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Original Research

cAMP Response Element-Binding Protein Controls the Appearance of Neuron-Like Traits in Chorion Mesenchymal Cells

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Abstract

Background: Mesenchymal stromal cells (MSC) from bone marrow have been reported to undergo the initial phases of neural differentiation in response to an increase of intracellular cAMP. We investigated the possibility that a similar effect applies to chorion-derived MSC. **Methods**: The intracellular concentration of cAMP was increased either by forskolin, to promote its synthesis, or by inhibitors of its degradation. The consequent reduction in the expression of mesenchymal markers was associated with the appearance of neuron-like morphology in a subset of cells. The effect was measured and characterized using biomarkers and an inhibitor of cAMP response element-binding protein (CREB). **Results**: The dramatic morphological change induced by all the treatments that promoted intracellular cAMP was transient and peaked on the third day. After that, cells returned to the typical fibroblast-like appearance within 24 hours. The distinctive morphology was associated to the expression of neuregulin 1, doublecortin, neuron-specific class III β-tubulin, and required cAMP response element-binding protein activity. Basic-fibroblast growth factor (b-FGF) treatment increased both the timeframe and number of cells undergoing the morphological change induced by the effect of forskolin. As opposite, arginine-vasopressin (AVP) and sphingosine-1-phosphate (S1P) reduced it. **Conclusions**: We conclude that cAMP and the ensuing CREB activation trigger a preliminary step towards neuronal differentiation of chorion-derived MSC. However, likewise other MSC, the stimulus is not sufficient to promote stable differentiation.

Keywords: cAMP; chorion mesenchymal stem cells; placenta; cAMP response element-binding protein; regenerative therapy; neural differentiation

1. Introduction

Full term placenta represents an abundant source of mesenchymal stromal cells (MSCs) and could offer an ideal source of cell precursors, particularly for pediatric autologous cell therapy. In addition to their self-renewal ability, immunomodulatory properties and plasticity, chorion derived MSCs (C-MSCs) are neither teratogenic nor liable of ethical limitations [1,2]. However, as compared to bone marrow MSCs (BM-MSCs) [3] and progenitor cells from other tissues, C-MSCs have been largely under-investigated for neural regenerative therapies.

Several authors described how an increase of the signaling molecule cAMP in the cytosol of MSCs derived from bone marrow [4–9], Wharton's jelly [10] amniotic fluid [11] produces a rapid morphological change and other features reminiscent of neuronal cells. Although the effect is transient [4] and does not lead to full commitment to neurons or glial cells phenotypes, it is likely to represent an early phase of the phenomenon [4]. Recently, IBMX-treated adipose derived MSCs were also shown to undergo morphological and phenotypic changes. Proteomic analysis supported an association to neuronal features deemed most promising in consideration of the resulting increase in b-FGF, a known neuronal growth factor for MSCs [12].

cAMP signaling is highly pleiotropic and is involved in the development of all three germ layers. cAMP formation is promoted by adenylyl cyclase, typically activated by stimuli acting via G-protein-coupled receptors. Its degradation to AMP is mediated by phosphodiesterases, crucial to control cAMP levels within the cell. cAMP activates several signaling pathways, most often mediated by the activation of protein kinase A (PKA) which in turn phosphorylates target proteins including other kinases, ion channels and transcriptional factors. Long-term effects are mediated by protein synthesis-dependent processes that involve the transcription factor cAMP response element-binding protein (CREB) [13]. cAMP is a universal second messenger and CREB role in neuronal development is well established. Being able to control this signaling pathway can be considered a crucial step in order to take full advantage of an untapped source of mesenchymal cells, like C-MSCs.

This study aims to reproduce and characterize the effect of cAMP on C-MSC. Three approaches known to increase intracellular cAMP produced a rearrangement of C-MSCs morphology that we prove to be reversible by time-lapse microscopy. The effect was prevented by a CREB-specific inhibitor and was modulated by potential regulators of neural development . These results support the potential for extending to C-MSCs the studies for the development of novel cell therapies for neural damages [14].

2. Materials and Methods

2.1 Materials

Forskolin, 3-isobutyl-1-methylxanthine (IBMX), and dibutyryl-cAMP (db-cAMP) were from Serva, Germany. Cell culture media and Pen Strep are from Life Technologies Europe BV. KG-501 was from Società Italiana Chimici, SIC, Italy. b-FGF was from Merck Darmstadt Germany. Sphingosine-1-phosphate (S1P) and arginine-vasopressin (AVP) were from Sigma Aldrich.

2.2 Cell Isolation and Culture

C-MSCs were enzymatically isolated from human term placenta and characterized as previously described [15]. In the experiments shown, cells were passaged 6–10 times. For neuron-like cell induction, C-MSCs were plated in 96 well plates (10^4/well) or on Ø12 mm coverslips coated with fibronectin for immunocytochemistry assay and grown at 37 °C, 5% CO_2 and 20% O_2 in DMEM 10% FCS and 1% Pen Strep. The medium was replaced after 24 hours with fresh growth medium reducing FCS to 1% and containing 50 μM forskolin (dissolved in DMSO), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX, dissolved in ethanol), or 1 mM dibutyryl-cAMP (db-cAMP, dissolved in water) or vehicle as reported in the text. The culture media remained the same for up to 6 days.

2.3 Immunofluorescence and Image Capture

After three days of treatment with the induction medium, C-MSCs were fixed using 4% paraformaldehyde (PFA) + 2% sucrose for 15 minutes and washed three times with phosphate buffer saline (PBS). Permeabilization and blocking was performed using PBS containing 10% FCS and 0.1% TritonX-100 for 30 minutes at room temperature. Coverslips were incubated overnight with primary antibodies raised against doublecortin (1:200 cat. #4645, Cell Signaling Technology, Inc. Danvers, USA), pro-neuregulin 1 (1:100 cat. CSB-PA09589A0Rb, Cusabio, Technology, Houston, USA), neuron-specific class III β-tubulin (1:500 Biolegend, 801202 San Diego, USA) for 3 hours at room temperature. After washing three times with PBS-0.05% Triton-X100, coverslips were incubated with secondary antibody (1:500 DyLightTM488-labeled IgG, KPL, Gaithersburg, USA) for 1 hour. Nuclei were stained with Hoechst 33342 (1:10000 SIC, Italy). The cytoskeleton was stained by phalloidin-alexa488 (1:500, SIC, Italy). Images were acquired by confocal microscopy (LSM710, Carl Zeiss) using a 20× Plan-Neofluor objective.

2.4 Images Analysis

Neuron-like cells were clearly different from fibroblasts because they manifested a number of protrusions and smaller nuclei, as compared to the larger, flat fibroblast phenotype. The number of neuron-like cells was quantified by manual counting using the Plugin Cell Counter of the software ImageJ Fiji (version 2.1.0/1.53c, NIH, Bethesda, Maryland, USA).

The fluorescent signal was quantified in confocal images and analyzed using ImageJ Fiji software. The background was measured in non-populated areas and subtracted. The specific signal was calculated by measuring the mean fluorescence intensity of the whole cell surface for over 30 cells. The intensity was normalized to the average signal of a comparable number of untreated cells stained and analyzed in parallel.

2.5 Time Lapse Analysis of Cell Morphology

C-MSCs were seeded in 96-well plates, after replacement of the growth medium with an induction medium, cells were placed in an EVOS® Onstage Incubator (Life Technologies) at 37 °C, 5% CO_2 and 20% O_2 under an EVOS-FL auto microscope (Life Technologies) for time lapse acquisition to monitor cell morphological changes. Images were taken with the high-sensitivity interline CCD monochrome (grayscale) camera in phase-contrast with a 10x Objective, fluorite, LWD, phase-contrast (Olympus AMEP 4681) every hour up to 6 days. The time lag of the neuron-like phase was determined for each cell in a number of representative fields (6 random fields at 10x, for each of triplicate well for each experiment).

2.6 Evaluation of Mesenchymal Markers by Real-Time PCR

Real-time PCR was performed on cells treated with forskolin (50 μM) and b-FGF (40 ng/mL) as indicated. Each condition was performed in triplicate wells. After treatment, cells were washed with PBS and total mRNA was extracted using the TRIzol extraction kit (Invitrogen) and reversed transcribed to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystem). cDNA from samples was mixed with the primers (the sequence is detailed in the **Supplementary File** S primers) and PowerUp SYBR Green Master mix (Applied Biosystems), and run on a CFX96Touch Real-Time PCR Detection System. Amplification was performed utilizing the following cycling parameters: 50 °C for 2 minutes, 95 °C for 2 minutes, 95 °C for 15 seconds and 60 °C for 1 minute; the last two steps were carried out for 40 cycles. Glyceraldhyde-3-phosphate dehydrogenase (GAPDH) was used as housekeeping gene. Experiments were conducted in triplicates. Data were transformed using the $\Delta\Delta$ct method.

2.7 Statistical Analysis

The statistical measurements values were presented as mean $\pm$ variance as indicated in the figure legend. n indicates the number of independent experiments. Normal distribution was analyzed applying Kolmogorov-Smirnov test. Normal distributed data were analyzed utilizing *t*-test. Not normal distributed data were analyzed utilizing non-parametric tests (Kruskal-Wallis and Dunn's multiple comparison as appropriate). In Fig. 3C nonparametric Kruskal-Wallis test was applied to compare the neuron-like cells number from day 2 to 4. Since at day 1 and 5 no neuron-like cell was present, the statistic was not applied because of the clear variation in respect to the other time point. To test at which day the effect was maximal we compared day 2 vs. day 3 and 4.

The p value was represented as follow: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. The details of the analysis for each figure are provided in the **Supplementary Data** (S statistics).

3. Results

3.1 Forskolin Applied to C-MSCs Induces the Appearance of Neuron-Like Cells

C-MSCs were isolated from human placentas at term and propagated *in vitro*. We characterized the mesenchymal properties of these preparations in terms of immunophenotype and potency in a previous paper [15].

Similarly to what was repeatedly documented for other MSCs typically derived from bone marrow [4–8], a subset of C-MSCs acquired a neuron-like morphology in response to a treatment aimed to raise the concentration of intracellular cAMP. The effect was unambiguous because in response to forskolin the large flat fibroblasts raised around the nucleus to form a central dense body surrounded by a branched cytoplasm (Fig. 1, **Supplementary Videos 1**,**2**).

The robust stress fibers, typical of mesenchymal fibroblast-like cells, remained well organized in the majority of the cells that did not respond to the treatment. On the contrary, actin filaments were completely disorganized in the cells that assumed the neuron-like morphology (Fig. 2A).

The effect on cell morphology was associated with an increase in the expression of three neuronal markers, doublecortin (DCX), neuregulin 1 (NRG1), and neuron-specific class III β-tubulin (TUJ1) (Fig. 2B–D). This increase mainly concerned neuron-like cells. In fact, despite the treatment with forskolin, the cells that retained a fibroblast-like morphology displayed a level of fluorescence comparable to untreated cells.

A component of FCS was suggested to inhibit the acquisition of a neuron-like morphology by BM-MSC [4]. Likewise, 10% FCS concentration in the growth medium of C-MSCs reduced the number of neuron-like cells as compared to 1% FCS (Fig. 3A).

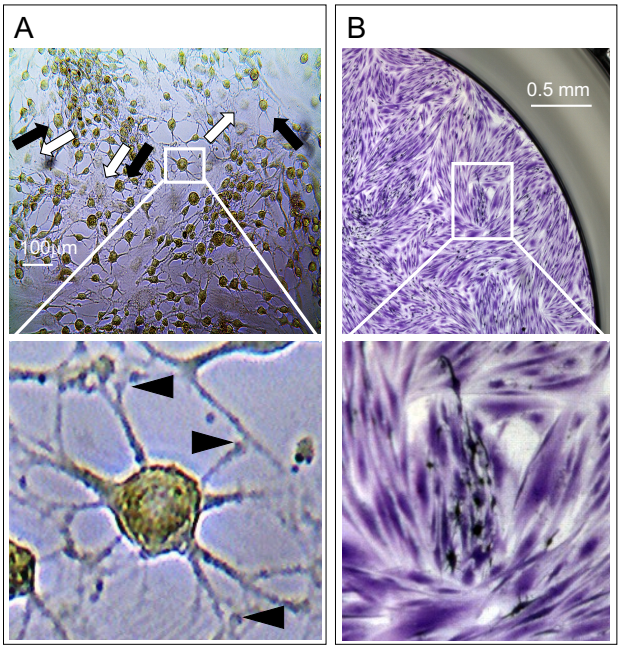

Fig. 1. Forskolin induces neuron-like morphology in C-MSC. (A) Phase-contrast image of C-MSCs after 3 days of treatment with 50 μM forskolin. Part of cells assumed a neuron-like morphology while others conserved a fibroblast-like shape. The upper panel shows a very large cluster of neuron-like cells. White arrows point to fibroblast-like cells, black arrows point to neuron-like cells. In the panel below a magnification of a neuron-like cell from the same cluster. Arrowheads indicate contacts with other cells. (B) Crystal violet staining of cells treated with forskolin. In the upper panel a cluster of neuron-like cells (cluster formation can also be observed in the **Supplementary Videos 1–5**).

3.2 The Appearance of Neuron-Like Cells is Transient and Prevented by CREB Inhibitor

The same dramatic change of morphology observed in response to forskolin was reproduced by two additional strategies, IBMX and the cAMP analog db-cAMP, that increase the intracellular concentration of cAMP acting on phosphodiesterases [9]. The three approaches replicated experimental settings applied by other authors to mesenchymal cells derived from other tissues [2,4–8,12,16] and produced an identical effect on cell morphology (Figs. 1,3B). Although the treatments demonstrated different efficacy, in all cases the peak was after three days (Fig. 3C). Eventually, neuron-like cells returned to their original morphology indistinguishable from all other fibroblast-like cells (**Supplementary Videos 1–4**), which excludes a toxic effect.

Further increasing the concentration did not show saturation of the effect, yet the morphological differences became less obvious, possibly as a consequence of toxic effects that over 100 μM reduced cell viability (**Supplementary Fig. 1**) while complete serum removal did not prevent morphological changes (not shown).

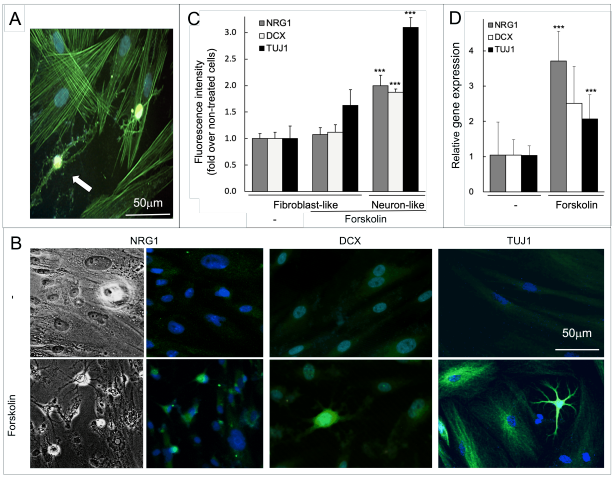

Fig. 2. Forskolin-induced rearrangement of the actin cytoskeleton and the expression of neuronal markers. (A) C-MSCs after 3 days of treatment with forskolin, phalloidin staining reveals the actin cytoskeleton in green, Hoechst-stained nuclei are in blue. The white arrow indicates a neuron-like cell. (B) Representative images of C-MSCs after 3 days of treatment with forskolin or vehicle. The panels on the left show phase-contrast images. All the other images present in panel (B) show cells stained for neuronal markers as indicated (green) or the nuclei (blue). (C) Fluorescence intensity was quantified in cells stained as in panel (B) and plotted as mean ± SD (unpaired *t*-test for the forskolin treated cells compares fibroblast-like vs. neuron-like, n = 3). (D) The expression of neuronal markers was measured by real-time PCR in C-MSC treated as indicated. For each condition, data are normalized to the gene expression of untreated cells.

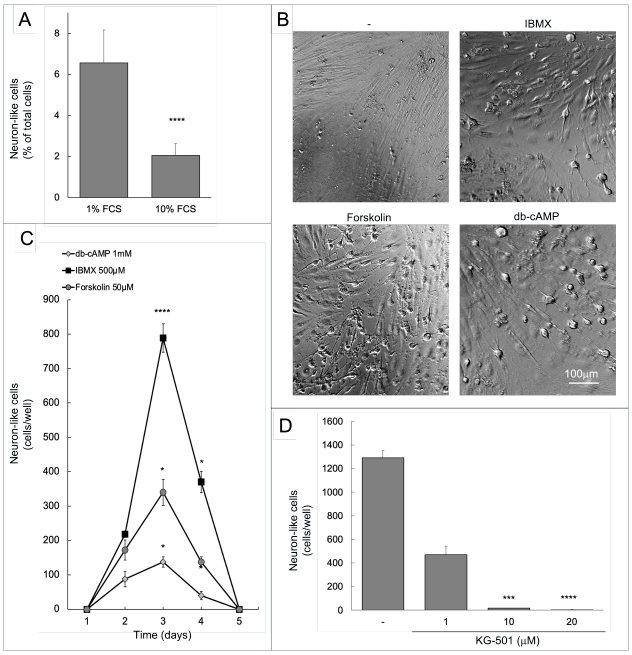

Fig. 3. Neuron-like morphology is transient and mediated by transcription. (A) Neuron-like cells were counted 3 days after stimulation with 50 μM forskolin comparing two concentrations of FCS (n = 3 performed in triplicate, mean ± SEM). (B) Representative phase-contrast images of clusters of C-MSCs after 3 days after stimulation (50 μM forskolin, 500 μM IBMX, 1 mM db-cAMP) as indicated in each panel. (C) Neuron-like cells were counted over 5 days starting the first day after addition of the indicated stimuli applied as in panels (A) and (B) (n = 3 performed in triplicate, mean ± SEM. Statistical tests and representative movie files are available in the **Supplementary Materials**). (D) KG-501 was applied concomitantly to 50 μM forskolin, neuron-like cells were quantified on the third day of differentiation (n = 3, performed in triplicate, mean ± SEM, one way ANOVA).

cAMP-responsive elements binding protein (CREB) phosphorylation by PKA controls neurogenesis by supporting newborn neurons and its role is conserved throughout evolution [17]. In order to verify whether the appearance of neuron-like cells involved CREB, the specific inhibitor KG-501 was simultaneously administered in culture with forskolin. In the presence of 1 μM KG-501, the number of neuron-like cells was already reduced by more than 50% and at 10 μM the morphology change was completely prevented (Fig. 3D) consistent with what previously reported for other cells [18].

3.3 Co-Stimulation with b-FGF Promotes the Appearance of Neuron-Like Cells

b-FGF is central to a highly orchestrated and complex sequence of cues required for the acquisition of a neuronal phenotype [19] and has often been combined with stimuli raising cAMP to promote MSCs neural differentiation [9,20]. Combining 40 ng/mL b-FGF with 50μM forskolin promoted the formation of a larger number of neuron-like cells (to 143 ± 32%, $p < 0.05$) and the effect lasted longer (from 26 hours to 33 hours with an average difference of 6.7 ± 0.9 h, Fig. 4).

3.4 Forskolin Treatment Reduces the Expression of Pluripotent Markers

The expression levels of Sox2 and Oct4 are tightly balanced to support self-renewal and pluripotency. Forskolin treatment produced a dramatic decrease in both transcription factors by the third day (Fig. 5). Expression remained low when all cells recovered the fibroblast-like appearance and the decrease was not prevented by b-FGF.

3.5 S1P Prevents the Appearance of Neuron-Like Morphology

S1P is a bioactive lysophospholipid with versatile biological effects, mainly exerted through the binding of their specific receptors [21]. S1P is a powerful stimulator of neurogenesis by binding to various G protein coupled receptors (GPCRs) to activate or inhibit signalling pathways and by cross-talking with other cytokine and growth factor receptors [22,23]. Our lab previously demonstrated that S1P ac-

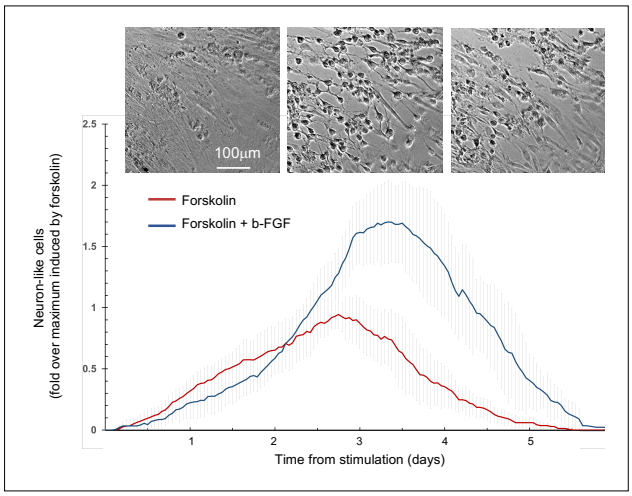

Fig. 4. b-FGF promotes neuron-like morphology. The appearance of cells characterized by a neuron-like morphology was monitored in time lapse documenting the time extent before the cell would return to a fibroblast-like aspect. For each experiment (n = 3) data are normalized to the maximum number of neuron-like cells achieved after stimulation with forskolin alone (mean ± SD). Each experiment was performed in triplicate wells for each condition. At least 6 fields/well were analyzed. In the inset panels, representative pictures in phase-contrast showing frames of cell cluster at the corresponding time (the full movie file is available in the **Supplementary Materials**).

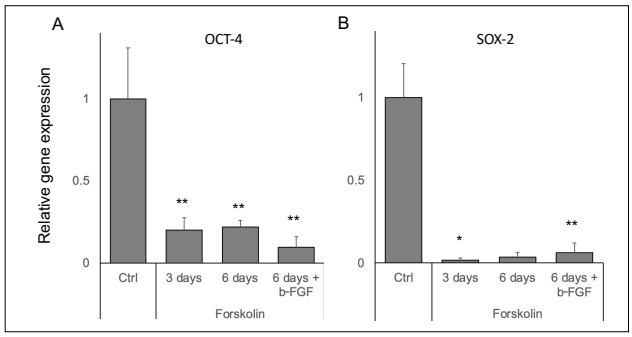

Fig. 5. Forskolin induced reduction in the expression of pluripotent markers. The expression of pluripotent markers was measured by real-time PCR in C-MSC treated as indicated. (A) OCT-4 mRNA expression. (B) SOX-2 mRNA expression. For each condition, data are normalized to the gene expression of untreated cells (n = 3, performed in duplicate, mean ± SEM, one-way ANOVA).

tivates ERK1/2 in C-MSCs in a dose-dependent manner. ERK1/2 activation was transient in time and it was completely inhibited by pertussis toxin, indicating that the pathway was fully Gi-dependent [15]. Combining 10 μM S1P with 50 μM forskolin was found to prevent the effect of forskolin (Fig. 6).

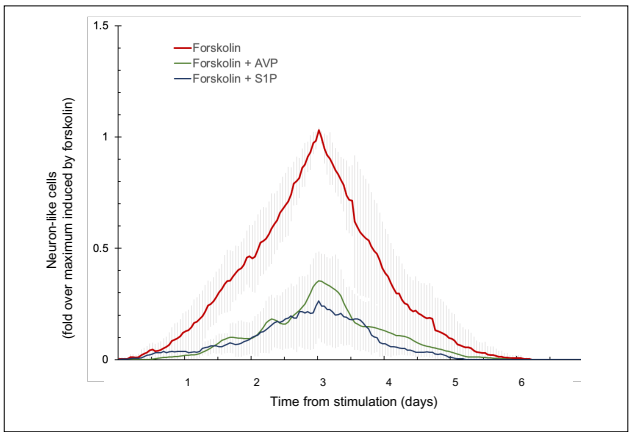

Fig. 6. S1P and AVP prevent neuron-like morphology. The appearance of cells characterized by a neuron-like morphology was monitored in time lapse. For each experiment (n = 6) data are normalized to the maximum number of of neuron-like cells achieved after stimulation with forskolin alone (mean ± SD). Each experiment was performed in triplicate wells for each condition. At least 6 fields/well were analyzed.

3.6 Arginine Vasopressin (AVP) Prevents the Appearance of Neuron-Like Morphology

AVP, a hormone synthesized from the AVP gene in hypothalamus neurons, regulates various aspects of stem cells life. All three subtypes of AVP receptors are found in mice adipose-derived MSCs (A-MSCs) whereas human A-MSCs expressed only AVPR1 subtype [24]. Activation of AVPR1 activates Gq-proteins and on turn PLC-β and calcium release [25], with a direct effect on CREB through the MAP-Kinase pathway. Similarly to S1P, 500 nM AVP prevented the effect of forskolin on the appearance of neuron-like cells (Fig. 6).

4. Discussion

The most common source of human MSCs for "cell therapy" is the bone marrow. However, only 1 out of 10,000 of nucleated cells in the marrow is mesenchymal and an invasive procedure, particularly in newborns, is required to obtain reasonable yield of cells.

Placenta could provide a virtually unlimited supply of MSCs from a tissue that is normally discarded at birth. C-MSCs exploitation could lead to highly innovative treatments, particularly for autologous therapies in newborns, including neurological disorders. We demonstrated that the exposure of C-MSCs to different agents that increase cytosolic cAMP reproduces very closely the neuron-like phenotype that has been thoroughly investigated for BM-MSCs [3–8].

Several authors reported the differentiation of MSCs into neural cell types and astrocytes, albeit the effective functional properties of the derived lineages remain highly debated.

The reliability of neural markers is also questionable considering the heterogeneity and relative abundance of transcripts and proteins expressed by MSCs [4]. Neural circuit development and regeneration is a highly complex process determined by a sequence of cues and interactions. In this sense, we hypothesize that our and others observations in a number of MSCs only represent a particular step triggered by cAMP, however, a detailed understanding of the cues that govern each step of neural differentiation is crucial in order to finally allow effective cellular therapies to treat damages of the neural tissue [6]. cAMP signalling is key to neural progenitor cells proliferation and differentiation, and consequently to nerve regeneration [26]. cAMP's effects on morphological maturation are believed to depend on PKA and CREB activation. Once activated, CREB can bind to the promoter region of genes containing CRE. Whereas phosphorylated CREB is basically absent in stem cells, it is transiently up-regulated in neural progenitor cells and young neurons during the first few weeks of differentiation. During this time the level of CREB phosphorylation correlates with the total dendritic length and the morphological maturity of the young neurons [27].

Alone or in combination with other agents, such as b-FGF and BDNF, cAMP has been reported to promote neuron-like morphology in BM-MSCs [3] with timeframes ranging from hours to days [6]. The rapid effect of other agents that produced dramatic morphological changes, i.e., β-mercaptoethanol, butylated hydroxianisole and dimethylsulfoxide, has been instead attributed to an art factual shrinkage of the cell. The effect of cAMP is mediated by PKA and, since it is typically transient [4,8], it has been interpreted as a partial replica of early steps of neuronal differentiation. Our results with C-MSCs support the same conclusion occurring with an even slower dynamic [4–8] that requires gene transcription. Indeed, the appearance of the first neuron-like cells took several hours and reached maximal number of dendrite-like protrusions by the third day. Since the half-life of the three approaches we utilized to raise cAMP ranged from few hours to several days [4], we assume the phenomenon reverts independently from the persistence of the cue or the substitution with fresh stimulus.

Albeit our initial characterization of these cells using a set of mesenchymal markers described a homogeneous population of precursors [15], we found that the morphological effect only occurred in part of the cells. Noticeably, neuron-like cells were not distributed randomly but in clusters (Fig. 1 and **Supplementary Videos**). It is thus possible that our preparation included a subset of cells with a specific potential to respond to cAMP by acquiring the neuron-like morphology. Additional research is required to understand the reason why only a well-defined subset of cells undertook the process.

If C-MSCs respond to cAMP similarly to BM-MSCs, the molecular basis of the phenomenon remains to be better characterized. KG-501 experiments suggest the involvement of CREB which was shown to mediate the differentiation of neuronal precursor cells in response to cAMP [16,28,29]. In addition, CREB obtained growing attention as a key regulator of synaptic plasticity in the adult brain [30]. Forskolin reduced the expression levels of SOX-2 and OCT-4 to an extent that was almost complete suggesting that not only it occurred to "neuron-like cells" but also to the large majority of the cells conserving the fibroblast-like shape. The expression levels of SOX-2 and OCT-4 play a pivotal role as lineage specifiers [31] including for neuronal commitment [32], and the treatment with forskolin most likely compromised the possibility of C-MSCs to undergo neuronal commitment. We suggest that a raise of cAMP in C-MSCs cytosol activates a transcription program that remains incomplete and it is thus turned off. As a consequence, cells do not die but revert to the fibroblast morphology as shown by our videos. A trophic effect of b-FGF leading to neuron-like phenotypic changes was previously reported not only for neural progenitors [33], but also for bone marrow [3] and other mesenchymal cells [34] and for pancreatic β-cells [35]. Other components previously included in differentiation media for MSCs should be combined to increase the effect of cAMP and further explore the potential of neuron-like cells derived from C-MSCs. Moreover, transcriptomic analysis could help to better characterize the significance of this phenotype.

S1P inhibited the appearance of neuron-like cells. The effect could be explained with the activation of Gi proteins and the consequent inhibition of adenylyl cyclase responsible for the production of cAMP. In this respect, AVP should not have produced the same effect since it signals through GPCRs that activate Gq/11 and Gs. Both, S1P and AVP, can promote PLCβ activity via Gq/11 and could play a pivotal role in the modulation of the neuron-like phenotype [36]. Additional experiments are unquestionably required to clarify the inhibitory effect.

Limitations

The authors recognize some limitations to this study. Time-lapse recording allows only to observe morphology. This approach proved that neuron-like cells can return to the original fibroblast-like aspect and indistinguishable from all other cells. However, it did not detail the nature of neuron-like phenotype. Transcriptomic and proteomic analysis of MSCs derived from several origins and treated with IBMX suggested that a similar morphology alteration coincide with neurite substructure development [12]. A similar analysis could be instrumental to define more precisely the significance of this process also in MSC derived from placenta. Microdissection strategies or better markers will allow to selectively tag the small cell population and explore the specific nature and physiological significance of neuron-like cells in future RNAseq studies.

A full commitment to neural phenotype could be tested with electrophysiological analysis by patch-clamp of single cells to proof the excitability, if any, associated with this neuron-like phenotype [11].

5. Conclusions

We conclude that, as previously described for other MSCs, agents promoting an increase of cAMP produce a dramatic reorganization of the cytoskeleton of a subpopulation of C-MSCs. The development of protrusions towards other cells is prevented by a CREB inhibitor and it is fully reverted within 72 hours from the stimulation.

The possibility to take advantage of C-MSCs for therapeutic use appears even more exciting than the exploitation of MSCs from other sources because of the several advantages mentioned above. Further characterization of the specific subset of C-MSC with a neuron-like phenotype and a deeper understanding of the underlying mechanism could allow to guide C-MSCs towards full neural commitment and differentiation.

Author Contributions

GI, FS and LG designed the research study. GI, GR, FS, AB and AD performed the research. GR, FS, AB, CB, AD, LO, MDC provided help and advice on this project. LO, and MDC provided materials. GI, GR, FS, AB, AD and LG wrote the manuscript. GI and LG supervised the project. LG contributed to funding acquisition. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was conducted according to the guidelines of 243 the Declaration of Helsinki and approved by the Ethics Committee of the Azienda Ospedaliera 244 Universitaria Integrata di Verona, no. 0054.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2708249.

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