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Research Article

Multilineage potential and proteomic profiling of human dental stem cells derived from a single donor

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ABSTRACT

Dental tissues provide an alternative autologous source of mesenchymal stem cells (MSCs) for regenerative medicine. In this study, we isolated human dental MSCs of follicle, pulp and papilla tissue from a single donor tooth after impacted third molar extraction by excluding the individual differences. We then compared the morphology, proliferation rate, expression of MSC-specific and pluripotency markers, and in vitro differentiation ability into osteoblasts, adipocytes, chondrocytes and functional hepatocyte-like cells (HLCs). Finally, we analyzed the protein expression profiles of undifferentiated dental MSCs using 2DE coupled with MALDI-TOF-MS. Three types of dental MSCs largely shared similar morphology, proliferation potential, expression of surface markers and pluripotent transcription factors, and differentiation ability into osteoblasts, adipocytes, and chondrocytes. Upon hepatogenic induction, all MSCs were transdifferentiated into functional HLCs, and acquired hepatocyte functions by showing their ability for glycogen storage and urea production. Based on the proteome profiling results, we identified nineteen proteins either found commonly or differentially expressed among the three types of dental MSCs. In conclusion, three kinds of dental MSCs from a single donor tooth possessed largely similar cellular properties and multilineage potential. Further, these dental MSCs had similar proteomic profiles, suggesting their interchangeable applications for basic research and call therapy.

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Introduction

Growing evidence in recent years emphasizes the promising regenerative potential of mesenchymal stem cells (MSCs) derived from dental tissues in clinical applications because of their ease of collection, and ability to undergo both self renewal and multilineage differentiation [1–4]. At present, a total of five different types of stem cells have been isolated and characterized from human dental tissues [5]. Besides showing MSC-like properties of fibroblastic morphology and expression of cell surface and intracellular markers, isolated cells from dental tissues were capable of differentiating into multiple lineages, such as osteocytes [6,7], adipocytes [2,4], chondrocytes [8], muscle cells [1], neural cells [2], and hepatocytes [3,9]. Importantly, MSCs from dental tissues shared similar features of surface markers expression and multilineage potential with MSCs derived from bone marrow (BMSCs) and skin [8,10]. Based on the fact that dental MSCs are isolated from extracted teeth, a medical waste discarded in routine dental procedures, their possible applications in stem cell based therapeutics are now being pursued with a great interest.

To realize the promising potential of MSCs in regenerative medicine, many attempts have been directed towards deciphering the cellular, molecular and functional properties based on transcriptional and proteomic profiling approaches [11–14]. Specifically, proteomic analysis has been efficiently employed to different types of MSCs to facilitate their definition through cellular phenotypic profile [11,15,16], identification of regulatory factors during lineage differentiation [17] and functional evaluation of secretory molecules as therapeutic vehicles [18,19]. Further, comparative analyses of protein expression have also been performed to distinguish the differences between MSCs from various tissue sources [18–20]. Despite largely similar phenotypic and cytological properties and the identification of specific marker genes or proteins of MSCs, differences were observed in expression profile possibly due to donor associated variability [4,13,14,20]. Supporting these observations, donor variation influenced the biological and molecular properties of MSCs, including dental stem cells [4,21]. Hence, a donor matched comparison of human dental stem cells in terms of multilineage potential and proteomic profiling was pivotal to evaluate their usefulness in clinical applications.

In this study, human dental MSCs of follicle, pulp and papilla tissue from a single donor tooth after impacted third molar extraction were compared on the basis of their cellular morphology, proliferation rate, expression of MSCs specific markers and pluripotent transcription factors. Further, ability to differentiate in vitro into osteoblasts, adipocytes, chondrocytes and functional hepatocyte-like cells (HLCs) was examined. Finally, we compared the protein expression profiles of undifferentiated dental MSCs of follicle, pulp and papilla using two-dimensional gel electrophoresis (2DE) coupled with mass spectroscopic (MALDI-TOF-MS) analysis. The present study identified nineteen proteins that were differentially expressed among the dental MSCs of follicle, pulp and papilla tissues derived from a single donor tooth, and all the three types of MSCs possessed typical features by displaying common and specific cellular proteomic profiles.

Materials and methods

Chemicals used in the present study were purchased from Sigma Chemical Company (St. Louis, MO, USA) and media from Gibco (Life Technologies, Grand Island, NY, USA), unless otherwise specified. Media used for human dental MSCs was advanced Dulbecco's modified Eagle's medium (ADMEM) supplemented with 10% fetal bovine serum (FBS), 4 mM glutamine and 1% penicillin–streptomycin (10,000 IU and 10,000 μg/ml, respectively, Pen–Strep). Washing medium consisted of Dulbecco's phosphate buffered saline (DPBS) supplemented with 0.1 mg/ml $CaCl_2$, 1 mg/ml poly vinyl alcohol and 1% Pen–Strep.

Isolation and culture of MSCs from human dental tissues

Human dental MSCs of follicle, pulp and papilla tissue from a single tooth sample were isolated and cultured as described previously [4,8], with minor modifications. Briefly, normal human third molars were collected from five adult male subjects (16 to 18 years of age), who were undergoing tooth extraction at the Dental Hospital of Gyeongsang National University under approved medical guidelines set by the GNUH IRB-2009-34, after obtaining the informed consents from the donors. Tooth samples were rinsed several times with DPBS containing 1% Pen–Strep. Dental follicle was separated from the surface of the tooth using a sterile scalpel and the papilla was plucked from the apical part of the tooth with a different sterile scalpel. Dental pulp tissue was gently separated from the pulp chamber of dental crown following fracture with bone forceps. Each tissue was minced into pieces and then digested by incubating in DPBS containing 1 mg/ml collagenase type I at 37 °C for 30 min with frequent gentle agitation. Tissue clumps were collected with ADMEM containing 10% FBS and passed through 100 μm nylon cell strainer in order to harvest single cell suspension. Following centrifugation at $500 \times g$ for 5 min, cell pellets were reconstituted in ADMEM and 1×10^5 cells were initially seeded into 10-cm culture dish containing ADMEM supplemented with 10% FBS. Culture dishes were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. Primary cell cultures were grown until they reached 80–90% confluence, cells were then harvested with 0.25% (w/v) trypsin-EDTA solution and either immediately passaged in a 1:4 ratio or frozen by mixing with freezing mixture (10% dimethyl sulfoxide in ADMEM supplemented with 10% FBS). MSCs at passage 3–5 were used for further characterization and proteomic analysis.

Characterization of MSCs from dental follicle, pulp and papilla

The proliferative capacity of MSCs was evaluated by population doubling time (PDT). Briefly, all three types of MSCs were plated at 2×10^3 cells in each well of the 24-well culture plate in triplicate. Cell number was determined every 2 days interval for 14 days. PDT of MSCs was calculated using a formula, $PDT = t(\log 2)/(\log N_t - \log N_0)$, where t represents the culture time, and N_0 and N_t are the initial and final MSCs numbers before and after seeding, respectively.

The DNA content, cell surface and intracellular antigens of MSCs were analyzed by flow cytometer (BD FACS Calibur; Becton Dickinson, NJ, USA) in triplicates. To detect DNA content, MSCs at a final density of 1×10^6/ml were fixed in 70% ethanol at 4 °C for 4 h. Post fixation, the cells were washed twice with DPBS and resuspended in 10 μg/ml propidium iodide solution for 15 min. DNA content of the each cell was measured and categorized as G_0/G_1, S or G_2/M phase of the cell cycle. To determine the surface