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Kamille Mercado
University of the Pacific

Nan (Tori) Xiao
University of the Pacific, nxiao@pacific.edu

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Comparison of Expression of the GDNF Family of Neurotrophic Factors in Dental Pulp Stem Cells Within Deciduous and Adult Teeth

Kamille Mercado, DDS, and Nan Xiao, M Ortho, PhD

ABSTRACT The glial cell line-derived neurotrophic factor (GDNF) family has a role in neuron growth within the peripheral and central nervous systems. This makes it a potential therapeutic agent for neurological conditions like Parkinson's disease and epilepsy. GDNF has also been implicated in murine odontogenesis. In permanent and deciduous human dental pulp stem cells, this study found that GDNF family members and receptors are expressed, indicating neurotrophic factors may play a role in dental pulp regeneration.

AUTHORS

Kamille Mercado, DDS, earned her bachelor of science in biology from the University of the Pacific in Stockton, Calif., and her Doctor of Dental Surgery in 2017 from the University of the Pacific, Arthur A. Dugoni School of Dentistry in San Francisco.
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Nan Xiao, M Ortho, PhD, is an assistant professor in the department of biomedical sciences at the University of the Pacific, Arthur A. Dugoni School of Dentistry in San Francisco.
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Neurotrophic factors were growth factors that can promote the survival and regeneration of the neurons. There are three major groups of neurotrophic factors: neurotrophins, including the nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and neurotrophins (NTs), the glial cell derived neurotrophic factors (GDNF) family and neuropoietic cytokines, such as ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF).¹⁻³ GDNF is a member of the glial cell derived neurotrophic factor ligands (GFL), which are also considered members of the transforming growth factor-beta (TGF-beta) family.⁴ GDNF is the well-studied member of the GFL family.

Besides GDNF, the GFL family also includes neurturin (NRTN), artemin (ARTN) and persephin (PSPN). GFLs bind to transmembrane receptors to activate the downstream signaling pathways. GDNF preferentially binds to the GDNF family receptor $\alpha 1$ (GFR $\alpha 1$), NRTN preferentially binds GFR $\alpha 2$, ARTN binds GFR $\alpha 3$ and PSPN binds GFR $\alpha 4$.^{4,5} GDNF is the most well-studied member of the GFL family. GFR $\alpha 1$ mediates the activation of the RET receptor tyrosine kinase and functions through the PI3K/AKT, MEK/ERK and SRC pathways.^{6,7} The pathways have been shown to regulate neuron survival, growth, differentiation and migration.⁵ GDNF was indicated to improve neural regeneration in neurodegenerative diseases, such as Parkinson's.⁸⁻¹⁰ It is also indicated to improve survival, proliferation

and migration of ureteric buds¹¹⁻¹³ and spermatogonial SCs.¹⁴ One recent study by Xiao et al. indicated that GDNF is also important in mediated salivary gland regeneration following radiation damage.¹⁵ More recently, it was reported that wnt signaling activation enhanced the expression of neural crest markers in human deciduous dental pulp cells.¹⁶ The neurotrophin receptor p75 was also found expressed in human dental pulp stem cells (DPSCs) and may denote a neurogenic subpopulation.¹⁷ These works suggest that neurotrophic factors can serve as potential therapeutic candidates in adult tissue regeneration in tissues other than neurons.

NGF, BDNF, GDNF, neurotrophin 3(NT3) and neurotrophin 4/5(NT4/5) mRNA were detected in the inner dental epithelium and dental follicle cells in developing human and rodent teeth.¹⁸⁻²¹ We hypothesized that neurotrophic factors are present in the human dental pulp stem cells and may promote dental pulp regeneration by regulating proliferation, migration and differentiation. In this study, we sought to compare the expression of the ligands of the GDNF family and their receptors in dental pulp stem cells found within deciduous (SHED) and adult (DPSC) teeth.

Materials and Methods

We cultured human DPSC cells and human SHED cells that were provided by collaborators at the University of Pennsylvania, School of Dental Medicine and the University of the Pacific, Arthur A. Dugoni School of Dentistry. DPSCs were cultured as previously reported.²² DPSCs were briefly cultured in Gibco Dulbecco's Modified Eagle medium (DMEM) (Thermo Fisher Scientific, Wilmington, Del.) supplemented with 10% FBS, antibiotics and ascorbic acids.

RNA was isolated from the stem cells using TRIzol Reagent (Life Technologies,

TABLE

List of Primer Pairs for PCR

Gene	5' primer sequence	3' primer sequence
β -actin	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTGGCGTACAG
Gdnf	TAGCTGCCGTTCTACCGACT	TCCCAATATGGCTTCCTCAG
Nrtn	TCCCATGTGATTATCGACCA	CACATCCAGATGGACAGCAC
Artn	AACTGACTAGCAGCCCCAGA	TGATGCCTCAGCCACTGTAG
Pspn	GTCTGAACAGGTGGCAAAGG	GCACTAGCGGAAGATGACCT
Gfr α 1	GAGATGAGTTGCTTGGCACA	GGCCACCAGTACTCGATCAT
Gfr α 2	GCTGAAACTGGCCTTGTAGG	AAACCTGGGAGGAGACAGGT
Gfr α 3	CCACACCCTACCTTTGCTGT	CTTCTGCTGCTGTCCTTTC
Gfr α 4	GAGCAGGTCAGAGACCATCC	CACAGGTGGCTACAATGGTG
Ret	GATCTCACAGGGGATGCAGT	CTGGCTCCTTTCACGTAGG
Etv4	TCATTGGGAAGGAAAAGTGG	GAGATCTGGGGAGCTCAGTG
Etv5	TTGTGCTTCTGCACCAGAC	TGACCAGGTTTCCAAGGAC

Camarillo, Calif.). Samples were homogenized in TRIzol. Chloroform was added to separate the RNA and isopropanol was used to precipitate the RNA. RNA quality was measured by 1000 spectrophotometer (NanoDrop, Thermo Fisher Scientific) in units of ng/ μ L. mRNA quality of SHED was 747.2 ng/ μ L and that of DPSC was 462.7 ng/ μ L. cDNA was transcribed from the purified RNA via reverse transcription. Reverse transcription was performed using random hexamer primers as indicated by the manufacturer (Applied Biosystems, Fisher Scientific, Pittsburgh, Pa.).

The cDNA samples were amplified by polymerase chain reaction (PCR) using a T100 thermal cycler (Bio-Rad, Hercules, Calif.) for 45 minutes. Agarose gel electrophoresis was carried out and the wells were loaded with cDNA in each PCR reaction. Primer sequences for the GDNF family ligands Gdnf, Nrtn, Artn, Pspn; the receptors Gfr α 1, Gfr α 2, Gfr α 3, Gfr α 4; co-receptor RET; and the downstream target transcription factors Etv4, Etv5 are listed in the **TABLE**. β -actin was used as an internal control. Agarose gel electrophoresis was carried out (100 V constant, ~40 minutes). Visualization of the samples was then done under UV light. Experiments were repeated three times.

Results

Using this semiquantitative analysis, the following genes were detected at respective base pair (bp) measurements for both DPSC and SHED tooth samples (**FIGURE**): actin at 150 bp, Gdnf at 250 bp, Nrtn at 200 bp, Artn at 175 bp, Pspn at 350 bp, Gfr α 1 at 175 bp, Gfr α 3 at 450 bp, Gfr α 4 at 150 bp and Etv5 at 175 bp. Gfr α 2 was present at 175 bp and RET at 800 bp for the adult tooth sample, but not for the deciduous sample. Neither the SHED nor DPSC sample showed a band for Etv4.

Discussion

The PCR results confirmed our hypothesis that GDNF family ligands and receptors are expressed in both adult and deciduous dental pulp stem cells. The four ligands of the GDNF family, Gdnf, Nrtn, Artn and Pspn, were expressed equally in both the deciduous and the adult teeth. Their receptors, Gfr α 1 and Gfr α 3, were also expressed equally in both samples. Receptors Gfr α 2 and Gfr α 4 and co-receptor RET seem to be more highly expressed in DPSC than in SHED. On the other hand, Etv5 is more highly expressed in SHED than in DPSC. This difference in expression could indicate a change in regulation of these

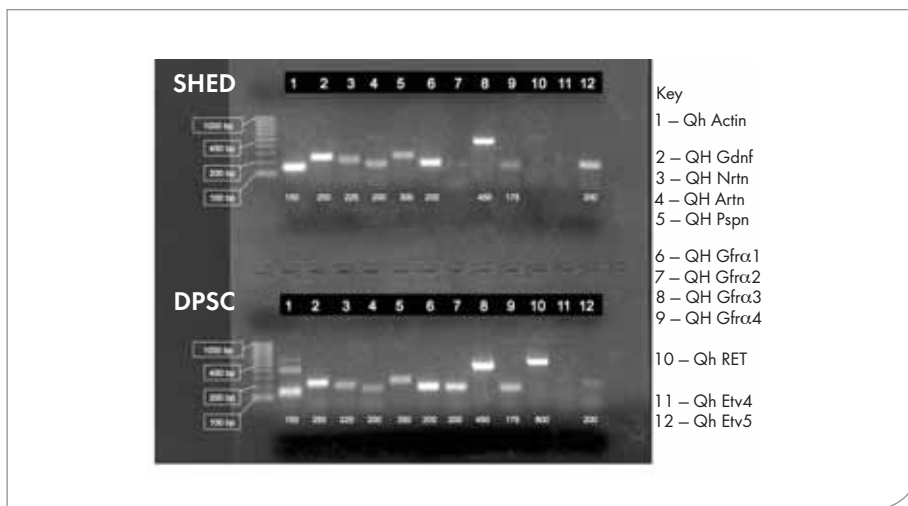


FIGURE. DNA gel comparison of the expression of four different know GDNF ligands (2–5), four receptors (6–9), one co-receptor (10) and two transcription factors (11–12) in human SHED and DPSC + actin (1) as an internal control.

genes during the progression from primary to permanent dentition. The results need to be further confirmed by quantitative PCR. We conclude that all the GDNF family ligands are expressed in the SHED and DPSC, although the expression of the receptors varies in the SHED and GDNF. The finding is consistent with previous reports that neurotrophic factors are expressed in developing human and rodent teeth. More work is required to further reveal the cellular localization of the GDNF family ligands and receptors in the DPSCs, as well as to further investigate the function of GDNF family ligands in regulating DPSCs activities.

Future experiments should include a qPCR of the samples and more replications of this experiment. Further studies into the signaling mechanism of the GDNF family to elucidate more about the population of dental pulp stem cells acted on should also be done. Due to GDNF's role in neuron growth, we ultimately hope to find a practical application for these neurotrophic factors in dental pulp regeneration, as well as in stem cell therapy and tissue regeneration in the head and neck area. ■

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THE CORRESPONDING AUTHOR, Nan Xiao, PhD, can be reached at nxiao@pacific.edu.