ORIGINAL ARTICLE

Human Respiratory Epithelial Cells from Nasal Turbinate Expressed Stem Cell Genes Even after Serial Passaging

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SUMMARY

Current development in the field of tissue engineering led to the idea of repairing and regenerating the respiratory airway through in vitro reconstruction using autologous respiratory epithelial (RE). To ensure the capability of proliferation, the stem cell property of RE cells from the nasal turbinate should be evaluated. Respiratory epithelial cells from six human nasal turbinates were harvested and cultured in vitro. The gene expression of FZD-9 and BST-1 were expressed in passage 2 (P2) and passage 4 (P4). The levels of expression were not significant between both passages. The RE cells exhibit the stem cell properties, which remains even after serial passaging.

KEY WORDS:
Human respiratory epithelial cells, Gene expression analysis, Stem cell marker, Tissue engineering, Nasal turbinate

INTRODUCTION

Tissue engineering is an emerging multidisciplinary field that can improve the quality of life by restoring and maintaining tissues and organs function. The respiratory epithelium (RE) generated *in vitro* can be used in the reconstructive surgery of the trachea[1,2]. However, for tissue engineered RE, the study of basic molecular properties of RE cells is equally important in regenerating superior quality epithelium, and most importantly, functional for in vivo uses. The epithelial cells from different tissue proved to contain cells that are capable of repopulating, either during normal life or at least under circumstances of tissue repair[3]. This multipotency, is often considered to be an aspect of stem cell behaviour. Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through cell division. Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions. In general, when a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with more specialized function. In order to use a certain cell type for tissue engineering purpose, the stem cell properties should be characterized. One of the proven ways to characterize the stem cell properties is the analysis of gene expression pattern. In this study, FZD-9 and BST-1 were chosen as the stem cell marker. Frizzled-9 (FZD-9) is involved in Wnt signaling pathways[4]. Frizzled-9 has been known to function as a Wnt receptor although there have been a number of mammalian Frizzled members identified. The Wnt signalling pathways describe a network of proteins most well known for their roles in embryogenesis and also involved in normal physiological processes in adult animals[5]. It is assumed that Wnt proteins can act as stem cell growth factors promoting the maintenance and proliferation of stem cells[6]. Bone marrow stromal cell antigen-1 is a glycosylphosphatidylinositol-anchored ectoenzyme belonging to the NADase/ADP-ribosyl cyclase family. It is abundantly expressed by myeloid lineage from precursors to differentiated stages of bone marrow stromal cells, synovial cells, endothelial cells, and other cell types[7].

In this study, expression of both FZD-9 and BST-1 in human respiratory epithelial cells at P2 and P4, were quantitatively evaluated.

MATERIALS AND METHODS

Nasal turbinates were obtained as discarded tissues from patients after turbinectomy and was approved by the Universiti Kebangsaan Malaysia Research and Ethical Committee. Six human nasal turbinate specimens were used for this study. Under aseptic conditions, the specimens were cleaned and cut into 2mm^3 pieces and digested in 0.3% Collagenase type I solution (Gibco/BRL, USA) for 4-12 hours. The cell suspension containing fibroblasts and respiratory epithelial cells were centrifuged at 6,500 rpm for 5 minutes. The cell pellet was then resuspended in 5-10ml trypsin EDTA (Gibco/BRL, USA) and kept in shaker incubator (Jouan, Guguay Trouin, SH) for 5 minutes at 37^{0}C. Following this, the same volume of Trypsin Inhibitor (Gibco/BRL, USA) was added to stop the action of trypsin EDTA. The addition of trypsin EDTA is a step to separate cell agglomerates into single cells. The mixture of respiratory epithelial cells and fibroblasts were cultured using Defined Keratinocytes Serum Free Medium (DKSFM) (Gibco/BRL, USA), F-12, and Dulbecco's Modified Eagle's Medium (DMEM) (Gibco/BRL, USA) with the 2:1:1 ratio, supplemented with 5% Fetal Bovine Serum (FBS) (Gibco/BRL, USA) [DKSFM:F-12:DMEM+5%FBS] decribed by Noruddin *et al*. and Mohd Heikal *et al*. Once confluence, the fibroblasts were trypsinised leaving colonies of RE cells. Respiratory epithelial cells at P2 were trypsinised once confluence and kept in TRI-REAGENT (Molecular Research Center, Inc., USA) at 1 million cells/ml for total RNA

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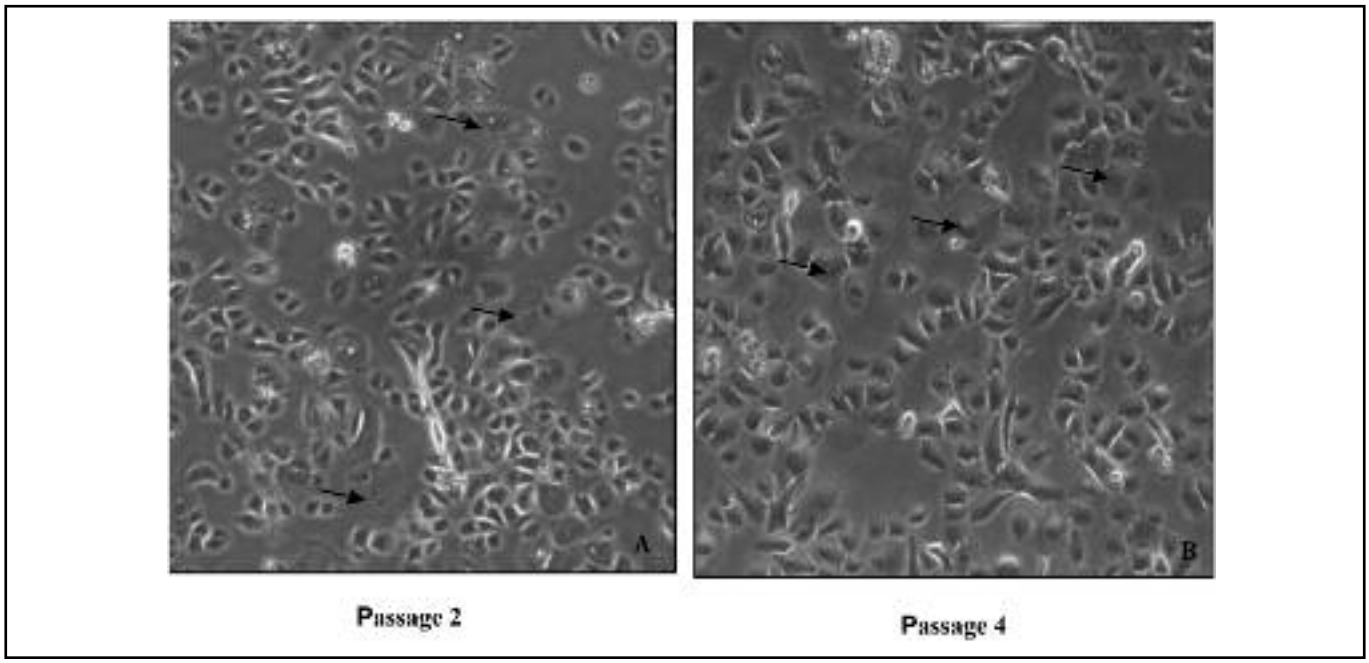

Fig. 1: A & B: Micrograph of cultured RE cells 6 days after seeding (100X magnification). Both passages demonstrated polygonal morphology. Binuclear cells which demonstrated proliferative cells were seen at both passages.

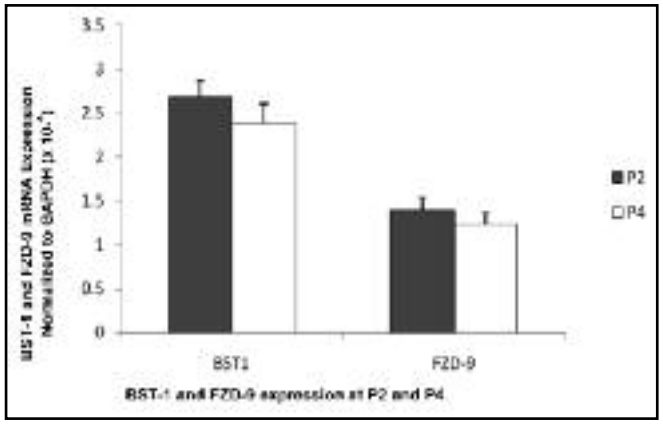

Fig. 2: BST-1 and FZD-9 gene expression of cultured RE cells for P2 and P4. No significant differences between P2 and P4 in both genes expression.

extraction. The remaining cells were sub-cultured at a density 1.0 x 10^4 cells/cm^2 until P4. The in vitro monolayer RE cells morphologic features were examined and observed daily using the inverted light microscope (Olympus, Shinjuku-ku, Tokyo). Photomicrographs were recorded for every passage. Cell count and viability was calculated using haemacytometer (Weber Scientific International Ltd, Middlx, England). The total RNA of cultured human RE cells was isolated using TRI REAGENT according to the manufacturer's recommendation. Primers were synthesized for the detection of two genes; FZD-9 and BST-1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene for data normalization. Polymerase chain reaction Master SYBR Green 1 mix was prepared using iScript™ One-Step RT-PCR Kit with SYBR® Green (Bio-Rad Laboratories 2000 Alfred Nobel Dr., Hercules, CA 94547) according to the manufacturer's protocol. The values of gene expression levels of FZD-9 and BST-1 were normalized to GAPDH for each RNA sample. The values were presented as mean ± standard error of mean (SEM). Student's t test was used to compare the data between the passages. Differences at the 5% level were considered significant.

RESULTS

The RE cells had the typical epithelial cell shape which is polygonal. The RE cells from nasal turbinate maintained the polygonal morphology from primary culture up to P4 in vitro. No significant changes were observed between the passages. Binuclear cells were observed in the majority of cells which mean that the cells were dividing and growing normally. Figure 1A and 1B shows the respiratory epithelial cells morphology at P2 and P4. Data showed that the total cell count at every passage for 6 samples is within the range of 8.0 x 10^5 to 1.2 x 10^6 cells. Real time RT-PCR demonstrated that BST-1 was expressed in respiratory epithelial cells at both P2 and P4, and the level of expression is not significant between the passages. FZD-9 also demonstrated similar pattern, without any significant differences (Figure 2).

DISCUSSION

In tissue engineering it is important to use cells with correct phenotype in order to guide the formation of a functional tissue. Stem cells have the remarkable potential to develop into many different cell types. In many tissues they serve as an internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. Frizzled-9 is a member of frizzled-receptor family that are expressed in stem cells and involved in the Wnt signaling

pathways. Wnt signaling is a key process in organogenesis and stem cell renewal. Battula *et al.*, 2007[8] describes FZD-9 as a key marker of primitive mesenchymal stem cells (MSCs). Recently, FZD-9 was found in human periodontal ligament MSCs[9] and placental derived MSCs[10]. The interaction of FZD-9 with Wnt signaling is important for the stem cells renewal[8]. BST-1 was originally identified as bone marrow stromal cell molecule[11] within the hematopoetic system and is prevalently expressed by the myeloid lineage[12]. Besides Ca^{2+} mobilization, BST-1 mediated signals are associated with a very rapid clustering of BST-1 and subsequent profound modifications in cytoskeletal organization and cell shape. The finding that BST-1 coordinates neutrophil adhesion and migration offers new perspectives for the design of treatment strategies in inflammatory conditions, in which aberrant recruitment of neutrophils results in tissue damage[13].

Our previous study by Noruddin *et. al* demonstrated that the human nasal turbinate respiratory epithelial cells using the co-culture system produces significant gene expression levels for different type of respiratory epithelial cells after serial passage[1]. In this study, respiratory epithelial cells obtained from human nasal turbinate expressed FZD-9 and BST-1 gene in serial passage, confirming that the stem cell genes were maintained without significant differences in between passages. This indicates that nasal turbinate is a potential respiratory epithelium sources for the development of tissue engineered respiratory epithelium construct for the treatment of tracheal defect[2]. The appropriate number of respiratory epithelial cells is important in the development of tissue engineered respiratory epithelium construct especially in large tracheal mucosal defect. The respiratory epithelium cells need to be cultured for few passages to obtain suitable number of cells before the construct can be made. In order to produce a good construct, the quality of the cultured respiratory epithelial cells should be maintained for proper differentiation and regeneration in-vivo.

CONCLUSION

Human RE cells derived from the nasal turbinate expressed stem cell properties even on serial passaging. Therefore, the nasal turbinate can be a potential cell source for the regeneration and restoration of the airway epithelium as well as tracheal tissue engineering.

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