Altering the intratumoral localization of macrophages to inhibit cancer progression

Andrea Casazza and Massimiliano Mazzone*

Laboratory of Molecular Oncology and Angiogenesis; Vesalius Research Center; VIB; Leuven, Belgium;
Laboratory of Molecular Oncology and Angiogenesis; Vesalius Research Center; Department of Oncology; K.U. Leuven; Leuven, Belgium

Hypoxia confers to macrophages angiogenic and immunosuppressive properties which promote tumor growth and progression. Preventing the migration of macrophages into hypoxic tumor regions hinders angiogenesis and restores the tumor-suppressive properties of these immune cells. We have recently uncovered a neuropilin 1- and semaphorin 3A-dependent signaling pathway that defines the repositioning of macrophages to hypoxic tumor niches, a discovery that generates new options for the development of complementary anticancer treatments.

The primary function of tumor-associated macrophages (TAMs) is presumably to work as a selective barrier against malignant progression. Nevertheless, the hypoxic tumor microenvironment modifies the expression of genes involved in metabolism, angiogenesis, and immunity, de facto altering the capacity of macrophages and other immune cells to control tumor growth.[1-3]

Hypoxic cancer cells release high amounts of vascular endothelial growth factor (VEGF) and semaphorin 3A (SEMA3A), both of which induce the activating phosphorylation of VEGF receptor 1 (VEGFR1). In particular, VEGF directly binds to VEGFR1 in a neuropilin 1 (NRP1)- independent manner, whereas SEMA3A interacts with NRP1 to prompt the clustering of Plexin A1, Plexin A4 and VEGFR1. VEGFR1 signaling attracts macrophages to hypoxic tumor niches but the most interesting phenomenon in this setting is the effect of SEMA3A on macrophages once they reach the hypoxic core. Hypoxia leads indeed to the stabilization of hypoxia-inducible factor 2 (HIF2) in macrophages, resulting in the activation of canonical NF-κB signaling and consequent NRP1 repression. In the absence of NRP1, SEMA3A elicits retention signals through Plexin A1 and Plexin A4, which impede the egression of macrophages from the hypoxic niche independently of VEGFR1.[3] Thus, our findings demonstrate that the downregulation of NRP1 converts SEMA3A from a guidance cue into a stop/retention signal. The NRP1/SEMA3A-dependent navigation system for macrophages is reminiscent of the mechanisms that guide the migration of endothelial tip cells or the outgrowth of neurites. This said, we were surprised to find that in TAMs SEMA3A can bind to and signal via Plexin A1/A4 in absence of NRP1, at odds with what reported in most cell types. Membrane-bound glycosaminoglycans are good candidates to present SEMA3A to plexins in the absence of NRP1, as it occurs in neurons,[4] but a direct (low-affinity) binding of SEMA3A to Plexin A1 and/or Plexin A4 is possible as well.[5]

The relocalization of TAMs to normoxic tumor regions and their exclusion from hypoxic niches (upon the deletion of *Nrp1* or the mutation of the NRP1 SEMA3A-binding site) results in the accumulation of macrophages with reduced angiogenic potential, increased tumoricidal activity, and limited immunosuppressive activity. In turn, this promotes the activation of T_H1 and cytotoxic T lymphocyte (CTL)-mediated antitumor immune responses, further perpetuating the acquisition of an M1 phenotype by TAMs (**Fig. 1**). Interestingly, SEMA3A, which is predominantly expressed in hypoxic tumor areas, inhibits the activation of T cells by blocking the production of interleukin-2 (IL-2).[6] This may explain why T_H1 and CTLs accumulate to normoxic tumor regions to exert optimal antineoplastic effects. In view of previous findings that we obtained with a different genetic model,[1] we can conclude that hypoxia finely tunes the angiogenic and immunoregulatory properties of TAMs even though it is not sufficient to determine their functional polarization.[1,3] The presence or absence of T cells, in particular of CD8+ CTLs, results in delivery to macrophages of the cytokine signals required to express M1 or M2 markers, respectively.[3]

These and previous results underline how different "topographic" distributions of the same signaling molecule can translate in distinct biological outcomes. Several studies have indeed shown that the administration of SEMA3A to tumor-bearing mice normalizes the intratumoral vasculature, thus improving the delivery of chemotherapeutic drugs, limiting disease burden and inhibiting metastatic dissemination.[7,8] Thus, the local
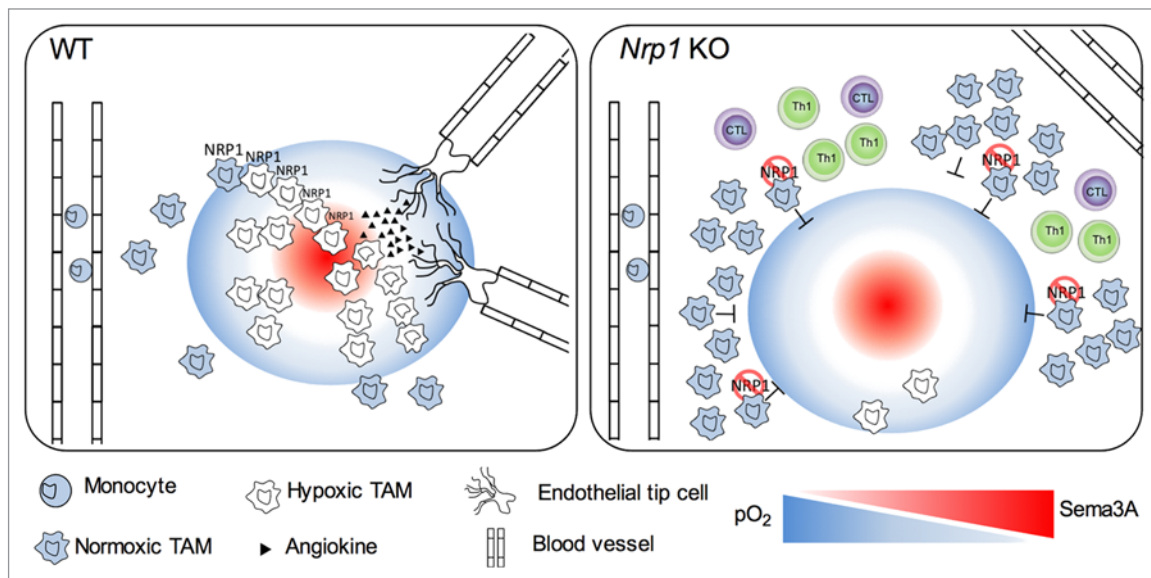
Figure 1. Entry of tumor-associated macrophages into hypoxic regions of solid tumors. Once monocytes extravasate and differentiate into macrophages, tumor-associated macrophages (TAMs) migrate from perivascular (normoxic) to avascular (hypoxic) areas of neoplastic lesions. Hypoxia-induced vascular endothelial growth factor (VEGF) or semaphorin 3A (SEMA3A) attracts TAMs via neuropilin 1 (NRP1)-independent or -dependent activation of VEGF receptor 1 (VEGFR1), respectively. The transcriptional repression of *NRP1* by low oxygen tensions converts SEMA3A in a stop signal that favors the retention of TAMs within hypoxic niches through the activation of Plexin A1 and Plexin A4. In hypoxic conditions, TAMs not only release angiokines that attract endothelial tip cells, thus favoring angiogenesis, but also promote the establishment of an immunosuppressive microenvironment. The loss of NRP1 by TAMs impedes their entry into hypoxic tumor regions because of the migration-inhibitory cues conveyed by SEMA3A, which in this setting potently antagonize the chemoattractive effects of VEGF. In normoxic conditions, NRP1-deficient TAMs exert limited angiogenic functions and promote antitumor immune responses that result from the recruitment of cytotoxic T lymphocytes (CTLs) and T_H1 cells and cytotoxic macrophages.

(hypoxia-dependent) induction of endogenous SEMA3A and the systemic administration of exogenous SEMA3A mediate reverse effects: pro-tumor in the former case and therapeutic in the latter.

Since NRP1-deficient TAMs do not enter hypoxic tumor niches, the vascular network in this setting remains poorly branched and intratumoral oxygen tension is low. Tumors are smaller but poorly metastatic despite hypoxia. This raises the important question on whether the dissemination of individual cancer cells might be fostered in this scenario but the inefficient angiogenesis and the restoration of antitumor immune responses would ultimately prevent the development/expansion of metastatic lesions, thus prolonging the survival of tumor-bearing hosts. This biological aspect might be relevant for the debate on the pros and cons of antiangiogenic agents in cancer therapy.[9]

Previous studies have tested the effects of chemical interventions or antibodies that deplete TAMs on tumor growth and metastasis.[10] The rationale for these strategies is that TAMs are generally viewed as a tumor-supporting cell population.

However, in settings in which TAMs appear to exert antitumor, rather than pro-tumor, effects, such an approach might even be harmful for patients. Conversely, strategies that convert M2 macrophages into their M1 counterparts might be relatively safe for the patients since they exploit the intrinsic nature of macrophages to eliminate harmful stimuli. This said, tumors might be able to circumvent these interventions and repolarize TAMs to serve their own needs. The blockade of SEMA3A or NRP1 shifts the phenotype of TAMs by impeding them to leave the perivascular sites via a molecular pathway that is otherwise naturally activated when TAMs encounter hypoxic conditions. Thus, this therapeutic intervention not only prevents angiogenesis and the establishment of an immunosuppressive microenvironment, but also restores the primitive functions of pro-inflammatory M1 macrophages.

The extent of tumor-infiltration by TAMs failed to convey prognostic information in patients affected by several types of cancer.[10] Perhaps, this reflects the fact that elevated amounts of TAMs in perivascular (normoxic) tumor regions is

beneficial, rather than detrimental, for the patient. It will be interesting to determine if the intratumoral distribution of TAMs can be used as a predictive marker of clinical responses to surgery or chemotherapy. It is tempting to speculate, yet remains to be formally demonstrated, that therapeutic interventions targeting NRP1 would be indicated for patients exhibiting the accumulation of TAMs at hypoxic tumor regions, but not for the treatment of tumors in which TAMs are accumulated only in the perivascular space.

In addition, there are pathological conditions other than cancer in which the entry of macrophages into hypoxic niches negatively influences disease outcome. For example, this is the case of choroidal neovascularization during age-related maculopathies or of neovascularization of atherosclerotic plaques. Future work will tell us if the blockade of NRP1 can be of any therapeutic utility in these contexts as well.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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