Polarization of Tumor-Associated Macrophages: A Novel Strategy for Vascular Normalization and Antitumor Immunity

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Vascular normalization is an emerging concept in cancer treatment. In this issue of *Cancer Cell*, Rolny et al. show that histidine-rich glycoprotein normalizes tumor vessels and promotes antitumor immunity by polarizing tumor-associated macrophages, leading to decreased tumor growth and metastasis. Placental Growth Factor deletion in macrophages phenocopies many of these effects.

The abnormal vasculature of tumors impedes the delivery of chemo- and immunotherapeutic agents and lowers the barrier for the escape of cancer cells from tumors. Moreover, the resulting abnormal microenvironment reduces the efficacy of radiation, chemo-, and immunotherapies, selects for more malignant clones, and fuels disease progression. Thus, restoration of the normal structure and function in blood vessels—coined as vascular normalization—is emerging as a new concept in cancer treatment (Jain, 2001). The normalization strategies developed to-date have targeted the abnormalities in the vascular endothelial cells and/or pericytes (e.g., Hamzah et al., 2008; Jain, 2005; Mazzone et al., 2009; Winkler et al., 2004). In this issue of *Cancer Cell*, Rolny et al. show that targeting abnormal polarization of tumor-associated macrophages (TAMs) can normalize tumor vessels and also enhance antitumor immunity (Rolny et al., 2011)(Figure 1).

TAMs represent a dominant myeloid population in many solid tumors, and their accumulation correlates with poor prognosis (Mantovani and Sica, 2010). TAMs usually exhibit M2-like phenotype, secreting immunosuppressive cytokines such as IL-10, CCL17, and CCL22 and producing proangiogenic and tissue remodeling factors such as VEGF, placental growth factor (PlGF), and MMP9 (Fischer et al., 2007; Mantovani and Sica, 2010). Phenotypic subgroups of TAMs often coexist in the same tumor microenvironment and can be modified by microenvironmental triggers such as hypoxia. However, a specific therapeutic agent that could polarize the protumor M2-like TAMs to tumor-inhibitory M1-like TAMs remains elusive. Rolny et al. offer compelling evidence that histidine-rich glycoprotein (HRG) is such an agent and that it induces polarization partly by downregulating macrophage-derived PlGF (Figure 1).

HRG is a multidomain plasma protein synthesized by hepatocytes and has important function in regulation of tumor angiogenesis and immunity (Blank and Shoenfeld, 2008). Tumor cells usually express low levels of HRG. When Rolny et al. overexpressed HRG in cancer cells, tumor vessels became normalized, resulting in decreased hypoxia and improved delivery of chemotherapeutic agents and decreased metastasis (Rolny et al., 2011). By measuring the production of cytokines in isolated macrophages and characterizing their surface markers, Rolny et al. also found that HRG has a direct effect on TAMs to polarize them away from protumoral M2-like phenotype (Figure 1) (Rolny et al., 2011). Hypoxia, a hallmark of tumors, is considered to be a major driving force to polarize macrophage to M2-like phenotype (Mantovani and Sica, 2010). The increase of oxygenation in HRG^+ tumors caused by vascular normalization seems to provide a stimulus for polarizing TAMs away from M2-like type, which could further sustain the normalized vasculature (Rolny et al., 2011). This positive feedback in the normalized tumor microenvironment may confer an additional advantage: extended window of normalization compared to blockade of VEGF signaling alone (Jain, 2005; Winkler et al., 2004).

Rolny et al. discovered that the sustained vascular normalization by HRG was also associated with substantially enhanced antitumor immunity, and, thus, could potentially explain the decreased growth of both primary and metastatic lesions. These effects were presumably mediated by redirected TAMs to relieve the immunosuppressive tumor microenvironment. Exposure of TAMs to HRG downregulated the M2 markers such as MRC1, Arg1, IL10, and CCL-22 and simultaneously elevated M1 markers such as IL6 and CXCL-9. Accordingly, tumor-infiltrated $CD8^+$ T cells, natural killer (NK) cells, and dendritic cells (DCs) increased and their functions improved in HRG^+ tumors (Rolny et al., 2011). Reduced levels of CCL22 could decrease the recruitment of T regulatory cells (Tregs) and, thus, improve DC and T cell function. Increased CXCL9 level could promote $CD8^+$ T cell and NK cell infiltration into tumor parenchyma. These observations indicate that re-education of TAMs links vascular normalization with restoration of tumor immune surveillance (Figure 1).

How does HRG skew TAMs away from M2-like phenotype? Tumors are heterogeneous, and numerous players shape TAM's phenotypes and function. Rolny et al. dissect the role of one potential player, Fcγ receptors (FcγRs), expressed in macrophages that HRG is known to bind to. Based on preliminary data, Rolny et al. propose that FcγR may mediate the polarization of TAMs by HRG. Given that

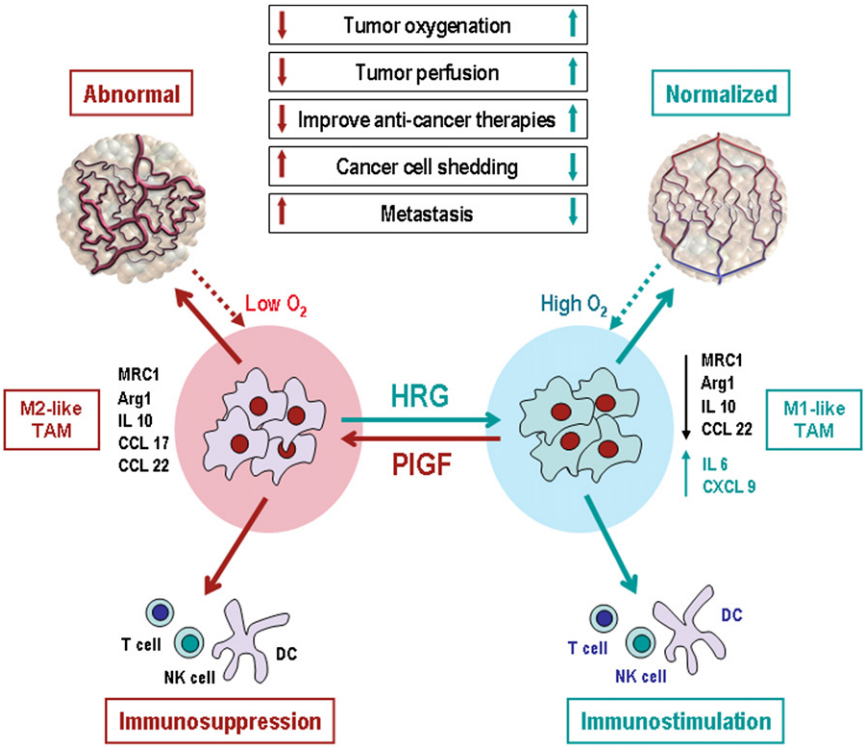

Figure 1. Targeting TAMs to Normalize Tumor Vessels and Promote Antitumor Immunity
TAMs with M2-like phenotype lead to abnormal tumor vasculature by producing angiogenic factors such as PlGF and M2-cytokines such as IL10 and CCL22. In addition, M2-cytokines suppress immune cell functions. Elevated levels of HRG polarize TAMs away from an M2-like phenotype to normalize tumor vessels and activate antitumor immunity. By fortifying tumor vessels, vascular normalization may decrease shedding of metastatic cells into circulation, resulting in decreased metastasis. Normalized vessels may also facilitate delivery of drugs and immune cells. Reduction in hypoxia, known to increase resistance to radiation and a number of therapeutics, also sensitizes tumors to various therapies, decreases selection pressure for more malignant clones, and promotes M1-like TAM phenotype. All these effects of HRG treatment may result in decreased tumor growth and metastasis and increased efficacy of various therapies. PlGF deletion in macrophages can phenocopy many effects of HRG treatment. (Schematics of abnormal and normalized tumor vasculature reproduced from Jain, 2001).

inactivation of FcγRs skews macrophages to M1-like phenotype (Andreu et al., 2010), these data suggest that HRG may serve as an antagonist of FcγR. It would be of interest to dissect how different ligands could also induce distinct context-dependent effects through different FcγR subtypes. Further work will answer these questions and improve our understanding of TAM polarization.

Rolny et al. also dissected the downstream mediators of the HRG activity in TAMs. By analyzing the cytokines and angiogenic factors produced by these cells, they found that HRG reduced PlGF production by TAMs. Indeed, the deletion of PlGF in macrophages phenocopied antitumor and vascular normalization effects of HRG treatment (Rolny et al., 2011). In a previous study by this team, blocking the TAM-chemo-attractant PlGF, produced by both tumor and stromal cells, led to a reduction in macrophages in tumors (Fischer et al., 2007). Since tumor-derived PlGF was not reduced by HRG and could rescue TAM infiltration in this study, the current findings also raise an interesting question—does the effect of HRG depend on the number of TAMs present in the tumor microenvironment? The tumor models used in this study have a relatively high number of TAMs. Since other tumors might have a lower number of TAMs, whether lower density of TAMs still offers a therapeutic opportunity for HRG to normalize vasculature remains to be explored. Careful dissection of players in different tumors and context will be crucial for optimal use of HRG.

In conclusion, this study provides not only unprecedented insight into the central role of M2-like TAMs in promoting vascular abnormalities, tumor metastasis, and tumor immunosuppression, it also offers further support for vessel normalization as a new strategy for cancer treatment. By normalizing tumor vasculature and enhancing antitumor immunity to suppress tumor growth and metastasis, HRG opens new avenues for more effective cancer treatment.

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