancer cells, the development of clones resistant to MTAs can happen easily by genetic mutations and modifications in the specific molecular pathways. Hence, additional chemotherapeutic agents would be required.

In contrast, immune checkpoint inhibitors (ICIs) play a role in tumor regression by a different mechanism from that of MTAs. They are known to interfere with the immunosuppressive mechanism to enhance the anti-tumor immune response. Because the target molecules of ICIs are primarily expressed in the stromal cells as well as the cancer cells, ICIs can be effective even for patients who fail to respond well to MTAs or acquire resistance to them, potentially enabling ICIs to complement treatment with MTAs. Although the clinical trial of anti-programmed cell death-1 (PD-1) monotherapy failed to show a significant difference in the survival of patients with advanced HCC compared with conventional MTAs, synergic effects of the combination of different kinds of agents can be expected in several ongoing clinical trials of ICI-based therapy. Based on a successful Phase III clinical trial, the combination of the ICI atezolizumab (an anti-PD-1 antibody) with MTA bevacizumab (an anti-VEGF-A antibody) was approved as a first-line therapy for unresectable HCC.

Because of the complexity of cancer immunity, where immune cells, tumor cells and other types of stromal cells affect each other, understanding the immune microenvironment of the tumor is difficult. While it has been considered that oncogenic mutations in tumor cells do not directly affect the outcome of ICI therapy, recent reports have suggested that mutation-induced changes in the tumor phenotype can affect the tumor–stroma interactions through alterations in the expression of immunosuppressive cytokines, chemokines, receptors and metabolites, thereby potentially affecting the tumor immune microenvironment. Thus, the anti-tumor effect of MTAs in combination therapy with ICIs can be attributed to the direct action of MTAs on the HCC cells, as well as the reduction in the immunosuppressive nature of the tumor microenvironment through the inhibition of specific oncogenic signals.

To understand the significance of oncogenic signaling in the establishment of an immunosuppressive tumor microenvironment, and for the application of this knowledge to the treatment of HCC, this review focused on the role of specific genetic mutations involved in the oncogenic pathways responsible for anti-tumor immunity, and the current status of and perspectives on the combination of ICIs and MTAs for the treatment of HCC.

2. Cellular Components and Molecules Associated with an Inhibitory Tumor Immune Microenvironment

Oncogenic signals affect the expression of several immune-related molecules, including immune regulatory receptors, ligands, growth factors and other humoral factors, which affect diverse stromal cells as well as cancer cells. The cellular components of tumors and their states are major players in the regulation of the tumor immune microenvironment.

Therefore, to better understand the impact of oncogenic signals on anti-cancer immunity, the functions of the stromal cells involved in the immune microenvironment of tumors are briefly discussed here.

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells that suppress tumor immunity and can be induced by VEGF. Via their increased arginase activity, degradation of arginine, and uptake of tryptophan, cysteine and other amino acids required for T-cell activation, MDSCs reduce the concentrations of these amino acids in the tissue microenvironment, thereby inhibiting the propagation and activation of T cells. In addition, MDSCs produce TGF-β and IL-10, inducing Tregs and inhibiting natural killer (NK) cell function. Furthermore, MDSCs induce the immunosuppressive M2 macrophages by secreting IL-10, which, in turn, downregulates IL-12 production by tumor-associated macrophages (TAMs).

As shown above, the concentrations of metabolites from cancer cells and stromal cells strongly affect the immune state of the tumor. Cyclic adenosine monophosphate (cAMP), which accumulates in tumor tissues, inhibits CD4 + and CD8 + T-cell responses and macrophage activation, and enhances the Treg response by binding to adenosine A2A receptors. In addition, due to the hypoxic environment in tumor tissues, cAMP upregulates the enzyme COX-2, which synthesizes PGE2 from arachidonic acid. Subsequently, PGE2 binds to prostaglandin E receptor 4 on T-cells and affects T-cell activation and cytokine production.

MDSC- and TAM-derived arginase hydrolyzes arginine in the urea cycle and inhibits the function of CTLs via this deficiency in L-arginine. In tumor tissues, a hypoxic environment results in the expression of hypoxia-inducible factor 1α (HIF-1α), which is known to activate arginase. Additionally, IDO is reported to be produced by DCs, macrophages, CAFs, vascular endothelial cells and HCC cells via inflammatory cytokines. As previously stated, IDO inhibits T-cell activation and amplification via depletion of tryptophan and stimulates the differentiation of naïve CD4 + T-cells into Tregs.

3. Signaling Pathways and the Immune Microenvironment of Tumors

Alterations in oncogenic signaling in cancer not only trigger abnormal differentiation and cell proliferation, but also play a crucial role in the immune evasion of tumors. Cancer-related signaling affects the state of the tumors' immune components via cytokine, chemokine and growth factor production. To date, genetic alterations in several signaling pathways observed in cancers have been reported to affect the tumor immune microenvironment.

Aside from the altered Wnt/β-catenin signaling in tumor cells, activation of this signaling pathway reportedly disturbs the effector function of CD8 + T-cells and induces the exhausted T-cell phenotype in HCC and colorectal cancers, which contributes to the establishment of immune suppressive tumor microenvironment. Interestingly, neutralization of a canonical Wnt ligand, Wnt 3a, enhances the T-cell response through the rescue of DC activation, resulting in tumor regression in a mouse model.

The JAK/STAT pathway, which transmits signals that are crucial for growth, differentiation, survival and immunity, is altered in many types of malignancy. The downstream transcription factor, STAT3, acts on the PD-L1 promoter, thereby inducing upregulation of PD-L1 in cancer cells. In melanomas, JAK1 and JAK2 mutations inhibit signals from interferon receptors and reduce antigen presentation on tumor cells, which results in resistance to ICI therap. Meanwhile, β2-microglobulin gene mutations have been reported to induce resistance to ICI treatment via the loss of MHC Class I antigen expression on the cell surface.

It is well known that cancers carrying mutations in DNA mismatch genes induce a large number of neoantigens that are attributed to the emergence of a variety of passenger mutations that occur in the microsatellite sequences of the DNA, where anti-tumor immunity is enhanced. Therefore, microsatellite instability is a biomarker for efficacy in the treatment of ICIs. Similarly, cancers with a high mutation burden (TMB) are also markers of tumors with an active immune microenvironment because of their high antigenicity. Recently, it was reported that loss-of-function mutations in the breast cancer susceptibility (BRCA) 1 and BRCA 2 genes, which are involved in the homologous recombination pathway of DNA repair, are also markers of a high TMB and could be predictors of the outcome of ICI-based treatment. From this perspective, alterations in DNA repair pathways are critical for the establishment of high antigenicity and "immune hot" status in cancer.

4. Signaling Pathway Abnormalities and the Immune Microenvironment in HCC

In a mouse model of HCC, it has been shown that activation of Wnt/β-catenin signaling induces reduced migration of CD103 + DCs and CD8 + TIL deficiency via downregulation of CCL5. Previous reports have also shown that Wnt/β-catenin activation is associated with the reduced expression of T cell-derived genes in HCC tissues. Therefore, HCC with activated Wnt/β-catenin signaling is unlikely to respond to ICIs because of the “immune cold” phenotype. In fact, post-ICI therapy outcomes are reported to be poor in cases of HCC with Wnt/β-catenin activation. Using a cohort of HCC cases from The Cancer Genome Atlas (TCGA), we determined that the expression of T cell-related genes was low in cases of HCC with activating mutations in Wnt/β-catenin. In addition, in an analysis of HCC tissues, we determined that HCCs with activating mutations in Wnt/β-catenin pathway genes are significantly deficient in CD8 + TILs. However, we did not find CD8 + TILs to be associated with mutations in any other oncogenic signaling pathways.

In a transcriptome analysis, we reported that Wnt/β-catenin signaling activation was associated with the decreased expression of gene sets related to T-cell priming/activation, IFN-γ response, immunosuppression and Tregs; it was most significantly associated with the downregulation of genes related to the IFN-γ response in multivariate analysis. These data are consistent with the deficiency in CD8 + T-cells in HCC tissues. In addition, we also reported that activating mutations in the Wnt/β-catenin pathway is negatively associated with PD-L1 expression in HCC. As the expression of PD-L1 can be induced by the stimulation of IFN-γ, the lack of PD-L1 expression in HCC with Wnt/β-catenin activation can probably be attributed to the low degree of CD8 + TILs that should secrete IFN-γ. On the other hand, a previous study found that mutations in genes involved in chromatin remodeling, such as AT-Rich Interaction Domain 2 (ARID2), were also associated with an immunosuppressive tumor microenvironment through the expression of genes involved in the induction of M2 macrophages, although there were no associations between mutations of the genes involved in chromatin remodeling and the degree of CD8 + TILs as well as PD-L1 expression. As mutations of ARID2 are reportedly associated with the TAM subclass of HCC, the immune suppressive mechanism in HCCs with an ARID2 mutation should be different from that of CTNNB1. In contrast, PD-L1-positive HCCs often have high levels of CD8 + TILs. This may be due to the fact that PD-L1 expression in HCC cells can be mainly attributed to stimulation by the IFN-γ from TILs. It is possible that, under continuous immune response to cancer cells, many CD8 + TILs are prone to expressing multiple inhibitory receptors (PD1, TIM-3, LAG-3) that result in the exhausted phenotype of T-cells. In many cases, PD-L1 expression is considered to be a favorable prognostic factor of ICI therapy, suggesting that blockade of the PD-1/PD-L1 response could, at least partially, activate the T-cell immune response, even if the immune cells express additional inhibitory receptors. Indeed, we found that the absence of activating mutations in Wnt/β-catenin pathway genes, a high CD8 + TIL volume and PD-L1 expression were associated with long progression-free survival of HCC patients on anti-PD-1 antibody therapy, regardless of the expression of other inhibitory receptors, such as TIM-3 and LAG-3. In this way, assessments of gene alterations in cellular signaling pathways are not only useful for finding suitable MTAs that act on the altered cellular signal, but may also, theoretically, serve to predict the response to ICI therapy, based on the tumor immune microenvironment.