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NRP1 function and targeting in neurovascular development and eye disease

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ABSTRACT

Neuropilin 1 (NRP1) is expressed by neurons, blood vessels, immune cells and many other cell types in the mammalian body and binds a range of structurally and functionally diverse extracellular ligands to modulate organ development and function. In recent years, several types of mouse knockout models have been developed that have provided useful tools for experimental investigation of NRP1 function, and a multitude of therapeutics targeting NRP1 have been designed, mostly with the view to explore them for cancer treatment. This review provides a general overview of current knowledge of the signalling pathways that are modulated by NRP1, with particular focus on neuronal and vascular roles in the brain and retina. This review will also discuss the potential of NRP1 inhibitors for the treatment for neovascular eye diseases.

Contents

Standard and non standard abbreviations: NRP1/2, neuropilin 1/2; VEGF, vascular endothelial growth factor; VEGFR1/2, vascular endothelial growth factor receptor 1/2; ERK1/2, extracellular signal-regulated kinases 1/2; AMD, age-related macular degeneration; PDR, proliferative diabetic retinopathy; ROP, retinopathy of prematurity; RVO, retinal vein occlusions; BRVO, branch RVO; CRVO, central RVO; DME, diabetic macular oedema; OIR, oxygen-induced retinopathy; CNV, choroidal neovascularisation; RPE, retinal pigment epithelium.

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Neuropilin 1 (NRP1) is expressed in many cell types, including neurons and blood vessels, and constitutive NRP1 knockout mice are embryonically lethal with both neural and vascular defects (Kawasaki et al., 1999; Kitsukawa et al., 1997; Lampropoulou and Ruhrberg, 2014; Schwarz and Ruhrberg, 2010). Since its discovery in 1987 as a cell adhesion molecule termed A5 in the frog nervous system (Takagi et al., 1987), an excess of 1775 PubMed citations have become available that have either examined the structure, expression or function of NRP1 in organ development or pathology. These studies have defined NRP1 roles in a range of signalling pathways that utilise diverse extracellular ligands. In particular, NRP1's ability to modulate vascular responses in tumour growth has sparked much interest in manipulating its function. Some of the emerging therapeutics to modulate NRP1 function have provided useful tools for experimental investigation and have clinical potential to treat ocular neovascular diseases such as retinopathy of prematurity (ROP), proliferative diabetic retinopathy (PDR) and age-related macular degeneration (AMD) (Foster and Resnikoff, 2005). This review provides a general overview of current knowledge of signalling pathways that are modulated by NRP1 and will discuss clinical potential of NRP1 targeting to treat neovascular eye disease.

1. Structure of the neuropilins (NRPs)

Both NRP1 and its homolog NRP2 are glycoproteins encoded by genes that are alternatively spliced into full-length transmembrane receptors and shorter soluble forms (Gagnon et al., 2000) (Fig. 1). The NRP1 transmembrane form is encoded by 17 exons and composed of an extracellular domain of about 840 amino acids, a single-pass transmembrane domain of 23 amino acids and a 44 amino acid cytoplasmic domain (Schwarz and Ruhrberg, 2010). The importance of NRP1 for a host of diverse developmental and pathological processes can be explained by its organisation into several structurally distinct domains (Fig. 1A) that mediate interactions with many different other proteins and are alternatively spliced. The extracellular NRP1 part consists of two domains called a1 and a2, which resemble the CUB (complement, Uegf, BMP) domain present in complement components. They are followed by the b1 and b2 domains, which are similar to coagulation factor V/VIII domains. The c domain, with homology to a MAM (meprin/antigen 5/receptor tyrosine phosphatase μ domain), separates the other extracellular domains from the transmembrane region. The short intracellular (cytoplasmic) domain is catalytically inactive, but contains a C-terminal SEA (serine-glutamine-alanine) motif that interacts with intracellular proteins containing a PDZ domain (Schwarz and Ruhrberg, 2010).

NRP2 was identified based on its homology to NRP1 (Chen et al., 1997). The amino acid sequences of the corresponding a, b and c domains of human NRP1 and NRP2 are 45%, 48%, and 35% identical (Fig. 1B). Two NRP2 alternative splicing variants exist, NRP2A and NRP2B (Gu et al., 2003; Nakamura et al., 2000; Schwarz and Ruhrberg, 2010) (Fig. 1B). Human NRP2A and NRP2B have identical a, b, and c domains, but their sequence differs after amino acid 808, which localises in the linker region connecting the c domain and transmembrane domains. The NRP2A cytoplasmic domain shares 53% identity with the NRP1 cytoplasmic domain and also has a SEA motif, whereas the NRP2B cytoplasmic domain lacks the SEA motif and is unable to interact with PDZ domain containing proteins (Rossignol et al., 2000).

Soluble forms of both NRP1 and NRP2 have also been described (Fig. 1A). The soluble forms s_{11}NRP1 and s_{12}NRP1 are encoded by the first 11 and 12 exons of the *NRP1* gene, respectively, and s_9NRP2 is encoded by the first 9 exons of the *NRP2* gene (Gagnon et al., 2000; Rossignol et al., 2000). More recently, transcripts for two additional soluble isoforms, named s_{III}NRP1 and s_{IV}NRP1, were identified in a human expression sequence tag (EST) clone library (Cackowski et al., 2004). s_{III}NRP1 contains the sequence encoded by the first 9 exons and exon 12, but skips exons 10 and 11, whereas the s_{IV}NRP1 mRNA contains the first 10 exons and exon 12, but lacks exon 11. As all soluble NRP1 isoforms lack the c, transmembrane and cytoplasmic domains (Fig. 1A), they can bind NRP1 ligands, but are unable to transduce signals and thus may serve as decoy receptors to sequester NRP1 ligands (Gagnon et al., 2000). Whilst soluble NRP1 is expressed in cells of the liver and kidney (Gagnon et al., 2000), little is known about its endogenous functions. In contrast, transmembrane NRP1, but not soluble NRP1 is expressed in blood vessels (Gagnon et al., 2000). Transmembrane NRP1 has been implicated in the development and function of numerous tissues, most notably blood vessels and neurons. The multiple neurovascular functions of the transmembrane isoform of NRP1 are therefore the main topic of this review.