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Mesenchymal Cells Hold the Key to Immune Cell Recruitment to and Migration within Melanoma

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Samaniego *et al.* (this issue) report on distinct tumor-associated mesenchymal cell (MC) populations in human melanomas. FAP^-CD90^+ peritumoral MCs may be involved in immune cell recruitment from the bloodstream. FAP^+CD90^- intratumoral MCs were associated with extracellular matrix fiber deposition, and their numbers correlated with high immune cell infiltration. Thus, different MC subsets modulate the cellular composition of the intratumoral and peritumoral melanoma microenvironment.

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Solid cancers are complex tissues composed of many specialized cell types, both normal and malignant. The intricate interplay between host-derived components of tumors, collectively known as the tumor stroma, and the transformed cells forge the tumor microenvironment (TME). The importance of the TME in shaping tumor development, progression, prognosis, and even response to therapies has been increasingly recognized. The tumor stroma has three main components: cells of mesenchymal origin, such as carcinoma-associated fibroblasts, blood and lymphatic vessels and associated cells, and infiltrating innate and adaptive immune cells (reviewed in Hanahan and Coussens, 2012).

Tumor-associated immune cells

Although most tumors arise from the organism's own cells, it is well accepted that the immune system has the potential to recognize and eliminate cancerous cells. How tumors escape immune responses is the subject of ongoing research, and many different mechanisms have been described. One important aspect of antitumor immunity is the positioning of immune cells within and around solid tumors. The mechanisms that regulate immune cell infiltration into the TME are incompletely understood. Nevertheless, immune cell infiltrates have been shown to both limit and promote tumor progression, depending on the nature of the immune cell subtypes and their functional state. Thus, the presence of effector T cells ($CD8^+$ CTL and $CD4^+$ helper T cells) is thought to constitute a good prognostic indicator in several human solid tumors, including melanoma, whereas other immune cells, such as macrophages and regulatory T cells, may promote tumor progression (reviewed in DeNardo *et al.*, 2010). A better understanding of immune cell recruitment into tumors is important in the development of therapeutic and vaccine strategies.

Recent observations using intravital multiphoton microscopy have shown that optimal migration of infiltrating effector T cells is essential for antitumor immune surveillance (Mrass *et al.*, 2008). As in other tissues, such as lymph nodes, lymphocyte motility within the TME is intimately linked to the architecture and composition of its extracellular matrix (ECM), and this may take the form of chemokine-driven and/or contact-guided migration (reviewed in Friedl and Weigelin, 2008). Although the association of migrating T cells with ECM fibers is relatively well established, excessive accumulation of ECM components may also limit access of T cells to the tumor cells and thereby contribute to cancer progression (Salmon *et al.*, 2012). Thus, in-depth characterization of the cellular and molecular factors that regulate ECM production in malignancy is important for our understanding of tumor survival and growth.

Tumor-associated mesenchymal cells

One of the major stromal cell types able to influence tumor progression is collectively known as carcinoma-associated fibroblasts. Carcinoma-associated fibroblasts are thought to originate from fibroblasts and other mesenchymal cell (MC) types, such as pericytes or mesenchymal stem cells, and are characterized by the expression of the myofibroblast marker α-smooth muscle actin or fibroblast activation protein (FAP) (reviewed in Polanska and Orimo, 2013). Cancer cells are able to recruit and stimulate the growth of normal MCs from the surrounding tissue, and MC phenotype and function may coevolve with tumor progression. Aside from their influence on cancer cell proliferation and invasion via secretion of growth factors/cytokines, regulation of neoangiogenesis, and ECM remodeling, MCs are also known to influence both immune cell recruitment and function via chemokine secretion and alterations in the tumor vasculature. However, a general shortcoming of investigating the different roles of MCs in tumor biology is that they represent a heterogenous population; thus, a better phenotypic definition of MC subsets would be useful.

In this issue of *JID*, Samaniego *et al.* (2013) report the characterization of novel tumor-associated MC subsets in primary and metastatic human melanoma specimens based on the expression of FAP and CD90 (cluster of differentiation 90; Thy-1). Using elegant multicolor immunostaining of tissue sections, three distinct MC groups were found: (1) $FAP^+CD90^{lo/-}$ MCs within

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Clinical Implications

- The tumor stroma in melanoma includes MCs with diverse and even competing capabilities.
- These MC subsets modulate the composition of infiltrating leukocytes into the intratumoral and peritumoral microenvironment.
- Importantly, leukocytes recruited into the tumor by these cells may compete by promoting or preventing its survival.
- Thus, therapies that inhibit or promote the activities of MCs in a selective manner may ultimately become useful therapeutic tools.
- In sum, this study corroborates the potential of MCs to serve as therapeutic targets or prognostic indicators in melanoma.

the intratumoral area; (2) FAP^+CD90^+ elongated MCs within peritumoral stroma; and (3) FAP^-CD90^{hi} perivascular MCs (pericytes). Whether these different MC phenotypes are derived from a single mesenchymal progenitor or represent distinct MC lineages was not investigated.

Establishment of $CD90^+$ MC cell lines isolated from melanoma tissues showed that these cells secreted high levels of CCL2 (chemokine (C–C motif) ligand 2) compared with MCs isolated from normal tissue. $CD90^+$ MC cells were able to induce CCL2-dependent chemotaxis of monocytes and, to a lesser extent, T lymphocytes. Most cells expressing CCR2 (the receptor for CCL2) in the melanoma tissues were leukocytes in proximity to peritumoral vessels, which suggests that leukocytes are recruited from the blood into the peritumoral stroma via CCL2. In addition, the density of peritumoral vessels and $CD90^+$ MCs correlated with leukocyte infiltration. This is consistent with a previous study in which microvessel density in the peritumoral stroma showed a correlation with leukocyte abundance (Kiss *et al.*, 2007). Nevertheless, the authors demonstrated further that other cells in the TME also expressed CCL2; therefore, it is unlikely that the perivascular MCs were the only cells involved in leukocyte recruitment into the peritumoral stroma via CCL2.

The authors found further that tumor-associated macrophages (TAMs) purified from melanoma tissues did not express CCR2, suggesting that leukocyte infiltration from the peritumoral region into the tumor itself was independent of CCL2. TAMs may downregulate CCR2, and the authors suggest that this could relate to macrophage differentiation triggered by the TME. Whether T cells also show downregulation of CCR2 in melanoma tissues was not investigated. A previous study in colorectal cancer described a mechanism by which tumors are able to escape T-cell infiltration by posttranslational modification of CCL2 (nitration) to prevent T-cell binding (Molon *et al.*, 2011). This may explain why T-cell infiltration does not correlate with CCL2 expression in melanoma.

Notably, Samaniego *et al.* found that leukocyte infiltration correlated positively with both the number of FAP^+ MCs within melanomas and the extent of the intratumoral ECM network, as shown by staining for collagen I. Thus, leukocyte density was significantly lower in melanomas with low numbers of FAP^+ MCs. In addition, intratumoral leukocytes were found to colocalize with collagen fibers, regardless of the density of the fiber network.

Importantly, the authors were also able to demonstrate that co-injection of patient-derived MCs with A375 human melanoma cell lines into immunodeficient mice resulted in the formation of a well-organized intratumoral ECM network when compared with tumor cells injected alone. Moreover, only in A375 cell–MC mixed tumors were abundant host-derived innate immune cells found. Elegant adoptive transfer studies with activated $CD8^+$ T cells revealed an increased T-cell infiltration into mixed tumors compared with A375 tumors. Finally, the authors showed that integrin-dependent T-cell migration on top of melanoma cell monolayers *in vitro* was enhanced significantly when the MCs were mixed with melanoma cells. Pharmacological inhibition of chemokine receptor signaling had no effect on T-cell motility, indicating that the enhanced migration was most likely chemokine independent, and was rather contact based.

In summary, this study supports a two-step model for immune cell recruitment and infiltration in human melanomas (see Figure 1). Peritumoral recruitment of immune cells is most likely supported by chemokine-secreting perivascular $CD90^+$ MCs. By contrast, infiltration of leukocytes into the melanoma itself requires the presence of FAP^+, ECM-producing MCs that generate a permissive environment for cell invasion and migration.

Concluding remarks

This study corroborates the potential of MCs to serve as therapeutic targets or prognostic indicators in melanoma. Previous work in murine cancer models showed that targeting FAP^+ stromal cells (by immunotherapy or ablation) would reduce tumor growth successfully by eliciting an antitumor immune response (Lee *et al.*, 2005; Kraman *et al.*, 2010). Targeting pericytes has also been tested in various cancer models, but whether such a strategy slows tumor progression is still uncertain (reviewed in Armulik *et al.*, 2011).

Although the data in this study have advanced our understanding of the role of MCs in leukocyte recruitment to the melanoma microenvironment, the authors did not investigate whether the number of FAP^+ MCs or ECM network density correlated with clinical outcome. They propose that melanomas can be classified into those with high intratumoral MC and leukocyte numbers as opposed to tumors that are low in both cell populations, and it will be important to determine whether the number of intratumoral MCs could be used as a prognostic factor. The most abundant immune cells associated with FAP^+ MCs were TAMs, followed by $CD8^+$ T cells. $CD8^+$ T-cell abundance correlates with a positive clinical outcome in a variety of malignancies,