In Vitro Selection of RNA Aptamers as Specific Inhibitors of P2Y2 Purinergic Receptors

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The development of the nervous system is one of the most important morphogenetic events occurring in the embryo. This process is accompanied by cell proliferation and differentiation as well as by tissue organization into a specific architecture. Although the specific molecular pathways that drive these events remain unresolved, it is widely believed that proliferation and differentiation programs of the neural progenitors require the interaction of extrinsic and intrinsic signals. While the function of growth factors in controlling neuronal differentiation is well documented, there also is increasing persuasive evidence for a role of neurotransmitters and their respective receptors in this process. One such neurotransmitter is ATP, whose biological actions are mediated by both ionotropic purinergic receptors (P2X) and G-protein-coupled metabotropic purinergic receptors (P2Y). Based on purinergic receptor pharmacology we have determined the participation of P2Y2 receptors in the formation of embryonic bodies as prerequisites for phenotype determination of P19 murine embryonal carcinoma progenitor cells. Final neuronal maturation of P19 cells in the presence or absence of purinergic receptors agonists and antagonists implicated the involvement of P2Y2 receptor subtypes in the determination of the final neuronal phenotype, such as expression of NMDA-glutamate and cholinergic receptors (Resende et al. Neuroscience,146: 1169, 2007). In order to further evaluate the function of this receptor and in view of the absence of specific inhibitor for this receptor subtype, we have used the SELEX technique (Systematic Evolution of Ligands by Exponential Enrichment) as a RNA-based combinatorial library approach for developing specific inhibitors of P2Y2 receptors. A protocol for reiterative in vitro selection has been established which allows the enrichment and identification of RNA aptamers with high affinity for human P2Y2 receptors.