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Identification of a novel small RNA encoded in the mouse urokinase receptor uPAR gene (*Plaur*) and its molecular targets

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The urokinase receptor (uPAR) is the glycosylphosphatidylinositol (GPI)-anchored receptor of urokinase (uPA), which is involved in brain development, nerve regeneration, wound healing and tissue remodelling. uPAR overexpression stimulates radial neuronal migration to the outer layers of the differentiating cortex, whereas uPAR knockout reduces the migration of parvalbumin-expressing GABA interneurons into the cerebral cortex. Recent papers have shown that mutations and polymorphisms in the *Plaur* gene or uPAR ligand *SRPX2* affect the formation of brain structures and induce severe developmental pathologies in humans (speech deficiency, mental weakness and autism spectrum disorders). Using a model of acute generalised seizures in mice, we revealed that *Plaur* operates as an immediate early gene, and is rapidly induced by neuronal activity in different brain regions independently of the *de novo* protein synthesis. This rapid and universal response confirms the important role of uPAR in regulating neuronal responses to excitation and/or damage.

Assumingly, diverse functions of *Plaur* might be attributed to hypothetical, unidentified microRNAs encoded within the introns of the *Plaur* gene. The *Plaur* gene consists of seven exons and seven introns. Prior to our study, miRNAs had not been identified in the *Plaur* gene sequence. The total *Plaur* gene size is 16,000 bp, while the mature mRNA (exons only) is composed of only 1,000 nt, suggesting that non-coding RNAs, including miRNAs, are encoded in the gene. We conducted a bioinformatic search and analysed the miRNAs that make up the *Plaur* gene. We identified a novel miRNA, which we named Plaur-miR1. Using wild-type uPAR-expressing Neuro2a cells, CRISPR-edited uPAR-deficient Neuro2a cells and *in vivo* model of endogenous induction of *Plaur* expression in the brain, we proved the existence of a new miRNA, Plaur-miR1-5p. *In silico* analysis of targets allowed us to identify its possible functions, namely determining cell fates in the developing central nervous system (plays a crucial role in neuronal apoptosis). We confirmed *Plaur*-dependent expression of Plaur-miR1 in the mouse brain and mouse neuroblastoma Neuro2a cells. Utilising the *in silico* MR-microT algorithm in

DianaTools, we selected two target genes – *Emx2* (is a transcription factor that plays an essential role in specifying cell fates in the embryonic central nervous system) and *Mef2d* (encodes a developmental protein that regulates large-scale gene expression programmes necessary in embryogenesis and tissue architecture maintenance, including the brain, and contributes to the regulation of neurogenesis, neuronal apoptosis and differentiation) – with high binding score. Moreover, sequencing of the mouse brain samples for Plaur-miR1 target genes revealed two other target genes – *Nrip3* (nuclear receptor interacting protein 3) and *Snrnp200* (U5 small nuclear ribonucleoprotein). The expression of *Emx2*, *Mef2d* and *Snrnp200* in mouse brain and *Mef2d* and *Snrnp200* in Neuro2a cells correlated with the expression of *Plaur* and Plaur-miR1.

In conclusion, we identified the novel Plaur-miR1 as a functional miRNA of *Plaur* intron 3. Furthermore, we revealed that Plaur-miR1 expression regulates *Emx2* and *Mef2d* expression. Taking into account our previously published data and the present results we suggest a novel role of *Plaur* as a morphogenetic factor in the brain development and a marker of brain disorders.

Accession numbers. Sequences from the figure 4 E (clones 1, 9, 11 and 16) for the reported microRNA Plaur-miR1-5p have been deposited with the GenBank® under sequences names and accession numbers as follows: BankIt2532817 Seq_1 OM105889, BankIt2532817 Seq_2 OM105890, BankIt2532817 Seq_3 OM105891, BankIt2532817 Seq_4 OM105892.

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