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Short communication

Establishment of cell lines that exhibit pluripotency from miniature swine periodontal ligaments

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ABSTRACT

Objective: The periodontal ligament (PDL) is a fibrous connective tissue composed of heterogeneous cell types, including PDL fibroblasts. It is not clear whether cells within the PDL fibroblast population retain the potency to differentiate into other cell types.
Design: In the present study, clonal cell lines, derived from Clawn miniature swine PDLs, were established by gene transfection for a human telomerase reverse transcriptase, and characterized.
Results: These cell lines, denoted TesPDL1–4, had PDL fibroblasts that showed fibroblastic morphology and expressed procollagen α1(I), osteopontin, periostin and alkaline phosphatase mRNA. Under the specific culture conditions, TesPDL3 cells also have the ability to express CD31, vascular endothelial cadherin, von Willebrand factor, osteocalcin, and to form extracellular mineralized nodules.
Conclusions: Our data indicate that TesPDL3 cells have unique properties of expressing several phenotype of fibroblasts, vascular endothelial cells and osteoblasts in cultures.

1. Introduction

The periodontal ligament (PDL) is a fibrous connective tissue between tooth cementum and surrounding alveolar bone and functions to support the teeth. The PDL tissue also contains microvessels and may have a role in tissue homeostasis and repair. To accomplish these functions, the PDL contains a range of heterogeneous cell types including PDL fibroblasts, osteoblasts, cementoblasts, epithelial cells (rests of Malassez), vascular endothelial cells.[1,2] The PDL has the capacity to reconstruct periodontal structure in response to pathological or physiological changes in the oral environment, such as,

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wound healing and regeneration due to periodontitis. Given this role in tissue reconstruction, it may be certain that the PDL contain pluripotent progenitor cells or putative stem cells. Mouse molar PDL has a slowly dividing population of progenitor cells in paravascular sites.[3] The paravascular zones in the adult PDL comprise the progenitors for the fibroblastic lineage and the osteoblastic and cementoblastic lineages that are responsible for formation of mineralized tissue.[3] Recently, several studies have indicated that PDL fibroblastic cells and bone marrow mesenchymal cells share common biological characteristics suggesting that the fibroblastic lineages and mineralized tissue-forming cells may originate from the same early progenitor cells.[4–6] Seo et al. also demonstrated that PDL cells show cementoblastic/osteoblastic and adipogenic differentiation *in vitro*, and have the potential to generate cementum/PDL-like tissue *in vivo*.[7] Although this suggests that mesenchymal stem cells are present in the PDL tissue, these have yet to be fully characterized.

Ligaments are a short bundle of tough fibrous connective tissue composed mainly of long, stringy collagen fibres. In a joint structure, bones were connected by ligaments. The specific character to PDL is highly vascularized compared to other ligaments, such as the cruciate and collateral ligaments of the knee. Vascular endothelial cells form the inner lining of blood vessels and provide an anticoagulant barrier between blood vessel walls and the blood, and play an important role in angiogenesis and vasculogenesis. Endothelial cells are thought to arise from the splanchnopleuric mesoderm and are characterized by expression of CD31 (termed platelet endothelial cell adhesion molecule-1), vascular endothelial-cadherin (VE-cadherin) and von Willebrand factor (vWF).[8] vWF is synthesized through a multistep process in endothelial cells and is stored in organelles called Weibel–Palade bodies.[9] vWF has two main functions in hemostasis: it mediates platelet adhesion to the injured vessel wall, and it carries and protects coagulation factor VIII.[9]

Recent studies on the reconstruction/regeneration of the PDL have suggested that endothelial cells have a role in this process. Angiogenesis in the PDL appears to be crucial for tissue reconstruction.[10] However, it is not shown that expression of phenotype of endothelial cells and such regulation of differentiation in PDL cells. To elucidate whether progenitor cells in the PDL tissue could differentiate into these endothelial cells, we isolated cell lines that were cultured from miniature swine PDL and were immortalized by overexpression of human telomerase reverse transcriptase (hTERT) and investigated expression of CD31, VE-cadherin and vWF in these cells. This is the first report of inducible expression of the endothelial cell and osteoblast phenotype by PDL fibroblasts in culture.

2. Materials and methods

2.1. Isolation of primary PDL cells and cell culture procedures

A premolar was extracted from the mandible of a Clawn miniature swine and PDL cells were obtained using the method of Somerman et al.[11] The donor swine, a 28-month-old male, was provided by the Japan Farm Co. (Kagoshima, Japan). Tissue from the central position of the resected root surface was immediately rinsed several times in minimum essential medium (MEM; Sigma) containing 1000 units/ml penicillin G (Banyu Pharmaceutical Co., Tokyo, Japan), 1000 μg/ml streptomycin (Meiji Seika, Tokyo, Japan) and 5 μg/ml amphotericin B (Banyu), and then transferred onto culture dishes coated with type I collagen (Sumilon Celltight multi-well plate; Sumitomo Bakelite Co., Tokyo, Japan). The tissue was cultured in MEM supplemented with 10% foetal bovine serum (FBS; Moregate Biotech, Bulimba, Australia), 100 units/ml penicillin G, 100 μg/ml streptomycin, and 1 ng/ml human recombinant fibroblast growth factor (FGF) 2 (Toyobo, Osaka, Japan). Outgrowth cells were cultured in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C for 5 weeks. The cells were treated with 0.05% trypsin and 0.53 mM EDTA (Invitrogen) and sub-cultured into dishes coated with type I collagen in F-12 HAM (Sigma) supplemented with 10% FBS, 100 units/ml penicillin G, 100 μg/ml streptomycin and anti-mycoplasmic antibiotic MC-210 (Dainihon-Sumitomo Pharmaceutical Co., Osaka, Japan), and 1 ng/ml of FGF2 in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C.

2.2. Transfection of primary cell cultures and isolation of single clones

After three passages, cells were seeded on a type I collagen coated dish at a density of 1×10^5 cells/ml and cultured until they were sub-confluent. The cells were transfected by the calcium phosphate procedure with pCI-Neo-hTERT plasmid (kind gift of Dr. Weinberg, Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, MA).[12] The pCI-Neo-hTERT plasmid carries a strong constitutive cytomegalovirus promoter and a neomycin gene to confer resistance to G418 sulphate to enable selection of stable transfectants. Seven days after transfection, the cells were replated at a low density and 1 mg/ml of geneticin (G418, Promega, Madison, WI) was added to the culture medium. Resistant clones were recovered after an additional 10 days of selection. Limiting dilution clones were obtained by plating the parental cells into 96-well tissue culture plates at one cell per well. In total, 27 clonal cell lines were obtained and designated TesPDL cells.

2.3. Culture of TesPDL cells

TesPDL3 cells were plated in type I collagen coated plastic culture dishes at 37 °C and 5% CO_2 in humidified air and maintained in F-12 HAM supplemented with 10% FBS, 100 μg/ml kanamycin (Meiji Seika, Tokyo, Japan) and 1 ng/ml of FGF2. Until the cells grew to confluence, they were cultured for the periods specified in the figure legends with FGF2; fresh medium was provided every 3 days. The mineralization microenvironment was created by treating TesPDL cells at a density of 1×10^5 cells/cm^2 with 50 μg/ml ascorbic acid (Kanto Kagaku, Tokyo, Japan), 10 mM β-glycerophosphate (Kanto Kagaku) and 5 μM dexamethasone (Wako, Osaka, Japan) for 14 days. Fresh medium was provided every 3 days.