Gene regulation of the receptor GPR17 in oligodendroglial cells: expression pattern and pathological alterations in a mouse model of multiple sclerosis

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GPR17 is a G protein-coupled receptor activated by both uracil nucleotides and cysteinyl-leukotrienes (Ciana et al., 2006). We have previously demonstrated that GPR17 is a key regulator of oligodendroglial differentiation. Under physiological conditions, the receptor starts to be expressed in oligodendrocyte precursor cells (OPCs) that also express the early marker NG2, it reaches its maximal peak of expression in immature oligodendrocytes, and is progressively downregulated during terminal maturation (Lecca et al., 2008; Fumagalli et al., 2011). At early differentiation stages, the activation of GPR17 with UDP-glucose induced OPC maturation, whereas inhibition with receptor antagonists or specific interfering RNAs impaired the correct differentiation. However, at later OPC stages, the forced overexpression of the receptor also led to impaired maturation, suggesting that the expression of the receptor GPR17 needs to be time regulated either at transcriptional, or post-transcriptional level.
The present work was aimed at understanding what is the molecular mechanism at the basis of Gpr17 gene expression, and to assess if receptor expression is altered in mouse spinal cord during a typical demyelinating disease.
To answer to the first issue, we cloned a putative promoter region of the Gpr17 gene into a reporter vector upstream to a gene encoding for an engineered firefly luciferase; then, we transfected this construct in Oli-neu cells, an immortalized oligodendroglial cell line, and we set up a dual luciferase reporter assay to evaluate the bioluminescence produced in response to an array of stimuli, including hormones and growth factors. Interestingly, incubation of cells with conditioned medium from cortical neurons was able to significantly induce Gpr17 promoter activity, suggesting that neurons release one or more factors triggering oligodendroglial differentiation through Gpr17 gene activation (Fratangeli et al., 2013).
Signals released by axons can profoundly influence the expression pattern of oligodendrocytes, and this is indeed responsible of their behaviour (proliferation, differentiation, migration, cell death), both in physiological and pathological conditions. For this reason, we investigated the expression of GPR17 in spinal cord after the induction of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. As already described for brain, immunohistochemistry analysis revealed that, also in spinal cord, GPR17 specifically decorates a subset of OPCs both in grey and white matter. After EAE induction, both the total number of mature CC1-positive, and GPR17-positive cells was reduced, whereas the number of NG2-positive cells was increased, suggesting disease-induced recruitment and proliferation of early progenitors. Interestingly, qRT-PCR showed that the mRNA of GPR17 is increased in EAE, and this seems to be in relation with the clinical score of the mice.
We hypothesize that, as demonstrated in brain ischemia, the expression of GPR17 is up-regulated in the disease to counteract demyelination, but this response is insufficient and may lead to dysregulations, eventually contributing to the disease. Understanding how the expression of GPR17 is finely regulated will not only advance basic knowledge in oligodendrogliogenesis, but will also help developing new pharmacological or biotechnological strategies to enhance the reparative potential of the quiescent OPCs that are still present in the adult brain.
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Ciana et al. (2006). *EMBO J.* 25, 4615-27
Lecca et al. (2008). *PLoS One.* 3, e3579
Fumagalli et al. (2011). *J Biol Chem*. 286, 10593-604
Fratangeli et al. (2013). *J Biol Chem*. 288, 5241-56