Triggering Tumor Immunity through Angiogenesis Targeting

Commentary on Manning et al., p. 3951

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Whereas nascent tumors fulfill their metabolic requirements for oxygen and nutrients through diffusion from the existing vasculature, advanced lesions engender the formation of new blood vessels to support progressive tumor growth and to effectuate tissue invasion and metastasis (1). Angiogenesis reflects the complex interplay of multiple cell types, whose functions are orchestrated by an array of growth factors, adhesion molecules, and extracellular matrix components. Among the many signals contributing to tumor blood vessel development, the major vascular endothelial growth factor isoform vascular endothelial growth factor (VEGF)-A subserves a crucial role (2). VEGF-A induces endothelial cell proliferation and vascular permeability through the receptor tyrosine kinase VEGF receptor 2 (VEGF-R2; KDR, Flk-1), while stimulating the recruitment and activation of hematopoietic precursors and mature myeloid cells through VEGF-R1 (Flt1; ref. 3). This elicited cross-talk of vascular elements and immune cells recapitulates physiologic mechanisms critical to wound healing, although tumor cells usurp these pathways to establish unrestrained angiogenesis (4, 5). In this issue of Clinical Cancer Research, Manning et al. (6) show that tumor destruction accomplished with antibody targeting of VEGF-R2 involves not only the compromise of the tumor vasculature but also the generation of antitumor T-cell responses. Moreover, in a recent volume of Clinical Cancer Research, Li et al. (7) similarly showed that the addition of anti–VEGF-A antibodies to tumor cell vaccinations intensified antitumor CTL responses, thereby resulting in increased tumor rejection. Together, these intriguing studies underscore the intimate connections between angiogenesis and tumor immunity and highlight the considerable clinical potential for combination therapies.

Endogenous host reactions mediate dual and seemingly opposing roles in cancer pathogenesis (8). In some cases, the activation of innate and adaptive cytotoxic lymphocytes results in tumor suppression or the elimination of immunogenic subpopulations. Whereas the formation of clinically evident disease implies the evasion of this spontaneous immunity, the development of intratumoral T-cell infiltrates is tightly correlated with the durable responses and prolonged survival effectuated with standard oncologic therapies. Nonetheless, in contrast to this protective function, compelling epidemiologic data delineate a strong link between unresolved inflammation and tumor susceptibility. In this setting of smoldering inflammation, the mixture of cytokines and immune cells present in the tumor microenvironment supports the malignant transformation initiated by cell-intrinsic mutations in oncogenes and tumor suppressor genes. The activation of nuclear factor-κB and signal transducer and activator of transcription-3 transcription factors in both tumor and host cells represents a key pathogenic event in this pathway of carcinogenesis (9, 10).

Multiple components of innate immunity including macrophages, neutrophils, and mast cells cooperate to establish a robust vascular network that drives tumor cell invasion, expansion, and metastasis (11). The dysregulated immune response at the site of tumors fosters the production of various angiogenic cytokines, growth factors, and matrix metalloproteinases. The important role of innate immune cells in this regard is underscored by the attenuation of tumor progression but not initiation in macrophage- and mast cell–deficient transgenic models of breast and skin carcinoma (12, 13). The coordinated recruitment of Tie-2 (the receptor for angiopoietins)–expressing macrophages, VEGF-R1–expressing hematopoietic precursors, circulating endothelial cells, and endothelial progenitor cells all contribute to the formation of an angiogenic niche (14–16). Moreover, the interleukin-23/interleukin-17 network, which functions as a master regulator of tumor promoting inflammation, concomitantly inhibits intratumoral T-cell infiltration and cytotoxicity, thereby further compromising host defense (17, 18).

The central role of VEGF-A/VEGF-R2 signaling in tumor angiogenesis has motivated the crafting of therapeutic strategies to antagonize this pathway. Bevacizumab (Avastin), a humanized anti–VEGF-A murine monoclonal antibody, is the first Food and Drug Administration–approved antiangiogenic agent for cancer. Bevacizumab as a single agent manifests only minimal antitumor activities in multiple diseases; however, in conjunction with cytotoxic chemotherapy, clinically meaningful effects are achieved. A large, randomized, phase III trial in metastatic colorectal carcinoma patients revealed an increased response rate and improved survival for the addition of bevacizumab to irinotecan, 5-fluorouracil, and leucovorin compared with chemotherapy alone (19). This important validation of VEGF-A as an antitumor target has catalyzed the exploration of many other approaches to blocking angiogenesis, including small-molecule inhibitors of the VEGF-A receptor tyrosine kinases and other biologics directed toward the ligand or receptors.

Within this context, the report of Manning and colleagues provides new insights into the therapeutic activities of anti–VEGF-R2 monoclonal antibodies. Rather than using human xenografts in immunodeficient hosts, as in earlier studies, these investigators examined the effects of anti–VEGF-R2 antibodies in immunocompetent mice harboring syngeneic breast cancer cells derived from Her2/neu transgenic animals (6). This experimental strategy revealed that although antibody administration reduced angiogenesis, as determined by immunohistochemistry for CD31, it also provoked an impressive CD4+...
and CD8+ T-cell intratumoral infiltrate. Depletion of either T-cell subset diminished the antitumor potency, establishing the requirement for endogenous immunity in tumor destruction. The anti-VEGF-R2 antibodies stimulated breast cancer–specific T-cell effector functions, including cytotoxicity and IFN-γ production, and these mediated protective immunity against subsequent tumor challenges. The anti-VEGF-R2 antibodies also augmented tumor destruction when administered in combination with granulocyte macrophage colony-stimulating factor (GM-CSF)–secreting tumor cell vaccines.

Intriguingly, the immunostimulatory effects of the anti-VEGF-R2 antibodies were evident despite a marked increase in the circulating levels of VEGF-A, a cytokine previously linked to immune suppression (20). Manning and colleagues found that the anti-VEGF-R2 antibodies failed to inhibit dendritic cell maturation or promote the expansion of Gr1+Mac1+ myeloid suppressor cells. These findings raise the possibility that blockade of VEGF-R2 might abrogate in some way the VEGF-R1–mediated immune suppression. Nevertheless, the antitumor efficacy of the anti-VEGF-R2 antibodies was diminished in Neu-N transgenic mice, which manifest tolerance to Her2/neu. In these animals, the provision of cyclophosphamide and Adriamycin, which might antagonize regulatory T cells (21), was required to unveil the therapeutic effects of anti-VEGF-R2 monoclonal antibodies and GM-CSF–secreting tumor cell vaccines.

How might antiangiogenic strategies promote tumor immunity and augment the potency of immunotherapy? One possibility is that destruction of the tumor vasculature renders endothelial cell or pericyte antigens accessible for presentation by dendritic cells and macrophages. Earlier work showed that vaccination with dendritic cells transfected with mRNA encoding VEGF-R2 or Tie-2 augmented the activity of tumor cell–based vaccines (22). Similarly, the targeting of the stromal cell antigen fibroblast activation protein also enhanced antitumor vaccination strategies (23, 24). Recent mRNA profiling experiments uncovered differences in the spectrum of genes expressed between normal and tumor-associated endothelial cells, further underscoring the possibility that vascular gene products might provoke immune recognition (25). Consistent with this idea, we found that the combination of vaccination with irradiated, autologous, GM-CSF–secreting tumor cells and antibody blockade of CTL-associated antigen-4 in advanced melanoma and ovarian cancer patients resulted in the selective destruction of tumor blood vessels and extensive hemorrhagic tumor necrosis (26). The patients that manifested these striking changes generated high titer antibodies to angiopoietins 1 and 2, VEGF-A, macrophage migration inhibitory factor, and galectin 3, all factors critical to supporting angiogenesis (27).

A second mechanism for the synergy of angiogenesis inhibition and immunotherapy might involve the transient normalization of the tumor blood supply. Aberrant angiogenic signaling in tumors results in structural and functional distortion of vessels, with incomplete endothelial cell monolayers.

alterations in the basement membrane, and impaired pericite coverage (28). The infusion of anti–VEGF-R2 antibodies transiently corrects these abnormalities by triggering the up-regulation of angiopoietins and modulating the matrix metalloproteinase profiles (29, 30). These effects might increase the intratumoral trafficking of various immune cells, analogous to the improved delivery of chemotherapy and radiolabeled conjugates. The significant intratumoral CD4+ and CD8+ T-cell infiltrate, as a consequence of anti–VEGF-R2 antibody administration, is in accord with this notion.

A third mechanism that might underlie the therapeutic synergy is the capacity of angiogenesis inhibition to overcome immunosuppressive networks. Li et al. (7) reported that anti–VEGF-A antibodies antagonized antitumor regulatory T cells, thereby empowering GM-CSF–secreting tumor cell vaccines to evoke higher levels of antitumor CTLs. This pathway might involve an alteration of dendritic cell function and/or the suppression of transforming growth factor-β production, key parameters for the maintenance of regulatory T cells. Consistent with this result, Manning and colleagues showed that cyclophosphamide and Adriamycin might also deplete or attenuate regulatory T cells in tolerant neu-N production, key parameters for the maintenance of regulatory T cells. Moreover, the authors reported that GM-CSF–secreting tumor cell vaccines can transiently correct these abnormalities by triggering the up-regulation of angiopoietins and modulating the matrix metalloproteinase profiles (29, 30). These effects might increase the intratumoral trafficking of various immune cells, analogous to the improved delivery of chemotherapy and radiolabeled conjugates. The significant intratumoral CD4+ and CD8+ T-cell infiltrate, as a consequence of anti–VEGF-R2 antibody administration, is in accord with this notion.

Cytotoxic therapies might contribute to tumor destruction in additional ways, however. Recent studies disclosed that anthracyclines promote the cell-surface expression of calreticulin on apoptotic tumor cells, resulting in the efficient uptake and cross-presentation of tumor antigens by dendritic cells that express CD91 (31). Cytotoxic agents also trigger the DNA damage response, which regulates the surface expression of natural killer group 2D ligands through a pathway involving ataxia telangiectasia mutated, ataxia telangiectasia mutated and Rad3-related, checkpoint kinase 1, and checkpoint kinase 2; natural killer group 2D signaling is a potent stimulus for innate and adaptive cytotoxic lymphocyte activation that culminates in tumor suppression (32). Together, these provocative investigations reveal unexpected immunostimulatory activities of particular chemotherapies.

The multiplicity of interactions among angiogenesis inhibitors, immunotherapy, and cytotoxic agents thus presents a wealth of opportunities for combinatorial treatments (Fig. 1). Deciphering the optimal mixtures and scheduling of these modalities will require thoughtful clinical experiments that incorporate detailed monitoring of both vascular and immune end points. The effective integration of these three promising therapeutic approaches should both reduce the likelihood for the emergence of lethal tumor escape variants and replenish host networks charged with the maintenance of immune homeostasis.

References


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