EVOLUTIONARY AND FUNCTIONAL LINKS BETWEEN NEUROPILINS AND VEGF

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INTRODUCTION:

Neuropilins are single-pass transmembrane proteins that are implicated in a range of processes including vasculogenesis, angiogenesis, cell adhesion and migration, immunomodulation, and axon guidance. Molecular mechanisms of regulation of these processes involve neuropilins' interactions with the range of diverse ligands that are supporting neuropilins' pleiotropic functions. One of the best-characterized group of ligands of neuropilins includes vascular endothelial growth factors (VEGFs). In this context, it was shown that neuropilin-1 interacts with VEGF-A165 form via its b1 domain. At the same time, VEGF-A165 also binds to its cognate receptor (VEGF-R2) forming a ternary complex that is essential for regulating angiogenesis. While the functions of VEGF-A165 and its main tyrosine kinase receptor are well-understood independently of neuropilins, the specific role of neuropilin-1 in supporting angiogenesis and other processes remains to be fully explained. The presence of neuropilins and the formation of the ternary complex is indispensable in the development, with neuropilin-1 gene deletion in mice resulting in embryonic lethality. In adult humans, overexpression of neuropilins is however linked to tumor development and the levels of expression inversely correlate with tumor prognosis.

OBJECTIVES:

In this project, we aimed to identify the evolutionary origin of neuropilins and to correlate their evolutionary emergence with a lifestyle niche or developmental stage that would be dependent on the neuropilins' function. Furthermore, we aimed to infer mechanistic and functional details of neuropilins, VEGF and VEGF receptor interactions, by comparing their phylogenic trees and protein sequence conservation.

METHOD / DESIGN:

We designed the workflow which included BLAST searches with the functionally coherent domain fragments as queries, followed by sequence filtering and name conversion. These sequences were then aligned using MAFFT protocol based on fast Fourier transform. The well-aligned sequences were identified and selected with BMGE tool, and a phylogeny tree was generated using FastTree tool, all with the NG phylogeny server (https://ngphylogeny.fr). Analysis and annotation of trees were carried out in iTOL (https://itol.embl.de) and the sequence conservation was calculated using the Scorecons Server.

RESULTS:

After the filtering of the initial BLAST hits, more than 3000 neuropilin sequences were used to generate trees which showed that neuropilin-1 and neuropilin-2 diverged from an ancestral sequence at an early stage. From the VEGF-like sequences that were identified and examined only a subset contained exons 7-8 that are known to be required for interaction with neuropilin protein as described for VEGF-A165 isoform. The phylogenetic tree demonstrated early separation between members of VEGF family with the placental growth factor and VEGFA, separated earlier from the clades containing VEGFB, VEGFC and VEGFD. While VEGF homologues are also identified in non-chordate invertebrates, full-length neuropilin proteins are only found in vertebrates.

CONCLUSIONS:

The earliest full-length neuropilins would have been present in cartilaginous fish, which are considered to have descended from some of the most ancient vertebrates, while VEGF homologues arose much earlier as they are found in various invertebrates such as insects but also the lancelets. As lancelets also contain some neuropilin-like sequences, it is speculated that gene fusion events from proteins in these 'fish-like' species could have led to the evolution of neuropilin. Conservation analysis of the b1 domain indicates that the early neuropilins could have been capable of mediating angiogenesis. Our findings suggest that the emergence of neuropilins correlates with the evolution of endothelial cells and that VEGF-dependent endothelial angiogenesis was necessary for the survival of the earliest jawed/jawless vertebrates. Further analysis should also include phylogenetic analysis of semaphorins that interact with neuropilins in a process of axon guidance as well as consideration of neuropilin coreceptors.

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