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Assessment of Dental Pulp Stem Cell (DPSC) Biomarkers Following Induction with Bone Morphogenic Protein 2 (BMP-2)

Joseph Cinelli[1], Erica Nguyen[2] and Karl Kingsley[3*]

[1]Department of Advanced Education in Orthodontics, University of Nevada, Las Vegas–School of Dental Medicine, USA.
[2]Department of Biological Sciences, University of Nevada, Las Vegas, USA.
[3]Department of Biomedical Sciences, University of Nevada, Las Vegas–School of Dental Medicine, USA.

Authors' contributions

This work was carried out in collaboration between all authors. Authors KK and JC designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JC and EN managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Tissue regeneration and biomedical engineering are the goals of modern research that have made tremendous strides in recent years. Dental pulp stem cells (DPSCs) have been demonstrated to exhibit functional multipotency, differentiating into neurons, adipocytes, and other cell types. The primary goal of this study was to investigate the ability of bone morphogenic protein (BMP-2) to induce proliferation and differentiation of DPSC isolates into mineral forming bone cell precursor lineages.
Study Design: This was a prospective study with the non-randomised experimental design.
Place and Duration of Study: This study was conducted at the University of Nevada, Las Vegas – School of Dental Medicine between May 2017 and August 2018.

**Corresponding author: E-mail: Karl.Kingsley@unlv.edu;*

Methodology: Eight previously isolated dental pulp stem cell (DPSC) isolates were grown in culture and treated with bone morphogenic protein (BMP-2) to evaluate any effects on growth, viability or biomarker expression.
Results: BMP-2 induced significant changes in cellular growth among a subset of DPSC with slow doubling times (sDT), which corresponded with similar increases in cellular viability. Also, BMP-2 was sufficient to induce mRNA expression of alkaline phosphatase (ALP) and other differentiation markers among the sDT isolates – although no significant changes were observed among the DPSC isolates with rapid or intermediate DTs (rDT, iDT).
Conclusions: This study may be the first to demonstrate not only the differential responsiveness of DPSC isolates to BMP-2, but also to identify the MSC biomarkers that may affect initial DPSC responsiveness to this stimulus. Although many studies have evaluated the role of the biomarkers NANOG, Sox-2 and Oct-4 in DPSC isolate, no other study of DPSC multipotency has evaluated the role of Nestin – which may be one of the key factors that potentiate or limits the responsiveness to BMP-2 and osteogenic potential among DPSCs.

Keywords: Dental pulp stem cells (DPSC); bone morphogenic protein (BMP-2); bioengineering; biotechnology.

ABBREVIATIONS

Dental pulp stem cell (DPSC), insulin-like growth factor (IGF), bone morphogenic protein (BMP), Office for the Protection of Research Subjects (OPRS), Institutional Review Board (IRB), University of Nevada Las Vegas (UNLV), School of Dental Medicine (SDM), cementoenamel junction (CEJ), phosphate buffered saline (PBS), bone morphogenic protein (BMP), doubling time (DT), Total RNA isolation reagent (TRIR), polymerase chain reaction (PCR), deoxyribonucleic acid (DNA), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), alkaline phosphatase (ALP), dentin sialophosphoprotein (DSPP), ANOVA (Analysis of variance), vascular endothelial growth factor (VEGF).

1. INTRODUCTION

Tissue regeneration and biomedical engineering are the goals of modern research that have made tremendous strides in recent years [1-3]. At the forefront of these efforts has been the use of stem cell-based therapies, which have demonstrated tremendous potential in these areas [4-6]. Although many studies have focused on embryonic and perinatal stem cells, the use of adult or mesenchymal stem cells may represent readily available, widely applicable and less controversial alternatives [7,8].

Many types of mesenchymal stem cells exist in a variety of tissues, including bone marrow, adipose tissue, and dental pulp [9-11]. Dental pulp stem cells (DPSCs) have been demonstrated to exhibit functional multipotency, differentiating into neurons, adipocytes, and other cell types [12,13]. Recent evidence has demonstrated considerable progress in new areas of research, such as DPSC use in the tissue engineering of bone [14-17].

Much the research focusing on DPSC induction into osteoblast cells or precursors has focused on the isolation and identification of DPSCs with strong osteogenic potential [18-20]. Another important area of research has been concentrated on the stimulus to direct DPSC differentiation towards these osteogenic lineages, including insulin-like growth factor (IGF) and bone morphogenic proteins (BMPs) [21-23]. Although BMPs have been known to facilitate dentin formation and regeneration among DPSCs, more recent evidence suggests these effects may also induce osteoblastic differentiation and bone regeneration potential [23-25].

Based on this information, the primary goal of this study was to investigate the ability of BMP-2 to induce proliferation and differentiation of DPSC isolates into mineral forming bone cell precursor lineages expressing the appropriate biomarkers.

2. METHODOLOGY

2.1 Study Approval

The protocol for this study was reviewed and approved by the Office for the Protection of Research Subjects (OPRS) and Institutional Review Board (IRB) OPRS#763012-1

"Retrospective analysis of dental pulp stem cells (DPSC) from the University of Nevada Las Vegas (UNLV) School of Dental Medicine (SDM) pediatric and adult clinical population". The original protocol for the collection and isolation of DPSC was approved by the IRB and OPRS#0907-3148 "Isolation of Non-Embryonic Stem Cells from Dental Pulp".

2.2 Study Design

The DPSC that were used and analysed in this study were previously obtained randomly from the UNLV-SDM pediatric patient population before the commencement of this study. The inclusion criteria for this project were patients – pediatric or adult – that were pre-orthodontic, of at least 7 years of age (mainly 12 – 15 years of age) who needed extraction of vital, non-carious teeth – mainly to relieve crowding of the anterior dentition. Both pediatric assent and parental consent were required to partake in the study. Informed consent was required for all adult patients. The exclusion criteria comprised of any individual who was not a patient of record at UNLV-SDM, patients whose teeth were extracted for any reason other than elective extraction including trauma, caries, or other pathology and any patients that declined to participate.

2.3 DPSC Collection (Initial)

Collection of DPSC began with participants of the study undergoing extractions – primarily third molars. Following extraction, the teeth were decoronated at the cemento-enamel junction (CEJ) using a high-speed dental handpiece and bur. The dental pulp was then removed from the canal with an endodontic broach and placed in a sterile microcentrifuge tube pre-filled with 1X phosphate-buffered saline (PBS) on ice for transport to a biomedical laboratory for further processing and screening.

De-identification of the samples through the use of randomly generated, non-duplicated numbers assigned to each sample and related patient demographic information was done to eliminate the possibility of revealing any information that could identify a participant or biasing the research. The researchers were not made privy to any of the non-deidentified participant information at any point during the study.

2.4 Culture and Propagation

In brief, two primary methods for establishing DPSC isolates are the enzymatic digestion and direct outgrowth methods [26,27]. Although methods were utilised, no results were found using the enzymatic digestion method to separate DPSC from the dental pulp, while n=31/40 or 77.5% of DPSC isolates were established using the direct outgrowth method – as previously described [28,29]. All viable samples were derived from patients aged 31 years and younger, which were equally distributed between males and females [26,27]. The rate of growth and doubling time (DT) were obtained by culturing and propagated over ten passages.

The split (passage ratio) for each DPSC sample was 1:2 and trypan blue and a BioRad TC20 automated cell counter (Hercules, CA) was used to determine confluence of the cell lines when used following the protocol established by the manufacturer. The data is comprised of total and live cell counts allowing for calculation of the percentage of viable cells available for analysis. The DPSC cell lines were further sorted based on the doubling time (DT) as either rapid (~2days) n=3 (dpsc-3882, dpsc-5653, dpsc-7089), intermediate or iDT (4-6 days) n=2 (dpsc-8124, dpsc-17322), and slow or sDT (10-12 days) n=3 (dpsc-11418, dpsc-11750, dpsc-11836). These doubling times may be functionally related to the proliferation and differentiation potential of the DPSC isolates, as previously described [26-29].

Rapid Doubling Time (rDT): dpsc-3882, dpsc-5653, dpsc-7089
Intermediate Doubling Time (iDT): dpsc-8124, dpsc-17322
Slow Doubling Time (sDT): 11418, dpsc-11750, dpsc-11836

2.5 Experimental Protocol

The various DPSC lines were plated in concentrations of $1.2x10^4$ cells/mL into 96-well tissue culture treated plates to assess the effects of bone morphogenic protein 2 (BMP-2), if any, on DPSC. The experimental cells, those dosed with BMP-2 from Fischer Scientific (RP-8638) at a concentration of 10 ng/mL, were compared to the control cells (non-dosed), similar to other studies of BMP-2 among MSC and DPSC [23-25]. A total of three experimental trials (n=24) were performed – eight DPSC isolates for each experimental condition and repeated three times throughout three weeks.

2.6 RNA Isolation

Total RNA was obtained from every sample through the use of Total RNA isolation reagent (TRIR) from Molecular Research Center (Cincinnati, OH) and following the manufacturer's protocol. Absorbance at wavelengths of 260 and 280nm (A260/A280 ratio) was used to screen the collected RNA for quality and quantity.

2.7 Polymerase Chain Reaction (PCR)

Evaluation of DPSC isolates for differences in the levels of mRNA expression was done using the ABgene Reverse-iT One-Step RT-PCR protocol and reagent kit under the following provisions: initial reverse transcription at 47C for 30mins followed by 30 cycles of denaturation at 95C for 10 minutes then annealing for 30 seconds at the appropriate temperature for each primer set and final extension at 60C for one minute. The following primers from Eurofins MWG Operon (Huntsville, AL) were synthesised:

Housekeeping gene Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), glycolytic pathway

Forward primer-
GAPDH, ATCTTCCAGGAGCGAGATCC; 20 nt, 55% GC, Tm 66C
Reverse primer-
GAPDH, ACCACTGACACGTTGGCAGT; 20 nt, 55% GC, Tm 70C

Annealing temperature 67C; Optimal temperature T(opt): Lower temperature – 5C = 61C

Osteogenic biomarker, Alkaline Phosphatase (ALP)

Forward primer-
ALP, CACTGCGGACCATTCCCACGTCTT;24 nt, 58% GC, Tm 74C
Reverse primer-
ALP, GCGCCTGGTAGTTGTTGTGAGCAT; 24 nt, 54% GC, Tm 72C

Annealing temperature: 72C; Optimal temperature T(opt): Lower temperature – 5C = 67C

Differentiation biomarker, dentin sialophosphoprotein (DSPP)

Forward primer-
DSPP, CAACCATAGAGAAAGCAAACGCG;23 nt, 48% GC, Tm 67C
Reverse primer-
DSPP, TTTCTGTTGCCACTGCTGGGAC; 22 nt, 55% GC, Tm 70C

Annealing temperature: 68C; Optimal temperature T(opt): Lower temperature – 5C = 62C.

2.8 Statistical Analysis

Descriptive statistics, including counts and percentages, for basic proliferation and viability of the isolated DPSC were compiled and analysed. The appropriate tests for parametric data analysis, t-tests, were used to calculate and compare changes in viability and proliferation. To limit Type I errors, the t-test results were confirmed via multiple ANOVA (Analysis of variance).

3. RESULTS

An assessment of the quality and quantity of RNA obtained from these assays under both control and experimental conditions was performed (Table 1). These data demonstrated that the average RNA concentration isolates from the rDT DPSC isolates under both conditions was similar and not significantly different (611.3, 618.2 ng/uL respectively), p= 0.588. Similar results were observed with the iDT (632, 628.1 ng/uL respectively) and sDT DPSC isolates (599.7, 649.4 ng/uL respectively), p=0.214. The quality of RNA assessed by the absorbance ratio of A260: A280 also demonstrated similar values between the control and experimental DPSC isolates: rDT (1.67, 1.69 respectively), iDT (1.72, 1.66 respectively) and sDT (1.75, 1.76 respectively).

The evaluate any potential effects on these DPSC isolates, 96-well growth assays were performed with and without the addition of BMP-2 (Fig. 1). These results demonstrated that the addition of BMP-2 (10 ng/mL) to the rapidly dividing (rDT) DPSC isolates (dpsc-3882, dpsc-5653, dpsc-7089) did not induce any significant effects on cellular proliferation over three days, p=0.388. Also, no measurable differences in cellular growth were observed with the intermediate doubling time (iDT) DPSC isolates (dpsc-8124, dpsc-17322) over this time period, p=0.411. However, the addition of BMP-2 significantly increased the growth of the slow doubling time (sDT) DPSC isolates (dpsc-11418, dpsc-11750, dpsc-11836), p=0.039.

Table 1. RNA analysis of control and experimental DPSC isolates

	Control DPSC RNA analysis	Experimental DPSC RNA analysis
Rapid (rDT) DPSC	RNA concentration Average = 611.3 ng/uL A260:A280 (purity) Average = 1.67 Range 1.52 – 1.99	RNA concentration Average = 618.2 ng/uL A260:A280 (purity) Average = 1.69 Range 1.55 – 1.86
Intermediate (iDT) DPSC	RNA concentration Average = 632.0 ng/uL A260:A280 (purity) Average = 1.72 Range 1.55 – 1.94	RNA concentration Average = 628.1 ng/uL A260:A280 (purity) Average = 1.66 Range 1.58 – 1.91
Slow (sDT) DPSC	RNA concentration Average = 599.7 ng/uL A260:A280 (purity) Average = 1.75 Range 1.58 – 1.91	RNA concentration Average = 649.4 ng/uL A260:A280 (purity) Average = 1.76 Range 1.62 – 1.88

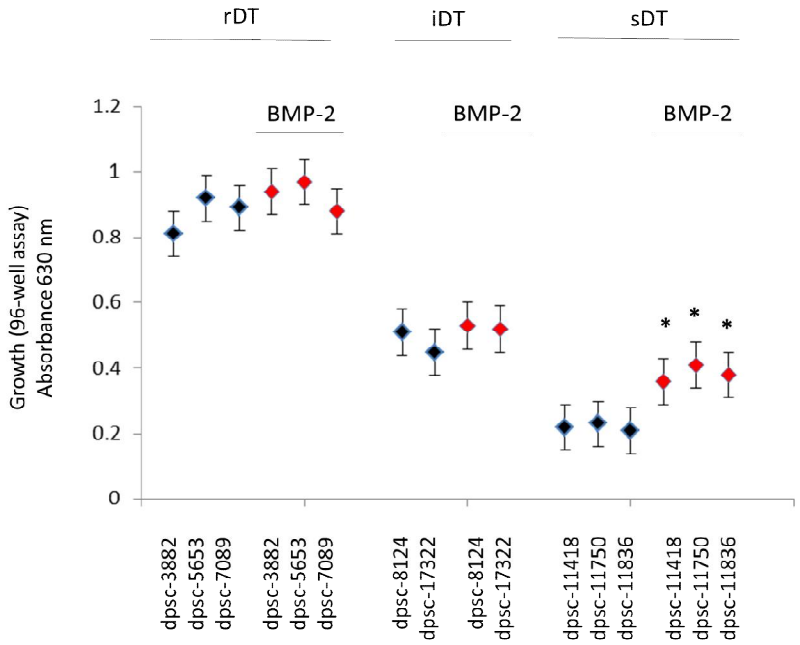

Fig. 1. DPSC growth following BMP-2 treatment. BMP-2 administration exhibited strong, positive effects on growth of the slow doubling time (sDT) DPSC isolates (dpsc-11418, dpsc-11750, dpsc-11836), without any significant effects on intermediate (iDT; dpsc-8124, dpsc-17322) or rapid (rDT; dpsc-3882, dpsc-5653, dpsc-7089) DPSC isolates (p=0.411, p=0.388, respectively)

To examine if these changes in cellular growth following BMP-2 administration were associated with any changes to cellular viability, Trypan Blue assays were performed on each DPSC isolate at the end of each experimental assay (Fig. 2). In brief, these data demonstrated that the addition of BMP-2 did not significantly alter cellular viability among the rDT (dpsc-3882, dpsc-5653, dpsc-7089) or iDT (dpsc-8124, dpsc-17322) DPSC isolates (p=0.512, p=0.399, respectively). However, distinct and significant positive effects were observed among the sDT DPSC isolates (dpsc-11418, dpsc-11750, dpsc-11836), p=0.022.

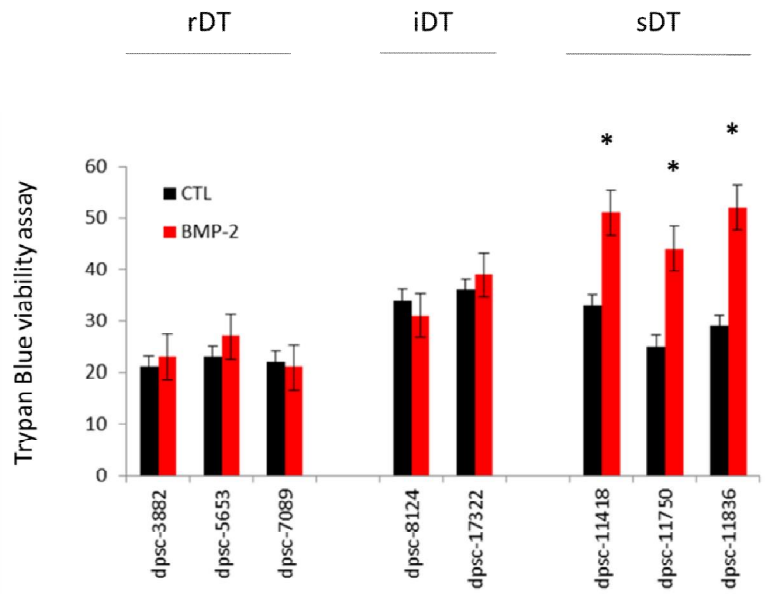

Fig. 2. BMP-2 effects on DPSC viability *in vitro*. Administration of BMP-2 did not result in any significant effects on rapid (rDT; -3882, -5653, -7089) or intermediate (iDT; -8124, -17322) DPSC isolates (p=0.512, p=0.399, respectively) but significantly increased viability among sDT (-11418, -11750, -11836), p=0.022

Due to the observed changes in both cell viability and growth following BMP-2 administration among the sDT DPSC isolates (-11418, -11750, -11836), an analysis of the DPSC biomarkers associated with osteoblastic differentiation were examined (Fig. 3). Total RNA isolated from all DPSC isolates following BMP-2 administration was screened using primers specific for alkaline phosphatase (ALP) and dentin sialophosphoprotein (DSPP)(Fig. 3A). This experiment revealed that one of the iDT DPSC isolates (-17322) and two of the sDT isolates (-11418-, -11750) exhibited differential mRNA production of these biomarkers. However, no expression of either ALP or DSPP has observed among the remaining sDT and iDT DPSC isolates (-11836, -8124, respectively) or any of the rDT isolates. Photomicroscopy of the sDT isolates revealed that BMP-2 exerted broad proliferative effects, but morphologic changes were observed only in dpsc-11418 and dpsc-11750 (Fig. 3B).

An additional screening of mesenchymal stem cell (MSC) biomarkers were then performed to determine if any of these changes to cellular phenotype (viability, growth, morphology) or biomarker expression (ALP, DSPP) were associated with the expression (or lack) of MSC biomarkers (Fig. 4). More specifically, the expression of the MSC markers Nestin, NANOG, Oct-4 and Sox-2 were evaluated (Fig. 4A). This analysis revealed the concomitant expression of two or more MSC markers among the rDT and iDT DPSC isolates, but only Nestin among the sDT isolates. Moreover, the expression of Nestin mRNA strongly correlated with DPSC response to BMP-2 (dpsc-11418, dpsc-11750) with the absence of response observed in the sDT with a relatively lower expression of Nestin (dpsc-11836 (Fig. 4B).

4. DISCUSSION

The primary goal of this study was to investigate the ability of BMP-2 to induce proliferation and differentiation of DPSC isolates into mineral forming bone cell precursor lineages expressing the appropriate biomarkers. These results demonstrated that some, but not all, DPSC isolates were capable of responding to BMP-2 with corresponding changes to growth, viability, and cellular morphology. Moreover, these changes were associated with sDT DPSC isolates not expressing multiple MSC biomarkers, but rather one specific MSC marker – Nestin [30,31].

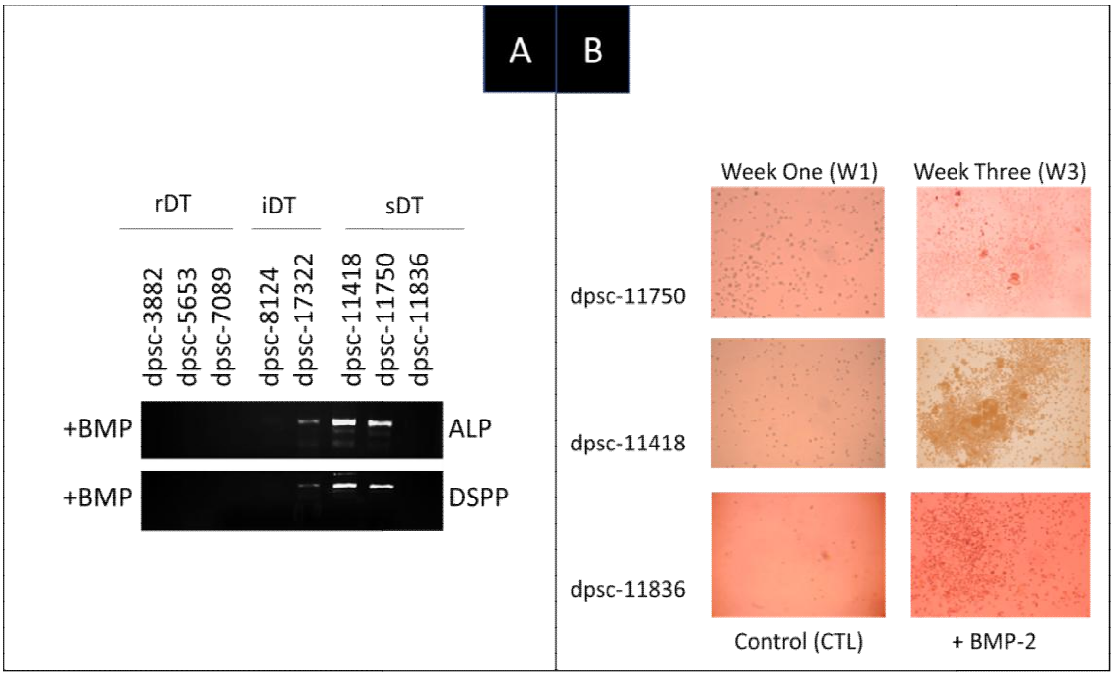

Fig. 3. DPSC mRNA biomarker induction following BMP-2 treatment. A) BMP-2 administration induced alkaline phosphatase (ALP) and dentin sialophosphoprotein (DSPP) mRNA expression among one iDT (dpsc-17322) and two sDT (dpsc-11418, dpsc-11750) DPSC isolates, but not all (iDT dpsc-8124 and sDT dpsc-11836 were both negative). No changes were observed among the rDT isolates (dpsc-3882, dpsc-5653, dpsc-7089). B) Photo microscopy of the sDT isolates revealed proliferative effects of BMP-2 with morphologic changes observed only in dpsc-11418 and dpsc-11750. All photomicroscopy was performed taking images from the centre of each well to minimise researcher selection bias. DPSC is mainly non-adherent, and their number may vary at different locations in each well as part of the normal experimental variation.

These data appear to confirm other experimental evidence that BMP-2 may exhibit the potential to induce ALP expression among some DPSC isolates [5,32]. However, there is a lack of experimental and observational evidence to evaluate the specific phenotypes and biomarkers associated with DPSC responsiveness – as few studies have compared the effects of BMP-2, DPSC differentiation, and MSC biomarkers [33]. The few studies to have evaluated these phenomena have also demonstrated differential results, with some DPSC isolates responding to BMP-2 (and others not) – although only Runx-2 and MEF2, a member of the myocyte enhancer factor-2 (MEF-2) box family appeared to be enhanced upon BMP-2 administration and ALP induction – although insufficient data were available to ascertain if these were upstream or downstream (cause or effect) changes [34,35].

Although these data provide novel insights into the properties and characteristics of DPSC isolates that may be responsive to BMP-2 administration, there are several limitations associated with this study that must also be considered. For example, new evidence has suggested that improved methods of culture may exist to differentially affect multipotency and stem cell-like properties of DPSC towards osteoblastic and osteogenic lineages [36,37]. Besides, some evidence has also suggested that the timing and administration of multiple stimuli (including BMP-2 in combination with vascular endothelial growth factor or VEGF) may also preferentially affect DPSC responsiveness to BMP-2 - although financial and timing constraints limited the scope of this initial study [33,35,38].

Finally, the selection of MSC and DPSC biomarkers to evaluate should also be carefully considered [39]. For example, many other studies of DPSC differentiation have evaluated NANOG, Sox-2 and Oct-4, which are known transcription factors that may directly influence

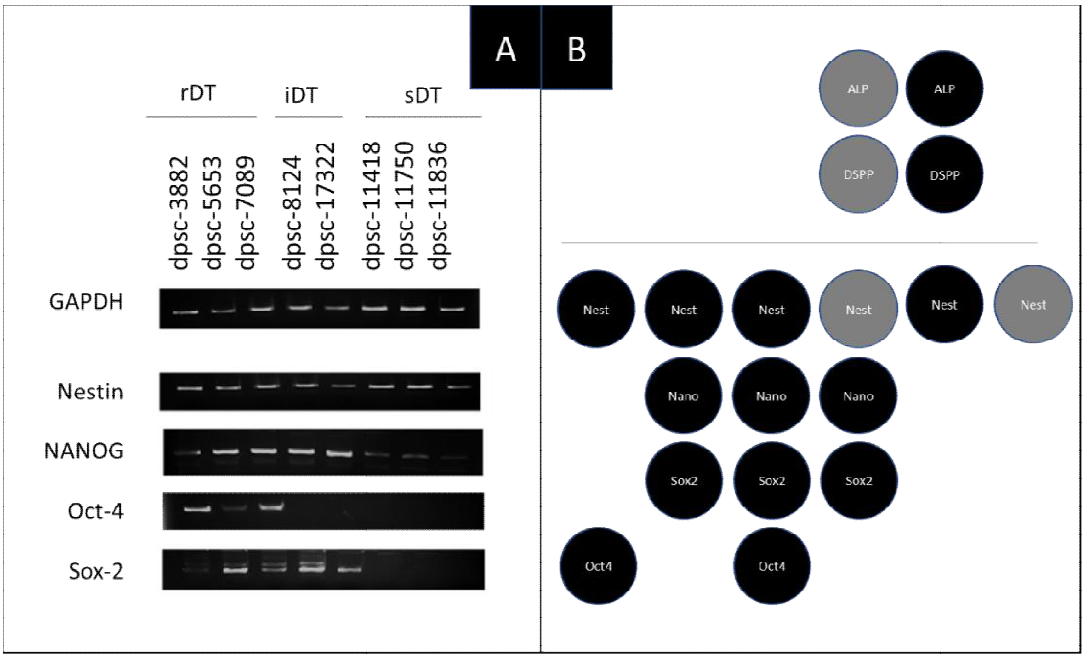

Fig. 4. Analysis of MSC biomarker expression among DPSC isolates. A) mRNA expression of Nestin, NANOG, Oct-4 and Sox-2 were differentially expressed among the DPSC isolates, with multiple biomarkers expressed among the rDT and iDT isolates but only Nestin expressed among the sDT isolates. B) Expression of ALP and DSPP induced by BMP-2 was associated with the sDT DPSC isolates with high Nestin expression. Weak or transient expression of Nestin (and the absence of other MSC biomarkers) was observed in the sDT isolate not responsive to BMP-2 treatment (dpsc-11836). Graphic representation of these results: Black circles denote strong mRNA expression and grey circles denote moderate mRNA expression

specific pathways related to cellular phenotypes [40,41]. However, the role of Nestin appears only to have been evaluated peripherally in studies of DPSC and neural differentiation without evaluation of this biomarker among studies of osteogenic differentiation and BMP administration [42,43].

These biomarkers may be critical indicators not only of differentiation status and may also directly or indirectly affect other phenotypic behaviours observed in this study, such as doubling time. For example, it was observed that rapid and intermediate doubling times of specific DPSC isolates were associated with the expression of mRNA for MSC biomarkers including Nestin, NANOG, Sox-2 and Oct-4 – confirming previous observations in these DPSC isolates [26,27]. Also, DPSC isolates with slow doubling times were associated with the expression of Nestin but not NANOG, Sox-2 or Oct-4 indicating the potential for partial differentiation – also confirmed in previous studies [28,29]. Although these observations must be confirmed by other studies using other DPSC isolates.

5. CONCLUSIONS

Based upon this information, this study may be the first to demonstrate not only the differential responsiveness of DPSC isolates to BMP-2, but also to identify the MSC biomarkers that may affect initial DPSC responsiveness to this stimulus. Although many studies have evaluated the role of the biomarkers NANOG, Sox-2 and Oct-4 in DPSC isolate, no other study of DPSC multipotency has evaluated the role of Nestin – which may be one of the key factors that potentiate or limits the responsiveness to BMP-2 and osteogenic potential among DPSCs. These results suggest more research into these phenomena may be needed to further the understanding of DPSC differentiation and bioengineering.

CONSENT

Both pediatric assent and parental consent were required to partake in the study. Informed consent was required for all adult patients.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Perez JR, Kouroupis D, Li DJ, Best TM, Kaplan L, Correa D. Tissue engineering and cell-based therapies for fractures and bone defects. Front Bioeng Biotechnol. 2018;6:105.
   DOI:10.3389/fbioe.2018.00105. eCollection 2018. Review. PMID: 30109228
2. Su P, Tian Y, Yang C, Ma X, Wang X, Pei J, Qian A. Mesenchymal stem cell migration during bone formation and bone diseases therapy. Int J Mol Sci. 2018;19(8).pii: E2343.
   DOI:10.3390/ijms19082343. Review. PMID: 30096908
3. Tavassoli H, Alhosseini SN, Tay A, Chan PPY, Weng Oh SK, Warkiani ME. Large-scale production of stem cells utilizing microcarriers: A biomaterials engineering perspective from academic research to commercialized products. Biomaterials. 2018;181:333-346.
   DOI:10.1016/j.biomaterials.2018.07.016. [Epub ahead of print] Review. PMID: 30098569
4. Leach JK, Whitehead J. Materials-Directed Differentiation of Mesenchymal Stem Cells for Tissue Engineering and Regeneration. ACS Biomater Sci Eng. 2018;4(4):1115-1127.
   DOI:10.1021/acsbiomaterials.6b00741. Epub 2017 Mar 14. PMID: 30035212
5. Wang S, Zhu R, Li H, Li J, Han Q, Zhao RC. Mesenchymal stem cells and immune disorders: From basic science to clinical transition. Front Med. 2018;30.
   DOI:10.1007/s11684-018-0627-y. [Epub ahead of print] Review. PMID: 30062557
6. Lin H, Sohn J, Shen H, Langhans MT, Tuan RS. Bone marrow mesenchymal stem cells: Aging and tissue engineering applications to enhance bone healing. Biomaterials. 2018;pii:S0142-9612(18): 30447-2.
   DOI:10.1016/j.biomaterials.2018.06.026. [Epub ahead of print] PMID: 29980291
7. Abbaspanah B, Momeni M, Ebrahimi M, Mousavi SH. Advances in perinatal stem cells research: A precious cell source for clinical applications. Regen Med; 2018.
   DOI:10.2217/rme-2018-0019. [Epub ahead of print] PMID: 30129876
8. Ayala-Cuellar AP, Kang JH, Jeung EB, Choi KC. Roles of Mesenchymal Stem Cells in Tissue Regeneration and Immunomodulation. Biomol Ther (Seoul). 2018;14.
   DOI: 10.4062/biomolther.2017.260. [Epub ahead of print] Review. PMID: 29902862
9. Francis SL, Duchi S, Onofrillo C, Di Bella C, Choong PFM. Adipose-Derived Mesenchymal Stem Cells in the Use of Cartilage Tissue Engineering: The Need for a Rapid Isolation Procedure. Stem Cells Int. 2018;2018:8947548.
   DOI: 10.1155/2018/8947548. eCollection 2018. Review. PMID: 29765427
10. Hollands P, Aboyeji D, Orcharton M. Dental pulp stem cells in regenerative medicine. Br Dent J; 2018.
    DOI:10.1038/sj.bdj.2018.348. [Epub ahead of print] PMID: 29725075
11. Le Blanc K, Davies LC. MSCs-cells with many sides. Cytotherapy. 2018;20(3):273-278.
    DOI:10.1016/j.jcyt.2018.01.009. Epub 2018 Feb 9. Review. PMID: 29434007
12. Rosa V, Dubey N, Islam I, Min KS, Nör JE. Pluripotency of Stem Cells from Human Exfoliated Deciduous Teeth for Tissue Engineering. Stem Cells Int. 2016;5957806.
    DOI: 10.1155/2016/5957806. Epub 2016 May 30. Review. PMID: 27313627
13. Potdar PD, Jethmalani YD. Human dental pulp stem cells: Applications in future regenerative medicine. World J Stem Cells. 2015;7(5):839-51.
    DOI: 10.4252/wjsc.v7.i5.839. Review. PMID: 26131314
14. Kaneko T, Gu B, Sone PP, Zaw SYM, Murano H, Zaw ZCT, Okiji T. Dental pulp tissue engineering using mesenchymal stem cells: A review with a protocol. Stem Cell Rev; 2018.

DOI:10.1007/s12015-018-9826-9. [Epub ahead of print] Review.

PMID: 29804171

15. Cristaldi M, Mauceri R, Tomasello L, Pizzo G, Pizzolanti G, Giordano C, Campisi G. Dental pulp stem cells for bone tissue engineering: A review of the current literature and a look to the future. Regen Med; 2018.

DOI:10.2217/rme-2017-0112. [Epub ahead of print] PMID: 29553875

16. Ercal P, Pekozer GG, Kose GT. Dental stem cells in bone tissue engineering: Current overview and challenges. Adv Exp Med Biol; 2018.

DOI:10.1007/5584_2018_171. [Epub ahead of print] PMID: 29498025

17. Leyendecker Junior A, Gomes Pinheiro CC, Lazzaretti Fernandes T, Franco Bueno D. The use of human dental pulp stem cells for in vivo bone tissue engineering: A systematic review. J Tissue Eng. 2018;9:2041731417752766.

DOI:10.1177/2041731417752766. eCollection 2018 Jan-Dec. Review. PMID: 29375756

18. Yasui T, Mabuchi Y, Morikawa S, Onizawa K, Akazawa C, Nakagawa T, Okano H, Matsuzaki Y. Isolation of dental pulp stem cells with high osteogenic potential. Inflamm Regen. 2017;37:8.

DOI:10.1186/s41232-017-0039-4. eCollection 2017. Review. PMID: 29259707

19. Nancarrow-Lei R, Mafi P, Mafi R, Khan W. A systemic review of adult mesenchymal stem cell sources and their multilineage differentiation potential relevant to musculoskeletal tissue repair and regeneration. Curr Stem Cell Res Ther. 2017;12(8):601-610.

DOI:10.2174/1574888X12666170608124303. Review. PMID: 28595566

20. Avinash K, Malaippan S, Dooraiswamy JN. Methods of isolation and characterization of stem cells from different regions of oral cavity using markers: A systematic review. Int J Stem Cells. 2017 May 30;10(1):12-20.

DOI:10.15283/ijsc17010. Review. PMID: 28531913

21. Teng CF, Jeng LB, Shyu WC. Role of Insulin-like Growth Factor 1 Receptor Signaling in Stem Cell Stemness and Therapeutic Efficacy. Cell Transplant. 2018;963689718779777.

DOI:10.1177/0963689718779777. [Epub ahead of print] PMID: 29882416

22. Guazzo R, Gardin C, Bellin G, Sbricoli L, Ferroni L, Ludovichetti FS, Piattelli A, Antoniac I, Bressan E, Zavan B. Graphene-based nanomaterials for tissue engineering in the dental field. Nanomaterials (Basel). 2018;8(5). pii: E349.

DOI:10.3390/nano8050349. Review. PMID: 29783786

23. Zhang F, Song J, Zhang H, Huang E, Song D, Tollemar V, Wang J, Wang J, Mohammed M, Wei Q, Fan J, Liao J, Zou Y, Liu F, Hu X, Qu X, Chen L, Yu X, Luu HH, Lee MJ, He TC, Ji P. Wnt and BMP signaling crosstalk in regulating dental stem cells: Implications in dental tissue engineering. Genes Dis. 2016;3(4):263-276.

DOI:10.1016/j.gendis.2016.09.004. Epub 2016 Oct 5. PMID: 28491933

24. Nakashima M. Bone morphogenetic proteins in dentin regeneration for potential use in endodontic therapy. Cytokine Growth Factor Rev. 2005;16(3):369-76. Review. PMID: 15878301

25. Chatakun P, Núñez-Toldrà R, Díaz López EJ, Gil-Recio C, Martínez-Sarrà E, Hernández-Alfaro F, Ferrés-Padró E, Giner-Tarrida L, Atari M. The effect of five proteins on stem cells used for osteoblast differentiation and proliferation: a current review of the literature. Cell Mol Life Sci. 2014;71(1):113-42.

DOI:10.1007/s00018-013-1326-0. Epub 2013 Apr 9. Review. PMID: 23568025

26. Alleman M, Low E, Truong K, Huang E, Hill CK, Chen TY, Deaton M, Kingsley K. Dental pulp-derived stem cells (DPSC) differentiation in vitro into odontoblast and neural progenitors during cell passaging is associated with alterations in cell survival and viability. International Journal of Medicine and Biomedical Research. 2013;2(2):133-141.

27. Hung E, Lee S, Fitzgerald B, Hill CK, Kingsley K. Dental pulp-derived stem cell (DPSC) survival and viability may correlate with specific patient demographics. Forum for Dental Student Research and Innovation (FDSRI). Winter. 2013,1(3):14-21.

28. Loveland K, Young A, Khadiv M, Culpepper M, Kingsley K. Dental Pulp Stem Cell (DPSC) pluripotency enhanced by transforming growth factor (TGF-beta1) in vitro may be inhibited by differentiation-inducing factors laminin-5 and dexamethasone. International Journal of Biological Sciences and Applications. 2014;1(3):55-61. [Epub ahead of print]

29. Burnett A, Kumar R, Westphal JD, Kingsley K. Dichloroacetate (DCA) Promotes a de-differentiated phenotype in dental pulp-derived stem cells in vitro. International Journal of Biological Sciences and Applications. 2015;2(3):25-32.

30. Heng BC, Gong T, Xu J, Lim LW, Zhang C. EphrinB2 signalling modulates the neural differentiation of human dental pulp stem cells. Biomed Rep. 2018;9(2):161-168.
DOI: 10.3892/br.2018.1108. Epub 2018 Jun 1. PMID: 29963307

31. Viña-Almunia J, Mas-Bargues C, Borras C, Gambini J, El Alami M, Sanz-Ros J, Peñarrocha M, Vina J. Influence of Partial O_2 Pressure on the Adhesion, Proliferation, and Osteogenic Differentiation of Human Dental Pulp Stem Cells on β-Tricalcium Phosphate Scaffold. Int J Oral Maxillofac Implants. 2017;32(6):1251–1256.
DOI:10.11607/jomi.5529. Epub 2017 Sep 22.
PMID: 28938034

32. Koyama N, Okubo Y, Nakao K, Bessho K. Evaluation of pluripotency in human dental pulp cells. J Oral Maxillofac Surg. 2009;67(3):501-6.
DOI:10.1016/j.joms.2008.09.011. PMID: 19231772

33. Atalayin C, Tezel H, Dagci T, Yavasoglu NU, Oktem G. Medium modification with bone morphogenetic protein 2 addition for odontogenic differentiation. Braz Oral Res. 2016;30. pii: S1806-83242016000100223. DOI:10.1590/1807-3107BOR-2016.vol30.0020. Epub 2016 Mar 8. PMID: 26981753-

34. Shen S, Huang D, Feng G, Zhu L, Zhang Y, Cao P, Zheng K, Zhang D, Feng X. MEF2 transcription factor regulates osteogenic differentiation of dental pulp stem cells. Cell Reprogram. 2016;18(4): 237-45.
DOI:10.1089/cell.2016.0016. PMID: 27459583

35. Hrubi E, Imre L, Robaszkiewicz A, Virág L, Kerényi F, Nagy K, Varga G, Jenei A, Hegedüs C. Diverse effect of BMP-2 homodimer on mesenchymal progenitors of different origin. Hum Cell. 2018;31(2):139-148.
DOI: 10.1007/s13577-018-0202-5. Epub 2018 Feb 13. PMID: 29442285

36. Fujii Y, Kawase-Koga Y, Hojo H, Yano F, Sato M, Chung UI, Ohba S, Chikazu D. Bone regeneration by human dental pulp stem cells using a helioxanthin derivative and cell-sheet technology. Stem Cell Res Ther. 2018;9(1):24.
DOI:10.1186/s13287-018-0783-7.
PMID: 29391049

37. Bakopoulou A, Apatzidou D, Aggelidou E, Gousopoulou E, Leyhausen G, Volk J, Kritis A, Koidis P, Geurtsen W. Isolation and prolonged expansion of oral mesenchymal stem cells under clinical-grade, GMP-compliant conditions differentially affects "stemness" properties. Stem Cell Res Ther. 2017;8(1):247.
DOI: 10.1186/s13287-017-0705-0.
PMID: 29096714

38. Aksel H, Huang GT. Combined effects of vascular endothelial growth factor and bone morphogenetic protein 2 on odonto/osteogenic differentiation of human dental pulp stem cells in vitro. J Endod. 2017;43(6):930-935.
DOI:10.1016/j.joen.2017.01.036. Epub 2017 Apr 27. PMID: 28457634

39. Di Scipio F, Sprio AE, Carere ME, Yang Z, Berta GN. A simple protocol to isolate, characterize, and expand dental pulp stem cells. Methods Mol Biol. 2017;1553:1-13. PMID: 28229403

40. Yang Y, Zhao Y, Liu X, Chen Y, Liu P, Zhao L. Effect of SOX2 on odontoblast differentiation of dental pulp stem cells. Mol Med Rep. 2017;16(6):9659-9663. PMID: 29039570

41. Ferro F, Spelat R, D'Aurizio F, Puppato E, Pandolfi M, Beltrami AP, Cesselli D, Falini G, Beltrami CA, Curcio F. Dental pulp stem cells differentiation reveals new insights in Oct4A dynamics. PLoS One. 2012;7(7): e41774. PMID: 22844522

42. Heng BC, Gong T, Xu J, Lim LW, Zhang C. EphrinB2 signalling modulates the neural differentiation of human dental pulp stem

cells. Biomed Rep. 2018;9(2):161-168. PMID: 29963307

43. Király M, Porcsalmy B, Pataki A, Kádár K, Jelitai M, Molnár B, Hermann P, Gera I, Grimm WD, Ganss B, Zsembery A, Varga G. Simultaneous PKC and cAMP activation induces differentiation of human dental pulp stem cells into functionally active neurons. Neurochem Int. 2009;55(5):323-32. PMID: 19576521

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