Immunosuppression in breast cancer: a closer look at regulatory T cells

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Neutrophils create a fertile soil for metastasis

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ABSTRACT

Neutrophils can facilitate the metastatic spread of cancer; however, how neutrophils are activated at metastatic sites remains poorly understood. In this issue of *Cancer Cell*, Xiao *et al.* demonstrate that the protease Cathepsin C secreted by breast cancer cells triggers neutrophils to form neutrophil extracellular traps in the metastatic niche, thereby promoting lung metastasis.

Upon arrival in distant organs, disseminated cancer cells can only form metastases when they succeed in creating a permissive environment that fosters their survival and outgrowth. While some members of the immune system can be harnessed to prevent metastatic spread, the role of others has proven to be context dependent, or even pro-metastatic. Among these immune cells are neutrophils. These granulocytic myeloid cells are well known for their key role in acute inflammation and immune regulation, and have recently gained much attention in the context of metastatic disease.

Neutrophil diversity, function and fate are shaped by environmental cues, enabling their quick and effective adaptation to a great diversity of homeostatic and pathological conditions[1]. During homeostasis, the phenotype and activity of these short-lived cells are mainly regulated by their tissue location, circadian oscillations and cellular aging[2]. Disruption of homeostasis, for instance during tumor development, can induce a drastic systemic mobilization of (partly immature) neutrophils from the bone marrow. Importantly, neutrophil accumulation in cancer patients has been associated with a worse prognosis[3]. In line with these clinical observations, preclinical studies have revealed that neutrophils can enhance metastasis formation through a variety of effector functions, including systemic suppression of T cells, preparation of the pre-metastatic niche, or promotion of cancer cell survival. In some preclinical settings, however, cancer-induced neutrophils inhibit metastasis, highlighting their functional plasticity [1]. As such, it is of vital importance to understand the molecular mechanisms that drive the functional adaptations of neutrophils towards a metastasis-supporting phenotype, as this may uncover novel therapeutic opportunities. In this issue, Xiao *et al.* describe an intriguing pathway exploited by breast cancer cells to enhance their metastatic potential through the co-option of neutrophils in the metastatic lung niche[4].

An important cause of breast cancer-related mortality is lung metastasis, which has limited treatment options, in part due to poor understanding of critical interactions between disseminated cancer and host cells that foster their outgrowth. Xiao *et al.* set out to profile the secretome of breast cancer cell lines with varying degrees of lung metastatic potential to identify secreted factors that are potentially involved in creating a permissive metastatic niche. They discovered that Cathepsin C (CTSC), a lysosomal cysteine protease, is consistently elevated in lung-tropic breast cancer cell lines. Cysteine cathepsin proteases are multifunctional proteolytic enzymes that act in a wide range of biological processes, and can exert their enzymatic activity both intracellularly (most notably in the lysosome) and extracellularly. Cathepsins are often dysregulated in cancer, and experimental evidence has specifically implicated CTSB, CTSK, CTSL, CTSS and CTSZ in breast cancer metastasis[5], whereas the role of CTSC has remained less clear, due to its context dependent role in carcinogenesis[6].

By using a variety of intravenously injected and orthotopically transplanted breast cancer cell lines in mice, Xiao et al. showed that CTSC overexpression in cancer cells exacerbates lung metastasis, whereas knock-down of CTSC reduces the metastatic burden in lungs. While modulation of CTSC has no direct effect on primary tumor outgrowth, CTSC critically increases cancer cell proliferation early upon their colonization of the lungs, thus suggesting that CTSC improves the adaptation of disseminated cancer cells to their new micro-environment. The authors observed that tumor-derived CTSC induces the recruitment of neutrophils into the lungs through paracrine communication. Strikingly, antibody-mediated depletion of neutrophils completely abrogates the pro-metastatic effect of CTSC, uncovering a crucial interaction between CTSC-expressing cancer cells and neutrophils.

Also in breast cancer patients, an association between high intratumoral CTSC expression and poor survival was observed, and CTSC expression levels are particularly high in lung metastases versus primary tumors. These data are in line with previous clinical studies that have linked high CTSC expression to increased incidence of both brain- and lung metastasis in breast cancer patients[5]. The current study from Xiao *et al.* provides insight into the pro-metastatic role of CTSC, and warrants further research into whether the same axis is relevant for metastasis formation in the brain.

In an impressive set of mechanistic studies, Xiao and colleagues showed that cancer cell-derived CTSC enzymatically activates the serine protease PR3 expressed on the membrane of neutrophils (Figure 1). This process induces the activation of IL-1β in lung neutrophils, which kick-starts an inflammatory cascade involving the secretion of IL-6 and CCL3 resulting in the recruitment of additional neutrophils from the circulation. In parallel, IL-1β activation also initiates intracellular production of reactive oxygen species (ROS) in neutrophils, which promotes the formation of neutrophil extracellular traps (NETs). NETs are extracellular web-like chromatin structures made of DNA fibres, histones and granule proteins, that are released from neutrophils primarily through an alternative cell death process called NETosis. These DNA traps play an important role in the defence against large pathogens by trapping microbes in place, but have recently also been observed in the microenvironment of various human cancer types including pancreatic, breast, lung, and liver cancer[7]. Notably, the authors demonstrated that the *in vivo* destruction of NETs through treatment of mice with DNAse I is sufficient to prevent the metastatic outgrowth of CTSC-expressing cancer cells in the lungs, highlighting a causal role for NETs in CTSC-enhanced metastasis formation. But how do these neutrophil-derived DNA traps enhance the metastatic potential of breast cancer cells?

Several mechanisms have been reported by which NETs can promote metastasis, including direct induction of cancer cell chemotaxis to the liver[8], and the awakening of dormant cancer cells in lungs [9]. Xiao and colleagues add a new mechanism to the list by demonstrating

that NETs induce the degradation of the matricellular protein thrombospondin-1 (TSP-1), which has been shown to be important for tumor spheroid outgrowth *in vitro*. Combined, this study reveals an intriguing novel pathway by which tumoral CTSC expression dictates metastatic potential by exploiting neutrophils in the metastatic lung niche (Figure 1).

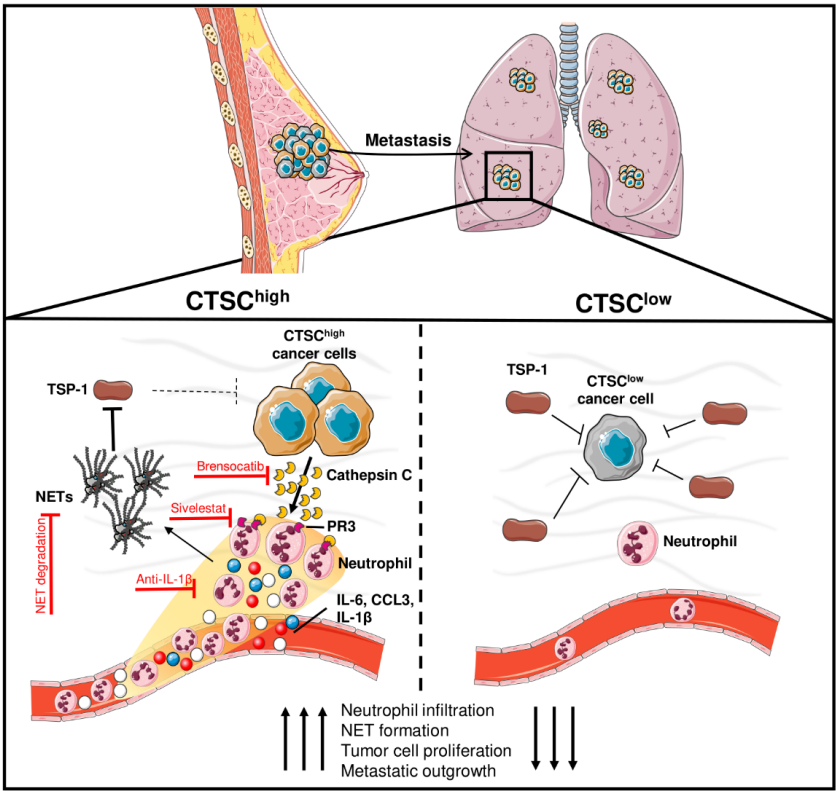

FIGURE 1. Breast cancer cells gain metastatic potential through expression of CTSC
In the metastatic lung niche, cancer cell-derived CTSC activates PR3 on neutrophils, leading to a signalling cascade that promotes the recruitment of neutrophils from circulation, and enhances NET formation via IL-1β. In turn, cancer cells gain a proliferative advantage through the NET-mediated degradation of TSP-1, resulting in enhanced metastatic outgrowth. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

These novel findings raise the question whether targeting the CTSC-PR3-IL1β-NET axis represents a viable therapeutic strategy to prevent metastatic spread of CTSC-expressing breast cancer. Excitingly, the authors showed that a small molecule inhibitor of CTSC, brensocatib, suppresses experimental lung metastasis. Besides CTSC, IL-1β might be an attractive actionable therapeutic target (Figure 1). Indeed, a recent clinical study has revealed that inhibition of IL-1β in patients with atherosclerosis reduces lung cancer

incidence, which associates with a reduction of tumor promoting inflammation[10]. As of yet, it is unclear whether the efficacy of targeting the CTSC-PR3-IL1β-NET pathway will be limited to preventing lung metastasis, or whether it may also prevent metastases in other tissues. This may be dependent on the tissue-specific expression levels of TSP-1, thereby highlighting a clinically relevant direction for future research.

The clinical relevance of this work is supported by complementary findings in samples of several human breast cancer cohorts, showing that tumoral CTSC expression strongly correlates with neutrophil and NET markers, as well as with lung metastasis. Interestingly, by analysing these cohorts, Xiao *et al.* found substantial interpatient heterogeneity of CTSC expression, and showed that CTSC is highest expressed in triple-negative breast cancer. It is unclear how CTSC is regulated in different subtypes of breast cancer, and looking forward, an important next step would therefore be to identify the patient population that is most likely to benefit from therapeutic exploitation of these findings. Taken together, this study reveals a novel mechanism that confers breast cancer cells with enhanced metastatic potential through co-option of the immune system. Importantly, these insights open new avenues for the future design of therapeutic strategies aimed at blocking a cancer cell's ability to create a permissive metastatic niche.

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