

Combination Therapies with Anti-angiogenesis and B7-H3 Blockade in Cancers

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Tumor angiogenesis, a hallmark of cancer, is a critical step in the tumorigenesis of solid cancers [1]. The process of tumor angiogenesis is orchestrated by a range of secreted factors, signaling pathways as well as non-endothelial cells [2]. Abnormal tumor vascular networks sometimes contribute to a decline in the efficacy of various therapies, such as chemotherapy, radiotherapy, and immunotherapy [3,4]. Anti-angiogenesis via targeting VEGF-mediated signaling has become one of the most promising therapies, which aimed at inhibiting VEGF activity to regress tumors by starvation [5]. In 2003, a clinical trial demonstrated that chemotherapy combined with bevacizumab (humanized neutralizing antibodies targeting VEGF) improved the clinical survival of metastatic colorectal cancer (CRC) patients [6]. After bevacizumab receiving the FDA (the Food and Drug Administration) approval [7], the FDA has approved various angiogenic inhibitors as cancer therapies. Unfortunately, emerging clinical evidence indicated that the benefit of these anti-angiogenic treatments have so far shown only modest clinical efficacy and remained to be suboptimal in patients who lack responses or acquire resistance.

Immune checkpoint inhibitors (ICIs) have shown a long-lasting clinical activity against a large number of malignancies [8], which re-start the anti-tumor immune responses of the host via inhibiting the negative

regulatory immune signals of T cells [9]. In the last decade, cytotoxic T lymphocyte antigen 4 (CTLA-4) and the programmed cell death receptor 1 (PD-1) and its ligand, PD-L1 inhibitors have made remarkable progress in the clinical application of cancer immunotherapies [10]. Ipilimumab, an anti-CTLA-4 antibody, was granted FDA approval in 2011 and improved the overall survival of patients with advanced melanoma [11]. Furthermore, pembrolizumab and nivolumab, anti-PD-1 antibodies, were approved for the advanced melanoma treatment in 2014 [12,13]. However, the response rates of single antibody blocking PD-1 or CTLA-4 pathway remain low in a majority of patients [14,15]. To overcome this problem, a rationale for the combination of therapies with ICIs and conventional therapies such as chemotherapy, radiotherapy, and anti-angiogenesis therapy, started to be considered.

Previous evidence showed that angiogenesis played a key role in regulating tumor immune response and lead to resistance to ICIs [16]. VEGF family, which induces physiological and pathological angiogenesis [17], has been reported to suppress tumor immune response by enhancing T cell exhaustion by upregulating PD-L1, CTLA-4, TIM3 and LAG3 expression on T-cells [18]. Besides, VEGF promoted the expansion of T-regulatory cells (T-regs) and Myeloid-derived suppressor cell (MDSCs) and the infiltration of tumor-associated

macrophages (TAMs) in tumors [19]. Conversely, the immune microenvironment is also able to affect the tumor angiogenesis. Huang et al. reported that IL-35 recruits monocytes via CCL5 and induces macrophages to promote angiogenesis through inducing CXCL1 and CXCL8 expression in pancreatic ductal adenocarcinoma cells [20]. CD11b⁺Gr-1⁺ MDSCs contributed to lung metastasis of breast cancer by inducing angiogenesis in a platelet-derived growth factor-BB dependent manner [21]. Therefore, the mutual interactions between tumor angiogenesis and immunosuppressive microenvironment may be a key cause of the failure of single anti-angiogenesis therapy or ICIs.

B7-H3 (B7 homolog 3 protein), also known as CD276, belongs to the B7-CD28 immune checkpoint family and takes part in cancer development and cancer immunity [22]. We have previously shown that B7-H3 is significantly upregulated in CRC tissue samples compared with normal adjacent tissues, and is positively associated with TNM stages [23]. Current research results also demonstrated that the upregulation of B7-H3 is closely related to lymph node metastasis in patients with CRC. Other groups have also shown that B7-H3 is overexpressed in multiple malignant tumors, and is associated with poor prognosis [24,25]. These results suggest that B7-H3 exerts crucial effects on tumor progression.

B7-H3 has been reported to exert a costimulating effect on the proliferation and IFN- γ production of T cells [26,27]. By contrast, other studies have shown that B7-H3 plays an inhibitory role in T cell proliferation [28]. Although the immunologic function of B7-H3 remains controversial, B7-H3 has been a potential target for cancer immunotherapy. Du and colleagues generated chimeric antigen receptor (CAR) T cells targeting B7-H3 (B7-H3-CAR-Ts) and found that B7-H3-CAR-Ts controlled the growth of pancreatic ductal adenocarcinoma, ovarian cancer, and neuroblastoma *in vitro* and in orthotopic and metastatic xenograft mouse models [29]. In addition, B7-H3-deficient mice or mice treated with an antagonistic antibody to B7-H3 showed reduced growth of multiple tumors, which depended on NK and CD8⁺ T cells [30]. In ID8 ovarian cancer mouse models, B7-H3 expressed on tumor cells, but not host cells, had a dominant role in suppressing the function of CD8⁺ T cells [31]. Moreover, B7-H3 blockade, but not PD-1 blockade, prolonged the survival of ID8 tumor-bearing mice [31]. Therefore, B7-H3 may be a promising immune therapeutic target for tumors.

Aside from its immunologic function, B7-H3 has been reported to participate in multiple non-immunological functions in cancers, such as proliferation, metastasis,

drug resistance and metabolism [32]. Previous reports from our group have shown that B7-H3 plays an important role in metabolism and chemoresistance in CRC [23,33]. Body of evidence has revealed that B7-H3 participates in the progression and metastasis of CRC, indicating that B7-H3 has become a new potential prognostic marker and therapeutic target for CRC [25]. More importantly, B7-H3 has been found to take part in the anti-angiogenesis in cancers. A prior study has shown that the expression of B7-H3 dramatically differs between physiological and pathological angiogenic vessels and is remarkably upregulated in the blood vessels of various human cancers [34]. In our current research, our data indicated that the expression level of B7-H3 is a positive association with CD31, a sensitive and specific endothelial marker for MVD in CRC tissue samples. Seaman et al. demonstrated that pyrrolobenzodiazepine-conjugated anti-B7-H3-drug can target both angiogenic vessels and non-angiogenic vessels, showing promising antitumor activity while little toxicity [35]. Furthermore, a series of *in vitro* and *in vivo* experiments showed that B7-H3 on CRC cells upregulated VEGFA expression and angiogenesis by activating the NF- κ B pathway. Therefore, B7-H3 may promote angiogenesis by upregulating the expression of VEGFA in CRC, showing its great prospect of a possible biomarker in personalized anti-angiogenic therapy.

Nowadays, it is believed that combining an immune checkpoint inhibitor (ICI) with anti-angiogenesis would become a promising strategy, which can overcome the treatment resistance and finally improve patients' prognosis [9]. We are aware that it seems to be a two-way street between anti-angiogenic therapies and immunotherapies, whose efficacy influence with each other [36]. Excessive levels of VEGF contribute to VEGF-induced immunosuppression in tumors [37]. The clinical success in the combination of VEGF inhibitors and ICI therapy attributed to the VEGF inhibitors, suppressing VEGF-induced immunosuppression and promoting an anti-tumor immune response [36]. Given that B7-H3 not only exerts crucial regulatory effects on the anti-tumor immune but also plays key roles in tumor angiogenesis, combination therapy with B7-H3 blockade and anti-angiogenesis may be a particularly valuable option for the treatment of cancers. In our current research, we showed that the combination therapy of B7-H3 inhibitor 3E8 with bevacizumab inhibited the tumor growth, showing a more inhibitory effect on the MVD and VEGFA expression in mouse xenograft models (Figure 1). As such, it is expected that combination therapy with B7-H3 blockade and anti-angiogenesis is applied to the clinical treatment of tumors.

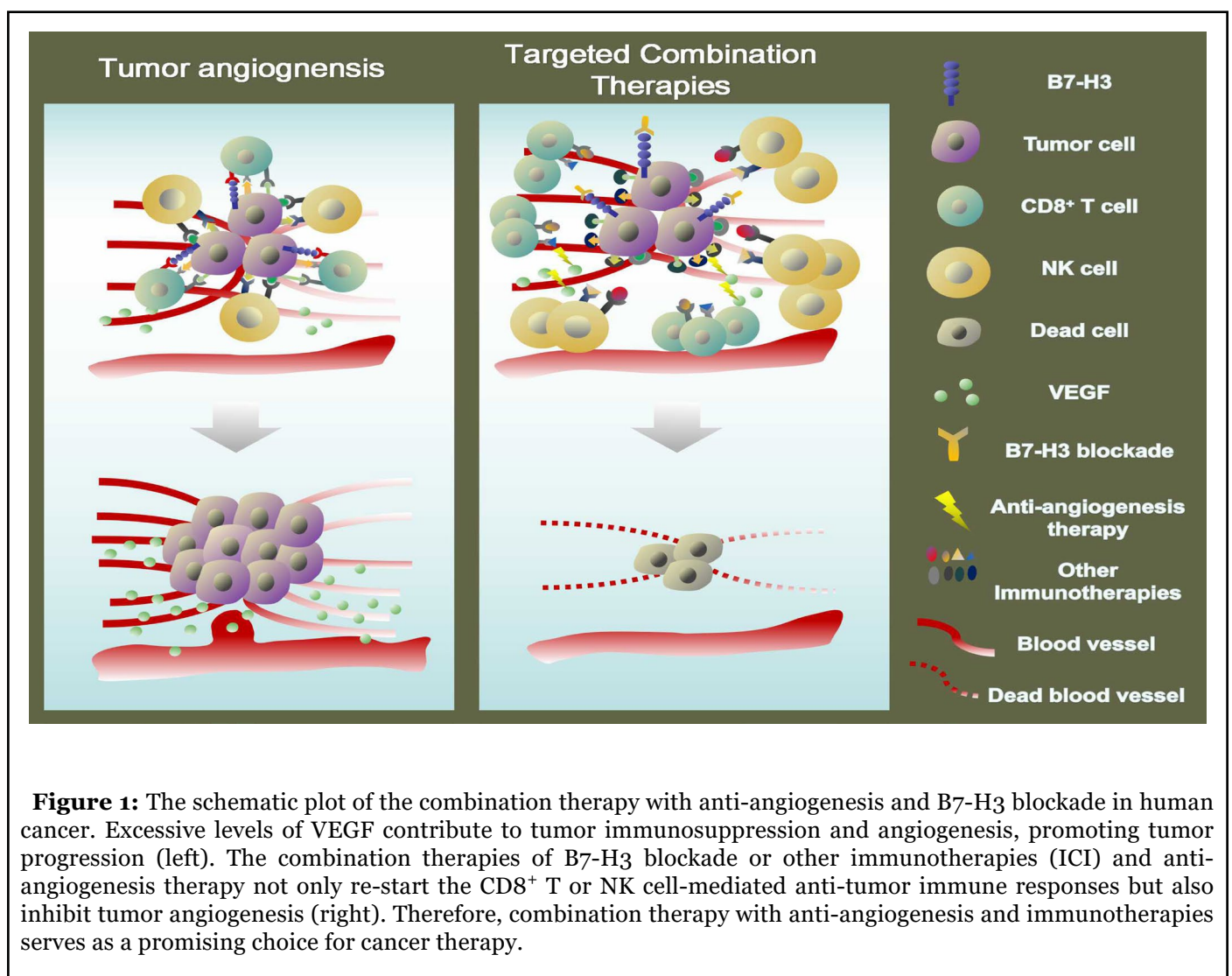


Figure 1: The schematic plot of the combination therapy with anti-angiogenesis and B7-H3 blockade in human cancer. Excessive levels of VEGF contribute to tumor immunosuppression and angiogenesis, promoting tumor progression (left). The combination therapies of B7-H3 blockade or other immunotherapies (ICI) and anti-angiogenesis therapy not only re-start the CD8⁺ T or NK cell-mediated anti-tumor immune responses but also inhibit tumor angiogenesis (right). Therefore, combination therapy with anti-angiogenesis and immunotherapies serves as a promising choice for cancer therapy.

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Conflict of Interest

The authors declare no conflict of interest.

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