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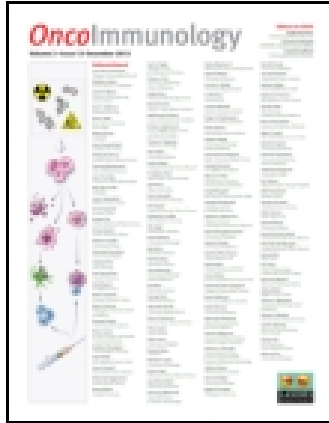
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Delineating CSF-1-dependent regulation of myeloid cell diversity in tumors

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Keywords: breast cancer, tumor microenvironment, M-CSF, tumor-associated macrophages, matrix metalloproteinase

Abbreviations: TAM, tumor-associated macrophages; MMP, matrix metalloproteinase

Myeloid cells contribute to increased malignancy and poor prognosis in breast cancer. We demonstrate that anti-CSF-1R therapy depletes a cell population sharing characteristics of tumor-associated macrophages (TAMs) and dendritic cells (DCs). Intravital imaging combined with cellular characterization has refined our understanding of anti-CSF-1R therapy on the tumor microenvironment.

Tumor-infiltrating Myeloid Cells Promote Tumor Progression

Tumors function as complex organs that shape their microenvironment through the interaction with stromal cells, consisting of fibroblasts, adipocytes, blood and lymph vessels, and immune cells.¹ As tumors progress, tumor-derived growth factors lead to evolving phenotypes of tumor-associated myeloid cells that can contribute to tumor initiation and progression by a wide range of mechanisms.² In human breast cancer and most other solid tumors, high numbers of tumor-associated myeloid cells, along with high expression of the myeloid colony-stimulating factor -1 (CSF-1),³ correspond to poor prognosis.

CSF-1 Dependent and Independent Tumor-associated Myeloid Cells

Due to the plasticity of macrophages, the complex spectrum of macrophage activation and the lack of cell surface markers that are distinct to subsets of macrophages,^{4,5} a clear understanding of macrophage diversity and function within the tumor and during tumor

progression cannot be delineated by immunohistochemistry and flow cytometry alone. In addition to these methods, we therefore utilized intravital microscopy, in combination with targeted therapy against CSF-1R, to provide unique insight into the cellular composition and real-time dynamics of the stromal microenvironment.⁶ Our goal was to characterize the evolving phenotype of TAMs and their dynamic interplay with the microenvironment during tumor progression. We utilized the MMTV-PyMT/*c-fms*-EGFP mouse model of breast cancer, which mimics the stages of tumor progression observed in humans, and the IgG₁ M279 blocking monoclonal antibody to the CSF-1R.

Using intravital imaging, we identified a *c-fms*-EGFP population of sessile myeloid cells that localized closely to the epithelium of tumor nodules and included subpopulations of cells with high endocytic and MMP proteolytic activity. Furthermore, we found these cells expressed macrophage cell surface markers F4/80 and CD115 (CSF-1R), along with classical DC markers CD11c and MHCII using flow cytometry. Accordingly, these cells were profoundly depleted with the anti-CSF-1R

antibody M279. In addition, we observed CSF-1 independent myeloid cell populations in the stroma, which were identified as CD11c⁺ F480⁻ MHCII^{hi} (classical DCs) and CD11c⁻ F4/80⁻ Gr1^{hi} (neutrophils) cells by flow cytometry. Further detailed characterization of these populations in two recent studies showed the CD11c⁺ F480⁻ MHCII^{hi} population is GM-CSF dependent⁷ and the Gr1^{hi} population, which consists predominantly of T cell-suppressive Ly6G⁺ Ly6C^{int} neutrophils, is G-CSF dependent,⁸ revealing distinct regulation of these myeloid cell populations in the PyMT model of breast cancer (Fig. 1).

CSF-1-dependent Regulation of Lung Metastasis During Early Tumor Progression

To determine whether depletion of CSF-1-dependent CD11c⁺ F4/80⁺ MHCII^{hi} myeloid cells was therapeutic, two treatment regimens were performed with M279 to evaluate efficacy of early (hyperplastic stage) and late (early carcinoma stage) treatment. We found that depletion of CSF-1R-dependent macrophages during early tumor

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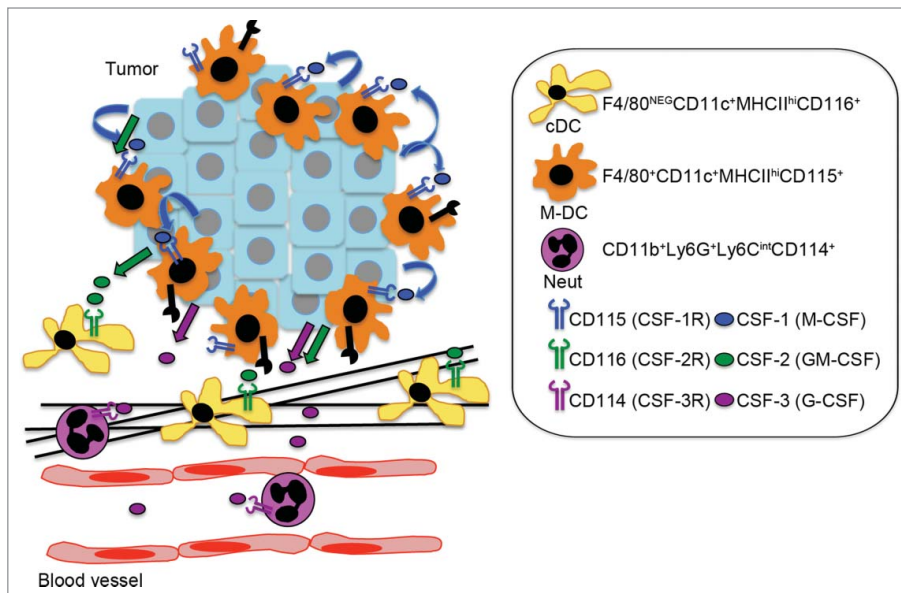


Figure 1. Proposed model for the distinct regulation of myeloid cell subpopulations in breast cancer. Tumor-derived hematopoietic growth factors CSF-1 (M-CSF), CSF-2 (GM-CSF), and CSF-3 (G-CSF) regulate distinct populations of myeloid cells in tumors. CSF-1-dependent $F4/80^+ CD11c^+ MHCII^{hi} CD115^+$ myeloid cells, referred to as macrophage-dendritic cells (M-DCs), showed high endocytic and matrix metalloproteinase (MMP) proteolytic activity and localized close to the epithelium of tumor nodules. CSF-1-independent $CD11b^+ Ly6G^+ Ly6C^{int}$ (neutrophils, CSF-3 dependent) and $F4/80^{neg} CD11c^+ MHCII^{hi}$ (classical DCs, CSF-2 dependent) localized in the stroma.

progression modestly reduced primary tumor growth and lung metastases, while it only affected primary tumor growth during late stage tumor progression, suggesting CSF-1R-dependent cells promoted the establishment of metastatic lesions. Surprisingly, we did not detect an accumulation of macrophage- or DC-like cells in lungs in this study, but, in contrast, observed only an expansion of $Gr1^{hi}$ neutrophils. The modest delay in tumor growth and metastasis suggests that other stromal cells contribute to the maintenance of the tumor trophic microenvironment. Alternatively, given the diversity of myeloid cells that are depleted with anti-CSF-1R blockade, the relatively modest effect on tumor growth may also suggest that CSF-1R dependent

myeloid cells may contribute both pro and antitumor functions.

M1 and M2 Characterization Should Be Avoided

While CSF-1 stimulation as a single agent polarizes macrophages away from an antigen presentation phenotype and induces a different gene expression profile than GM-CSF, CSF-1 dependent macrophages should not be classified as M2 polarized, since their heterogeneity is likely regulated by the microenvironment.⁴ Furthermore, while TAMs have been termed M2 or alternatively activated macrophages (AAMs), $IL-4^{-/-}$ PyMT mice had normal proportions of TAMs (characterized as $CD11b^{lo}$

$MHCII^{hi} VCAM^+$ cells that highly expressed *itgax*, the gene that encodes CD11c) and TAM differentiation was intact in the absence of lymphocytes, suggesting TAMs are not the same as classical AAMs.⁹ Thus, it has been suggested that macrophage heterogeneity should not be simplified on a linear scale between M1 and M2, but should be described in terms of the diversity of points on a color wheel.⁴

Future Therapy With CSF-1 Blockade in Cancer

CSF-1 blockade depletes macrophage populations in various tumor models, but the effects as a single agent therapy on primary tumor growth is variable.³ This variability may result from the heterogeneity and relative proportions of macrophage subpopulations in different tumor types and their role (s) in disease progression. Of interest, CSF-1 blockade in combination with radiation, chemotherapy, and immunotherapy has shown synergistic effects.³ However, one study reported a neutrophil-dependent enhanced metastasis with CSF-1 blockade.¹⁰ A better understanding of the complex interplay between tumor-associated myeloid cells and disease progression in different tumor types is warranted and should provide predictive and prognostic value.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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