MOLECULAR BASIS OF DIFFERENTIATION OF STEM AND NEURAL PROGENITOR CELLS

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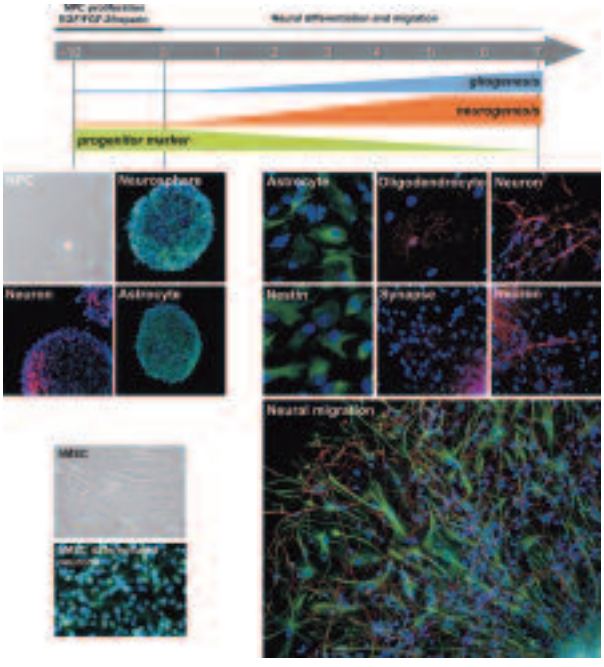


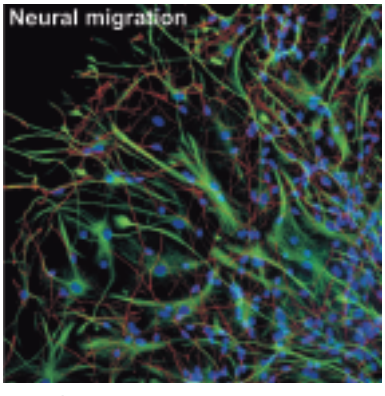
The immense phenotypic variety of cells in the central nervous system (CNS) is due to the differential development of stem and progenitor cells which give origin to neurons, astrocytes and oligodendrocytes

The nervous system is formed by a network formed by trillions of neurons with different phenotypes and other supporting cell types. The process of neurogenesis is directed by the activation of innumerous receptors on cell surfaces of differentiating cells. We have shown in previous studie the participation of kinin-B2, purinergic and cholinergic receptors in neuronal differentiation using P19 embryonic carcinoma cells as an *in vitro* model.

Cholinergic receptor expression was altered when cells were differentiated in the presence of inhibitors of purinergic or kinin-B2 receptors. Based on these results, we are proposing to study the participation of these receptors in the neural differentiation of mouse embryonic and mesenchymal stem cells, as well as, in the process of maturation of rat progenitor cells (neurospheres). DNA aptamers will be identified using the SELEX technique from a combinatorial DNA library, which is capable to specifically recognize stem cells, in order to purify these cells from contaminating ones. Gene expression and receptor activities of kinin-B2, purinergic and nicotinic receptor activity, during differentiation of embryonic and mesenchymal stem cells and neurospheres, will be determined. The fates of neuronal differentiation will be evaluated in the presence of a specific antagonist of kinin-B2 receptors and subtype-specific inhibitors of muscarinic and nicotinic acetylcholine receptors. RNA aptamers and RNA interference will be used for subtype-specific inactivation of purinergic P2Y1, P2X2,4,7 receptor activity or gene expression, respectively. This study will contribute to the functional analysis of stem cells differentiated *in vitro*, and furthermore, to verify the participation of kinin-B2 and purinergic receptors in directing neuronal differentiation. Furthermore, the capability of stem and progenitor cells to give origin to regenerative processes, in the peripheral nervous system, will be evaluated.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

By using P19 embryonic carcinoma cells as an *in vitro* model, we have demonstrated the participation of kinin-B2, purinergic and cholinergic receptors in neuronal differentiation. Cholinergic receptor expression was altered when cells were differentiated in the presence of inhibitors of purinergic or kinin-B2 receptors. Nicotinic acetylcholine receptors were active during all stages of neuronal differentiation, including in embryonic cells, whereas muscarinic receptor function was only identified beginning from the neural progenitor stage. Muscarine induced proliferation in progenitor cells, by activation of Gαq/11-coupled M1, M3 and M5 receptors, whereas Gαi/o-protein M2 receptors contributed to acceleration of differentiation in the presence of muscarine. Purinergic receptor expression was modulated during differentiation, and P2Y1 and P2Y2 receptors participated in proliferation and differentiation induction of embryonic and neural-progenitor P19 cells as judged from pharmacological analysis. Kinin-B2 receptor expression and activity, as well as bradykinin secretion into the culture medium, increased during the course of neuronal differentiation. These studies led to conclude that numerous ionotropic and metabotropic receptors interact in a network and are activated at specific checkpoints of differentiation, thereby contributing to a pattern of calcium transients involving influx of extracellular calcium as well as calcium, mobilization from intracellular pools. Based on the results obtained with P19 cells, the objective of the on-going thematic project is to study participation of these receptors in the neural differentiation of mouse embryonic and mesenchymal stem cells, as well as, in the process of maturation of rat progenitor, cells (neurospheres). We could already identify the presence of components of the kallikrein-kinin system as well as bradykinin secretion and active kinin-B2 receptors during neurosphere differentiation. Treatment of differentiating neurospheres, with the kinin-B2 receptor inhibitor HOE-140, resulted in a loss of purinergic receptor activity. Ongoing studies will reveal the functions of cholinergic, purinergic and kinin receptors in neural differentiation of embryonic and mesenchymal stem cells, as well as, of neural progenitor cells, thereby providing insights in how to direct differentiation to a specific neural phenotype.



Neural migration

MAIN PUBLICATIONS

Resende RR, Majumder P, Gomes KN, Britto LRG, Ulrich H. 2007. P19 embryonal carcinoma cells as *in vitro* model for studying purinergic receptor expression and modulation of N-methyl-D-aspartate-glutamate and acetylcholine receptors during neuronal differentiation. *Neurosci*. **146**:1169-81.

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