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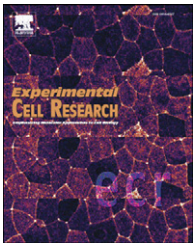

Research Article

Limited Ca^{2+} and PKA-pathway dependent neurogenic differentiation of human adult mesenchymal stem cells as compared to fetal neuronal stem cells

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ABSTRACT

The ability of mesenchymal stem cells to generate functional neurons in culture is still a matter of controversy. In order to assess this issue, we performed a functional comparison between neuronal differentiation of human MSCs and fetal-derived neural stem cells (NSCs) based on morphological, immunocytochemical, and electrophysiological criteria. Furthermore, possible biochemical mechanisms involved in this process were presented. NF200 immunostaining was used to quantify the yield of differentiated cells after exposure to cAMP. The addition of a PKA inhibitor and Ca^{2+}

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Abbreviations: NSCs, fetal-derived neural stem cells; MSCs, mesenchymal stem cells; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; CREB, cAMP response element binding protein; NeuN, neuronal-specific nuclear protein; MAP2, microtubule-associated protein 2; NF-M, neurofilament of medium molecular weigh; NF-200, neurofilament with 200KD; βIII tubulin the same as Tuj1, microtubule protein; TTX, tetrodotoxin; FACS, fluorescence-activated cell sorting; CD, cluster of differentiation; PBS, phosphate-buffered saline; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; DMEM, Dulbecco's modified Eagle medium; F12, Ham's culture medium; B27, N2, serum supplements; PDGF, platelet-derived growth factor; GDNF, glial-derived neurotrophic factor; NGF, nerve growth factor; IBMX, 3-isobutyl-1-methylxanthine; BDNF, brain-derived neurotrophic factor; TGFβ, transforming growth factor beta; FGF8, fibroblast growth factor 8; NT3, neurotrophin 3, LIF, leukaemia inhibitory factor; BrdU, bromodeoxyuridine; PFA, paraformaldehyde; DAPI, 4,6-diamidino-2-phenylindole dihydrochloride; PSA-NCAM, polysialylated neuronal cell adhesion molecule; GFAP, glial fibrillar acidic protein; GABA, gamma aminobutyric acid; ACSF, artificial cerebral spinal fluid

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blockers to the differentiation medium significantly reduced the yield of differentiated cells. Activation of CREB was also observed on MSCs during maturation. Na^+-, K^+-, and Ca^{2+}-voltage-dependent currents were recorded from MSCs-derived cells. In contrast, significantly larger Na^+ currents, firing activity, and spontaneous synaptic currents were recorded from NSCs. Our results indicate that the initial neuronal differentiation of MSCs is induced by cAMP and seems to be dependent upon Ca^{2+} and the PKA pathway. However, compared to fetal neural stem cells, adult mesenchymal counterparts are limited in their neurogenic potential. Despite the similar yield of neuronal cells, NSCs achieved a more mature functional state. Description of the underlying mechanisms that govern MSCs' differentiation toward a stable neuronal phenotype and their limitations provides a unique opportunity to enhance our understanding of stem cell plasticity.

Introduction

Many neurological conditions, such as Parkinson's and Huntington's diseases, stroke, neuropathic deafferentation pain, and trauma, create severe functional deficits in patients; the currently available treatments for these conditions are insufficient or even lacking. Along these lines, cell therapy has become a promising restorative treatment option that promotes anatomical reconstruction and functional recovery in animal models of neurological disorders [1]. Fetal neural tissue has been used extensively for this purpose in the past, but despite neuronal differentiation observed in the host brain, amelioration of functional deficits, and long-term survival of the implanted cells [2–4], limited tissue availability has motivated the search for alternative types of embryonic or adult stem cells. Although promising strategies have arisen from these research activities, the ability of many of these cell types to generate real functional neurons (i.e., cells able to fire action potentials, form electrophysiologically active synaptic contacts, and be integrated into functional neuronal circuits), the biological safety of these cells, and the practical feasibility of their use remain largely unknown and are currently topics of intense investigation [5].

Mesenchymal stem cells (MSCs) are being advocated as a promising cell source for brain repair, but a description of their neurogenic potential, especially in comparison with other stem cells, is still missing. In general, MSCs can be easily obtained from adult tissue and isolated from other cell types in the bone marrow. They are biologically safe and have been used extensively in bone marrow transplantation in patients suffering from hematological cancer [6,7]. Furthermore, the availability of MSCs allows for the transplantation of a patient's own cells, which eliminates the need for immunosuppression and its inconvenient side effects. To date, many authors have described the *in vitro* differentiation of MSCs into neuron-like cells [8–11]. Nevertheless, complete functional maturation with electrophysiological confirmation was only demonstrated under co-culture with neurons or astrocytes, and no previous work has shown the occurrence of action potentials and spontaneous synaptic activity in MSCs cultivated alone. Reports presenting evidences for transdifferentiation of MSCs into a neuronal lineage are usually based on upregulation of neuronal genes, expression of some few markers, and even synthesis of some neurotransmitters, but often weak morphological/immunocytochemical criteria and poor or no electrophysiology [12–16]. Moreover, some authors claimed that the initial morphological changes undergone by MSCs during differentiation were due to the increased osmolarity of the culture media and did not imply neuronal differentiation [17,18].

On the other hand, reports with electrophysiologically verified differentiation were based on co-culture conditions that included mature neurons or astrocytes in the system [19,20]. Because mesenchymal cells can fuse and incorporate genomic material, it is important to determine whether neuronal differentiation is an intrinsic characteristic of MSCs or the result of cellular fusion with neurons that are present in the culture system [21]. Ruling out the possibility of cell fusion is of utmost significance for the further interpretation of MSC multipotency. Additionally, taken into consideration that in co-culture systems mature neuronal tissue not derived from mesenchymal cells is always present, the correct targeting of a cell derived from an MSC during electrophysiological recording becomes a central issue.

Based on what was exposed, the real neurogenic potential of MSCs is still a matter of controversy [22–26]. If transdifferentiation of MSCs indeed occurs, it would be interesting to know at which rate functional neurons are generated in comparison to other stem cell types. This is an important issue for planning a restorative approach based on cell therapy and still needs further elucidation.

The present study was designed to examine whether MSCs have a limited potential to acquire traits of neurons in a monoculture system in comparison to fetal neural stem cells and in fact represents the first report showing a functional comparison between MSCs and another cell type known to provide high yield of neuronal cells. For this, neuronal differentiation was assessed by using morphological, immunocytochemical, and electrophysiological methods. Further, we sought to identify possible biochemical mechanisms that might play an important role in the differentiation of MSCs. The electrophysiological maturation of MSCs was compared to that of fetal-derived neural stem cells (NSCs) differentiated under identical culture conditions. First, we determined the ideal neuronal marker for quantification of the differentiating cells based on (1) its specificity to stain cells with typical neuronal morphology and (2) the fluorescence expression levels before and after differentiation. Second, we selected an optimal culture condition for MSCs in which the higher proportion of neuronal cells was found (this was under increased cAMP). This condition was then reproduced for neural stem cells. Co-culture with rodent astrocytes failed to promote a higher yield of neuronal cells on MSCs. On the basis of morphological, immunocytochemical, and electrophysiological studies, we conclude that MSCs acquire some characteristics of neuronal cells, such as elongated and ramified processes, expression of NeuN, MAP2, NF-M, NF-200, βIII tubulin, and voltage-dependent ionic currents like TTX-sensitive Na^+, K^+, and Ca^{2+} currents. Nevertheless, MSC-derived cells were not able to reach the maturation stage achieved by NSCs,