From Stem Cell to Astrocyte: Decoding the Regulation of GFAP
R. Kanski

Summary

The research presented in this thesis focuses on the Glial Fibrillary Acidic Protein (GFAP), the main intermediate filament (IF) protein in astrocytes and astrocyte subpopulations such as neural stem cells (NSCs). The GFAP gene is alternatively spliced, and the canonical isoform GFAPα and an alternative spliced form GFAPδ represent the most abundant isoforms. In contrast to GFAPα, GFAPδ is assembly compromised and its expression levels are a crucial determinant of IF network assembly. Expression of GFAP is highly regulated during development and upon brain injury. After brain damage and in neurodegenerative diseases, astrocytes respond to an injury with an upregulation of IF proteins such as GFAP. During development of the mammalian brain, initiation of GFAP expression induces astrocyte differentiation. Unraveling the regulatory pathways that control the initiation of GFAP and the generation of astrocytes is crucial for transplantation of NSCs which currently is investigated as treatment for different brain diseases. To date, directing NSC differentiation towards a specific cell type such as neurons, astrocytes, and oligodendrocytes remains challenging. In this respect, investigation of cell fate determination during development is an important tool to understand specification of NSCs in the adult brain. The mechanisms of how GFAP expression is initiated at the onset of astrogenesis are reviewed in **Chapter 1**.

In **Chapter 2** we identified that histone acetylation in astrocytes is essential for GFAP transcription, splicing, and the assembly of the GFAP network. Epigenetic modifications such as histone acetylation are crucial regulators of gene transcription. As demonstrated here in human primary astrocytes and astrocytoma cells, suppression of histone acetylation using histone deacetylase inhibitors (HDACi) reduced GFAP expression and decreased the ratio between the canonical isoform GFAPα and the alternatively spliced isoform GFAPδ. As shown by different RNA interference (RNAi) approaches, expression of GFAPδ is dependent on the presence and binding of the splicing factors of the SR protein family. Consistent with a crucial impact of GFAPδ expression levels on the assembly of the IF network, a shift in the GFAP isoform ratio towards GFAPδ expression was associated with an aggregation of the whole IF network including GFAP, nestin, and vimentin in astrocytes.

Specific silencing of the canonical isoform GFAPα in **Chapter 3** revealed that modulation of the GFAP isoform expression affects astrocyte function. The GFAPδ/α ratio increased in favour of GFAPδ expression upon silencing of GFAPα, an effect that was enhanced by upregulation of GFAPδ by the cell, itself. Modulation of the GFAP network composition in favour of GFAPδ increased the production of the extracellular matrix component laminin and decreased the motility of astrocytes. In contrast, knockdown of all GFAP isoforms mainly acted on adhesion and morphology of astrocytes, demonstrating that altering the GFAP network composition affects astrocyte functions and that a specialized IF composition affects them differentially. An alternative approach to investigate a specialized GFAP network is presented in **Chapter 4**, a technical note

describing the design of a GFAPδ-specific shRNA. Silencing of GFAPδ in future experiments will provide important insights into the specific function of this isoform in relation to its distinct expression pattern in astrocyte subpopulations in the brain.

This idea was followed up in **Chapter 5** where we aimed to silence GFAPδ *in vivo* in the SVZ of adult mice, a brain area with high GFAPδ expression. In contrast to the human brain, GFAPδ is expressed in all astrocytes of the developing and adult mouse brain. However, GFAPδ is an integral part of the IF network of NSCs in both species and silencing of GFAPδ is an essential tool to investigate its function in the neurogenic system *in vivo*. Two separate RNAi approaches, shRNA and exon skipping constructs, were designed to specifically target Gfapδ transcripts. The ability of the two RNAi approaches together with three different viral vector delivery systems was employed to silence GFAPδ expression. Both Lentivirus VSV-G and AAV5 were successfully able to transduce the SVZ. However a stable downregulation of GFAPδ failed due to an unspecific tropism of the viral particles. Moreover, off-target effects might have masked the effects of the RNAi approach *in vivo*. All together, this study highlights the importance of an in depth *in vitro* RNAi screening and proper, cell type-specific construct delivery for successful RNAi in the SVZ.

Alternatively in **Chapter 6**, we focused on the investigation of the regulation and function of GFAP in human NSCs. We demonstrated that Notch activity, a key regulator of NSC differentiation, controls human GFAP expression in NSCs and glial progenitors. Inhibition of Notch signaling reduced GFAP expression in undifferentiated primary human fetal NSCs (fNSCs), *in vivo* in zebrafish embryos, as well as in glial progenitors differentiated from immortalized fNSCs.
Differentiation of NSCs into glial progenitors was induced by human post-mortem ventricular cerebrospinal fluid, a very potent stimulus of GFAP expression in human NSCs as shown here. Upon initiation of differentiation, increased GFAP expression was associated with a decreased expression of Notch downstream targets, suggesting an inhibitory role of GFAP. Promoter assays of the Notch target gene Hes-1 confirmed a negative modulation of Notch signaling by GFAP, which is further supported by inhibition of Notch downstream target Hes-5 by GFAP overexpression. Together, this data indicates that GFAP, itself, silences Notch signaling forming a negative feedback loop which might regulate astrogenesis during brain development. Importantly, a dysregulation of GFAP expression in NSCs leads to a dramatic impairement of the sphere forming capacity of NSCs, indicating that a balanced GFAP expression is critical for NSC self-renewal.
Confirming our finding that Notch signaling is a crucial regulator of GFAP expression, we showed in **Chapter 7** that an upregulation of GFAP in the context of reactive gliosis is dependent on Notch activity.
Previously our group demonstrated reduced GFAP expression upon inhibition of the proteasome. Consistantly, we report here on the ability of a proteasome activator to enhance GFAP levels.

Consistent with a regulatory role of Notch, the upregulation of GFAP was dependent on Notch activity. The proteasome is one of the major protein degradation systems in the cell and its dysregulation is implicated in many brain diseases. Intriguingly, specific inhibition of the immunoproteasome, a variant of the proteasome induced by inflammatory signaling, was sufficient to prevent Notch activity and, in turn, the upregulation of GFAP. Since recent data in our group revealed that enhanced immunoproteasome activity is characteristic for reactive astrocytes in the diseased brain, inhibition of immunoproteasome activity might represent an attractive approach to prevent Notch activation and GFAP upregulation in reactive astrocytes. Future research will reveal whether preventing GFAP induction reduces reactive gliosis.

In the final **Chapter 8**, we discuss our main findings of the preceding chapters in relation to current research. We emphasize that alternative splicing of GFAP might represent an important mechanism to regulate GFAP function in different astrocyte subpopulations. Moreover, we speculate that GFAP might act as a signaling platform in the cell that binds and regulates the activity of signaling molecules in order to influence vital cellular functions.

GFAP expression is widely used as a marker for astrocytes and specific astrocyte subpopulations, such as NSCs. However, very little is known about its function and the consequences of a specialized IF network including the alternative isoform GFAPδ. Considering the widespread GFAP network as signaling platform in the cell that can regulate signaling pathway activities, will significantly enhance our knowledge on the function of the broadly used astrocyte marker GFAP.