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Hypoxia-driven immunosuppression contributes to the pre-metastatic niche

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Primary tumor cells create favorable microenvironments in secondary organs, termed pre-metastatic niches, that promote the formation of metastases. Using immune competent syngenic breast cancer mouse models, we have recently demonstrated that factors secreted by hypoxic tumor cells condition pre-metastatic niches by recruiting CD11b$^+$/Ly6C^{med}/Ly6G$^+$ myeloid cells and suppressing natural killer cell functions.

Stromal and bone marrow-derived cells (BMDCs) are critical components of the tumor microenvironment, promoting tumor growth, survival, proliferation and invasion. Until recently, the process of invasion and metastasis has been defined by the so-called metastatic cascade: a series of discrete steps that tumor cells must undertake in order to successfully colonize a distant tissue. These steps include invasion of the local tissue and adjacent blood and lymphatic vessels, survival and evasion of immune responses in the circulation, extravasation at secondary organs, and induction of proliferation and angiogenesis to allow the outgrowth of macroscopic metastases.[1] As numerous mutations appear to be necessary for tumor cells to accomplish all these steps, metastasis has been considered a late event in tumorigenesis. It is now clear that the timing of metastatic invasion may differ depending on tumor type, and that metastasis may in fact occur early during tumorigenesis.[2]

Pre-metastatic niches are supportive microenvironments established in secondary organs by primary neoplastic lesions prior to tumor cell dissemination. Various pro-angiogenic factors and cytokines secreted from primary tumor cells initiate the mobilization and recruitment of BMDCs to distant organs, where they create pre-metastatic niches to allow for the seeding of disseminating tumor cells.[3] Many different tumor-derived secreted factors (TDSFs) have been demonstrated to be in the formation of pre-metastatic niches, though previous studies have focused on the functional roles of a few specific factors.[3] The recruitment of CD11b$^+$ myeloid cells has also been implicated in this process, though the specific subpopulations involved have not been well characterized. Furthermore, the use of xenograft models has complicated the study of the interactions between different BMDC populations in the pre-metastatic niche.

Hypoxia within the primary tumor is one of the factors that are causally associated with metastatic progression. The main downstream regulator of the hypoxic response in tumor cells is hypoxia-inducible factor (HIF)-1α.[4] Elevated HIF-1α expression correlates with increased tumor stage and poor prognosis in a variety of cancer types[1,4] and has recently been linked to the formation of pre-metastatic niches in breast cancer,[5] mainly through the hypoxia-induced production of lysyl oxidase.

Recently, we demonstrated that hypoxia within primary breast cancer cells leads to the secretion of multiple previously identified as well as unidentified pre-metastatic niche TDSFs, and that conditioned media from such hypoxic cells promotes the recruitment of BMDCs to lungs in immunocompetent bone marrow chimeric mice (**Fig. 1**).[6] As previously demonstrated by Kaplan et al.,[7] in mice intraperitoneally injected with hypoxic conditioned medium (HCM), these BMDCs cluster at the terminal bronchioles of the lung. Treatment of mice with HCM, as compared with normoxic conditioned medium (NCM), has also been shown to increase the metastatic burden in an experimental breast cancer metastasis model.[6] Therefore, factors secreted by hypoxic tumor cells appear to alter the lung microenvironment to make them more permissive for metastatic tumor cell growth. Interestingly, HCM from breast tumor cells also increased lung metastases in mice injected with B16F10 melanoma cells, pointing to the establishment of a pre-metastatic niche with decreased immune surveillance.

One of the major aims of our work was to investigate BMDCs populating the pre-metastatic niche in greater detail. Among previously identified CD11b$^+$ myeloid cells is a heterogeneous population of CD11b$^+$/Gr-1$^+$ myeloid derived-suppressor cells

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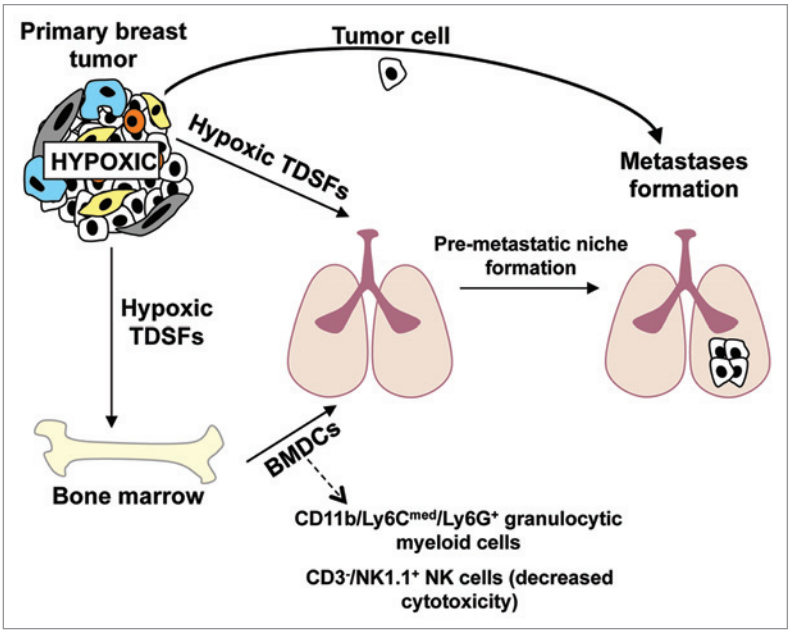

Figure 1. Hypoxia at the primary tumor promotes the formation of an immunosuppressive pre-metastatic niche. Tumor-derived secreted factors (TDSFs) produced by hypoxic primary breast tumor cells promote the formation of pre-metastatic niches in the lung by recruiting $CD11b^+/Ly6C^{med}/Ly6G^+$ granulocytic myeloid cells and $CD3^-/NK1.1^+$ natural killer (NK) cells from the bone marrow. NK cells in the pre-metastatic lungs have reduced cytotoxic effector functions, resulting in an immunosuppressed microenvironment that allows for the formation of metastases.

(MDSCs).[3] These include precursors of macrophages, granulocytes, dendritic cells and myeloid cells at various stages of differentiation.[8] Examination of BMDCs for $Ly6G^+$ and $Ly6C^+$ subtypes (both recognized by the Gr-1 antibody) revealed that only a subpopulation of granulocytic $CD11b^+/Ly6C^{med}/Ly6G^+$ cells was significantly enriched in the pre-metastatic niche.

In a search for secreted factors that might induce the recruitment of these cells, we found that one of the most consistent and strongly upregulated factors in the HCM from breast cancer cells is monocyte chemotactic protein-1 (MCP-1/CCL2). MCP-1 attracts and activates mononuclear cells during inflammation and has been shown to attract MDSCs in cancer.[9] The sole neutralization of MCP-1 in HCM resulted in decreased $CD11b^+/Ly6C^{med}/Ly6G^+$ myeloid cells in the pre-metastatic niche and reduced metastatic burden in vivo.[6] This suggests that MCP-1 produced by hypoxic breast tumor cells regulates the recruitment of $CD11b^+/Ly6C^{med}/Ly6G^+$ myeloid cells to the pre-metastatic niche, whose presence promotes the formation of metastases.

$CD3^-/NK1.1^+$ natural killer (NK) cells were the only other BMDC population significantly accumulating in the pre-metastatic lung upon HCM administration. Interestingly, although NK cell abundance was increased, these NK cells were not fully mature and exerted limited cytotoxic effector functions. The ablation of NK cells using anti-GM1 in vivo did not change the metastatic burden of HCM-treated mice, as compared with isotype/HCM-treated control mice. Instead, in NCM-treated mice, in which NK cells were active and the metastatic burden low, the ablation of NK cells resulted in significantly increased metastasis. Therefore, we concluded that NK cell-activity in the pre-metastatic niche controls the development of metastases.

Overall, our findings demonstrate that hypoxic-primary breast tumor cells are an important source of TDSFs that drive the formation of the pre-metastatic niche (**Fig. 1**).[6] Furthermore, we identified a specific population of granulocytic $CD11b^+/Ly6C^{med}/Ly6G^+$ myeloid cells as well as immature NK cells with reduced cytotoxicity in the pre-metastatic niche (**Fig. 1**).[6] In this setting, the potential relationship between NK-cell function and granulocytic MDSCs is of interest. Specific TDSFs control myelopoiesis in the bone marrow to promote the accumulation of immunosuppressive, immature MDSCs during cancer progression.[10] Hypoxic TDSFs, including MCP-1, may promote the differentiation and accumulation of $CD11b^+/Ly6C^{med}/Ly6G^+$ myeloid cells that in turn suppress NK-cell function, most probably in a contact-dependent manner. Our work provides novel therapeutic options to inhibit the pre-metastatic niche, including the restoration of NK-cell functions and the targeting of immunosuppressive mechanisms engaged by MDSCs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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