CANCER

Vascular regulation of antitumor immunity

Improving vascular function provides opportunities to enhance immunotherapy

By **Lance L. Munn** *and* **Rakesh K. Jain**

Immunotherapy with immune checkpoint blockers (ICBs) has revolutionized cancer treatment. Unfortunately, ICB therapy usually benefits <15% of patients and causes immune-related adverse events in a substantial number of patients. Another immunotherapy—engineered chimeric antigen receptor (CAR) T cells that specifically target tumor-associated antigens—has transformed the treatment of multiple hematological cancers and exhibited therapeutic potential in solid tumors. Emerging data show that the function of blood vessels associated with tumors is critical in the response to these immunotherapies (*1*, *2*).

The vasculature in tumors is abnormal, which impairs blood flow and limits the delivery of oxygen, nutrients, and therapeutics, including antibodies and immune cells (*3*) (see the figure). The resulting hypoxia (low oxygen concentration) and low pH can in turn induce the production of immunosuppressive molecules, such as transforming growth factor–β (TGFβ), vascular endothelial growth factor (VEGF), and adenosine in the tumor microenvironment (TME). Some of these molecules lower the expression of adhesion molecules on tumor vascular endothelium, the lining of vessel walls that normally supports immune cell adhesion and transmigration into tissues. Reducing the expression of adhesion molecules therefore interferes with the ability of immune cells to adhere to and migrate across the vessel wall, preventing their entry into the tumor.

In addition, pro-angiogenic molecules produced by cancer, stromal, and immune cells up-regulate Fas ligand on endothelial cells and thereby induce the apoptosis of infiltrating immune cells. VEGF can hinder maturation of dendritic cells (DCs), which are needed to present tumor-associated antigens and activate effector immune cells. Furthermore, hypoxia directly up-regulates the expression of immune checkpoint molecules [e.g., programmed cell death protein ligand 1 (PD-L1)], which suppress effector T cell activation, on various cells in the TME, including myeloid-derived suppressor cells (MDSCs) and DCs. Hypoxia also promotes the recruitment of immunosuppressive regulatory T lymphocytes (T_{reg} cells) and reprograms tumor-associated macrophages (TAMs) from an antitumor to a protumor phenotype. Notably, the immunosuppressive molecules generated in the TME can enter the circulation and cause systemic immunosuppression.

Given the consequences of malfunctioning blood vessels, multiple therapeutic strategies to improve the function of the tumor vasculature have been developed (*4*). Because excess VEGF contributes to vascular abnormalities, it was proposed that the judicious use of anti-VEGF agents could normalize tumor vessels and thereby improve oxygenation and drug delivery. This was supported by experiments showing that low-dose anti–VEGF receptor 2 (VEGFR2) treatment enhances trafficking of immune cells to tumors, reprograms TAMs to an antitumor phenotype, and improves outcomes of vaccine therapy in mouse breast cancer models (*5*). A number of preclinical studies have now shown the benefit of combining anti-VEGF agents with various immunotherapies, including ICBs and adoptively transferred engineered CAR T cells (*3*).

Anti-VEGF–induced vascular normalization is transient, which limits efficacy. Thus, a number of strategies are emerging to extend the window of normalization. One such strategy is to target angiopoietin-2 (ANG-2), which begins to increase toward the end of the normalization window. Importantly, dual blockade of VEGF and ANG-2 prolonged the duration of normalization and survival in a number of mouse tumor models, compared with inhibiting either molecule alone. Relevant for immunotherapies, VEGF and ANG-2 dual targeting reprogrammed TAMs to an antitumor phenotype (*6*) and enhanced tumor response to ICBs (*7*). Furthermore, circulating ANG-2 concentrations are initially elevated or increase in melanoma patients with an unfavorable response to ICBs. Moreover, blood ANG-2 concentrations inversely correlate with response to ICBs with or without anti-VEGF therapy in melanoma patients (*3*), providing additional rationale for VEGF and ANG-2 dual inhibition.

A number of other agents that target endothelial cells and/or pericytes, which support the structure and function of blood vessels, have been shown to transiently normalize tumor vessels. These targets include the receptor tyrosine kinase TIE-2, Notch signaling, endothelial cell metabolism, and oxygen sensors (*4*). Other strategies that can induce vessel normalization—either directly or indirectly—include restoring perivascular nitric oxide gradients, some chemotherapeutics such as eribulin, and aerobic exercise. Notably, two recent studies showed that ICBs can normalize tumor vessels in breast cancer models in mice (*8*, *9*), which is intriguing because it implies that T cells can directly affect vascular function. These studies showed that the increased vessel perfusion by ICB is positively correlated with its efficacy (*9*). Future efforts will determine whether these agents can be combined with particular ICBs in specific cancer types.

Physical forces generated by cells and the extracellular matrix (ECM) in tumors can also impair the function of blood and lymphatic vessels through compression (*4*). This is particularly the case in desmoplastic tumors, which are characterized by excessive fibrosis and activated stroma [e.g., pancreatic ductal adenocarcinoma (PDAC) and triple-negative breast cancer] and which generally have abundant carcinoma-associated fibroblasts (CAFs) and ECM. Killing cells with cytotoxic agents or depleting specific ECM components by using enzyme-based drugs can reopen these vessels. However, as cancer cells become resistant to cytotoxic agents and begin to proliferate, vessels collapse again. Similarly, enzymes that target a single ECM component (e.g., hyaluronan), become ineffective when other ECM components (e.g., collagen I) begin to contribute to compressive forces.

Agents that reprogram CAFs so that they stop producing multiple ECM components can overcome this compression. For example, angiotensin system inhibitors (ASIs) are a class of molecules commonly used to treat hypertension that also reprogram CAFs and have been shown to improve the function of tumor vessels as well as the delivery and efficacy of various therapeutics (*10*). Moreover, these widely used drugs can activate both innate and adaptive antitumor immune responses in mouse models and patients (*10*). On the basis of a successful phase II clinical trial, this approach is now being tested in a randomized clinical trial in patients with locally advanced PDAC (NCT03563248). Because ASIs can cause hypotension, new formulations are needed to lower the systemic exposure of these drugs. Recently,

Edwin L. Steele Laboratories, Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA. Email: jain@steele.mgh.harvard.edu

Published by AAAS

Downloaded from http://science.sciencemag.org/ on May 12, 2021

valsartan—an ASI linked to pH-sensitive polymers—was shown to reprogram CAFs in the acidic tumor environment to alleviate antitumor immunosuppression and improve T lymphocyte activity without adverse hypotension. This approach improved the response to ICBs in mice bearing primary and metastatic breast tumors (*2*).

Other strategies are also emerging for targeting CAF-mediated desmoplasia and vessel compression. For example, blocking C-X-C motif chemokine 12 (CXCL12)-C-X-C chemokine receptor 4 (CXCR4) signaling in mouse models of primary and metastatic breast cancer reduces fibrosis, vessel compression, and hypoxia while alleviating immunosuppression, which enhances the efficacy of ICBs (*1*). Similarly, the activity of tumor-associated immunosuppressive cells and the highly desmoplastic stroma in PDACs are fueled by cancer cell focal adhesion kinase (FAK) activity. Selective FAK inhibitors normalized the fibrotic TME, improved T cell infiltration of the tumor, and increased survival of tumor-bearing mice (*11*).

Endothelial cells present in tertiary lymphoid organs (organized structures consisting of lymphocytes and stromal cells that form outside of the spleen or lymph nodes) within tumors can also regulate antitumor immunity. By combining VEGFR2 and PD-L1 antibodies, it is possible to induce the formation of specialized vessels called high endothelial venules (HEVs) in some tumor models, which improved survival (*12*). HEVs facilitate the entry of immune cells into lymph nodes and might serve a similar function in tumors. Moreover, depletion of T_{reg} cells can induce the formation of HEVs outside of lymph nodes and the spleen, where they are normally found. When HEVs form in tumors, they can support lymphocyte infiltration and subsequent tumor cell killing by cytotoxic T lymphocytes (*13*).

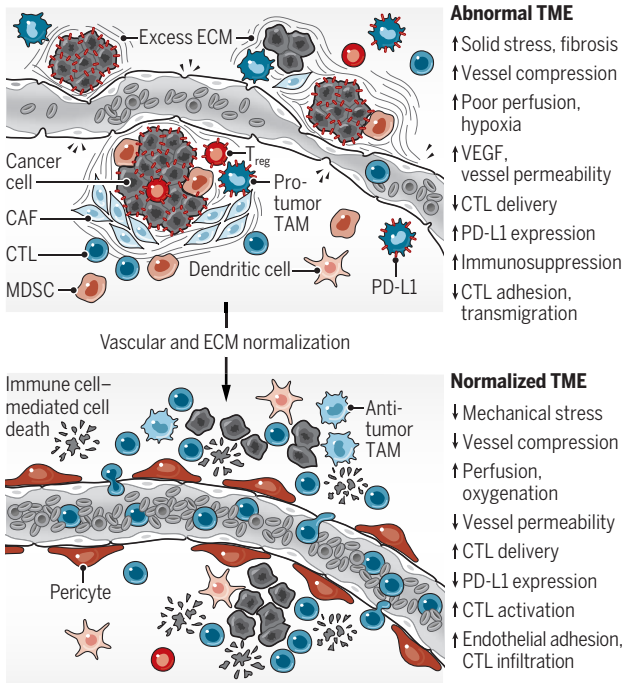
Lymphatic vessels are the routes from tissue to lymph nodes and back to the blood circulation. Therefore, lymphatic endothelial cells (LECs) are among the first cells to contact antigens that drain from tissue. LECs can control DC maturation and migration as well as directly present antigens on major histocompatibility complex class I (MHC I) and MHC II molecules to T cells (*14*). They also produce cytokines that modulate the immune response. Numerous lines of evidence demonstrate that LECs help limit and resolve effector T lymphocyte responses, modulate leukocyte function, and maintain peripheral tolerance, which prevents autoimmune responses (*14*). In cancer, LECs also suppress immune cell antitumor responses (*14*). Paradoxically, tumors that contain LECs (even in the absence of functional lymphatic vessels) show better responses to ICBs. Tumors with more intratumoral LECs have more T cells, but they become quiescent. These dysfunctional yet antigen-educated T cells can be reawakened by ICBs to produce antitumor immunity.

Immune-related toxicities are a major problem with ICB and CAR–T cell immunotherapies. However, discontinuing or reducing the dose of these agents can reduce or even abrogate these toxicities. Improving the function of tumor blood vessels can improve the delivery of antibodies and cells and thus may lower the required immunotherapy dose. Such approaches hold promise for reducing toxicities while improving efficacy. However, one of the biggest challenges in realizing this goal is the lack of validated biomarkers for guiding the dose and schedule of drugs that directly or indirectly target blood vessels. This is further complicated by the recently described "paradoxical effects of obesity." Obesity is known to fuel tumor progression, desmoplasia, hypoxia, immunosuppression, and resistance to various therapies, including chemotherapies and anti-VEGF therapies (*3*). Yet in mouse models of melanoma and lung cancer, and in various human cancers, obesity is associated with improved response to ICBs (*15*). Given the epidemic of obesity, a better understanding of the mechanisms underlying these paradoxical effects are likely to yield strategies to improve immunotherapy further.

Combining strategies that improve function of tumor vessels with ICBs holds promise. For example, three recent phase III trials have shown the benefit of combining PD1 or PD-L1 antibodies with anti-VEGF or anti-VEGFR agents, leading to U.S. Food and Drug Administration approvals for the combination in lung and kidney cancers (NCT02684006, NCT02853331, and NCT02366143). However, the relative contribution to patient survival from immunomodulatory versus nonimmunomodulatory effects of VEGF blockade with ICBs in these trials is not known. The ongoing trial that aims to test the role of adding losartan—a safe, inexpensive, and widely prescribed antihypertensive ASI shown to decompress tumor blood vessels—to chemo-radiation and PD1 antibody therapy will reveal the potential of this approach in improving the treatment outcome in patients with PDAC (NCT03563248). ■

Normalizing the tumor microenvironment

Excess angiogenic molecules produced by cancer or stromal cells cause abnormal tumor vasculature. ECM-normalizing drugs can decompress the blood vessels, and anti-angiogenesis therapies can reverse many of the TME abnormalities, resulting in improved perfusion and immune cell infiltration and creation of an immunostimulatory microenvironment.



CAF, cancer-associated fibroblast; CTL, cytotoxic T lymphocyte; ECM, extracellular matrix; MDSC, myeloid-derived suppressor cell; PD-L1, programmed cell death protein ligand 1; TAM, tumor-associated macrophage; TME, tumor microenvironment; T_{reg}, regulatory T cell; VEGF, vascular endothelial growth factor.

GRAPHIC: KELLIE HOLOSKI/*SCIENCE*

REFERENCES AND NOTES

1. I. X. Chen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **116**, 4558 (2019).
2. V. P. Chauhan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **116**, 10674 (2019).
3. D. Fukumura *et al.*, *Nat. Rev. Clin. Oncol.* **15**, 325 (2018).
4. J. D. Martin *et al.*, *Annu. Rev. Physiol.* **81**, 505 (2019).
5. Y. Huang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 17561 (2012).
6. J. Kloepper *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **113**, 4476 (2016).
7. M. Schmittnaegel *et al.*, *Sci. Transl. Med.* **9**, eaak9670 (2017).
8. L. Tian *et al.*, *Nature* **544**, 250 (2017).
9. X. Zheng *et al.*, *J. Clin. Invest.* **128**, 2104 (2018).
10. M. Pinter, R. K. Jain, *Sci. Transl. Med.* **9**, eaan5616 (2017).
11. H. Jiang *et al.*, *Nat. Med.* **22**, 851 (2016).
12. E. Allen *et al.*, *Sci. Transl. Med.* **9**, eaak9679 (2017).
13. C. F. Sautès-Fridman *et al.*, *Nat. Rev. Cancer* **19**, 307 (2019).
14. K. Maisel *et al.*, *Adv. Drug Deliv. Rev.* **114**, 43 (2017).
15. Z. Wang *et al.*, *Nat. Med.* **25**, 141 (2019).

ACKNOWLEDGMENTS

L.L.M. and R.K.J. thank Z. Amoozgar, D. G. Duda, D. Fukumura, Y. Huang, and T. Padera for helpful comments. R.K.J. received honorarium from Amgen; consultant fees from Chugai, Enlight, Merck, Ophthotech, Pfizer, SPARC, and SynDevRx; owns equity in Enlight, Ophthotech, and SynDevRx; and serves on the Boards of Trustees of Tekla Healthcare Investors, Tekla Life Sciences Investors, Tekla Healthcare Opportunities Fund, and Tekla World Healthcare Fund. The Massachusetts General Hospital has applied for patents related to concepts discussed in this article. L.L.M. owns equity in Bayer AG.

10.1126/science.aaw7875

Downloaded from http://science.sciencemag.org/ on May 12, 2021

Published by AAAS

Science

Vascular regulation of antitumor immunity

Lance L. Munn and Rakesh K. Jain

Science **365** (6453), 544-545.
DOI: 10.1126/science.aaw7875

ARTICLE TOOLS http://science.sciencemag.org/content/365/6453/544

RELATED CONTENT
http://stm.sciencemag.org/content/scitransmed/10/432/eaag0945.full
http://stm.sciencemag.org/content/scitransmed/9/410/eaan5616.full
http://stm.sciencemag.org/content/scitransmed/9/385/eaak9679.full
http://stm.sciencemag.org/content/scitransmed/9/385/eaak9670.full

REFERENCES This article cites 15 articles, 6 of which you can access for free
http://science.sciencemag.org/content/365/6453/544#BIBL

PERMISSIONS http://www.sciencemag.org/help/reprints-and-permissions

Use of this article is subject to the Terms of Service

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.

Copyright © 2019, American Association for the Advancement of Science

Downloaded from http://science.sciencemag.org/ on May 12, 2021