White matter really matters in cerebral small vessel disease

Chapter 7

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Impact

Widespread risk factors such as hypertension can lead to functional and structural changes of the cerebral small blood vessels, ultimately leading to development of cerebral small vessel disease (cSVD) [1]. It is difficult to estimate the exact prevalence of cSVD, as the disease is often asymptomatic, underdiagnosed, and heterogeneous, and often presents with several comorbidities. The disease contributes to approximately 25% of strokes and 45% of dementia cases, causing a substantial global social and economic burden [2]. After ageing, hypertension as well as most of the other risk factors associated with cSVD are modifiable (e.g. smoking, diabetes, physical inactivity), which gives rise to a window of opportunity for potential prevention and treatment [3, 4]. However, primary prevention therapies are lacking as cSVD progression is usually asymptomatic and diagnosis relies on MRI markers, while primary treatment is based on late-stage symptom relief [3]. Thus, the aim of this thesis was the identification of cellular and molecular mechanisms that may ultimately be used for the prevention and treatment of cSVD. This thesis particularly focuses on the interplay between oligodendrocytes precursor cells (OPCs) and vascular endothelial cells (ECs). Unravelling mechanisms involved in this interplay not only clarifies current knowledge gaps, but also paves the way for new diagnostic strategies, including fluid biomarkers, and treatment options to tackle the growing global cSVD burden.

Scientific impact

In Chapter 2, our systematic review revealed Wnt7a as a potential mediator of OPC-EC crosstalk in cSVD pathology. Studies demonstrated that hypoxia increases the expression and secretion of Wnt7a by hypoxic OPCs and thereby mediates EC proliferation and angiogenesis [5–9]. Furthermore, Wnt7a is also known to promote the migration of OPCs by utilising the vasculature through inducing CXCL12-CXCR4 signalling pathway [5, 10, 11]. However, our findings in Chapter 3 and 4 are partly contradicting these previous findings. In Chapter 3, Wnt7a decreased the expression of TJ proteins and increased blood-brain barrier (BBB) permeability through activation of β-catenin, which contradicts studies showing the Wnt/β-catenin mediated increase in these TJ proteins and the decrease in BBB permeability [12, 13]. It is thus important that future studies investigate these findings and unravel its involvement in pathological mechanisms leading to BBB impairments and white matter lesions (WMLs) seen in cSVD patients [14].

In vitro studies utilising human pluripotent stem cells (hPSCs) hold the potential to elucidate these discrepancies, as they provide a valuable tool for disease modelling. Additionally, OPC migration via the vasculature has previously been shown to be mediated by increased CXCL12-CXCR4 interaction as a consequence of Wnt7a stimulation [10], which also contradict our findings in Chapter 4 where we found decreased *Cxcl12* expression. Genetic mouse models for Wnt7a knockdown can elucidate these findings in vivo. A previous study generated a Wnt7a/b-deficient mutant mouse by intercrossing Olig2-Cre to conventional Wnt7a null and conditional Wnt7b (*fl*/*fl*) alleles to study the role of OPC-derived Wnt7a/b in development and hypoxic injury [8]. Inducing hypoperfusion with BCAS in similar models could lead to better understanding the role of OPC-derived Wnt7a in BBB permeability and OPC migration, and its implication in hypoxic brain injury.

Lastly, our in vitro findings in Chapter 5 indicate that hypoperfusion mediated hypoxia in OPCs leads to increased VEGFA rather than Wnt7a expression, supporting other studies that did not find Wnt7a regulation in OPCs under hypoxic conditions [15, 16].

Although these findings may identify novel molecular pathways relevant to cSVD development, addressing knowledge gaps regarding the underlying dysfunctional mechanisms contributes to the complexity of cSVD pathology.

Clinical implications

Highlighting the clinical implications of research on cSVD is crucial given that treatment strategies primarily focus on symptom relief rather than addressing the underlying cause. Efficacy of current treatments may be compromised due to the presence of multiple risk factors and the various conditions contributing to disease development. Understanding molecular mechanisms in cSVD can advance diagnosis with biomarkers, improve cognitive outcomes through novel prevention and treatment strategies, and enable personalised therapies targeting high-risk individuals. Current approaches for the prevention and treatment of cSVD encompass a combination of pharmacotherapy and lifestyle modifications. Pharmacotherapy involves the use of various medications, blood pressure-, cholesterol-, and glucose-lowering drugs, aimed at limiting the impact of cardiovascular risk factors. Additionally, anti-dementia drugs, like the NMDA-receptor partial antagonist memantine and cholinesterase inhibitors, as well as drugs such as cytidine-diphosphocholine and dl-3-n-butylphthalidle may be utilised [17]. Memantine can improve cognitive function by antagonising glutamate-induced neurotoxicity [18, 19], while cholinesterase inhibitors increase the availability of acetylcholine, an important neurotransmitter associated with memory, in neuromuscular junctions by inhibiting its enzymatic breakdown [20, 21]. Cytidine-diphosphocholine show neuroprotective effects by increasing noradrenaline and dopamine levels in the central nervous system (CNS) [22]. Dl-3-n-butylphthalidle can improve outcome after stroke due to its vasodilative effects by promoting NO production of ECs, its anti-thrombotic effects, or most importantly, by upregulating VEGFA and HIF1α expression [23].

More recently, clinical trials assessing efficacy of drugs in restoring cerebral blood flow (CBF) and limiting blood flow pulsatility, with the use of e.g. cilostazol, isosorbide mononitrate, tadalafil, and pentoxifylline, are currently ongoing. In conjunction with pharmacotherapy, lifestyle modifications play a significant role in cSVD management. Quitting smoking, maintaining a healthy diet, and engaging in aerobic exercise are crucial for reducing risk factors and promoting cardiovascular well-being [24]. Taken together, this emphasising the importance of fundamental research in elucidating the link between vascular pathology and cognitive impairment in cSVD.

Wnt7a signalling modulation

Modulating the Wnt7a signalling pathway might be a potential therapeutic strategy, as our results show that Wnt7a stimulation could lead to EC permeability and suggest a role in OPC migration. Modulating these processes post-stroke might be beneficial for patients to reduce ischaemic injury and facilitate remyelination in damaged regions. Recently, clinical trials have explored novel approaches to modulate Wnt signalling pathways by combining technologies to cope with the pleiotropic nature of Wnt, which plays diverse roles in various biological processes [25]. Moreover, pre-clinical advancements demonstrate promising outcomes by utilising Wnt7a derivatives as a potential approach to mitigate CNS disorders by repairing the BBB. These derivatives specifically target Gpr124/Reck signalling and show neurovascular protective properties in stroke and glioblastoma models in mice without Wnt activation in other tissue [26]. However, Wnt signalling-targeting drugs are not clinically available yet because of associated side effects, and thus other therapeutic strategies must be considered.

VEGFA signalling modulation

As previously mentioned, the mechanism of action of dl-3-n-butylphthalidle includes modulation of ECs and VEGFA and HIF1α expression [23]. Thus, offering a potential target to promote vascular repair, inhibit brain inflammation, and prevent white matter (WM) damage by exploring the role of VEGFA in the interaction between ECs and OPCs. Following up on our retrospective findings in Chapter 5 with large cohort prospective studies that measure BBB permeability in different WM regions and VEGFA blood plasma concentration overtime could reveal an association between WML development and VEGFA blood plasma concentration. Blood plasma levels of VEGFA might then potentially be used as a biomarker for the early diagnosis for risk of WML development. It would also present a potential target for modulating VEGFA to decrease the risk of WML development. However, it is essential to acknowledge the potential dual nature of modulating VEGFA signalling. While beneficial angiogenic properties may facilitate compensatory vascular network growth in the brain, reducing hypoxia and CBF deficits, there is a risk of increased BBB permeability due to VEGFA ability to loosen tight junctions, which might ultimately contribute to inflammation, and WM damage [27, 28]. Another potentially adverse aspect of stimulating angiogenesis by systemic interventions is the risk of promoting the vascularisation of any malignancies that may be present in the patient. A solution would be the local delivery to the brain or specific modulation of VEGF receptors in the brain. The use of VEGFA isoforms, which elicit different biological effects, has been suggested as a potential solution for these unwanted side effects [28]. Thus, the therapeutic potential of VEGFA must be considered carefully.

Timing of VEGFA administration is also critical. Angiogenesis induction in the early silent stages of cSVD may help prevent ischaemic injury, while post-stroke stimulation of new vessel formation and increased BBB permeability could enhance the inflammatory response and contribute to WML progression and cognitive decline, with previous studies indicating an association between VEGFA levels and stroke severity [29, 30]. Preclinical studies involving VEGFA administration

have shown promising effects on stroke recovery, such as reducing lesion size, decreasing infarct size, promoting angiogenesis, and improving cognitive function. However, the effectiveness of VEGFA modulation in humans is yet to be proven [28, 31, 32].

Future directions

Future studies should prioritise the validation of the hypothesis regarding OPC-derived VEGFA in the interaction between OPCs and ECs in cSVD. By investigating the specific intracellular signalling pathways associated with VEGFA, such as HIF1α/VEGFA and Wnt/VEGFA, we can gain insight into the underlying mechanisms driving VEGFA-related changes in the brain vasculature and WM, which contribute to the development of cSVD. Manipulating these pathways using more accurate and sophisticated preclinical in vivo and in vitro models will allow the identification of novel biomarkers and the development of targeted therapeutic strategies. To facilitate the translation of preclinical findings into clinical applications, further research should focus on conducting well-designed clinical trials to test the safety and efficacy of VEGFA signalling modulation-based therapies. Ultimately, these efforts have the potential to improve patients' quality of life.

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