Myeloid TGF-β Responsiveness Promotes Metastases

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Summary: Tumor-induced immune suppression is a major impediment to many potentially effective cancer therapies. TGF-β has previously been described as having both tumor-promoting and tumor-suppressive characteristics. In this issue of Cancer Discovery, Pang and colleagues show that myeloid-specific TGF-β signaling is a critical mediator in tumor metastasis. These findings point toward a more specific means to reduce cancer immune-suppression, prevent metastasis, and minimize treatment-related adverse events. Cancer Discov; 3(8); 846–8. ©2013 AACR.

See related article by Pang et al., p. 936 (3).

Immune evasion represents one of the most contemporary hallmarks of cancer, and myeloid cells are one of the most important cell types in the suppression of host immune surveillance, consequent tumour progression (1), and premetastatic niche formation (2). These tumor-infiltrating myeloid cells include tumor-associated macrophages (TAM, CD11b+F4/80+), and Gr-1−CD11b+ myeloid-derived suppressor cells that can be divided into myeloid monocytes (CD11b+Ly6C−) or myeloid neutrophils (CD11b+Ly6G+). All of these cell types potentially produce TGF-β within the tumor microenvironment. TGF-β is also overexpressed in advanced human cancers, and its expression correlates with metastasis and poor prognosis. In addition to the fact that many cell types can both produce and respond to TGF-β, another great challenge in our understanding of TGF-β cancer biology and the successful application of TGF-β-targeted therapy is that TGF-β works as both a tumor suppressor and a tumor promoter.

Consequently, there has been a great need to understand which TGF-β-sensitive cell type is the most important in tumor promotion and spread. Now, Pang and colleagues (3) report that genetic deletion of TGF-β type II receptor (Tgfr2) specifically in mouse myeloid cells using the LysM-cre strain (Tgfr2^myko^) significantly inhibited tumor metastasis. Importantly, the Tgfr2^myko^ mice had no alteration in the number or percentage of T, B, NK, or Gr-1−CD11b+ and F4/80+ cells. Reconstitution of tumor-bearing mice with Tgfr2^myko^ bone marrow recapitulated the reduced metastasis phenotype. Reduced metastasis was mediated through decreased production of type II cytokines, TGF-β1, arginase 1, and iNOS, which promoted IFN-γ production and improved systemic CD8+ T-cell systemic immunity in the Tgfr2^myko^ mice (Fig. 1).

This work follows on from other intensive efforts in this area. In particular, by crossing LysM-Cre and Tgfr2 floxed mice, Novitskiy and colleagues (4) also achieved specific deletion of Tgfr2 in myeloid cells in the C57BL/6 strain background. In contrast to Pang and colleagues (3), where no, or only modest, effects on orthotopic tumor growth were noted, Novitskiy and colleagues (4) showed the slower growth of a variety of subcutaneously transplanted syngeneic tumors in Tgfr2^myko^ mice. They also reported an increase in the percentage of T cells (CD3+) in the primary tumors in Tgfr2^myko^ mice, but no change in the myeloid cell infiltrates. The dendritic cells from tumor tissue of Tgfr2^myko^ mice had increased antigen-presenting properties and an enhanced ability to stimulate antigen-specific T-cell proliferation. Taking advantage of Tgfr2^myko^ mice on a BALB/c background, Pang and colleagues (3) focused some attention on the role of myeloid cell TGF-β responsiveness in 4T1 mammary metastasis to the lung. Both in this model and in other experimental metastasis models (B16 and Lewis lung carcinoma), they were able to contrast the significantly greater protumorigenic effect of TGF-β signaling in myeloid cells on metastases versus primary tumor growth.

Previously, using the 4T1 model, Li and colleagues (5) showed that depletion of Gr-1−CD11b+ cells diminished the antitumor effect of TGF-β neutralization and that TGF-β neutralization significantly decreased the expression of Tregs cytokines and arginase 1. They suggested that peripheral blood Gr-1−CD11b+ cell number and function could be good biomarkers for TGF-β-targeted therapy. Now, they have shown that Gr-1−CD11b+ cells derived from Tgfr2^myko^ mice have reduced levels of interleukin (IL)-4, IL-10, TGF-β1, arginase 1, and iNOS, but similar type I cytokine production (e.g., IL-12 and TNF-α), compared to the same populations from control mice (3). These outcomes are consistent with the known regulatory effects of TGF-β1 on myeloid cell products. There was also an increased percentage of IFN-γ-positive CD8+ T cells in the spleen of tumor-bearing Tgfr2^myko^ mice compared with control mice. Pang and colleagues (3) were able to show that depletion of CD8+ T cells or neutralization of IFN-γ reduced the antimetastatic effect observed in Tgfr2^myko^ mice. These functional experiments in vivo lend a lot of weight to the proposed mechanism. More revealingly, the authors went on to assess infiltrates in the lung metastases themselves, and found that specifically a CD11b+Ly6C− (also F4/80+ and Ly6G+) population had increased IFN-γ production and expressed CD86. Other data, including adoptive transfer rescue experiments,
Figure 1. Possible consequences of TGF-β signaling in tumor-infiltrating myeloid cells in (A) wild-type mice and (B) Tgfb1KO mice. TGF-β1 production is enhanced in myeloid cells through autocrine and/or paracrine (from tumor, Treg, or stromal fibroblasts) mechanisms. Deletion of TGF-βRII in myeloid cells decreases the production of TGF-β1, type II cytokines, arginase, and iNOS, but not IL-12 and TNF-α, shifting the cytokine balance to in turn increase IFN-γ production by antimetastatic CD8+ T cells and subsets of macrophages and neutrophils. Treg, regulatory T cell.
suggested that TGF-β signaling affected the properties of both CD11b+Ly6G+ (neutrophils) and CD11b+Ly6C+ (monocytes), and that their relative impact may depend on the tumor composition of these myeloid subsets. The CD11b+Ly6G+ population had a higher TGF-βRII expression level and produced TGF-β1 in a TGF-βRII-dependent manner, suggesting an autocrine and/or paracrine loop that enhances TGF-β1 production. It is not clear whether TGF-β1 production directly converts the myeloid cells from a type I to type II phenotype or is a result of type II myeloid cell polarization.

Previously, when TGF-β signaling was blocked in T cells using CD4dnTGF-βRII transgenic mice, tumors were also inhibited, but mice concurrently developed an autoimmune pathology (6). As myeloid cells are significantly expanded under tumor conditions, the neutralization of TGF-β signaling in them is possibly more specific. Using the LysM-cre donor, the deletion of the transcription factors ETS2 or c-MYC in myeloid cells inhibits a gene program for angiogenesis and decreases the frequency and size of carcinoma metastases in the lung. However, in contrast, myeloid deletion of VEGF-A resulted in accelerated tumor progression (7). The context and cell type in which the gene/protein is deleted may be very important, given the very broad effects of LysM-cre. Myeloid-specific deletion of HuR, a RNA-binding protein, displayed increased susceptibility to colitis-associated cancer (CAC; ref. 8), although in this context metastasis was not assessed. Very recently, others have shown that TGF-β signaling in colonic myeloid cells is significantly involved in the development of CAC (9). Myeloid TGF-βRII deficiency markedly decreased the production of IL-6 and TNF, two proinflammatory cytokines that are essential for colonic tumorigenesis, and a marked myeloid cells is significantly involved in the development of CAC.

Consistent with these mouse studies, myeloid cells from patients with advanced-stage cancer showed increased TGF-βRII expression. Pang and colleagues (3) used CD33, CD34, or CD15 markers to identify immature myeloid cells in the peripheral blood of 16 patients with metastatic non-small cell lung cancer and showed that their TGF-βRII expression was higher than in normal controls. Combined with other analyses in other cancer types, the data suggested that increased TGF-βRII expression correlated with cancer progression in a clinical setting. Collectively, these studies show that myeloid-specific TGF-β signaling is an important component of the metastasis-promoting function of TGF-β, and TGF-β signaling is critical in shaping the type I/type II phenotype of myeloid cells.

How might these new data be translated therapeutically? TGF-β is a powerful metastasis promoter in the later stages of cancer progression; however, it mediates growth inhibition in early stages. The factors driving the functional change of TGF-β are largely unknown, and this poses significant challenges to our successful application of TGF-β-targeted therapy. It is not impossible to think that in the context of bone marrow transplantation current technology can allow myeloid-specific reduction in TGF-β signaling in humans. But one would not expect widespread application of such an approach. Anti-TGF-β antibody has been shown to reduce tumor burden in mice and rescue tumor-associated bone loss in metastatic breast cancer (10). Thus, anti-TGF-β treatment may offer a novel therapeutic option for tumor-induced bone disease. But generally, results with anti-TGF-β antibodies have been disappointing to date, and TGF-β-neutralizing antibodies may have adverse effects in normal healthy tissues. Ligation of Toll-like receptor 7, which is expressed on TAMs, but not tumor cells, in the presence of TGF-βRII inhibitor, reprogrammed the phenotype of the TAMs to M1-type with increased tumorcell activity and elevated expression of iNOS, CD80, and MHC class II, whereas TGF-β secretion was reduced (11). This combination also increased tumor apoptosis and elevated the number of CD4+, CD8+, and CD19+ cells, as well as neutrophils infiltrating the tumor. Overall, these recent studies provide a strong rationale for targeting TGF-β signaling in TAMs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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REFERENCES
