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INSIGHTS

Lymph node metastasis: An immunological burden

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Lymph node metastasis in breast cancer depends in part on the acquisition of an IFN-dependent, MHC-II$^+$ state that induces regulatory T cell expansion and local immune suppression (Lei et al. 2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20221847).

A new study by Lei et al. (2023) reveals a dynamic interplay between breast cancer cells and the lymph node (LN) microenvironment that sets up local immune suppression. Interest in the role of LN metastasis is re-emerging along with a shifting clinical landscape where prophylactic LN removal is less common and neoadjuvant immunotherapy shows clinical progress (Patel et al., 2023). The question has remained as to whether LN metastasis directly promotes systemic disease or rather acts as a harbinger of aggressive tumor behavior. While preclinical studies demonstrated that tumor cells can access the hematogenous vasculature in LNs to mediate systemic spread (Brown et al., 2018; Pereira et al., 2018), genomic sequencing of synchronous LN and distant metastases in both colorectal (Naxerova et al., 2017) and breast cancer indicate that only a minority of patients share clonal ancestry between their LN and distant metastases (Ullah et al., 2018; Venet et al., 2020). These data appear to indicate that tumor cells may often take separate, parallel paths as they metastasize from the primary. Still, LN metastasis remains a poor prognostic indicator in many solid tumors and recent work may indicate that LN metastasis accelerates parallel hematogenous metastasis through effects on systemic immune surveillance (Reticker-Flynn et al., 2022). How the clonal and phenotypic evolution of the metastasizing tumor directs the immune suppressive context of the LN remains largely unknown.

Lei et al. (2023) tested the hypothesis that intratumoral heterogeneity established upon LN invasion directs local immune suppression. Single-cell sequencing of mouse 4T1 primary tumors and their associated LN metastases revealed transcriptional states specific to the LN, including the emergence of mesenchymal-like state and high expression of the major histocompatibility complex II (MHC-II). High expression of MHC-II was also observed in human LN metastases and associated with an IFN-γ signature. Using ligand-receptor prediction algorithms, they found that MHC-II–expressing breast cancer cells were likely to interact with regulatory T cells (Treg), which were increased in number and exhibited enhanced suppressive potential in metastatic LNs in both mouse and human. Loss- and gain-of-function studies demonstrated a causal link between MHC-II expression on tumor cells, Treg accumulation, and LN metastasis. The authors therefore suggest a model whereby IFN-γ signaling induced upon LN entry upregulates MHC-II, leading to the direct education of Treg, local immune suppression, and thereby enhanced LN seeding (see figure).

Pseudotime tracing of breast tumor cells from primary to LN indicated that activation of epithelial to mesenchymal transition in the primary generated an invasive population of mesenchymal cells that first seeded the LN. Indeed, when LN burden was low, mesenchymal-like cells predominated. With metastatic outgrowth, however, a spatially segregated epithelial state re-emerged in LNs, which specifically expressed high levels of MHC-II leading to enhanced LN seeding and ultimately poor survival in preclinical models (Lei et al., 2023). Spatially segregated, genomically and transcriptionally distinct tumor cell states are observed across primary and metastatic tumors (Barkley et al., 2022); however, much less is known about the tumor-intrinsic states necessary for LN metastasis and the environmental pressures that induce or maintain them. In a recent study of human breast cancer, sub-clonal territories were identified in LN metastases having distinct histological features and transcriptional programs that associated both with intrinsic and extrinsic factors (Lomakin et al., 2022). Notably, two clones occupied distinct immune microenvironments, one proximal to germinal center B cells and another within LN sinuses and infiltrated by myeloid cells.

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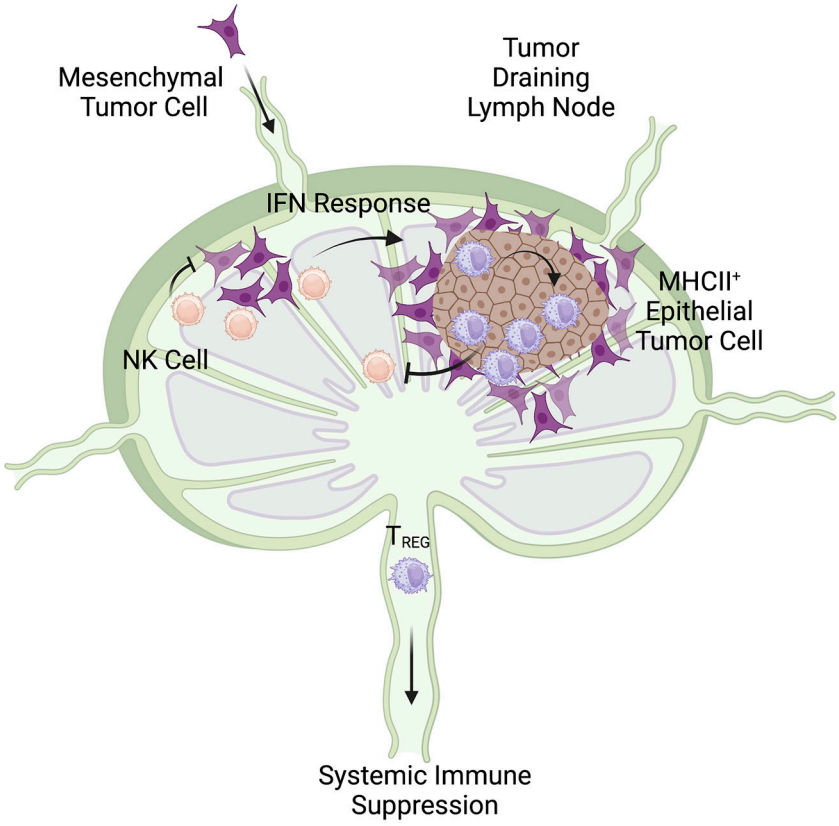

An emerging model of LN metastasis and immune suppression. Mesenchymal metastatic tumor cells arrive in the tumor-draining LN via afferent lymphatic vessels where exposure to IFNs released by NK cells may activate transcriptional and epigenetic reprogramming leading to the establishment of heterogenous cell states that suppress local cytotoxic activity. IFNs stimulate an MHC-II+ epithelial tumor cell state that evades NK cell killing and directly educates local Tregs, which further suppress NK cell function. LN Treg expansion and circulation drives systemic immune suppression.

The LN, a highly compartmentalized immunological organ, may therefore provide distinct sub-anatomical niches that extrinsically shape tumor adaptation. Interestingly, the distribution of heterogenous states may have prognostic significance. Again in an analysis of breast cancer LN metastases, the intermixing of cell states histologically, rather than their spatial segregation, strongly associated with better patient outcome even when controlling for clinical features (Fischer et al., 2023).

Here the authors propose that a source of this intratumoral heterogeneity is the expression of IFN-γ in the LN, which is sufficient to drive high expression of MHC-II in vitro (Lei et al., 2023). IFNs are interestingly a cornerstone of the cancer immunoediting hypothesis, which describes the pressures placed on nascent tumors to avoid targeted killing, but may also play paradoxical roles in both immune regulation and intrinsic tumor cell metastatic behavior. The single-cell RNA sequencing supports the interpretation that an IFN-γ–induced state emerges specifically as a function of the LN microenvironment but does not directly test the extrinsic nature of the IFN-γ signaling in vivo, and the location and/or source of the ligand remains unclear. Similar IFN-associated states are observed in preclinical melanoma LN metastases (Reticker-Flynn et al., 2022) and in a pan-cancer analysis of cell states across anatomical sites and tumor types (Barkley et al., 2022). Interestingly, an alternative explanation for the data could be that tumor-intrinsic IFN responses accounts for the transcriptional changes observed and the enrichment for MHC-II expression over time. Chromosomal instability correlates with tumor metastasis to the lungs, bone, and brain, and directly promotes metastasis by sustaining tumor cell autonomous responses to cytosolic DNA, activation of cGAS-STING, and NF-κB (Bakhoum et al., 2018). Consistently, in melanoma, an epigenetically reinforced, tumor-intrinsic IFN program was required for LN metastasis, and while exogenous IFNs induced initial adaptation, the IFN state was maintained in their absence (Reticker-Flynn et al., 2022).

One source of extrinsic IFN-γ could be natural killer (NK) cells, which play an important role in controlling LN metastasis across several preclinical models. In melanoma, tumor cells adapted for LN outgrowth first evade NK cell–mediated killing (Reticker-Flynn et al., 2022). Similarly, suppression of NK cell activity is necessary for breast cancer metastasis to LNs, and mediated by Tregs that are enriched in LNs draining primary breast cancers in mice (Kos et al., 2022). While Treg depletion reduced LN metastasis in a preclinical breast cancer model, simultaneous depletion of NK cells reversed the effect, promoting metastatic outgrowth. In the current study, the IFN-dependent, MHC-II+ state is necessary and sufficient to educate and expand local Tregs, leading to more efficient seeding and outgrowth (Lei et al., 2023). These data all together support a model whereby initial NK cell encounter could provide the IFNs necessary for adaptive expression of MHC-II leading to enhanced Tregs, NK cell suppression, and tumor outgrowth (see figure). Treg expansion specifically in the tumor-draining LN is reported in both preclinical models and clinical biospecimens, with the abundance of Tregs increasing progressively with first tumor drainage and then LN invasion (Núñez et al., 2020; Reticker-Flynn et al., 2022). Whether the systemic rewiring of the Treg population (Núñez et al., 2020; Kos et al., 2022) stems specifically from the tumor-draining LN and interactions with metastasizing tumor cells, as has been proposed (Reticker-Flynn et al., 2022), would have important implications for regional and systemic disease management.

These data support the growing model that even if not necessary for sequential seeding of distant organs, LN metastasis plays a critical role in preparing the systemic host for hematogenous spread. Indeed, while only 25% of LN metastases in breast cancer patients shared a common clonal origin with synchronous distant

metastatic lesions, those that did exhibited significantly poorer prognosis (Venet et al., 2020), indicating perhaps that tumor cell adaptation through, or education of the LN has important clinical implications for patients. Consistent with this, lymph may protect metastasizing tumor cells from ferroptosis and thereby offer an adaptive advantage for sequential seeding (Ubellacker et al., 2020). Perhaps this suggests that the minority of tumors that do progress through an LN state might be poised for more aggressive behavior through both tumor-intrinsic and immune-mediated mechanisms, despite the fact that clinical data argues that this is not an absolute prerequisite for systemic disease (Naxerova et al., 2017; Ullah et al., 2018; Venet et al., 2020).

Overall, this study demonstrates the important and ongoing interplay between tumor cells and immune system that determines metastatic tropism. Together with existing literature, these data indicate that the establishment of LN metastasis requires local immune suppression that then subsequently remodels the systemic immune response to cancer, positioning LN metastasis as a critical cornerstone of disease progression. The necessary co-adaptations that permit metastatic seeding could, therefore, present important targets for therapeutic intervention that would have the advantage of both managing regional disease and preserving systemic immune surveillance.

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