Immunohistochemical study on the CD45 marker in human periodontal ligament stem cells.

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Abstract
Aim: The present study was undertaken to evaluate immunohistochemically the presence or absence of CD45 in human periodontal ligament cells.
Methodology: In the present study, samples were obtained from the periodontal ligament of 10 extracted premolars of five healthy patients aged 15 to 28 years, after obtaining written informed consent from the patient. The teeth were extracted for orthodontic reasons. These were then processed and stained immunohistochemically for detection of cell surface marker namely CD 45.
Results: The results clearly showed the absence of CD45 in the periodontal ligament stem cells in all the samples.
Conclusion: It may be inferred from the above study the absence of CD45 markers is noted in the periodontal ligament stem cells and is one of their characteristic features.
Index Terms: Immunohistochemical, periodontal, stem cells

I. INTRODUCTION.

Loss of teeth resulting from decay, periodontal diseases, trauma, or surgery negatively affects quality of life [1]. During recent years, the quest for identifying the ideal stem cell to regenerate tooth has attracted increased attention. Earlier studies have shown that cells in bone marrow, which contains both hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), can differentiate into odontoblast-like cells [1,2] and regenerate dental pulp [3]. Recently, it has been shown that compressive forces in the scaffolds can induce adult bone marrow stem cells to undergo a lineage switch and begin to form dentin-like tissue [4]. Local transplantation of bone marrow cells regenerated periodontal ligament (PDL) [5-8], and their migration after systemic transplantation into periodontal tissues was increased by mechanical stress [9]. Enhanced green fluorescent protein (EGFP)-expressing cells were observed around periodontal defects after systemic transplantation of bone marrow derived cells [10,11], which were capable of participating in tissue repair [12]. GFP+ bone marrow cells have been shown to differentiate into dental-specific cells and expressed dental-specific proteins after systemic transplantation [1,13].

A few studies have suggested that PDL-derived MSC may have phenotypic differences with other MSCs [14,15]. Studies to address this question are hampered by the absence of any specific markers for either PDL cells or tissues. Nevertheless, a number of proteins are reported to be highly expressed in PDL and have been proposed as PDL markers in studies investigating differences between MSC phenotypes, even though they may not be uniquely expressed in that tissue only. These putative candidate PDL markers include periostin, asporin (periodontal ligament associated protein-1, PLAP-1), cementum protein-1 (CEMP-1) and the neural crest-derived tissue marker Nestin [16]. The aim of the study here was to test the CD45 marker in PDL-derived MSC which were phenotypically distinct from MSCs derived from other sources. Specifically, the aim was to test the hypothesis that PDL-derived MSC (PDLSC) will not express high staining levels of the CD45 marker.

II. MATERIALS AND METHODS

A total of 10 extracted premolar teeth of 5 healthy male patients, who underwent the extraction for orthodontic purposes were included in the study based on a protocol approved by the Institutional Review Board and Ethics Committee. Informed consent was obtained from the patients to use the extracted the teeth. The PDL tissue was obtained from patients, who had no chronic diseases or history of smoking, alcohol consumption or medication use. Included teeth had no endodontic infections and no lucent or opaque lesions observed on radiographs, and fully erupted teeth from patients who exhibited no periodontal infection and had no history of previous treatment. The following inclusion criteria were used for tooth selection: (1) only patients without systemic alterations, (2) suitable periodontal health, (3) first and second premolar teeth from right and left upper and lower jaws. Exclusion criteria were: (1) patients afflicted with systemic diseases, (2) patients with gingivitis and/or periodontal disease, (3) premolars with presence of dental plaque and/or presence of caries and/or periapical reaction.

Root surfaces of extracted teeth were scraped with a back-action chisel and isolated tissue samples were placed in a nutrient solution for a minimum of 20 minutes at 4°C, avoiding contact with the walls of the dish. The solution, which contained 15mL of Roswell Park Memorial Institute medium combined with penicillin, streptomycin, gentamicin and amphotericin B, was then transferred to the research laboratory within three hours. Serial sections, 5 μm in thickness, prepared from archived formalin-fixed, paraffin-embedded (FFPE) tissue blocks, were used for immunohistochemical analysis. CD45 is a transmembrane glycoprotein which is expressed at high levels on the cell surface, and its presence distinguishes leukocytes from non-hematopoietic cells. The CD45 isoforms play complex roles in T-cell and B-cell antigen receptor signal transduction. This antibody is specific for mouse CD45 and suitable for immunohistochemical staining of Zinc-fixed and formalin fixed paraffin sections as well as acetone-fixed, frozen sections. The obtained sections were allowed to incubate at room temperature overnight. The sections were counterstained with hematoxylin and then mounted in aqueous media before light microscopic analysis.

The expression of CD45 on the tissue samples were analyzed. The immunoreactivity of the specimens was interpreted based on the intensity of the staining. Score ranks usually lie in a range from 'negative' (mostly marked as '-') to 'positive', which was signed with different amount of '+' depending upon the intensity of the stain. The samples were examined with conventional light microscope. As previously described by Shetty et.al [10], the membranous immunohistochemical staining was considered negative for CD45. The staining intensity was graded as (+) weak, (++) moderate and (+++) intense according to the overall appearance at different powers of magnification, in other words, 4×, 10× and 40×, respectively and (-) for no staining, as described by Shetty et. al.[10]

III. RESULTS

The first adherent cells appeared 2 h after the primary culture and reached 80% of confluence at about day 10. Under optical microscopy, all the primary cells appeared spindle-shaped fibroblast-like morphology. After subculture, cells grew rapidly and appeared radial or whorled arrangement. Immunocytochemistry staining showed that the cultured cells were detected negative for CD45 in 9 out of 10 samples. All the cells from only one sample were weakly positive for the marker CD45. Immuno-histochemical examination, as shown in Table-1, demonstrated that CD45 was not expressed.

Table:1 Immunohistochemistry examination of cells with CD45

MEMBRANOUS IMMUNOHISTOCHEMICAL MARKER	DEGREE OF STAINING			ABSENCE OF STAINING
	Weak (+)	Moderate (++)	Intense (+++)	
CD 45	1	0	0	9

The above table shows the degree of staining in the 10 sample teeth. CD45 was not stained.

IV. DISCUSSION

Phenotypic differences between MSCs derived from different tissues have been proposed as potentially of importance in selecting cell sources for optimal tissue engineering strategies. Some studies have tried to address this issue in dental stem cells and there is at least some evidence from non-human sources that there are quantitative (but not qualitative) differences in protein expression patterns between PDLSCs, BMSCs and DPSCs [17,18].

Advances in tissue engineering offers exciting translational opportunities for new therapies for dental regeneration. There has been considerable interest in recent years in the use of stem cells for repair of dental tissues such as pulp, PDL and Alveolar Bone. Increasing evidence suggests that stem cells from sources such as dental tissue itself (PDL stem cells, pulp stem cells), stem cells from human exfoliated deciduous teeth, stem cells from apical papilla and dental follicle precursor cells, adipose tissue as well as bone marrow can differentiate into cells in pulp and periodontium.

Zhou et al.[19] reported that bone marrow derived cells preferentially migrate into pulp and periodontium over other organs and a small fraction of these migrating cells became dental tissue specific stem cells with a greater proliferative potential than resident stem cells. Thus, bone marrow can be a beneficial source of stem cells for dental repair. Bone marrow contains two types of stem cells, MSCs and HSCs. It is mostly believed that MSCs are responsible for the generation of tissue stromal cells but studies from our group have shown that HSCs can give rise to many types of mesenchymal cells such as fibroblasts/myofibroblasts and adipocytes as reviewed by Ogawa et al. [20]. We have demonstrated in this study that PDLSCs do not stain for CD45. The low percentage of CD45+ cells seen in culture can be explained by the fact that cells are thought to lose the expression of CD45 when grown in culture [21].

PDL-MSCs lack expression of haematopoietic markers, CD34 (primitive haematopoietic progenitor marker), CD45 (pan-leucocyte marker), CD14 (monocyte/macrophage marker), and CD19 (B cell marker) [34]. Additionally, they were negative for CD40, CD80, and CD86 (markers associated with hematopoietic cells) [22]. The absence of these hematopoietic markers is known to be essential for defining mesenchymal cells. However, the specific markers for the identification of PDL-MSCs have not been discovered and the lack of a PDL-MSC-specific marker limits their precise isolation and characterization.

PDL-MSCs are promising cells; however, their rarity prevents their application for use in studies of PDL regeneration. In addition, elderly people need PDL regeneration than young people do because the prevalence and severity of periodontal disease are increasing with age. However, the multipotent and self-renewal capacity of PDL-MSCs decreases while donor aging. Therefore, easily obtaining large numbers of PDL from elderly people is significantly important to achieving the development of PDL regenerative therapy.

IV. CONCLUSION

We were able to successfully demonstrate the absence of CD45 staining in characterized stem cells from the PDL of the freshly extracted teeth. Further studies are required to elucidate the regenerative potential of PDLSC's.

V. ACKNOWLEDGMENT

None

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