Exploiting pleiotropic activities of semaphorins as multi-target therapies for cancer

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Semaphorins (SEMAs) are a superfamily of secreted or membrane-associated glycoproteins implicated in the control of axonal wiring and involved in angiogenesis and cancer progression. Class-3 SEMAs are the only secreted vertebrate SEMAs and several of them are regulated by protease-mediated cleavage (Capparuccia & Tamagnone, 2009). Their high-affinity receptors, Plexins and co-receptor Neuropilins, are expressed in a wide variety of cell types including endothelial and tumour cells. Plexins show an intrinsic R-Ras GAP activity, but interestingly also form complexes with additional transmembrane molecules, including certain receptor tyrosine kinases (RTKs) such as c-Met, ErbB2 and vascular endothelial growth factor receptor 2 (VEGFR2), that are transactivated by Plexins and initiate critical signalling pathways. These functional interactions with transactivated kinase receptors are key to define the cellular activities of SEMAs and convert the SEMAs into pleiotropic molecules. Thus, SEMAs can positively or negatively modulate many intrinsic properties of tumour cells, such as proliferation, cell survival, alteration in cell adhesion and tumour invasiveness, but also modulate several stromal components including endothelial cell migration and survival (Capparuccia & Tamagnone, 2009; Serini et al, 2009).

Sema3E is one of the SEMAs implicated in tumour invasion and metastatization and its expression correlates with the metastatic process. Sema3E is synthesized as a full-length precursor molecule and its proteolytic maturation by Furin proprotein-convertase produces the active fragment p61-Sema3E, which is required and sufficient for the function of Sema3E in tumour invasiveness and metastasis (Casazza et al, 2010). Furthermore, Sema3E exerts pleiotropic activities through its specific receptor, Plexin-D1 (PlxnD1), including a collapsing pro-apoptotic response in endothelial cells and a pro-invasive and pro-metastatic effect on tumour cells. This leads to a paradoxical dual effect where the overexpression of Sema3E in one hand reduces the tumour burden by counteracting tumour angiogenesis, but on the other increases the metastatic spread of the tumour. The dual activities of p61-Sema3E depend on the specific transactivated molecules recruited by the complex Sema3E–PlxnD1 in the different types of cells. In endothelial cells, the intrinsic R-Ras GAP activity of PlxnD1 promotes a cell-collapsing response as well as an Arf6 GTPase-mediated integrin-beta1 endocytosis and decreased cellular adhesion to the extracellular matrix (Casazza et al, 2010; Sakurai et al, 2010). In tumour cells, on the contrary, ErbB2 plays a master role in the pro-invasive, pro-metastatic properties of p61-Sema3E with PlxnD1 forming a complex with ErbB2 that results in its transactivation and further activation of EGFR-ErbB2-mediated signalling pathways (Casazza et al, 2010; Fig 1A). Thus, the well-documented negative effect on endothelial cells exerted by Sema3E makes it a good candidate to block angiogenesis in tumours. However, the heightened tumour aggressiveness induced by this pleiotropic molecule precludes its possible exploitation as a therapeutic molecule.

In this issue of EMBO Molecular Medicine, Casazza et al deeply explore the pleiotropic dual activities associated to Sema3E. They identify a point-mutated uncleavable Sema3E isoform (Uncl-Sema3E) that selectively competes with p61-Sema3E for the binding to PlxnD1 (Casazza et al, 2012). This molecule retains the same anti-angiogenic activity, but also exerts an unexpected anti-invasive and anti-metastatic effect on the tumour (Fig 1B). Similar to the endogenous p61-Sema3E isoform, Uncl-Sema3E binds to PlxnD1 in endothelial cells and induces the expected Sema-driven anti-angiogenic collapsing response. On the contrary, in tumour cells, the Uncl-Sema3E–PlxnD1 complex fails to elicit the ErbB2-mediated pro-invasive and pro-metastatic pathway. Moleculary, Casazza et al demonstrate that Uncl-Sema3E not only interferes with the endogenous Sema3E signalling, but is also unable to induce the association of PlxnD1 with ErbB2 in the tumour cell context.
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The study from Casazza et al points out a remarkable pleiotropic anti-cancer potential of Uncl-Sema3E but also reveals several relevant therapeutic opportunities dually targeting angiogenesis and invasion/metastasis. On the one hand, based on the observation that the uncleavable molecule demonstrates a dominant-negative function over the endogenous Sema3E signalling, one specific therapeutic approach could be the use of a recombinant Uncl-Sema3E as a drug that would maintain the known anti-angiogenic activity of Sema3E without activating the pro-invasive and pro-metastatic process. Interestingly, the receptor PlxnD1 is generally low expressed in normal adult tissue but it is elevated in tumour vessels (endothelial cells) and in tumour cells (Roodink et al., 2009). Thus, with this tumour-restricted expression pattern one would expect to find lower toxicities of this recombinant drug, but this has to be experimentally determined.

Another specific therapeutic opportunity arising from the study is the development of new drugs blocking the convertase Furin that is responsible for the proteolytic cleavage of the full-length Sema3E into p61-Sema3E. By inhibiting Furin the endogenous full-length Sema3E would not be effectively cleaved, and an uncleaved form would accumulate in Sema3E-expressing tumours. Uncleaved Sema3E accumulation by Furin inhibition would lead to the competitive blockage of p61-Sema3E pro-invasive and pro-metastatic activity without impairing the anti-angiogenic effects of cleaved Sema3E. To further support this possibility, Furin is frequently expressed in human cancers and its expression correlates with aggressiveness and metastasis, thus making it a potential target for anti-cancer therapies (Christensen et al., 2005).

A crucial point in the putative applicability of Sema3E-related therapies is the critical functional link between Sema3E–PlxnD1 and its transactivated associated molecules. The repertoire of transactivated receptor tyrosin-kinases expressed in the cells (among them c-Met, ErbB2 and VEGFR2) could indeed provoke variability in the cell responses to Sema3E-related therapies, limiting their effects to a specific cancer or more importantly resulting in opposite outcome in the different tumour types. Therefore, it would be critical to profile the specific repertoire of transactivated molecules and how they interact with the Sema3E–PlxnD1 complex in order to anticipate the consequences of recombinant Uncl-Sema3E treatment or Furin cleavage inhibition.

In the context of other anti-tumour therapies, the article from Casazza et al further emphasizes the need of a careful reflection on the use of anti-angiogenic therapy in the treatment of cancer. In a variety of experimental systems, several previous studies have indeed pointed out an unexpected side effect of anti-angiogenic treatments promoting tumour aggressiveness (Casazza et al., 2012).
Sema3E as well as VEGF/R inhibitors increase tumour invasiveness but while for Sema3E this effect is achieved by the activation of specific pathways in tumour cells, the malignization induced by anti-VEGF therapy is associated with vascular trimming and tumour hypoxia. Due to the implication of different mechanisms involved in the spread of tumour cells promoted by these two distinct treatments, the inhibition of pro-invasive and pro-metastatic process by Uncl-Sema3E strongly supports a possible added therapeutic potential of this molecule in anti-VEGF/R treated tumours. Indeed, results from Casazza et al demonstrate Uncl-Sema3E anti-tumour activity is maintained in models refractory to VEGF therapy. Thus, further efforts have to be made to define the anti-cancer outcome of combinatorial Uncl-Sema3E together with standard anti-angiogenic therapy to determine whether there could be additive anti-angiogenic effects and compensatory anti-invasive and anti-metastatic effects.

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References
Capparuccia L, Tamagnone L (2009) Semaphorin signaling in cancer cells and in cells of the tumor microenvironment-two sides of a coin. J Cell Sci 122: 1723-1736
Casazza A, Finisguerra V, Capparuccia L, Camperi A, Swiercz JM, Rizzolio S, Rolny C, Christensen C, Bertotti A, Sarotto I, et al (2010) Sema3E–Plexin D1 signaling drives human cancer cell invasiveness and metastatic spreading in mice. J Clin Invest 120: 2684-2698
Casazza A, Kigel B, Maione F, Capparuccia L, Kessler O, Giraudo E, Mazzone M, Neufeld G, Tamagnone L (2012) Tumor growth inhibition and anti-metastatic activity of a mutated furin-resistant semaphorin 3E isoform. EMBO Mol Med DOI: 10.1002/emmm.201100205
Christensen C, Ambartsoumian N, Gilestro G, Thomsen B, Comoglio P, Tamagnone L, Guldberg P, Lukandin E (2005) Proteolytic processing converts the repelling signal Sema3E into an inducer of invasive growth and lung metastasis. Cancer Res 65: 6167-6177
Ebos JML, Kerbel RS (2011) Antiangiogenic therapy: impact on invasion, disease progression, and metastasis. Nat Rev Clin Oncol 8: 210-221
Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, Inoue M, Bergers G, Hanahan D, Casanovas O (2009) Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell 15: 220-231
Roodink I, Verrijp K, Raats J, Leenders WP (2009) Plexin D1 is ubiquitously expressed on tumor vessels and tumor cells in solid malignancies. BMC Cancer 9: 297
Sakurai A, Gavard J, Annas-Linhares Y, Basile JR, Amornphimoltham P, Palmby TR, Yagi H, Zhang F, Randazzo PA, Li X, et al (2010) Semaphorin 3E initiates antiangiogenic signaling through plexin D1 by regulating Arf6 and R-Ras. Mol Cell Biol 30: 3086-3098
Serini G, Maione F, Giraudo E, Bussolino F (2009) Semaphorins and tumor angiogenesis. Angiogenesis 12: 187-193