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Review article

Bidirectional ephrin signaling in bone

Charles H. Rundle [a,b,*], Weirong Xing [a,b], Kin-Hing William Lau [a,b], Subburaman Mohan [a,b]

[a] *Musculoskeletal Disease Center, VA Loma Linda Healthcare System, 11201 Benton St, Loma Linda, CA 92357, USA*
[b] *Department of Medicine, Loma Linda University, Loma Linda, CA 92354, USA*

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Abstract

The interaction between ephrin ligands (efn) and their receptors (Eph) is capable of inducing forward signaling, from ligand to receptor, as well as reverse signaling, from receptor to ligand. The ephrins are widely expressed in many tissues, where they mediate cell migration and adherence, properties that make the efn-Eph signaling critically important in establishing and maintaining tissue boundaries. The efn-Eph system has also received considerable attention in skeletal tissues, as ligand and receptor combinations are predicted to mediate interactions between the different types of cells that regulate bone development and homeostasis. This review summarizes our current understanding of efn-Eph signaling with a particular focus on the expression and functions of ephrins and their receptors in bone.

Keywords: Ephrins; Signaling; Bone; Osteoblast; Osteoclast

1. Introduction

Ephrin ligands (also known as efn) are a family of proteins that serve as the ligands of ephrin receptors (Eph), which represent the largest known subfamily of receptor tyrosine kinases (RTKs). Both efn ligands and Eph receptors are membrane bound proteins that exert many important functions in a variety of tissues during development and adulthood. In bone, a number of efn ligands and Eph receptors are known to be expressed in many cell types and have been shown to play important roles in communication between osteoblasts and osteoclasts that regulate their differentiation [reviewed in Ref. [1]]. In addition to propagating Eph receptor mediated forward signaling, one unique property of the efn ligands is that they all have the capacity to initiate a "reverse" signal that is distinct from the "forward" signal associated with activation of their corresponding receptors. Efn-Eph signaling is also known to interact with other growth factor signaling pathways that have been implicated in skeletal development [2–4] and maintenance [5,6]. In this review, we will focus on expression patterns of efn ligands and Eph receptors in bone and their mechanism of action in regulating skeletal development and homeostasis.

2. Ephrin ligand and receptor families

The efn ligands are comprised of two subfamilies, the GPI-anchored efnA family and the transmembrane efnB family. Eph receptors (EphA and EphB) belong to the RTK superfamily. In general, efnA ligands bind to EphA receptors while, with a few exceptions such as EphA4 and A5, efnB ligands bind to EphB receptors. However, the affinities between ligand and receptor pairs can vary depending on the ligand or receptor (Table 1). Interaction of the efnA ligand with its receptor displays a "lock-and-key" binding, while efnB ligand binding proceeds through conformational changes that result in an "induced fit" with its receptors [7]. The promiscuity of binding suggests that there is

* Corresponding author. Musculoskeletal Disease Center, Research Service (151), Jerry L. Pettis Memorial Veterans Administration Medical Center, 11201 Benton St., Loma Linda, CA 92357, USA.
E-mail address: Charles.Rundle@va.gov (C.H. Rundle).
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Table 1
Mammalian ephrin ligand-receptor binding specificity.

Ligand		efnA						efnB		
		A1	A2	A3	A4	A5	A6	B1	B2	B3
Receptor	EphA1	++	–	–	–	–	–	–	–	–
	A2	++	+	+++	B	B	B	–	–	–
	A3	+	+++	++	++	+++	B	+	+	B
	A4	+++	++	++	B	+++	B	–	++	B
	A5	++	++	++	B	+++	B	–	–	–
	A6	B	B	B	B	B	B	–	–	–
	A7	++	+++	++	B	B	B	–	–	–
	A8	B	++	++	B	+++	B	B	B	B
	A10	ND	ND	ND	ND	ND	ND	ND	ND	ND
	EphB1	++	–	++	++	–	ND	++	++	B
	B2	–	–	–	–	–	ND	+++	+++	B
	B3	ND	–	ND	ND	ND	ND	+++	+++	++
	B4	ND	–	ND	ND	ND	ND	+	+++	B
	B6	ND	ND	ND	ND	ND	ND	B	B	B

Binding affinities determined by ligand-receptor binding assays [reviewed in 72].
+: low, Dissociation Constant (K_d) greater than 10 nM.
++: medium, K_d 1 nM–10 nM.
+++: high, K_d less than 1 nM.
–: no binding detected.
B: ligand-receptor binding observed, but no data on binding affinity [19,72].
ND: no data on binding [72].
EphA9 and EphB5 are avian. EphA10 is not yet characterized for ligand binding.

considerable biological redundancy in efn-Eph functions within the ephrin family. The complexity of signaling is also dependent on ligand and receptor expression in *cis* versus *trans* signaling, which can involve clustering of ligands and receptors into higher order structures within and between cells to further modulate signaling [8,9]. Interactions with other intracellular signaling pathways also occur. Signaling is terminated through the cleaving of the extracellular ligand-receptor domains or the endocytosis of the complex [reviewed in Ref. [10]]. Efn-Eph signaling is therefore complex and exerts multitude functions beyond simple expression and binding of cognate ligands and receptors.

The interaction of ephrin ligands and receptors can activate a bidirectional signal in which receptor (forward) or ligand (reverse) signaling activate downstream signaling cascades to produce various outcomes [reviewed in Refs. [11,12]]. Forward signaling is generally mediated through phosphorylation and activation of the receptor protein-tyrosine kinase activity. While reverse signaling is known to be mediated through the PDZ (postsynaptic density 95/discs-large/zona occludens-1) binding domain in efnB ligands, it is not well characterized in the efnA ligands, which lack an intracellular domain. EfnA ligand reverse signaling is therefore thought to proceed through associations with non-ephrin intracellular partners. These efn-Eph bidirectional signaling mechanisms can mediate complex functions within and between tissues, and are therefore of great interest in tissue development and repair.

3. Role of efn-Eph signaling in tissue development

Efn-Eph signaling is predicted to be an important mediator of tissue development and patterning, where efn-Eph interactions define the spatial boundaries between tissues and maintain segment boundaries. The efn-Eph system have been established to regulate both cell adherence and cell migration during development, and can act as attractants and repellants between cells within and between tissues, but these functions can depend on the cell context in which they are expressed [reviewed in Ref. [10]].

During embryonic development, efnB2 patterns the somites and regulates neural crest cell migration [13,14]; these structures contribute to the formation of a diverse set of tissues, including skeletal tissues. Efn-Eph regulation of neurogenesis has been one of the better studied areas of tissue development [reviewed in Ref. [15]], and several ephrin ligands and receptors expressed in neural tissues have been identified in bone. Studies in knockout mice identified efnB-EphB forward [16] and reverse [17] signaling as key in regulating axon fasciculation and guidance, respectively. The efnA family also regulates axon guidance in chick embryos. In this case the relative cell surface levels of efnA ligand and EphA receptor expression determine the migration of axon growth cones, with forward signaling generally inhibiting and reverse signaling generally promoting the growth cone survival [8]. It is probable that the efn-Eph regulation of neural tissue development might also be important for bone development, as the periosteum, which mediates bone growth and repair, is a highly innervated tissue.

Ephrin regulation of the vasculature has also received considerable attention, and is especially important in bone, a normally highly vascular tissue that must establish the blood supply during development and re-establish it under the hypoxic conditions that result from tissue injury. The ossification process itself requires the entry of osteogenic cells to the