Neuroimmune guidance cues in vascular (patho)physiology

Vreeken, D.

Chapter 1

General introduction and scope of the thesis

1. General introduction

1.1 The vascular system

With a total length of approximately 100.000 kilometers, our vascular system enables transport of blood to every part of our body. The vascular system, comprised of the arteries, veins and capillaries, is a closed system that delivers oxygen and nutrients to, and removes waste products from tissues. While the capillaries are composed of a single layer of endothelial cells stabilized by pericytes, arteries and veins have three distinct layers. From the luminal side, the first layer, the tunica intima, is comprised of a single layer of endothelial cells covered by a layer of glycoproteins, the glycocalyx. The middle layer, the tunica media is composed of vascular smooth muscle cells and elastic tissue that provide support and can regulate blood flow and pressure. The precise composition of this layer depends on the location of the vessel. The outermost layer, the tunica adventitia, is a layer of fibro-elastic connective tissue on the outer side of the vessel providing anchorage and protection of the vessel (1).

Being the first interface between blood and surrounding tissues, endothelial cells are critical in regulating and maintaining tissue homeostasis. The endothelium regulates processes like vascular tone, coagulation, inflammatory and immunological processes, endothelial permeability to plasma proteins and cells and tissue regeneration and remodeling. Endothelial dysfunction, characterized by a pro-inflammatory and procoagulant phenotype and decreased availability of nitric oxide, can result in impaired endothelial function. Dysfunction of the endothelium including increased vascular permeability and loss of vascular tone, can cause a diversity of pathologies such as cancer, diabetes mellitus and cardiovascular disease including atherosclerosis (1-3).

1.2 Atherosclerosis

Atherosclerosis is a slow progressing pathophysiological process often resulting in cardiovascular disease (4), one of the leading causes of death worldwide (5). Atherosclerotic lesions can develop at sites of a dysfunctional endothelium and are characterized by the deposition of oxidized fractions of low-density lipoproteins (ox-LDL) within the vessel wall. The local inflammatory response activates endothelial cells and enhances the recruitment of monocytes that engulf and clear up the LDL deposits. Excessive amounts of LDL deposits result in the formation of dysfunctional lipid-laden foam cells that are retained within the developing plaque. The chronic inflammatory state of the area promotes continued growth of the atherosclerotic plaque, eventually resulting in a necrotic lipid core covered by a fibrous cap composed of vascular smooth muscle cells and extracellular matrix. Ultimately, rupture of (unstable) plaques or

occlusion of an artery by the atherosclerotic plaque cause cardiac events such as ischemia or stroke (6-8). The development of atherosclerosis can often be contributed to one or more traditional risk factors, such as dyslipidemia, age, smoking, diabetes mellitus, hypertension, obesity or a family history of cardiovascular disease (9). However, a substantial amount of environmental and genetic risk factors remain unaccounted for.

1.3 Neuroimmune guidance cues

In the past decades, evidence is accumulating indicating an important role for neuroimmune guidance cues (NGCs) in inflammation and vascular biology. NGCs, also known as neuronal or axonal guidance cues, were originally discovered during embryonic development, where they are involved in the patterning of the nervous system as well as the vascular system. Functioning as attractive and/or repulsive cues, NGCs modulate cell growth and migration and therewith 'guide' the development of neuronal and vascular networks (10, 11). More recent studies, suggest that NGCs go beyond embryogenesis and remain important in such processes post-embryonically (7, 12, 13).

NGCs comprise four major families, the Ephrins, Netrins, Semaphorins and Slits that include around 70 proteins (Figure 1). In humans the erythropoietin-producing hepatocellular receptors (EPHs) and their EPH receptor interacting protein (Ephrin) ligands comprise a large family of receptor tyrosine kinases with 14 EPH receptors and 8 Ephrin ligands that are all membrane bound (Figure 1A). The Ephrin ligands are subdivided into class A and class B based on their GPI anchor or transmembrane domain, respectively. The EPH receptors are also subdivided into class A and B based on their ligand binding preference. In addition to inducing receptors signaling (forward signaling), binding of an ephrin ligand to an EPH receptor also induces ligand signaling (reverse signaling) (14, 15). The Netrin family consists of a total of 4 secreted and 2 GPI-anchored Netrin (NTN) ligands (Figure 1B), in human. NTNs induce signaling through different receptors including Neogenin, deleted in colorectal cancer (DCC) and UNC5A-D (16). The Semaphorin family comprises a large group of 20 Semaphorin ligands (SEMAs) that are subdivided in Classes 3 to 7 (in human). Class 4-7 are membrane bound through either a transmembrane domain or a GPI-anchor. Class 3 Semaphorins are secreted proteins (Figure 1C). Semaphorin signaling mainly occurs via membrane-bound plexin receptors (PLXNs), which are subdivided in classes A-D. Class 3 secreted Semaphorins also need Neuropillins (NRPs) as a co-receptor to mediate signaling. The Slits (SLITs) are a relatively small family consisting of 3 secreted ligands (Figure 1D). Slit

ligands can initiate signaling by binding to one of the 4 transmembrane Roundabout (ROBO) receptors, in human (17).

NGC ligand-receptor interactions can regulate a variety of downstream signaling pathways, including small GTPases and focal adhesion kinases, of which most are involved in regulation of the cellular cytoskeleton. By regulating the cellular cytoskeleton, NGCs are known to be involved in processes such as cell morphology, cellular adhesion (cell-cell and cell-matrix), cell migration and proliferation (11, 18).

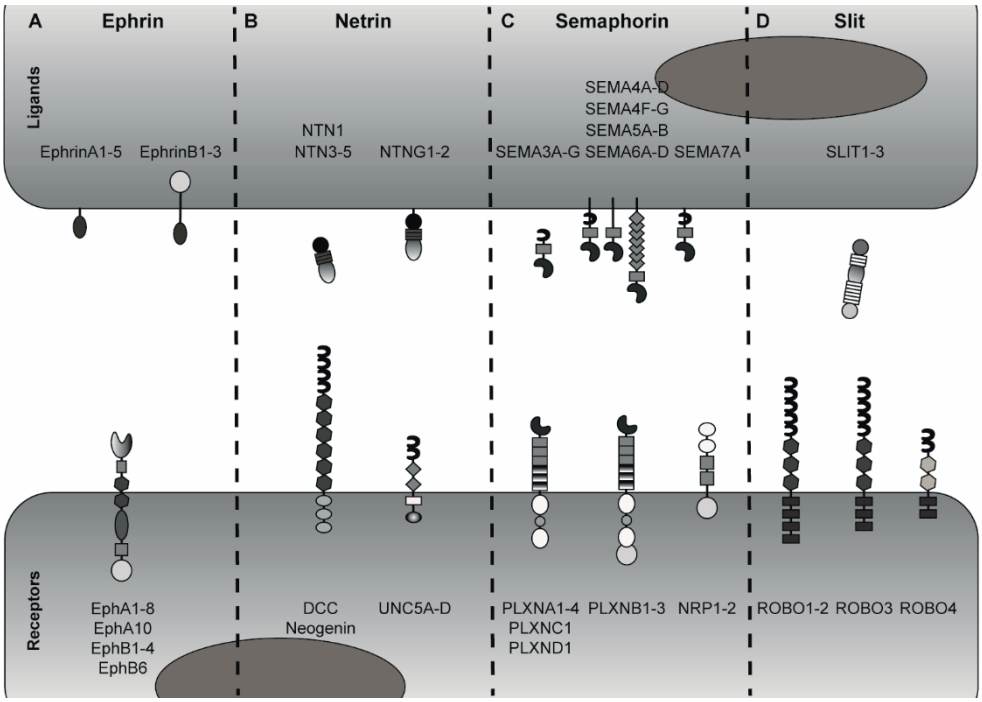

Figure 1: Schematic overview of vertebrate Neuroimmune Guidance Cues. (A) Ephrin ligands are either GPI-anchored (Class A) or transmembrane (Class B) proteins that interact with EphA or EphB receptors. (B) Netrin ligands (soluble or GPI-anchored) can bind to different receptors, including DCC, Neogenin and UNC5 receptors. (C) The Semaphorins exist as soluble, transmembrane and GPI-linked ligands that can interact with Plexin receptors. In some cases Neuropillins are required as co-receptors to initiate signaling. (D) Secreted SLIT ligands interact with transmembrane ROBO receptors.

1.4 Neuroimmune guidance cues in atherosclerosis

Due to its effect on a variety of cellular processes, NGCs have been implicated in several diseases including (cardio)vascular disease. In endothelial cells, expression of NGCs is different in regions that easily develop atherosclerosis compared to regions that are protected against atherosclerotic plaque development. For example, the disturbed

flow in atheroprone regions decreases the expression of NTN-1 by endothelial cells, thereby negating NTN-1's anti-adhesive and -migratory effect on leukocytes (19). In contrast, SEMA7A expression is increased in areas with disturbed flow and knockdown of this protein in mice reduced leukocyte adhesion and atherosclerotic plaque formation (20). NGCs are also affected by inflammation, but can also influence inflammation themselves. Endothelial activation with proinflammatory cytokines, for example, increases the expression of the EPHA2 receptor and the EphrinA1 ligand, while induction of EPHA2 signaling induced expression of proinflammatory genes (21), suggesting a role in atherosclerotic plaque formation. Indeed, knockdown of EPHA2 in APOE-/- mice, diminished atherosclerotic plaque formation not only by reducing pro-inflammatory gene expression and monocyte adhesion but also by regulating smooth muscle cell proliferation and extracellular matrix deposition (22). More other NGCs, have been shown to modulate leukocyte adhesion and migration and endothelial cell function (23-27). In addition, a number of NGCs, including EPHA2, EPHB2 and SEMA3E have been detected in mice and/or human plaque tissue (21, 23, 25), further supporting a role for NGCs in the pathogenesis of atherosclerosis.

2. Scope of this thesis

Despite the fact that NGCs are emerging as regulators of inflammation and vascular (patho)physiology, many questions are still unanswered. Only a small subset of NGCs have been investigated leaving a potential role for other NGCs unexplored. In addition, many of the studies so far have been performed in animals and little human validation is available. Therefore, this thesis aims to explore the role of NGCs in human (patho)physiology of the vascular system by 1) studying the expression of NGCs in (atherosclerosis-related) cells under pro-inflammatory conditions, 2) exploring the role of NGCs in cellular function and atherosclerosis-related processes and 3) validate the effect of genetic variations in NGCs discovered in a patient cohort with unexplained premature atherosclerosis. The results obtained from these studies could identify NGCs as potential novel therapeutic targets for cardiovascular disease. ***Chapter 2*** starts with an overview of the expression of neuroimmune guidance cue genes in endothelial cells and monocytes under inflammatory conditions. In addition, an important role for PLXNA4, which expression is decreased upon inflammation, in endothelial cell function is described. ***Chapter 3*** gives an overview of the function of Ephrin ligands and Eph receptors in adult endothelial cell biology, summarizing the currently available data of the effect of Eph family members on proliferation, migration, barrier formation and cell-cell interactions in health and disease. In ***Chapter 4*** the (atherogenic) role of EphB2, which is increasingly expressed in more severe atherosclerotic lesions, in monocyte

function is explored. After establishing involvement of NGCs in inflammation-related processes and vascular (patho)physiology, ***Chapter 5 and 6*** focus on unreported genetic variations in neuroimmune guidance cue genes in families suffering from premature atherosclerosis and their potential implications for the disease. In ***Chapter 7*** the results of my studies are summarized and discussed and future perspectives are provided.

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