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RESEARCH HIGHLIGHT

Arresting supporters: targeting neutrophils in metastasis

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It is becoming increasingly clear that leukocytes dynamically regulate cancer progression and metastasis, and among leukocytes, granulocytic cells abundantly accumulate in metastatic organs; however, their function in metastasis remains controversial. In a recent report in *Nature*, Wculek and Malanchi clarify the role of mature neutrophils as mediators of metastatic initiation and provide a targeted approach to prevent the pro-metastatic activity of neutrophils in breast cancer models.

Metastasis remains the primary cause of death in solid tumors. Metastatic tumors often respond poorly to standard therapies, underscoring the need for new and effective drugs targeting metastasis [1]. Several factors are thought to determine the survival and growth of disseminated cancer cells in a distant microenvironment. These include cell-autonomous processes such as tumor-reinitiating capacity at the metastatic site and cell-extrinsic interactions with non-cancer cells in the tumor microenvironment.

Research over the past decades has demonstrated that leukocytes dynamically regulate cancer progression and metastasis [2]. Some of these paracrine interactions are tumor suppressive (NK or $CD8^+$ T cells), while many others (macrophage subsets or platelets) support metastatic progression [3]. Among leukocytes, neutrophils represent the most abundant class of circulating immune cells. Although their role as the first responders in infection is well characterized, their function in metastasis is poorly understood and remains controversial. Some studies have demonstrated a tumor-promoting effect of neutrophils during metastatic progression [4-6], whereas others have found a contrasting, anti-tumor function of these cells [7]. Other close cousins of neutrophils, granulocytic myeloid-derived suppressor cells (G-MDSCs) and $Ly6G^+Ly6C^+$ granulocytes, have been shown to promote metastatic progression [8-10]. It is currently unclear whether subpopulations of granulocytic cells with such distinct functions coexist in metastatic tissues, whether they are localized differentially within the metastatic organ or regulated temporally in metastasis. In a recent paper in *Nature*, Wculek and Malanchi [11] clarified some of these open questions and defined the role of neutrophils as mediators of metastatic initiation.

In line with previous findings [6], Wculek and Malanchi confirmed that $CD11b^+Ly6G^+$ neutrophils accumulated in the pre-metastatic lung before the arrival of tumor cells in the genetically engineered MMTV-PyMT mammary cancer mouse model, and the number of these neutrophils subsequently increased with metastatic progression [11]. Since granulocyte-colony stimulating factor (G-CSF) regulates neutrophil production and mobilization from the bone marrow, neutrophil accumulation and lung metastasis were expectedly impaired in *Gcsf*-null mice. Surprisingly, G-CSF deficiency in MMTV-PyMT tumor cells did not alter either lung neutrophil accumulation or metastasis, although these tumor cells are known to secrete G-CSF [10]. However, lung metastasis was reduced when neutrophils were depleted in mice that were genetically engineered with an inducible, neutrophil-targeted diphtheria toxin. As a complementary strategy, the authors showed that depletion of neutrophils using anti-Ly6G blocking antibody in the pre-metastatic phase decreased spontaneous metastasis. Using labeled MMTV-$PyMT^+$ tumor cells, the authors showed reduced lung colonization of these tumor cells upon neutrophil depletion and further delineated that the neutrophils support metastatic initiation. One of the strengths of this study lies in the demonstration of the role of neutrophils in metastasis using multiple experimental strategies and models.

One question that arises is how neutrophils support metastatic initiation. Using the MMTV-PyMT model, the authors showed that neutrophil-derived signals promote metastatic initiation by increasing the number of metastatic initiating cancer cells (MICs). Among the neutrophil-derived factors, the authors identified high levels of leukotrienes (LTs). LTs are eicosanoids that belong to a family of molecules that are derived from arachidonic acid. LTs mediate inflammatory processes and increase vasodilation and vascular permeability [12]. Interestingly, LT stimulation of mouse MMTV-$PyMT^+$ breast cancer cells enhanced tumor initiation potential of these cancer cells both *in vitro* and *in vivo*. In addition, the authors showed that the LT receptors (LTRs; BLT2 and CysLT2) are enriched among the MICs in mouse and human breast cancer cell lines. LTs are products of the arachidonate 5-lipoxygenase (Alox5) enzyme. Therefore, to address the role of the neutrophil Alox5-LT pathway in metastasis, the authors generated bone marrow chimeric mice using the *Alox5*-

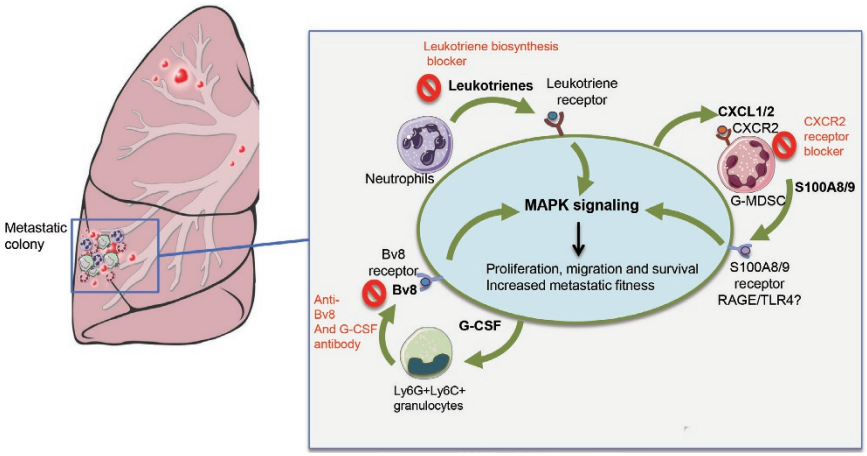

Figure 1 Diverse paracrine inputs from granulocytic cells activate the MAPK signaling pathway in cancer cells, which increases their metastatic fitness. Drugs that inhibit these paracrine inputs and reduce metastasis in mouse models are also shown.

null mice, implanted MMTV-PyMT mammary cancer cells and quantified lung metastasis. Alox5 inhibition decreased spontaneous lung metastasis.

LTs are important mediators of inflammatory and allergic responses [12]. As such, Alox5 inhibitors such as Zileuton (Zil) are in use for treating asthma. Using Zil, this study revealed a therapeutic strategy to block the pro-metastatic activity of neutrophils in multiple mouse models of breast cancer. The authors showed that lung metastasis was reduced with Zil treatment; however, the primary tumor load or lung neutrophil accumulation remained unaffected. It needs to be explored how LT inhibition selectively affects pro-metastatic neutrophils and whether there are any side effects of long-term LT inhibition in preclinical cancer models. From a translational aspect, combination therapy with standard care will further determine the efficacy of this asthma drug as an anti-metastatic agent in breast cancer.

A corollary question is how LTs produced by neutrophils benefit the MICs. The authors showed that LT treatment increased MIC proliferation through ERK/MAPK activation. Interestingly, activation of the MAPK pathway in cancer cells is a common feature of several paracrine inputs from granulocytic cells (Figure 1). Granulocytic myeloid derived suppressor cells (G-MDSC) recruited by CXCL1/2 chemokines secrete pro-survival, S100A8/9 factors that activate MAPK signaling in metastatic cancer cells [9]. Metastatic tumors secrete G-CSF that mobilizes $Ly6G^+Ly6C^+$ granulocytes to the lungs [10]. $Ly6G^+Ly6C^+$ cells produce Bv8, a small protein belonging to the prokineticin family, which binds to cancer cell Bv8/prokineticin receptor and activates the MAPK signaling pathway, thereby promoting cancer cell migration. Therefore, different granulocyte-derived factors ranging from lipid eicosanoids to Bv8 and S100A8/9 proteins trigger MAPK pathway activation in metastatic cancer cells, which subsequently promotes their proliferation, migration and survival in metastatic organs (Figure 1).

Based on these encouraging experimental results, it is necessary to evaluate the clinical importance of these findings. In this regard, Wculek and Malanchi showed that LTR expression was detected in a cohort of human breast primary and lymph node metastasis. Further investigation is warranted to explore how the neutrophil paracrine LT-LTR-MAPK pathway might be linked to lung metastasis and survival in breast cancer patients. Validation of these experimental findings from mouse models in patient samples would pave the way towards clinical translation of these important findings. In summary, the study by Wculek and Malanchi addresses the functional contribution of a key player, neutrophils, in the metastatic microenvironment, and provides a targeted approach to prevent pro-metastatic activity of these cells in breast cancer models.

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