Tumors often contain abundant macrophage infiltrates, which are largely derived from circulating monocytes. After tumor colonization, the macrophages acquire specialized phenotypes that appear to be instructed by the distinct signals they are exposed to in different tumor microenvironments, such as perivascular, stromal, or necrotic tumor areas (1). For example, perivascular macrophages that express the angiopoietin receptor TIE2 are proangiogenic (2, 3) and molecularly distinct from other tumor macrophage subpopulations (4). In this issue of Cancer Discovery, Harney and colleagues (5) use intravital microscopy to analyze vascular permeability at sites where TIE2-expressing macrophages (TEM) make contacts with the endothelial cells (EC) that line the tumor blood vessels. They found higher vascular permeability at these sites, compared with vessel segments lacking macrophages. Furthermore, cancer cell invasion of the blood vessels (intravasation)—the first step in the metastatic cascade—occurred selectively at the macrophage–EC contacts. These new findings provide insight into the regulation of cancer cell dissemination by perivascular macrophages.

Previous work by these authors identified perivascular microanatomical structures, called “tumor microenvironment of metastasis” (TMEM), in which a macrophage, a specialized cancer cell, and a blood vessel establish tripartite contacts (ref. 6; Fig. 1). To study the properties of the TMEM ensemble in live tumors, Harney and colleagues (5) performed intravital imaging of mouse mammary tumors after the intravenous delivery of a high–molecular weight marker, which would not diffuse across the EC layer of the tumor blood vessels. Video imaging of the tumor blood vessels indeed showed that most of the injected marker had remained entrapped in the vessels. However, the authors also identified discrete sites, in fact, the TMEM structures, where the marker could leak from the vessels. Computer-assisted analysis of the recorded videos revealed that leakage of the marker, which is indicative of high vascular permeability, occurred at different time points after its injection, depending on which TMEM structure was imaged. Also, vascular permeability at each TMEM site was only transient; indeed, it peaked and declined rapidly, showing a pulsating nature. Together, these observations indicate that although permeability appears erratic and ephemeral throughout the tumor vasculature, it occurs specifically at vascular sites harboring TMEM structures.

An important result of the study (5) was that the peak of vascular permeability at the TMEM site was both spatially and temporally associated with the attraction of neighboring cancer cells, some of which could enter the bloodstream and leave the tumor. Diffusion of plasma proteins from leaky vessels is known to attract cancer cells and enhance their motility (1). Furthermore, the authors noted that the molecular junctions that normally seal adjacent ECs to limit vascular leakiness were more frequently disrupted at the hyperpermeable TMEM sites (5). Therefore, the cancer cells could more easily find their way to the systemic circulation through these temporarily opened gates. But how did the TMEM structures increase vascular permeability? The macrophage component of the TMEM seemed to play a key role here. Indeed, the authors found that the TMEM-associated macrophages express high levels of VEGFA, a protein that promotes EC proliferation, vascular growth, and permeability by loosening EC junctions (7). The genetic inactivation of VEGF specifically in the macrophages was sufficient to decrease vascular permeability, restore EC junctions, and abate cancer cell extravasation at the TMEM sites (5). Therefore, perivascular macrophage–derived VEGFA functions as a gatekeeper for the dissemination of cancer cells via the tumor-associated vascular network.

Similar to macrophage–specific VEGF inactivation, the physical elimination of the tumor macrophages decreased the number of circulating cancer cells in the mice (5). This finding is in line with previous observations and supports the notion that macrophages facilitate the initial steps of tumor metastasis, at least in mouse models of breast cancer (1). Several clinical studies have shown that high macrophage numbers in primary breast carcinomas are associated with a worse outcome, suggesting, although indirectly, that tumor macrophages may have a prometastatic role also in human breast...
Further studies are now warranted to see if neutralizing angiopoietin-2 in tumors decreases the density of TMEM structures, and whether this correlates with the tumor’s metastogenic capacity.

Several antimacrophage drugs are currently being investigated in the clinic. However, preclinical studies in mice have shown that tumor-associated macrophages display a spectrum of functions that range from protumoral to antitumoral (1, 4, 9). The former include, as discussed above, the production of proangiogenic factors, but also the secretion of growth and prosurvival cytokines for the cancer cells, or the activation of regulatory cues for immune suppression. The latter may involve tumor-antigen presentation and other immunostimulatory activities that would promote immune surveillance against cancer cells. Of note, such variegated (and potentially opposing) functions may be accomplished by distinct macrophage subpopulations (1, 4) and be heavily modulated by the effects of anticancer therapies on the tumor microenvironment (1, 9). Furthermore, besides mouse studies, our understanding of the roles of macrophages in human tumors is still limited. So, the application of antimacrophage drugs to a broad spectrum of cancer types will have to contemplate such complexities and current uncertainties.

Perivascular TEMs were shown recently to promote tumor relapse after chemotherapy via VEGFA (10), so their specific elimination or inhibition may abolish the proangiogenic and metastatic functions of macrophages in tumors treated with chemotherapy and, possibly, other anticancer drugs. This selective approach would spare macrophage subpopulations that may have immunostimulatory or other antitumoral functions (9). However, the nature of the perivascular macrophages and their precise functions in human cancer remain elusive. The challenging task now is to discover the identifying traits of such cells as a prelude for their effective therapeutic targeting.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**REFERENCES**

6. Robinson BD, Sica GL, Liu YF, Rohan TE, Gertler FB, Condeelis JS, et al. Tumor microenvironment of metastasis in human breast cancer (1). Importantly, the study by Harney and colleagues (5) also involved the examination of human breast cancer biopsies. They found that EC junctions were less intact at vascular sites containing a TMEM structure, similar to findings in mice. Moreover, previous work from these authors had established a relationship between the number of TMEM structures in primary breast carcinomas and the risk of distant metastasis (8). Collectively, these results suggest that the therapeutic targeting of TMEM-associated macrophages may help inhibit the spread of breast cancer cells to distant sites. It remains to be seen whether bona fide TMEM units also exist—and can predict metastasis risk—in other human cancer types.

Harney and colleagues (5) noted that the TMEM-associated macrophages expressed Tie2 and other markers characteristic of perivascular TEMs (4). TEMs were shown previously to promote angiogenesis in mouse tumors by interacting with and facilitating the growth of immature blood vessels, likely via the secretion of growth and proangiogenic factors, including VEGFA (2, 4). The “TEM nature” of the TMEM-associated macrophage (5) now assigns a prometastatic role to TEMs. In this regard, previous studies showed that the pharmacologic blockade of the Tie2 ligand angiopoietin-2 impeded the association of TEMs with the tumor blood vessels, decreased tumor angiogenesis, and dramatically abated the incidence of spontaneous lung metastasis in a breast cancer model (3). Because blocking angiopoietin-2 also had direct antiangiogenic effects on the tumor blood vessels, it could not be unambiguously established whether disrupted TEM-EC contacts had a role in blocking metastasis (3). The new findings of Harney and colleagues (5) add weight to the notion that blocking angiopoietin-2 may limit breast cancer metastasis, at least in part, by impeding TEM association with tumor blood vessels.

Because angiopoietin-2 is secreted specifically in the perivascular space by activated tumor ECs, it can be envisioned that it plays a role in establishing and maintaining the TMEM structures (3, 5). According to this view, a paracrine and reciprocal cross-talk may exist between the TMEM EC and TEM that involves angiopoietin-2 (from EC to TEM) and VEGFA (from TEM to EC). Further studies are now warranted to see