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Research Article

Folic Acid-Modulated Growth of Dental Pulp Stem Cells (DPSCs)

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Folates are associated with a wide variety of human health benefits, including reduced risk for stroke, heart failure, and coronary heart disease as well as chemo-preventive and anti-tumor actions of folates. Recent evidence has suggested folic acid (FA) may facilitate growth and protect cellular viability in mesenchymal stem cells, although no studies have yet examined this potential in dental pulp-derived stem cells (DPSCs). Using multiple DPSC isolates, FA was administered at supraphysiologic levels corresponding with bioavailable concentration following FA supplementation [0-400 ug/mL]. Cellular growth and proliferation, as well as viability were assessed. In addition, key enzymes involved in FA metabolism were also assessed using RT-PCR. These data reveal that FA administration is sufficient to induce significant increases in cellular proliferation at both low and high FA concentrations [100, 400 ug/mL]. In addition, dose-dependent increases in cellular viability were also observed. These changes correlated with an increase in mRNA expression of dihydrofolate reductase (DHFR) and thymidylate synthetase (TS), which are responsible for distinct intracellular pathways of folate metabolism. These observations may provide novel information regarding the ability of FA to stimulate growth and increase viability in DPSC, which may be critical for future studies of regeneration and bioengineering for clinical and translational therapies. These data may provide novel information to facilitate positive homeostatic microenvironments useful to develop and differentiate DPSCs for therapeutic and clinical treatments.

Keywords: Dental Pulp Stem Cells (DPSC), Folic Acid (FA), growth, viability

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Introduction

Folates are associated with a wide variety of human health benefits, including improved insulin resistance and glycemic control, improved cognitive functions and reduced symptoms associated with Alzheimer-related dementia, as well prevention of neural tube defects in pregnancy [1-4]. Other studies have focused on the cardiovascular disease prevention aspects of folate intake and dietary supplementation, demonstrating reduced risk for stroke, heart failure, and coronary heart disease [4,5].

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Moreover, evidence continues to accumulate regarding the chemo-preventive and anti-tumor actions of folates, with substantial evidence supporting these effects to prevent and ameliorate colorectal cancers [6,7].

Despite the breadth and depth of research regarding the positive outcomes and benefits of folate intake and dietary folate supplementation, lack of dietary folate continues to be a risk factor for many health problems, including congenital defects, developmental disorders, and many human cancers [8-12]. Although much of these efforts have focused on colorectal cancer, risk for many other cancers including breast cancers and leukemia may also be linked to folate deficiency [13-15]. In fact, some evidence now suggests that folate deficiency may affect the specific response of cancer stem cells thereby increasing the risk of chemotherapeutic resistance and metastatic potential [16-18].

These observations may provide support for observations of mesenchymal stem cells, which may also respond differentially to folate supplementation and deprivation [19-23]. Although these studies have evaluated the potential effects of folic acid (FA) to facilitate growth and protect cellular viability in mesenchymal stem cells, to date no studies have examined this potential in dental pulp-derived stem cells (DPSCs). Based upon the paucity of evidence, the goal of this study was to assess the effects of FA supplementation on human DPSCs.

Materials and Methods

Human subjects

Dental pulp stem cells were obtained from patients at the University of Nevada, Las Vegas – School of Dental Medicine (UNLV-SDM) dental clinic. This study was reviewed and approved by the Institutional Review Board (IRB) in the Office for the Protection of Research Subjects (OPRS). Approval for the original study "Isolated of Non-Embryonic Stem Cell s from Dental Pulp" was obtained in February 2010 (OPRS 0907-3148).

In brief, subjects were recruited from clinic patients of record at UNLV-SDM that were previously scheduled for tooth extraction of one or more healthy, vital intact teeth – mainly for Orthodontic treatment. Prior to participation, all potential subjects provided Informed Consent. Any patients that were found to have injured or compromised (fractured, diseased) teeth and those patients that refused to participate were excluded.

Cell culture and DPSC identification

Following extraction, each tooth was sectioned to expose the dental pulp that was subsequently extracted and deposited into a sterile microcentrifuge tube containing sterile saline solution for transfer to the laboratory for culture using the direct outgrowth method, as previously described [24-26]. Subsequent culture was facilitated using RPMI-1640 culture media with 2 mM L-Glutamine containing 4.5 g/L glucose, 1.5 g/L sodium bicarbonate, 10 mM HEPES, 1.0 mM sodium Pyruvate, and 1% Penicillin-Streptomycin (10,000 unit/mL), as previously described [24-29]. Analysis of the resulting DPSC isolates after 10 passages demonstrated each patient isolate exhibited a distinct proliferation rate or doubling time (DT), which were characterized as rapid or rDT (less than three days), slow or sDT (greater than one week, usually 8 – 10 days), and a few with intermediate or iDT [25,29,30]. Most of the isolates exhibited rDT (n=25), while fewer exhibited iDT (n=2) or sDT (n=3) [29,30]. Cultures with greater than 1.0 x 10^6 cells/mL were considered confluent and were

passaged or split 1:2. All DPSC isolates were screened for the expression of Sox-2, NANOG, and Oct4 – biomarkers for DPSC and MSC [24,25,27-30].

Cell proliferation and viability

Cells were assessed for viability using the Trypan Blue method, which involves mixing an aliquot of non-adherent DPSC for analysis in a BioRad TC20 automated cell counter and hemocytometer using the protocol recommended by the manufacturer, which provides information regarding cell number, relative abundance (confluence) and percent viability [31,32]. Cultures with greater than 1.0 x 10^6 cells/mL were considered confluent and were passaged or split 1:2. All cultures utilized for each DPSC isolate were P10 or greater.

Folic acid

Folic acid (folate) molecular weight (MW) 441.40 was obtained from Fisher Scientific (Fair Lawn, NJ) and suspended in the appropriate cell culture complete media at concentrations between 100 – 400 ug/mL that approximated the supraphysiologic range 0.23 mM to 0.91 mM, which is observed under conditions of folate supplementation [33,34]. Cells were plated in 96-well assay plates at 1.2 x 10^5 cells/mL with eight (n=8) replicates for each condition. Three (n=3) separate, independent experiments were performed for each isolate, folate concentration and experimental condition, yielding a total of n=24 data points (8 replicates x 3 experiments) for each experimental condition. Cell number, relative confluence and percent viability were assessed to determine any changes to proliferation rate (doubling time, DT) and viability.

100 ug/mL = 100 mg/L = 0.1 g FA/441.4 MW = 0.0002265 or 0.23 mM

400 ug/mL = 400 mg/L = 0.4 g FA/441.4 MW = 0.0009062 or 0.91 mM

Statistics

Basic descriptive statistics were provided for cellular viability and proliferation rate (doubling time) and were compiled. Changes in cellular viability were measured using two-tailed Student’s t-tests with significance set at alpha level of α=0.05, which provide robust analysis for samples with even moderate samples sizes (n~20) [35,36].

RNA isolation and PCR

RNA was extracted from negative controls and experimental cells at each folic acid concentration (100, 400 ug/mL) using the ABgene total RNA isolation reagent (Surrey, UK) using the protocol recommended by the manufacturer, as previously described [24,25,27,28,33]. The quality (purity) and quantity of RNA was determined using spectrophotometric analysis at 260 and 280 nm. Ratio of the absorbance readings (A260, A280) will provide estimated purity, which should be approximately 1.70 or higher. RNA concentration can then be determined using the formula:

A260 (absorbance) x 40 (RNA standard) x dilution factor (50) = RNA yield per mL

RT-PCR was then used to screen the RNA from negative control and experimental cells to determine if any changes to mRNA expression were induced by the addition of folic acid. Equal amounts of RNA (1 ng) from each isolate used to screen for the positive control internal cell standard, which was glyceraldehyde-phosphate dehydrogenase or GAPDH (glycolytic enzyme). Each sample was then subsequently screened using primers specific for dihydrofolate reductase (DHFR), which is required to

convert folate for the *de novo* synthesis of purines and thymidylate synthetase (TS) – the sole *de novo* pathway to produce dTMP in DPSC [34,37,38].

GAPDH forward primer, GGTCGGAGTCAACGGATT; 18 nt, 56% GC, Tm = 64C
GAPDH reverse primer, ATCGCCCCACTTGATTTTG; 19 nt, 47% GC, Tm = 63C
Annealing temperature = 64C
Optimal Tm (GAPDH-F: GAPDH-R) Lower Tm – 5C = 58C

β-actin forward primer, AGGGCAGTGATCTCCTTCTGCATCCT; 26 nt, 54% GC, Tm = 74C
β-actin reverse primer, CCACACTGTGCCCATCTACGAGGGGT; 26 nt, 62% GC, Tm = 77C
Annealing temperature = 72C
Optimal Tm (β-actin -F: β-actin -R) Lower Tm – 5C = 69C

DHFR forward primer, GGCGGCAAGCTTATGGTTGGTTCGCTAAACTGC; 33 nt, 55% GC, Tm = 78C
DHFR reverse primer, GCCGCCGGATCCATCATTCTTCTCATACTTC; 31 nt, 52% GC, Tm = 74C
Annealing temperature = 72C
Optimal Tm (DHFR-F: DHFR-R) Lower Tm – 5C = 69C

TS forward primer, GCCTCGGTGTGCCTTTCA; 18 nt, 61% GC, Tm = 69C
TS reverse primer, CCCGTGATGTGCGCAAT; 17 nt, 59% GC, Tm = 66C
Annealing temperature = 67C
Optimal Tm (TS-F: TS-R) Lower Tm – 5C = 61C

Results

Four DPSC isolates were selected for inclusion in this initial pilot study (Table 1). Due to the higher prevalence of DPSC isolates with rDT (n=25/30 or 83.3%), two of the isolates chosen for inclusion exhibited rDT of approximately two days (dpsc-17322, dpsc-11836). Due to the lower prevalence of DPSC isolates with slower doubling times, the remaining two isolates chosen for inclusion each exhibited iDT of approximately six days (dpsc-5653) and sDT of nearly nine days (dpsc-7089). Baseline viability following cryopreservation, thawing and cell culture varied between 58% and 63%.

Table 1. Dental Pulp Stem Cell isolates

	DT (doubling time)	**Viability (baseline)**
dpsc-17322	2.2 days rapid or rDT	63% +/- 1.7% SD
dpsc-11836	2.1 days rapid or rDT	61% +/- 1.9% SD
dpsc-5653	5.9 days intermediate or iDT	58% +/- 2.1% SD
dpsc-7089	8.7 days slow or sDT	59% +/- 2.0% SD

To evaluate the effects of FA the four DPSC isolates were plated in 96-well plates with or without the addition of FA at low [100 ug/mL] and high [400 ug/mL] concentrations and proliferation was observed over four days (Figure 1). The addition of FA at both low and high concentrations was sufficient to induce changes to

proliferation in all four DPSC isolates. More specifically, the low concentration of FA increased growth in the rDT DPSC isolates dpsc-11836 (Fig. 1A) and dpsc17322 (Fig. 1B) between 1.56- and 1.70-fold, which was statistically significant, $p<0.05$. This same concentration also increased growth in the iDT and sDT DPSC isolates dpsc-5653 (Fig. 1C) and dpsc-7089 (Fig. 1D) between 1.40- and 1.61-fold, which was also statistically significant, $p<0.05$.

The additional of FA at the higher concentration (400 ug/mL) increased growth in the rDT DPSC isolates dpsc-11836 (Fig. 1A) and dpsc17322 (Fig. 1B) between 2.61- and 2.63-fold, which was statistically significant, $p<0.01$. This same concentration also increased growth in the iDT and sDT DPSC isolates dpsc-5653 (Fig. 1C) and dpsc-7089 (Fig. 1D) between 2.81- and 2.23-fold, which was also statistically significant, $p<0.01$.

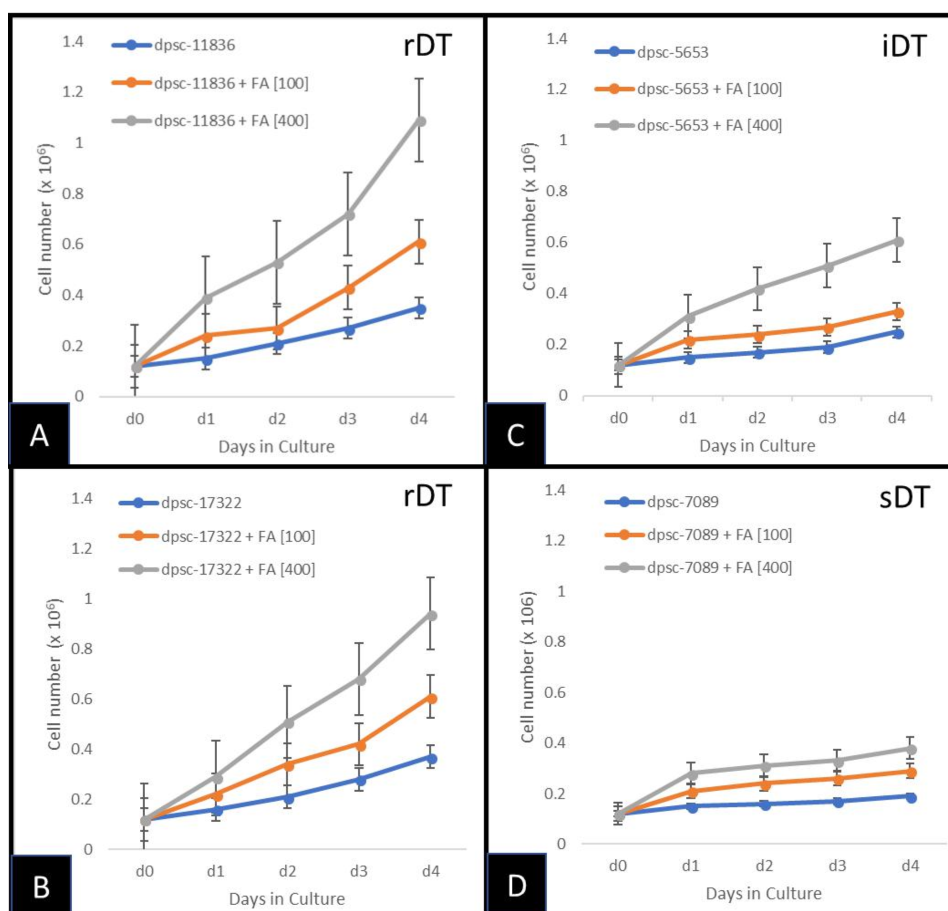

Figure 1. FA-mediated changes in proliferation of DPSC isolates. A) Low (100 ug/mL) and high (400 ug/mL) concentrations of FA induced significant increases in rDT dpsc-11836, 1.56- and 2.61-fold. B) Low and high FA concentrations induced similar increases in rDT dpsc-17322, 1.70- and 2.63-fold. C) The addition of FA also stimulated iDT growth in dpsc-5653 by 1.40- and 2.81-fold. D) FA induced similar changes to sDT dpsc-7089, 1.61- and 2.23-fold. All concentrations evaluated induced statistically significant changes compared with negative controls, $p<0.05$.

To assess whether these changes to growth and proliferation observed in all DPSC isolates in response to FA administration also correlated with changes in cell survival, viability was also assessed at each time point and for each concentration (Figure 2). In brief, FA at the low concentration of 100 ug/mL induced changes in DPSC viability, increasing on average between 10% – 21%, which was statistically significant, $p<0.05$. In addition, FA at the higher concentration of 400 ug/mL induced greater increases in DPSC viability between 26% – 31%, which was also statistically significant, $p<0.01$.

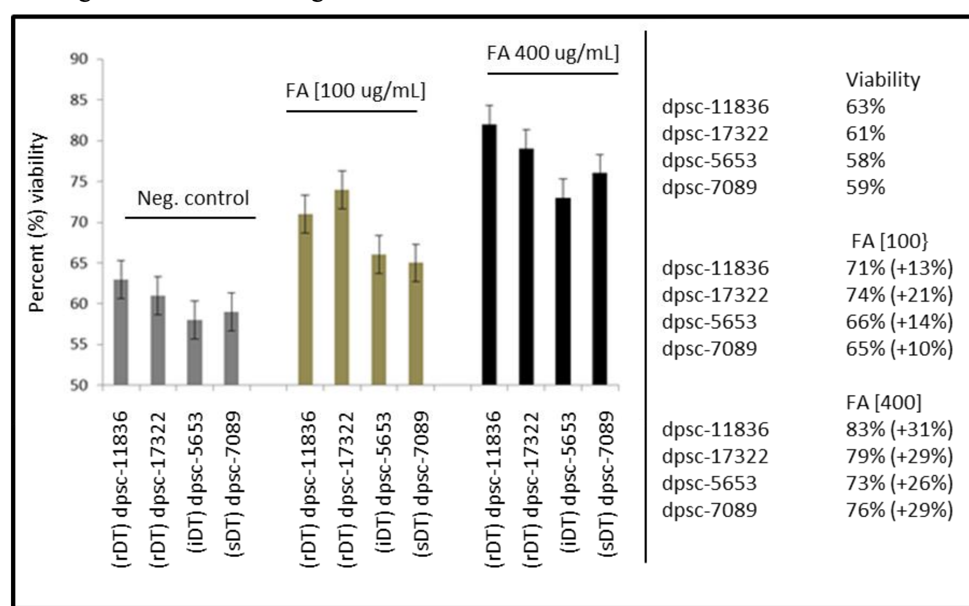

Figure 2. FA-induced changes to DPSC viability. Low concentrations of FA [100 ug/mL] increased DPSC viability between 10% – 21% compared with baseline and negative controls. High concentrations of FA [400 ug/mL] increased DPSC viability between 26% - 31% compared with baseline and negative controls. These changes were statistically significant, $p<0.05$.

To determine how FA may be inducing these changes in cellular growth and viability, the two main metabolism enzymes responsible for folate metabolism were assessed using RT-PCR, dihydrofolate reductase (DHFR) – which is required to covert folate for the de novo synthesis of purines, and thymidylate synthetase (TS) – the sole de novo pathway to produce dTMP in DPSC (Figure 3). In brief, these results demonstrated that DHFR is produced in all four DPSC isolates, but that mRNA expression of DHFR is strongly enhanced following FA supplementation at either low [100 ug/mL] or high [400 ug/mL] FA concentrations (Fig. 3A). Similar results were observed for TS mRNA, which is also produced in all four DPSC isolates, but is strongly enhanced following FA supplementation at both low and high FA concentrations (Fig. 3B). Internal controls for structural (actin) and enzymatic mRNA included for reference standards to normalize DHFR and TS expression (Fig. 3C).

Discussion

Although many studies have evaluated the potential effects of FA to facilitate growth and protect cellular viability in mesenchymal stem cells [19-23], to date no

studies had examined this potential in dental pulp-derived stem cells (DPSCs). Based upon the paucity of evidence, the goal of this study was to assess the effects of FA supplementation on human DPSCs. These results clearly demonstrated that FA is sufficient to induce significant increases in cellular growth and proliferation, which are also correlated with increases in cellular viability. Moreover, these increases appear to be, dose-dependent with higher concentrations of FA correlated with larger increases in growth and higher levels of viability.

In addition, this study also provides some of the first mechanistic evidence to demonstrate that FA administration is also inducing changes to enzymes directly responsible for the metabolism of FA within the cell. Without these data, it might have been possible to suggest that FA could induce these changes through interactions with other intracellular pathways. However, the observed changes in mRNA for separate pathways of FA metabolism clearly suggest that the increased availability of FA may be directly linked with increased bioavailability and metabolism, which in turn may be directly altering cellular growth and viability.

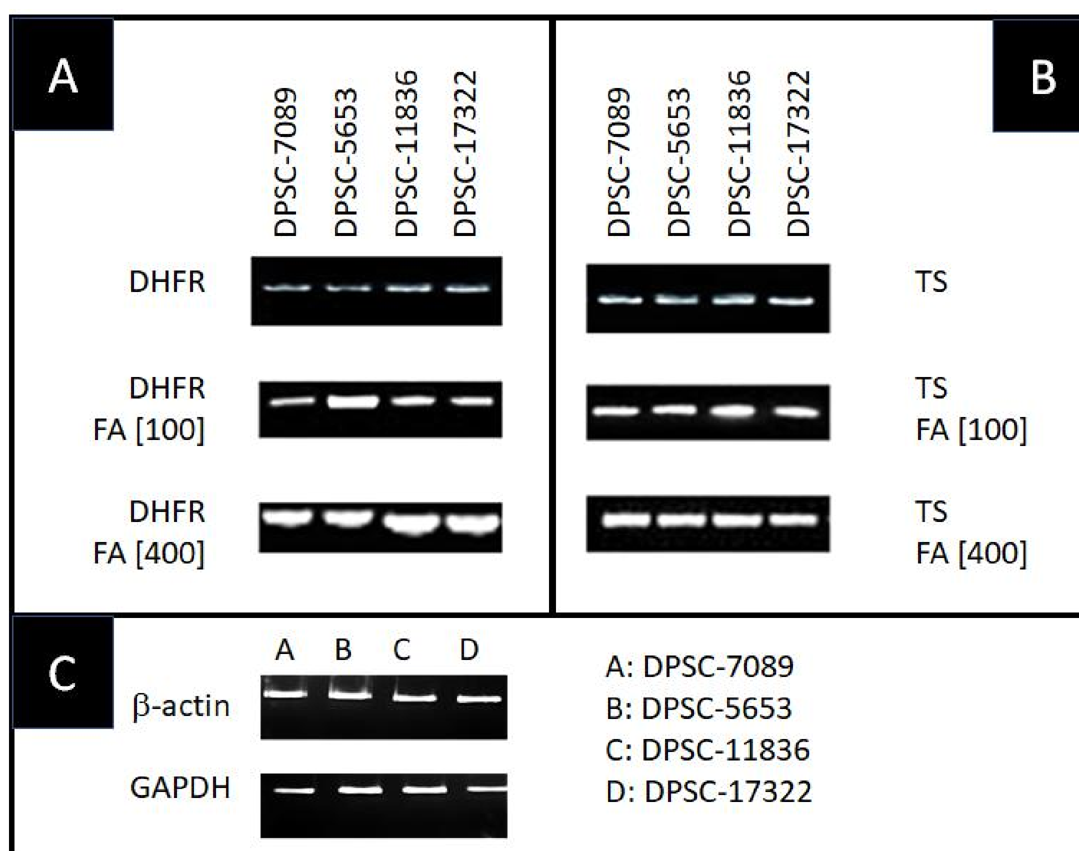

Figure 3. FA-induced changes to folate metabolism enzymes. A) FA administration induced significant increases in mRNA expression of dihydrofolate reductase (DHFR), with an average increase in signal band intensity of approximately 3.24-fold at 100 ug/mL and 7.68-fold at 400 ug/mL. B) FA administration also induced significant increases in mRNA expression of thymidylate synthetase (TS), with an average increase in signal band intensity of 1.76-fold at 100 ug/mL and 2.71-fold at 400 ug/mL.

Despite these observations, there are some limitations of this study which should be considered. First, this is an *in vitro* study of previously collected DPSC isolates [24,25]. These same observations may not be applicable *in vivo* and therefore may not represent a true representation of how DPSC may react to FA supplementation in the body. Furthermore, this study sample size was limited due to funding considerations – which suggests that more robust analyses of larger numbers of DPSC isolates will be needed in order to confirm and corroborate these findings.

Conclusions

New research has suggested DPSC may be used to repair or replace injured and aging tissues, such as nerves and bone – in addition to dental tissues and structures [39-42]. Any method proven to stimulate growth or increase viability may be critical for future studies regarding the regeneration and bioengineering of DPSC for clinical and translational therapies. These data may provide novel information to facilitate positive homeostatic microenvironments to develop and differentiate DPSCs for therapeutic and clinical treatments.

Competing interests

The authors declare that they have no competing interests.

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References

1. Zhao JV, Schooling CM, Zhao JX. The effects of folate supplementation on glucose metabolism and risk of type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials. Ann Epidemiol. 2018;28(4):249-257.e1.

2. Butler M, Nelson VA, Davila H, Ratner E, Fink HA, Hemmy LS, McCarten JR, Barclay TR, Brasure M, Kane RL. Over-the-Counter Supplement Interventions to Prevent Cognitive Decline, Mild Cognitive Impairment, and Clinical Alzheimer-Type Dementia: A Systematic Review. Ann Intern Med. 2018;168(1):52-62.

3. US Preventive Services Task Force, Bibbins-Domingo K, Grossman DC, Curry SJ, Davidson KW, Epling JW Jr, García FA, Kemper AR, Krist AH, Kurth AE, Landefeld CS, Mangione CM, Phillips WR, Phipps MG, Pignone MP, Silverstein M, Tseng CW. Folic Acid Supplementation for the Prevention of Neural Tube Defects: US Preventive Services Task Force Recommendation Statement. JAMA. 2017 ;317(2):183-189.

4. Viswanathan M, Treiman KA, Kish-Doto J, Middleton JC, Coker-Schwimmer EJ, Nicholson WK. Folic Acid Supplementation for the Prevention of Neural Tube Defects: An Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2017;317(2):190-203.

4. Colin-Ramirez E, Arcand J, Ezekowitz JA. Dietary Self-management in Heart Failure: High Tech or High Touch? Curr Treat Options Cardiovasc Med. 2017;19(3):19.

5. Li Y, Huang T, Zheng Y, Muka T, Troup J, Hu FB. Folic Acid Supplementation and the Risk of Cardiovascular Diseases: A Meta-Analysis of Randomized Controlled Trials. J Am Heart ssoc.

2016;5(8). pii: e003768.

6. Danenberg PV, Gustavsson B, Johnston P, Lindberg P, Moser R, Odin E, Peters GJ, Petrelli N. Folates as adjuvants to anticancer agents: Chemical rationale and mechanism of action. Crit Rev Oncol Hematol. 2016;106:118-31.

7. Dulai PS, Singh S, Marquez E, Khera R, Prokop LJ, Limburg PJ, Gupta S, Murad MH. Chemoprevention of colorectal cancer in individuals with previous colorectal neoplasia: systematic review and network meta-analysis. BMJ. 2016;355:i6188.

8. Harris BS, Bishop KC, Kemeny HR, Walker JS, Rhee E, Kuller JA. Risk Factors for Birth Defects. Obstet Gynecol Surv. 2017;72(2):123-135.

9. Li K, Wahlqvist ML, Li D. Nutrition, One-Carbon Metabolism and Neural Tube Defects: A Review. Nutrients. 2016;8(11). pii: E741.

10. Pearl PL. Amenable Treatable Severe Pediatric Epilepsies. Semin Pediatr Neurol. 2016;23(2):158-66.

11. Desai A, Sequeira JM, Quadros EV. The metabolic basis for developmental disorders due to defective folate transport. Biochimie. 2016;126:31-42.

12. Obeid R, Oexle K, Rißmann A, Pietrzik K, Koletzko B. Folate status and health: challenges and opportunities. J Perinat Med. 2016;44(3):261-8.

13. Choi JH, Yates Z, Veysey M, Heo YR, Lucock M. Contemporary issues surrounding folic Acid fortification initiatives. Prev Nutr Food Sci. 2014;19(4):247-60.

14. Cantarella CD, Ragusa D, Giammanco M, Tosi S. Folate deficiency as predisposing factor for childhood leukaemia: a review of the literature. Genes Nutr. 2017;12:14.

15. Kotsopoulos J, Kim YI, Narod SA. Folate and breast cancer: what about high-risk women? Cancer Causes Control. 2012;23(9):1405-20.

16. Chen WJ, Huang RS. Low-folate stress reprograms cancer stem cell-like potentials and bioenergetics metabolism through activation of mTOR signaling pathway to promote in vitro invasion and in vivo tumorigenicity of lung cancers. J Nutr Biochem. 2018;53:28-38.

17. Feng HC, Lin JY, Hsu SH, Lan WY, Kuo CS, Tian YF, Sun DP, Huang RS. Low folate metabolic stress reprograms DNA methylation-activated sonic hedgehog signaling to mediate cancer stem cell-like signatures and invasive tumour stage-specific malignancy of human colorectal cancers. Int J Cancer. 2017;141(12):2537-2550.

18. Cipolleschi MG, Marzi I, Rovida E, Olivotto M, Dello Sbarba P. Low-dose methotrexate enhances cycling of highly anaplastic cancer cells. Cell Cycle. 2017;16(3):280-285.

19. Xiao WL, Wu M, Shi B. Folic acid rivals methylenetetrahydrofolate reductase (MTHFR) gene-silencing effect on MEPM cell proliferation and apoptosis. Mol Cell Biochem. 2006;292(1-2):145-54.

20. Katsuno T, Ozaki T, Saka Y, Furuhashi K, Kim H, Yasuda K, Yamamoto T, Sato W, Tsuboi N, Mizuno M, Ito Y, Imai E, Matsuo S, Maruyama S. Low serum cultured adipose tissue-derived stromal cells ameliorate acute kidney injury in rats. Cell Transplant. 2013;22(2):287-97.

21. Fan CW, Chen T, Shang YN, Gu YZ, Zhang SL, Lu R, OuYang SR, Zhou X, Li Y, Meng WT, Hu JK, Lu Y, Sun XF, Bu H, Zhou ZG, Mo XM. Cancer-initiating cells derived from human rectal adenocarcinoma tissues carry mesenchymal phenotypes and resist drug therapies. Cell Death Dis. 2013;4:e828.

22. Liang SQ, Marti TM, Dorn P, Froment L, Hall SR, Berezowska S, Kocher G, Schmid RA, Peng RW. Blocking the epithelial-to-mesenchymal transition pathway abrogates resistance to anti-folate chemotherapy in lung cancer. Cell Death Dis. 2015;6:e1824.

23. Su YH, Huang WC, Huang TH, Huang YJ, Sue YK, Huynh TT, Hsiao M, Liu TZ, Wu AT, Lin CM. Folate deficient tumor

microenvironment promotes epithelial-to-mesenchymal transition and cancer stem-like phenotypes. Oncotarget. 2016;7(22):33246-56.

24. 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.

25. 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.

26. Bakopoulou A, Leyhausen G, Volk J, Tsiftsoglou A, Garefis P, Koidis P, Geurtsen W. (2011) Assessment of the impact of two different isolation methods on the osteo/odontogenic differentiation potential of human dental stem cells derived from deciduous teeth. Calcif Tissue Int. 2010;88(2):130-41.

27. 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.

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.

29. Young A, Kingsley K. Dental Pulp Stem Cells: A review of factors that influence the therapeutic potential of stem cell isolates. Biomaterials and Biomedical Engineering. 2015;2(2): 61-69.

30. Tomlin A, Sanders MB, Kingsley K. The effects of cryopreservation on human dental pulp-derived mesenchymal stem cells. Biomaterials and Biomedical Engineering. 2016;3(2): 103-112.

31. Tiku V, Todd C, Kingsley K. Assessment of Oral Human Papillomavirus Prevalence in a Multi-ethnic Pediatric Clinic Population. Compendium of Continuing Education in Dentistry Nov/Dec 2016;37(11): Online only.

32. Row L, Repp MR, Kingsley K. Screening of a pediatric and adult clinic population for the dental caries pathogen Scardovia wiggsiae using saliva. Journal of Clinical Pediatric Dentistry 2016;40(6):438-444.

33. McCabe J, Chang S, Hajibandeh J, Tran MD, Meeder CA, Sharma K, Nguyen DH, Keiserman MA, Bergman CJ, Kingsley K. Folate supplementation induces differential dose-dependent modulation of proliferative phenotypes among cancerous and non-cancerous oral cell lines in vitro. Journal of Dietary Supplements. 2010;7(4):325-340.

34. Kingsley K. Potential effects of dietary folate supplementation on oral carcinogenesis, development and progression. Journal of Dietary Supplements. 2010;7(1):51-59.

35. Glaser AN. High-Yield Biostatistics, 3rd Ed. Lippincott Williams Wilkins. 2004.

36. Jekel, Katz, and Elmore. Epidemiology, Biostatistics and Preventive Medicine, 2nd Ed. 2001

37. Nazki FH, Sameer AS, Ganaie BA. Folate: metabolism, genes, polymorphisms and the associated diseases. Gene. 2014 Jan 1;533(1):11-20. Epub 2013 Oct 1. Review. PMID: 24091066

38. Sadaghiani L, Gleeson HB, Youde S, Waddington RJ, Lynch CD, Sloan AJ. Growth Factor Liberation and DPSC Response Following Dentine Conditioning. J Dent Res. 2016;95(11):1298-307.

39. Seong JM, Kim BC, Park JH, Kwon IK, Mantalaris A, Hwang YS. Stem cells in bone tissue engineering. Biomed Mater. 2010;5(6):062001.

40. 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. pii:

S1806-83242014000100401. Epub 2014 Aug 21. Review. PMID: 25166769

41. Aurrekoetxea M, Garcia-Gallastegui P, Irastorza I, Luzuriaga J, Uribe-Etxebarria V, Unda F, Ibarretxe G. Dental pulp stem cells as a multifaceted tool for bioengineering and the regeneration of craniomaxillofacial tissues. Front Physiol. 2015;6:289.

42. Mead B, Logan A, Berry M, Leadbeater W, Scheven BA. Concise Review: Dental Pulp Stem Cells: A Novel Cell Therapy for Retinal and Central Nervous System Repair. Stem Cells. 2017;35(1):61-67.

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