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Comparison of expression of the GDNF family neurotrophic factors in dental pulp stem cells with deciduous and adult teeth

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

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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

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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 α1 (GFRα1), NRTN preferentially binds GFRα2, ARTN binds GFRα3 and PSPN binds GFRα4.[4,5] GDNF is the most well-studied member of the GFL family. GFRα1 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 buds[11–13] 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, 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/uL. mRNA quality of SHED was 747.2 ng/ul and that of DPSC was 462.7 ng/ul. 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 Gfrα1, Gfrα2, Gfrα3, Gfrα4; 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.

TABLE

List of Primer Pairs for PCR

Gene	5′ primer sequence	3′ primer sequence
β-actin	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG
Gdnf	TAGCTGCCGTTCTACCGACT	TCCCAATATGGCTTCCTCAG
Nrtn	TCCCATGTGATTATCGACCA	CACATCCAGATGGACAGCAC
Artn	AACTGACTAGCAGCCCCAGA	TGATGCCTCAGCCACTGTAG
Pspn	GTCTGAACAGGTGGCAAAGG	GCAGTAGCGGAAGATGACCT
Grfα1	GAGATGAGTTGCTTGGCACA	GGCCACCAGTACTCGATCAT
Grfα2	GCTGAAACTGGCCTTGTAGG	AAACCTGGGAGGAGACAGGT
Grfα3	CCACACCCTACCTTTGCTGT	CTTCCTGCTGCTGTCCTTTC
Grfα4	GAGCAGGTCAGAGACCATCC	CACAGGTGGCTACAATGGTG
Ret	GATCTCACAGGGGATGCAGT	CTGGCTCCTCTTCACGTAGG
Etv4	TCATTGGGAAGGAAAAGTGG	GAGATCTGGGGAGCTCAGTG
Etv5	TTGTGCTTTCTGCACCAGAC	TGACCAGGTTTCCAAAGGAC

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, Gfrα1 at 175 bp, Gfrα3 at 450 bp, Gfrα4 at 150 bp and Etv5 at 175 bp. Gfrα2 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, Gfrα1 and Gfrα3, were also expressed equally in both samples. Receptors Gfrα2 and Gfrα4 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

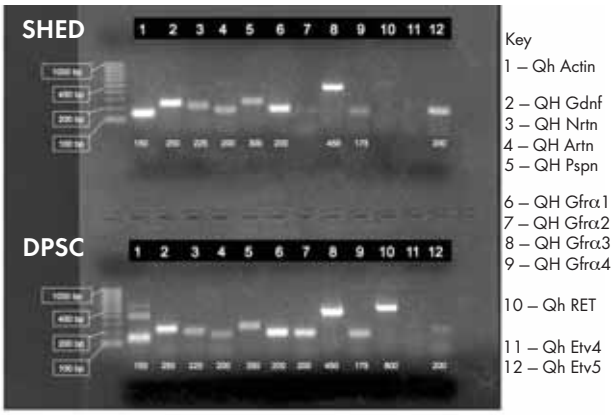

FIGURE. DNA gel comparison of the expression of four different know GDNF ligands (2–5), four receptors (6–9), one co-receptor (10) and two transcription factors (11–12) in human SHED and DPSC + actin (1) as an internal control.

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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