Acute infection induces a metastatic niche:
a double menace for cancer patients

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Running Title: Acute inflammation promotes tumor metastasis

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Summary:

Tumor-derived factors can induce a pre-metastatic niche, yet little is known about how metastatic microenvironments are influenced by external insults, such as acute infections commonly seen in cancer patients. New findings reveal increased metastasis to lung after acute bacterial infection via the CXCR4 Ubiquitin axis, suggesting new targets for anti-metastasis therapeutics.
In this issue of *Clinical Cancer Research*, Yan and colleagues evaluated the role of acute lung inflammation on tumor metastasis in mice and observed enhanced tumor cell recruitment via the ubiquitin-CXCR4 chemokine axis as well as increased lung metastatic lesions (1). Treating acute inflammation with the anti-biotic amoxicillin or the CXCR4 blocking molecule AMD3100 dramatically diminished tumor cell recruitment and metastatic growth.

The contribution of *chronic* inflammation to primary tumor formation and progression is well supported by a large body of evidence (2), and additional studies have demonstrated that factors secreted from the primary tumor can systemically alter the microenvironment of secondary organ sites to establish a pre-metastatic niche (3). In particular, cytokines secreted by tumors can activate inflammatory and mesenchymal stem cells within secondary tissue environments or recruit bone marrow-derived cells, which promote metastasis by altering local protein and cytokine expression patterns. Such factors include vascular endothelial growth factor (VEGF)-A, TGF-β, and TNF-α, which have been shown to up-regulate the expression of S100A8 and S100A9 to attract tumor cells (4). Other studies demonstrate that suppressive immune cell populations at secondary organ sites can promote regional inflammation that fosters metastatic seeding. For instance, Gr1⁺CD11b⁺ myeloid cells suppress IFN-γ production and increase local inflammatory cytokines in the pre-metastatic niche as well as MMP9 expression, which alters surface protein expression on vascular cells to promote adhesion of circulating breast tumor cells (5). Primary tumor induction of S100A8 and S100A9 expression has also been shown to recruit Mac1⁺ myeloid cells via TLR4 to pre-metastatic sites (4). These myeloid cells further alter the microenvironment through the secretion of inflammatory and immunosuppressive cytokines to promote metastasis. Indeed, multiple lines of evidence suggest that both local and systemic inflammation play a key role in the metastatic cascade.

The work of Yan and colleagues now provides a new mechanism by which *acute* inflammation in the lung can foster metastatic seeding (Figure 1). Previous studies of acute inflammation and metastasis have only tested the systemic effects on mice injected with the bacterial cell wall component, lipopolysaccharide (LPS), which was found to increase the metastatic potential of colon cancer cell lines and 4T1 breast cancer seeding to the lung (6, 7). In some instances, acute inflammation and induction of immune responses have actually been shown to manifest *anti*-tumor effects. In fact, a common treatment for non-invasive bladder cancer is the bacillus Calmette-Güérin (BCG) vaccine (8). While this treatment does indeed activate acute inflammatory states, the accepted mechanism by which this therapy kills tumor cells is through the anti-tumor activity of cytotoxic effector cells, which may overshadow any pro-tumor effects of the inflammation.

To evaluate the impact of acute lung inflammation on tumor metastasis, the authors combined two well established acute infection models, the LPS-induced acute lung injury/inflammation (ALI) and the DH5α bacterial pneumonia, with experimental metastasis models in mice. Both tail vein injection and an orthotopic tumor cell model resulted in enhanced lung metastasis in mice with bacterial lung infections. These
findings potentially have broad applicability for the treatment of metastasis in cancers of different histological origin since melanoma, lung, prostate, and colorectal cancer cell lines were all evaluated in this study.

The mechanistic basis for bacterial-induced tumor cell recruitment to the lungs was investigated in a series of assays utilizing bronchoealveolar lavage fluid (BALF) from control, LPS-, or bacteria-injected mice. Yan and colleagues observed increased migration of CXCR4+ tumor cells towards the BALF from infected mice. Since LPS itself was not found to be responsible for the enhanced tumor cell migration, and because differential cytokine expression patterns were not observed in BALF from infected and control mice, the researchers evaluated tumor cell migration via the well established CXCR4/SDF-1 signaling axis. This receptor/ligand interaction is known to be responsible for the recruitment and homing of normal haematopoietic stem cells to the bone marrow, and for the homing of metastatic tumor cells to distant organs (9). While SDF-1 was the most likely CXCR4 candidate for tumor cell recruitment to the inflamed lungs, the investigators instead revealed that extracellular ubiquitin, a recently identified alternative ligand for CXCR4, was responsible for this chemotaxis. Very little is known about the role of extracellular ubiquitin in tumor cell recruitment. It would be informative to evaluate human patients with pneumonia to determine if they similarly express high levels of extracellular ubiquitin in lung fluid and whether this could contribute to the CXCR4-mediated tumor recruitment. It would also be important to characterize the expression of this protein and its properties of tumor cell recruitment in environments other than the lung. Because tumor cell migration could be efficiently blocked using the CXCR4 inhibitor molecule AMD1300, this signaling axis could prove to be a valuable therapeutic target if it was indeed used to recruit metastatic cells to multiple sites of acute inflammation in patients.

CXCR4 can be modulated by multiple components of the inflammatory environment, including stress, inflammation, and tissue damage, which can alter CXCR4 surface expression (10). Since CXCR4 incorporates into lipid rafts, statin drugs, which block cholesterol synthesis and have been associated with decreased malignancy, may be utilized to reduce CXCR4 expression on the tumor cell surface (11). Therefore, statin drugs should also be evaluated as therapeutics to block the CXCR4 axis and recruitment of tumor cells to the sites of acute inflammation. Moreover, the CXCR4/Ub axis was found to activate AKT signaling within tumor cells to mediate efficient migration (1), suggesting another possibility of using AKT inhibitors to reduce the risk of metastasis during acute inflammation.

In summary, this study clearly establishes that infection-induced inflammation, rather than tumor-induced inflammation, plays a role in the formation of a metastatic niche-like environment, and a novel ubiquitin-CXCR4-AKT signaling axis has been identified as a key mediator of tumor cell recruitment. Non-steroidal anti-inflammatory drugs (NSAIDS) have already been shown to reduce the risk of cancer, particularly colorectal carcinoma, and much work is currently being conducted to assess ways to prevent or treat chronic inflammation as a cancer therapy (12). Cytokines and growth factors secreted by chronically inflamed tissues are often not shared in common with acute inflammatory
environments and further evaluation of acute inflammation could reveal new targets to prevent or treat metastasis and tumor relapse. These results provide evidence that multiple types of inflammation can contribute to metastasis and could potentially be translated to clinical practice where antibiotics or agents such as AMD3100 could be administered to cancer patients to prevent infection-induced risk of metastasis. In particular, the timing of acute inflammation appeared to be critical for metastatic formation in the pneumonia model, as only tumor cells injected within six hours of induced inflammation were recruited significantly to the lung (1). This finding may highlight the importance to prevent or provide prompt treatment for bacterial infections to avoid metastatic relapse. Treatment to block such acute inflammation would be particularly important for patients who have undergone surgery or immunosuppressive therapy and are at greater risk for pneumonia and other bacterial infections.
Figure 1. Acute lung infection and inflammation enhances lung metastasis via CXCR4/Ub axis.
C57BL/6 and BALB/c mice were inoculated with LPS or DH5α bacteria to induce acute lung inflammation, and subsequently challenged with melanoma, breast, prostate, or lung carcinoma cell lines via tail vein injection. Acute inflammation in the lung microenvironment resulted in increased secretion of extracellular ubiquitin. CXCR4+ tumor cells were found to migrate toward ubiquitin via CXCR4/Ub ligand-mediated chemotaxis. Lung metastasis was dramatically enhanced in mice with acute lung inflammation compared to controls and this effect could be ameliorated with the CXCR4 blocking molecule AMD3100 and the anti-biotic amoxicillin.
References:


Figure 1:

Legend key:
- DH5α/LPS
- Ubiquitin
- CXCR4
- AMD3100
- Normal lung epithelial cell
- Inflamed cell
- Tumor cell
- Metastatic lesion

Bacterial infection

AMD3100
Amoxicillin

Lung inflammation & metastasis

Circulating tumor cells
Clinical Cancer Research

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