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Original articles

THE EFFECT OF COMBINATIONS OF GROWTH FACTORS AND HYALURONIC ACID ON THE PROLIFERATION AND CHONDROGENIC DIFFERENTIATION OF HUMAN SYNOVIUM DERIVED MESENCHYMAL STEM CELLS

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ABSTRACT

Growth factors and hyaluronic acid (HA) have been shown to stimulate functional activity of mesenchymal stem cells (MSCs) and thus may influence damaged tissue healing, including cartilage. In this study we investigated the effects of individual and combinatorial application of HA, platelet derived growth factor–BB (PDGF-BB), transforming growth factor β1 (TGF-β1), bone morphogenetic protein-4 (BMP-4) and fibroblast growth factor-2 (FGF-2) on the proliferation and chondrogenic differentiation of human synovium derived MSCs (SD MSCs). Isolated SD MSCs were expanded in α-MEM medium and characterized using immunocytochemistry, multilineage differentiation assay and CFU-assay. Proliferation activity of MSCs was examined with MTT-assay. Chondrogenic differentiation and glycosaminoglycan level in micro-pellet culture of MSCs were evaluated with histochemical and biochemical analysis. Our results showed that the individual use of FGF-2 and PDGF-BB significantly increased the rate of MSC proliferation compared to the control. At the same time, TGF-β1 did not affect cell growth. Combinations of growth factors: TGF-β1+FGF-2, TGF-β1+PDGF-BB, and FGF-2+PDGF-BB were able to significantly increase the proliferative activity of MSCs. The greatest synergistic effect was found with combination of all three factors TGF-β1, FGF-2, and PDGF-BB. Histochemical and biochemical analysis revealed that combination of TGF-β1 and BMP-4 have a significant effect on chondrogenic differentiation and the synthesis of glycosaminoglycans in micro-pellet culture of MSCs. The addition of HA to growth factors has minor effect on the proliferation and does not affect the chondrogenic differentiation of MSCs. This study demonstrated that combination of growth factors can promote proliferation and chondrogenic differentiation of SD MSCs and may be effective in enhancing cartilage healing.

Key words: synovium derived mesenchymal stem cells, cell proliferation, chondrogenic differentiation, growth factors, hyaluronic acid.

INTRODUCTION

Mesenchymal stem cells (MSCs) are multipotent adult stem cells that can be isolated from various tissues of humans and animals. MSCs attracted the attention of scientists due to their immunomodulatory properties and multilineage differentiation potential and active participation in the regeneration of damaged tissues [1-3]. When stimulated by appropriate growth factors or stimuli, MSCs are able to differentiate into a number of specialized cell types, such as adipocytes, chondrocytes and osteoblasts [4].

Growth factors are signaling molecules that affect the functional activity of MSCs. A number of these growth factors affect cartilage regeneration including platelet derived growth factor–BB (PDGF-BB), transforming growth factor β1 (TGF-β1), bone morphogenetic protein-4 (BMP-4) and fibroblast growth factor-2 (FGF-2) [5]. Rosier and colleagues have shown that TGF-**β**1 stimulates proliferation and induces chondrogenic differentiation of bone marrow MSCs [6]. Moreover, it has been shown that *in vitro* treatment of human bone marrow and adipose tissue-derived MSCs with recombinant TGF-β1 leads to a downregulation of collagen type I gene expression and to upregulation of collagen type II and aggrecan gene expression typical for hyaline cartilage formation [7]. It was revealed that BMP-4 as TGF-**β**1 also increases the production of aggrecan and type II collagen, induces initial differentiation of MSCs to chondroblasts and facilitates a differentiation into mature chondrocytes [8]. FGF-2 or basic fibroblast growth factor (bFGF) is mitogenic factor for mesodermal and neuroectodermal cells in vitro. FGF-2 is involved in various biological processes, including embryonic development, cell growth, morphogenesis and tissue repair [9]. It was shown that FGF-2 promotes the proliferation and chondrogenic potential of MSCs as well as enhances proteoglycan production by adipose tissue-derived MSCs [10]. PDGFs are important mitogens for various types of mesenchymal cells such as fibroblasts, osteoblasts, tenocytes, vascular smooth muscle cells and mesenchymal stem cells [11]. PDGFs are dimeric glycoproteins that can be composed of two A subunits (PDGF-AA), two B subunits (PDGF-BB) or one of each (PDGF-AB). These growth factors stimulate the proliferation, differentiation, survival, chemotaxis and directed migration of mesenchymal cells [12]. For example, it was demonstrated that PDGF-BB can protect various cell types from apoptosis and can induce cell division and growth of mesenchymal cells [13, 14].

Hyaluronic acid (HA) is one of the main components of the extracellular matrix, found in many biological fluids, including synovial fluid. Recent studies showed that HA plays an important role in cell proliferation and migration of MSCs [15]. In addition HA is used as therapeutic agent providing additional shock absorption and prevent articular cartilage degeneration.

Therefore, these growth factors and HA have been reported to promote *in vitro* proliferation and chondrogenic differentiation of bone marrow and adipose tissue-derived MSCs in combination with TGF-β. However, there is no literature data on the effects of combinations of growth factors and HA on proliferation and chondrogenesis of human synovial-derived MSCs (SD MSCs).

The present study was designed to test the hypotheses (1) that proliferation of human SDMSCs can be markedly promoted by using a combination of TGF-β1, FGF-2, and PDGF-BB, (2) chondrogenic differentiation can be enhanced by using a combination of TGF-β1 and BMP-4, and (3) whether HA in combination of growth factors can promote proliferation and chondrogenic differentiation of human SD MSCs. The aim of this study was to find the optimal combination of growth factors and HA promoting the proliferation and chondrogenic differentiation of human SD MSCs.

MATERIALS AND METHODS

Isolation and cultivation of SD MSCs. Human synovial membrane biopsy was taken aseptically from the knee joint during arthroscopic procedures at the National Scientific Center for Traumatology and Ortopedics (Nur-Sultan) with patient's informed consent for biopsy. Pieces of the synovial membrane were placed in 50 ml centrifuge tubes with DMEM medium containing 100 IU/ml of penicillin, 100 μg/ml of streptomycin, and 0,25 μg/ml of amphotericin B. Biopsy was delivered at a temperature of 2-8°C in a special shipping box. Then, the synovial membrane was washed twice with antibiotic-containing phosphate buffered saline (PBS) and minced into small pieces (1-2 mm^3). Pieces of the synovial membrane were incubated in 0.3% collagenase type II solution (Life Technologies, UK) for 4 hours or overnight at 37°C. Obtained cell suspension was filtered through a 70 μm nylon cell filter (Becton-Dickenson, USA) to remove the remaining tissue fragments, resuspended in a complete culture medium (DMEM medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin) and cultured at 37°C and 5% CO_2. After 2 days, unattached cells were removed and a fraction of adherent cells were cultivated until the confluence of cells reached about 80-90%. Cells were subcultured using recombinant trypsin (TrypLE™ Express) with an interval of 5-7 days. The culture medium in MSC culture was changed every two days.

Colony-forming unit (CFU) assay. Cells isolated from the synovial membrane of humans and rabbits were seeded into Petri dishes of 1 cell/cm^2 and cultured in a complete culture medium for 14 days at 37°C, 5% CO_2. At the end of the cultivation, the cells were washed with PBS and stained with 0.5% crystal violet solution for 5 min at room temperature. After washing twice with PBS, colonies were counted using an SZ61 stereomicroscope (Olympus, Germany).

Crystal violet staining. The cell monolayer was washed twice with phosphate buffer and fixed for 2 min with ethanol:PBS (1:1) solution. Next, the cells were incubated for 10 minutes in freshly prepared ethanol. After removal of ethanol, Petri dishes with cells were dried and stained with 0.5% crystal violet for 25 minutes. Petri dishes with stained cells were washed in running water, then in deionized water, dried, and analyzed using an inverted microscope.

Chromosomal analysis. Before fixation, the cells were checked for the presence of metaphases under a phase-contrast microscope. 5 μl of ethidium bromide (stock 1 mg/ml) per 1 ml of culture medium was added to the cell culture containing a sufficient amount of metaphases and incubated for 15 minutes at 37°C. After that, 1 μl of colcemid solution (stock 1000x) per 1 ml was added for 2-4 hours and incubated at 37°C. After treatment with colcemid, cells were washed with PBS and trypsinized with 0.25% trypsin solution containing 0.037% EDTA-Na_2 for 5 minutes at 37°C. Then trypsin was inactivated and the cell suspension was centrifuged for 5 minutes at 1000 rpm. A warm hypotonic solution of 0.38M KCl was added to the cell pellet and gently resuspended by pipetting. After 15 min incubation at 37°C, the cells were fixed in a freshly prepared fixative solution (1:3, ethanol:glacial acetic acid). After fixation, the resuspended cells were placed on clean glass slides and allowed to air-dry. Prepared specimens were checked under a light microscope. In the presence of metaphases, the samples were stained with Giemsa. Chromosomes were analyzed using the Metasystem software.

Multilineage differentiation assay. For differentiation into chondrocytes 1.25×10^6 cells/ml of MSCs were resuspended in a differentiation medium consisting of high glucose DMEM medium, 1% ITS+Premix (BD Biosciences), 100 μM ascorbate-2-phosphate (Sigma, USA), 10^{-7} M dexamethasone (Sigma), and 10 ng/ml TGFβ1 (Sigma, USA). To obtain chondrogenic micro-pellets, 2.5×10^5 cells were seeded in 96 well v-bottom polypropylene plate (Phenix, Hayward, CA), then centrifuged at 500 g, and transferred to a CO_2 incubator at 37°C, 5% CO_2. The medium was changed 3 times per week. On day 21 of differentiation, cell micro-pellets were harvested and fixed in 10% neutral buffered formalin. Samples were embedded in paraffin, cut on a microtome, and processed for staining with hematoxylin-eosin and toluidine blue.

For osteogenic differentiation of MSCs, an induction medium containing 10^{-7} M dexamethasone, 10 mM β-glycerol-phosphate, and 50 μM ascorbate-2-phosphate was used. Cells were cultivated for 3 weeks, after which the cells were stained with alizarin red. Differentiation of MSCs into adipocytes was performed by culturing them in an induction medium containing 10^{-6} M dexamethasone, 0.5 μM 3-isobutyl-1-methylxanthine, and 10 ng/ml insulin for 3 weeks. At the end of cultivation, the cells were stained with Oil Red O.

Immunocytochemical analysis. The cells were fixed with a freshly prepared solution of 4% paraformaldehyde in PBS (pH 7.2) for 20 min. After five minutes of treatment with Triton X-100, the cells were washed three times for 5 minutes with PBS, and then a 1% solution of albumin in phosphate buffer was added for 30 min. Next, the cells were incubated with CD90, CD105, CD73, STRO-1 antibodies. To prepare the required concentration, antibodies were diluted in a solution containing 1% albumin and 0.1% Tween 20 in phosphate buffer. Primary mouse monoclonal CD90, CD105, and CD45 antibodies were diluted at 1:100 (BD Biosciences, USA), polyclonal CD73 rabbit antibodies at 1:200 (Abcam, UK), and mouse STRO-1 monoclonal antibodies at 1:50 (Life Technologies, UK). Cells were incubated at 37°C for 1 hour. After incubation, cells were washed 3 times for 5 minutes with PBS containing 0.2% Tween 20, and secondary goat anti-rabbit and anti-mouse antibodies (1:500) conjugated with Alexa Fluor 488 (Life Technologies, UK) were added. Cells were incubated for 45 min at 37°C in the dark and then washed 3 times for 5 minutes with PBS containing 0.2% Tween 20. Specimens were dried and 10 μl of antifade medium with DAPI (Life Technologies, UK) was added to the slide. Specimens were analyzed using an inverted microscope Axio Observer

A1 (Carl Zeiss, Germany) and Zen 2011 software. Obtained images were analyzed using Image J software.

Cell proliferation assay. The proliferative activity of SD MSCs was measured using the MTT assay, which was previously described by Mosman et al. [16]. To do this, cells were resuspended in a complete medium and seeded 5 x 10^3 cells/100 µl per well into a flat-bottomed 96-well plate. The next day, the medium in wells was carefully removed and complete medium with various growth factors such as TGFβ1 (10 ng/ml), FGF-2 (10 ng/ml), PDGF-BB (5 ng/ml), and 0,25% low molecular weight HA (TRB Chemedica AG, Germany) was added, individually or in various combinations. All recombinant growth factors were purchased from Life Technologies. After 48 hours, 20 µl of MTT (5 mg/ml) were added to the wells and incubated for 4 hours at 37°C, 5% CO_2. After incubation, the medium was carefully removed and 150 µl of DMSO was added to the wells to dissolve the crystals of dye. Optical density was measured at wavelength 570 nm using a plate spectrophotometer (Biorad, Model 680, France).

Chondrogenic differentiation of SD MSCs in the presence growth factors and HA. For this experiment, the following growth factors were used: 10 ng/ml TGF-β1, 100 ng/ml BMP-4 and 0,25% low molecular weight HA. The culture medium without growth factors and HA was used as a control. Chondrogenic differentiation of SD MSCs after the addition of various combinations of growth factors and HA was assessed by the formation of chondrogenic micro-pellets as described in subsection "Multilineage differentiation assay" .

Measurement of DNA Content. DNA content was analysed in SD MSC micro-pellet. 500 µL of papain solution was added to the micro-pellet and they were incubated in dry oven at + 60°C for 16 h. After digestion of the micro-pellets biochemical assays were carried out. Measurement of DNA content was performed using Hoechst 33258 DNA assay, following a previously described protocol [17]. DNA after staining Hoechst 33258 dye was measured by multifunctional plate reader (SpectraMax M5, Molecular Devices, USA) at excitation of 350 nm and emission of 450 nm.

Measurement of GAG Content. The chondrogenic differentiation of SD MSC in micro-pellet culture was evaluated by measuring the GAG content on the papain digested samples and dimethylmethylene blue (DMB) dye with 10 mg/mL bovine derived chondroitin-4-sulfate (Sigma-Aldrich) that was used as a standard, according to a previously described protocol [18]. Briefly, 40 µL of each sample was added to a flat bottom 96 well-plate and 125 µL of the DMB was added. After staining, GAG content in the samples was measured on the multifunctional plate reader at an optical density of 595 nm.

Statistical analysis. All data are presented as mean ± SD. The statistical significance was calculated using one-way ANOVA with Bonferroni's multiple comparison tests. P <0,05 was considered statistically significant. Statistical analysis was conducted with software Statistica 6.0 (StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Before studding the effects of growth factors and HA on the proliferation and chondrogenic differentiation of human SD MSCs, we first have isolated the cells from human synovium and characterized them according recommendation of the International Society for Cell&Gene Therapy. It was found that cells isolated from human synovium have high proliferative activity and the ability to form fibroblastic colonies (Figure 1).

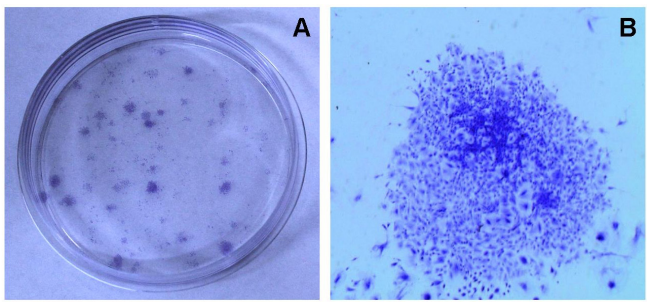

Figure 1 - Colony formation of human SD MSCs. A - MSC colonies in a Petri dish after staining with crystal violet. B - Enlarged image of MSC colonies.

Moreover, the chromosomal analysis showed that the cells isolated from the synovial membrane had a normal karyotype (46; XX) without visible chromosomal rearrangements, as shown in Figure 2.

To prove that obtained culture of SD MSCs is a phenotypically homogeneous cell population, an immunocytochemical analysis was performed using antibodies to MSC markers:

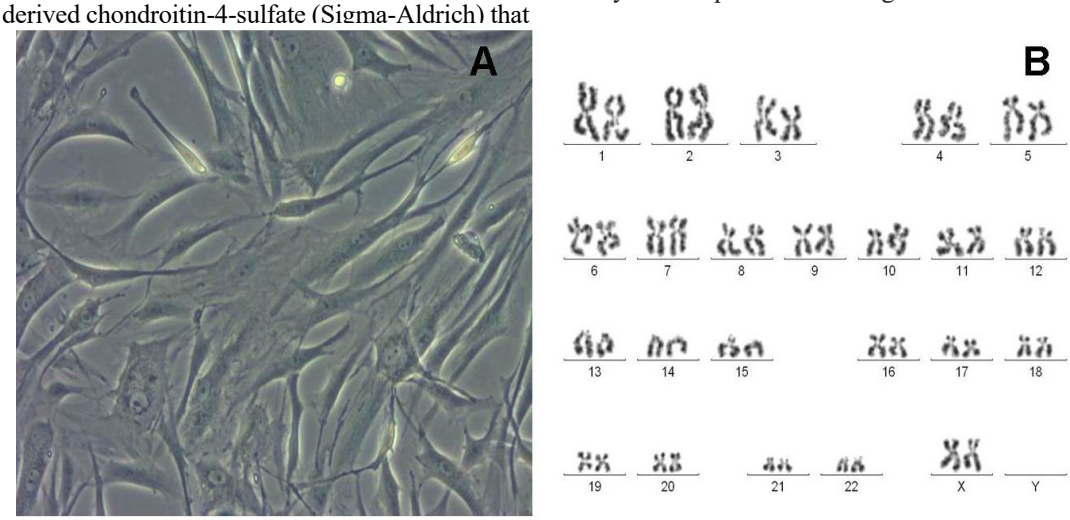

Figure 2 - Morphological and chromosomal analysis of human SD MSCs. A - Phase-contrast image of a live culture of human MSCs. B - Metaphase chromosomes of MSCs stained with Giemsa stain.

CD105, CD90 CD73, and STRO-1. The results of the analysis showed that cells isolated from human synovial membrane have the MSC phenotype with a significantly pronounced expression of CD105, CD90 CD73, and STRO-1 (Figure 3).

In addition, these cells were found to have the ability to multilineage differentiation into adipocytes, chondrocytes, and osteoblasts, as shown in Figure 4.

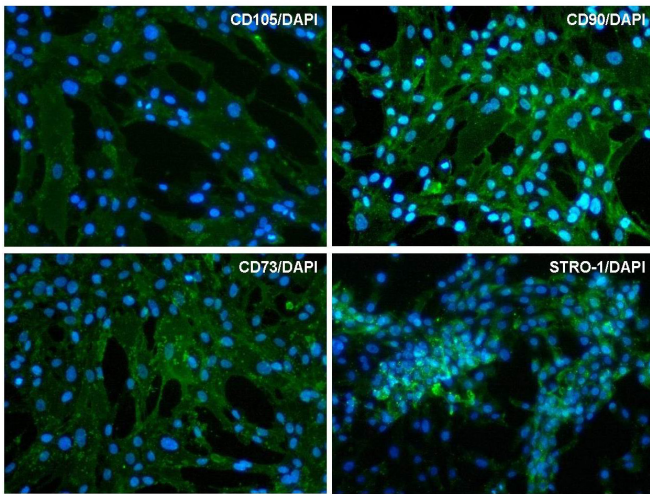

Figure 3 – Immunocytochemical analysis of human SD MSCs. The cells express mesenchymal stem cell markers CD105, CD90 CD73, and STRO-1 (green). The nuclei (blue) are labeled stained with DAPI.

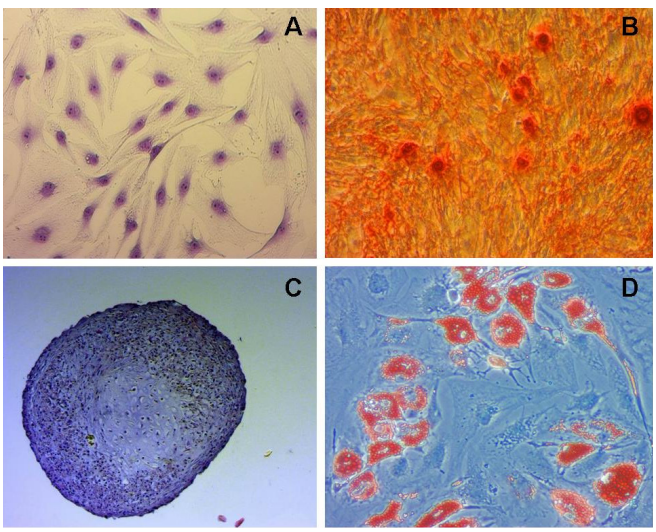

Figure 4 – Multilineage in vitro differentiation potential of human SD MSCs. A – Undifferentiated human MSCs stained with crystal violet. B - Osteoblasts after staining with Alizarin red S. Orange-red deposits are visible in the osteoblasts, which indicates a significant accumulation of calcium. C – Cross-section of chondrogenic micro-pellet stained with toluidine blue. D - Adipocytes containing lipid vacuoles stained with Oil Red O.

Thus, the presence of the above-listed properties in the cells isolated from human synovial membrane confirms their belonging to the class of multipotent MSCs.

To define the proliferation capacity of human SD MSCs we carried out the experiments on individual and combined effects of growth factors TGF-β1, FGF-2, PDGF-BB and HA on SD MSC proliferation. In the study, SD MSCs were seeded after 3rd passage into a 96-well plate at $5x10^3$ cells per well. After 24 hours, when the cells adhered to the surface of the wells, complete culture medium was replaced with a medium with the following factors: FGF-2 (10 ng/ml), TGF-β1 (5 ng/ml), PDGF-BB (10 ng/ml), as well as a combination of these growth factors with hyaluronic acid. The proliferation of SD MSCs in each well was measured using the MTT assay. Our results showed that the individual application of FGF-2 and PDGF-BB significantly increased the rate of human SD MSC proliferation compared to the control (Figure 5).

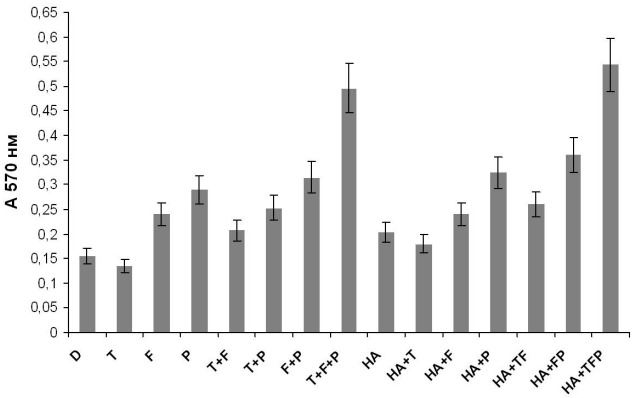

Figure 5 - Effects of growth factors TGF-β1, FGF-2, PDGF-BB and HA on human SD MSC proliferation: D - DMEM medium without growth factors (control), T - TGF-β1, F - FGF-2, P - PDGF-BB, HA - hyaluronic acid.

At the same time, the addition of TGF-β1 did not affect cell growth. Although combinations of growth factors: TGF-β1+FGF-2, TGF-β1+PDGF-BB, and FGF-2+PDGF-BB were able to significantly increase the proliferative activity of SD MSCs, the greatest synergistic effect was found after application of a combination of all three factors TGF-β1, FGF-2, and PDGF-BB. It should also be noted that incubation with three growth factors changed a morphology of SD MSCs dramatically. In the presence of growth factors, MSCs acquired a slight fusiform morphology, while MSCs grown in complete culture medium without growth factors had a flatter, fibroblast-like morphology (Figure 6).

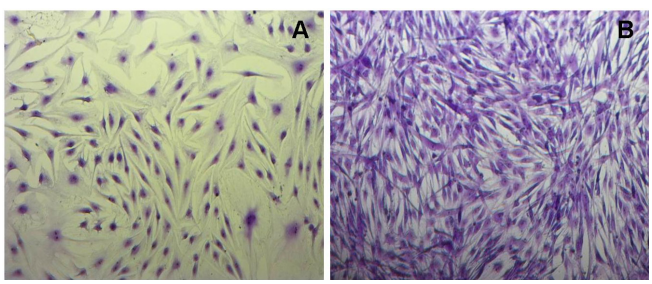

Figure 6 – Morphology of MSCs after cultivation in culture media without (A) and with the addition of growth factors TGF-β1, FGF-2, and PDGF-BB (B).

In previous experiments, it was shown that low molecular weight HA at a concentration of 0,25% caused an increase in the proliferation of SD MSCs [20]. In this regard, it was interesting to study the effect of HA in a combination of growth factors on the growth of SD MSCs. In this series of experiments, the same combinations of growth factors with the addition of 0,25% HA were used. MTT assay showed that the addition of 0,25% HA with combination growth factors promoted proliferation of SD MSCs (Figure 5). However, stimulating effects of combined application HA and growth fac-

tors on MSC proliferation were insignificant compared to the effects of growth factors.Thus, based on the data, it can be concluded that the combination of growth factors TGF-β1+FGF-2+PDGF-BB significantly increases the proliferation of human synovial MSCs. The addition of HA to growth factors can slightly increase the proliferative activity of SD MSCs.

Next, to define the differentiation capacity, micro-pellet culture of human SD MSCs were treated with TGF-β1, BMP-4 and HA. Cultivation of chondrogenic micro-pellets makes it possible to determine cell differentiation, but also the production of extracellular matrix. The choice of the above-listed growth factors was based on the literature data on the role of these factors in chondrogenesis. In our study, growth factors from TGF-β superfamily (TGF-β1 and BMP-4) were selected because of their capability to stimulate cartilage tissue regeneration by inducing differentiation into chondrocytes and the synthesis of extracellular matrix [5]. Thus, in this study, 6 different combinations of growth factors and HA were studied for chondrogenic differentiation and extracellular matrix production.

It was found that the combination of TGF-β1 + BMP-4 had the greatest effect on chondrogenic differentiation and production of the extracellular matrix (Figure 7).

This can be seen both in the size of the micro-pellets and in the morphology of the obtained chondrocytes (Figure 8). In another series of experiments, the same combinations of factors were studied, but with the addition of 0.25% HA.

Our results showed that TGF-β1+BMP-4 with the addition of HA more effectively induces extracellular matrix production and chondrogenic differentiation in micro-pellets. When comparing the sizes of micro-pellets formed in the presence of TGF-β1 + BMP-4 and the same factors but with the addition of HA, it was found that the sizes of the micro-pellets were approximately the same. Thus, the results of our study showed that the combination of growth factors TGF-β1 and BMP-4 has a significant effect on chondrogenic differentiation and synthesis of extracellular matrix in micro-pellets.

Based on these obtained data, a biochemical analysis was performed to determine the level of glycosaminoglycans (GAGs) and DNA in micro-pellets after cultivation with TGF-β1 (10 ng/ml) and BMP-4 (100 ng/ml). As can be seen in Figure 9, the GAG content after treatment with BMP-4 was significantly higher in comparison to TGF-β1.

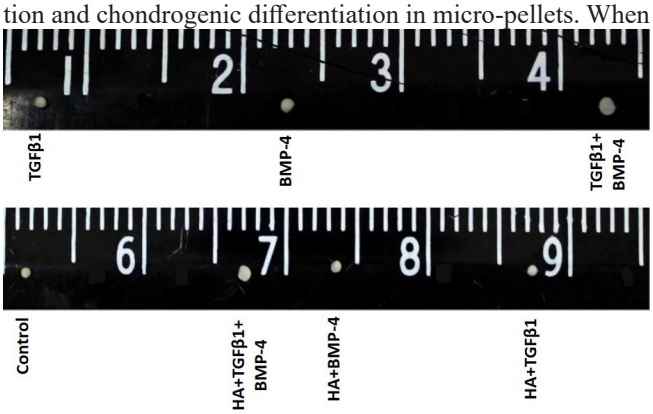

Figure 7 – Effects of growth factors and HA on formation chondrogenic micro-pellets. Scale bar 1 mm.

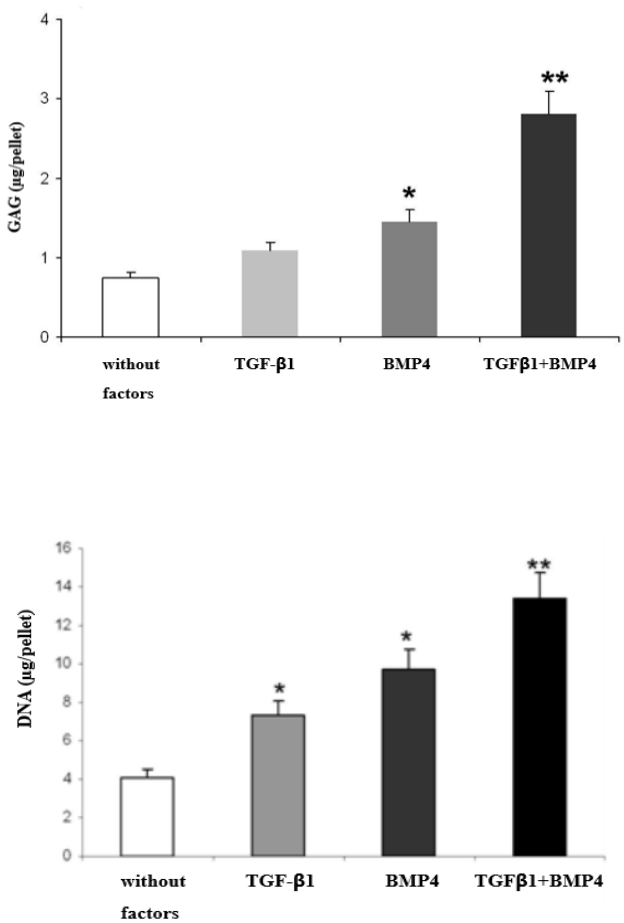

Figure 9 - Effects of TGF-β1 and BMP-4 on the synthesis of GAGs and DNA content in micro-pellet culture of human SD MSCs.

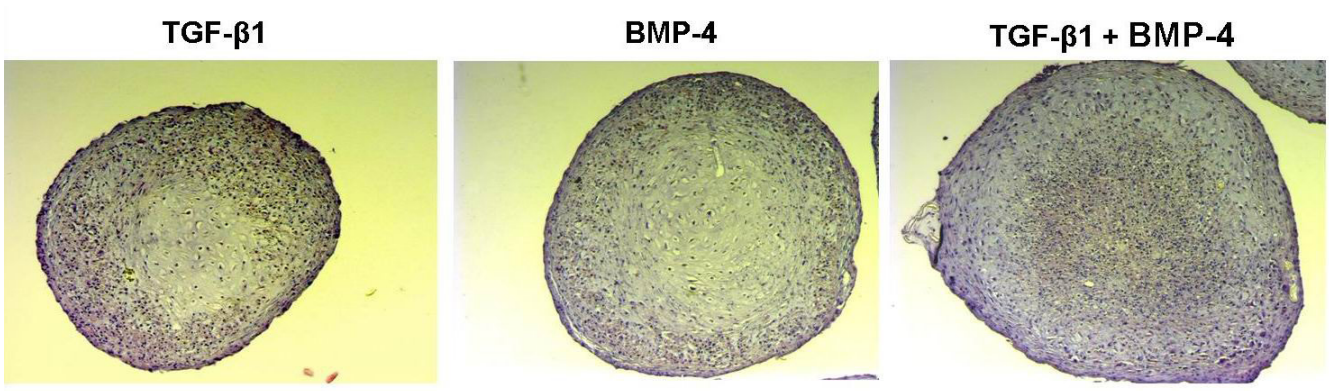

Figure 8 - Histological staining of formed chondrogenic micro-pellets with toluidine blue after cultivation of human SD MSCs with TGF-β1 and BMP-4.

However, when these two growth factors were combined, the level of GAG content in chondrogenic micro-pellets increased significantly up to 2,9 μg. Similar results were obtained when the content of DNA in micro-pellets after treatment with TGF-β1 and BMP-4 were measured (Figure 9).

Thus, the combined action of TGF-β1 and BMP-4 can not only enhance chondrogenic differentiation and synthesis of the extracellular matrix but also increase the level of GAG in chondrogenic micro-pellets.

As already known, in normal joints, cartilage provides a smooth, load-bearing surface that allows the joint to move freely. Since cartilage tissue is practically devoid of vessels and nerves, non-penetrating cartilage defects are poorly repaired, since there is no mobilization of chondrocytes near the injury site and bone marrow progenitor cells are not available. Therefore, after damage, the cartilage is unable to regenerate normally, which can eventually lead to the development of osteoarthritis [21]. Currently, in many foreign clinics, instead of surgical treatments, an alternative reparative strategy is used using transplantation of autologous chondrocytes to restore cartilage defects [22]. Although the method of transplantation of autologous chondrocytes can improve the regeneration of cartilage defects to some extent, it has several certain disadvantages, the main of which is trauma (when a transplant is taken from an adjacent healthy cartilage area), difficulties in obtaining a sufficient number of chondrocytes and their expansion in culture, as well as incomplete recovery - transplanted chondrocytes often form fibrocartilage instead hyaline tissue [22]. Due to these shortcomings, the focus of scientific research has recently shifted from autologous chondrocytes to the application of mesenchymal stem cells, biocompatible scaffolds and growth factors, and their various combinations [21].

In this study, we conducted a series of experiments to study the influence of certain key growth factors and HA on the proliferation and chondrogenic differentiation of human SD MSCs. Table 1 presents growth factors and their main effects on cartilage regeneration. One such key growth factor is TGF-β1, which plays a central role in chondrogenesis [23, 24]. This factor stimulates the synthetic activity of chondrocytes and acts against the catabolic activity of the inflammatory mediator IL-1 [25], and also increases the proliferation and chondrogenic differentiation of bone marrow MSCs. Moreover, the cultivation of MSCs with the addition of TGF-β1 led to the suppression of the expression of the collagen I gene, and at the same time, activated the expression of collagen II, which is usually produced during the formation of hyaline cartilage. Despite these literature data, in our experiments, we found that individual application of TGF-β1 did not have any stimulatory effect on the proliferation of human SD MSCs.

Table 1 – List of selected growth factor and their effects on MSCs.

Growth factor	Main effects
BMP-2	Increases extracellular matrix production Increases aggrecan degradation
BMP-4	Promotes the formation of cartilage tissue, inducing the differentiation of MSCs into chondroblasts and the maturation of chondrocytes Increases extracellular matrix production
FGF-2	Increases aggrecan degradation Inhibits the synthesis of proteoglycans Activates matrix metalloproteinase
IGF-I	Stimulates the production of extracellular matrix Reduces the catabolism of the extracellular matrix
PDGF	Chemotactic factor for MSCs Suppresses cartilage destruction caused by IL-1
TGF-β1	Stimulates the production of extracellular matrix Suppresses cartilage breakdown by reducing levels of interleukins and matrix metalloproteinase

However, in combination with FGF-2 and PDGF-BB, a powerful synergistic effect was observed leading to the active proliferation of SD MSCs in culture. These results are in agreement with the data obtained by Felicia et al., who showed that TGF-β1, FGF-2, and PDGF-BB are important growth factors for the activation of proliferation and maintenance of bone marrow MSC growth [26]. Other researchers have shown that pretreatment with a combination of TGF-β1/IGF-I/FGF-2 can also significantly increased the proliferation of synovial MSCs.

Other factors that play a very important role in chondrogenesis and osteogenesis are BMPs, which are homodimeric molecules belonging to the superfamily of TGF-β. In our study, we used BMP-4 and TGF-β1 to determine the optimal combination of these factors for chondrogenic differentiation of SD MSCs. It was found that TGF-β1 in combination with BMP-4 has a significant effect on both chondrogenic differentiation and the synthesis of extracellular matrix and GAG in chondrogenic micro-pellets. With separate use of these factors, the effect was significantly lower. BMP-4 acts synergistically with TGF-β1, stimulating chondrogenesis in SD MSCs and chondroprogenitor cells. Similar results have been obtained by other researchers who have shown that the combination of TGF-β3 and BMP-4 factors is required to stimulate

chondrogenesis, while chondoprogenitor cells can differentiate into chondrocytes in the presence of BMP-4 alone [27]. This implies that under *in vitro* conditions TGF-β3 and TGF-β1 are important in the early stages of chondrogenic MSC differentiation, while BMP-4 plays a major role in the slightly late stages of chondrogenesis. Moreover, TGF-β1 and BMP-4 can stimulate and increase the synthesis of extracellular matrix components such as type II collagen, aggrecans, and GAGs [5].

Conclusion

In conclusion, our study demonstrated that the combination of growth factors TGF-β1, FGF-2 and PDGF-BB significantly increases the proliferation of human SD MSCs, while TGF-β1 and BMP-4 have a significant effect on chondrogenic differentiation and the synthesis of glycosaminoglycans in micro-pellet culture. Combinatorial treatment of human SD MSCs with HA and growth factors insignificantly enhances cell proliferation and does not affect promotion of chondrogenic differentiation of SD MSCs. We suggest that our obtained data could be useful for development innovative cell therapy and tissue engineering techniques for repair of damaged cartilage tissue using human SD MSCs.

Acknowledgements

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ВЛИЯНИЕ КОМБИНАЦИИ ФАКТОРОВ РОСТА И ГИАЛУРОНОВОЙ КИСЛОТЫ НА ПРОЛИФЕРАЦИЮ И ХОНДРОГЕННУЮ ДИФФЕРЕНЦИАЦИЮ МЕЗЕНХИМАЛЬНЫХ СТВОЛОВЫХ КЛЕТОК, ПОЛУЧЕННЫХ С СИНОВИАЛЬНОЙ ОБОЛОЧКИ ЧЕЛОВЕКА

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АБСТРАКТ

Было показано, что факторы роста и гиалуроновая кислота (ГК) стимулируют функциональную активность мезенхимальных стволовых клеток (МСК) и таким образом могут влиять на заживление поврежденных тканей, включая хрящи. Были исследованы эффекты индивидуального и комбинированного применения ГК, тромбоцитарного фактора роста BB (PDGF-BB), трансформирующего фактора роста β1 (TGF-β1), костного морфогенетического белка 4 (BMP-4) и фактора роста фибробластов (FGF-2) на пролиферацию и хондрогенную дифференцировку МСК, полученных из синовиальной оболочки человека (МСК СО). Выделенные МСК были размножены в среде α-МЕМ и охарактеризованы с использованием иммуноцитохимии, анализа мультилинейной дифференцировки и анализа КОЕ. Пролиферативную активность МСК проверили с помощью МТТ-анализа. С помощью гистохимического и биохимического анализа оценили хондрогенную дифференцировку и уровень гликозаминогликанов в микрошариках МСК. Наши результаты показали, что индивидуальное использование FGF-2 и PDGF-BB значительно увеличило скорость пролиферации МСК по сравнению с контролем, в то время как TGF-β1 не влиял на рост клеток. Комбинации факторов роста TGF-β1+FGF-2, TGF-β1+PDGF-BB и FGF-2+PDGF-BB значительно повысили пролиферативную активность МСК. Наибольший синергетический эффект был обнаружен при сочетании всех трех факторов TGF-β1, FGF-2 и PDGF-BB. Гистохимический и биохимический анализ показал, что комбинация TGF-β1 и BMP-4 оказывает значительное влияние на хондрогенную дифференцировку и синтез гликозаминогликанов в микрошариках МСК. Добавление ГК к факторам роста оказало незначительное влияние на пролиферацию и не влияло на хондрогенную дифференцировку МСК. Это исследование показало, что комбинация факторов роста может способствовать пролиферации и хондрогенной дифференцировке МСК и может быть эффективна для улучшения заживления хряща.

Ключевые слова: мезенхимальные стволовые клетки синовиальной оболочки, пролиферация клеток, хондрогенная дифференцировка, факторы роста, гиалуроновая кислота.

ӨСУ ФАКТОРЛАРЫНЫҢ КОМБИНАЦИЯСЫ ЖӘНЕ ГИАЛУРОН ҚЫШҚЫЛЫНЫҢ АДАМНЫҢ СИНОВИАЛДЫҚ ДІҢГЕК ЖАСУШАСЫНЫҢ ПРОЛИФЕРАЦИЯСЫНА ЖӘНЕ ХОНДРОГЕНДІК ДИФФЕРЕНЦИАЦИЯСЫНА ӘСЕРІ

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ТҮЙІН

Өсу факторлары мен гиалурон қышқылы (ГҚ) мезенхималық дің жасушалардың (МДЖ) функционалдық белсенділігін ынталандырады және осылайша зақымдалған тіндердің, соның ішінде шеміршектің сауығуына әсер етуі мүмкін екендігі көрсетілген. Адамның синовиалдық жарғақынан (СЖ МДЖ) алынған жасушалар пролиферациясына және хондрогенді дифференциациясына ГҚ, тромбоциттерден алынған өсу факторы BB (PDGF-BB), трансформациялаушы өсу факторы β1 (TGF-β1), сүйек морфогенетикалық ақуызы 4 (BMP-4) және фибробласт өсу факторы (FGF-2) жеке және біріктірілген қолданудың әсері зерттелді. Оқшауланған мезенхималық дің жасушалары α-МЕМ-де өсірілді және иммуноцитохимия, көп сызықты дифференциация талдауы және CFU талдауы арқылы сипатталды. МДЖ пролиферативті белсенділігі МТТ талдауы арқылы тексерілді. МДЖ микробалондарындағы хондрогендік дифференциацияны және гликозаминогликандардың деңгейін бағалау үшін гистохимиялық және биохимиялық талдау қолданылды. Біздің нәтижелеріміз FGF-2 және PDGF-BB жеке қолдануы бақылаумен салыстырғанда МДЖ пролиферациясының жылдамдығын айтарлықтай арттырғанын көрсетті, ал TGF-β1 жасушаның өсуіне әсер етпеді. TGF-β1+FGF-2, TGF-β1+PDGF-BB және FGF-2+PDGF-BB өсу факторларының комбинациясы MSC пролиферативті белсенділігін айтарлықтай арттырды. Ең үлкен синергиялық әсер TGF-β1, FGF-2 және PDGF-BB үш факторының үйлесімімен табылды. Гистохимиялық және биохимиялық талдау TGF-β1 және BMP-4 комбинациясы МДЖ микробалондарындағы гликозаминогликандардың хондрогендік дифференциациясына және синтезіне айтарлықтай әсер ететінін көрсетті. Өсу факторларына ГҚ қосу пролиферацияға аз әсер етті және МДЖ-ның хондрогендік дифференциациясына әсер етпеді. Бұл зерттеу өсу факторларының комбинациясы МДЖ СЖ пролиферациясы мен хондрогендік дифференциациясына ықпал ете алатынын және шеміршектің жазылуын жақсартуда тиімді болуы мүмкін екенін көрсетті.

Негізгі сөздер: синовиалдық мезенхималық дің жасушалары, жасуша пролиферациясы, хондрогендік дифференциация, өсу факторлары, гиалурон қышқылы.