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Targeting immunosuppression after standard sorafenib treatment to facilitate immune checkpoint blockade in hepatocellular carcinoma – an auto-commentary on clinical potential and future development

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Immunotherapy has shown great promise to transform solid cancer treatment. The challenge is to optimally incorporate novel immunotherapeutics, such as immune checkpoint blockers, with standard therapies. This is well exemplified by multimodal therapies recently developed for liver cancer in which immunomodulation using CXCR4 inhibition prevented immunosuppression and enhanced sorafenib and anti-PD-1 therapeutic outcome.

Immunotherapy has emerged as an attractive therapeutic modality for cancer due to durable responses and remissions seen with immune checkpoint (*e.g.*, CTLA-4 and PD-1) blockade in a fraction of patients with certain malignancies such as advanced melanoma and lung carcinoma.[1] This has generated great excitement and renewed promise for solid tumor treatment in general but has also raised concerns about how to best incorporate these new drugs in the clinic with the current standards of care. Effective immunotherapies depend on their ability to activate tumor-specific effector (cytotoxic) $CD8^+$ T cells and increase their accumulation in tumors and/or inhibit immunosuppressive cues. However, most cancers have insufficient intratumoral infiltration of activated antitumor T cells and an immunosuppressive microenvironment. These traits could be exacerbated in tumors after treatment with standard therapies. In these contexts, increased hypoxia, infiltration of pro-inflammatory/immunosuppressive bone marrow-derived cells (BMDCs) and regulatory T cells, and the secretion of immunosuppressive cytokines can impair activation of lymphocytes in tumors, thereby hindering immunotherapy. Furthermore, immunosuppressive tumor-associated BMDCs secrete cytokines and interact with the surrounding cancer cells or other stromal cells in the tumor microenvironment to promote cancer cell survival and proliferation, angiogenesis and metastasis. Thus, new combinatorial therapeutic strategies need to take into consideration these intricate paracrine interactions and treatment-induced effects.

In advanced hepatocellular carcinoma (HCC), the current standard-of-care is systemic treatment with the multi-kinase inhibitor sorafenib. Sorafenib is considered an antiangiogenic drug due to its inhibition of vascular endothelial growth factor (VEGF) signaling. We recently developed orthotopic (implanted and genetically engineered) mouse models of HCC using chemically induced liver fibrosis to reproduce some of the features of human disease. In these clinically relevant models, we demonstrated that sorafenib therapy, while delaying HCC growth, further polarized the microenvironment toward a pro-fibrotic and immunosuppressive phenotype culminating in treatment evasion.[2,3] We also showed that sorafenib treatment in HCC reduced tumor vascular density and increased hypoxia, which was associated with increased expression of the immune checkpoint inhibitor programmed cell death-ligand 1 (PD-L1/CD274) as well as the chemokine (C-X-C motif) ligand 12 (CXCL12, also known as SDF-1α). The activation of the SDF-1α/CXCR4 axis incited tumor immunosuppression through recruitment of BMDCs, such as $Gr\text{-}1^+$ myeloid cells, M2-type macrophages and regulatory T cells. In addition to pro-angiogenic, pro-inflammatory and pro-immunosuppressive effects, hypoxia can induce epithelial-to-mesenchymal transition (EMT) in cancer cells, which plays a key role in promoting metastasis and cancer progression. Indeed, sorafenib treatment did not reduce metastasis despite delaying HCC growth in this preclinical model.

Given these findings, we next examined the effects of implementing checkpoint inhibition with anti-PD-1 antibody in HCC. We found that this immunotherapy

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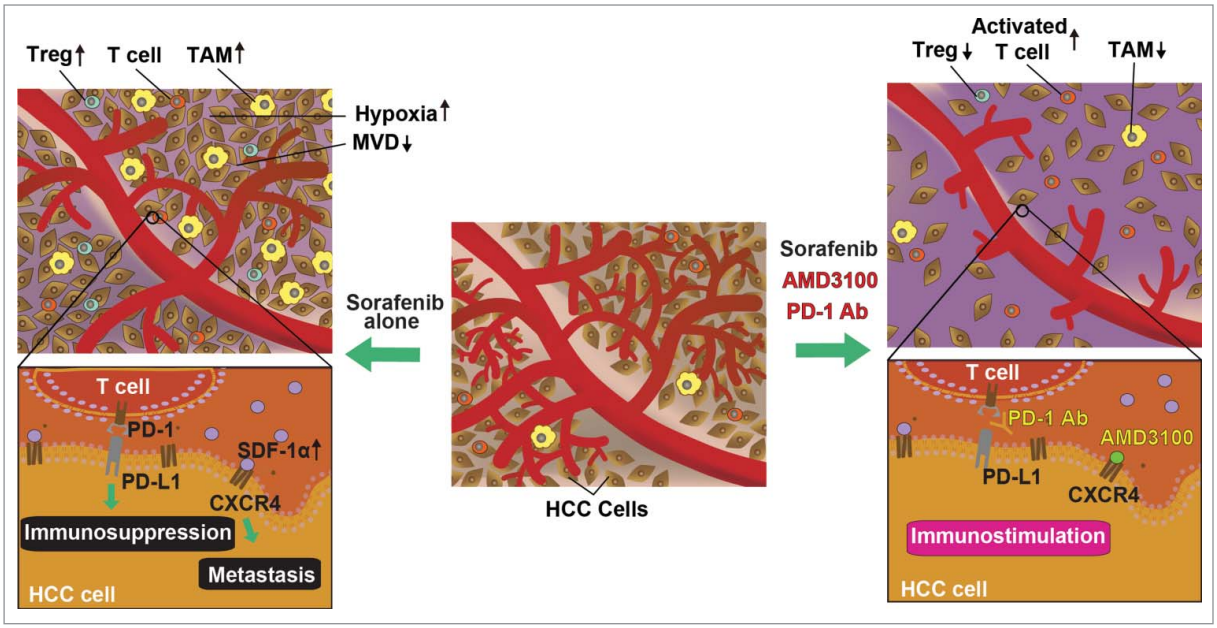

Figure 1. Combination therapeutic strategy modulating the immunosuppressive microenvironment for cancer treatment. Increased intratumoral hypoxia after sorafenib treatment–caused by reduced microvascular density (MVD)–increased expression of PD-L1 and SDF-1α, and the recruitment of immunosuppressive bone marrow-derived cells (BMDCs) and regulatory T cells (Tregs) in hepatocellular carcinoma (HCC). These effects were prevented when combining sorafenib with AMD3100, a CXCR4 antagonist, which facilitated immunotherapy with anti-PD-1 antibodies.

is active against both grafted and spontaneous tumors. However, anti-PD-1 blockade did not significantly delay tumor growth or metastasis when combined with sorafenib, likely owing to the increased immunosuppression seen after sorafenib treatment. Sorafenib plus anti-PD-1 antibody significantly delayed HCC growth and reduced lung metastasis only when combined with anti-CXCR4 therapy to prevent the increase in immunosuppression (**Fig. 1**). Triple combination treatment was safe and associated with increased tumor penetration by activated $CD8^+$ T lymphocytes and accompanying increased HCC cell apoptosis. Moreover, CXCR4 blockade inhibited HCC metastasis by preventing EMT, despite the persistent intratumoral hypoxia.

These findings may have direct implications for the development of targeted therapies in HCC in general, and for the translation of immune checkpoint blockers in particular.[4] Approval of sorafenib has prompted aggressive development of other antiangiogenic agents against HCC, a highly vascularized tumor.[5] Unfortunately, all Phase III trials of more potent or specific antiangiogenic agents conducted so far have failed. Our data indicate that increased intratumoral hypoxia after sorafenib treatment fuels resistance to treatment by promoting fibrosis and immunosuppression in HCC,[2,3] in line with findings on the role of hypoxia in other cancers.[6-9] This also raises the concern that the efficacy of immunotherapies against HCC could be hampered by antiangiogenic treatment. Indeed, our results showed a lack of efficacy of sorafenib when simultaneously administered with anti-PD-1 antibody. They also show that this unwanted antagonism may be thwarted by concomitantly targeting CXCR4 as an immuno-modulatory and anti-metastatic approach, which rendered anti-PD-1 treatment efficacious in HCC models.

Cancer immunotherapy has benefited greatly from the development of modified cancer cell-based vaccines, immunogenic gene or peptide delivery systems, and immune checkpoint blockades over the past 2 decades. One potential strategy to combine these approaches with anti-angiogenic therapy is to use "vascular normalizing" doses that alleviate hypoxia and enhance the efficacy of immunotherapy.[10] Our study suggests another potential strategy for such combinatorial therapies against HCC, by adding an agent that targets a hypoxia-induced pathway, SDF1α/CXCR4. This approach facilitated immunotherapy in the face of persistent hypoxia, and should be tested in combination with other immunotherapeutic agents, such as a cancer cell-based vaccine. Irrespective of the approach, our results clearly highlight the importance of rationally developing immunotherapies for HCC, which should be based on the mechanism– and biomarker-driven approaches and appropriate modulation of the immunosuppressive tumor microenvironment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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