

## KNOCK DOWN OF P2X<sub>2</sub> RECEPTOR EXPRESSION IN MOUSE EMBRYONAL CARCINOMA P19 CELLS BY RNA INTERFERENCE

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The P2X<sub>2</sub> receptor is a subtype of ionotropic ATP receptors and plays a significant role in regulating synaptic transmission in the nervous system. Purinergic signaling has also been implicated in neuroprotection and early neuronal development. The aim of this study was to develop small-interfering (si)-RNA against the receptor P2X<sub>2</sub> in P19 embryonal carcinoma cells which are a well-accepted model for early neuronal development. Following 24h of induction of RNA interference (RNAi) in the presence of siRNA-9 or siRNA-34, P2X<sub>2</sub> receptor gene-expression was reduced by 64% or 42%, respectively. Immunocytochemical staining revealed decreases of 70% or 55% in receptor protein-expression in the presence of siRNAs-9 or -34. We demonstrated that the selected siRNAs are specific for down-regulating P2X<sub>2</sub> receptor expression, as gene-expression analysis of other P2X receptor subtypes (P2X<sub>1, 3-7</sub>) by real-time PCR did not indicate any significant reduction. Expression profiling of P2X<sub>2</sub> receptors was performed during *in vitro* neuronal differentiation of P19 cells. P2X<sub>2</sub> receptor mRNA transcription increased during initial induction to differentiation followed by a reduction of gene-expression until P19 cells became functional neurons. Using RNAi for suppressing gene expression of P2X<sub>2</sub> receptors during differentiation of P19 cells, we will be able to study the participation of this receptor in an model of early neuronal development.

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