EXPERIMENTAL CELL RESEARCH 319 (2013) 1306–1316

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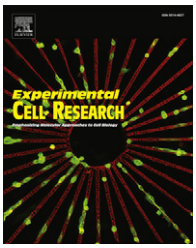

Review Article

The role of semaphorins and their receptors in vascular development and cancer

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ARTICLE INFORMATION

Article Chronology:
Received 1 February 2013
Accepted 6 February 2013
Available online 17 February 2013

Keywords:
Semaphorin
Plexin
Neuropilin
Angiogenesis
Cancer
Tumor
Development
Vasculature

ABSTRACT

Semaphorins (Semas) are a large family of traditional axon guidance molecules. Through interactions with their receptors, Plexins and Neuropilins, Semas play critical roles in a continuously growing list of diverse biological systems. In this review, we focus on their function in regulating vascular development. In addition, over the past few years a number of findings have shown the crucial role that Semas and their receptors play in the regulation of cancer progression and tumor angiogenesis. In particular, Semas control tumor progression by directly influencing the behavior of cancer cells or, indirectly, by modulating angiogenesis and the function of other cell types in the tumor microenvironment (*i.e.*, inflammatory cells and fibroblasts). Some Semas can activate or inhibit tumor progression and angiogenesis, while others may have the opposite effect depending on specific post-translational modifications. Here we will also discuss the diverse biological effects of Semas and their receptor complexes on cancer progression as well as their impact on the tumor microenvironment.

Contents

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http://dx.doi.org/10.1016/j.yexcr.2013.02.003

Semaphorins and their receptors

The Semas are a large family of secreted and membrane-bound proteins originally identified as axon guidance cues and later shown to be key regulators in many biological processes including vascular development and cancer. Based on structural similarities, the Semas are grouped into eight classes, all of which carry a N-terminal Sema domain. Semas signal via two major receptor families, Plexins and Neuropilins (Nrps or Npns). Membrane-bound Semas bind and signal directly through Plexins whereas most of the class 3 secreted Semas (Sema3A–3G) are known to bind to a holoreceptor complex consisting of Nrps as the ligand binding subunit and Plexins as the signal transducing subunit [1–3]. The exception to this rule is Sema3E, which binds its receptor Plexin-D1 directly and independently of the Nrps [4]. There are four groups of Plexins (A, B, C, and D) that have been identified so far, and similar to Semas, all Plexins have Sema domains in their extracellular region. In addition, Plexins have Met-related sequences (MRS) and glycine/proline-rich motifs in the extracellular region. The intracellular domains of Plexins do not have obvious enzymatic activity, but most of them have weak sequence similarity to GTPase activating proteins (GAPs) and exhibit GAP activity towards the small GTPase R-Ras [5]. Moreover, their intracellular regions all contain two highly conserved intracellular domains known together as the SEX-plexin domain. Recent structural work has revealed that binding of each homodimer arrangement of Sema and Plexin forms a heterodimer complex that then elicits a conformational change in the complex. This structural alteration transmits signals to the intracellular domain of Plexins [6]. In contrast to Plexins, Nrps (Nrp1 and Nrp2) have a very short cytoplasmic domain with a PDZ-binding motif at the C-terminal. The extracellular domains of Nrps contain two complement-binding domains (a1 and a2), two coagulation factor V/VIII homology domains (b1 and b2), and a MAM domain (c). Importantly, besides binding to Sema3s, Nrps also bind to a structurally different family of ligands, the VEGF family, and serve as their co-receptors [7]. Although the a1 domain is only required for Sema3 binding, the b1 domain is required for both Sema3 and VEGF binding [8]. Thus, determining the precise contributions of Sema3s and Nrps is complex because Nrps also control VEGF receptor signaling.

Semaphorins in developmental angiogenesis

It was initially suggested that Sema3s compete with VEGF to bind to Nrp1 and therefore Sema3s inhibit VEGF-induced angiogenesis. Some *in vitro* data also suggest that Sema3s negatively regulate endothelial migration, for example Sema3A inhibits endothelial cell migration and survival [9,10]. However, *in vivo* studies using knockout mice thus far show very little evidence for the role of Sema3s in developmental angiogenesis except in the case of Sema3E (see below). In the case of Sema3A, no obvious developmental vascular patterning defect were found in mice selectively lacking Sema3-Nrp1 signaling [11] or mice lacking Sema3A [12]. In addition, Sema3B-, Sema3F-, and Sema3G-null mice are also viable and fertile with no overt vascular defects. Sema3C null mice die perinatally from cardiac defect [13]. Therefore, most of the Sema3s are perhaps not required for the early stages of developmental angiogenesis, but rather play a role during vessel remodeling and pathological angiogenesis (see the other sections of the review). For example, Sema3A is expressed in endothelial cells and in an autocrine manner regulates endothelial cell migration and vessel remodeling by inhibiting integrin function [14]. However, the molecular mechanisms by which Sema3A regulates integrins are not fully understood. Moreover, recent work demonstrated that genetic deletion of Sema3A in mice results in severe renal vascular patterning defects, further support the role of Sema3A in angiogenic remodeling [15] Most Sema3s have an inhibitory function on *in vitro* endothelial cell migration assays, but whether this inhibitory effect is due to competitive Nrp binding and therefore the inhibition of VEGF signaling as was originally proposed is still unclear (see more discussion in the Nrp section).

The most obvious Sema3 member to play a key role in regulating developmental angiogenesis is Sema3E and its receptor Plexin-D1. Unlike other class 3 secreted Semas that require Nrps as an obligatory ligand-binding subunit in the Plexin/Nrp holoreceptor, Sema3E can bind and signal through Plexin-D1 independent of Nrps. As a novel ligand–receptor pair, Sema3E-Plexin-D1, plays a critical role in shaping the vascular network during development [4]. During intersomitic vessel formation, Sema3E is expressed in the caudal region of each developing somite, whereas Plexin-D1 is expressed in the intersomitic blood vessels adjacent to the somite boundary in the rostral region of each somite. Sema3E acts as a repulsive cue to restrict vessel growth and branching in the intersomitic space. As a result, both Sema3E and Plexin-D1 knockout mice exhibit severe intersomitic vessel patterning defects. Specifically, the intersomitic vessels are no longer excluded from the normal Sema3E-expressing caudal region of the somite and extended ectopically throughout the somites, resulting in exuberant growth and a loss of the normal segmented pattern [4,16]. Plexin-D1 morphant zebrafish exhibit similar defects in intersomitic vessel patterning [17]. In addition to its function in intersomitic vessel patterning, recent work also demonstrates that Sema3E plays a role in the initial formation of the dorsal aorta. Sema3E secreted from the notochord and lateral plate mesoderm are required for the formation of avascular regions that coordinate to sculpt the mouse dorsal aorta. In Sema3E knockout embryos, a branched aortic plexus develops abnormally with a markedly narrowed avascular midline [18]. Therefore, the repulsive gradient generated by Sema3E in the mouse somite as well as the notochord and lateral plate mesoderm determines the proper patterning of Plexin-D1-expressing intersomitic vessels and the dorsal aorta, respectively.

The molecular mechanism underlying Sema3E-Plexin-D1 signaling in vascular patterning has been linked to the well-known VEGF pathway by several studies in both mouse and zebrafish [19,20–22]. In the developing retinal vasculature, Sema3E is expressed uniformly by retinal ganglion cells (RGCs),