Project Proposal for a PhD position granted by the International Graduate School in Molecular Medicine Ulm

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2. Project Title

Role of the G-protein coupled receptor 17 (GPR17) and the GPR17^{+} subset of NG2-glia in the adult brain under physiological and pathological conditions

3. Keywords (max. five)

- oligodendrocyte progenitor cells (OPCs)
- myelination
- repair
- knockout and transgenic mice
- oligodendrocyte differentiation

4. Research Training Group

X Neurobiology

☐ Aging and Degeneration

☐ Oncology and Endocrinology

☐ Virology, Microbiolgy, Biotechnology and Systems Biology

☐ Development and Regeneration

X Trauma, Regeneration and Immune Modulation

☐ Pulmosens

5. Project description

Date and Signature: 02.07.2017; Prof.Dr. Leda Dimou

Project background

NG2-glia, also known as oligodendrocyte progenitor cells, resemble 5-10% of the cells in the adult brain. The widespread interest in this glial cell population raises from their unique properties, as they represent the only proliferating cell type in the adult brain parenchyma outside the neurogenic niches (Simon et al., 2011) and generate mature, myelinating oligodendrocytes also at adult stages, well after the end of the major myelination process (Dimou et al., 2008; Vigano et al., 2013). Genetic ablation of proliferating NG2-glia and subsequent block of oligodendrocytes´ generation not only leads to structural changes of the nodes of Ranvier but also to deceleration of conduction velocity resulting in progressive motor deficits. These results demonstrated for the first time that axon function is not only controlled by the reliable organization of myelin in the adult brain but also requires a dynamic and continuous formation of myelin (Schneider et al., 2016). Analysis of the areas of myelination revealed a region dependent ability of NG2-glia to differentiate, pointing to strong heterogeneity between cells. By transplantation experiments we could show that the regional differences are mainly the result of intrinsic differences between grey (GM) and white matter (WM) NG2-glia, as only the WM-cells have the capacity to differentiate in both the more supportive (WM) and the less supportive (GM) environment (Viganó et al., 2013). The concept of heterogeneity among NG2-glia became more complex when heterogeneity was observed also in the same cortical area. Indeed, together with our collaborators, we could show that the membrane G-protein coupled receptor GPR17 is transiently expressed by a subpopulation of NG2-glia in the adult brain (Boda et al., 2011). This receptor is suggested to be an intrinsic timer of myelination during development. It is expressed by oligodendrocyte progenitors and its modulation *in vitro* or at postnatal ages *in vivo* can influence the rate of their differentiation (Chen et al., 2009; Fumagalli et al., 2011). However, so far nothing is known neither about the function of GPR17 nor about the role of the GPR17-expressing subset of NG2-glia in the adult brain.

Scientific objectives

Within this project, we are aiming to:

- Reveal the role of the GPR17 protein in the function of the subset of NG2-glia expressing this receptor under physiological and pathological conditions
- Understand the role of this subset of NG2-glia in the adult brain

Preparatory work

To label and monitor the fate of $GPR17^+$ cells without affecting the physiological expression and function of GPR17, we generated a novel BAC-transgenic mouse line for fate mapping (GPR17-$iCreER^{T2}$; Vigano et al., 2016). With this mouse model we were able to demonstrate that although NG2-glia in the adult cerebral cortex can differentiate into mature oligodendrocytes, cells expressing GPR17 remain longer in an undifferentiated state compared to NG2-glia not expressing the receptor. More interestingly, after challenging the cortical environment by inducing acute injury or ischemia, recombined $GPR17^+$ glia that under physiological conditions are reluctant to differentiation, immediately react to damage and undergo maturation, suggesting that they represent a reserve pool of adult progenitors maintained for repair purposes. To understand the molecular differences between the $GPR17^+$ and $GPR17^-$ NG2-glia in order to identify molecules important and necessary for the differentiation of NG2-glia we performed a RNAseq analysis that revealed major differences in gene expression between these two cell populations. GO-term analysis revealed differences e.g. in binding, catalytic activity, signal transduction, translation/transcription regulation (Unger et al., in preparation). These results not only give new insights in the function of this NG2-glia subset in the adult brain but also open the exciting possibility to modulate and reinforce their differentiation for e.g. therapeutic purposes.

Working programme

We could recently identify the $GPR17^+$ cell population as a subset of NG2-glia that is not differentiating (Vigano et al., 2016). However, it is still unclear if the maturation differences observed between recombined and non-recombined cells are dependent on GPR17 itself or if

this receptor is “only” a marker specifically labeling a subpopulation of NG2-glia that is retained for a long time in an undifferentiated state. In this project we are planning to understand 1/ the role of the GPR17 in NG2-glia and 2/ the role of the GPR17$^+$ subset of NG2-glia in the adult brain.

1/ understanding the role of GPR17 in NG2-glia

Towards this aim, we are planning to analyse the proliferation and differentiation properties of NG2-glia in a GPR17-knockout mouse line under physiological and pathological conditions. Chen et al. (2009) could show an embryonic premature myelination in these mice but nobody ever analysed the properties of these cells in adult mice. Following experiments are planned:

a/ adult intact and lesioned (stab wound injured) wildtype and knockout mice will receive the thymidine analogue BrdU for different durations (14 and 28 days), to label proliferating cells as well as to recognize newly generated oligodendrocytes.

b/ lesioned animals will be analyzed at different timepoints after stab wound and/or traumatic brain injury (2, 4, 7 and 14 days) in regard to the reaction of other cell types (oligodendrocytes, astrocytes, microglia, invading immune cells, neurons) in the area of the injury as well as of the wound closure properties in these mice.

c/ In a parallel approach we will apply agonists and/or antagonists of GPR17 provided by our collaborator Prof. Mariapia Abbracchio (Milan, Italy) by osmotic minipumps into the ventricle of adult wildtype mice for 2 or 4 weeks and study the above described parameters with and without an injury.

2/ understanding the role of GPR17$^+$ NG2-glia in the adult brain

In order to specifically understand the role of proliferating GPR17$^+$ cells in the adult brain we will cross the GPR17-iCreER^{T2} to Esco2-floxed mice (Whelan et al., 2012) to specifically kill GPR17$^+$ cells after induction with tamoxifen. Therefore, after induction, the cell cycle protein Esco2, an important part of the mitosis cohesion apparatus, will not be functionally expressed anymore leading to a block of the separation of the chromatids and a consequent cell apoptosis. For analysis, mice will be induced in adult ages and sacrificed at different timepoints thereafter (5, 30, 90, 180days). Fixed brains will then be immunohisctochemicaly analyzed for cells of the oligodendrocyte lineage, astrocytes, microglia and neurons as well as for their proliferative capacity. The proliferation and the differentiation properties of recombined cells will be compared to the non-recombined ones and the number of newly generated oligodendrocytes will be analyzed. To unravel possible myelination deficits in these mice we will also perform electron microscopical analysis of animals after long recombination times as performed and described previously (Schneider et al., 2016). Additionaly, mice will underfo behavioral studies in regard to their motorical abilities (e.g. grid walk, beam crossing, open field, irregular ladder, rotarod tests). When we deleted Esco in the complete NG2-glia population, mice developed behavioral deficits due to the lack of newly generated oligodendrocytes in the white matter. Here, we will specifically ablate the brain from NG2-glia that under physiological conditions are not differentiating, making the results even more interesting.

Timetable and milestones

	6 mo	12 mo	18 mo	24 mo	30 mo	36 mo
Analysis of GPR17-KO under physiological conditions	**X**	**X**	**X**			
Analysis of GPR17-KO after injury		**X**	**X**	**X**		
Analysis of mice after application of GPR17-ant/agonists under physiological conditions				**X**	**X**	
Analysis of mice after application of GPR17-ant/agonists after injury					**X**	**X**
Immunohistochemical analysis of mice after depletion of GPR17$^+$ cells				**X**	**X**	**X**
Behavioral analysis of mice after depletion of GPR17$^+$ cells		**X**	**X**	**X**		

Funding of the project: Institutional budget of the group and partially DFG (Schwerpunkt-Projekt)