Are TEMs Canceled? Questioning the Functional Relevance of Tie2-Expressing Macrophages

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Inflammatory cells are a vital component of the tumor stroma and, of these, tumor-associated macrophages (TAM) are the major cell type. TAMs are recruited early in tumorigenesis and generally promote metastasis, stimulate tumor angiogenesis, and drive immunosuppression. TAMs have been shown to express the endothelial cell markers that enable chemotaxis and proangiogenic capacity. In this issue of *Cancer Research*, Jakab and colleagues challenge the functional significance of Tie2-expressing monocytes/macrophages (TEM) in the context of tumor growth and progression. By employing myeloid-specific deletion of the angiopoietin receptor Tie2 and comprehensive analysis of myeloid cell single-cell RNA sequencing datasets, they provide compelling data that Tie2-positive macrophages do not contribute to tumor angiogenesis or relapse after chemotherapy, two major biologic processes previously attributed to tumor-associated TEMs. The study highlights that the concept of macrophage-expressed Tie2 as a therapeutic target or prognostic indicator needs reconsideration.

See related article by Jakab et al., p. 1353

Angiogenesis, the formation and growth of new blood vessels from preexisting vasculature, is a critical process mediating oxygen and nutrient delivery necessary for tumor growth as well as immune cell trafficking. VEGFA and angiopoietin are major growth factors that function through VEGF receptors (VEGFR) and angiopoietin receptors (e.g., Tie2), respectively, to induce endothelial cell proliferation and orchestrate the angiogenic process. The expression of 'angiogenic' receptors, once considered to be restricted to endothelial cells and hematopoietic stem cells, is now appreciated to be broader and to include immune cells such as tumor-associated macrophages (TAM). Given that TAMs can have multiple phenotypes and these phenotypes are associated with distinct patterns of gene/protein expression, determining if expression of Tie2 or VEGFR2 is critical for the function of specific TAM populations during tumor progression is important. De Palma and colleagues identified a population of monocytes from bone marrow and peripheral blood that express Tie2 and coined them as Tie2-expressing monocytes (TEM; ref. 1). TEMs were found to be selectively recruited to the tumor stroma and function as prominent proangiogenic myeloid cells. Remarkably, by generating transgenic mice expressing the conditionally toxic gene thymidine kinase under the control of Tie2 regulatory elements, De Palma and colleagues provided robust data showing that TEMs are required for tumor neovascularization and contribute to tumor progression, thus illustrating that TEMs represent a potential therapeutic target (1, 2). Further studies extended these findings by using a small molecule multi-receptor tyrosine kinase inhibitor that has activity against Tie2, rebastinib, which reduced tumor growth and metastasis (3). Additional studies supported the importance of myeloid Tie2 expression for blood vessel reconstruction and tumor relapse after chemotherapy by exploiting a myeloid Tie2-specific knockout model (LysM^{Cre};*Tie2*$^{-/fl}$ mice; ref. 4). In addition, Tie2^{hi} macrophages were shown to secrete VEGFA to induce transient vascular permeability, creating an entry for tumor cells to extravasate and metastasize (5).

In the current issue of *Cancer Research*, Jakab and colleagues present a systematic investigation on the function of TEMs, especially in the process of tumor vessel reconstruction and relapse after chemotherapy (6). They also exploited a myeloid cell–specific Tie2 homozygous deletion model by crossing LysM^{Cre} mice to *Tie2*$^{fl/fl}$ mice. The resulting LysM^{Cre};*Tie2*$^{fl/fl}$ mice were used to examine the growth of MCA205 fibrosarcoma, Lewis lung carcinoma, B16F10 melanoma, and E0771 breast cancer models. To their surprise, myeloid Tie2 deletion did not alter or influence therapy response or tumor relapse in any of the syngeneic models evaluated. Similarly, lack of Tie2 expression on macrophages did not affect tumor vascularization or the extent of necrosis induced by tumor growth or therapy. Moreover, increasing the dose of chemotherapy did not result in differences between control and *Tie2* myeloid–specific knockout mice with respect to tumor vessels, progression, or metastatic burden. Accordingly, tumor-infiltrating macrophages in these models (Cre$^-$ or Cre$^+$) did not show significant Tie2 expression at the transcriptional or translational level. To validate the ability of macrophages to express Tie2, they analyzed murine macrophage-like cell lines (RAW267.4 and J774) and bone marrow–derived myeloid cells *in vitro* and revealed that neither hypoxia nor angiopoietin-2 (Ang2) stimulation induced Tie2 expression. Because inflammation has been linked to Tie2 expression on TAMs, the authors interrogated Tie2 expression on myeloid cells in an inflammatory model (peritonitis) in wild-type and *Tie2* myeloid–specific knockout mice. Consistent with their previous findings, Tie2 expression was not observed on macrophages in the inflammatory site in either genetic background. In addition, the authors performed unbiased analysis of publicly available single-cell RNA sequencing data of tumor-infiltrating CD11b$^+$ myeloid cells. However, they failed to identify a transcriptionally distinct population of macrophages expressing Tie2 despite rigorous and in-depth analysis.

Jakab and colleagues (6) discuss potential reasons for the distinction between their results and prior studies. They highlight mouse genetic background as a potential source of the discrepancy in studies that also used genetically engineered mouse models to investigate TEMs. In

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particular, prior work by Chen and colleagues (4), which seemed to support the importance of TEMs for blood vessel reconstruction and tumor relapse after chemotherapy exploited mice that were globally heterozygous for *Tie2*, a situation that likely clouded interpretation, as mice with *Tie2* haploinsufficiency display inherent vascular defects. While in the current study, Jakab and colleagues employed a mouse model that allowed for homozygous deletion of *Tie2* only in the myeloid lineage (6). Further, earlier studies by De Palma and colleagues (1, 2) used a viral vector to drive a transgene expressing either a reporter or a suicide gene under control of Tie2 regulatory elements. This is a distinct scenario that is not replicated by a constitutive tissue selective Cre. In addition, a small molecule inhibitor that has activity against Tie2 and multiple other kinases is difficult to use as validation for the importance of Tie2 specifically in myeloid cell function during tumor progression. Unless additional rigorous studies are performed validating the function of Tie2 in myeloid cells, it is likely that TEMs will no longer be seen as drivers in the tumor microenvironment.

Although results from Jakab and colleagues (6) cast doubt on the existence and functional significance of TEMs, accumulating studies have reported that myeloid cells express other angiogenic receptors, specifically, VEGFR2. For example, VEGFR2 is expressed on dendritic cells (DC), where it is associated with differentiation and maturation of DCs (7). VEGFR2 expression on bone marrow–derived plasmacytoid DCs is responsible for type I IFN production and cell proliferation (8). Recently, multiple groups including ours have shown selectively elevated expression of VEGFR2 on myeloid-derived suppressor cells (MDSC) and macrophages in tumors (9, 10) where it contributes to VEGF-induced infiltration and the immunosuppressive capacity of MDSCs.

In summary, Jakab and colleagues (6) argue against the importance of previously well-described Tie2^{+} macrophages and question the critical function of these macrophages for tumor angiogenesis and relapse after chemotherapy. At the same time, VEGFR2 expression has been confirmed to be elevated on myeloid cells including macrophages in tumor-bearing animals and to be involved in regulating tumor vascularization and the immune microenvironment. The concept that myeloid cells express functional markers that were initially described to be endothelial cell–specific is a fascinating and potentially impactful area of ongoing investigation that benefits from rigorous investigations such as those by Jakab and colleagues.

Authors' Disclosures

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