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Innate molecular signature of stem cells from carious teeth influences differentiation toward endodermal endpoint

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ABSTRACT

The aim of this study was to characterize cells from carious teeth (DPSCs-CT) in terms of proliferation, mesoderm differentiation and gene expression profile as compared to DPSCs. Up-regulated genes in DPSCs-CT was detected via qPCR array and downstream trans-differentiation toward hepatocyte-like cells was performed. Additionally, qPCR array was employed to describe the genes pertaining to EMT and their possible mechanism. Despite basic characterizations favoured DPSCs, peculiarly DPSCs-CT had expressed a tremendous expression of hepatocyte growth factor gene (HGF; > 20 fold). To ascertain the notion that DPSCs-CT can be utilized for generating hepatic-like cells, we further de-toured the cells into hepatic lineage. As expected, DPSCs-CT expressed higher (>3 fold) hepatic markers such as SOX17, HNF3β, GATA4, AFP, TAT, TDO, AAT and ALB at both gene and protein levels. Improved homing capacity of DPSCs-CT and overall liver function were observed in bile duct ligation (BDL) treated rats. Mesenchymal-epithelial transition (MET) profiling was further conducted to elucidate the role HGF in promoting differentiation of DPSCs-CT and surprisingly, more than 40 genes related to MET were highly expressed in DPSCs-CT. To conclude, this information highlighted the potential of DPSCs-CT to differentiate into putative hepatocyte and subsequent usage for liver regeneration.

1. Introduction

The discovery of dental pulp stem cells (DPSCs) has opened up an array of possibilities in regenerative medicine.[1,2] These cells, which can either originate from deciduous (stem cells from human exfoliated deciduous teeth: SHED) or permanent tooth, have shown to have stem-cell-like qualities, including self-renewal and multi-lineage differentiation capability. As of the time this was written, at least 800 journal articles are available in Pubmed related to studies involving DPSCs. Some of these studies are dedicated to characterizing SHED[1,3,4] while others explored the prospects of these cells in various facets such as dental-related diseases,[5] tooth regeneration,[6] bone regeneration,[7] neurogenic differentiation,[8,9] hepatic differentiation,[10,11] craniofacial tissue engineering[12] and many more.

Despite having been explored thoroughly, all of the above mentioned studies have employed healthy DPSCs as their test subject upon rigorous selection criteria, making their procurement for research purposes to be extremely challenging. It should be noted then, that apart from healthy DPSCs, the second most viable source worth exploring are those that originate from carious teeth (DPSCs-CT). However, this group of cells were considered as waste simply due to their deficiency in proliferation and multi-lineage capacity.[13] Thus, there is a gap in terms of understanding the mechanisms beyond these disadvantages. Among the methods employed to understand these mechanisms, gene expression profiling is considered to be quite reliable, as it gives an overall view on the biology and the ontogeny of the cells.[14,15] Despite the presence of post-translational modifications which could alter the ultimate phenotype of a cell, the intrinsic molecular properties of cells still carries the importance as they govern the biological fate of a particular cell. Hence, in the present study, we have compared the proliferation, mesoderm differentiation and more specifically, gene expression profiling of DPSCs and DPSCs-CT. With the added knowledge obtained from this study, it is expected to bring us a step

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a closer to translating stem cell research from bench to bedside.

2. Methodology

2.1. Sample population and tooth selection

The study was conducted using samples from paediatric donors (4–12 years old). Healthy pulp tissue (n = 10) was obtained from participants with healthy impacted third molars while extracted pulp tissue from deciduous teeth diagnosed with reversible pulpitis (n = 10) was aseptically collected under pulpectomy. Prior to the commencement of subject recruitment, the procedures for using human samples were conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee, Faculty of Dentistry, University of Malaya (Medical Ethics Clearance Number DF CO1107/0066[L]). Written informed consent was obtained from all minors' legal guardians/parents and assent from the children.

2.2. Pulp collection and isolation of cells

Stem cell cultures derived from healthy (DPSCs) and inflamed deciduous teeth (DPSCs-CT) were established as previously described.[13,16,17] Digested pulp tissues were neutralized with FBS and centrifuged before seeded in culture flasks. The medium was replaced every alternate days until cells reached confluency of 80–90%. It should be noted that human hepatocellular carcinoma cell line (HepG2, ABM, Biorev) was utilized as positive control in the present study. These cells are highly differentiated and display many genotypic features of normal liver cells.[10,18]

2.3. Growth kinetics and multilineage differentiation

The proliferation rate for both cell lines by means of population doubling time (PDT) was evaluated using methods as described previously.[4,17] Determination of *in vitro* multilineage differentiation of both cell lines was performed as previously established.[16,20,21] The presence of lipid droplets, build-up of proteoglycan, and calcium accumulation was assessed via Red Oil staining, Alizarin Blue staining, and Von Kossa staining, respectively. Analysis of important genes related to fat, cartilage and bone development were also carried out and the primers employed are listed in Table 1.

2.4. Induction of DPSCs and DPSCs-CT into hepatic lineage

The *in vitro* hepatic differentiation protocol was executed as previously described with some modifications.[22,23] Fresh media was added every 3 days for 22 days throughout differentiation period. The morphological changes of both DPSCs and DPSCs-CT were captured using phase-contrast microscope with HepG2 cell line as positive control.

2.5. Quantitative gene expression via Polymerase Chain Reaction (PCR) and PCR array platforms

Gene expression studies were performed using our in-house protocol as described previously.[9,10,17] Gene expression levels were analysed by means of comparative C_T method (ΔΔCT) and were normalized to 18s rRNA. The primer sequences are listed in Table 1. Additionally, gene expression of both DPSCs and DPSCs-CT were carried out in parallel using RT^2 Profiler™ PCR Array Human Mesenchymal Stem Cells (MSC; SABiosciences, Qiagen) as well as RT^2 Profiler™ PCR Array Epithelium to Mesenchymal Transition (EMT; SABiosciences, Qiagen). A total of 1 μg RNA was reverse-transcribed into cDNA using RT^2 First Strand Kit (Qiagen, Venlo, Limburg, Netherlands) and RT^2 SYBR Green qPCR Mastermix (Qiagen, Venlo, Limburg, Netherlands) was then added prior to real-time PCR as per manufacturer's protocol.

2.6. Hepatocyte Growth Factor detection, immunocytochemistry and Western blot analysis

The detection of HGF was employed by using customized human cytokine antibody array (Panomics, Redwood City, CA) as described previously.[13] For immunocytochemistry analysis, both differentiated and undifferentiated cells were fixed for 20 min in 4% ice-cold paraformaldehyde. Primary antibodies [AFP (mouse, Abcam), HNF4 (mouse, Abcam), Albumin (mouse, Sigma), SOX17 (rabbit, Abcam) using a dilution factor of 1:400, except for SOX17 where the dilution factor was 1:500] were incubated overnight at 4 °C, washed with Dulbecco's Phosphate Buffered Saline (DPBS; Invitrogen), and then incubated with secondary antibodies (either fluorescein isothiocyanate [FITC]-conjugated IgG or rhodamine-conjugated IgG) for 90 min at RT. Slides were counterstained with 4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI, Chemicon,

Table 1
List of genes with primer sequence and the product size.

Gene name	Forward (5′-3′)	Reverse (5′-3′)	Base pair	Accession No.
RUNX2	CGCCTCACAAACAACCACAG	TCACTGTGCTGAAGAGGCTG	225	NM_001015051.3
OSTEOCALCIN	CAGAGGTGCAGCCTTTGTGTC	TCACAGTCCGGATTGAGCTCA	150	NM_001199662.1
PPARγ2	ACAGCAAACCCCTATTCCATGCTGT	TCCCAAAGTTGGTGGGCCAGAA	159	NM_015869.4
LPL	TGGACTGGCTGTCACGGGCT	GCCAGCAGCATGGGCTCCAA	167	NM_000237.2
AGGRECAN	AGGGCGAGTGGAATGATGTT	GGTGGCTGTGCCCTTTTTAC	68	NM_001135.3
COLLAGEN 2A1	CTGCAAAATAAAATCTCGGTGTTCT	GGGCATTTGACTCACACCAGT	101	NM_001844.4
18s rRNA	CGTCGTCCTCCTCGCTTG	TAGGTAGAGCGCGGCGA	90	NR_046235.1
GATA4	ACACCCCAATCTCGATATGTTTGA	GCACAGATAGTGACCCGTCC	112	NM_002052.3
GATA6	GAGCGCT GTTTGTTTAGGGC	CTGGAAAGGCTCTGGAGTCG	337	NM_005257.5
SOX17	GGACCGCACGGAATTTGAAC	GGATCAGGGACCTGTCACAC	186	NM_022454.3
HNF4a	AGGGATTAACTCTGCCCAGC	ACATCCTCCTCCTGCTGCTA	124	NM_001287184.1
HNF3b	TGCACTCGGCTTCCAGTATG	CATGTTGCTCACGGAGGAGT	110	NM_021784.4
AFP	CATCCAGGAGAGCCAAGCAT	CGCCACAGGCCAATAGTTTG	209	NM_001134.2
Albumin	ACCTAGGAAAAGTGGGCAGC	CTGAAAAGCATGGTCGCCTG	183	NM_000477.5
TAT	GCTAAGGACGTCATTCTGACAAG	GTCTCCATAGATCTCATCAGCTAAG	354	NM_000353.2
CYP3a4	TCACCCTGATGTCCAGCAGAAACT	TACTTTGGGTCACGGTGAAGAGCA	251	NM_017460.5
TDO	GGTTTAGAGCCACATGGATT	ACAGTTGATCGCAGGTAGTG	425	NM_005651.3
AAT	CTTGAGGAGAGCAGGAAAGCCTC	ATGCCCCACGAGACAGAAGA	150	NM_001127707.1
CYP7a1	GAGAAGGCAAACGGGTGAAC	ATCGGGTCAATGCTTCTGTG	276	NM_000780.3
TO	GGCAGCGAAGAAGTACAAATC	TCGAACAGA ATCCAACTCCC	220	NM_005651.3
HGF	TGATACCACACGAACACAGC	GCAAGAATTTGTGCCGGTGT	261	NM_001010932.1