Human Endometrial Adult Stem Cells May Differentiate Into Odontoblast Cells

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Tooth regeneration is a biological technique used to solve problems of tooth loss. The detection of various stem cells in unerupted tooth buds, dental pulp or bone marrow has provided opportunities for their management in dentin-pulp repair. However, these cell types are limited by availability, invasiveness of extraction and in some cases limited proliferative capacity. What is currently required is a source of stem cells that overcomes these deficiencies and the possibility of angiogenesis. Herein we hypothesize that endometrial adult stem cells may be induced into odontoblasts and applied as suitable seeding cells for tooth regeneration.

Citation: Ai J, Tabatabaei FS, Jafarzadeh Kashi TS. Human Endometrial Adult Stem Cells May Differentiate Into Odontoblast Cells. *Hypothesis* 2009, **7**(1): e6.

Introduction

A. Missing teeth and tooth regeneration

TOOTH LOSS usually accompanies a diversity of oral diseases and physiological causes, including dental caries, periodontal disease, trauma, genetic disorders and aging, and can lead directly to physical and mental distress that noticeably lowers an individual's quality of life (1-3). For centuries, dentistry has been dedicated to the healing of defects with durable materials. However, there is a possibility of failure and a limited service time for dental materials such as amalgam, composites and titanium dental implants (4). Developing a technique which permits replacement teeth to grow in mouths for permanent teeth regeneration, *in situ*, has a great importance. Tooth regeneration is a hopeful biological technique that aims to regenerate natural tooth-like mineral tissues in terms of histology, morphology, and function (5).

Finding an appropriate source for seeding cells that can be used in the clinic for tooth regeneration is a challenge. Stem cells from dental pulp, deciduous teeth and bone marrow and ectomesenchymal cells from the first branchial arch and cranial neural crest–derived ectomesenchymal cells showed odontogenic differentiation potential in basic study, but these cells are not practical to harvest in a clinic (6-11). It has also been reported that bone marrow mesenchymal stem cells (MSC) are not perfect seeding cells for old patients because these cells lose their differentiation capacity with the increase of the donor age (12).

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Received: 2009/07/18, Accepted: 2009/09/01
Posted online: 2009/09/25

B. Human endometrial adult stem cells

The human endometrium is a dynamic remodeling tissue undergoing more than 400 cycles of regeneration, differentiation, and shedding during a woman's reproductive years (13). Recent studies have confirmed that stem cells are persistent in the uterine endometrium (14). These cells were isolated from human endometrium, using co-expression of two perivascular cell markers, CD146 and PDGF-receptor β (PDGF-Rβ) (15). The extra enrichment of colony-forming endometrial stem cells with co-expression of these markers suggests that the reservoir of stem cells in human endometrium may be similar to bone marrow or dental pulp (16). Previous studies have shown the potential differentiation of endometrial stem cells into chondrogenic and osteoblastic lineages when cultured in appropriate induction medium (17-20). In addition, other studies have shown that stromal cells from endometrial explants can proliferate and then generate new vessels (21, 22).

C. Tooth development

Tooth development occurs through mutually inductive signaling between interacting oral epithelial and ectomesenchymal cells (23, 24). Many studies have indicated that the fetal oral epithelium provides direct signals for the initiation and morphology confirmation during tooth development (25, 26). When these signals are received by ectomesenchymal cells, the biological process of odontogenesis is initiated (27, 28). Following differentiation, the post-mitotic odontoblasts secrete an extracellular matrix including collagens, non-collagenous proteins and growth factors (29). In the mature tooth, injury due to caries and other causes, in the case of cell necrosis, can lead to differentiation of a new generation of odontoblast-like cells (30). The signaling processes responsible for this up-regulation of dentinogenesis appear to be growth-factor mediated and indicate recapitulation of developmental events (31). Thus, the aim of the present hypothesis is to investigate the ability of human endometrial adult stem cells to respond to inductive signals from a preparation of dentin matrix components and to differentiate into cells showing characteristics of odontoblast-like cells.

Hypothesis

In previous research concerning tooth regeneration, odontogenesis induced by a combination of oral epithelium and bone marrow stem cells represents adequate plasticity of non-dental adult mesenchymal stem cells to react with signals secreted by the oral epithelium (9, 11). Also, previous studies have demonstrated that adipose-derived stem cells can differentiate into functional odontoblast-like cells by overexpression of dentin sialophosphoprotein (32). Other studies have confirmed effects of transforming growth factor β-1 (TGFβ-1) and dentin non-collagenous proteins (DNCP) on human embryonic ectomesenchymal cells (33), and *in vitro* odontoblast-like cell differentiation of cranial neural crest cells induced by fibroblast growth factor 8 (FGF8) and DNCP (34).

The potential for bone marrow stem cells to differentiate into odontoblast-like cells is previously well-established (35, 36). However, it should be noted that this has several drawbacks, including the necessity for anesthesia during the process of obtaining these cells in the clinic. Also, bone marrow MSC do not seem to be perfect seeding cells for elderly patients due to the fact

that the cells lose their differentiation capacity with the increase of donor age (12). Recent studies have confirmed the existence of stem cells in the uterine endometrium with expression of stem cell markers such as CD146 and PDGF-Rβ in human endometrium. These cells that displayed excellent pluripotency potential (37) also exist in the basal layer of endometrium of menopausal women (38). Previous studies concerning long-term follow-up of animals treated with endometrial regenerative cells, and the karyotypic normality of these cells after extended passage (68 doublings), confirmed lack of tumorigenicity (39). In addition, the percentage of colony-forming mesenchymal cells present in human endometrial stroma appears greater than in bone marrow or dental pulp, with a baseline colony-forming capacity of 1.2% (40, 41), compared with 0.1–0.01% for dental pulp and bone marrow (16). Furthermore, endometrial adult stem cells are more accessible for treatments than the dental stem cells which need to be isolated under specific circumstances (40). Moreover, the requirement of excellent vascularization is an important factor for vitality of tissue construction. Previous studies have shown that stromal cells from endometrial explants can proliferate and generate new vessels (21, 22).

Endometrium may therefore be an attractive source of stem cells for tooth regeneration, accessible with no extra morbidity than that required for bone marrow particularly in elderly female patients (42, 43). Therefore, we postulate a hypothesis for the reconstruction of teeth by a challenging novel approach which considers the possibility of inducing the odontoblast differentiation of endometrial adult stem cells in the presence of FGF8, TGFβ-1 and DNCP.

Our Strategy

Endometrial biopsies will be obtained from patients referred to the hospital for infertility treatment. Exclusion criteria will include any endometrial abnormality (polyps, hyperplasia, or cancer) and administration of any hormones, GnRH agonist therapy, or intrauterine device within the last 3 months. A written informed consent form describing the procedures and aims of the study will be obtained from each donor in compliance with regulations concerning the use of human tissues.

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Endometrial samples will be collected from a total of ten normal ovulating women on cycle days 19-24. The biopsies will be obtained from the fundal region of the uterine cavity using an endometrial sampling device. In all patients, accurate menstrual dating will be carried out according to the last menstrual period in the early proliferative phase of the cycle. The biopsy tissue will be washed in Dulbecco's phosphate buffered saline (DPBS), minced and treated with collagenase. Following tissue digestion, epithelial and stromal cells will be separated using filtration. The cells will then be centrifuged and will undergo Ficoll purification. Isolated stromal cells will be examined to be free from endothelial cells using CD31 staining. The cells will then be mixed with 1 mL/well fibrinogen solution, plated in culture dishes (24 wells) and 15 uL thrombin will be

added. After gel formation, each well will be covered with 1 mL α-MEM supplemented with fetal bovine serum (FBS), glycerophosphate, ascorbic acid and dexamethasone. The following study groups will be examined: Treatment Group A, medium supplemented with TGFβ-1 and FGF8 and DNCP; Treatment Group B, only DNCP added to the medium; Treatment Group C, only TGFβ-1 and FGF8 added to the medium; and a Control Group in which TGFβ-1, FGF8 and DNCP are absent from the medium. The cells will be cultured at 37 °C in 5.5% CO_2 and 95% humidity up to 3 weeks. The medium will be changed every 3 days and cultures will be observed twice weekly with an inverted microscope to evaluate their appearance. Immunocytochemistry, reverse transcription-PCR (RT-PCR) for DSPP, alkaline phosphatase activity assay and calcium nodule staining will be used for evaluation of differentiation.

The combination of endometrial adult stem cells with specific growth factors could enhance the differentiation of endometrial adult stem cells...

Conclusion

It may be concluded that endometrial stem cells in tooth regeneration are more convenient than other sources of stem cells due to the following properties: 1) obtaining bone marrow stem cells in the clinic is not easy, because of the requirement for anesthesia, 2) bone marrow MSC are not perfect seeding cells for elderly patients since these cells lose their differentiation capacity significantly with increased donor age, 3) endometrial stromal cells produce a higher overall clonogenicity of 1.25% in comparison to the clonogenic activity of stromal cells in bone marrow and dental pulp, 4) karyotypic normality of endometrial stromal cells after extended passage (68 doublings) demonstrates lack of tumorigenicity, 5) endometrial stromal cells can proliferate and generate new vessels, 6) comparison between endometrial stem cells and bone marrow-derived MSC demonstrate superior immunomodulatory potential of endometrial stem cells, and 7) endometrial stromal cells can be obtained by a simple, safe and painless procedure, in contrast to bone marrow aspiration. By contemplating attractive advantages of endometrial adult stem cells over other stem cells, we speculate that the combination of endometrial adult stem cells with specific growth factors, which could enhance the differentiation of endometrial adult stem cells, might be a feasible way for tooth regeneration. The tooth primordial formed *in vitro* can form the basis of transplants into the adult mouth.

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