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Semaphorin signaling in bone

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ABSTRACT

Semaphorin molecules regulate cell adhesion and motility in a wide variety of cell types and are therefore involved in numerous processes including axon guidance, angiogenesis, cardiogenesis, tumor growth, and immune response. Increasing evidence points to a role of transmembrane, membrane-associated and soluble semaphorins during bone development as well as in the control of normal bone homeostasis. Within bone, semaphorins are implicated in the communication between different cell types by relaying signals in an autocrine or paracrine way. Semaphorins are not only involved in bone resorption but also in bone formation. Therefore, targeting semaphorin-induced signaling in bone may constitute an interesting new therapeutic strategy in osteoporosis. However, all the pioneering research on semaphorins is performed in mice and it remains to be established to what extent semaphorin signaling pathways are conserved between mice and men. In addition, knowledge of semaphorin signaling in bone mostly arises from loss/gain of function studies of one single semaphorin and/or receptor. However, different semaphorin molecules are co-expressed in bone and their signaling pathways are likely to interact in a complex and coherent way that needs proper understanding before targeting semaphorin signaling can be therapeutically exploited.

1. Semaphorin-mediated signaling

1.1. Semaphorin ligands

Semaphorin molecules constitute a pleiotrophic family of more than 20 glycoproteins that are able to direct migration of cells and growing neurites. Moreover, they influence other cellular processes including cell proliferation, differentiation, and survival. As a consequence, they are involved in a variety of biological processes, such as axonal guidance, cardiogenesis, angiogenesis, oncogenesis, immune cell regulation, and recently also bone homeostasis (Castellani and Rougon, 2002, Epstein et al., 2015, Jongbloets and Pasterkamp, 2014, Takamatsu et al., 2010). Semaphorins are categorized into 8 classes based on sequence and structure similarities. Semaphorins class 1 and 2 are expressed in invertebrates, classes 3–7 are present in vertebrates whereas class V contains the viral semaphorins. Semaphorins from classes 1, 4, 5, and 6 are transmembrane proteins while class 7 semaphorins contains proteins that are membrane-associated through a glycosylphosphatidylinositol (GPI) anchor. Class 2, 3, and V semaphorins are secreted proteins. Proteolytic cleavage of some of the transmembrane (class 4) or membrane-linked (class 7) semaphorins results in the formation of soluble proteins, which creates an even greater diversity in the semaphorin family. Within each class, the different semaphorin molecules are indicated with a letter code (Castellani and Rougon, 2002).

The common and defining feature of the semaphorin family is the presence of the approximately 400 amino acid extracellular sema domain and the cysteine-rich plexin, semaphorin, and integrin domain (PSI domain), which are both encoded by the aminoterminal part of the protein. The sema domain, with the structure of a seven-blade β-propeller fold, is not only required for homodimerization of semaphorin molecules but also for the binding to the high affinity receptors plexins and neuropilins (Fig. 1). The presence of additional C-terminal domains, such as immunoglobulin-like domains (in class 3, 4, and 7 semaphorins) and thrombospondin domains (in class 5), is class-specific (Siebold and Jones, 2013).

1.2. Semaphorin receptors: plexins and neuropilins

The predominant class of semaphorin receptors is the plexin family of single-pass transmembrane proteins, which are auto-

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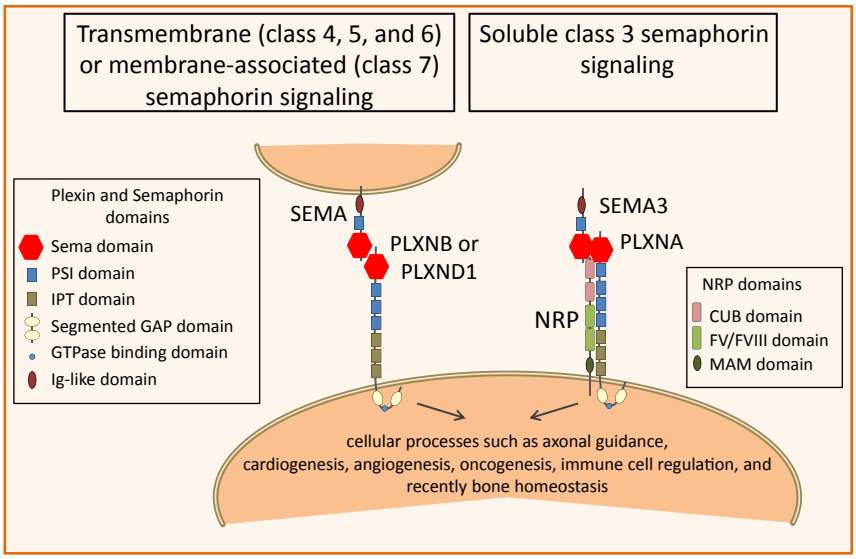

Fig. 1. Composition of vertebrate semaphorin-signaling complexes. Signal transduction of most transmembrane and membrane-associated semaphorins is executed by binding to predominantly PLXNB and PLXND1 receptors, which may form functional holoreceptor complexes through functional association with other proteins. Class 3 semaphorins (except SEMA3E) mostly bind to PLXNA receptors in which the NRP1 or NRP2 receptor stabilizes the receptor complex. Upon binding the transmembrane receptor PLXN becomes active and signal transduction takes place affecting diverse processes like axonal guidance, cardiogenesis, angiogenesis, oncogenesis, immune cell regulation, and bone homeostasis. The extracellular region of plexin receptors consists of a Sema domain, three (for PLXNB and PLXND1) or four (for PLXNA) Ig-like, plexins and transcription factor (IPT) domains, and three plexins, semaphorin, and integrin (PSI) domains. The intracellular plexin receptor consists of a GTPase activating protein (GAP) domain that is segmented by a GTPase binding domain. The neuropilin receptors NRP1 and NRP2 have a very short intracellular domain and a large extracellular region that consists of two complement binding factors C1s/C1r, VEGF, bone morphogenetic protein (BMP) 1 (CUB) domains, two coagulation factor V/VII homology domains, and a meprin, A5 antigen, receptor tyrosine phosphatase μ (MAM) domain.

inhibited in the absence of semaphorin ligands to prevent ligand-independent signaling. In vertebrates, these evolutionarily conserved proteins are categorized in four subfamilies based on overall homology (plexin A1-A4, B1-B3, C1, and D1), whereas in invertebrates PlexA and PlexB are responsible for semaphorin binding (Hota and Buck, 2012). The extracellular region of plexin receptors is made up of a sema domain, three PSI domains, and three Ig-like, plexins and transcription factor domains (IPT domains). The Sema domain within this extracellular region binds the semaphorin ligand. For signal transduction, plexins can interact in a cell- and tissue specific manner with one another or with other proteins to form holoreceptor complexes. As such extracellular interaction partners include neural cell adhesion molecule L1 (L1CAM) and the receptor tyrosine kinases vascular endothelial growth factor receptor (VEGFR), ErbB2, Met, and Ron (Hota and Buck, 2012, Gay et al., 2011, Messina and Giacobini, 2013). In their intracellular domain, plexins contain a GTPase-activating protein (GAP) domain that is segmented by a Rho-GTPase-binding domain (RBD). This GAP domain allows plexins to directly control the activity of small GTPases, such as R-Ras, M-Ras and Rap1, which are master regulators of divergent cellular processes, among which the control of cell motility and morphology through regulation of actin and microtubule dynamics (Hota and Buck, 2012, Pascoe et al., 2015). In contrast to other plexins, B plexin family members contain next to this GAP domain also a C-terminal PDZ-domain interaction motif that, after semaphorin binding, interacts with leukemia-associated Rho guanine nucleotide exchange factor (RhoGEF) (LARG) and PDZ-RhoGEF, two related RhoGEF proteins. This interaction subsequently leads to the activation of RhoA and RhoC. Plexin A family members on the other hand can interact with two other GEF proteins being FERM, RhoGEF, and pleckstrin homology domain protein (FARP) 1 and FARP2. The PLXNA/FARP interaction is implicated in various biological processes including dendrite growth, axonal repulsion and osteoprotection (Pascoe et al., 2015).

Whereas most semaphorin ligands rely on plexin receptors for signal transduction, all class 3 semaphorins, except for SEMA3E, require neuropilin receptors (NRP1 and 2) for downstream signaling (Sharma et al., 2012). Class 3 semaphorins also bind to the N-terminal Sema domain within the extracellular region of plexins, whereas the neuropilin receptor in this complex serves to stabilize the interaction between plexins and semaphorins (Janssen et al., 2012). The highly conserved neuropilins are single-pass transmembrane glycoproteins that contain a large aminoterminal extracellular domain (containing CUB, FV/FVIII, and MAM domain), a short membrane-spanning domain and a small cytoplasmic domain (Fig. 1) (Pellet-Many et al., 2008). The extracellular domain contains different motifs through which it cannot only bind to semaphorin ligands but also to other growth factors such as vascular endothelial growth factor (VEGF) A-D, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β, hepatocyte growth factor (HGF), placenta growth factor (PlGF)-2 and galectin. As a result, various signaling complexes can be generated and this versatility permits the neuropilins to be involved in a high number of signaling pathways. The approximately 40 amino acid intracellular domain lacks any recognizable functions, suggesting that neuropilin receptors cannot transmit signals on their own (Nasarre et al., 2014). As the neuropilin receptors NRP1 and NRP2 only have a 44% homology at the amino acid level, with the highest homology in the transmembrane domain, it is not surprising that they display different semaphorin binding patterns.

Finally, semaphorins may also signal independently from plexin receptors as demonstrated for SEMA7A, which can signal through β-integrins, for SEMA4A, which can functionally interact with T-cell immunoglobulin and mucin-containing protein 2 (TIM2), and also for SEMA4D that interacts with CD72 (Jongbloets and Pasterkamp, 2014, Worzfeld and Offermanns, 2014). Also SEMA3A may confer