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Adult lung side population cells have mesenchymal stem cell potential

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Background

The development of stem cell therapy for pulmonary diseases remains a challenge. Many diverse cell types reside within the lung and a common stem cell has not yet been identified. A basic understanding of lung stem cell fate during disease may prove important for drug intervention as well as autologous therapies. Niches for resident mesenchymal stem cells (MSC) have been identified in many adult tissues and more recently in the lung. We present data to confirm the observation that non-hematopoietic CD45^{neg} lung side population (SP) cells contain MSC, single cells capable of multilineage differentiation.

Methods

We carried these observations forward by analyzing the MSC potential of single-cell clones, as well as their chromosomal stability and telomerase activity.

Results

The expression of MSC markers was characterized in mouse CD45^{neg} lung SP by flow cytometry on freshly isolated or cultured clonal populations. The karyotype of these cells was subsequently assayed by banding analysis, and telomerase activity was assessed using quantitative polymerase chain reaction. MSC differentiation potential was confirmed by the characteristic ability of single-cell clones to differentiate into cells of three mesenchymal lineages, chondrocytes, adipocytes and osteocytes. Differentiation was confirmed by histochemical analysis. All analyzed populations of CD45^{neg} lung SP expressed mesenchymal markers (CD44, CD90, CD105, CD106, CD73 and Sca-I) and lacked hematopoietic markers (CD45, c-kit, CD11b, CD34 and CD14). The cultured and clonal CD45^{neg} lung SP had normal chromosomal structures and expressed high levels of telomerase. After being expanded and cultured in differentiation medium, all populations of CD45^{neg} lung SP demonstrated adipogenic, osteogenic and chrondrogenic potential. Adult CD45^{neg} lung SP cells are a source of MSC.

Discussion

In defining this tissue-specific stem cell population in the lung, we are now better able to clarify a potential role for them in lung diseases.

Keywords

adult stem cells, cell therapy, lung side population cells, mesenchymal stem cells.

Introduction

Pulmonary hypertension, chronic obstructive pulmonary disease, interstitial pulmonary fibrosis and other adult lung conditions are a major cause of morbidity and mortality. Deaths as a result of these conditions have doubled in the last decade. Interestingly, while the lung exhibits tremendous regenerative capacity following tissue resection, regeneration and restoration of pulmonary function do not occur in many adult lung diseases. There is an increasing emphasis on the development of cell-based therapies to address these conditions, but the lung is a recalcitrant candidate for these strategies because of the diverse cell types and functions. We are focused on identifying and manipulating the endogenous lung stem cells.

Both the origins of stem cells for cell-based therapy and contributions of stem cells to pulmonary remodeling are currently under intense investigation. Specialized

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microenvironments, or niches, for resident multipotent mesenchymal stem cells (MSC) have been identified in many adult tissues [1–3]. The normal differentiation processes of these stem cells may be disrupted by pathologic micro-environmental stimuli during disease, epigenetic changes or genetic mutation, which program their contribution to pathologic expansion at the expense of functional tissue regeneration.

Side population cells (SP) were first identified in bone marrow (BM) and are characterized based on their lateral location on a cytometric dot plot as a side arm off the main population [4]. SP cells demonstrate this unique profile when stained with the Hoechst 33342 vital dye, which fluoresces in red and blue when excited by ultraviolet (UV) laser. These cells decrease in fluorescence as the dye is pumped out of their cytoplasm via an ABCG2 transporter mechanism [5,6]. Functional analyzes have defined the BM SP population as multipotent and enriched for hematopoietic stem cells (HSC), distinguished by uniform expression of CD45 [4,7,8]. In other adult tissues, CD45 expression varies and has been used to distinguish between hematopoietic SP and the non-hematopoietic ($CD45^{neg}$) [9–12]. HSC ability is limited to the $CD45^{pos}$ SP fraction [8,9].

SP cells have been reported to reside in many adult tissues other than BM, as well as tumors [9,13]. Recent data suggest $CD45^{neg}$ SP cells may represent an enriched source of organ-specific pulmonary precursors with endothelial, epithelial and mesenchymal potential [2,14,15]. Endothelial precursors arise from the mesenchyme during development [16]. MSC have been isolated from tracheal aspirates from neonates in respiratory distress and from adult bronchoalveolar lavage [17–20]. The origin of MSC from these subjects and their significance to lung pathology is presently unknown.

We sought to characterize a source of lung MSC by isolating $CD45^{neg}$ lung SP single-cell clones, measuring their telomerase expression over time, and analyzing their chromosomal stability. We then assayed the mesenchymal differentiation potential of these cells over time using cultures at passage 3, as well as single-cell clones at passage 50. We hypothesized that these lung SP clones would exhibit stem cell characteristics and mesenchymal differentiation potential *in vitro* and thereby represent a potential source of MSC in the lung that may be used to study the fate of lung stem cells during pulmonary disease.

Methods

Isolation and immunophenotyping of lung SP cells

SP cells were isolated from 12-week-old murine adult C57Bl6 lungs using a 0.2% collagenase digest of lung tissue to obtain a single-cell suspension. Hoechst 33342 staining was performed to identify SP cells, as described previously using a DAKO MoFlo [4,7,9,14,21]. Isolated lung SP cells in these experiments were analyzed for expression of CD45 to separate the CD45 positive (hematopoietic) from negative (mesenchymal) subpopulations [9]. The gates were set using whole BM SP cells. Freshly isolated $CD45^{neg}$ lung SP cells were sorted and expanded in Miltenyi NH medium (Miltenyi Biotech, Auburn, CA, USA), specific for expansion of MSC. Initially, to obtain single-cell clones we sorted freshly isolated SP (passage 0) as a single cell per well in a 96-well plate. They did not give rise to clonal colonies or appear to survive. Because stem cells by definition progress through the cell cycle extremely slowly, we sorted cultured cells. The rationale behind culturing cells and then sorting a single cell per well is to get the cells into active cell cycle in order to increase their odds of survival. Single-cell clones were obtained by separating actively proliferating cultures during passage 3 using trypsin digest into single $CD45^{neg}$ lung SP cells, which were sorted into wells of a 96-well plate. Approximately 1000 clones were isolated. Clones were expanded and analyzed between passages 36 and 50. Cells were isolated from plastic using trypsin prior to analyzes. Cultures were fixed with methanol and morphology assessed by Giemsa staining (Sigma, St Louis, MO, USA).

Analysis of cell-surface hematopoietic and MSC markers (Table 1) expressed by freshly isolated or cultured lung SP was performed by incubating primary antibodies directly conjugated to phycoerythrin (PE), fluorescein isothiocyanate (FITC) or allophycocyanin cells (APC) (BD Pharmingen, San Jose, CA, USA) with 1×10^5 cells for 10 min on ice. The cells were then washed and resuspended for analysis in cold Hanks' containing 2% fetal calf serum (FSC) with propidium iodide (PI) to exclude dead cells [4]. Analysis of freshly isolated SP was performed using a DAKO MoFlo and clones using a Beckman Coulter FC-500. Gates were set using isotype controls, known positive and negative markers. As a control for removal of cell-surface antigens (Ag) by collagenase