EXPERIMENTAL CELL RESEARCH 334 (2015) 160–172

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/yexcr

Research Article

Transdifferentiation of mouse adipose-derived stromal cells into acinar cells of the submandibular gland using a co-culture system

*Jingu Lee, Sangkyu Park, Sangho Roh**

Cellular Reprogramming and Embryo Biotechnology Laboratory, Dental Research Institute, School of Dentistry, Seoul National University, Jongno-gu, Seoul 110-744, South Korea

ARTICLE INFORMATION

Article Chronology:
Received 27 November 2014
Received in revised form
10 March 2015
Accepted 12 March 2015
Available online 20 March 2015

Keywords:
Adipose-derived stromal cell
Acinar cell
Co-culture system
Transdifferentiation
Conditioned medium

ABSTRACT

A loss of salivary gland function often occurs after radiation therapy in head and neck tumors, though secretion of saliva by the salivary glands is essential for the health and maintenance of the oral environment. Transplantation of salivary acinar cells (ACs), in part, may overcome the side effects of therapy. Here we directly differentiated mouse adipose-derived stromal cells (ADSCs) into ACs using a co-culture system. Multipotent ADSCs can be easily collected from stromal vascular fractions of adipose tissues. The isolated ADSCs showed positive expression of markers such as integrin beta-1 (CD29), cell surface glycoprotein (CD44), endoglin (CD105), and Nanog. The cells were able to differentiate into adipocytes, osteoblasts, and neural-like cells after 14 days in culture. ADSCs at passage 2 were co-cultured with mouse ACs in AC culture medium using the double-chamber (co-culture system) to avoid mixing the cell types. The ADSCs in this co-culture system expressed markers of ACs, such as α-amylases and aquaporin5, in both mRNA and protein. ADSCs cultured in AC-conditioned medium also expressed AC markers. Cellular proliferation and senescence analyses demonstrated that cells in the co-culture group showed lower senescence and a higher proliferation rate than the AC-conditioned medium group at Days 14 and 21. The results above imply direct conversion of ADSCs into ACs under the co-culture system; therefore, ADSCs may be a stem cell source for the therapy for salivary gland damage.

Abbreviations: ACs, acinar cells; ADSCs, adipose-derived stromal cells; CD29, integrin beta-1; CD44, cell surface glycoprotein; CD105, endoglin; SMG, submandibular gland; SLG, sublingual gland; PG, parotid gland; DCs, ductal cells; MCs, mucous cells; SCs, serous cells; AQP5, Aquaporin5; Amyl, α-amylase; NaK, Na^+/K^+-ATPase; CK7, cytokeratin7; CK19, cytokeratin19; BMSCs, bone marrow stem cells; hAECs, human amniotic epithelial cells; SGCs, salivary gland cells; SVFs, stromal vascular fractions; ACM, AC-conditioned medium; PBS, phosphate-buffered saline; FBS, fetal bovine serum; AM, Acinar cell medium; ADSCM, ADSCs medium; CCA, co-culture ADSCs with ACs; E-cad, E-cadherin; EpCAM, Epithelial cell adhesion molecule; Krt18, Keratin18; N-cad, N-cadherin; Vim, Vimentin; Fn, Fibronectin; PCNA, proliferating cell nuclear antigen; MET, mesenchymal–epithelial transition; PPAR-g, peroxisome proliferator-activated receptor gamma; LPL, lipoprotein lipase; OC, osteocalcin; Runx2, runt-related transcription factor 2; Col1, type-I collagen; Tubb3, tubulin, beta 3 class III; Olig2, oligodendrocyte lineage transcription factor 2; Gapdh, glyceraldehyde 3-phosphate dehydrogenase

**Corresponding author.* Tel.:+822 740 8681.
E-mail address: sangho@snu.ac.kr (S. Roh).

http://dx.doi.org/10.1016/j.yexcr.2015.03.006
0014-4827/

Introduction

Salivary glands are essential for the maintenance of a healthy oral environment, and saliva plays an essential role in digestion, lubrication, oral homeostasis, and defense of the oral cavity. Hypofunction of salivary glands and xerostomia often occur after radiotherapy targeting head and neck tumors, which can cause diverse problems such as dry mouth, oral discomfort, periodontal disease, and taste disturbances [1]. The damaged salivary glands can also affect oral mucosal function and sense of taste; therefore, a plan for prevention and treatment should be developed for salivary gland damage [1,2]. Cell transplantation may be an effective method of treatment for repairing tissue in radio-damaged salivary glands.

In mammals, there are three major salivary glands. The submandibular (SMG) and sublingual (SLG) glands are located under the tongue, while the parotid gland (PG) is located underneath the ear between the upper and lower jaw. These major salivary glands produce approximately 90% of saliva. The components of salivary glands are classified as acinar cells (ACs), ductal cells (DCs), endothelial cells, nerves, and myoepithelial cells; the ACs are categorized as serous, mucous, or seromucous. Mucous cells (MCs) secrete mucin, which contributes to the viscosity of saliva, while serous cells (SCs) secrete many proteins such as α-amylase. Although the component of cells varies in mammals, ACs mainly exist in three major salivary glands [3]. SCs are purely in the PG and MCs are mainly in the SLG, whereas both SCs and MCs exist together in the murine SMG. The PG develops from the oral epithelium of the ectodermal lineage, whereas the SMG and SLG develop from the oral epithelium of the endodermal lineage during embryonic development. The salivary gland structure develops through mesenchymal–epithelial interaction [4]. Previous studies have suggested that Aquaporin5 (AQP5), α-amylase (Amyl), and mucins can be used as AC specific markers, while Na^+/K^+-ATPase (ATP1a1, NaK), cytokeratin7 (CK7), and cytokeratin19 (CK19) can be used as ductal cell-specific markers in mouse submandibular glands [5,6].

Recently, adult stem cells have been isolated and applied in cell-based therapies to restore and regenerate salivary gland tissues. Lin and colleagues showed that rat bone marrow stem cells (BMSCs) have the potential to differentiate into ACs at the rate of 30–50% after co-culture with ACs for 1 to 3 weeks [7]. Huang and colleagues proposed that human amniotic epithelial cells (hAECs) can differentiate into ACs, and the cells are positive for mucins after two weeks of co-culture with ACs [8]. Human adipose-derived mesenchymal stem cells (hADSCs) can differentiate into salivary gland cells (SGCs) when transplanted into radiation-damaged salivary glands. In an *in vitro* experiment, 13–18% of hADSCs transdifferentiated into SGCs when hADSCs were co-cultured with SGCs [9]. Most studies in the field of transdifferentiateion into SGCs have only focused on the expression of a few specific markers, such as α-amylase and mucins. However, it is yet to be investigated whether those predecessor cells primarily transdifferentiate into the acinar or ductal form of SGCs and whether these SGCs have potential in the context of cell therapy.

ADSCs are considered to be multipotent adult stem cells and are able to transform into various types of cells such as adipocytes, osteoblasts, neuronal, neural, endothelial cells, and hepatocytes [10–15]. ADSCs are a good stem cell source for repairing damaged tissues and for drug screening, and these cells can be obtained by high yield and are easily collected from stromal vascular fractions (SVFs) of adipose tissues [16]. The outcome is approximately 5000 cells/g, compared to 100–1000 cells/ml when BMSCs are used [17]. These reports indicate that ADSCs can be efficiently recovered from SVFs, in addition to the advantage of versatile differentiation potential.

Culture of two different types of cells using a double-chamber (co-culture system) is an appropriate method to investigate the communication between two types of cells without mixing them [7,8]. The communication between different types of cells may include unknown secreted factors which are essential for (trans) differentiation of the cells [18].

We investigated the transdifferentiational potential of mouse ADSCs into ACs after co-culture with ACs from the SMGs. The co-culture system was compared with AC-conditioned medium (ACM) because ACM obtained after culturing ACs may contain various growth factors and signaling molecules originating from the secretory ACs [19]. The factors in ACM may facilitate differentiation of ADSCs into ACs, like the factors secreted by ACs in a co-culture system. To demonstrate the transdifferentiation potential of ADSCs in a co-culture and ACM system and the change of their morphology from mesenchymal to epithelial lineage, we assessed cell proliferation and senescence as well as the expression of AC-specific markers.

Materials and methods

Animals

Eight-week-old C57BL6 X DBA2 F1-hybrid (B6D2F1) female and male mice were used in the following experiments. All animal work was approved and performed under the guidelines and regulatory standards of the Institutional Animal Care and Use Committee (IACUC) of Seoul National University (approval number: SNU-130123-3).

Isolation and culture of ACs

ACs from submandibular glands were obtained from female mice by the explants outgrowth technique without collagenase treatment, as previously described with a few modifications [20,21]. The thin fascia covering the submandibular glands was carefully removed to expose the glandular tissues. After the glands were dissected and washed 4 times in phosphate-buffered saline (PBS; Gibco, Grand Island, NY, USA) with 1% penicillin–streptomycin (Sigma-Aldrich, St. Louis, MO, USA), they were cut into 1 mm^3 fragments. The tissue fragments were then cultured at 37 °C in a 5% CO_2 atmosphere in a humidified incubator with Dulbecco's modified Eagle's medium (DMEM)/F12 culture medium (1:1 mixture, v/v, Gibco) supplemented with 10% fetal bovine serum (FBS; Atlas Biologicals, Inc., Fort Collins, CO, USA), 20 ng/mL epidermal growth factor (Peprotech, Rocky Hill, NJ, USA), 1% penicillin–streptomycin and 0.1% Fungizone™ (Gibco), hereafter referred to as Acinar cell medium (AM). Cells released from the tissues grew to confluence in 4 to 6 days of culture. At approximately 90% confluence, the tissue fragments in suspension were removed along with the medium, and only the adherent cells