Remote control: the cancer cell-intrinsic mechanisms that dictate systemic inflammation and anti-tumor immunity

Wellenstein, M.D.

Fatty acids corrupt neutrophils in cancer

Max D. Wellenstein[1] & Karin E. de Visser[1,*]

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Affiliations

[1] Division of Tumor Biology & Immunology, Oncode Institute, Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands

* Corresponding author

Abstract

Understanding how tumors escape from immune attack may offer novel therapeutic opportunities. Veglia *et al.* demonstrate in *Nature* that Fatty acid transport protein 2 (FATP2) endows neutrophils with immunosuppressive capabilities that promote cancer growth. This receptor can be targeted to unleash anti-tumor immunity and to potentiate immune checkpoint blockade.

Main text

Despite recent major advances in the treatment of cancer with immunotherapy, which is aimed at boosting the patients' immune system to fight cancer, a large proportion of patients does not benefit from this therapy. Tumors have evolved various mechanisms to escape immune attack. There is emerging experimental and clinical evidence that immune escape of solid tumors is facilitated by myeloid immune cells that are actively mobilized and reprogrammed by developing tumors. One such group of myeloid cells are the immunosuppressive neutrophils, which –in the context of cancer– is often referred to as the polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC). While neutrophils have an indispensable role in protection against pathogens and tissue repair, these cells can be pathologically activated in cancer patients and have been shown to the progression of tumors via several mechanisms, including the induction of immunosuppression[1]. Given their prominent tumor-promoting functions, neutrophils represent attractive targets for therapeutic intervention. Targeting tumor-associated neutrophils can be envisioned to occur in three ways: 1) depletion, 2) targeting downstream effector molecules, or 3) reprogramming/ normalization of these cells. Evidently, depletion would be too toxic for patients due to the essential function of neutrophils in anti-microbial responses. Targeting their downstream effector molecules remains challenging because of the diversity of effector functions and the high degree of plasticity of these cells, especially in cancer. However, this plasticity could also be used as an advantage: reprogramming neutrophils could be a potential therapeutic avenue to relieve immunosuppression in tumor-bearing hosts, but little is known about how we can reprogram neutrophils in tumor-bearing hosts. Veglia *et al.* describe in *Nature* an intriguing role of fatty acid uptake in the pathological activation of neutrophils by cancer[2], thus mediating the acquisition of immunosuppression and promoting tumor progression.

Adaptation to diverse environments is a common feat of immune cells, including neutrophils. To meet the demands of adaptation to different contexts and to fuel effector functions, immune cells can undergo metabolic reprogramming[3]. Also in cancer, metabolic signaling pathways in immune cells can be altered to contribute to the induction of pro-tumoral effector functions[3]. However, the molecular basis of metabolic reprogramming of tumor-associated neutrophils is largely unresolved. Veglia *et al.* reveal how metabolites of fatty acids can contribute to PMN-MDSC-mediated immunosuppression. By using a variety of subcutaneously transplanted and transgenic mouse tumor models, they demonstrate that lipids accumulate in splenic and intratumoral PMN-MDSCs, mainly in the form of triglycerides that contain arachidonic acid. Assessing various receptors that have been reported to facilitate lipid uptake, they found that Fatty acid transport protein 2 (FATP2, encoded by the *Slc27a2* gene) was significantly up-regulated exclusively in PMN-MDSCs of tumor-bearing mice, compared to neutrophils from healthy mice and to other cell types present in the tumor microenvironment. By studying tumor-bearing FATP2-deficient mice, or mice lacking FATP2 in neutrophils, the authors showed that this transporter is crucial for the expression of pro-inflammatory genes and the suppression of $CD8^+$ T cells by PMN-MDSCs, thereby promoting the growth of transplanted tumors.

Immunosuppression by tumor-induced PMN-MDSCs can occur via a variety of molecules, including inducible nitric oxide synthase (iNOS) and Arginase-1 (Arg-1)[4,5]. Deletion of FATP2 however, did not alter expression of these enzymes. Rather, the arachidonic acid that was trafficked by FATP2 was metabolized into another potent immunosuppressive mediator, Prostaglandin E2 (PGE_2). In line with this, co-culture of T cells and PMN-MDSCs

demonstrated increased immunosuppression in the presence of free arachidonic acid, but not when MDSCs were generated from mice deficient for the enzyme PGE_2 synthase (PTGES2). In a series of mechanistic studies, the authors showed that the transcription factor STAT5 could activate transcription of *Slc27a2* by binding its promoter, which was activated by GM-CSF signaling. Neutrophil-specific deletion of STAT5 decreased FATP2 expression and consequently led to decreased tumor growth. This collectively demonstrated that the metabolites of the fatty acids that are taken up by PMN-MDSCs can be used to suppress T cells (**Fig. 7.1**). It remains to be elucidated whether these same mechanisms also occur in tumors that are low in either GM-CSF or arachidonic acid, or that these tumors employ alternative mechanisms of activation of immunosuppression.

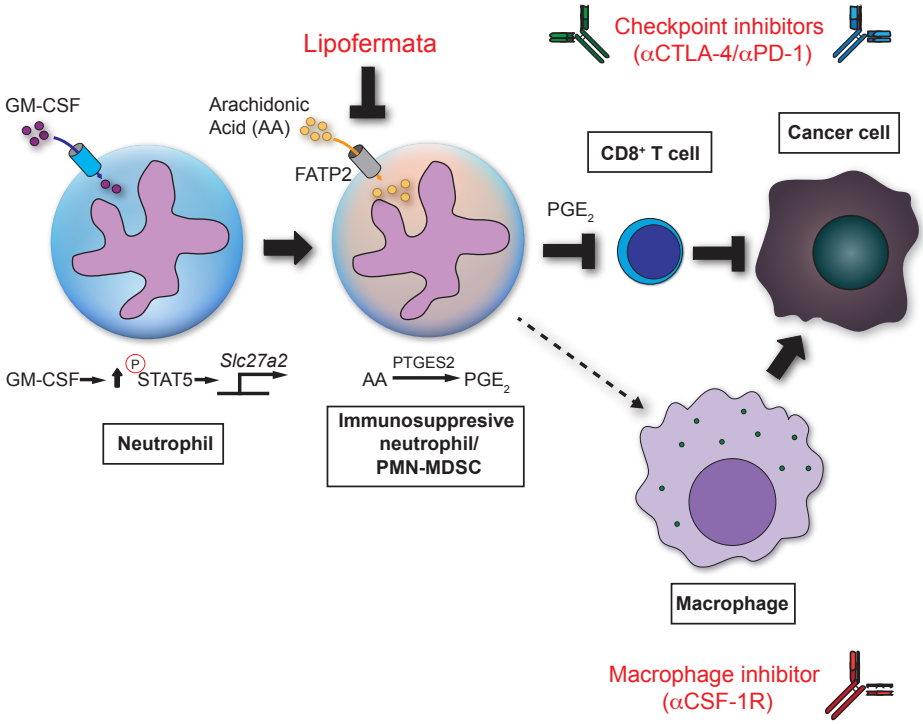

Figure 7.1: Fatty acid uptake by PMN-MDSCs drives immunosuppression in cancer. Suppression of $CD8^+$ T cells in the tumor microenvironment is mediated by immunosuppressive neutrophils/PMN-MDSCs. In tumors, GM-CSF activates STAT5, leading to transcriptional activation of *Slc27a2*, which encodes the Fatty acid transport protein 2 (FATP2). This facilitates increased uptake of arachidonic acid (AA)-containing triglycerides that are metabolized into Prostaglandin E_2 (PGE_2) by the Prostaglandin E Synthase 2 (PTGES2) enzyme. PGE_2 mediates suppression of $CD8^+$ T cells. Targeting fatty acid transport using Lipofermata increases responses to anti-CTLA-4, anti-PD-1 and anti-CSF-1R immunotherapies in mice.

Notably, the authors demonstrated that lipids also accumulate in PMN-MDSCs in blood of patients with various cancer types. In addition, of the lipid transport proteins expressed by PMN-MDSCs in cancer patients, *SLC27A2* was increased most dramatically. Moreover, GM-CSF could induce STAT5 activation and FATP2 expression in human PMN-MDSCs, and these cells showed higher levels of PGE_2 than control neutrophils. These findings suggest

that the mechanisms governing the fatty acid-induced immunosuppressive phenotype of neutrophils are conserved between mice and humans and add an important piece to the puzzle of how PMN-MDSCs acquire their immunosuppressive function. Importantly, this work also demonstrates that FATP2-mediated activation of T cell suppression is specific to PMN-MDSCs, and that immunosuppression by monocytic (M)-MDSCs is independent of this signaling pathway.

The Veglia *et al.* study complements recent findings showing a key role for fatty acids in the immunosuppressive programming of PMN-MDSCs[6,7]. Intriguingly, while previous work demonstrated that fatty acid transporter CD36 mediates GM-CSF/STAT5-mediated fatty acid uptake and immunosuppression by PMN-MDSCs[6],Veglia *et al.* did not observe a role for this receptor, underscoring the plasticity of these cells in different settings. Nonetheless, these papers clearly demonstrate that the induction of immunosuppressive capacities of PMN-MDSCs can be abrogated by targeting fatty acid trafficking, opening an attractive therapeutic avenue to reprogram these harmful cells in the tumor context. Indeed, treatment of mice bearing subcutaneously transplanted tumors with Lipofermata, a selective inhibitor of FATP2-mediated fatty acid transport, slowed tumor growth in a $CD8^+$ T cell-dependent manner. Next, the authors assessed how Lipofermata may affect the therapeutic benefit of several immune-modulatory therapies. Interestingly, not only did Lipofermata improve the therapeutic benefit of T cell-activating immune checkpoint blockers, such as anti-PD-1 and anti-CTLA-4, but also increased response to the macrophage-targeting treatment anti-CSF-1R. These pre-clinical functional data, combined with the observed increase in lipid accumulation and *SLC27A2* expression in human PMN-MDSCs suggest that targeting this pathway represents a promising therapeutic strategy to improve current immunotherapies for cancer patients.

The impact of pathologically activated neutrophils in cancer is undisputed, and the vast majority of literature indicates these cells as adversaries of a successful anti-tumor immune response[1]. Studies like that performed by Veglia *et al.* add important insights into the mechanisms of neutrophil reprogramming and provide therapeutic targets to curb their tumor-promoting functions. However, in order to target these cells successfully in cancer patients, we need an improved understanding of their context-dependent phenotypes, as these highly plastic cells continuously adapt to their anatomical and molecular environments. This complexity is exemplified by the observation that neutrophils in early- and late-stage tumors can behave quite differently[8,9], and that the tumor-infiltrating neutrophil population can be composed of different subsets, based on single-cell gene expression profiles[10]. Whether this diversity in neutrophil phenotype and behavior is steered by differences in lipid metabolism remains to be uncovered. In addition, since neutrophils play an indispensable role in metastasis, future studies should evaluate how metabolic signaling pathways are reprogrammed in different metastatic niches, and how this depends on the bio-availability of lipids and other metabolites in these anatomical locations. Together, these insights will help us utilize signaling pathways such as lipid trafficking and metabolism as therapeutic targets to improve anti-tumor immune responses and immunotherapies in cancer patients.

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