UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI
PENYELIDIKAN
LAPORAN AKHIR

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENE/S OBSERVED IN STEM CELLS DERIVED EXTRACTED HUMAN TOOTH

PENYELIDIK

DR. KHAIRANI IDAH MOKHTAR

PENYELIDIK BERSAMA

DR. ZULIANI MAHMOOD
DR. SITI NOOR FAZLIAH MOHD NOOR
PN. NOR SHAMSURIA OMAR

2012

1. **Nama Ketua Penyelidik:** Dr. Khairani Idah Mokhtar
Name of Research Leader

☐ Profesor Madya/ *Assoc. Prof.* ☒ Dr./ *Dr.* ☐ Encik/Puan/Cik *Mr/Mrs/Ms*

2. **Pusat Tanggungjawab (PTJ):**
School/Department

School of Dental Sciences, Health Campus, USM

3. **Nama Penyelidik Bersama:**
Name of Co-Researcher

Dr Zuliani Mahmood, Dr. Siti Noor Fazliah Mohd Noor, Pn. Nor Shamsuria Omar

4. **Tajuk Projek:**
Title of Project

Identification of differentially expressed gene/s observed in stem cells derived from extracted human tooth

5. **Ringkasan Penilaian/***Summary of Assessment*:	Tidak Mencukupi *Inadequate*		Boleh Diterima *Acceptable*	Sangat Baik *Very Good*	
	1	2	3	4	5
i) **Pencapaian objektif projek:** *Achievement of project objectives*	☐	☐	☐	☐	☒
ii) **Kualiti output:** *Quality of outputs*	☐	☐	☐	☐	☒
iii) **Kualiti impak:** *Quality of impacts*	☐	☐	☐	☐	☒
iv) **Pemindahan teknologi/potensi pengkomersialan:** *Technology transfer/commercialization potential*	☐	☐	☐	☐	☒
v) **Kualiti dan usahasama :** *Quality and intensity of collaboration*	☐	☐	☐	☒	☐
vi) **Penilaian kepentingan secara keseluruhan:** *Overall assessment of benefits*	☐	☐	☐	☐	☒

6. Abstrak Penyelidikan
(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris.** Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Abstract of Research
(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English). This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Attached separately

7. Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.
[Sila gunakan kertas berasingan]
Applicant are required to prepare a Comprehensive Technical Report explaning the project. (This report must be appended separately)

Senaraikan kata kunci yang mencerminkan penyelidikan anda:
List the key words that reflects your research:

Bahasa Malaysia	*Bahasa Inggeris*
Kepelbagaian ekspresi gen	Differentially expressed genes
Sel stem	Stem cells
Gigi manusia	Human dental pulp

8. Output dan Faedah Projek
Output and Benefits of Project

(a) * **Penerbitan Jurnal**
Publication of Journals
(Sila nyatakan jenis, tajuk, pengarang/editor, tahun terbitan dan di mana telah diterbit/diserahkan)
(State type, title, author/editor, publication year and where it has been published/submitted)

Attached separately

(b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**
State other benefits such as product development, product commercialisation/patent registration or impact on source and society.

* Sila berikan salinan/*Kindly provide copies*

(c) **Latihan Sumber Manusia**
Training in Human Resources

i) Pelajar Sarjana:
Graduates Students
(Perincikan nama, ijazah dan status)
(Provide names, degrees and status)

Name : Muhammad Fawwaz bin Abdullah
Degree : Master in Science (Thesis submission phase)

ii) Lain-lain: Final year project for undergraduate student- Siti Fadilah Bt Abdullah
Others
Bachelor of Science (Biology)

9. Peralatan yang Telah Dibeli:
Equipment that has been purchased

Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat
Comments by the Research Committees of Schools/Centres

This research project has been successfully completed with achievements of the set objectives. An MSc research student graduated from this project and had a number of conference presentations. However, a few manuscripts were submitted are under review. The researcher should continue and ensure that the submitted manuscripts will be ultimately published.

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG PENYELIDIKAN GERAN USM(304)
PENYATA PERBELANJAAN SEHINGGA 31 OGOS 2012

Jumlah Geran:	RM	38,320.00	Ketua Projek:	Khairani Idah Mokhtar, Dr/Zuliani Mahmood, Dr
Peruntukan 2009 (Tahun 1)	RM	25,380.00	Tajuk Projek:	Identification of Differentially Expressed Gene/s Observed in Stem Cells Derived from Extracted Human Tooth
Peruntukan 2010 (Tahun 2)	RM	12,940.00		
			Tempoh:	01 Julai 2009- 31 Dis 2011 (DILANJUTKAN SEHINGGA 30 JUN 2012)
			No.Akaun:	304/PPSG/6139051

Kwg	Akaun	PTJ	Projek	Donor	Peruntukan Projek	Perbelanjaan T'kumpul Hingga Tahun Lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
304	11000	PPSG	6139051		-	-	-	-	-	-	-
304	14000	PPSG	6139051		-	-	-	-	-	-	-
304	15000	PPSG	6139051		-	-	-	-	-	-	-
304	21000	PPSG	6139051		5,000.00	-	5,000.00	-	448.50	448.50	4,551.50
304	22000	PPSG	6139051		-	-	-	-	-	-	-
304	23000	PPSG	6139051		300.00	-	256.15	-	54.85	-	201.30
304	24000	PPSG	6139051		-	-	-	-	-	-	-
304	25000	PPSG	6139051		-	-	-	-	-	-	-
304	26000	PPSG	6139051		-	-	-	-	-	-	-
304	27000	PPSG	6139051		26,820.00	21,326.35	(4,306.40)	1,360.00	1,333.20	2,693.20	(6,999.60)
304	28000	PPSG	6139051		-	-	-	-	-	-	-
304	29000	PPSG	6139051		6,200.00	714.15	2,675.95	-	397.95	397.95	2,278.00
304	32000	PPSG	6139051		-	-	-	-	-	-	-
304	35000	PPSG	6139051		-	-	-	-	-	-	-
304	A11559	PPSG	6139051		-	-	-	-	-	-	-
304	A11102	PPSG	6139051		-	-	-	-	-	-	-
					38,320.00	22,040.50	3,625.70	1,360.00	2,234.50	3,539.65	31.20

Identification of differentially expressed gene/s observed in stem cells derived from extracted human tooth

ABSTRACT

The aim of this study was to identify the differentially expressed genes (DEGs) in SHED and DPSCs by GeneFishing™ DEG method using the arbitrary primer pairs provided. Two bands which were highly expressed in SHED and three bands in DPSCs were isolated purified and sent for sequencing. Sequencing analysis revealed these to be *TIMP Metallopeptidase Inhibitor 1* (*TIMP1*), (A09), and *ribosomal protein s8,* (*RPS8*), (A16) in SHED and *collagen, type I, alpha 1*, (*COL1A1*), (A20), *follistatin-like 1* (*FSTL1*), (A17), *lectin, galactoside-binding, soluble, 1,* (*LGALS1*), (A16) in DPSCs. *TIMP1* is involved in degradation of the extracellular matrix, cell proliferation and anti-apoptotic function and *RPS8* is involved as a rate-limiting factor in translational regulation; *COL1A1* is involved in the resistance and elasticity of the tissues; *FSTL1* is an autoantigen associated with rheumatoid arthritis; *LGALS1* is involved in cell growth, differentiation, adhesion, RNA processing, apoptosis, and malignant transformation. The gene expression patterns of SHED and DPSCs might be useful in determining the detailed functional roles of the relevant genes applicable to stem cell therapies, which paves way to be used as multipotent cell sources for genetic and tissue engineering technology.

Pengenalpastian Kepelbagaian Ekspresi Gen Sel Stem daripada Gigi Manusia

ABSTRAK

Tujuan kajian ini adalah untuk mengenalpasti kepelbagaian ekspresi gen sel stem daripada gigi susu (SHED) dan gigi kekal (DPSCs) oleh GeneFishing™ DEG menggunakan kaedah pasangan primer arbitrari yang tersedia. Dua jalur yang sangat terang dalam SHED dan tiga jalur dalam DPSCs telah diasingkan, ditulenkan dan dijujukan. Hasil analisis jujukan mendedahkan *TIMP Metallopeptidase Inhibitor 1* (*TIMP1*), (A09), dan *ribosomal protein s8,* (*RPS8*), (A16) dalam SHED dan *collagen, type I, alpha 1*, (*COL1A1*), (A20), *follistatin-like 1* (*FSTL1*), (A17), *lectin, galactoside-binding, soluble, 1,* (*LGALS1*), (A16) dalam DPSCs. *TIMP1* terlibat dalam degradasi matriks extracellular, proliferasi sel dan fungsi anti-apoptotic dan *RPS8* terlibat sebagai faktor kadar penghad dalam kawalatur translasi; *COL1A1* terlibat dalam ketahanan dan keanjalan tisu; *FSTL1* adalah autoantigen dikaitkan dengan reumatoid artritis; *LGALS1* terlibat dalam pertumbuhan sel, pembezaan, perlekatan, pemprosesan RNA, apoptosis, dan transformasi malignan. Corak gen SHED dan DPSCs mungkin berguna dalam menentukan peranan fungsi gen yang berkaitan secara terperinci untuk kegunaan dalam terapi sel, yang membuka jalan untuk digunakan sebagai sumber sel multipotent bagi teknologi kejuruteraan genetik dan tisu.

General objective

To identify the differentially expressed genes observed in stem cells derived from human exfoliated deciduous teeth (SHED) and human permanent dental pulp stem cells (DPSC).

Specific objectives

1. To screen and identify differential expressed gene/s from SHED and DPSC using differential display techniques
2. To isolate and confirm the sequence of the detected differentially expressed gene/s observed from stem cells derived from SHED and DPSC

1.0 Introduction

SHED and DPSCs are rich with mesenchymal stem cells (MSCs). These adult stem cells have high proliferation, and are able to differentiate into a variety of cell types including neural cells, adipocytes, and odontoblasts (Gronthos *et al.*, 2000, Gronthos *et al.*, 2002, Miura *et al.*, 2003). SHED and DPSCs were proved to be clinically useful as other types of stem cells. Knowledge of human genome and its expression might greatly enhance in tissue engineering and regenerative medicine. Hence, the gene expression profile and functional pathway of SHED and DPSCs need to be evaluated to determine their biological functional activity, describe, and compare the gene expression patterns of more specialized adult stem cells for cell characterization which can be used in determining the usefulness of these stem cells for cell-based regeneration therapy.

Improved techniques have been designed to identify differentially expressed genes (DEGs) in cells under various physiological stages or experimental conditions (Kim *et al.*, 2004). Using

this technique, a T-cell marker gene has been identified from dental pulp tissue-derived cell lines (Suguro *et al.*, 2008) suggesting the existence of different gene expression profiles present in the subpopulation cell lines. With this view, it would be interesting to identify differentially expressed genes observed in SHED in comparison to DPSC. The identification of differential displayed genes observed in these two types of stem cells might give additional information; for example, on the molecules involved in controlling their growth and characteristics.

2.0 Materials and Methods

2.1 Sample collection

Four teeth were collected from patients aged between 4 and 7 years with at least one carious teeth class 1 (for SHED analysis) and two teeth from patients aged 10 to 40 years with at least one carious teeth extracted or undergoing extraction of teeth for orthodontic and periodontal reasons (for DPSCs analysis). The dental pulp was obtained, cultured and characterized accordingly. The confluent cells from passage 4 were used for RNA isolation and differential display analysis.

2.2 Dissection of tissue and isolation of stem cells from dental pulp

Within 24 hours, the samples of teeth were cut at enamel-cementum junction using hard material cutter. Cut teeth were briefly immersed in 75% ethanol followed by soaking in Phosphate Buffer Saline (PBS). Then, the pulp was separated from a remnant crown and digested in a solution of 3 mg/ml collagenase type I (Worthing Biochem, Freehold, NJ) overnight at 37°C. Single-cell suspensions were obtained by passing the cells through a 70μm strainer (BD Falcon™, USA).

2.3 Culture of stem cells from dental pulp

Single-cell suspensions were cultured in alpha modified Eagle's medium (αMEM), (BioWhittaker™, Walkersville, USA) supplemented with 20% Fetal Bovine Serum (FBS) (GIBCO™, Invitrogen, USA), 100μM L-ascorbic acid 2-phosphate (Stem Cell Technologies, Canada), 2mM L-glutamine (GIBCO™, Invitrogen, Japan), 100 U/ml penicillin and 100μg/ml streptomycin (Gronthos *et al.*, 2000). The culture was incubated at 37°C in 5% CO_2. The stem cell lineage was confirmed using antibodies specific for stem cell markers using immunocytochemistry.

2.4 Mesenchymal stem cell characterization by immunocytochemistry

Immunocytochemistry staining to detect human mesenchymal stem cell characterization was performed by immunoperoxidase secondary detection system with primary antibodies mouse monoclonal anti-human endoglin/CD105 and mouse monoclonal anti-human CD166 (Chemicon, USA). The samples were plated on sterile cell culture cover slip, termanox plastic in 24 multiwell plate with cell density of 1 X 10^4 cells per well.

2.5 GeneFishing reverse transcription-polymerase chain reaction (RT-PCR)

2.5.1 First-strand cDNA synthesis

Total RNA (3 μg) was added into a tube on ice along with following reagents, 2 μl of 10μM dT-ACP1 and RNase-free water in a final volume of 9.5 μl. The reagents were mixed by tapping the tube followed by incubation at 80°C for 3 minutes. Then, the tube was chilled on ice for 2 minutes. After that, 8 μl of 10X RT Buffer was added along with 5 μl 2mM dNTP PreMix, 0.5 μl ScriptGuard RNase Inhibitor and Moloney Murine Leukemia Virus (MMLV) Reverse Transcriptase (Epicentre® Biotechnologies, USA) for a total volume of 20 μl. The mixture was incubated at 42°C for 90 minutes. Then the tube was heated at 94°C for 2 minutes. After incubation, the tube was chilled again on ice for 2 minutes. Finally, the first-strand cDNA was diluted by adding 80 μl of RNase-free water and stored at -20°C until use.

2.5.2 ACP (annealing control primer) based GeneFishing PCR

For second-strand cDNA synthesis, 7 µl of the first-strand cDNA was diluted and placed in a PCR tube on ice with 2 µl of an arbitrary ACP, 1 µl of dT-ACP2 and 10 µl of 2X SeeAmp™ ACP™ Master Mix for a total volume of 20 µl. The tube was placed in a preheated (94°C) for 1 minute, followed by 50°C for 3 minutes and 72°C for 1 minute. This was followed by the second-stage PCR amplification protocol which was 40 cycles (94°C) for 40 seconds, then 65°C for 40 seconds, 72°C for 40 seconds and 5 minutes of final extension at 72°C. The PCR products were electrophoresed on 2% agarose gel and the differentially expressed band was visualized under UV light.

2.5.3 DNA purification and sequencing

The differentially expressed band was extracted from gel by using QIAquick® Gel Extraction Kit (Qiagen, USA) and the purified DNA was sent for sequencing. The sequences obtained were analyzed using Basic Local Alignment Search Tool (BLAST) software for confirmation of specific genes.

2.5.4 RT-PCR Confirmation

The differential expression of DEGs was confirmed by RT-PCR using gene specific primer pair. The first-strand cDNA was normalized to the human *β-actin*. The normalized cDNA was used as a template.

3.0 Results

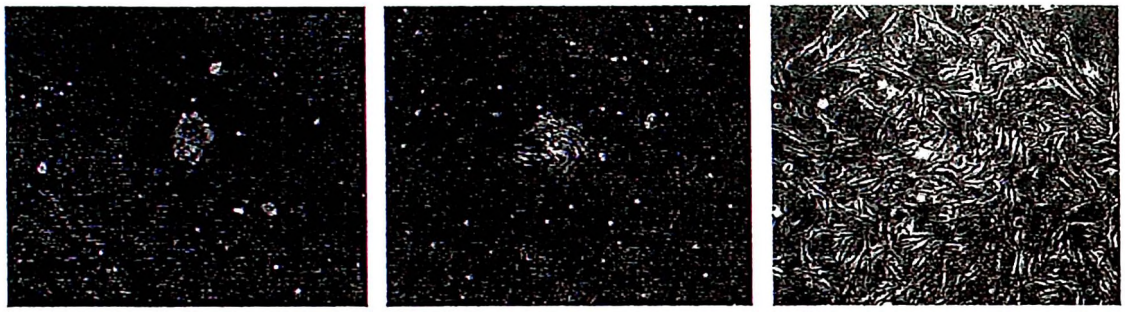
3.1 Culture of stem cells from dental pulp

Adherent cells grown on culture flask with the complete media used in this study displayed the typical morphology of SHED and DPSCs (Fig. 1). The colonies were seen to arise from single cells and contained both spindle shaped cells and large flat cells. The primary cultures

were grown to a confluence of 80% to 100% before being trypsinized and passaged into flask for further applications.

1.1 Mesenchymal stem cell characterization and proliferation rate of SHED and DPSCs

The characterization of stem cells from SHED and DPSCs results showed the presence of the brownish color indicating positive reactivity for CD105 and CD166 primary antibodies on stem cells derived from SHED and DPSCs (Fig. 2).



Day 0 Day 2 Day 11

Figure 1: On day 0, the digested dental pulp including fibroblast, mesenchymal cells and macrophages were seen floating inside the flask at this early stage. On day 2, small rounded and spindle shaped cell was seen attached to the flask and at day 11, the spindle-shaped cells have become 80% confluent (x 40).

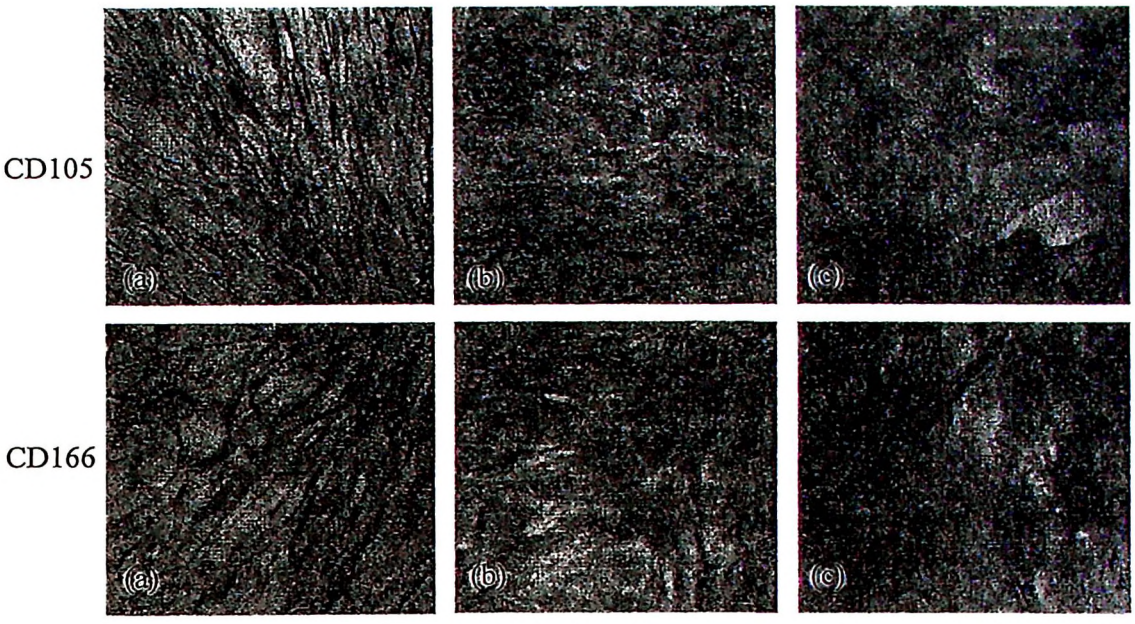


Figure 2: Characterization of stem cells from dental pulp by immunocytochemistry staining using CD105 and CD166 antibodies viewed at 100X. The CD105 and CD166 expressions were positive on stem cells derived from the (a) SHED, (b) DPSC and (c) hMSC as positive control.

1.2 Differentially expressed gene in SHED and DPSCs

Two genes were highly expressed in SHED and three genes in DPSCs (Fig. 3). The BLAST results showed that the genes highly expressed in SHED were *TIMP Metallopeptidase Inhibitor 1* (*TIMP1*), (A09) (with 82 % query coverage and 93 % identity), and *ribosomal protein s8,* (*RPS8*), (A16) (with 90 % query coverage and 99 % identity) while genes that were highly expressed in DPSCs were *collagen, type I, alpha 1*, (*COL1A1*), (A20) (97 % query coverage and 99 % identity), *follistatin-like 1* (*FSTL1*), (A17) (96 % query coverage and 100 % identity), *lectin, galactoside-binding, soluble, 1,* (*LGALS1*), (A16) with 75 % query coverage and 96 % identity). To confirm the efficacy of the ACP system, the DEGs were confirmed by RT-PCR using gene specific primer pairs (Table 1). The Arbitrary A09

(*TIMP1*), A16 (*LGALS1, RPS8*), A17 (*FSTL1*) and A20 (*COL1A1*) were subjected to confirmation with RT-PCR, based on results showed in fig. 3 (Fig. 4).

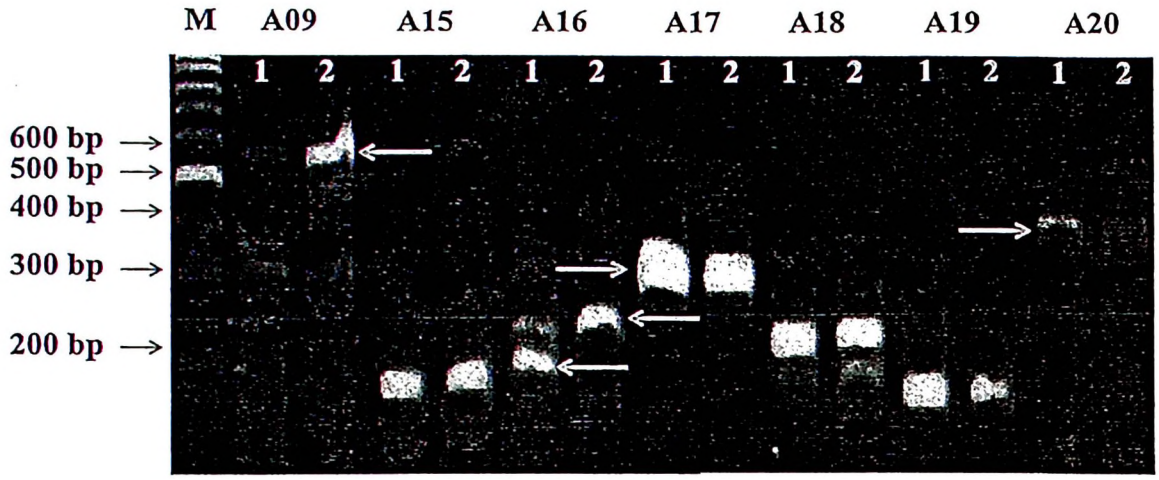


Figure 3: GeneFishing™ DEG screening results. The arrows show the genes which were highly expressed in SHED and DPSCs. (1. DPSCs, 2. SHED, M = markers).

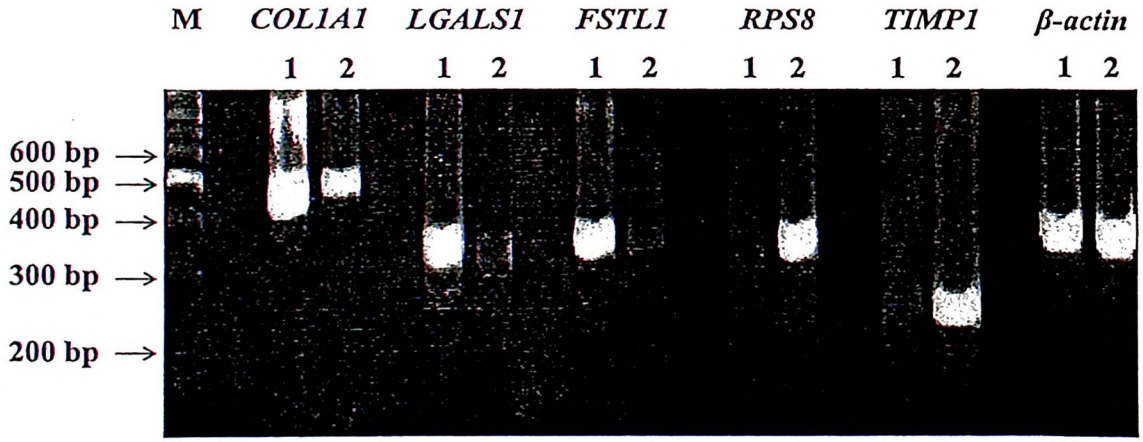


Figure 4: Arbitrary A09 (*TIMP1*), A16 (*LGALS1, RPS8*), A17 (*FSTL1*) and A20 (*COL1A1*) were confirmed with RT-PCR and confirmed ACP observation. (1. DPSCs, 2. SHED, *β-actin,* a housekeeper gene, served as a PCR amplification control, M = markers).

Table 1 RT-PCR primer sets of BLAST genes

Primer	Arbitrary	Forward	Reverse	Size
COL1A1	A20	5'ACAGTGATTGAATACAAAACCA'3	5'GTGGAGAAAGGAGCAGAAAG'3	496
FSTL1	A17	5'TAGCATCTGTTAAGATCCAGTG'3	5'TCCACTCTTAGGAAGTAAATGG'3	391
LGALS1	A16	5'TGGATACGAATTCAAGTTCC'3	5'TACTATGTGCCAAACTCTGTGT'3	347
TIMP1	A09	5'CAACAGATGTATAAAGGGTTCC'3	5'CCTTCTGATAGACTGAAATTGG'3	270
RPS8	A16	5'GTTGTGCTAAGGATCACCTACT'3	5'GAGTTGAGAACAGGGACTTTAC'3	350
β-actin	-	5'TGGCACCACACCTTCTACAATGAG C3'	5'GCACAGCTTCTCCTTAATGTCACG C3'	395

Sequencing results? DNA alignment

2.0 Discussion

SHED can differentiate plastically into neuronal cells, adipocytes and odontoblasts. SHED also show higher proliferation rate than DPSCs and can form significant amounts of alveolar and orofacial bone for tissue regeneration (Gronthos *et al.*, 2000, Miura *et al.*, 2003). It is essential for tissue engineering and clinical applications to isolate the postnatal stem cells from an easily accessible source. The isolation of mesenchymal progenitors from the pulp of human deciduous incisors found that *in vivo* SHED can induce bone or dentin formation but, in contrast to DPSCs, SHED failed to produce a dentin-pulp complex (Miura *et al.*, 2003). DPSCs are stem cells derived from dental pulp which are not active and shack in a specific perivascular microenvironment where they hold their stem cells (SCs) characteristics. DPSCs are similar to mesenchymal stem cells (MSCs) by showing multipotential differentiation ability. These DPSCs express MSC markers including Stro-1 and CD146 and undergo colony forming *in vitro* and can regenerate the dentin/pulp complex *in vivo* (Gronthos *et al.*, 2000, Gronthos *et al.*, 2002).

Research shows that DPSCs are able to differentiate into odontoblast and adipocytes as well as expressing nestin and GFAP and form a dentin/pulp-like complex after *in vivo* transplantation (Gronthos *et al.*, 2002). Dental pulp progenitors have not been clearly identified, but according to some data, the pericytes, which are able to differentiate into osteoblasts, could also differentiate into odontoblasts (Shi and Gronthos, 2003, Lovschall *et al.*, 2007). MSCs might exist in adult dental pulp because tooth repair is a lifetime process. And because of that, the *in vivo* therapeutic targeting of these adult stem cells remains to be explored and discovered.

Characterization of SHED and DPSCs was performed by immunocytochemistry staining because of its stability, sensitivity, clear cytomorphological details, ease of use, cost-effectiveness and low technology need (Leong, 1996). CD105 is also known as human endoglin (ancillary TGF-beta receptor), a transmembrane glycoprotein expressed by vascular endothelial cells and activated macrophages (Cheifetz *et al.*, 1992) while CD166 (active leukocyte cell adhesion molecule (ALCAM)), is a member of the Ig superfamily and is expressed primarily in the spleen, palcenta, liver (weakly) and activated T-cells, B-cells, thymic epithelial cells, fibroblasts, keratinocytes and neurons (Bowen *et al.*, 1995). In this study, both SHED and DPSCs were found positive for both antibodies against human antigens CD105 and CD166. Similar results have been reported where MSCs isolated from placenta/umbilical cord cells population were positive for CD54, CD29, CD73, CD13, CD44, CD105 and CD166 which suggested that the immunophenotypical and morphological profiles of these cells are the same as those of MSCs isolated from bone marrow (Sato *et al.*, 2005, Yen *et al.*, 2005, Kadivar *et al.*, 2006).

GeneFishing™ DEG Premix Kits are designed to overcome the weakness of previous gene expression profiling-related methodologies, like the microarray and differential display techniques. The most critical factors in success of PCR amplification can be determined by specificity and sensitivity with which a primer anneals to its target sequence. GeneFishing™ DEG Premix Kits consist of three steps which are reverse transcription (RT) and two-stage PCR (GeneFishing™ PCR) which uses primers that anneal specifically to the template and amplified only genuine products. The GeneFishing™ system can overcome the difficulty in identifying the genes responsible for a specialized function during a certain biological stage; this is because the gene is expressed at low levels, whereas most mRNA transcripts within a cell are abundantly expressed (Kim *et al.*, 2004, Hwang *et al.*, 2003). With this technique, we identified 5 DEGs that are highly expressed in comparable samples.

DEGs in SHED

TIMP1: Tissue inhibitors of metalloproteinases (TIMPs) family have been recognized as intrinsic inhibitors of matrix metalloproteinases (MMPs) and *TIMP1* has been shown to regulate cell proliferation by interacting with the ninth zinc finger domain of promyelocytic leukemia zinc finger protein (PLZF) (Rho *et al.*, 2007). Over-expression of *TIMP1* was also shown to inhibit tumor growth and metastasis of T-cell lymphoma in transgenic mice (Guedez *et al.*, 1998, Henriet *et al.*, 1999). *TIMP1* also possesses anti-apoptotic and differentiation properties in B cells as well as in breast cancer cells (Guedez *et al.*, 2001, Li *et al.*, 1999).

RPS8: 40S ribosomal protein S8 is a protein in humans that is encoded by the *RPS8* gene (Davies and Fried, 1993). Ribosomes, the organelles that catalyze protein synthesis, consist of a small 40S subunit and a large 60S subunit. Together these subunits are composed of 4 RNA species and approximately 80 structurally distinct proteins. This gene encodes a

ribosomal protein that is a component of the 40S subunit. The protein belongs to the S8E family of ribosomal proteins. It is located in the cytoplasm. Increased expression of this gene in colorectal tumors and colon polyps compared to matched normal colonic mucosa has been observed. This gene is co-transcribed with the small nucleolar RNA genes U38A, U38B, U39, and U40, which are located in its fourth, fifth, first, and second introns, respectively. As is typical for genes encoding ribosomal proteins, there are multiple processed pseudogenes of this gene dispersed through the genome. As a component of 40S ribosome, *RPS8* is in an mRNP granule complex containing untranslated mRNAs (Jonson *et al.*, 2007), while the precise function of *RPS8* is still not clear. In a study by Hao et al. (Hao *et al.*, 2011), they found either over-expression or knockdown of *RPS8* decreased luciferase activity through suppressing translation, suggesting *RPS8* as a rate-limiting factor in translational regulation. Previous reports show that though eIF6 is essentially required for 60S subunit biogenesis (Basu *et al.*, 2001, Wood *et al.*, 1999), over-expression inhibits assembly of 80S ribosome (Ceci *et al.*, 2003). The role of RPS8 in controlling translation still needs to be clarified in the future.

DEGs in DPSCs

FSTL1: Follistatin-related protein 1 is a protein that in humans is encoded by the *FSTL1* gene. This gene encodes a protein with similarity to follistatin, an activin-binding protein. It contains a Follistatin module, a follistatin-like sequence containing 10 conserved cysteine residues. This gene product is thought to be an autoantigen associated with rheumatoid arthritis (Tanaka *et al.*, 1998).

LGALS1: Galectin 1 (GAL1), a b-galactosyl binding lectins, is a ubiquitous protein expressed in many animal tissues (Hirabayashi and Kasai, 1993, Barondes *et al.*, 1994) and has pleiotropic biological functions implicating in cell growth, differentiation, adhesion, RNA

processing, apoptosis, and malignant transformation (Barondes *et al.*, 1994, Perillo *et al.*, 1995, Vyakarnam *et al.*, 1997). Numerous studies have shown that cell adhesion is critical in regulating cell proliferation per se, and cell proliferation stimulated by growth factors (Assoian and Marcantonio, 1997, Giancotti, 1997). Normal cells must adhere to extracellular matrix (ECM) components in order to proliferate *in vitro* (Moiseeva *et al.*, 2000). GAL1 has been shown to affect cell proliferation. However, the effect of GAL1 on cell proliferation seems to be a cell type specific response (Wells and Mallucci, 1991, Yamaoka *et al.*, 1991, Adams *et al.*, 1996, Yamaoka *et al.*, 1996). It inhibited growth of mouse embryo fibroblast, but stimulated proliferation of the mouse fibroblast BALB/3T3 cell line. GAL1 also stimulated or inhibited cell growth in some types of tumor cells, depending on the GAL1 concentrations.

COL1A1: belongs to the superfamily of genes encoding collagen; it encodes the a1 chain of collagen type 1. Such genes are of particular interest because of their involvement in resistance and elasticity of the tissues. They have been associated with a large spectrum of diseases such as Ehlers-Danlos syndrome, osteogenesis imperfecta, chondrodysplasia and low bone mineral density (Kuivaniemi *et al.*, 1997). Moreover, biochemical studies have shown that congenital displacement of the hip (CDH) is associated with alterations in the metabolism of collagen, which could explain the joint laxity observed (Skirving *et al.*, 1984). The involvement of these two genes in CDH has been suggested by a Chinese study which, later on, refuted the implication of COL1A1 (Jiang *et al.*, 2003, Jiang *et al.*, 2005).

When Miura et al. (Miura *et al.*, 2003) found that SHED have been identified as a novel population of stem cells, Nakamura et al. (Nakamura *et al.*, 2009) compared the gene expression between SHED and DPSCs based on proliferation pathways. They observed that genes which were highly expressed in SHED participated in pathways related to cell

proliferation and extracellular matrix, including several cytokines such as fibroblast growth factor and tumor growth factor beta. On the other hand, *LGALS1* was highly expressed in DPSCs compared to SHED and also involved in cell proliferation, but *LGALS1* had multiple effects (Barondes *et al.*, 1994, Perillo *et al.*, 1995, Vyakarnam *et al.*, 1997).

This study showed that gene variations also occurred within the different sources of the same stem cells, and these variations determine their future lineage tendency towards a specific goal. By using GeneFishing technique™, Annealing Control Primer (ACP) system, the screening results for DEGs are more secure and valid. By identifying these differentially expressed genes, we believe that SHED and DPSCs can be applied in cell-based regeneration therapy. However, further studies need to be done to ascertain their functional roles *in vitro* and *in vivo*.

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