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Membrane Cholesterol Regulates Macrophage Plasticity in Cancer

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The pro-tumoral diversion of macrophages remains an unresolved paradox of tumor immunology and a conceptual gap in the understanding of tumor biology. Goossens et al. (2019) identify a new level of cross-regulation by which tumors increase the membrane cholesterol efflux of macrophages to enhance their pro-tumoral activation in response to IL-4.

It is well known that in many human solid tumors, a high number of tumor-associated macrophages (TAMs) is associated with poor prognosis (Bingle et al., 2002), although in few types of cancers, including ovarian cancer, macrophage content has been reported as a favorable prognostic index. In addition to their number, the M1 versus M2 paradigm of macrophage polarization has established that it is the activation state of TAMs that is a critical determinant of tumor progression. In particular, it has been shown that relevant pathways exist by which tumors promote the M2-like, tumor-promoting or the M1-like, tumor-inhibiting phenotypes of macrophages. For example, it is believed that IL-4 signaling leads to an M2-polarizing, pro-tumoral effect, while IFNγ promotes the M1-polarizing, anti-tumoral action of macrophages (Porta et al., 2018). Accumulating evidence indicates that the phenotype of TAMs is intricately regulated by several additional actors, including microphysiological conditions (e.g., hypoxia, pH, and glucose levels) and point to myeloid cell metabolism as an essential part of the signaling network that orchestrates their phenotype. As examples, while the immunosuppressive functions of myeloid-derived suppressor cells (MDSCs) are supported by increased fatty acid uptake and reverted upon silencing of the fatty acid translocase CD36 (Al-Khami et al., 2017), mice with genetic deletion of the *Abcg1* gene, a cholesterol efflux transporter, showed strong resistance to tumor growth in models of bladder cancer and melanoma (Sag et al., 2015). Metabolic events not only influence the activation and polarization state of terminally mature cells but may also affect the fate of myeloid progenitors. Indeed, the retinoic-acid related orphan receptor (RORC1/RORγ), which requires cholesterol precursors and metabolites (i.e., oxysterols) as coactivators, was recently shown to orchestrate emergency myelopoiesis in human and mouse tumor bearers, supporting both expansion of MDSCs and differentiation of M2-polarized, pro-tumor TAMs (Strauss et al., 2015).

This evidence supports a direct connection between lipid metabolism and the pro-tumoral differentiation of myeloid cells,

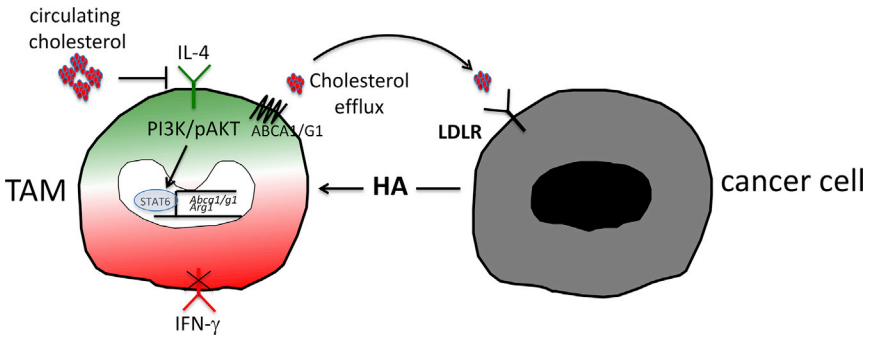

Figure 1. Alteration of Membrane Cholesterol Efflux Controls the Phenotye of TAMs
Tumor-derived hyaluronic acid enhances membrane cholesterol efflux from TAMs to amplify their IL-4-dependent and tumor-promoting M2 polarization in response to IL-4. By contrast, enhanced cholesterol efflux hinders IFNγ-mediated gene transcription, supporting a M1 to M2 skewing of TAM phenotype. Enhanced IL-4 activity results in increased activation of STAT6 and PI3K-Akt signaling, which drive expression of immunosuppressive genes (i.e., Arg1). Cancer cells acquire cholesterol from TAMs, which express high levels of the ABCA1 and ABCG1, thus diminishing their energetic cost for cholesterol biosynthesis. Finally, high levels of circulating cholesterol may impair cholesterol efflux, restoring inflammatory responses by TAMs.

which represents the focus of the study by Goossens et al. (2019). By using a mouse model of ovarian cancer, characterized by cancer cell colonization of the peritoneum, the authors show that tumor progression promotes a gradual replacement of resident peritoneal macrophages (embryonic-derived large macrophages: F4/80^{high}MHCII^{low}) with CCR2-dependent, bone-marrow-derived intermediate macrophages (F4/80^{int}MHCII^{int}*Cx3r1*$^{+}$). Such an event was paralleled by an early enhanced expression of pro-inflammatory genes (5 days after tumor cells implantation), assessed in a bulk TAM population, which declined at later stages of tumor progression (21 days after tumor cell implantation). These later stages were also marked by increased expression in TAMs of genes encoding for cholesterol efflux transporters, such as ABCA1 and ABCG1, displaying a reduction in the number of cholesterol-rich membrane micro-domains. Further, the authors observed that the extracellular matrix component hyaluronic acid, released by cancer cells, promoted depletion of membrane cholesterol content in macrophages and consequently increased IL-4R activity, including STAT6 and PI3K-mTORC2-Akt signaling, while impairing expression of IFNγ-induced genes. However, they did not clarify how HA sensing by macrophages occurs and how this is connected to the reprogramming of IL-4R- and IFN-γR-dependent signaling activity. Nor did the authors identify the intratumoral source of IL-4. Despite these limitations, the tumor-promoting role of IL-4 was demonstrated *in vivo* using a monoclonal antibody against the IL-4 receptor (αIL-4ra), which recapitulated the tumor growth inhibition observed in chimeric mice harboring hematopoietic genetic deficiency of either STAT6 or PI3K.

The results reported by Goossens et al. (2019) are in line with the observation that membrane cholesterol levels strongly affect receptor stability and signaling pathways. They also dovetail with previous reports showing that while ABCA1-deficient macrophages become hyperresponsive to M1-polarizing signals (e.g., TLR4 agonists), they acquire hyporesponsiveness to M2-polarizing signals (e.g., IL-4 and IL-13) (Pradel et al., 2009). The possibility of acquiring cholesterol from TAMs, which display high activity of the ABCA1 and ABCG1 efflux transporters, offers a clear energetic advantage to cancer cells. Indeed, this ''feeding'' of tumor cells by TAMs can diminish the energetic costs of tumor cells, as it reduces the need for their own endogenous cholesterol biosynthesis. But, importantly, the effect on macrophage reprogramming by membrane cholesterol depletion is reverted by the addition of exogenous cholesterol. In addition, alterations in cholesterol metabolism may affect metastasis formation by widely altering functions of other types of innate and adaptive immune cells (Baek et al., 2017). Moreover, HA-mediated inhibition of IFNγ-inducible genes might have relevant impact on current anticancer immunotherapies, since several immunosuppressive molecules (e.g., PD-L1, indoleamine 2,3 dyoxygenase, and NOS2) are prototypically induced by IFNγ, and clinical-grade immune checkpoint inhibitors (i.e., anti-PD-L1 and anti-CTLA4) entrust their antitumor activity on the reactivation of Th1-mediated antitumor immunity (Porta et al., 2018). However, while preclinical data might support a clinical role for serum cholesterol in cancer, this has not been defined yet. Some epidemiological cohorts suggest an increased risk and more recurrences of some types of malignancies such as prostate cancer, and some trials suggest a risk reduction by using statins in several cancers such as colon cancer, melanoma, endometrial cancer, breast cancer, and lymphomas (Nielsen et al., 2013). On the other hand, other trials exclude a direct role of cholesterol and of the lowering of cholesterol on the risk of cancer (Kuzu et al., 2016). Similar debatable findings are recently found also for a diet with an increased fat intake (Christ and Latz, 2019). All these data can be hampered on both sides of the argument by several factors, such as the retrospective nature of the observations, the non-controlled analyses for multifactorial disease, and the association to non-cancer related deaths, rendering an interpretation of the role of cholesterol homeostasis in cancer still an open clinical issue. The work by Goossens et al. (2019) has the merit to emphasize cholesterol metabolism as a crucial crossroads in the regulation of immune responses (Figure 1) and urges new efforts to clarify the interplay between lipid metabolism and antitumor immunity.

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DECLARATION OF INTERESTS

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advisory role, Novartis, Pfizer, Inivata, and Sanofi-Aventis.

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Metabolic Adaptation Sets the Fate of Regulatory Macrophages

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In this issue of *Cell Metabolism*, Du et al. (2019) describe how insulin-like growth factor 2 (IGF-2), a protein with structural similarity to insulin, induces an anti-inflammatory phenotype in maturing macrophages through reprogramming of their mitochondrial metabolism. These anti-inflammatory properties are maintained upon secondary stimulation and alleviate experimental autoimmune encephalomyelitis (EAE) *in vivo*.

Macrophages have important roles for tissue repair and regeneration as well as host defense components against invading microorganisms. They are plastic cells that can differentiate and adapt toward specific phenotypes: a more proinflammatory phenotype is needed for host defense, whereas an anti-inflammatory or regulatory phenotype is necessary for the resolution of inflammation and tissue repair. These processes are under complex regulation. Much has been learned about the capacity of exogenous (especially microbial) and endogenous ligands to activate immunological, metabolic, and epigenetic pathways resulting in a proinflammatory phenotype (Lachmandas et al., 2016). However, much less is known about whether similar processes are also important for the induction of a regulatory phenotype of macrophages. In this issue of *Cell Metabolism*, Du, Lin, and colleagues take an important step toward understanding these processes by describing that insulin-like growth factor 2 (IGF-2), a protein hormone with structural similarity to insulin, induces a strong anti-inflammatory phenotype in maturing macrophages through reprogramming of their mitochondrial metabolism (Du et al., 2019). These anti-inflammatory properties are maintained upon secondary stimulation and alleviate experimental autoimmune encephalomyelitis (EAE) *in vivo*.

The immune system is constantly challenged by exogenous and endogenous stimuli. In recent years, it has been shown that not only lymphocytes but also myeloid cells from the innate immune system are able to "remember" the stimuli they encounter and undergo functional metabolic and epigenetic reprogramming, facilitating secondary inflammatory or anti-inflammatory responses upon restimulation (Netea et al., 2016). These innate immune memory mechanisms (also termed "trained immunity") were originally described to be triggered by microbial stimuli, such as LPS from Gram-negative bacteria or β-glucan from fungi, that respectively skew macrophages toward