SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Investigation of osteoblastic differentiation of DPSC stem cells isolated from human wisdom tooth pulp under the influence of BMP-2 growth factor and substances that modify epigenetic characteristics

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UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF DENTAL SCIENCES

Debrecen, 2025.

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The Examination takes place at University of Debrecen, Faculty of Dentistry Dean's conference room on 01.10.2025. at 11:00.

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Debrecen at 1 PM, 1st of October, 2025.

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1. Introduction

In the course of dental practice, we meet patients who have different size of bone deficiency. In the fields of dental implantology, surgery and periodontal tissue regeneration, the treatment of bone defects is a crucial problem. Many bone replacement techniques and bone replacement materials can be found in the literature, but in addition to their many advantages, the use of these materials usually also has disadvantages, e.g.: they are not available in sufficient quantities, such as autologous bone grafts or their transformation into bone tissue and thus their absorption is not perfect. The latest trend in bone regeneration is the usage of the patient's own mesenchymal stem cells for the treatment of bone defects, either by combining the transplanted stem cells with a biodegradable scaffold or by *in vitro* differentiating them into osteoblasts before transplantation. There are many types of mesenchymal stem cells in the oral cavity, e.g.: stem cells that can be isolated from the pulp (DPSC-permanent tooth, SHED- deciduous tooth), stem cells isolated from the follicle (DFPC), from the periodontal ligaments (PDLSC), from the apical papilla (SCAP), from the gingiva (GMSC), stem cells from the buccal fat pad (BFPSC) or stem cells that can be extracted from the bone marrow. (1,2) Of these the isolation of DPSC cells from the pulp of surgically removed wisdom teeth is the simplest. DPSCs express cell surface markers characteristic for MSCs and they can be differentiated into many cell types, e.g.: odontoblast, cementoblast, osteoblast, nerve cell, muscle cell, cartilage cell, endothelial cell and even fat cell. (2,3) Thus, their therapeutic application has great potential in tissue regeneration in the field of dentistry.

In addition, the effects of many bioactive organic molecules are investigated in the literature, which may also be suitable for perfecting bone regeneration by using these substances in combination with a solid carrier/scaffold, or by promoting the osteoblastic differentiation of stem cells. Such molecules can be extracellular matrix proteins (collagen, chondroitin sulfate), adhesion molecules involved in cell-cell interaction (integrins), growth factors promoting the differentiation of osteoblast progenitor cells and the proliferation of osteoblast cells (BMP-2, BMP-7, parathyroid hormone. (4) These materials may also be suitable to modify the surface of titanium dental implants in order to promote the osseointegration of them and minimize complications. Bone morphogenetic proteins (BMPs), TSA, EZH2 inhibitor, and 5-azacitidine may aslo appropriate for this purpose.

2. Literature review

The process of bone formation is called osteogenesis, which begins between the sixth and seventh week of embryonic development and lasts until approximately twenty-five years of age. Two types of bone development are distinguished: intramembranous and endochondral ossification. All of these processes begin with a mesenchymal tissue precursor, but the way they transform into bone is different. With intramembranous ossification, the flat bones of the calvaria, clavicle and skull are also formed, the mesenchymal tissues are directly transformed into bone. During endochondral ossification, the mesenchymal tissue first transforms into a cartilage intermediate and then into bone. This is the way, how the axial skeleton and long tubular bones are formed. The bone is a dynamic organ, it continuously renews itself even after its formation, and is able to rebuild itself under stress, this process is called remodelling. Bone homeostasis is also a very complex process, the quantity and quality of the formed bone is influenced by several factors. It is regulated by several signaling processes. Such signaling pathways include the TGF-β, BMP, WNT/β-catenin, Notch, Hedgehog, and NELL signaling pathways. But the differentiation of stem cells is not only regulated at the genetic level by proteins directly binding to different DNA sequences (e.g. transcription factors), but also through epigenetic mechanisms. These mechanisms include DNA methylation and histone post-translational modifications. Several epigenetic-modifying agents have been developed for clinical use in cancer therapy to inhibit cell proliferation by modulating the function of chromatin remodeling complexes. (5-7) These drugs also affect the fate of stem cells (8) and accelerate the osteogenic differentiation and mineralization of DPSCs (8), which opens new possibilities for the usage of these drugs. Given the promising use of DPSCs in tissue regeneration in dentistry and general medicine, it would be beneficial if we could enhance their osteogenic differentiation. Epigenetic modifying agents can be used for this purpose when DPSCs are differentiated *in vitro* (9) or when drug treatment is combined with bio-degradable polymers (10,11) used for drug delivery system.

3. Objective

Understanding the mechanisms that regulate the osteogenic and odontogenic differentiation of DPSCs is extremely important for the clinical application of these cells. The osteogenic commitment of cells requires not only specific transcription factors, but also epigenetic regulatory mechanisms. In order to reproducibly test the effect of substances that induce osteoblastic differentiation (BMP-2, TSA, EZH2i and 5-AZA), reliable *in vitro* systems are needed. **During my work, I chose human DPSC cells as such a system, which cells are also potentially suitable for tissue regeneration purposes aimed at bone replacement.**

The aims of my research were:

- Examination of the effect of the BMP-2 homodimer on bone-oriented differentiation of DPSC cells.
- Examination of the individual effect of the components of the OIM medium and their combination with BMP-2.
- Comparison of the ability of DPSC cells to differentiate into osteoblasts as a result of BMP-2 treatment with other cell lines with osteogenic differentiation potential (Saos-2, HEPM).
- Examination of the combined effect of molecules influencing epigenetic characteristics (TSA, EZH2i and 5-AZA) on osteogenic differentiation of DPSCs.
- Comparison of the short- and long-term (1-3 weeks) effects of TSA, EZH2i and 5-AZA, on osteogenic differentiation of DPSCs.

4. Materials and methods

4. 1. Culturing of cells and induction of osteoblastic differentiation

Dental pulp stem cells (DPSCs) were isolated from the pulp tissue of impacted wisdom teeth that were not communicate with the oral cavity, they were removed for other reasons, according to the protocol previously used by Kerényi et al. (ethical license no. F0102/1ST). (12) Human embryonic palate mesenchymal preosteoblasts (HEPM, ATCC No.: CRL-1486) were cultured in Eagle's Minimum Essential Medium (EMEM, Sigma Aldrich, M5650), Saos-2 osteosarcoma cells (ATCC No.: HTB-85) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma Aldrich, D6046) and DPSC cells were cultured in Dulbecco's Modified Eagle's Medium/F12 (DMEM-F12, ThermoFisher Scientific, 1132003). In each case the medium was supplemented with 10% FBS (Sigma Aldrich, F9665), 100 U/ml penicillin and 100 mg/ml streptomycin (Sigma Aldrich, P0781), and 1% GlutaMAX (Life technology, 10567014), and the cells were cultured at 37 °C and 5 % CO_2 in a humidified atmosphere. Further on, in the figures and in the text, I marked "CM" (culture medium) for treatments in general media used for cell culture, and "OIM" (osteo inductive medium) for treatments in osteoinductive media. 10 mM β-glycerophosphate (Sigma Aldrich, G9891), 50 μg/ml ascorbic acid (Sigma Aldrich, 1043003), 0.1 μM dexamethasone (Sigma Aldrich, D4902) and 50 nM vitamin D3 were added to OIM medium compared to CM medium. (Sigma Aldrich, 740292) In the case of the samples indicated as β-GLY, AA, DEX and D3 VIT on the figures, the CM medium was supplemented with 10 mM β-glycerophosphate (β-GLY), 50 μg/ml ascorbic acid (AA), 0.1 with μM dexamethasone (DEX) or 50 nM vitamin D3 (D3 VIT) respectively. BMP-2 (Antibodies-online GmbH, Z00327) was used at a final concentration of 0.1 μg/ml. The BMP-2 stock solution was prepared from lyophilized recombinant human BMP-2 according to the manufacturer's instructions, at a concentration of 1 mg/ml and stored in 10 μl aliquots at -80 °C until use. To induce epigenetic changes in the chromatin, CM and OIM medium were supplemented with 5 μM EZH2i (ab269816; Abcam, Cambridge, UK), 10 μM TSA (ab146598; Abcam) or 1 μM 5-AZA (ab142744) separately or in different combinations. In the case of 5-AZA, a 24-hour treatment was used on day 0; for TSA and EZH2i, the treatment was continuous. The media on the samples was changed every 2 days.

4. 2. Alamar blue assay

Into each well of a 96 well cell culture plate (VWR, 89093-608), 1000 cells in the case of HEPM and Saos-2 and 7000 cells in the case of DPSC were seeded in 200 µl CM. Cells were allowed to attach overnight, then CM was removed (day 0), cells were washed with 200 µl colourless DMEM (Sigma Aldrich, D5921) and were incubated in 200 µl alamar blue reagent (Life Technologies, DAL 1100) diluted 1:10 in colourless DMEM. Incubation was for 3 h in the case of HEPM and Saos-2 cells and 2 h in the case of DPSC at 37 °C in 5% CO2. Alamar blue assay was previously optimized for each cell type (data not shown). After incubation, fluorescence of the reduced alamar blue was measured by Hidex Sense Microplate reader using 535 nm excitation light and 595 nm emission filter. After the measurement, alamar blue was changed to the appropriate media and culturing was countinued. Measurements were repeated at every second day during an 8-day long interval. Fluorescence intensities were normalized to the fluorescence measured at day 0.

4. 3. Qantification of Ca2+

After osteogenic induction, the cells were washed twice with 1 mL of 1 × PBS (P5493; Sigma-Aldrich). The samples were fixed in 1 mL of ice-cold methanol (322415; Sigma-Aldrich) for 30 min at room temperature. Afterwards, the samples were dried for 5 min at room temperature and stained with 2 % (w/v) Alizarin Red S (pH 7; A5533; Sigma-Aldrich). For staining quantification, Alizarin Red S–calcium complexes were extracted with 10 % cetylpyridinium chloride (C0732; Sigma-Aldrich) diluted in 10 mM sodium phosphate buffer adjusted to pH 7 (P5244; Sigma-Aldrich), and the absorbance was measured at 570 nm using a Hidex Sense Microplate reader. For standardisation, the protein concentration of cell lysates was determined with the Pierce BCA Protein Assay (23227; Thermo Fisher Scientific) according to the manufacturer's instructions from parallel samples that were not stained with Alizarin Red S. Calcium deposition was expressed as A570 nm/µg protein.

4. 4. Alkaline phosphatase activity

After osteogenic induction, the cells were washed twice with 1 mL of 1 × phosphate-buffered saline (PBS) and lysed in lysis buffer (10 mM Tris-HCl pH 7.4, 100 mM NaCl, 1 mM ethylenediaminetetraacetic acid [EDTA], 1 % Triton X-100, and 1 % protease inhibitor cocktail [PIC]). Scraped lysates were transferred to sterile Eppendorf tubes. After centrifugation (10,000×g for 10 min at 4 °C), the supernatant was used to determine ALPL activity and protein concentration (Pierce BCA Protein Assay; 23227; Thermo Fisher Scientific). To determine ALPL activity, 0.1 % p-nitrophenyl phosphate (N7653; Sigma-Aldrich; in 0.1 M glycine, 1 mM MgCl2, ZnCl2, pH 10.4) was added to the samples, and the absorbance was measured at 405 nm with a Hidex Sense Microplate reader (Hidex, Turku, Finland). The kinetic measurements were performed every 3 min for 2 h.

4. 5. Real time quantitative PCR

After osteogenic induction, the cells were washed twice with 2 mL of 1 × PBS. Total RNA was extracted using a Quick-RNA Miniprep Kit (R1054; Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. The High-Capacity cDNA Reverse Transcription Kit (4368814; Thermo Fisher Scientific) was used to reverse transcribe 0.5 µg of RNA per sample into cDNA. Gene expression levels were determined using TaqMan gene expression assays for *RUNX2* (Hs00231692_m1; Applied Biosystems, Waltham, MA, USA), *BMP2* (Hs00154192_m1; Applied Biosystems), and *ALPL* (Hs01029144; Applied Biosystems), and were normalised to the reference housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*; Hs02758991; Applied Biosystems) in each sample. The 5x HOT FIREPOL Probe qPCR Mix Plus (no ROX; 08-15-00001; Solis BioDyne, Tartu, Estonia) was used for qPCR.

4. 6. Statistical analysis

The error bars in the figures represent the standard deviation (SD) calculated from three parallel (figures 1-5) or three independent (figures 6-8) measurements. The statistical analysis was performed with ANOVA and Bonferroni's post hoc statistical test for multiple comparisons (figures 1-5), and with

ANOVA and Dunnett's post hoc statistical test for comparison with a control measurement (figures 6-8). The level of significance was 5% in all cases ($\alpha=0.05$).

5. Results

In the course of my work, I first studied the effect of BMP-2 on cell proliferation of DPSC, Saos-2 and HEPM cells, after 8 days of treatment with different of BMP-2 concentrations diluted in the cell culture medium (CM) or the differentiation medium (OIM). The rate of cell growth in all three cell types decreased in a time-dependent manner when CM and OIM media were used, with the exception of Saos-2 cells, where cell growth stopped on the 4th day in OIM media, and then began to decrease again as time progressed. Examining the components of OIM separately, I found that only β-GLY had an effect on all three cell lines, reducing cell proliferation. DEX alone had an inhibitory effect only on the division of DPSC cells, but not on the other two cell lines.

Further, I investigated bone-oriented differentiation by measuring the ALP activity of the cells and the mineralization (calcium deposits) of the extracellular matrix. Mineralization was measured in CM, OIM, and CM supplemented with β-GLY, which proved to be the most effective among the OIM components during the proliferation measurements, after 6 days of incubation. After 6 days, calcium phosphate, which stains well with Alizarin Red S staining, was observed in the extracellular matrix of Saos-2 cells cultured in OIM. A small amount of staining was also visible on DPSC cells, however, during the quantitative analysis of the released dye, there was no statistically significant difference compared to the control CM medium. Examining the effect of BMP-2, a significant increase was observed in the mineralization of DPSC and Saos-2 cells when the OIM medium was supplemented with BMP-2, and in the case of Saos-2 cells, also due to the effect of the combination of β-GLY and BMP-2.

Another method of detecting osteogenic differentiation is the measurement of the ALP activity of the cells, which was also significantly increased in the OIM medium in Saos-2 cells, and as a result of the OIM+BMP-2 treatment in the case of Saos-2 and DPSC cells. A significant increase in ALP activity was also observed in Saos-2 cells treated with β-GLY and β-GLY+BMP-2 combination. The osteoblastic differentiation was examined for 12 and 18 days. This longer study could not be performed with Saos-2 cells cultured in OIM, as after day 6 the number of live cells was significantly lower. In the case of DPSC cells cultured in OIM for 12 days regardless of whether BMP-2 was present, we did not measure an increase in mineralization, and alkaline phosphatase activity also seemed to decrease. However, this difference was not statistically significant. After 18 days, however, the amount of calcium deposits and alkaline phosphatase activity of DPSCs increased again as a result of the OIM

treatment, in addition, when the OIM medium was supplemented with BMP-2, a synergistic effect between OIM and BMP-2 was observed.

I also examined the early marker genes (RUNX2, BMP-2) regulating osteoblastic differentiation using the real-time qPCR method. In the case of Saos-2, the expression level of *RUNX2* did not change on the fourth day for either treatment. However, endogenous BMP-2 levels were significantly increased in β-GLY-treated cells as well as in OIM. BMP-2 also exerted a synergistic effect in OIM medium. Since the differentiation of DPSC cells takes a longer time based on mineralization and alkaline phosphatase activity measurements, I examined the gene expression levels after 6, 12 and 18 days of differentiation. The cells were grown in CM, OIM, CM + BMP-2 and OIM + BMP-2. Based on my results, the expression level of *RUNX2* was the highest on the 6th day, which then gradually decreased to the 12th and 18th days. This trend was also observed in the case of OIM, CM+BMP-2 and OIM+BMP-2 treatments, and BMP-2 acted synergistically on the expression of *RUNX2* in OIM medium. In contrast, the level of BMP-2 gradually increased from day 6 to day 18 in the case of OIM, CM+BMP-2 and OIM+BMP-2 treatments. BMP-2 also showed synergism with the OIM medium in this case.

The individual and combined effect of the molecules, EZH2i, TSA and 5-AZA that influence epigenetic characteristics, on osteoblastic differentiation was investigated on DPSC cells by examining the ALP activity and mineralization of the treated cells, as well as the expression of osteogenic marker genes (*RUNX2*, *BMP-2* and *ALPL*).

The ALP activity of the cells was measured after 7 and 21 days of treatment in CM or OIM supplemented with different combinations of EZH2i, TSA or 5-AZA. As a result of treatment in CM medium, ALP activity increased on the 7th day when I supplemented the CM medium with EZH2i alone or with combinations of EZH2i+TSA or EZH2i+5-AZA. Also in CM medium, after 21 days of treatment, ALP activity remained significantly higher in DPSCs treated with EZH2i alone, while 5-AZA treatment had a negative effect on ALP activity alone or in any combination with the other two substances. In CM, short-term (7 days) or long-term (21 days) treatment with TSA had no effect on the ALP activity of the cells. In OIM medium, EZH2i increased ALP activity similarly to CM after 7 days of treatment. TSA alone or in combination with other agents was ineffective at this time point, while 5-AZA had a negative effect. After 21 days of treatment, only TSA-treated DPSCs showed increased ALP activity in OIM medium, on the other hand, EZH2i and 5-AZA reduced the enzyme activity.

Regarding ALP activity, no synergistic effect was observed for any drug combination, regardless of whether the treatment was done in CM or OIM medium.

I examined the mineralization of DPSCs treated with epigenetic-modifyingl agents only after treatment in OIM medium, not in CM, because based on my previous experiments in CM, due to the lack of inorganic phosphate, mineralization of DPSC cells is not expected even if CM is supplemented with an osteogenic differentiation-inducing factor e.g.: BMP-2. (13) Surprisingly, mineralization was not increased by any drug or drug combination treatment at days 7 and 21 compared to cells grown in OIM medium. In the presence of EZH2i, a significant decrease was observed on day 21, this was also observed when using in combination with other drugs, which is consistent with the negative effect observed when measuring ALP activity.

I also examined the effect of epigenetic-modifying drug molecules on osteoblastic differentiation by measuring the expression level of the osteogenic marker genes *RUNX2*, *BMP2* and *ALPL*. The measurements were performed after 21 days of treatment, as the goal was to detect increased gene expression as a result of long-term epigenetic changes. In CM medium, the treatment with EZH2i alone or in combination with other active ingredients had a positive effect on the expression level of the tested genes. In the samples treated in OIM medium, only TSA alone increased the expression level of all three tested genes, while EZH2i alone and in combination with TSA and 5-AZA inhibited *BMP2* and, to a non-significant extent, *RUNX2*. These results are consistent and correlated with ALP activity measurements. We did not observe a synergistic effect in any of the investigated drug combinations.

6. Discussion, major results and conclusions

- BMP-2 is known to enhance osteogenic differentiation. However, treatment of cells with BMP-2 may result in different outcomes depending on the cell type and the osteoinductive molecules (molecular environment). (14,15)
- BMP-2 inhibited the proliferation of all three cell types in a concentration-dependent manner alone, in CM and in combination with osteoinductive substances.
- β-glycerophosphate also inhibited cell proliferation in all three cell types. In combination with BMP-2, we found a synergistic effect to inhibit proliferation in HEPM and Saos-2 cells. According to published data, the decrease in the proliferation rate may be related to the differentiation process. (16-19)
- In Saos-2 cells, osteoblastic differentiation presumably occurs faster and from the 4th day, the late phase of differentiation took place, where mineralization by terminally differentiated osteoblasts began and later some of the cells died by apoptosis. The decrease in the proliferation of Saos-2 cells that we experienced in an environment inducing osteogenic differentiation is the same as the observations of other authors. (20)
- Among the components of the OIM medium, it is enough to supplement the cell culture medium with β-GLY, which serves as a source of inorganic phosphate, to reduce cell proliferation.
- BMP-2 alone had no effect on alkaline phosphatase activity. Combining BMP-2 with β-GLY or OIM medium, on the other hand, synergistic effects were detected in the case of Saos-2 and DPSC cells.
- As expected, the increased ALP activity showed a positive correlation with the amount of deposited calcium phosphate crystals.
- In DPSC cells, the effect of OIM medium on differentiation was more pronounced when the differentiation was carried out for 18 days.
- The positivity given to the staining proves differentiation in the direction of bone cells, which is assumed based on the decrease in proliferation.
- During the study of osteogenic marker genes, OIM increased the expression of *RUNX2*, which was further increased when combined with BMP-2 in DPSC cells. With continued treatment, the *RUNX2* level gradually decreased as maturation progressed, which is consistent with literature data. (21)

- The expression level of endogenous *BMP-2* correlated with increased alkaline phosphatase enzyme activity.
- BMP-2 alone is not sufficient to induce the maturation of osteoblast progenitors. However, in an appropriate molecular environment that allows osteogenic differentiation and includes molecules such as β-GLY, BMP-2 can act synergistically on the osteoblastic differentiation of DPSC and Saos-2 cells.
- Among the molecules that influence epigenetic characteristics that I investigated, TSA and EZH2i proved to have a positive effect on the osteoblastic differentiation of DPSC cells in terms of gene expression, where, in accordance with literature data (22,23), both substances increased the expression of *RUNX2, BMP2* and *ALPL* genes level.
- However, the two substances were effective under different conditions, EZH2i only had an effect on the tested genes when the treatment was carried out in CM medium, which did not contain other factors that affect the bone-oriented differentiation of DPSC cells.
- TSA had a positive effect on the expression of the studied genes in culture conditions combined with OIM, which promotes osteogenic differentiation, but not in CM.
- Among the three tested substances, 5-AZA did not have a positive effect during the differentiation of DPSC cells, either alone or in combination with TSA or EZH2i, which differs from literature data on DPSC. (24)

7. Summary

Among stem cells from the oral cavity, DPSC cells have great potential for clinical use, due to their simple isolation and multipotent properties. DPSCs, thanks to their ability to differentiate into osteoblasts, can also be suitable in the field of dentistry in stem cell therapy aimed at replacing bone deficiency and better osseointegration of dental implants. Substances that promote osteogenic differentiation would greatly contribute to their use in this direction. To this end, I investigated the effect of a commercially available growth factor approved for clinical use, the human recombinant BMP-2 protein, as well as the combined effect of drugs affecting epigenetic characteristics, TSA, EZH2i and 5 –AZA, that stimulate the expression of endogenous *BMP-2* and other osteogenic regulatory genes on the differentiation of DPSC cells into osteoblasts.

The effect of BMP-2 was investigated during the comparative analysis of different preosteoblast cell lines: Saos-2 and HEPM, as well as primary DPSC cell culture isolated from the pulp tissue of wisdom teeth. BMP-2 treatment was investigated in different time intervals and in different molecular environments, in general cell culture medium (CM) and in cell culture media supplemented with osteogenic differentiation factors (OIM). To monitor the progress of differentiation, I measured cell proliferation, ALP activity, mineralization and the expression of *RUNX2* and *BMP-2* among the osteogenic regulatory genes. Based on my results, BMP-2 inhibited cell proliferation in a concentration-dependent manner for all three cell types tested, but it was not sufficient to activate osteogenic differentiation by itself. In the OIM medium supplemented with components favorable to osteogenic differentiation, BMP-2 promoted the differentiation of DPSC and Saos-2 cells better than the OIM medium alone, and in the case of Saos-2 cells, only the phosphate source among the OIM components was sufficient for differentiation. In DPSC cells, BMP-2 was therefore only effective in conjunction with the co-activation of appropriate signaling pathways.

I investigated the individual and combined effects of TSA, EZH2i and 5-AZA on DPSC cells. The treatments were carried out for different periods of time in CM and OIM media, and their effect on differentiation was measured by examining the cells' ALP activity and mineralization, as well as the expression of the *RUNX2, BMP-2* and *ALPL* genes. Among the three substances, I observed positive effects when using TSA and EZH2i alone. Based on my results, TSA and EZH2i act in different ways on the genes regulating the osteoblastic differentiation of DPSC cells: EZH2i acts directly on the target genes, while TSA indirectly interacts with the proteins of the signal transduction pathways involved in

bone-oriented differentiation. During the joint application of TSA, EZH2i and 5-AZA, I did not observe synergism in any active ingredient combination.

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Registry number: DEENK/492/2024.PL
Subject: PhD Publication List

Candidate: Edit Hrubi
Doctoral School: Doctoral School of Dental Sciences

List of publications related to the dissertation

1. **Hrubi, E.**, Imre, L., Hegedűs, C.: Effects of EZH2 inhibitor, trichostatin A, and 5-azacytidine combinatorial treatment on osteogenic differentiation of dental pulp stem cells.
Heliyon. 10 (12), 1-7, 2024.
DOI: http://dx.doi.org/10.1016/j.heliyon.2024.e32553
IF: 3.4 (2023)

2. **Hrubi, E.**, Imre, L., Robaszkiewicz, A., Virág, L., Kerényi, F., Nagy, K., Varga, G., Jenei, A., Hegedűs, C.: Diverse effect of BMP-2 homodimer on mesenchymal progenitors of different origin.
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DOI: http://dx.doi.org/10.1007/s13577-018-0202-5
IF: 2.333

List of other publications

3. Kerényi, F., Tarapcsák, S., **Hrubi, E.**, Baráthné Szabó, Á., Hegedűs, V., Balogh, S., Bágyi, K., Varga, G., Hegedűs, C.: Fogbél eredetű őssejtek fluoreszcens és mágneses válogatásának összehasonlító vizsgálata.
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4. Bakó, J., Kerényi, F., **Hrubi, E.**, Varga, I., Daróczi, L., Dienes, B., Csernoch, L., Gáll, J., Hegedűs, C.: Poly-[gamma]-Glutamic Acid Nanoparticles Based Visible Light-Curable Hydrogel for Biomedical Application.
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IF: 1.871

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5. **Hrubi, E.**, Imre, L., Bacsó, Z., Biró, S., Jenei, A., Hegedűs, C.: BMP-2 hatása a humán embrionális szájpadlásból származó mesenchymalis preosteoblast sejtek morfológiájára és proliferációjára.
Fogorv. Szle. 108 (3), 99-105, 2015.

Total IF of journals (all publications): 7,604
Total IF of journals (publications related to the dissertation): 5,733

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

25 September, 2024

DEBRECENI EGYETEM
Egyetemi és Nemzeti Könyvtár

9. Keywords

Őssejt, DPSC, oszteoblaszt, differenciáció, BMP-2, Trichostatin A, 5-azacitidnin, EZH2i.

Stem cells, DPSC, osteoblast, differentiation, BMP-2, Trichostatin A, 5-azacitidnine, EZH2i

10. Acknowledgements

I would like to express my gratitude to Dr. Kinga Bágyi, head of the University of Debrecen Dental Clinic and Faculty of Dentistry.

In addition, I would like to thank my supervisor, Prof. Dr. Csaba Hegedűs, for his professional guidance, helpful advice, and support during my PhD work. I also thank to Prof. Dr. Attila Jenei, head of the DE FOK Institute of Basic Medical Knowledge, under whose co-leadership I was able to gain an insight into the world of research as a TDK student.

I am grateful to Prof. Dr. Gábor Varga, University Professor of the Oral Biology Department of the Semmelweis University Faculty of Dentistry, and to Dr. Dr. Simonné Dr. Krisztina Nagy, a staff member of the Semmelweis University Department of Biophysics and Radiobiology, from whom I had the opportunity to learn about the isolation and cultivation of dental pulp stem cells. I also thank to Dr. Agnieszka Robaszkiewicz, a former colleague of the University of Debrecen, Faculty of Medicine, Institute of Medical Chemistry, and her supervisor Prof. Dr. László Virág, who made it possible for me to learn the implementation and evaluation of Real-time qPCR analyses. Saos-2 osteosarcoma cell line was also provided by Prof. Dr. László Virág.

In addition, I would like to thank to the former and current members of our research group who supported me and my work with their advice: Dr. Melinda Szalóki, Dr. Andrea Keczánné Üveges, Dr. Farkas Kerényi, Dr. József Bakó, Dr. Ferenc Tóth.

Finally, I am grateful to my family, my children and my husband Dr. László Imre, who stood by me with patience and love and a lot of support even in the most difficult times.