Neuroimmune guidance cues for vascular health

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Chapter 1

General introduction and scope of the thesis

1.1 Current cardiovascular disease burden and intervention strategies in brief

Despite improvements in surgical and drug treatment, cardiovascular diseases (CVD) remain the leading cause of death in modern societies. CVDs account for 3.9 million death or 45% of all death in Europe with cost of €210 billion on economy per year [1]. A major part of CVD is attributable to atherosclerosis. Correcting dyslipidemia (mostly lowering blood low density lipoprotein (LDL) cholesterol levels) and mitigating immune response are the 2 main strategies to attenuate risk for vascular mortality caused by atherosclerosis [2, 3]. The most successful and widely used intervention is inhibition of endogenous cholesterol synthesis by targeting HMGCoA (β-hydroxy β-methylglutaryl-coenzyme A) reductase using statins. Monoclonal antibody for PCSK9 can further decrease LDL levels [4]. However, there is still substantial risk after LDL levels are corrected, prompting the need for managing inflammatory response in atherosclerosis.

Comparing to LDL cholesterol lowering strategy, evidence of efficacy of interventions targeting immune response is still emerging. IL-1β antibody canakinumab was shown to reduce cardiovascular disease endpoint by 16% [5]. Nonetheless, there was the side-effect of increasing lethal infection episodes, resulted from impaired host defense. On the other hand, patients with atherosclerosis could display normal blood cholesterol levels [6]. Moreover, it is well-known that atherosclerosis lesions profoundly affect vascular bifurcations and areas with low wall shear stress, despite universal exposure to the one's blood lipid levels, suggesting an independent role of endothelial dysfunction in atherogenesis [7]. The above evidence has motivated research efforts to gain deeper insights in endothelial dysfunction and inflammatory responses in pathogenesis of atherosclerosis to further tackle the residual risk after correction of dyslipidemia. A series of recent research has identified novel functions of neuronal guidance cues in atherogenesis and related pathophysiological process (reviewed in detail in **Chapter 2**).

1.2 Pathogenesis of atherosclerosis: a brief recall

Atherosclerosis, literally meaning "artery hardening" from Greek, is the complex pathology of formation of plaques on the artery walls. The pathological process starts as early as teenage with forming of initial lesion, featured by continued exposure of the endothelium to metabolic and hemodynamic risk factors leading to a state of chronic systemic activation

of the endothelial cells [8]. Endothelial dysfunction results in enhanced macrovascular endothelial permeability, an event that augments low-density lipoprotein (LDL) accumulation in the subendothelial space. Oxidation of LDL leads to further activation of the endothelium and potentiation of pro-inflammatory responses, including upregulation of various adhesion molecules and chemokines that actively recruit inflammatory cells like monocytes [9]. Recruited monocytes can differentiate to macrophages with stimulation of various inflammatory cytokines and other regulatory factors. The monocytes and macrophages can endocytose the pro-inflammatory oxidized lipids, leading to lipid-laden cells called "foam cells" (initial lesion – fatty streak). Following the years, the lesions grow mainly by thickening of intima by deposition of lipoprotein by formation of "foam cells" and later proliferation of vascular smooth muscle cells. The foam cells in the plaques can eventually die via apoptosis or necrosis process, allowing forming of extracellular lipid core (intermediate lesion - fibroatheroma). The plaques can further growth in size, fibrosis content and histological compositions into fibrous plaques and complicated lesions. Atherosclerosis remains largely asymptotic before the size of atherosclerotic lesion causes significant stenosis, formation of atherothrombosis or plaque rapture, causing decrease or sudden loss of perfusion to the organs. Stenosis in coronary artery leads to angina due to the restrict blood supply, while occlusion of coronary artery or arteries in brain causes myocardial infarction or stroke, respectively. Essentially, pathways involved in endothelial function and dysfunction, monocyte-macrophage biology are likely to be involved in development of atherosclerosis.

1.3 What are neuronal guidance cues?

Neuronal guidance cues (NGCs) are proteins originally identified in the nervous system for their ability to directing growth of axons to the correct targets. Depending on the context, they are also called neuronal guidance proteins (to specify the molecular property), axonal guidance cues (to specify the original function) or even neuroimmune guidance cues (to emphasize on their later-found roles in immunology). It is generally accepted that NGCs consist of 4 families of ligands, namely netrin, semaphorin, slit and ephrin (Reviewed in [10]). The ligands bind to their various receptors with different specifies and affinities, exerting complex downstream signals to the target cells (See **Table 1** for a list of NGC ligands and receptors). A large part of the downstream signals results in altered activities of small-GTPases, which in turn regulates organizations of cytoskeletons: the underlying mechanism of repulsive or attractive effect of NGCs.

Class	Ligand	Receptor
Semaphorin	SEMA3A-3G SEMA4A-4G SEMA5A-5B SEMA6A-6D SEMA7A	PLXNA1-A4 PLXNB1-B3 PLXNC1 PLXND1
Netrin	NTN1 NTN3-5 NTNG1-2	UNC5A-D DCC DSCAM MCAM
Ephrin	EFNA1-A5 EFNB1-B3	EPHA1-A8 EPHA10 EPHB1-B4 EPHB6
Slit	SLIT1-3	ROBO1-3

Table 1. List of NGC ligands and receptors

1.4 Scope of this thesis

The nervous and vascular systems of vertebrates share many similarities and often overlap in anatomy. These notions led to one of the major recent insights in developmental vascular biology being that the coordinated patterning of nerves and vessels is achieved by each system separately using the same cues and signals [9]. Next to roles of NGCs during development, functions of NGCs have been described in both mature endothelium and immune cells, which is the focus in this thesis. With the knowledge in this thesis, I demonstrate the relevance of NGCs in vascular biology from bench to bedside. Previous knowledge on NGCs showed that NGCs are regulated by hemodynamic conditions and inflammations, thereby affecting endothelial cell function. In **Chapter 2**, a detailed literature review of functions of semaphorins and netrins in mature endothelial cells is given as the background of NGC research in endothelial cells. Next, in **Chapter 3** and **Chapter 4** are the studies describing the regulation and function of SEMA3F and NTN4 in mature endothelial cells, 2 of most abundantly expressed NGCs in endothelial cells. We tried to push the boundary by understanding the mechanism underlying the function of SEMA3F and NTN4. We describe a role for both SEMA3F and NTN4 in preventing endothelium dysfunction and trans-endothelial migration of monocytes, 2 critical mechanisms in development of early atherosclerotic lesions. NGCs have also been found to play a role in monocyte biology. In this regard, I move on to a comprehensive analysis of NGC gene expression and change of NGC pathway during monocyte to macrophage differentiation in **Chapter 5**. I first quantified regulation of NGC expression during monocyte to macrophage differentiation. And secondly, the role of the RNA-binding protein quaking, a regulator of monocyte-macrophage function, in regulation of NGC expression was investigated and discussed. In **Chapter 6**, I seek to prove the clinical relevance of NGCs by building predictive modeling models using NGC expression in circulating monocytes to infer the health status of CVD patients. Finally, in **Chapter 7**, I discuss the results from my studies in relevance to the current knowledge and give directions for future research.

References

1. *Timmis, A., N. Townsend, C. Gale, et al., European Society of Cardiology: Cardiovascular Disease Statistics 2017. Eur Heart J, 2018. 39(7): p. 508-579.*

2. *Bertrand, M.J. and J.C. Tardif, Inflammation and beyond: new directions and emerg-*

ing drugs for treating atherosclerosis. Expert Opin Emerg Drugs, 2017. 22(1): p. 1-26.

3. *Bruikman, C.S., R.M. Stoekenbroek, G.K. Hovingh, et al., New Drugs for Atherosclerosis. Can J Cardiol, 2017. 33(3): p. 350-357.*
4. *Robinson, J.G., M. Farnier, M. Krempf, et al., Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. N Engl J Med, 2015. 372(16): p. 1489-99.*
5. *Ridker, P.M., B.M. Everett, T. Thuren, et al., Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med, 2017. 377(12): p. 1119-1131.*
6. *Fernández-Friera, L., V. Fuster, B. López-Melgar, et al., Normal LDL-Cholesterol Levels Are Associated With Subclinical Atherosclerosis in the Absence of Risk Factors. Journal of the American College of Cardiology, 2017. 70(24): p. 2979-2991.*
7. *Souilhol, C., J. Serbanovic-Canic, M. Fragiadaki, et al., Endothelial responses to shear stress in atherosclerosis: a novel role for developmental genes. Nat Rev Cardiol, 2020. 17(1): p. 52-63.*
8. *Deanfield, J.E., J.P. Halcox, and T.J. Rabelink, Endothelial function and dysfunction: testing and clinical relevance. Circulation, 2007. 115(10): p. 1285-95.*
9. *Ross, R., Atherosclerosis–an inflammatory disease. N Engl J Med, 1999. 340(2): p. 115-26.*
10. *Melani, M. and B.M. Weinstein, Common factors regulating patterning of the nervous and vascular systems. Annu Rev Cell Dev Biol, 2010. 26: p. 639-65.*