

NEURON-LIKE CELLS DERIVED FROM BONE MARROW STROMAL CELLS: EXPRESSION AND FUNCTIONING OF GABA_B RECEPTORS

Cervetto Chiara¹, Paluzzi Paola¹, Guarnieri Stefania¹, Marcoli Manuela¹, Castagnola Patrizio², Tonachini Laura², Mastrogiacomo Maddalena², Monticone Massimiliano², Pestarino Mario³, Candiani Simona³, Maura Guido^{1*}

¹Dep. Experimental Medicine, ³Dep. Biology, *Centre of Excellence for Biomedical Research, University of Genova and ²Ist Naz Ricerca sul Cancro, Genova, Italy

Bone Marrow Stromal Cells (BMSCs) are reported to differentiate in neuronal-like cells, based on expression of neuron markers and morphologic features; nevertheless, doubts are raised on the true neuronal differentiation potential of mesenchymal stem cells and on their ability to attain functional neuron features. To investigate on functional relevance of BMSC differentiation, we focus on receptors for the neurotransmitter GABA, acting as a trophic factor during neurogenesis. Briefly, rat BMSCs and fibroblasts were exposed to neuron differentiating procedure according to Woodbury; semi-quantitative RT-PCR and Real-Time PCR was performed and relative gene expression calculated; intracellular cyclic AMP accumulation was measured by radioimmunoassay. After 24-hour induction BMSCs displayed neuronal-like morphological phenotype and increased gene expression for Nestin, Neurofilament-M and Tau: we refer to these cells as to BMSC Derived Neuron Like cells (BDNL cells). GABA-A β 3 mRNA was detected in BDNL cells; GABA-A β 2, GABA-A β 3, GABA-A α 1, GABA-A ϵ subunits were expressed at higher level in BDNL cells, suggesting possible assembly of heterotrimeric GABA_A receptors. GABA-B1 mRNA was present in BMSCs and BDNL cells, BDNL cells also expressing GABA-B2 subunit. Inhibition of forskolin-evoked cAMP accumulation by the GABA_B receptor agonist baclofen, ineffective in BMSC (and baclofen antagonism by phaclofen, CGP 35348, or CGP 52432), confirmed the presence of functional GABA_B receptors negatively coupled to cAMP production in BDNL cells. Fibroblasts undergoing induction protocol expressed GABA-A β 2, GABA-A β 3, GABA-A α 1, and GABA-A ϵ ; GABA-B1, but not GABA-B2, transcript was induced; forskolin-stimulated cAMP accumulation was unaffected by baclofen. To conclude, GABA-B1 and GABA-B2 subunit mRNAs were detected in BDNL cells, meeting requirements for functional GABA_B receptor expression; functional analysis confirmed that expression and assembly of GABA_B receptors (exhibiting the profile of classical phaclofen- CGP 35348-, CGP 52432-sensitive GABA_B receptors) coupled to transduction mechanism was selectively induced in BMSCs, and not in other cells tested. GABA_B receptors seem to play roles in neuron migration and in modulation of neuron activity during development; presence of functional GABA_B receptor in BDNL cells would suggest that BMSCs can be converted in cells equipped with appropriate receptors, potentially responding to the neurotransmitter GABA. **Supported by PRIN 2004057732**