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

Keywords: angiogenesis, hypoxia, immunity, neuropilin, semaphorin, tumor-associated macrophages

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-kB 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 VEGFR1.4 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, a hypothesis that is supported by our recent findings.1,3 Nevertheless, it remains unclear 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...

*Correspondence to: Massimiliano Mazzone; Email: massimiliano.mazzone@vib-kuleuven.be
Submitted: 01/14/2014; Accepted: 01/15/2014; Published Online: 02/14/2014
Citation: Casazza A, Mazzone M. Altering the intratumoral localization of macrophages to inhibit cancer progression. OncolImmunology 2014; 3:e27872; http://dx.doi.org/10.4161/onci.27872
(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.

Previous studies have tested the effects of chemical interventions or antibodies that deplete TAMs on tumor growth and metastasis. 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. 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. 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.
References

1. Laoui D, Van Overmeire E, Di Conza G, Aldeni C, Keirse J, Morias Y, Movahedi K, Houbrechen I, Schoupe E, Elkim Y, et al. Tumor Hypoxia Does Not Drive Differentiation of Tumor-Associated Macrophages but Rather Fine-Tunes the M2-like Macrophage Population. Cancer Res 2014; 74:24-30; PMID:24220244; http://dx.doi.org/10.1158/0008-5472.CAN-13-1196.

2. Fang HY, Hughes R, Murdoch C, Coffelt SB, Biswas SK, Harris AL, Johnson RS, Imityaz HZ, Simon MC, Fredlund E, et al. Hypoxia-inducible factors 1 and 2 are important transcriptional effectors in primary macrophages experiencing hypoxia. Blood 2009; 114:844-59; PMID:19454749; http://dx.doi.org/10.1182/blood-2008-12-195941.

3. Casazza A, Laoui D, Wenes M, Rizzolio S, Bassani N, Mambretti M, Deschoemaeker S, Van Ginderachter JA, Tamagnone L, Mazzone M. Impeding macrophage entry into hypoxic tumor areas by Sema3A/Nrp1 signaling blockade inhibits angiogenesis and restores antitumor immunity. Cancer Cell 2013; 24:695-709; PMID:24352099; http://dx.doi.org/10.1016/j.ccr.2013.11.007.

4. De Wit J, De Winter F, Klooster J, Verhaagen J. Semaphorin 3A displays a punctate distribution on the surface of neuronal cells and interacts with proteoglycans in the extracellular matrix. Mol Cell Neurosci 2005; 29:40-55; PMID:15866045; http://dx.doi.org/10.1016/j.mcn.2004.12.009.

5. Nogi T, Yasui N, Mihara E, Matsunaga Y, Noda M, Yamashita N, Toyofuku T, Uchiyama S, Goshima Y, Kumanogoh A, et al. Structural basis for semaphorin signalling through the plexin receptor. Nature 2010; 467:1123-7; PMID:20881961; http://dx.doi.org/10.1038/nature09473.

6. Catalano A, Capparri P, Moretti S, Faronato M, Tamagnone L, Procopio A. Semaphorin-3A is expressed by tumor cells and alters T-cell signal transduction and function. Blood 2006; 107:3321-9; PMID:16380453; http://dx.doi.org/10.1182/blood-2005-06-2445.

7. Maione F, Capano S, Regano D, Zentilin L, Giacca M, Casanovas O, Bussolino F, Serini G, Giraud E. Semaphorin 3A overcomes cancer hypoxia and metastatic dissemination induced by antiangiogenic treatment in mice. J Clin Invest 2012; 122:1832-48; PMID:22484816; http://dx.doi.org/10.1172/JCI58976.

8. Casazza A, Fu X, Johansson I, Capparuccia L, Andersson F, Giustacchini A, Squadrito ML, Venneri MA, Mazzone M, Larsson E, et al. Systemic and targeted delivery of semaphorin 3A inhibits tumor angiogenesis and progression in mouse tumor models. Arterioscler Thromb Vasc Biol 2011; 31:741-9; PMID:21205984; http://dx.doi.org/10.1161/ATVBAHA.110.211920.

9. De Bock K, Mazzone M, Carmeliet P. Antiangiogenic therapy, hypoxia, and metastasis: risky liaisons, or not? Nat Rev Clin Oncol 2011; 8:393-404; PMID:21629216; http://dx.doi.org/10.1038/nrclinonc.2011.83.

10. De Palma M, Lewis CE. Macrophage regulation of tumor responses to anticancer therapies. Cancer Cell 2013; 23:277-86; PMID:23518347; http://dx.doi.org/10.1016/j.ccr.2013.02.013.