ORIGINAL ARTICLE

Pluripotent Stem Cell Markers and microRNA Expression May Correlate with Dental Pulp Stem Cell Viability and Proliferation Rates

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ABSTRACT

Background: Recent evidence has demonstrated that dental pulp stem cells (DPSC) may represent a source of pluripotent progenitors capable of differentiating into many cell and tissue types. Although microRNAs are known to modulate differentiation and function in human dental tissues, much of this research has focused selectively on tooth development. The primary objective of this study was to evaluate the expression of microRNA in dental pulp stem cell isolates to compare with classical biomarkers of cellular phenotypes and pluripotency. **Materials and Methods:** Using eight previously isolated and characterized DPSC isolates, growth and viability were evaluated and RNA was extracted for mRNA screening. DPSC biomarker and microRNA expression were analyzed for comparison with cellular phenotypes. **Results:** Evaluation of the growth and proliferation rates of each cell line resulted in the categorization of DPSC isolates into rapid, intermediate, and slow doubling times, which demonstrated higher viability among the most rapidly proliferating DPSCs. Analysis of DPSC biomarkers (Oct-4, Sox-2, NANOG) revealed an association with total live cell count, while microRNA expression (miR-27, miR-218, miR-124, and miR-16) appeared to be more closely associated with cellular viability. **Conclusions:** Although this study was limited to a small number of DPSC isolates, these results suggest a more thorough investigation and evaluation of biomarkers and microRNA expression may be necessary to elucidate the associations and complex interconnections with DPSC viability, proliferation, differentiation, and pluripotency.

Key words: Biomarkers, dental pulp stem cells, microRNA, pluripotency

BACKGROUND

Recent evidence has demonstrated that dental pulp stem cells (DPSC) may represent a source of pluripotent progenitors capable of differentiating into many cell and tissue types,[1,2] some of which may have therapeutic potential. Several key markers of pluripotency in DPSC have been identified, including the transcription factors Oct-4, Sox-2, and NANOG.[3,4] Although many studies have evaluated the functional effects of these transcriptional activators and repressors, many factors that contribute to maintaining pluripotency among DPSC remain undiscovered.[5-7]

For example, recent evidence now suggests that mesenchymal stem cell (MSC) differentiation may be regulated not only by classical stem cell-associated transcription factors (Sox-2, Oct-4,

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and NANOG) but also through transcriptional modulation by long, non-coding RNA known as microRNA.[8,9] In fact, there is some evidence that specific microRNAs may also be useful as biomarkers to identify and distinguish stem cells with differing therapeutic potential.[10-12] Although an extensive body of evidence has accumulated regarding the role of microRNAs and MSC, a few (if any) studies have focused more specifically on the role of DPSC.[13]

Although microRNAs are known to modulate differentiation and function in human dental tissues, much of this research has focused selectively on tooth development.[14-16] Developmental biology requires a thorough understanding of these mechanisms; however, recent studies now suggest that DPSC may also be useful in bioengineering and therapeutic applications for regenerative medicine.[17,18] Promising areas of interest include bone and neural tissue engineering using DPSC although much remains to be discovered regarding the mechanisms that control these processes.[19,20]

Research from our group has evaluated classical biomarkers and other factors that may influence the therapeutic potential of primary explants and DPSC isolates.[21,22] The most recent study from this group may have also identified a limited number of microRNAs that may influence DPSC pluripotency and differentiation, including miR-27, miR-124, and miR-218.[23] Many other studies have examined the role of these microRNAs in MSC although only a single, recent study has examined miR-218 in DPSC and none have evaluated the concomitant presence (or absence) of traditional biomarkers.[24-26]

To advance the evidence in this area, the primary objective of this study was to evaluate the expression of microRNA in DPSC isolates to compare with classical biomarkers of cellular phenotypes and pluripotency.

MATERIALS AND METHODS

Human subjects

This project was reviewed and approved by the Institutional Review Board (IRB) and Office for the Protection of Research Subjects (OPRS) under protocol OPRS#763012-1 "retrospective analysis of dental pulp stem cells (DPSC) from the University of Nevada, Las Vegas – School of Dental Medicine pediatric and adult clinical population." DPSC was originally collected using protocol OPRS#0907-3148 "isolation of non-embryonic stem cells from dental pulp."

In brief, patients scheduled for extraction of third molars for orthodontic treatment (primarily indicated for spacing and crowding) were asked to participate. Informed consent (and pediatric assent) were provided. Exclusion criteria included refusal to participate, refused to provide Informed Consent/Assent, or compromised dental pulp due to injury, fracture, infection or disease.

Following cross-sectioning of the extracted tooth at the cementoenamel junction, DPSC was extracted from the pulp chamber using an endodontic broach and transferred to the laboratory for culture and analysis, as previously described.[27,28] Growth for a minimum of 10 passages was allowed for each DPSC isolate, as part of the direct outgrowth method.[29,30]

Growth and proliferation

Doubling time (DT) was established for each DPSC isolate using the growth rate measured between 1:4 passaging and achieving confluence. These data were used to characterize three distinct categories of DPSC growth: Rapid DT (rDT) of 2–3 days, intermediate DT (iDT) 5–7 days, and slow DT (sDT) of 10–14 days.[21,22]

Cellular viability and photomicroscopy

Analysis of cellular viability was performed using the Trypan blue exclusion method and the BioRad TC20 automated cell counter, using the manufacturer recommended protocol. These data provided both live and total cell counts, allowing for the calculation of the percentage of viable cells in each sample, as previously described.[27,28,31] Digital images of DPSC isolates were captured using the Lionheart LX Automated Microscope and ×20 objective lens.

Statistical analysis

Rates of proliferation and cellular viability were measured, and these descriptive statistics were collected and analyzed using the Student's two-tailed *t*-test. Statistical significance was set using an alpha level (α) =0.05.

RNA isolation and polymerase chain reaction (PCR)

RNA was extracted from each DPSC isolate using total RNA isolation reagent from Molecular Research Center and the manufacturer recommended protocol.[23,27] RNA was analyzed to determine the purity using spectrophotometric absorbance readings at 260 and 280 nm (A260:A280 ratio). All samples were required to meet the standard A260:A280 >1.65.

Screening for mRNA expression was facilitated using the ABgene Reverse-iT One-Step reverse transcription (RT)-PCR kit and protocol. The basic specifications were an initial RT at 47°C for 30 min and 30 cycles of the following: Denaturation 95°C, annealing 30 s at selected primer temperature (see below), and final extension 60°C for 60 s. Results were obtained using gel electrophoresis and visualized using ethidium bromide with a Kodak Gel Logic 100 Imaging System. Primers were synthesized from Eurofins MWG Operon, as follows:

DPSC biomarker primers

Oct-4 forward, 5'-TGGAG AAGGAGAAGC TGG AGCAAAA-3'; 25 nt: 48% GC; Tm 70C Oct4 reverse,5'-GGCAGATGGTCGT TTGGCTGAATA-3'; 24 nt; 50% GC; Tm 70C Optimal Tm: 71C.

Sox2 forward, 5'-ATGGGCT CTGTGG TCAAGTC-3'; 20 nt: 55% GC; Tm 67C Sox2 reverse, 5'-CCCTCCCAA TTCCCTTGTAT-5'; 20 nt; 50% GC; Tm 64C Optimal Tm: 65C.

NANOG forward, 5'-GCTGAGA TGCCTCACAC GGAG-3'; 21 nt; 62% GC; Tm 71C NANOG reverse, 5'-TCTGTTTCTTGACTGGGACCTTGTC-3'; 25 nt: 48% GC; Tm 69C Optimal Tm: 70C.

Nestin forward, 5'-CGTTGGAAC AGAGGTTGGAG-3'; 20 nt; 55% GC; Tm 66C Nestin reverse, 5'-TCCTGAAAGCT GAGGGAAG-3'; 19 nt; 53% GC; Tm 64C Optimal Tm: 65C.

MicroRNA screening primers

miR-27 forward, 5'-ATATGAGA AAAG AGCTT CCCTGTG-3'; 24 nt; 42% GC; Tm 64C miR-27 reverse, 5'-CAAGGCC AGAGGAG GTGAG-'3'; 19 nt; 63% GC; Tm 64C Optimal Tm: 65C.

miR-218 forward, 5'-TCG GGCTT GTGCTT GATCT-3'; 19 nt; 53% GC; Tm 67C miR-218 reverse, 5'-GTGCAGGG TCCGAGTG-3'; 16 nt; 69% GC; Tm 66C Optimal Tm: 67C.

miR-124- forward, 5'-ATGAATTC TCGCCA GC TTTT TCTT-3'; 24 nt; 38% GC; Tm 65C miR-124 reverse, 5'-ATGAATTCA TTTGCAT CTGCACAAACCC-3'; 28 nt; 39% GC; Tm 65C Optimal Tm: 66C.

miR-16 forward, 5'-TAGCAGCA CGTAAATAT TGGCG-3'; 22 nt; 45% GC; Tm 65C miR-16 reverse, 5'-TGCGTG TCGTGGAGTC-3'; 16 nt; 63% GC; Tm 65C Optimal Tm: 66C.

RESULTS

Evaluation of the growth and proliferation rates of each DPSC isolate resulted in categorization of DPSC isolates into rDT, iDT, and sDT [Table 1]. The average DT for the rapid DPSC was 2.2 days, which was significantly less than the average DT for the intermediate (6.25 days) and slow DPSC isolates (12.1 days), $P = 0.0241$.

Cellular viability was then measured for each DPSC isolate to determine if any correlations could be found with growth rates and DT [Table 2]. In brief, the cellular viability for the rDT DPSC isolates averaged 56%, ranging between 52% and 59%. The average was considerably higher than those observed among the iDT DPSC isolates (37%, range 35–39%) or sDT DPSC isolates (31%, range 29–34%), $P = 0.311$.

To more closely evaluate cellular phenotypes and morphology as well as to evaluate the presence of both viable and non-viable cells, digital micrographs were obtained from each DPSC isolate [Figure 1]. Briefly, these data demonstrated multiple, non-adherent DPSC cells with fewer cells displaying signs of apoptosis (intense blebbing) among the rDT isolates [Figures 1a and 1c]. Higher proportions of these non-viable cells were observed among the iDT [Figure 1d and h] and sDT isolates [Figure 1e and g].

To evaluate the association between growth and viability, DPSC biomarkers (Oct-4, Sox-2, NANOG, and Nestin) and microRNAs (miR-27, miR-218, miR-124, and miR-16) were evaluated using RT-PCR for comparison with graphs of live and viable cells [Figure 2]. More specifically, the total live counts from each cell line were plotted, which revealed the highest numbers among the rDT and iDT isolates [Figure 2a]. These data appear to correlate with the expression of NANOG, Sox-2, and Oct-4 among the rDT and iDT isolates although some variability in mRNA expression was observed [Figure 2b].

Although mRNA expression of at least two DPSC biomarkers was noted in all rDT and iDT isolates, the expression of all DPSC biomarkers was noted in only one DPSC isolate (DPSC-7089), which was observed to have the highest overall live cell count and cell viability [Figure 2b and c]. In addition, Oct-4 expression was not observed in either of the iDT isolates (DPSC-8124 and DPSC-17322). However, the expression of Sox-2 and NANOG was observed in all of the iDT isolates, unlike the variable expression observed among the rDT isolates.

The highest overall viability was verified among the rDT isolates (DPSC-3882, DPSC-5653, and DPSC-7089) with an average exceeding 50% [Figure 2c]. These data appear to correlate with the expression of miR-27, which was observed only among the rDT isolates [Figure 2d]. Expression of miR-124 was restricted

Table 1: Analysis of DPSC growth rates

DPSC isolate	DT (days)	Categorization
DPSC-3882	2.6	rDT
DPSC-5653	2.1	rDT
DPSC-7089	1.9	rDT
	rDT average: 2.2 range: 1.9–2.6	
DPSC 8124	5.9	iDT
DPSC-17322	6.6	iDT
	iDT average: 6.25 range: 5.9–6.6	
DPSC-11418	10.2	sDT
DPSC-11750	13.1	sDT
DPSC -11836	12.9	sDT
	sDT average: 12.1 range: 10.2–13.1	

DPSC: Dental pulp stem cells, DT: Doubling time, sDT: Slow DT, rDT: Rapid DT, iDT: Intermediate DT

Table 2: Trypan blue exclusion assay test for DPSC viability

DPSC isolate	Cellular viability	Categorization
DPSC-3882	1.62×10^5 cells/mL TC 0.84×10^5 cells/mL LC Viable: 52%	rDT (2.6 days)
DPSC-5653	1.90×10^5 cells/mL TC 1.12×10^5 cells/mL LC Viable: 59%	rDT (2.1 days)
DPSC-7089	3.35×10^5 cells/mL TC 1.90×10^5 cells/mL LC Viable: 57%	rDT (1.9 days)
rDT	Ave: 2.29×10^5 cells/mL TC Ave: 1.29×10^5 cells/mL LC Average viability: 56%	
DPSC 8124	3.68×10^5 cells/mL TC 1.45×10^5 cells/mL LC Viable: 39%	iDT (5.9 days)
DPSC-17322	2.90×10^5 cells/mL TC 1.00×10^5 cells/mL LC Viable: 35%	iDT (6.6 days)
iDT	Ave: 3.29×10^5 cells/mL TC Ave: 1.23×10^5 cells/mL LC Average viability: 37%	
DPSC-11418	3.24×10^5 cells/mL TC 0.948×10^5 cells/mL LC Viable: 29%	sDT (10.2 days)
DPSC-11750	3.24×10^5 cells/mL TC 1.00×10^5 cells/mL LC Viable: 31%	sDT (13.1 days)
DPSC-11836	1.62×10^5 cells/mL TC 0.558×10^5 cells/mL LC Viable: 34%	sDT (12.9 days)
sDT	Ave: 2.70×10^5 cells mL TC Ave: 0.835×10^5 cells/mL LC Average viability: 31%	

DPSC: Dental pulp stem cells, DT: Doubling time sDT: Slow DT, iDT: Intermediate DT, rDT: Rapid DT

to the sDT isolates, which appeared to have the lowest overall viability (approximately 30%), with variable expression observed with miR-218. It was noted that only miR-16 (control) expression was observed among the iDT isolates, with no expression of miR-27, miR-124, or miR-218 observed.

DISCUSSION

The primary objective of this study was to evaluate the expression of microRNA in DPSC isolates to compare with classical biomarkers of cellular phenotypes and pluripotency. Evaluation of the growth and proliferation rates of each cell line resulted in the categorization of DPSC isolates into rDT, iDT, and sDT, which demonstrated higher viability among the most rapidly proliferating DPSCs. Analysis of DPSC biomarkers (Oct-4, Sox-2, and NANOG) revealed an association with total live cell count, while microRNA expression (miR-27, miR-218, miR-124, and miR-16) appeared to be more closely associated with viability.

These data appear to confirm recent studies that demonstrated proliferation and growth potential among DPSC isolates to be closely linked with expression of Sox-2 and other DPSC biomarkers, such as Oct-4.[32-34] The results of this study also support previous findings from this group regarding the association between DT (growth) and these biomarkers. [21,22,27] In addition, these data also confirm one of the only studies to demonstrate an association between microRNA expression (miR-218) and DPSC viability and differentiation potential.[35]

Although only limited evidence regarding DPSC and microRNAs may be available, a growing body of evidence regarding microRNA expression and stem cell differentiation may suggest that further research in this area is warranted.[36,37] For example, miR-124 is a key regulator of osteogenic, myogenic, and neuronal differentiation in MSC although this role has yet to be confirmed among DPSC.[38-40] miR-218 may also function in a similar capacity, as a key regulator of osteogenic differentiation in MSC.[41]

The role of miR-27 in the differentiation of other MSC may be the most thoroughly examined of these microRNAs.[24,25] For example, miR-27 has been shown to promote osteoblast differentiation by directly modulating transcription of several key components of the beta-catenin/Wnt pathway.[42] miR-27 may also function to promote myeloblast differentiation through direct and indirect modulation of Runx1 transcription.[43,44] However, miR-27 has also been shown to inhibit adipose differentiation and mitochondrial function through multiple pathways in other types of adipose-derived MSC.[45-47]

CONCLUSIONS

Although this study was limited to a small number of DPSC isolates, these results suggest a more thorough investigation and evaluation of biomarkers and microRNA expression may be necessary to elucidate the associations and complex interconnections with DPSC viability, proliferation, differentiation, and pluripotency.

Declarations

This project was reviewed and approved by the IRB and OPRS under protocol OPRS#763012-1 "Retrospective analysis of DPSC from the University of Nevada, Las Vegas – School of Dental Medicine pediatric and adult clinical population".

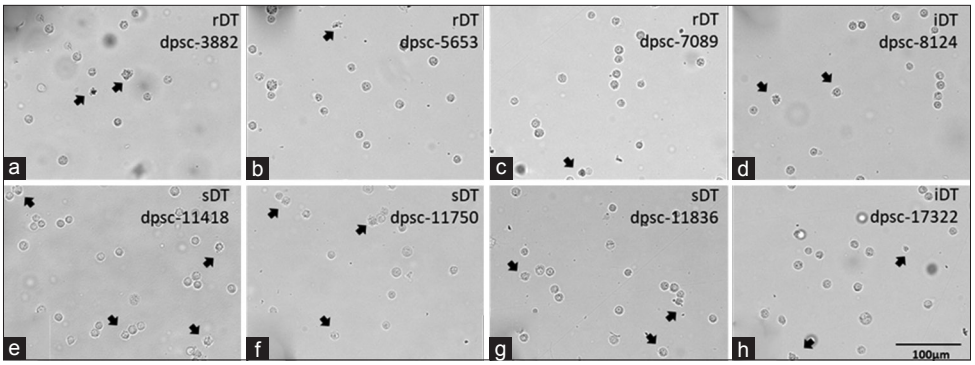

Figure 1: X DIC digital photomicrographs of freshly dispersed and cultured dental pulp stem cells (DPSCs). (a-c) rapidly dividing rapid DT (rDT) DPSC, (e-g) slowly dividing (slow DT) DPSC, (d-h) intermediately dividing DPSC. Arrows showing intense cellular blebbing (apoptosis), scale bar = 100 µm

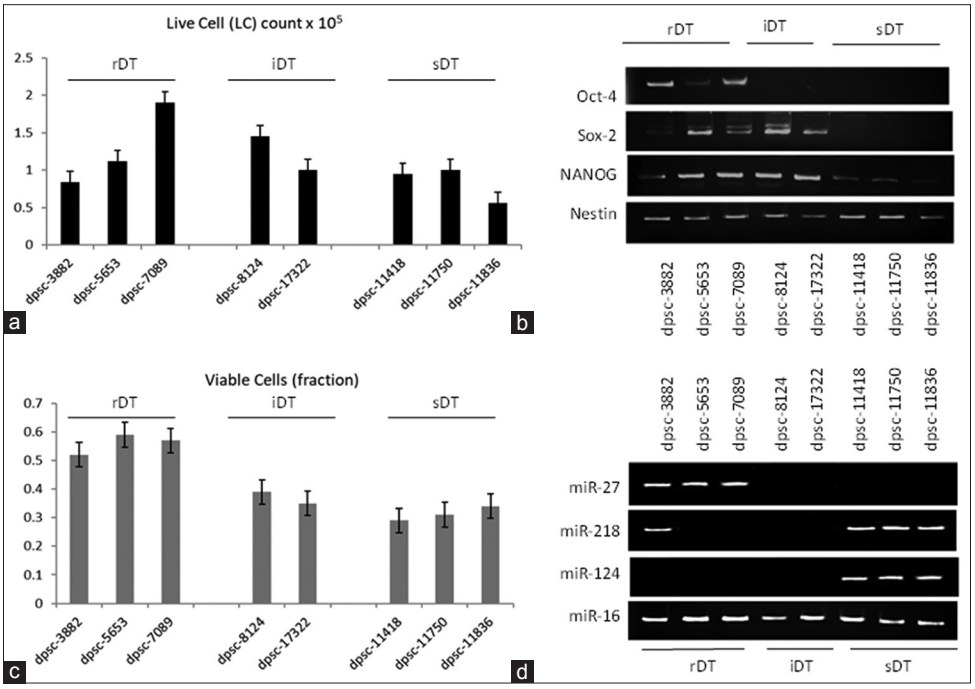

Figure 2: Live and viable cell counts correlate with dental pulp stem cells (DPSC) biomarkers and microRNA expression. (a) Live cell counts from DPSC isolates demonstrated the highest levels among rapid DT (rDT) and iDT isolates. (b) mRNA expression of DPSC biomarkers revealed Oct-4, Sox-2, and NANOG among rDT isolates (variable) and only Nestin expression among sDT isolates. (c) Percentage of viable cells revealed higher percentages among rDT isolates compared with either iDT or sDT. (d) MicroRNA expression of miR-27 was observed among rDT only, while miR-124 and miR-218 were observed mainly among the sDT isolates

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AUTHORS' CONTRIBUTIONS

KK and MW were responsible for experimental protocols, including RNA isolation and PCR screening, as well as project design and funding. WJH was responsible for photo microscopy and cell imaging. All authors participated in data analysis and manuscript preparation.

REFERENCES

1. Lima RL, Holanda-Afonso RC, Moura-Neto V, Bolognese AM, DosSantos MF, Souza MM, *et al.* Human dental follicle cells express embryonic, mesenchymal and neural stem cells markers. Arch Oral Biol 2017;73:121-8.
2. Shetty H, Kakade A, Shetty S, Neelakantan P, Nagar S, Desai RS, *et al.* Immunohistochemical characterization of stem cell and differentiation markers of the dental pulp of human

natal teeth. Future Sci OA 2018;4:FSO342.
3. Liu L, Wei X, Ling J, Wu L, Xiao Y. Expression pattern of oct-4, sox2, and c-myc in the primary culture of human dental pulp derived cells. J Endod 2011;37:466-72.
4. Kaukua N, Chen M, Guarnieri P, Dahl M, Lim ML, Yucel-Lindberg T, *et al.* Molecular differences between stromal cell populations from deciduous and permanent human teeth. Stem Cell Res Ther 2015;6:59.
5. Liu L, Wu L, Wei X, Ling J. Induced overexpression of oct4A in human dental pulp cells enhances pluripotency and multilineage differentiation capability. Stem Cells Dev 2015;24:962-72.
6. Yuan J, Liu X, Chen Y, Zhao Y, Liu P, Zhao L, *et al.* Effect of SOX2 on osteogenic differentiation of dental pulp stem cells. Cell Mol Biol (Noisy-le-grand) 2017;63:41-4.
7. Huang CE, Hu FW, Yu CH, Tsai LL, Lee TH, Chou MY, *et al.* Concurrent expression of oct4 and nanog maintains mesenchymal stem-like property of human dental pulp cells. Int J Mol Sci 2014;15:18623-39.
8. Krstic J, Trivanovic D, Obradovic H, Kukolj T, Bugarski D, Santibanez JF, *et al.* Regulation of mesenchymal stem cell differentiation by transforming growth factor beta superfamily. Curr Protein Pept Sci 2018;19:1138-54.
9. Huang C, Gou S, Wang L, Huang K, Liu L, Zhao W, *et al.* MicroRNAs and peroxisome proliferator-activated receptors governing the differentiation of mesenchymal stem cells. Curr Stem Cell Res Ther 2016;11:197-207.
10. Martin EC, Qureshi AT, Dasa V, Freitas MA, Gimble JM, Davis TA, *et al.* MicroRNA regulation of stem cell differentiation and diseases of the bone and adipose tissue: Perspectives on miRNA biogenesis and cellular transcriptome. Biochimie 2016;124:98-111.
11. Sekar D, Saravanan S, Karikalan K, Thirugnanasambantham K, Lalitha P, Islam VI, *et al.* Role of microRNA 21 in mesenchymal stem cell (MSC) differentiation: A powerful biomarker in MSCs derived cells. Curr Pharm Biotechnol 2015;16:43-8.
12. Clark EA, Kalomoiris S, Nolta JA, Fierro FA. Concise review: MicroRNA function in multipotent mesenchymal stromal cells. Stem Cells 2014;32:1074-82.
13. Collino F, Bruno S, Deregibus MC, Tetta C, Camussi G. MicroRNAs and mesenchymal stem cells. Vitam Horm 2011;87:291-320.
14. Sehic A, Tulek A, Khuu C, Nirvani M, Sand LP, Utheim TP, *et al.* Regulatory roles of microRNAs in human dental tissues. Gene 2017;596:9-18.
15. Zhang R, Li T. Modulation of microRNAs in tooth root and periodontal tissue development. Curr Stem Cell Res Ther 2018;13:118-24.
16. Jin Y, Wang C, Cheng S, Zhao Z, Li J. MicroRNA control of tooth formation and eruption. Arch Oral Biol 2017;73:302-10.
17. Aurrekoetxea M, Garcia-Gallastegui P, Irastorza I, Luzuriaga J, Uribe-Etxebarria V, Unda F, *et al.* Dental pulp stem cells as a multifaceted tool for bioengineering and the regeneration of craniomaxillofacial tissues. Front Physiol 2015;6:289.
18. Daltoé FP, Mendonça PP, Mantesso A, Deboni MC. Can SHED or DPSCs be used to repair/regenerate non-dental tissues? A systematic review of *in vivo* studies. Braz Oral Res 2014;28:S1806-83242014000100401.
19. Kim BC, Bae H, Kwon IK, Lee EJ, Park JH, Khademhosseini A, *et al.* Osteoblastic/cementoblastic and neural differentiation of dental stem cells and their applications to tissue engineering and regenerative medicine. Tissue Eng Part B Rev 2012;18:235-44.
20. Seong JM, Kim BC, Park JH, Kwon IK, Mantalaris A, Hwang YS, *et al.* Stem cells in bone tissue engineering. Biomed Mater 2010;5:62001.
21. Young A, Kingsley K. Dental pulp stem cells: A review of factors that influence the therapeutic potential of stem cell isolates. Biomater Biomed Eng 2015;2:61-9.
22. Tomlin A, Nelson B, Kingsley K. Dental pulp stem cell biomarkers for cellular viability following long-term cryopreservation. Int J Cell Sys Dev Biol 2018;1:1-6.
23. Whiting M, Kingsley K. Expression of microRNA among dental pulp stem cell (DPSC) isolates. Curr Res Dent 2019;9:12-8.
24. Ma Y, Yao N, Liu G, Dong L, Liu Y, Zhang M, *et al.* Functional screen reveals essential roles of miR-27a/24 in differentiation of embryonic stem cells. EMBO J 2015;34:361-78.
25. Xue Y, Qian H, Hu J, Zhou B, Zhou Y, Hu X, *et al.* Sequential regulatory loops as key gatekeepers for neuronal reprogramming in human cells. Nat Neurosci 2016;19:807-15.
26. Chang K, Chen RS, Chang FH, Chen MH. Promoting dentinogenesis of DPSCs through inhibiting microRNA-218 by using magnetic nanocarrier delivery. J Formos Med Assoc 2018. DOI: 10.1016/j.jfma.2018.10.018.
27. Alleman M, Low E, Truong K, Huang E, Hill CK, Chen TY, *et al*. Dental pulp stem cells (DPSC) differentiation *in vitro* into odontoblast and neural progenitors during cell passaging is associated with alterations in cell survival and viability. Int J Med Biomed Res 2013;2:133-41.
28. 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 Dent Stud Res Innov 2013;1:14-21.
29. Karamzadeh R, Eslaminejad MB, Aflatoonian R. Isolation, characterization and comparative differentiation of human dental pulp stem cells derived from permanent teeth by using two different methods. J Vis Exp 2012;69:4372.
30. Bakkar M, Liu Y, Fang D, Stegen C, Su X, Ramamoorthi M, *et al.* A simplified and systematic method to isolate, culture, and characterize multiple types of human dental stem cells from a single tooth. Methods Mol Biol 2017;1553:191-207.
31. Cinelli J, Ngueyn E, Kingsley K. Assessment of dental pulp stem cell (DPSC) biomarkers following induction with bone morphogenic protein (BMP-2). J Adv Biol Biotechnol 2018;19:1-12.
32. Xie H, Dubey N, Shim W, Ramachandra CJ, Min KS, Cao T, *et al.* Functional odontoblastic-like cells derived from human iPSCs. J Dent Res 2018;97:77-83.
33. Yang Y, Zhao Y, Liu X, Chen Y, Liu P, Zhao L, *et al.* Effect of SOX2 on odontoblast differentiation of dental pulp stem cells. Mol Med Rep 2017;16:9659-63.
34. Liu P, Cai J, Dong D, Chen Y, Liu X, Wang Y, *et al.* Effects of SOX2 on proliferation, migration and adhesion of human dental pulp stem cells. PLoS One 2015;10:e0141346.
35. Gay I, Cavender A, Peto D, Sun Z, Speer A, Cao H, *et al.* Differentiation of human dental stem cells reveals a role for microRNA-218. J Periodontal Res 2014;49:110-20.
36. Gu H, Xu J, Huang Z, Wu L, Zhou K, Zhang Y, *et al.* Identification and differential expression of microRNAs in 1, 25-dihydroxyvitamin D3-induced osteogenic differentiation of human adipose-derived mesenchymal stem cells. Am J Transl

Res 2017;9:4856-71.
37. Hao Y, Ge Y, Li J, Hu Y, Wu B, Fang F, *et al.* Identification of microRNAs by microarray analysis and prediction of target genes involved in osteogenic differentiation of human periodontal ligament stem cells. J Periodontol 2017;88:1105-13.
38. Qadir AS, Um S, Lee H, Baek K, Seo BM, Lee G, *et al.* MiR-124 negatively regulates osteogenic differentiation and *in vivo* bone formation of mesenchymal stem cells. J Cell Biochem 2015;116:730-42.
39. Qadir AS, Woo KM, Ryoo HM, Yi T, Song SU, Baek JH, *et al.* MiR-124 inhibits myogenic differentiation of mesenchymal stem cells via targeting dlx5. J Cell Biochem 2014;115:1572-81.
40. Mondanizadeh M, Arefian E, Mosayebi G, Saidijam M, Khansarinejad B, Hashemi SM, *et al.* MicroRNA-124 regulates neuronal differentiation of mesenchymal stem cells by targeting sp1 mRNA. J Cell Biochem 2015;116:943-53.
41. Xu JF, Yang GH, Pan XH, Zhang SJ, Zhao C, Qiu BS, *et al.* Altered microRNA expression profile in exosomes during osteogenic differentiation of human bone marrow-derived mesenchymal stem cells. PLoS One 2014;9:e114627.
42. Wang T, Xu Z. MiR-27 promotes osteoblast differentiation by modulating wnt signaling. Biochem Biophys Res Commun 2010;402:186-9.
43. Feng J, Iwama A, Satake M, Kohu K. MicroRNA-27 enhances differentiation of myeloblasts into granulocytes by post-transcriptionally downregulating runx1. Br J Haematol 2009;145:412-23.
44. Lozano-Velasco E, Contreras A, Crist C, Hernández-Torres F, Franco D, Aránega AE, *et al.* Pitx2c modulates pax3+/Pax7+ cell populations and regulates pax3 expression by repressing miR27 expression during myogenesis. Dev Biol 2011;357:165-78.
45. Chen KD, Goto S, Hsu LW, Lin TY, Nakano T, Lai CY, *et al.* Identification of miR-27b as a novel signature from the mRNA profiles of adipose-derived mesenchymal stem cells involved in the tolerogenic response. PLoS One 2013;8:e60492.
46. Kang T, Lu W, Xu W, Anderson L, Bacanamwo M, Thompson W, *et al.* MicroRNA-27 (miR-27) targets prohibitin and impairs adipocyte differentiation and mitochondrial function in human adipose-derived stem cells. J Biol Chem 2013;288:34394-402.
47. Chen SZ, Xu X, Ning LF, Jiang WY, Xing C, Tang QQ, *et al.* MiR-27 impairs the adipogenic lineage commitment via targeting lysyl oxidase. Obesity (Silver Spring) 2015;23:2445-53.

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