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Anti-angiogenic immunotherapy

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Tumors stimulate angiogenesis to meet increasing nutrient and oxygen demands. In addition to their role in vascular remodeling, pro-angiogenic cytokines and effector cells contribute to an immune-inhibitory environment associated with advanced malignancies. Despite the critical role of angiogenesis in tumor growth and dissemination, most anti-angiogenic cancer therapies have had only limited success selectively targeting one of the many factors implicated in this process. Similarly, the effectiveness of tumor immunotherapies has been limited by tumor-mediated escape mechanisms and immune suppression. By combining the two strategies, however, anti-angiogenic immunotherapy offers the possibility to more robustly inhibit tumor angiogenesis and simultaneously impact the immune-inhibitory effects of the pro-angiogenic tumor milieu. These potential synergies make the combination of immunotherapy and anti-angiogenic treatment a promising avenue for future research.

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Tumor Angiogenesis

Angiogenesis is a critical part of tumor growth and dissemination. Tumors greater than two to three millimeters have oxygen and nutrient requirements that exceed those that can be met by diffusion alone.[1] Consequently, they recruit, remodel and expand the existing vasculature to meet their metabolic demands. This process is critical to development,[2] but occurs only rarely in the healthy adult: during endometrial proliferation through the menstrual cycle, and during the process of wound healing. Thus, the continued cycle of angiogenesis that occurs in the tumor microenvironment has been likened to "wounds that never heal."[3] Chronic inflammation, inhibition of cellular immune responses, and a dysfunctional and abnormal vasculature are all hallmarks of this pathologic environment.

Under normal conditions, angiogenesis is a complex process involving regulated changes in endothelial cell growth, survival, proliferation, migration and tube formation.[4] These multiple steps require the concerted action of numerous cytokines, cell surface receptors and intracellular signaling cascades. Vascular endothelial growth factor-A (VEGF-A), originally identified as a vascular permeability factor,[5,6] is an important cytokine mediator of tumor-driven angiogenesis.[7] The remainder of the VEGF family of proteins (VEGF-B, C, D and E), placental growth factor (PlGF) and the angiopoietins are additional cytokines that have also been implicated in this process.[8,9]

The dependence of growing tumors on new blood vessel formation has made angiogenesis an appealing target for anticancer therapies. Most notably, a VEGF-A blocking antibody, bevacizumab, has demonstrated clinical benefit, improving survival in metastatic colon cancer.[10] Bevacizumab has also demonstrated promise and benefit in other malignancies including lung, breast, renal cancers and glioblastoma.[11-14] Tyrosine kinase inhibitors that impact angiogenesis such as sorafenib and sunitinib have also proved efficacious in diseases such as hepatocellular carcinoma and renal cell cancer.[15-17] Other anti-angiogenic strategies, including monoclonal antibodies, kinase inhibitors, IgG fusion proteins, RNA-aptamers

and RNA-interference are being actively pursued for these and many additional malignancies.[9]

Despite their promise and encouraging initial results, the benefits of existing anti-angiogenic therapies have been modest, with limited improvements in survival. There are many potential explanations for this short-term overall benefit. Existing therapies generally target only one or a single family of angiogenic mediators. In response, tumors may evade this inhibition by utilizing redundant pro-angiogenic pathways and cytokines, thus eventually resuming angiogenesis unabated.[18-20] Moreover, tumors can potentially bypass angiogenic inhibitors by using autocrine loops and angiogenic factors sequestered in the tumor microenvironment, and thereby inaccessible to exogenous blockade.[21,22]

Tumor Immunotherapy

Evidence supporting the immune system's potential role in the treatment of established malignancy is rapidly expanding. Tumor-infiltrating lymphocytes have been associated with improved outcomes in various malignancies including melanoma and cancers of the ovary, esophagus, prostate, breast, kidney and colon.[23-32] Two drugs used in the treatment of high-risk and advanced melanoma, interferon-α-2b and interleukin-2, are hypothesized to work by augmenting anti-tumor immunity,[33,34] and an additional drug, the immunomodulatory anti-Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) antibody ipilimumab, was recently approved by the FDA based on its ability to increase survival in metastatic melanoma patients refractory to previous therapy.[35] Finally, a prostate cancer dendritic cell vaccine targeting prostatic acid phosphatase was also recently approved by the FDA after a randomized trial demonstrated its ability to increase median survival by approximately 4 months in metastatic castrate-resistant prostate cancer patients.[36]

Although our ability to manipulate the immune system and identify potential tumor antigens has increased, tumor immunotherapies are still limited by the ability of advanced malignancies to evade immune recognition. Many potential tumor antigens are also present on normal tissue and can therefore induce immune tolerance; moreover, tumors can mutate and shift their antigenic profile, thereby evading immune attack.[37] Additionally, in part because of a significant tumor burden, patients with advanced malignancies are often relatively immunocompromised. Cancers can themselves be immunosuppressive in a variety of ways: by releasing immunosuppressive cytokines; by inhibiting antigen processing and presentation; by increasing numbers of inhibitory T-regulatory cells; by recruiting tumor-infiltrating macrophages and myeloid-derived suppressor cells; and by attenuating immune-mediated cytotoxicity.[37-50]

Angiogenesis as a Target for Tumor-Immunotherapy

Pro-angiogenic cytokines, receptors and effector molecules may be particularly good targets of immune-based cancer therapy. As mentioned above, although angiogenesis is a normal physiologic process, it is tightly regulated[4] and infrequently occurs in the healthy, non-menstruating adult. Consequently, anti-angiogenic immunotherapy can target a wide variety of tumor types yet be tumor-specific. In contrast to other tumor-specific therapies, targeting the tumor-associated vasculature in addition to the tumor itself may make anti-angiogenic immunotherapy more resistant to immune-escape mechanisms.[51] The mediators of angiogenesis, including a number of normal cell types such as the vascular endothelium, do not likely possess the same degree of mutability as cancer cells.

Immune-based cancer therapies that target angiogenesis may potentiate a broader anti-tumor immune response[52] by interfering with tumor-mediated immune inhibition. VEGF may functionally inhibit the immune system in part by preventing the maturation of dendritic cells[53,54] and inhibiting early T-cell development.[55] In a study of 19 patients with colon cancer, inhibiting VEGF with bevacizumab increased the antigen-presenting capacity of peripheral blood dendritic cells.[56] The angiopoietins impact inflammation[57] and affect immune trafficking due to their ability to increase expression of platelet endothelial cell adhesion molecule-1 (PECAM-1) and vascular endothelial (VE)-cadherin and decrease expression of vascular-cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and endothelial leukocyte adhesion molecule 1 (ELAM-1).[58] Consequently, inhibiting angiopoietins or other angiogenic mediators may restore normal immune cell trafficking and increase numbers of tumor-infiltrating lymphocytes.[59,60] For instance, mice with tumors engineered to express the inhibitory soluble angiopoietin receptor, tie-2, demonstrated increased survival dependent on increased numbers of tumor-infiltrating leukocytes.[58]

Angiogenic cytokines also help recruit and stimulate immune-inhibiting monocytes and myeloid suppressor cells. Angiopoietin-2 stimulates tie-2 expressing monocytes to suppress T-cell activation and promote regulatory T-cell activity.[61,62] Populations of myeloid-suppressor cells that release immunosuppressive cytokines can be recruited by VEGF expression.[63,64] Inhibition of VEGF with bevacizumab can reduce levels of these cells in the peripheral blood.[56]

In addition to the potential for anti-angiogenic therapy to increase anti-tumor immunity, there is also evidence to suggest that immunologic approaches may be a more successful way of inhibiting tumor angiogenesis. As previously mentioned, existing anti-angiogenic therapies that only target a single agent are limited when tumors eventually utilize other pro-angiogenic mediators.[65] Anti-angiogenic immunotherapy, in contrast, could potentially target multiple angiogenic mediators and attack tumor-associated stroma in addition to tumor cells.[66,67] Indeed, poor penetration and resistance of tumor-associated stroma may contribute to the limited effectiveness of systemic anti-angiogenic therapy.[22] Targeting immune-inhibiting monocytes[68] and myeloid-suppressor cells could also more successfully inhibit tumor angiogenesis, as these cell types have been associated with resistance to anti-VEGF therapies.[69] Finally, anti-angiogenic treatment may be most successful when administered constantly, preventing the re-growth of tumor vasculature during

breaks in treatment.[70] Immunotherapy could deliver this constant therapy without the need for repeated drug administration.

Evidence Supporting Anti-Angiogenic Immunotherapy

Studies have demonstrated the potential effectiveness of using immune therapy to target mediators of angiogenesis. Endothelial cell vaccines inhibited tumor growth and led to tumor destruction in mice.[71-74] Other studies have used a variety of means to generate a specific anti-VEGF immune response that was successful in inhibiting implanted tumor cell-types including: colorectal, rhabdomyosarcoma, fibrosarcoma, hepatoma, melanoma, lung, ovarian, pancreatic and mammary cancer cells in mice[75-78] and spontaneously arising sarcomas in dogs.[79] A number of other pro-angiogenic molecules and receptors including VEGF receptor-2 (VEGFR2), tie-2, fibroblast growth factor (FGF) receptor-1, integrin Beta-3, vascular endothelial (VE)-cadherin and matrix-metalloproteinase-2 (MMP-2) were similarly targeted with success.[77,80-85]

Combined treatment with angiogenic inhibitors and anti-tumor immunotherapy has demonstrated initial efficacy in animal models. Vaccination with dendritic cells transfected with angiogenic cytokine and receptor mRNA in addition to total tumor mRNA demonstrated a synergistic anti-tumor effect.[86] In a murine prostate cancer model, concomitant treatment with the anti-angiogenic tyrosine kinase inhibitor SU6668 and the recombinant immunomodulatory B7.2-IgG fusion protein significantly inhibited tumor growth to a greater degree than either treatment alone.[87] Lymphocytes obtained from mice that received the combined treatment demonstrated a higher proliferative response to CD3 stimulation. Administration of a GM-CSF-secreting tumor cell vaccine in combination with VEGF-blockade significantly increased the survival of mice implanted with B16 melanoma and CT26 colon carcinoma cells, increasing overall numbers of tumor-infiltrating T-cells while decreasing tumor-infiltrating regulatory T cells.[88] Treatment of mice with implanted NT2.5 breast cancer cells with DC101, an anti-angiogenic monoclonal antibody targeting VEGFR-2, increased the numbers of tumor-infiltrating lymphocytes. When combined with Her2/neu vaccination, DC101 induced tumor regression by augmenting anti-tumor immune responses,[89] an effect that was not present in mice tolerant to Her2/neu. In another study, VEGF inhibition increased the infiltration of adoptively transferred T cells into tumor implants, increasing the efficacy of this treatment.[90] Adding the CTLA-4 inhibiting antibody 9H10 to treatment of mice with DC101 and a dendritic cell vaccine led to rejection of established tumors, an effect that required the combination of all three treatments.[91] Finally, the use of the immune-stimulating cytokines GM-CSF and IL-12 with anti-angiogenic factors endostatin and pigment epithelium-derived factor had synergistic anti-tumor effects in an established woodchuck hepatoma model.[92] The combined treatment was able to induce activation of natural-killer cells and reduce expression of immune inhibitory CTLA-4 and programmed death (PD)-1 receptors compared with animals treated with immunotherapy alone.

In humans, the use of an autologous tumor cell vaccine engineered to express high levels of GM-CSF has demonstrated success in generating a coordinated anti-tumor immune response including a lymphocytic infiltrate in tumor metastases.[93,94] Interestingly, some long-term surviving patients were also noted to develop tumor-associated vasculopathy with associated lymphocytic and granulocytic invasion. Further investigation identified VEGF family members and the angiopoietins as targets of immune recognition in these and other patients who demonstrated a prolonged response to the autologous vaccine.[95] These generated antibodies demonstrated functional abilities to inhibit binding to the tie-2 receptor, downstream signaling, endothelial cell tube formation and macrophage chemotaxis. Of note was the ability of this autologous tumor cell vaccine to generate antibodies directed against a panel of angiogenic cytokines, potentially preventing the tumor-associated vasculature from eventually escaping immune recognition and angiogenic blockade in these patients. Autologous vaccination also led to antibodies directed against macrophage inhibitory factor (MIF) that functionally inhibited MIF-induced expression of tie-2 on monocytes and also inhibited the production of MMP-9. Thus, functional inhibition of monocytes as a result of this anti-MIF antibody response may act in concert with antibodies directed pro-angiogenic cytokines to inhibit tumor angiogenesis.

Future Prospects and Conclusion

The number of anti-angiogenic therapies available or undergoing active investigation has rapidly expanded in recent years, as has the potency and potential impact of anti-tumor immunotherapies. Whether these existing and investigational therapies can be used in tandem for a synergistic benefit is of much interest. Combining anti-angiogenic treatment with tumor immunotherapy may help circumvent the weaknesses of either treatment administered individually (**Fig. 1**).

Future work should delineate the specific types of anti-angiogenic treatments and anti-tumor immunotherapies that work best together and the optimal timing of each therapy when used in combination. Alternatively, angiogenic mediators can be targeted directly with immunotherapeutic approaches. Anti-angiogenic immunotherapy may also be successful when combined with conventional tumor-directed treatments such as chemotherapy and radiation, thus increasing the ability to target the tumor and tumor-stroma simultaneously.[96] Although the anti-tumor effects of these different treatments may be additive or even synergistic, their toxicities are likely to be very different, thereby increasing the potential therapeutic benefit.

Research continues to identify the complex interactions that exist between cancer, angiogenesis and the immune system. As wounds that never heal, cancer's continued angiogenic drive incites inflammation and recruits systemic mediators to aid in tumor growth and metastasis. These factors contribute to the abnormal tumor microenvironment and at least partially mediate the resistance of advanced malignancy to conventional and experimental treatments. Anti-angiogenic immunotherapy could potentially circumvent multiple

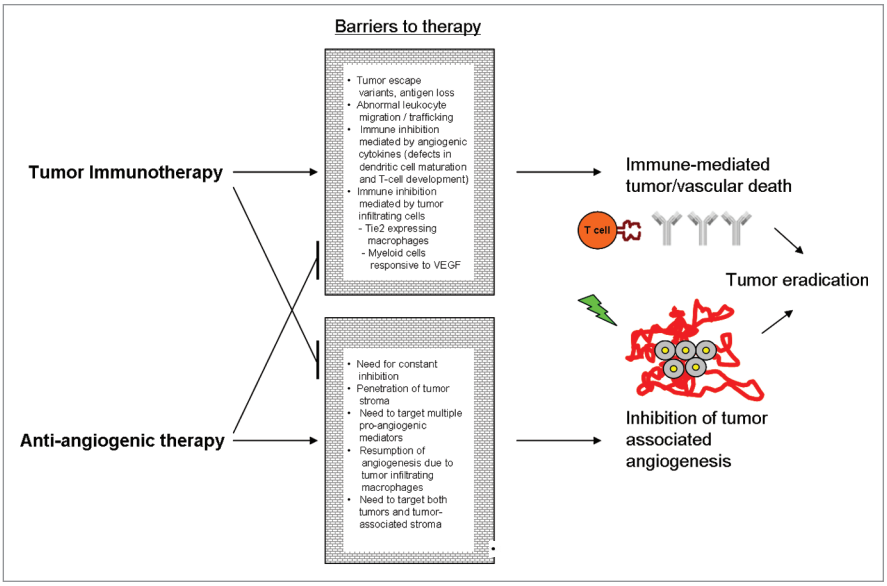

Figure 1. Combined use of tumor immunotherapy and anti-angiogenic therapy may help overcome barriers to successful tumor eradication.

aspects of this resistance to provide more effective cancer treatment.

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