Be Easy and Chill: Melanoma Cells Tell Lymph Node Fibroblasts to Relax

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Melanomas coopt tumor-draining lymph nodes to support metastatic potential and install immunosuppression. The specific mechanisms that mediate lymph node education, however, remain incompletely understood. In this issue, Rovera and colleagues describe the deactivation of contractile lymph node fibroblasts by dedifferentiated melanoma cells, leading to lymph node expansion and enhanced melanoma invasive potential. Fibroblastic reticular cell relaxation depended upon inhibition of constitutive JAK1/STAT3 and YAP/TAZ signaling, which was mediated in part by tumor secretion of the inflammatory cytokine, IL1. These data support an emerging hypothesis that intrinsic melanoma heterogeneity feeds forward to drive microenvironmental adaptations that mediate invasion and progression.

See related article by Rovera et al., p. 1774

In this issue of *Cancer Research*, Rovera and colleagues (1) explore cross-talk between melanoma cells and the fibroblastic architecture of the tumor-draining lymph node (LN). Melanomas engage LNs through lymphatic drainage early in their development. Consequently, LNs are often the first site of metastasis for melanoma and micrometastatic seeding of the sentinel LN is a well-established poor prognostic factor. Though the mechanisms that enable LN colonization remain incompletely understood, recent literature points to concerted stromal and immunologic changes that induce a prometastatic and immunosuppressive LN environment (2). LN fibroblast reticular cells (FRC) are specialized myofibroblasts with unique immunologic function (3). FRCs construct a physical scaffold that actively relaxes in response to inflammation to permit leukocyte expansion (4), and secrete homeostatic and inflammatory chemokines that govern leukocyte localization. Notably, FRCs are structurally and transcriptionally impacted by tumor drainage (2, 5), yet the functional relevance of these changes and the molecular mechanisms that drive them remains largely unclear. Rovera and colleagues use primary cultures of both LN and dermal fibroblasts to model the unique physical characteristics of the LN FRC network *in vitro* and its response to melanoma-derived factors (1). They find that, in contrast to dermal fibroblasts that acquire a contractile phenotype in response to TGFβ, *in vitro* LN FRC maintains a constitutive, TGFβ-independent contractile phenotype ($PDPN^{+}FAP^{+}\alpha SMA^{+}$). FRC contraction is instead dependent on ROCK and active YAP/TAZ, consistent with *in vivo* murine models that implicate YAP/TAZ activity in FRC commitment and maturation (6).

To identify mechanisms that mediate melanoma and FRC cross-talk, the authors asked whether signals secreted from melanoma cells impact FRC function (1). In melanoma, intratumoral heterogeneity generates novel phenotypic states that may be intrinsically resistant to targeted therapy (7) and exhibit enhanced invasive potential (8). Comparing a panel of human melanoma cell lines and short-term cultures characterized for their expression of the differentiation marker, MITF, and dedifferentiation marker, AXL, they find that $MITF^{lo}AXL^{hi}$ (dedifferentiated and invasive), but not $MITF^{hi}AXL^{lo}$ (differentiated) melanomas, inhibit FRC contraction (**Fig. 1A**). FRCs treated with dedifferentiated conditioned media exhibited reduced nuclear YAP and a downregulation of TEAD2-dependent transcripts and YAP deletion was sufficient to recapitulate the relaxed, tumor-conditioned phenotype. The JAK1/STAT3 pathway activated both the nuclear localization of YAP and phosphorylation of MLC2, linking JAK1/STAT3 signaling directly to actomyosin contractility and the *in vitro* contraction phenotype (**Fig. 1B**). Notably, *in vivo* administration of conditioned media from dedifferentiated melanoma cells, both mouse and human, was sufficient to inhibit nuclear YAP in LN FRCs, and FRC relaxation increased melanoma cell invasion into collagen gels *in vitro*.

These data point to an interesting link between the emergence of intratumoral heterogeneity, which imparts a tumor intrinsic capacity for invasion and metastasis (8), and the microenvironmental changes that support invasive behavior. The emergence of intratumoral heterogeneity and stromal adaptation both at local and distant sites are often studied as separate processes but likely coordinate to inform progression. The data here would seem to suggest that melanomas influence LN FRCs from a distance, mediated by lymph transport of secreted factors, but whether the mechanisms described here precede or follow initial metastatic seeding of the LN remains unclear. Still, these data are consistent with the hypothesis that FRC relaxation provides a supportive, compliant niche for arriving tumor cells. Interestingly, recent evidence indicates that macrometastatic LN outgrowth conversely drives progressive fibrosis that increases LN solid stress and reduces structural plasticity (9). These data together suggest that there is a progressive relationship between adapting tumor cells and FRCs that requires further mechanistic and longitudinal investigation.

In search of individual factors that might explain the effects of the conditioned media, the authors performed cytokine profiling and revealed elevated secretion of IL1α, IL1β, IL6, and IL8 by dedifferentiated melanomas. Interestingly, only IL1α and IL1β were sufficient to inhibit FRC contraction and STAT3 phosphorylation *in vitro*, though the exact mechanism of this inhibition remains unclear. The

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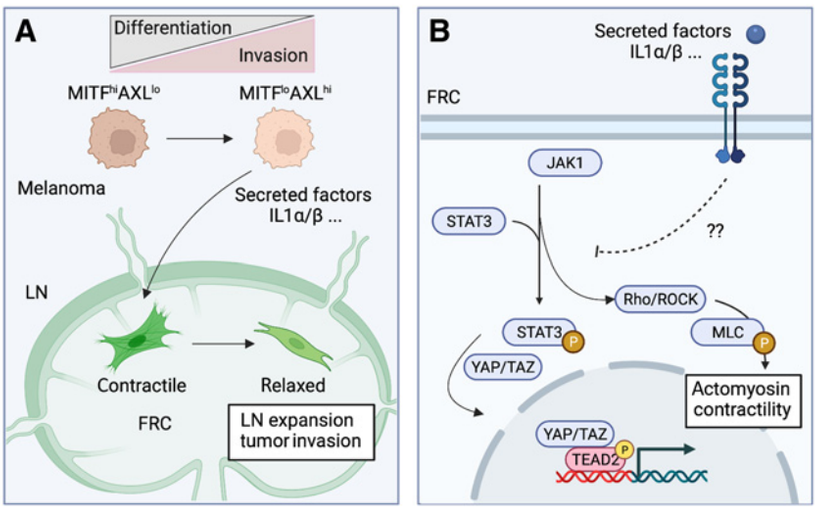

Figure 1.

Dedifferentiated melanoma cells inhibit FRC contraction to promote LN invasion. **A,** Tumor-secreted factors from MITF^{lo} dedifferentiated melanoma cells inhibit FRC contraction, allowing for LN expansion and tumor invasion. **B,** Constitutive JAK1/STAT3 signaling enhances Rho/ROCK- and YAP/TAZ-dependent actomyosin contractility in LN FRCs to maintain a myofibroblast state. MITF^{lo} tumor-secreted factors, including IL1α/β, inhibit JAK1/STAT3 signaling and actomyosin contraction, and convert FRCs from a myofibroblast to a relaxed, IL6-producing inflammatory state. (Created with BioRender.com).

level of IL1α/β in supernatants across multiple cell lines and primary cultures predicted the relative contractile effect of conditioned media on FRCs, and IL1 transcripts inversely correlated with MITF expression in human datasets. Interestingly, IL1 is also implicated in the activation of an IL6-producing, inflammatory cancer-associated fibroblast (iCAF) in solid nonlymphoid tumors (e.g., pancreas; ref. 10). FRCs exposed to the invasive melanoma secretome enrich transcriptionally for an established iCAF signature, supporting the idea that these cells shift from a myofibroblast to an inflammatory state. These data highlight important parallels between lymphoid and nonlymphoid fibroblast responses to cancer and indicate that melanomas may induce an iCAF-like state in myofibroblast precursors that influences both the biophysical and biochemical LN microenvironment.

Importantly, though not explicitly explored here, relaxation of the FRC network is also required for optimal adaptive immune responses (4). Migratory dendritic cells express high levels of the PDPN receptor CLEC2, which dismantles PDPN-ERM interactions, inhibits actomyosin contractility, and thereby expands the LN to permit lymphocyte influx and robust priming (4). Conversely, hyperactivation of YAP/TAZ signaling (6) and metastatic outgrowth (9) both drive a fibrotic response that limits leukocyte recruitment and expansion, suggesting that the state of the FRC is a key feature of LN immunity. Despite the prevailing hypothesis that tumor-draining LNs are immunosuppressed (2), it remains unclear from this work, whether melanoma-induced FRC relaxation would be permissive of, or rather a barrier to tumor antigen presentation. Importantly, the impact of these physical changes likely intersect with additional molecular remodeling of the FRC secretome, including upregulation of the inflammatory cytokine IL6 (1) and downregulation of CCL21 and IL7 (5), which are critical homeostatic signals that govern leukocyte position and survival within the LN, respectively. Therefore, understanding the impact of FRC remodeling on melanoma progression requires future investigation in context with spatial and temporal resolution.

While much remains to be validated and explored in the immunocompetent setting, these data take an important step toward integrating intrinsic and extrinsic mechanisms that govern melanoma invasion. Rovera and colleagues describe functional coupling between emergent melanoma cell states and LN expansion that enhances melanoma invasion and likely impacts the activation of antitumor adaptive immune responses. The disparate LN responses observed between murine models (1, 5) further support the hypothesis that intrinsic tumor biology (e.g., genetics, phenotypic state) will drive distinct FRC responses that contribute to progression. Whether these and other mechanisms of early tissue adaptation to melanoma provide functional targets for early disease interception remains an exciting possibility to be explored moving forward.

Authors' Disclosures

No disclosures were reported.

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