Semaphorin 4D (Sema4D) plays a role in various cell types including B lymphocytes, differentiating neurons, endothelial cells, and cancer cells. Preclinical and in vitro studies have shown that Sema4D-directed antibodies in combination with immune checkpoint inhibitors reshape the tumor microenvironment by promoting recruitment of effector lymphocytes and antigen-presenting cells, while reducing immunosuppressive cell types, which ultimately leads to tumor rejection. Hence, early-stage clinical trials with combination therapies including anti-Sema4D antibodies are ongoing. In this issue of Cancer Research, Zuazo-Gaztelu and colleagues report an unexpected proinvasive effect induced by anti-Sema4D antibodies in a preclinical model of neuroendocrine pancreatic cancer (Rip1-Tag2), mediated by retrograde signaling of transmembrane Sema4D in macrophages, which increases their recruitment to tumors, SDF-1 secretion, and metastasis-promoting phenotype.

See related article by Zuazo-Gaztelu et al., p. 5328

Targeting Semaphorin 4D in Cancer: A Look from Different Perspectives

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While originally identified as signaling cues for axonal navigation, semaphorins are actually involved in the regulation of a wide spectrum of developmental processes and human diseases; moreover, accumulating evidence indicates that a subset of semaphorins play a relevant role in physiologic and pathologic immune responses (1). In particular, semaphorin 4D (Sema4D) was originally described in immune cells as CD100 antigen and was the first semaphorin family member found to have immunoregulatory activity. Sema4D is synthesized as a transmembrane molecule, but its soluble extracellular portion can be shed upon metalloprotease-mediated cleavage. Notably, Sema4D has three known receptors, the high affinity receptor PlexinB1, an intermediate affinity receptor PlexinB2 [both expressed at various levels by tumor and stromal cells of the tumor microenvironment (TME)], and a low affinity receptor CD72, which is mainly expressed by immune cells. Like other transmembrane semaphorins, Sema4D is expected to function as both a receptor and a ligand, and initiate poorly known retrograde (“reverse”) signaling cascades via its own intracellular domain (2).

Previous studies have consistently suggested that Sema4D expression in the TME promotes tumor progression, even if its distribution and that of its receptors have not been fully elucidated. In fact, both tumor cells and cells in the microenvironment express these molecules, potentially establishing a complex signaling network. According to Sierra and colleagues (3) and the study by Zuazo-Gaztelu and colleagues (4), Sema4D is mainly found on tumor-associated macrophages (TAM), which are known to play a major role in suppressing antitumor immune responses and promoting tumor angiogenesis, cancer cell invasiveness, and metastatic dissemination. This is consistent with the fact that Sema4D receptors are correspondingly expressed by endothelial cells, lymphocytes, and cancer cells. Sierra and colleagues showed that tumor growth and metastatic progression were strongly impaired in Sema4D knockout mice (3); in this study, TAM-derived Sema4D was shown to be particularly critical for tumor angiogenesis.

More recently, Younis and colleagues showed in vitro that soluble Sema4D released by cancer cells polarizes myeloid cells toward an immune suppressor phenotype (MDSC, CD33+ CD11b− HLA-DR2/low); in turn, these inhibit T-cell proliferation and IFNγ production, likely through production of immunosuppressive factors (5). Downregulating Sema4D in cancer cells inhibited MDSC regulation of T lymphocytes, promoting the differentiation of effector T cells and decreasing the number of suppressive regulatory T cells (Treg).

Utilizing an anti-Sema4D mAb (MAb 67-2), Evans and colleagues independently demonstrated the efficacy of targeting Sema4D to induce tumor immune rejection in different murine syngeneic colon and mammary carcinoma models (6). In this study, Sema4D expression was particularly high at the border of the tumor, concurrent with the confinement of anticancer immune cells in the same zone. Antibody-mediated targeting of Sema4D disrupted this localization and enhanced tumor infiltration of CD11c+ monocytic/ APC cells and CD68+ T-effector lymphocytes responsible for tumor rejection and disease control. Moreover, in association with anti-Sema4D antibodies, the therapeutic efficacy of immune checkpoint inhibitors (ICI) was enhanced and elicited a durable antitumor response. In this work, the previously reported anti-angiogenic effects of Sema4D interference were not investigated. In a separate study, Fisher and colleagues confirmed that MAb 67-2 interferes with Sema4D-PlexinB1 binding (7), however, the identity of plexin-expressing cells in the TME and the targeted Sema4D-dependent molecular mechanism preventing tumor rejection was not clarified.
Similarly, Clavijo and colleagues recently showed that treatment with anti-Sema4D MAb 67-2, in combination with ICI, enhanced tumor rejection in murine syngeneic models of oral carcinoma (MOC1) and Lewis lung cancer (8). Tumor growth control was not due to significant changes in tumor vascularity, or the inhibition of cancer cell proliferation or survival. However, following Sema4D targeting, a significant reduction in the number of infiltrating Ly6G<sup>hi</sup> Ly6C<sup>int</sup> myeloid cells with immunosuppressive activity (MDSC) was observed, which is consistent with the above-mentioned findings from Younis and colleagues. The reduction of Ly6G<sup>hi</sup> Ly6C<sup>int</sup> myeloid cells was due to inhibition of Sema4D-dependent production of CXCL-1/2/5 chemotactic chemokines, released by cancer cells or other undefined cells in the TME; the signaling mechanism targeted by the antibody, however, remains unclear. Notably, MDSC themselves express high levels of PlexinB1, and soluble recombinant Sema4D directly upregulates their immunosuppressive activity, which can be blocked by the antibody, leading to higher counts of CD8<sup>+</sup> tumor-infiltrating lymphocytes and enhanced anticancer immune response in vivo (8).

Zuazo-Gaztelu and colleagues (4) investigated the impact of targeting Sema4D using MAb67-2 in a well-known transgenic mouse model of spontaneous pancreatic neuroendocrine tumors (PanNET), RIP1-Tag2. Despite inhibition of tumor growth and significant increase in mouse survival, surprisingly increased invasion and metastasis to the liver was observed (4). The RIP1-Tag2 model has been widely used to investigate the impact of aberrant angiogenesis on tumor progression, and previous studies based on VEGF signaling blockade have demonstrated tumor shrinkage, accompanied by hypoxia-driven cancer cell invasion and metastasis. However, targeting Sema4D with MAb67-2 did not exert a classical antiangiogenic effect. In fact, no major changes in tumor vasculature could be detected in response to anti-Sema4D MBAs, apart from an increase in vascular leakage (indicated by microhemorraging). It is not clear whether this anomaly can account for the reduced tumor burden, or whether additional activities mediated by the antibodies may have impacted cancer cell growth. Importantly, and unlike what was observed with VEGF2 blockade, anti-Sema4D-treated tumors were not hypoxic. Therefore, the increased invasive and metastatic behavior of the cancer cells could not be the result of hypoxia-induced gene expression reprogramming.

Interestingly, a subset of Sema4D-positive TAMs was selectively enriched inside tumors treated with anti-Sema4D, as though the antibodies were capable of attracting or stabilizing the presence of these macrophages in the TME. Indeed, the authors found in vitro that anti-Sema4D antibodies induced the directional migration of two independent Sema4D<sup>+</sup> macrophage cell lines (RAW264.7 and THP-1), which was not observed with soluble Sema4D. In a previous study, Delaire and colleagues showed that soluble recombinant Sema4D could inhibit spontaneous and chemokine-induced migration of human monocytes (9), which was putatively attributed to interaction with an unidentified surface receptor. Notably, in Rip1-Tag2 tumors, only the transmembrane full-length form of Sema4D and not its soluble extracellular fragment could be detected. Moreover, gene knockout experiments demonstrated that the ability of the antibodies to induce macrophage migration was dependent on the target Sema4D, but independent of its receptors PlexinB2 and CD72 expressed by these cells. This led to the working hypothesis that antibody treatment induces bona fide Sema4D retrograde signaling in macrophages. It has been previously reported that Sema4D can mediate a reverse signaling pathway via the RAC1 activator protein TIAM1, leading to enhanced proliferation, migration, and invasion of oral squamous carcinoma cells (10). Further investigation is required to elucidate the cascade downstream of the Sema4D intracellular domain in macrophages; however, these findings underscore a major issue, which is not novel for antibodies directed against transmembrane signaling molecules, that they potentially trigger intracellular pathways besides interfering with ligand–receptor binding.

Mechanistically, the engagement of surface-exposed Sema4D by MAb67-2 induced a change in macrophage phenotype and secretome, which possibly promoted cancer progression through increased release of stromal cell–derived factor 1 (SDF1/CXCL12), known to elicit invasion and metastasis via its receptor CXCR4 expressed on tumor cells. It should be noted, however, that TAMs are subject to diverse regulatory mechanisms in vivo, and it is unknown whether this novel Sema4D reverse signaling cascade in TAMs can produce prominent effects across diverse TME.

Notably, targeting Sema4D with MAb67-2 resulted in only a marginal increase in the number of infiltrating T lymphocytes in PanNETs (4). This finding contrasts the observations of Evans and colleagues after treating murine colorectal Colon26 and ERBB2<sup>+</sup> mammary carcinoma models with the same antibody (6). However, this regulatory mechanism was postulated to be indirect (i.e., mediated by Sema4D-dependent regulation of immune suppressor TAMs or MDSCs); thus it likely depends on the specific makeup of the TME and can be antagonized by peculiar immune regulatory signals. Notably, ICI are known to have limited success in patients with pancreatic cancer due to the immunosuppressive environment of these tumors. In general, a significant fraction of all tumors are resistant to ICI, due to overarching immune suppressor mechanisms, which may not necessarily be influenced by Sema4D regulation. Potential reasons for this conflicting data include use of different mouse strains for tumor models and the regulatory impact of gut microbiota, which is relevant both for mouse and human immune systems. These aspects call for the identification of markers that can help to predict cancer responsiveness to anti-Sema4D treatment in patients.

A humanized version of anti-Sema4D that has the antigen recognition domain of MAb67-2 has been developed and is named VX15/2503 (7) or pepinemab. This putative new drug is currently in early phase clinical trials for the treatment of human cancers and Huntington disease. Interim results of a phase Ib/II study in patients with advanced stage non–small cell lung cancer were recently reported at the 2019 American Society of Clinical Oncology annual meeting. Of the 5 patients who had been treated previously with ICI for 6–18 months before progression, two showed partial responses and two had stable disease after receiving combination therapy of pepinemab and the anti-PD-L1 inhibitor avelumab. Furthermore, in a small group of patients who progressed shortly after immunotherapy, about half had stable disease after receiving the combination of pepinemab and avelumab, while the other half continued progressing. HIC tumor analysis following combination therapy demonstrated increased CD8<sup>+</sup> T-cell influx into tumors and increased T effector/regulatory (Teff/Treg) ratio, suggesting a favorable immunopheno...
the TME. The combination of pepinemab and avelumab was found to be well-tolerated and no concerning safety signals have been reported to date.

The findings by Zuazo-Gaztelu and colleagues confirm that anti-Sema4D treatment may be beneficial in terms of inhibiting the tumor and increasing patient survival, however, they raise concerns that the Sema4D-targeting antibodies may potentially trigger a reverse signaling mechanism that promotes tumor invasion and metastasis. The relevance of these findings for clinical translation may be limited to PanNET or similar tumors. Nevertheless, the existence of a signaling pathway triggered by anti-Sema4D antibodies in macrophages should be further investigated in additional settings and tumor models. Moreover, future studies should focus on identifying factors that predict cancer response to anti-Sema4D treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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