Seminars in Cell & Developmental Biology 24 (2013) 172–178

Contents lists available at SciVerse ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Neuropilin signalling in vessels, neurons and tumours

Claudio Raimondi, Christiana Ruhrberg*

UCL Institute of Ophthalmology, University College London, 11-43 Bath Street, London EC1V 9EL, UK

ARTICLE INFO

Article history:
Available online 11 January 2013

Keywords:
Angiogenesis
Neuron
Neural crest cell
Cancer
VEGF
Semaphorin
Neuropilin

ABSTRACT

The neuropilins NRP1 and NRP2 are transmembrane proteins that regulate many different aspects of vascular and neural development. Even though they were originally identified as adhesion molecules, they are most commonly studied as co-receptors for secreted signalling molecules of the class 3 semaphorin (SEMA) and vascular endothelial growth factor (VEGF) families. During nervous system development, both classes of ligands control soma migration, axon patterning and synaptogenesis in the central nervous system, and they additionally help to guide the neural crest cell precursors of neurons and glia in the peripheral nervous system. Both classes of neuropilin ligands also control endothelial cell behaviour, with NRP1 acting as a VEGF-A isoform receptor in blood vascular endothelium and as a semaphorin receptor in lymphatic valve endothelium, and NRP2 promoting lymphatic vessel growth induced by VEGF-C. Here we provide an overview of neuropilin function in neurons and neural crest cells, discuss current knowledge of neuropilin signalling in the vasculature and conclude with a summary of neuropilin roles in cancer.

Contents

1. Introduction 172
2. VEGF-A and its receptors in the vasculature 173
3. Neuropilin signalling in endothelial cells 173
4. VEGF-A signalling through neuropilins in neurons 174
5. Semaphorins and their receptors 175
6. Semaphorin signalling through neuropilins in neurons 175
7. Semaphorin signalling through neuropilins in the vasculature 175
8. Semaphorin signalling through neuropilins in neural crest cell development 175
9. Neuropilin interactions with unconventional co-receptors 176
10. Neuropilin and its ligands in cancer 176
11. Conclusions 176
Acknowledgements 176
References 177

1. Introduction

During vertebrate development, shared families of growth factors and guidance molecules regulate the development of vascular and neural networks (reviewed in [1,2]). These include the netrins and their UNC5 and DCC receptors, slits and their robo receptors, ephrins and their Eph receptors, and semaphorins, which signal through plexins with or without their neuropilin co-receptors (reviewed in [1]). Additionally, the vascular endothelial growth factor VEGF-A regulates both neuronal and vascular development by acting through neuropilins and VEGF receptor tyrosine kinases (reviewed in [2]). Neuropilin (NRP) 1 was originally identified as an adhesion molecule in the nervous system, but is since studied mostly as the ligand binding subunit of the semaphorin 3A (SEMA3A) receptor in axon patterning and an endothelial co-receptor for the VEGF receptor tyrosine kinase VEGFR2 (reviewed in [3]).

NRP1 is a single-pass trans-membrane protein with an N-terminal extracellular domain that consists of two complement-binding homology domains, termed a1 and a2, two coagulation factor V/VIII homology domains, named b1 and b2, and a C domain that separates the b2 domain from the transmembrane domain

* Corresponding author. Tel.: +44 7608 4017; fax: +44 7608 6810.
E-mail address: c.ruhrberg@ucl.ac.uk (C. Ruhrberg).

1084-9521/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.semcdb.2013.01.001

and mediates interactions with other receptors (reviewed in [3]). Whilst the a1 and 2 domains are essential for semaphorin binding, the b1 domain promotes binding of the VEGF165 isoform of VEGF-A [4,5]. The NRP1 intracellular domain contains a PSD-95/DIg/ZO-1 (PDZ)-binding motif at its C-terminus, which binds synectin, also known as GAIP-interacting protein GIPC1 or neuropilin interacting protein NIP (reviewed in [3]).

NRP2 was identified based on its sequence homology to NRP1. NRP2 has two major alternative splice variants, NRP2A and NRP2B (reviewed in [3,6,7]). The amino acid sequence of its CUB, FV/FVIII and MAM domains is 45%, 48% and 35% similar to the corresponding domains of NRP1, respectively. The cytoplasmic domains of NRP1 and NRP2A also share 49% identity, and NRP2A carries a PDZ-binding motif at its carboxyl terminus, like NRP1. Both neuropilins exhibit different specificities for class 3 semaphorins. Whilst NRP1 predominantly binds SEMA3A and SEMA3C, NRP2 preferentially binds SEMA3F and SEMA3C, but also SEMA3B, SEMA3D and SEMA3E.

We now know that NRP1 and NRP2 are both essential for many different aspects of normal nervous system development, where they can exert complementary functions, for example by acting as semaphorin receptors on distinct subsets of neurons or neural crest cells (see below). They also act in different types of endothelial cells, as NRP1 is required for blood vessel patterning and normal lymphatic valve development, whilst NRP2 regulates the growth of lymphatic blood vessels, but not lymphatic valve development [8–10]. Recently, the boundaries between the neural and vascular roles of the neuropilins have become blurred, because neural roles for VEGF165/NRP1 and vascular roles for SEMA3A/NRP1 signalling have been identified (reviewed in [1,2]). Here, we discuss the contribution of NRP1 and NRP2 to neural development, vascular growth and cancer, with particular emphasis on NRP1 and its semaphorin and VEGF-A ligands in the vasculature.

2. VEGF-A and its receptors in the vasculature

The vasculature is the first organ to develop in the vertebrate embryo and provides a steady supply of oxygen to growing organs, thereby counteracting the limitations imposed on physical diffusion of gases in tissues. Several studies have demonstrated that a vascular supply is required to support the growth of tumours beyond 1–2 mm^3 (reviewed in [11]). This tissue volume corresponds in size approximately to a mouse embryo on embryonic day (E) 9.5 after conception. Accordingly, embryo and tumour growth alike depend on the formation and expansion of a functional vasculature to distribute oxygen and other blood constituents.

VEGF-A plays essential roles at all stages of vascular development and contributes to physiological and pathological blood vessel growth in adults, for example during wound healing, diabetic retinopathy and solid tumour growth (e.g. [12–14]). In particular, hypoxic tissue environments upregulate VEGF-A production and therefore neovessel growth and arteriogenesis, which is often accompanied by VEGF-A-stimulated vascular permeability. The specific signalling mechanisms that convey these different VEGF-A mediated responses are only poorly understood, but may benefit from the complexity of VEGF-A expression as several different isoforms (Fig. 1A), and the differential affinity of these isoforms for different VEGF-A receptors (Fig. 1B; reviewed in [15]).

The various VEGF-A isoforms are produced by alternative splicing from a single *VEGFA* gene comprised of 8 exons in the human genome (Fig. 1A) [16]. The main human VEGF-A isoforms are termed VEGF189, VEGF165 and VEGF121 (Fig. 1A), whilst the corresponding mouse isoforms are known as VEGF188, VEGF164 and VEGF120. The presence of exon 6 and 7 confers to VEGF-A the ability to bind heparin in vitro (Fig. 1A) and has been associated with the

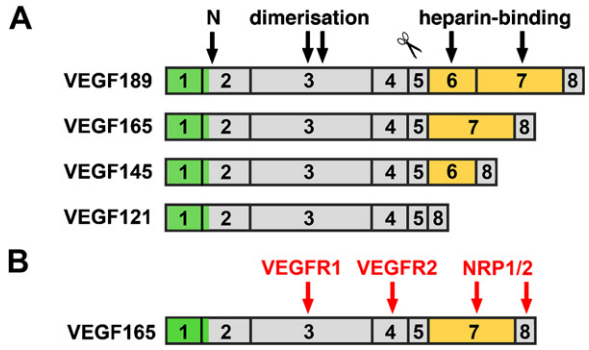

Fig. 1. The major human VEGF-A isoforms and their receptors. (A) The human *Vegfa* gene encodes three major splice forms, VEGF121, VEGF165, VEGF145 and VEGF189. The yellow boxes represent domains that can promote binding to heparin in vitro and are thought to mediate binding to heparin sulfate proteoglycans (HSPG) in the extracellular matrix (ECM) in vivo. VEGF121 does not possess these domains. Green indicates the signal sequence that is cleaved to release the mature protein. Black arrows indicate the N-terminus of the mature protein, the dimerisation sites and the heparin binding sites, respectively; scissors indicate protease cleavage sites that release ECM-bound VEGF-A isoforms. (B) The VEGF-A receptors tyrosine kinase VEGFR1 (FLT1) and VEGFR2 (FLK1/KDR) bind VEGF-A domains encoded by exon 3 and 4, respectively, whilst the binding sites for the non-tyrosine kinase neuropilin receptors NRP1/2 overlap with the heparin-binding VEGF-A domains; neuropilin binding additionally utilises the exon 8-encoded domain. Red arrows indicate the receptor binding sites. Because it lacks the domain encoded by 7, VEGF121 binds to the neuropilins with low affinity.

sequestration of VEGF-A to extracellular matrix (ECM). VEGF189 has the domains encoded by exon 6 and 7, VEGF165 contains the exon 7 domain and VEGF120 lacks both exons 6 and 7 (Fig. 1A). Thus, these isoforms distribute differentially in the environment of VEGF-A secreting cells in vitro [17,18] and in vivo [19].

All VEGF-A isoforms bind two tyrosine kinase receptors in endothelial cells, VEGFR2, which is the main signalling receptor in endothelial cells, and VEGFR1, which fine-tunes VEGF-A signalling during vascular development and additionally serves as a receptor for other VEGF family member, including VEGF-B and PGF (reviewed in [15]). The alternative name for VEGFR1 is FLT1, whilst VEGFR2 is also known as FLK1 or KDR. The VEGF-A isoforms bind VEGFR1 and VEGFR2 through distinct domains (Fig. 1B). VEGF165 also binds NRP1, but with lower affinity than VEGFR1 or VEGFR2 (Fig. 1B). VEGF189 was recently shown to also bind NRP1 [20]. In contrast, VEGF121's affinity for NRP1 is low when directly compared to that of VEGF165 (e.g. [21]). In agreement, expression of the VEGF120 isoform alone cannot rescue neural defects caused by loss of VEGF189 and VEGF164 in mice [22–24]. NRP2 can bind VEGF165 (Fig. 1B) and the less abundant VEGF145 isoform [25], but the physiological significance of VEGF-A binding to NRP2 is not known.

3. Neuropilin signalling in endothelial cells

VEGFR2 forms a receptor complex with NRP1 upon VEGF165 binding (reviewed in [15]). Complex formation is mediated by the exon 4-encoded cysteine knot motif of VEGF165, which contacts VEGFR2, and the exon 7/8-encoded C-terminal domain that contacts the NRP1 b1 domain (Fig. 1B). The cytoplasmic NRP1 tail is also essential for complex formation [26]. To investigate the structural requirements and identify functional roles for NRP1 and VEGFR2 interactions in vascular endothelial cells, many studies have taken advantage of porcine aortic endothelial (PAE) cells, which lack endogenous expression of either protein, but respond to VEGF165 when transfected with expression vectors for these VEGF-A receptors [26,27]. For example, co-expressing NRP1 and VEGFR2 in PAE cells showed that NRP1 enhances the VEGF165-induced phosphorylation of extracellular-signal-regulated kinase (ERK) and