Dissecting the role of Gpr158 in the brain

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Summary

Gpr158 is an orphan G protein-coupled receptor that has been implicated in cognition, depression, ocular hypertension and cancer progression. The interaction of Gpr158 with RGS7 and the physiological responses of this interaction have been described in multiple studies. Increasing evidence has shown that *Gpr158* plays an important role in synaptic transmission and synapse formation. Recently, Gpr158 has been found as a binding partner for the heparan sulfate proteoglycan glypican 4 (GPC4). GPC4 is enriched on hippocampal granule cell axons. Gpr158-induced presynaptic differentiation in contacting axons requires cell-surface GPC4 and the co-receptor LAR. In this thesis, I characterized Gpr158 using different approaches at the molecular, cellular and behavioral level. Furthermore, I provided information on the deorphanization process of Gpr158. Future studies should shed light on the precise signaling mode of Gpr158 in a neuronal context, how this relates to neuronal network activation and behavioral output.

In chapter 2, I described the role of *Gpr158* in neuronal development (*in vitro* and *ex vivo*) and synaptic transmission in the CA1 region of the hippocampus. Our *in vitro* analysis revealed that *Gpr158* KD reduced cell viability and impaired dendritic architecture (i.e. reduced neurite length, bifurcation and extremities) in primary hippocampal neurons. In line with this, we found impaired dendritic architecture in the CA1 pyramidal neurons of *Gpr158* KO mice and thereby uncovered a previously unknown role of the *Gpr158* in neuronal development. Synaptic transmission (both basal excitatory and inhibitory transmission) was not disrupted in the CA1 hippocampal region of *Gpr158* KO mice. However, we found increased action potential frequency and input resistance while having a more depolarized resting membrane potential. In addition, CA1 pyramidal neurons were more excitable and SC input to CA1 pyramidal neurons was reduced in absence of *Gpr158*. Finally, I investigated the behavior of *Gpr158* KO mice using hippocampal dependent learning and memory paradigms. *Gpr158* KO mice showed deficits in spatial learning and memory in the MWM paradigm and in the acquisition of extinction memory in the PA test. Moreover, the observed morphological impairments in CA1 pyramidal neurons were positively correlated with the spatial memory acquisition deficit in the MWM.

In chapter 3, I focused on the Gpr158 interactome in a region-specific manner (hippocampus *vs.* striatum). In previous studies, RGS7 and Gβ5 were identified as interactors of Gpr158 using whole brain lysates. In the Gpr158-RGS7 interaction, Gpr158 regulates the expression and localization of RGS7 in the brain and affects anxiety behavior in mice. In our study, I found the previously known interactors (RGS7 and Gβ5) and Gαz as a new interactor in both brain regions for Gpr158. In addition, I investigated the protein regulation in the hippocampal synaptosome fractions of *Gpr158* KO mice using a quantitative proteomics

approach. Based on our SWATH-MS analysis, I found significant down-regulation of RGS7 and Gβ5 proteins in *Gpr158* KO synaptosomes. Furthermore, the regulation of neurodevelopment-related proteins was putatively affected in absence of Gpr158, a finding that supports the neuronal development role of Gpr158 described in chapter 2. In this chapter, I also assessed the behavioral flexibility of *Gpr158* KO mice using a reward-based discrimination task (CognitionWall test). *Gpr158* KO mice showed reversal learning deficit in this test. This finding suggests that Gpr158 plays a role in different forms of learning that likely involves multiple brain regions.

In chapter 4, I investigated the adhesion role of Gpr158 in hippocampal primary neurons using artificial synapse formation and density assays. Gpr158 contains structural features of aGPCRs (e.g. EGF-like and a leucine-rich repeat domains) at the N-terminal protein domain, which makes it likely to act in cell adhesion. In the synapse formation assay, I co-cultured Gpr158 overexpressed HeLa cells with primary hippocampal neurons and found that Gpr158 induces presynaptic synapsin accumulation in the contacting area of Gpr158 overexpressed HeLa cells and axons of primary hippocampal neurons. This suggests that Gpr158 has a role in synapse formation. In the synapse density assay, I knocked-down (KD) *Gpr158* in hippocampal primary neurons and measured the density of pre- and post-synaptic protein co-localization per neurite. The *Gpr158* KD reduced the synapse density through decreasing the density of the post-synaptic marker PSD-95. Altogether, these assays indicate the importance of Gpr158 in the synapse formation.

In chapter 5**,** I describe the use of a brain-extract-based reverse pharmacology approach to search for endogenous Gpr158 ligand(s). A luciferase reporter gene assay was used to investigate the activation of G-protein signaling pathways for Gpr158 in HEK293T cells in the absence and presence of potential ligand(s). We followed a proteomic- and a lipidomic- based approach for identifying potential ligand(s). In both approaches, mouse brain-extracted biomolecules were fractionated based on size and hydrophobicity and subsequently assessed for bioactivity by measuring G-protein signaling in the luciferase reporter assay. We found that Gpr158 induces NFAT RE activation and this activation is further increased with the incubation of protein/peptide and lipid fractions. Since increased NFAT RE activation was observed in multiple fractions, Gpr158 most likely has multiple endogenous ligands that induce the same G-protein signaling pathway. However, neither protein/peptide- nor lipid-like ligands of Gpr158 have been previously identified. Our lipidomic-based approach suggested that the most parsimonious chemical structure of the bioactive compound of interest (*m/z* 537.3 Da) is a formylated form of the detergent *N*-Dodecyl-D-Maltoside (DDM, 510.62 Da) used during tissue extraction. Taken together, we provided the first evidence of the existence of Gpr158 ligands in the mouse brain and the induction of NFAT RE activation through a non-classical $G\alpha_{q/11}$ pathway in HEK293T cells.

In chapter 6, I summarized the findings and their implications in light of the literature. I highlighted the different roles of Gpr158 in neuronal development, synapse formation, synaptic transmission, learning and memory. Furthermore, I provided future research perspectives for a further characterization of Gpr158.