Identification of microRNAs potentially regulating the expression of GPR17, a receptor involved in the development of oligodendroglial precursor cells

D. Marangon[1], G.T. Coppolino[1],A. Finardi[2],R. Furlan[2], M.P. Abbracchio[1,*], D. Lecca[1,*]

[1]Laboratory of Molecular and Cellular Pharmacology of the Purinergic Transmission, Dept. of Pharmacological and Biomolecular Sciences, University of Milano
[2]Neuroimmunology Unit, Institute of Experimental Neurology (INSpe), Division of Neuroscience, San Raffaele Scientific Institute, Milano
[*]Equally contributed

GPR17 is a G protein-coupled receptor activated by both uracil nucleotides and cysteinyl-leukotrienes. Under physiological conditions, the receptor starts to be expressed in early oligodendrocyte precursor cells (OPCs), reaches its maximal expression in immature oligodendrocytes, but then it has to be progressively down-regulated to allow terminal maturation. Its activation with agonists promotes, whereas its blockade with antagonists impairs oligodendroglial differentiation. Any alteration in this expression pattern results in delayed maturation, suggesting the importance of this receptor as a checkpoint during myelination.
However, the genic and epigenetic mechanisms regulating GPR17 expression are still unknown. This work was aimed at assessing if the expression of GPR17 can be regulated at post-transcriptional level by microRNAs (miRNAs), a class of small, highly conserved, non-coding RNA molecules that control gene expression by binding to complementary sequences in the 3' untranslated regions of their target messenger RNAs, therefore resulting in either translational repression or degradation. First, we analyzed the 3'UTR of mouse and human GPR17 in silico with six different prediction tools and we identified three candidates miRNA. Then, we found that one of them (named miR-X), was putatively able to bind to transcripts involved in oligodendroglial differentiation, such as NG2, GalC and APC. Thus, we characterized the expression pattern of this miRNA in neural cells, in particular in oligodendrocytes, showing that it is progressively up-regulated during in vitro differentiation. Interestingly, we also found that overexpression of this miRNA by the transfection of its "mimic" in cultured OPCs differentiated with triiodothyronine (T3) strongly reduced the number of mature oligodendrocytes expressing the myelin basic protein MBP, whereas, the number of GPR17-positive cells was not affected. However, the transfection of miR-X in milder differentiating conditions (in the absence of T3), caused a strong reduction in the number of GPR17-positive cells, suggesting that in these conditions, it can also repress OPC differentiation at earlier stages.
In order to understand if GPR17 down-regulation is the result of a direct effect on its mRNA, we set up a luciferase reporter assay on the GPR17 3'UTR, that contains 7 hypothetical binding sites for this miRNA. We selected a region including the 3 consecutive more conserved sites between mouse and rat species, and we cloned it in a construct downstream to the luciferase gene, under the control of a constitutive promoter. The co-transfection of this construct with the miRNA mimic in HEK293 cells did not lead to a significant decrease in luciferase activity, suggesting that the active binding sites are not present in the cloned region or that the effects on GPR17 expression are indirect.
To evaluate the pathways potentially involved in the reduction of the expression of MBP, we took advantage of Ingenuity Pathway Analysis tool (IPA), building a model network containing GPR17, known players of oligodendrogliogenesis and the already validated targets of miR-X.
This analysis revealed that intracellular kinases such as Fyn and p38, cytoskeletal proteins and neuregulins can directly or indirectly promote MBP expression, suggesting that this miRNA, through the simultaneous inhibition of several targets, leads to a decrease in MBP expression and a delay in oligodendroglial maturation.
Taken together, these results suggest that miR-X synergically inhibits different mechanisms that normally promote the expression of myelin genes, such as GPR17 and MBP, and that, besides the identification of new targets, integration of the mechanisms connecting all the validated targets of a miRNA could shed light on a very complex and redundant regulatory network.
Sponsored by Fondazione Italiana Sclerosi Multipla (FISM)2013/R-1 project to MPA and by Fondazione Cariplo, grant n° 2014-1207 to DL.