Molecular and mechanical functions of the intermediate filament protein GFAP

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Scope and Outline
The brain is an extremely complex organ, consisting of a diversity of neuronal and non-neuronal cells. Each cell type plays its specific role, with various classes of neurons specialized in rapid communication, oligodendrocytes providing myelination, and astrocytes involved in maintenance of blood brain barrier, energy homeostasis, neurotransmitter homeostasis, and modulation of neuronal signalling. For all these neural cell types there are stem and progenitor cells in varying degrees of multipotency/differentiation, involved in the maintenance and plasticity of the brain and of great interest for potential therapeutic applications. In addition to these cells of the neural lineage, there are microglia providing the brain's innate immune system and endothelial cells forming vessels for blood perfusion.

This thesis is centred on astrocytes, which is a main type of macroglia. Astrocytes play an important role in the communication between the different cell types in the brain and are considered as integrators of information in the brain. These cells are known to react to brain injury and are activated in neurodegeneration (i.e. reactive gliosis), which coincides with a change in shape and function. It is now recognized that astrocytes are a heterogeneous cell population, and different classes are being identified. A clear subtype are the astrocytes in the neurogenic niches, which are the stem cells of the adult brain. An accumulation of genetic mutations in these cells during cell division is thought to be an origin of astrocytic brain tumours.

A hallmark protein of astrocytes is the cytoskeletal protein Glial Fibrillary Acidic Protein (GFAP), which is highly upregulated in reactive gliosis. GFAP is a member of the intermediate filament family and its gene encodes at least 10 different splice isoforms. One of these isoforms, GFAPδ, is enriched in stem cell niches in the human brain, whereas the canonical GFAPα isoform is expressed in astrocytes throughout the brain. Both isoforms have also been described in astrocytoma, and they have been linked to tumour malignancy. This thesis describes the role of GFAP in astrocyte biology in health and disease from cell biological, molecular biological, and biophysical perspectives.

In Chapter 1 we reviewed GFAP and other members of the intermediate filament family as integrators of mechanics, cell-cell communication and key molecules in shaping the structure of complex tissues. GFAP is a highly regulated gene and a prominent marker protein of astrocytes in brain injury and pathology.

In Chapter 2 we studied the transcriptomic changes in an astrocyte cell line in an in vitro injury model. Surprisingly, we found that the injury model we applied (stretching cells) only induced minor transient changes in the cell astrocytoma cells. To pinpoint what could be the cause of this low response, we varied the extracellular
matrix coating of the injury model, we attempted to induce injury response by means of purinergic signalling, and we studied primary human astrocyte response to injury. However, none of the conditions showed the expected astrocytosis response.

To gain more insight in the changes in cells induced by modulation of the GFAP network, we performed functional studies on cells with a specific recombinant expression of GFAP isoforms in Chapter 3. Here we showed that GFAPδ has a very prominent effect on the state of the IF-network, as at high expression levels the network is disrupted and the intermediate filaments form a juxtanuclear collapse, whereas GFAPα is incorporated into the network. Using GFP-tagged GFAP isoforms, we also showed GFAPδ has a different exchange rate between GFAP in solution and in the network, both when the network had collapsed and when the network was still intact. In addition, an effect of GFAPδ expression on cell shape was shown, and this was linked to a difference in focal adhesion size. Surprisingly, despite the prominent effect on the network, no effect of GFAPδ expression was found on cellular motility or proliferation compared to control, although a distinction was found between the proliferating fraction of GFAPα expressing primary human astrocytes, and GFAPδ primary astrocytes.

In Chapter 4 we performed an analysis of GFAP isoform expression in relation to astrocytoma malignancy based on the Cancer Genome Atlas patient database. This revealed that the GFAPδ/α ratio increases with increasing astrocytoma grade. To find whether this isoform ratio controls gene expression in astrocytoma, we modulated GFAP isoforms in astrocytoma cell lines by recombinant expression or knockdown and performed transcriptome analysis on a microarray platform. Overlapping these GFAP regulated genes with the genes correlating in astrocytoma with GFAPδ/GFAPα ratio, resulted in 8 GFAP regulated targets relevant for tumour biology and/or prognosis.

In Chapter 5 we studied the effect of the absence of GFAP and vimentin on gene expression in the healthy mouse brain. We studied the transcriptome of astrocytes, which were acutely isolated from the cortex of wild type, $GFAP^{+/-}$ (GFAPko), and $GFAP^{+/-} Vim^{-/-}$ (VIM-GFAPko) mice. Because of the crosstalk between astrocytes and microglia, we also studied the effect of the absence of GFAP on microglia, which surprisingly resulted in more pronounced changes in gene expression than in the GFAPko astrocytes themselves. This showed in a nutshell that GFAP might have more impact through modulation of cell-cell signalling and modulation of cellular interaction with the ECM, than in the astrocytes themselves. This was corroborated by our observation that in the astrocytes the extracellular region and cellular surface/periphery were amongst the most significantly regulated annotations.
The effects of GFAP modulation on the viscoelastic properties of the IF network were studied in Chapter 6. Intermediate filaments are implied in cellular mechanics, therefore we anticipated that modulating the GFAP content of the IF network would change the viscoelastic properties of the IF cytoskeleton. We took an approach of extracting the IF network from cells and to remove other major cytoskeletal components to study the network properties in a relatively defined setting. Before extraction we introduced fluorescent beads in the cells, which were captured inside the network structures. The motions of these beads were analysed using particle tracking microrheology. As a proof of method study, we found this procedure is viable, and particles are restricted in their mobility with addition of GFAP isoforms to the network, although further definition, control of the system and more sensitive methods are required to make quantitative statements about the effect of different GFAP isoforms on the network.

Finally, in Chapter 7, we discuss the most promising targets from our transcriptome analyses. We highlighted a couple of the recurring targets of GFAP modulations to crystallize which cellular processes are related to GFAP. This shows that GFAP has broad functions in cellular biology, but seems to be most important in cell-cell organization through ECM remodelling and growth factor signalling both in astrocytoma and in astrocytes. We identified two unexpected genes as possible targets downstream of GFAP modulation, and discuss how GFAP modulation might mechanistically exert its downstream effects.