GROWTH FACTORS EXPRESSION IN DENTAL STEM CELLS: DECIDUOUS PERIODONTAL LIGAMENT AND DENTAL PULP

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INTRODUCTION

Stem cell research has become a promising field for tissue regeneration and implementation of regenerative medicine. Since the discovery and characterisation of multipotent mesenchymal stem cells (MSC) from bone marrow, similar populations from other tissues have now been characterised including dental origin.

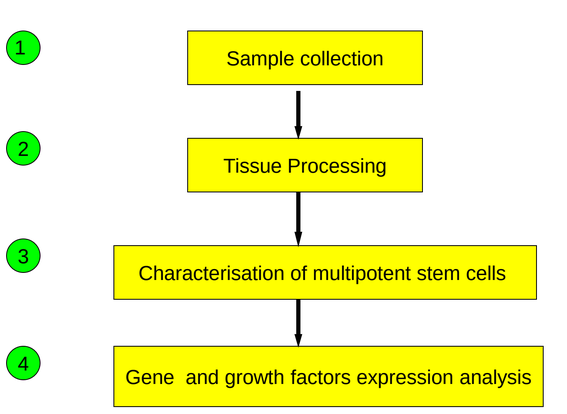
Various studies were conducted and demonstrated a number of growth factors involved on tissue healing, which leads to tissue regeneration, particularly in pulp-dentine complex and periodontium. It is logical that different tissue sources might generate different stem cell products producing different growth factors that more suited for clinical applications.

AIM

To examine the expression of growth factors of stem cells from deciduous periodontal ligament (PDLSC) and dental pulp (SCD), and to establish possible regenerative capacity potential for tissue engineering.

MATERIALS AND METHOD

Experimental design was consist of four phases



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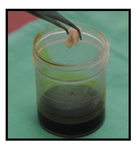


Figure 1

Molar and incisor of deciduous teeth were collected and decontaminated with povidone-iodine (Figure 1)

2

A B C D E

Figure 2

Dental tissue processing was done under sterile environment. The extracted pulp and PDL tissues were transported in media for culturing and expansion [(Figure 2(A-E)]

3

Figure 3

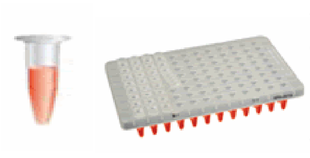
Both SCD and PDL were cultured in six-well plate and confirmed by staining-method using Van Kossa, Oil red O and Alician Blue (Figure 3).

Subsequently, immunophenotyping were carried out by using flowcytometry analysis

Cell surface antigen	Manufacturer
CD34-phycoerythrin	BD Pharmingen
CD44-phycoerythrin	BD Pharmingen
CD73-phycoerythrin	BD Pharmingen
CD90-phycoerythrin	BD Pharmingen
CD166- phycoerythrin	BD Pharmingen
CD45-Fluoro-isothyocyanate	BD Pharmingen
HLA-DR-FITC	BD Pharmingen

Table 3.1:
The list of labeled antibodies used for immunophenotyping of human dental stem cells

4



A B

Figure 4

Total RNA from dental stem cells were isolated and converted to cDNA [Figure 4(A)] to be used as a template for amplification in PCR.

Following that, samples were loaded into 89 well of PCR array (Figure (B) for gene expression analysis related to human growth factors [Figure 4(B)]

RESULTS

The descriptive analyses were performed using the software SPSS for windows version 11. Graphical representation was obtained using SAB software (www.SABiosciences.com/pcrarraydatanalysis.php) and Ingenuity Pathway Analysis (www.ingenuity.com) to generate the gene list. Statistical comparison was made by the software using Fisher's Exact test. The level of significance was set at p=0.05.

1 Characterization of mesenchymal stem cells (MSC) derived from dental origin

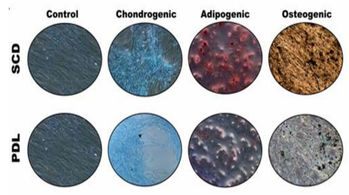


Figure 5

Both samples were shown the multilineages capasity of mesenchymal stem cells (MSC) (Figure 5).

Based on the flowcytometry analysis, both samples were mutually expressed MSC markers CD44, CD73, CD90 and CD 166 (Figure 6)

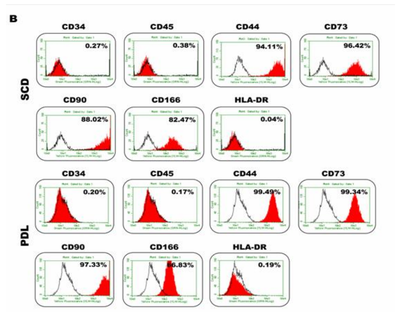
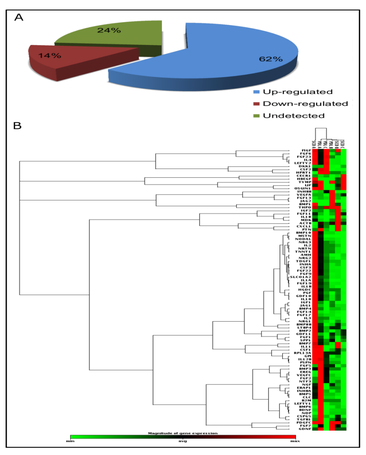


Figure 6

2 Comparison of the gene expression profile between SCD and PDLSC of human growth factor

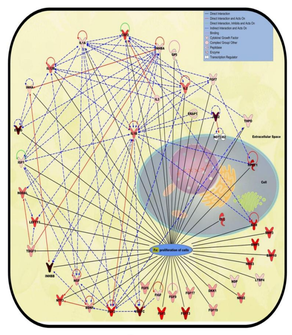


A pie-chart illustrated that a total of 62% genes were up-regulated in PDLSC [figure 7(A)]

A clustergram represent a total of genes expression in both samples. This result indicated that the ages or genders did not affect the inherent propensity of the cell lines as shown in figure 7(B)

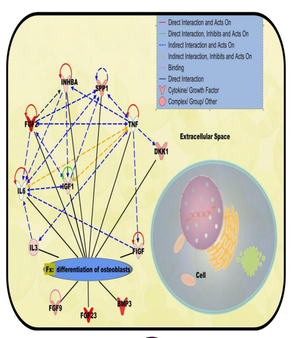
Figure 7

A The highlights of ingenuity pathway analysis



i

A high number of genes and growth factor receptors were highly up-regulated in PDLSC for cell proliferation and osteoblast differentiation as shown in Figure 8A (i) and (ii) such as EREG, JAG2, FGF2, VEGF, BMP2



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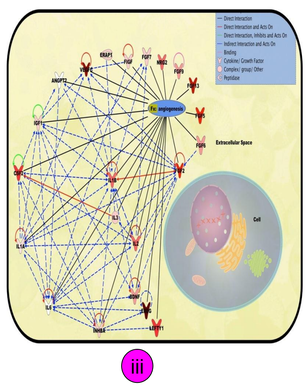
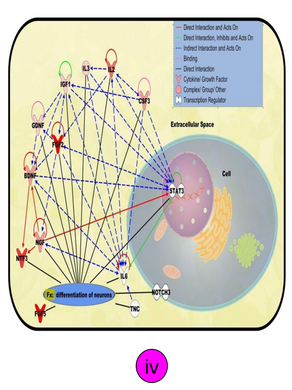


iii

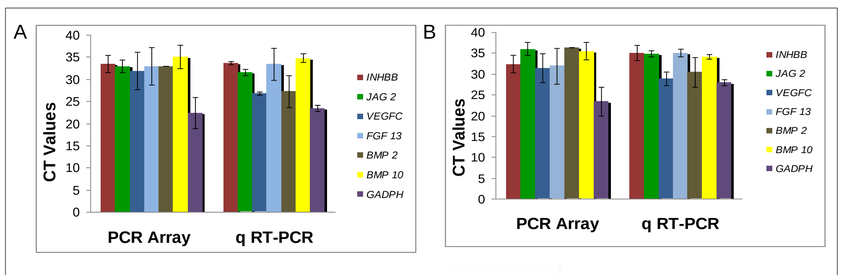
Higher genes expression associated with angiogenesis pathway and neuroectodermal lineage were found in PDLSC as compared to SCD such as VEGF-c, FGF13, IGF, NGF. [Figure 8A(iii) and (iv)]



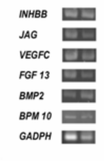
iv

Figure 8

3 Quantitative validation of PCR array



The expression of selected genes and growth factors were significantly up-regulated in PDLSC (Figure 9A) as compared to SCD (Figure 9B)



Gel electrophoresis image of semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) of selected growth factors and genes (Figure 9B)

Figure 9

DISCUSSION

The goal of regenerative medicine is to restore functions of damaged organs and tissues. It is apparent that dentistry, which long has embraced the concept of restoring function of damaged teeth has practised this goal from beginning. Among the dental tissues involved in dental regenerative are dentine, pulp and periodontium; and stem cells are now been discovered as the main component driving the whole concept.

This study showed that both stem cells from deciduous dental pulp (SCD), and periodontal ligament (PDLSC) cultures were capable of multilineage cell differentiation and mutually expressed specific phenotype profile of human MSC namely CD 44, CD 73, CD 90 and CD 166 which were consistent with the criteria set by The International Society for Cell Therapy (ISCT). (1),(2),(7).

This experiment showed several growth factors were highly up-regulated in PDLSC as compared to SCD. This included EREG, JAG2, INHβB, VEGF-C, FGF2, FGF13, BMP2, BMP3, BMP5 and BMP10. The SCD and PDL were mutually exhibited INHβB, JAG2, VEGFC, BMP2 and BMP3.

Based on the ingenuity pathway, the higher expression of JAG2 in PDLSC has been well demonstrated during bone and tooth development via NOTCh signaling pathway. The deletion of this gene will leads to abnormal tooth mprphology (4).

In this study, the expressions of BMPs family were also found to be higher in PDLSC. The remarkable capacity of BMP is to induce bone formation in inherited craniofacial anomalies or acquired bony defects (6), (8).

Secretions of angiogenic growth factors by both dental stem cells were observed in the experiment including EREG, VEGF-C, FGF2 and FGF13. Nevertheless, PDLSC has shown to express higher expression of these genes. These growth factors hold important functions in repair damaged tissues by disease or trauma (5), (9).

The other dynamic feature of dental stem cells is the neurogenic potential. The migrating cranial neural crest cells contribute the formation of dental papilla, dental pulp, PDL and other tissues in tooth mandible (3). Other growth factors has also discovered in this study such as NGF, IGF and GDNF.

SUMMARY

This study shows that both dental stem cells exhibited the characteristics of mesenchymal stem cells and established their cell lineages and were differentiated into osteogenic, chondrogenic and adipogenic differentiation. The greatest expression of growth factors by periodontal stem cells was shown in this study and would be more advantages in tissue regeneration and engineering in cell therapy.

ACKNOWLEDGEMENT

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