Emerging roles of Semaphorins in the regulation of epithelial and endothelial junctions

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Tissue barriers maintain homeostasis, protect underlying tissues, are remodeled during organogenesis and injury and limit aberrant proliferation and dissemination. In this context, endothelial and epithelial intercellular junctions are the primary targets of various cues. This cellular adaptation requires plasticity and dynamics of adhesion molecules and the associated cytoskeleton, as well as the adhesive-linked signaling platforms. It is therefore not surprising that the guidance molecules from the Semaphorin family arise as novel modifiers of epithelia and endothelia in development and diseases. This review will focus on the actions of Semaphorins, and their cognate receptors, Plexins and Neuropilins, on epithelial and endothelial barrier properties.

Historically characterized for their roles in the developing central nervous system (CNS), Semaphorins have emerged as multifaceted guidance proteins that control biological responses in the epithelia and endothelia under physiological and pathological circumstances. In this review, we discuss their influence and mode of action in the regulation of tissue barriers, especially at the level of intercellular junctions.

Vertebrate Semaphorins and their Receptors

Semaphorins were identified more than 20 years ago as proteins, which provide a wide repertoire of attractive and repulsive signals that orchestrate axon outgrowth.[1,2] A large number of these guidance molecules combines with multiple receptors and co-receptors, increasing thereby both specificity and complexity of the Semaphorin mode of action (**Fig. 1**). Therefore, the elucidation of signaling cascades activated by these molecules is the subject of intense research.

Semaphorins. In vertebrates, there are 20 semaphorin genes,[2] sub-divided into classes III–VII. Class III semaphorins (Sema3A–G) are secreted, while Semaphorins from subclasses IV–VI exist as transmembrane proteins and Sema7A contains a glycosylphosphatidylinositol (GPI) anchor. Each Semaphorin possesses a 500 amino acid extracellular domain,

called semaphorin (sema) composed of a seven-bladed β propeller domain, essential for signaling. This domain is followed by a cysteine-rich domain (PSI), involved in receptor binding ability.[3] Conversely, the C-terminal tail features additional sequence motifs that diverge among classes: class III contains both an immunoglobulin-like and a basic residue-rich domains, whereas class V are distinguished by thrombospondin repeats[4] (**Fig. 1**). Semaphorins co-opt two kinds of transmembrane receptors (Plexins and Neuropilins), which mediate downstream signaling.

Plexins. Plexins are the main cell surface receptors for Semaphorin signal transduction. Plexins comprise nine members in mammals and are subdivided into four classes: class A (A1–4), class B (B1–3), PlexinC1 and PlexinD1[5] (**Fig. 1**). Unlike Semaphorins, Plexin architecture is conserved throughout the family. The extracellular region is composed by one sema domain and two or three PSI and IPT (immunoglobulin shared by Plexins and transcription factors) repeats. Class III and VI Semaphorins mainly bind to PlexinA. Most of the class III Semaphorins requires a co-receptor from the Neuropilin family (NRP, see paragraph 1.3), while the class VI Semaphorins directly interact with PlexinA through their respective sema domains, which in turn conveys intracellular signaling.[5] The Plexin cytoplasmic region contains a Rho and Ras-family-specific GTPase-activating protein (GAP) domain.[6,7] Although several models have been proposed, the exact role of the Ras-GAP activity in Semaphorin signaling remains obscure.[5,8,9] However, it has recently been characterized that this GAP domain can be activated by dimerization after Semaphorin binding to Plexin ectodomains.[10] This process could in turn inhibit Rap GTP-binding proteins and affect downstream pathways. Additionally, Plexins B and D bear PDZ (post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1) and zonula occludens-1 protein (ZO-1))-binding motifs that are most likely involved in protein-protein interactions and downstream signaling.

Neuropilins. The Neuropilin receptors, namely NRP-1 and -2 are only found in vertebrates, where they exist as multiple isoforms, including soluble ones. They operate as receptors or co-receptors for diverse ligands, ranging from class III Semaphorins, heparin-binding proteins and growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF) and placental growth factor 2 (PlGF).[11-13] These single transmembrane proteins include five extracellular domains and a short cytoplasmic tail. Their extracellular region contains two complement-like[6] (CUB) domains, also designated a1 and a2
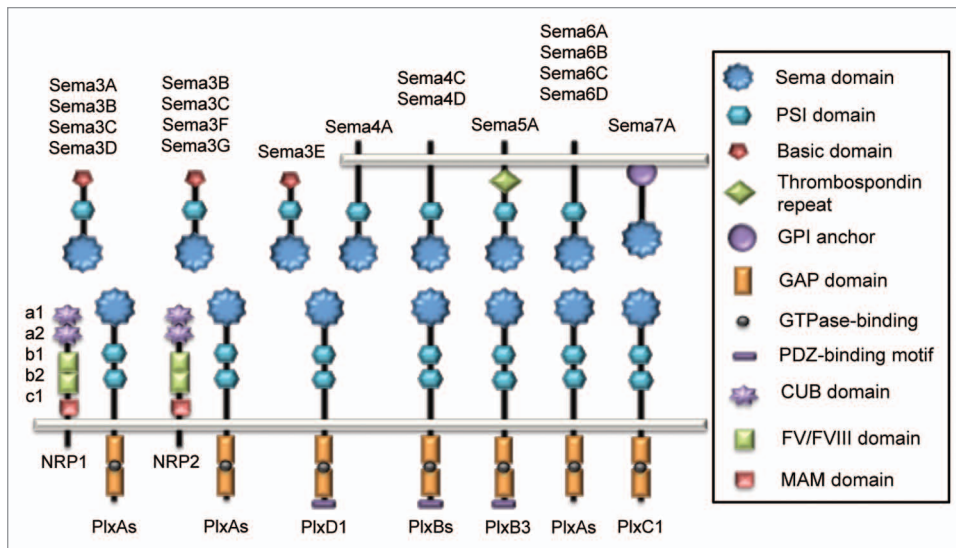
Figure 1. Schematic representation of vertebrate Semaphorins and their receptors, Plexins and Neuropilins. Each semaphorin contains one sema and one PSI domains. Class III Semaphorins are secreted and possess a C-terminus basic-rich domain. They signal through Plexin (Plx) A and Neuropilins (NRP), except for Sema3E that only required PlxD1. Class IV Semaphorins are transmembrane proteins and operate through PlxD1 and PlxB. Sema5A is also transmembrane and characterized by thrombospondin repeats, and binds to PlxB3. Transmembrane class VI Semaphorins use PlxA, while GPI-anchored Sema7A signals through PlxC1.

domains, two FV/FVIII coagulation factor-like domains, also known as b1 and b2, and a single meprin/A5-protein/PTPμ (MAM) domain, also called c. The a1/a2 domains are involved in Semaphorin binding, while the b1/b2 part is necessary for VEGF binding.[11,14] More specifically, the domain a1 is essential for the stabilization of the class III Sema3-PlexinA interaction and subsequently allows Semaphorins signaling through PlexinA[14] (**Fig. 1**); with the exception of Sema3E which acts via PlexinD1 and can signal in the absence of Neuropilins in endothelial cells.[15] The cytoplasmic tail does not harbor enzyme activity per se but rather a PDZ-binding motif, whose function requires further investigation.

General mode of action of Semaphorins. Semaphorins have been implicated in a wide variety of functions instrumental in the development of the CNS. These include neuron migration,[16] axonal pruning,[17,18] synapse formation[19] and synaptic transmission.[20]

Neurons, as with epithelial or endothelial cells, are polarized cells. Indeed, neurons harbor a unique long axon, which is directed by a highly motile structure, named the growth cone, and, multiple shorter dendrites, whose formation is controlled, among others, by Semaphorin 3A (Sema3A). Indeed, this decisive Semaphorin was first characterized for its ability to promote dendrite formation via the inhibition of axon specification.[21] The Sema3A co-receptor NRP-1 favors the accumulation of cyclic GMP (cGMP) over AMP and the further inhibition of PKA and GSK3β activities, two crucial signaling pathways for axon development.[21] Through this cGMP/AMP balance, Sema3A repels growth cone movement, while stimulating dendrite outgrowth. This action is thought to rely on a protein named Nervy that links PKA to the Sema3A receptor PlexinA. Alternatively, it employs a different set of neuropilin co-receptors.[22,23]

Another important aspect of Semaphorin action in neuron biogenesis is its impact on the actin cytoskeleton and extracellular matrix adhesion. For instance, class III Semaphorins released into the surrounding environment of a growth cone, triggered Plexin GAP activity upon receptor engagement, which in turn decreases R-Ras activation and integrin attachment.[24] This results in the reduction of outgrowth, illustrating the repellent activity of Semaphorins. On the other edge of the growth cone, the absence of Semaphorins allows higher level of R-Ras activity, and therefore the upregulation of integrin binding that facilitates growth.[7,25]

The leading edge of migrating cells, such as endothelial or epithelial cells, is comparable to the axon growth cone. Any abnormal changes in the expression of Semaphorins might trigger CNS diseases by affecting neuronal structure,[26] as demonstrated in the case of regeneration failure where Sema3A inhibits axon growth at sites of injury.[27] It also appears that Semaphorins can contribute to tumor progression,[28] a point that will be particularly discussed in this review.

Effects of Semaphorins on Epithelial Junctions

Epithelia are located at the boundaries of tissues where they form regulated sealing barriers controlling solute diffusion and immune cell passage. To assume their roles, epithelial cells establish specific junctions that maintain barrier integrity. For instance, in the case of the intestine, epithelial cells protect underlying tissues from luminal content, while permitting the absorption and secretion of fluids and ions. In this context, epithelial cells are polarized; i.e cell-cell contacts are organized in complexes distributed specifically along the apical-basal axis. Both tight and adherens junctions, as well as desmosomes are found within epithelial intercellular junctions (**Fig. 2**).

Epithelial adherens junctions. *Structure and function of E-cadherin.* The key protein of adherens junctions (AJ) that is found accumulated at epithelial cell-cell contacts is the transmembrane glycoprotein E-cadherin from the classical cadherin family. Structurally, classical cadherins consist of three different domains: the extracellular domain (ECD), the transmembrane (TM) and the intracellular domain (ICD).[29] The ECD is formed by the repetition of five cadherin repeats called EC 1 to 5, from the N-terminal to the C-terminal end. Each EC consists of 110 amino acids organized in β-sheets.[30,31] The EC1 domain contains the HAV sequence and is suspected to bear the adhesive specificity and thus to promote homophilic trans-association with adjacent
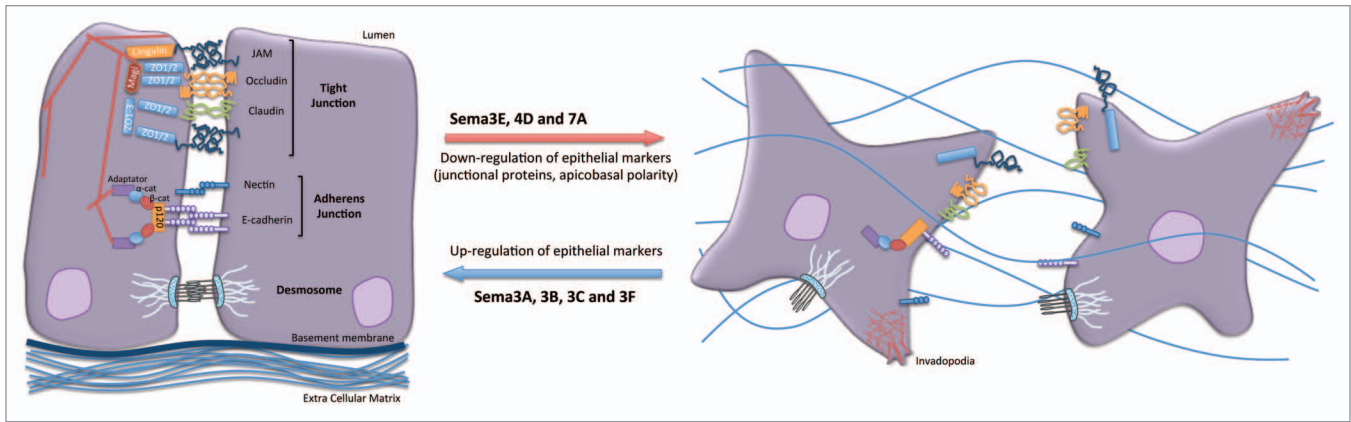
Figure 2. Impact of Semaphorins on the organization of epithelial cell-cell junctions. Specific and distinct adhesion proteins structure the epithelial cell junctions: tight junctions (JAM, occludin and claudins) and adherens junctions (nectin and E-cadherin) are linked to actin cytoskeleton and multiple intracellular adaptors. Upon exposure to Sema3A, 3B, 3C and 3F, cell adhesion can be strengthened, while Sema3E, 4D and 7A provoked dramatic cell-cell junction remodeling, which may ultimately favor tumorigenesis.

cells,[32] while the entire ECD likely engages in heterophilic interaction.[33] The ICD is highly conserved among vertebrate cadherins, in terms of sequence, length and cytoplasmic interacting partners. This cytosolic part modulates strength, dynamics and signaling abilities of cadherins at the cell-cell junctions.

E-cadherin cytoplasmic partners. The E-cadherin ICD is connected to the actin cytoskeleton through its association with β-catenin, which in turn binds to α-catenin. Finally, α-catenin interacts with actin and several actin-binding adaptors, such as formin, vinculin, α-actinin, afadin and ZO-1,[34,35] that can modulate actin organization, dynamics and polymerization. Cell-cell contacts can also be strengthened through E-cadherin cis-interaction involving the juxtamembrane region where the p120 catenin serves as a linker.[36] Importantly, epithelial cell-cell contacts still remain plastic, as E-cadherin can undergo endocytosis, recycling, lateral movements and shedding.[37] Tight junctions (TJ) presented in the paragraph below, delimited the apical pole of epithelial cells and accumulated above AJs. At the opposite, basolateral proteins, such as desmosomes, are found below AJs (**Fig. 2**). Thus, E-cadherin is not uniformly distributed over the cell surface but rather clustered in specific membrane domains within AJs, which serve as signaling platforms.[36] Indeed, AJs can also signal through proteins such as Rho GTPases, tyrosine kinase receptors and other lipid modifications.[38,39] These interactions contribute to the organization of membrane trafficking and promote polarized growth in regions that can be immediately adjacent or distant from AJs. In this scenario, AJs modulate TJ formation and epithelial polarization and therefore discriminate apical and basolateral subcellular areas.

Overall, one should keep in mind that AJs are not a rigid structure but rather a complex that can integrate and adapt to external changes and morphogenetic movements, including delamination, cell division and epithelial-to-mesenchymal transition (EMT).

Epithelial tight junctions. The epithelium is fastened apically by TJs, which almost completely obstruct the paracellular exchange pathway. TJs therefore contribute to the regulation of the ion and fluid passage, while restricting the diffusion of large molecules. In addition to their role as a barrier, TJs can regulate numerous cellular processes such as polarity, proliferation, differentiation and migration. First identified by electronic microscopy in epithelial cells,[40] TJs form typical structures of close apposition between membranes of two adjacent cells. The freeze-fracture method had allowed the observation of focal hemifusion sites associated with intracellular fibrils. This highlights the interplay between transmembrane proteins, cytosolic partners and the cytoskeleton.

Structure and functions of transmembrane proteins. TJs are enriched with many transmembrane proteins that associate to each other and link to scaffolding proteins and the actin cytoskeleton. Three protein families are found in TJs: claudins, occludin and junctional adhesion molecules (JAMs).

Claudins are calcium independent cell-cell adhesion proteins, comprising at least 24 members, which regulate paracellular permeability. They are composed of two extracellular loops that mediate homo- and hetero-typic intercellular junctions, as well as ion and fluid passage selectivity. They are instrumental in the maintenance of barrier integrity, as demonstrated by severe barrier defects in knockout mice lacking individual claudin family members.[41] Interestingly, claudins exhibit organ and tissue specific expression patterns, thus forming a large repertoire of TJs with different strength, size and ion specificity.

Occludin was the first protein identified as a TJ component.[42] Occludin is a tetraspan membrane protein bearing a MARVEL domain (MAL and related proteins for vesicle trafficking and membrane link).[43] Its role is not fully understood, but in vitro studies agree that this junctional protein operates at the epithelial barrier and functioned as a signaling protein.[44-46]

Finally JAMs, the third group of proteins found at TJs, consist of single transmembrane proteins that belong to the immunoglobulin superfamily.[47] They are composed of two extracellular immunoglobulin-like domains, a single transmembrane region and a C-terminal part. Among them, JAM-A, -B, -C and -4[48] and the Coxsackie and adenovirus receptor (CAR), are found at

epithelial junctions. JAMs can be engaged either in either homo- or hetero-philic adhesions within the TJs. However, they cannot form TJs by themselves when expressed in fibroblasts.[49] Although their exact function is still uncertain, JAMs, similarly to claudins and occludin, can bind to cytoplasmic scaffolding partners.

Scaffolding proteins. Among all cytosolic proteins involved at the cytoplasmic surface of TJs, the major components are the zonula occludens (ZO), designated as ZO-1, ZO-2 and ZO-3.[50-52] These proteins share sequence similarity and molecular organization, as they all contain three PDZ domains and one Src-homology 3 (SH3) domain followed by one guanylate kinase-like (GUK) domain involved in protein-protein interactions. Through the N-terminal PDZ domains, ZOs interact directly with JAMs, occludin and claudins, while they are connected to the cytoskeleton via their C-terminal tail.[53] ZO-1 was found to be crucial for epithelial barrier function.[54] This protein also serves as scaffold for regulatory proteins such as kinases, GTP exchange factors (GEF) and many transcription factors.[55] Many other PDZ-containing proteins have since been discovered to be cytoplasmic components of the TJs, including membrane-associated guanylate kinases (MAGI-1, -2, -3), Multi-PDZ domain protein 1 (MUPP1), cingulin and polarity proteins PAR3/6, and will not be discussed in details here.[56]

These TJ components, much like the cytoplasmic partners for AJs, are pivotal for the epithelial biology. They exhibit the ability to link many transmembrane proteins to the cytoskeleton, promote signals toward other cellular compartments and adapt the cellular responses to external cues.

Semaphorins and the epithelial barrier function. At present, knowledge concerning Semaphorins in epithelial barrier function, and in particular their direct action on junctional components, is quite limited. This review will instead present selected cases that support for a role of individual Semaphorin in the reinforcement of epithelial barrier components, or, at the opposite end, favor barrier dismantlement, especially in the course of cancer progression (**Fig. 2**).

Pro-barrier Semaphorins. During lung organogenesis, layers of progenitors lining the developing airways maintain their epithelial characteristics, while proliferating and differentiating into multiple cell types.[57] Many genes that establish and maintain cell polarity and/or cell junctions are regulated.[58,59] In this context, it has been shown that class 3 Semaphorins, namely Sema3A, Sema3C and Sema3F, display a specific spatiotemporal distribution in lung epithelial cells. Organ culture analyses have shown that Sema3C and Sema3F positively regulate branching and promote epithelial proliferation, whereas Sema3A leads to a reduced number of terminal pulmonary buds.[60] Altogether these data suggest that a repertoire of Semaphorins may orchestrate lung epithelial morphogenesis, a process that might culminate in the regulation of the epithelial barrier architecture.

As Sema3B is concerned, this gene was characterized to be inactivated in lung cancer[61,62] and metastatic variants;[63] while its expression in lung and breast cancers induces growth inhibition and apoptosis activation.[64] In a more recent study, the Grainyhead-like (Grhls) transcription factors were proposed to function upstream of Semaphorins in lung development.[65]

These factors regulate cell-cell adhesion molecule expression, for instance, Grhl3 controls claudin1 and occludin transcription, while Grhl2 positively affects E-cadherin and claudin4 levels. Grhl2 was also described to modulate Sema3B and Sema3C expression, both directly and indirectly, plus that of their corresponding receptor NRP-2. Indeed, Grhl2 downregulation correlated with Sema3B, Sema3C and NRP-2 decrease that in turn could impact gene expression of apical junction proteins. Although the signaling mechanisms involved have not been fully characterized, Sema3B and Sema3C might emerge as important positive mediators of lung epithelial barrier integrity.

As mentioned above, the lung epithelium is also modulated by Sema3F, which is expressed in lung epithelial cells together with NRP-1 and -2. Interestingly, Sema3F and Sema3B map into 3p21.3, a region frequently downregulated in small cell lung cancer[66-68] and breast carcinoma.[69] In their study, Brambilla E. and colleagues[70] describe Sema3F distribution in normal lung and several lung epithelial cancer cell lines. In normal epithelial cells, Sema3F localizes predominantly at the apical face. In the case of low-grade tumor cells, with little to no locomotion, Sema3F accumulated at the interface of adjacent cells, in a region evocative of AJs. In contrast, in high-grade tumor cells, Sema3F expression was considerably reduced and remained mainly in the cytoplasm and in motile regions, such as lamellipodia and protrusions. Nasarre and colleagues[71] have further focused on the functional role of Sema3F during the course of breast cancer progression. Using poorly to highly motile cancer cells, a different expression pattern of Sema3F and its receptors was first highlighted. Low levels of Sema3F, NRP-1 and NRP-2 were found in motile cells, whereas less motile cells release more Sema3F and NRP-1 was the unique receptor being expressed. Interestingly Sema3F is thought to have a repulsive activity on highly motile cells, operating in an NRP-2-dependent manner. In less motile cancer cells, addition of Sema3F induced an NRP-1-dependent delocalization of membrane E-cadherin and β-catenin. No impact on N-cadherin expression was however detected. It has thus been hypothesized that Sema3F could dampen tumor progression. Although not completely elucidated, the mechanism underlying the switch from high to low Sema3F expression in cancer cells, and its associated redistribution, might involve VEGF, a potential Sema3F binding competitor for Neuropilins. Indeed, loss of Sema3F membrane expression correlated with high levels of VEGF.[72] In this scenario, Sema3F reduction and cytoplasmic sequestration could facilitate VEGF receptor activation and associated migratory responses.

Similarly to its putative role in the lung epithelium barrier morphogenesis, Sema3A could affect as well differentiation processes in the corneal epithelium. Sema3A, together with its receptors PlexinA1 and NRP-1 are expressed in the cornea.[73] The works by Nishida group[74,75] demonstrate that Sema3A released from surrounding corneal fibroblasts triggers an upregulation in the membrane expression of E- and N-cadherins in corneal epithelial cells. In a wound-healing model, NRP-1 and Sema3A levels were increased, in association with epithelial thickening. However, Sema3A was not colocalized within junctional proteins such as ZO-1, occludins, E-cadherin and β-catenin.[74]

Beside their impact on adhesion molecules, Semaphorins also regulate actin at motile structures, comparable to neuron growth cones.[76] Altogether these observations suggest that Semaphorins might emerged as modulators of cell-cell adhesion and migration during development and maturation of specialized epithelia, while their aberrant expression and/or function is involved in the course of cancer progression.

Anti-barrier Semaphorins. In opposition to the roles of class III Semaphorins in epithelial junction reinforcement are other Semaphorins that participate in epithelial junction dissociation (**Fig. 2**).[12]

Normal and tumor epithelial liver cells express PlexinB1, which mediates cell scattering upon Sema4D stimulation.[77] This mechanism triggers cell-cell dissociation and subsequent cell migration, in a manner similar to the effect of scatter factor (SF-1), the ligand for the receptor encoded by the Met proto-oncogene.[78] Indeed, while Sema4D acts primarily through PlexinB1, the signal transduction leading to cell dissociation and invasive growth rather implied the tyrosine kinase activity of Met. Moreover, this mechanism was not restricted to the liver but was also unveiled in pancreatic, lung and mammary epithelial tumor cell lines.[28] This original signaling complex, which is formed between tyrosine kinase receptors and Plexins is modulated by Semaphorins, and impacts on epithelial cell junctions and therefore aggressiveness of tumor cells.

In a comparable manner, Sema7A was described to be instrumental for EMT in mammary epithelial cells.[79] Indeed, Sema7A was suggested to be required for TGF-β-induced EMT through E-cadherin downregulation. Furthermore, the Sema7A promoter contains tandem Ets-binding sites, which can be repressed by Ets-2-repressor factor (ERF), suggesting a point of convergence with TGF-β signaling, which is also inhibited by ERF. Additionally, Sema7A might play a role in cell motility through β1-integrin interaction[80] and PlexinC1 signaling.[81] Thus, Sema7A appears to be an important modifier of epithelial cell adhesion.

Finally, the role of Sema3E, also known as Semaphorin H, was recently highlighted in epithelial barrier integrity. This signaling molecule, together with its receptor PlexinD1, are dramatically upregulated in high-grade tumor cells, when compared with normal and low-grade tumors,[82,83] especially in ovarian[84] and colon epithelial cancer cells.[85] It has been suggested that Sema3E expression drives cancer and metastasis progression through several crucial steps such as EMT, invasion into the extracellular matrix, extravasation and metastatic colonies formation. For instance, Sema3E and PlexinD1 signaling have been demonstrated to drive Snail1 nuclear relocalization, in correlation with E-cadherin downregulation and vimentin upregulation, crucial events in cancer EMT.[84] In addition, Sema3E can activate the ErbB2 tyrosine kinase receptor via its phosphorylation, which in turn triggers MAPK, PLC-γ and PI3K pathways that collectively induce massive cytoskeletal rearrangement and increased cell motility.[85] Notably, Sema3E-expressing tumor cells also provoke extracellular matrix degradation, in areas that colocalize with actin-rich structures characterized as invadopodia.[85] Likewise, such cells possess filopodia and lamellipodia, a round-shaped morphology and increased migration capacity. Overall,

these data converge on the idea that Sema3E can affect epithelial barrier integrity by disrupting among others, E-cadherin.

To conclude, Semaphorin expression is modulated during physiological and pathological epithelial morphogenesis. Although molecular mechanisms are still under investigation, their signaling activity impacts on cell-cell junction organization. These molecules could thus represent promising candidates for novel therapeutic targets in cancers.[86]

Effects of Semaphorins on Endothelial Junctions

The vascular endothelium forms an interface between circulating blood and irrigated tissues. Endothelial cells are paramount to vascular biology and control the distribution of molecules and cells, as well as waste release throughout the body. The intercellular endothelial junctions primarily contain TJs and AJs, and restrict bidirectional passage between the blood compartment and underlying tissues. Unlike epithelial junctions, endothelial junctions exhibit a more flexible organization, where TJs and AJs are found intertwined along the cell-cell contact zone (**Fig. 3**).

Endothelial tight junctions. Collectively TJs formed approximately 20% of the total cellular junctions in endothelial cells,[87] and allow strong electrical resistance and low permeability.[88] Endothelial TJs are organized around adhesion proteins from the same families than those found in epithelial cells, although the isoforms expressed and the associated partners diverge. For instance, the claudin family comprises more than 20 members, however only a few are found in endothelial cells; such as only claudin-3, -5 and -12 that are expressed in the brain endothelium.[89] Of note, claudin5 knockout mice have normal TJs with an increased permeability in brain vasculature territories.[90]

As in epithelial junctions, other transmembrane proteins compose TJs, namely occludin and JAMs. Occludin is particularly enriched in the brain microvasculature,[44] in corroboration with stronger tissue barrier function. However, loss of occludin alone in mice is not sufficient in mouse model to impact on vascular permeability.[91] Endothelial JAMs, namely JAM-A, -B, -C, ESAM (endothelial cell specific adhesion molecule), CD146 (melanoma cell adhesion molecule MCAM or cell surface glycoprotein MUC18) and PECAM (platelet endothelial cell adhesion molecule or CD31) are also recruited to cell-cell contacts. Their level of expression, localization and interaction with extracellular ligands might vary in response to permeability factors, involved in either angiogenesis and/or immune responses. For instance, histamine and VEGF promote JAM-C relocalization into cell-cell contacts, where it could in turn modulate the stability of the vascular cell-to-cell adhesion molecule, Vascular Endothelial (VE)-cadherin.[92]

Endothelial adherens junctions. AJs contribute to the initiation and stabilization of cell-cell adhesion, as well as regulation of the actin cytoskeleton and intracellular signaling. They are mainly composed by VE-cadherin, which belongs to the calcium-dependent adhesion molecule family (see **Fig. 3**). Similarly to classical cadherins, VE-cadherin binds to p120-catenin and β-catenin, which bridges the cadherin-catenin complex to the actin cytoskeleton via α-catenin. VE-cadherin is ubiquitously and
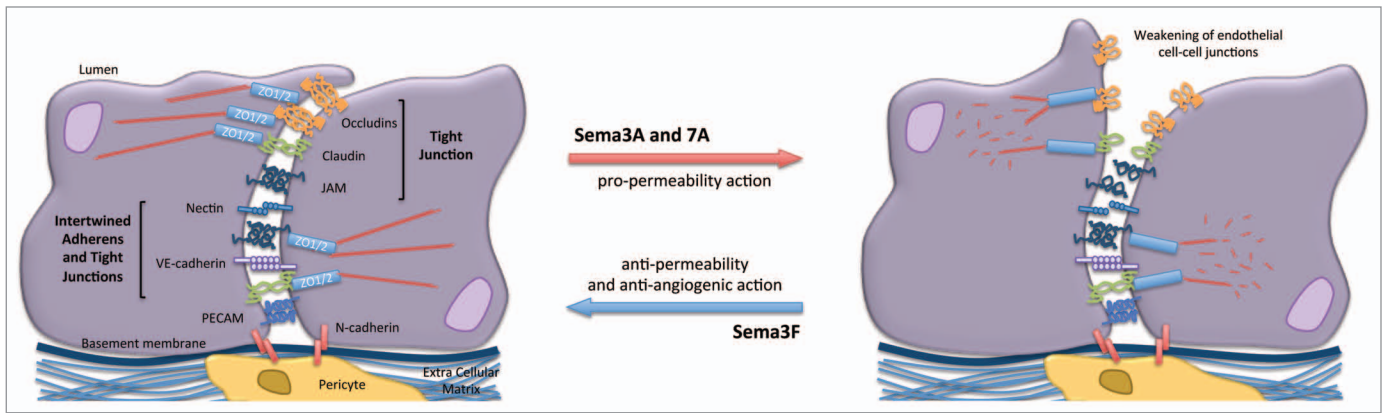
Figure 3. Impact of Semaphorins on the organization of endothelial cell-cell junctions. Endothelial cell-cell junctions are organized around inter-twined adherens (nectin and VE-cadherin) and tight junctions (JAM, occludin and claudins). Other non-junctional adhesive proteins, such as PECAM, an endothelial marker, and N-cadherin, which anchors pericytes, might collectively contribute to the endothelial barrier properties. Semaphorins exert either pro- or anti-barrier actions on the endothelial junctions, which in turn alter vascular properties including permeability and angiogenesis.

specifically expressed in the vascular endothelium. Importantly, VE-cadherin deletion in mice induces embryonic death because of vascular system defects; in other words, VE-cadherin is essential for the formation of stable, mature vessels.[93] Moreover, VE-cadherin blocking antibodies provoke an increase of micro-vascular permeability,[94] while its stability at the junctions oppose VEGF-mediated permeability.[95] In addition, VE-cadherin modulates signaling pathways that affect the overall TJ architecture and organization.[96,97] Hence VE-cadherin is the target of many molecular pathways involved in vascular leakage.[98]

Vascular permeability is finely tuned in quiescent vessels and adapts to many challenges in the perivascular microenvironment including angiogenesis, shear stress, flow and inflammation.[99] The intercellular junctions, and especially VE-cadherin, actively contribute to these modifications in the vascular barrier. Indeed, VE-cadherin integrity can be altered by multiple means, including phosphorylation, destabilization of catenin interaction, internalization, shedding and acto-myosin contractility, all of which ultimately converge on increased endothelial permeability.[95,100]

Semaphorins and the endothelial barrier. Semaphorin family members have emerged as important molecular modifiers of both neural fate and endothelial biology, largely by operating a fine tuned combination of attractive and repulsive signals.[101] Indeed, cancer cells release pro-angiogenic Semaphorins, such as Sema4D, that are able to promote endothelial migration and vascular invasion within the tumor mass.[28,102-104] By contrast, class III Semaphorin expression is generally reduced in cancers; they normally function as general inhibitors of angiogenesis.[72,105-109]

Semaphorin 3A. Beside its axon guidance properties, Sema3A controls vessel formation by inhibiting integrin function. It is also suspected to act as an anti-angiogenic factor.[110] However, while most anti-angiogenic proteins oppose permeability, Sema3A, paradoxically, increases vascular permeability (**Fig. 4**). The signaling mechanisms mediating this effect involve the destabilization of VE-cadherin through tyrosine and serine phosphorylations.[111,112] From a molecular standpoint, Sema3A permeability action depends on NRP-1 and PlexinA1 receptors,

independently of VEGF. However, divergent downstream molecular pathways have been reported depending on the endothelial context, i.e. macro- vs. micro-vascular cells.[111,112] In a first study, Sema3A was found to induce permeability via the PI3Kγ-δ/Akt pathway, independently of Src via NRP-1.[111] Another study from our lab established that tumor-released Sema3A provoked serine 665 phosphorylation of VE-cadherin and endothelial junction disorganization, thereby leading to increased endothelial permeability.[112] In microvascular brain endothelial cells, Sema3A operates via Src phosphorylation and activation, recruitment of Set, a potent inhibitor of the serine/threonine Protein Phosphatase 2A (PP2A), and inhibition of PP2A phosphatase activity.[112] The Sema3A pathway is then subsequently involved in the dissociation of a constitutive PP2A/VE-cadherin interaction that might operate to secure VE-cadherin junctions in quiescent endothelial cells.[112] To conclude, Sema3A exerts pro-permeability action through targeting VE-cadherin at the endothelial junctions as a means to modulate local disruption of the endothelial barrier.

Semaphorin 3F. Sema3F is a well-characterized repulsive molecule in endothelial cells,[72,113,114] and could be a potential candidate for anti-angiogenic therapies. However, it has been recently proposed that hypoxia opposes Sema3F anti-angiogenic action and signaling activity in endothelial cells by downregulating the levels of NRP-2[115]. This ultimately favors VEGF-paracrine function and therefore angiogenesis. Nonetheless, in poorly vascularized tumors, such as schwannomas, Sema3F overexpression could enhance pericyte coverage and decrease vascular permeability.[115,116] Therefore, these findings suggest that depending on the tumor context, Sema3F could act as a vascular normalization agent.

Semaphorin 7A. Sema7A is reported to take part in immune responses and inflammation.[117,118] Interestingly, Sema7A increases transmigration of polymorphonuclear neutrophils across endothelial layers.[119] This effect relies on the endothelial expression of Sema7A, rather than a direct action on leukocytes. Moreover, Evan's blue experiments demonstrated that hypoxia-induced vascular leakage was attenuated in Sema7A knockout mice.[119] Likewise, mice lacking Sema7A exhibited decreased blood-brain

barrier permeability upon West Nile virus infection and were thus protected.[120] These results were also recapitulated in human cell models for West Nile virus infection using a Sema7A blocking antibody, thus pointing toward a role of Sema7A at the crossroads between endothelial biology and immunology.

Class III Semaphorins in particular were recently highlighted for their anti-angiogenic properties, as well as their remarkable ability to impact on endothelial barrier properties (**Fig. 3**). Although the signaling mechanisms are not fully elucidated, this interplay between Semaphorins and intercellular junctions opens a new field of investigation in endothelial biology.

Epithelial and endothelial junctions function as keystones in the organization and integrity of tissue barriers. They accomplish this by modulating the biology of the epithelia and endothelia through a finely tuned combination of multiple inside-out and outside-in signals. In pathological conditions, such as cancer progression, misexpression of junctional proteins and/or their dysfunction are frequently observed. In this context, Semaphorins could guide the dismantlement of cell-cell contacts, or, at the opposite end, reinforce the intercellular junctions. Although research efforts are still required to better understand the combinatory actions of Semaphorins, Plexins and Neuropilins and their associated molecular signaling in epithelial and endothelial barriers, targeting them will offer new opportunities for future cancer therapies.

Disclosure of Potential Conflicts of Interest

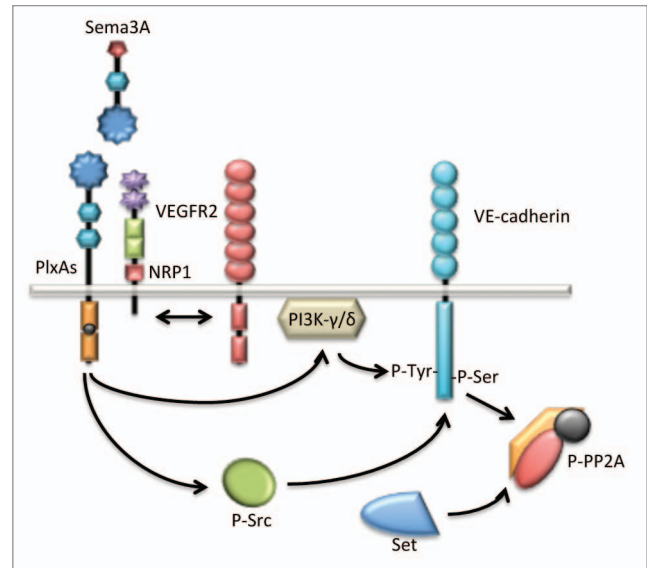The authors have declared that no competing interests exist.

Figure 4. Sema3A enhances endothelial permeability through VE-cadherin destabilization. Sema3A directly augments endothelial permeability by the mean of PlxA1 and NRP1 receptors. Further downstream signaling relies on the activation of the kinase activities bear by PI3K-γ/δ and Src. These pathways culminate at the phosphorylation and destabilization of VE-cadherin. Interestingly, in quiescent endothelial cells, VE-cadherin is associated with the phosphatase PP2A, a mechanism possibly involved in locking the adherens junctions. In this model, Src-directed PP2A phosphorylation and inactivation contribute in turn to VE-cadherin destabilization upon Sema3A challenge.

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