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Human induced pluripotent stem cell–derived lung progenitor and alveolar epithelial cells attenuate hyperoxia-induced lung injury

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Abstract

Background aims. Bronchopulmonary dysplasia (BPD), a chronic lung disease characterized by disrupted lung growth, is the most common complication in extreme premature infants. BPD leads to persistent pulmonary disease later in life. Alveolar epithelial type 2 cells (AEC2s), a subset of which represent distal lung progenitor cells (LPCs), promote normal lung growth and repair. AEC2 depletion may contribute to persistent lung injury in BPD. We hypothesized that induced pluripotent stem cell (iPSC)-derived AECs prevent lung damage in experimental oxygen-induced BPD. *Methods.* Mouse AECs (mAECs), miPSCs/mouse embryonic stem sells, human umbilical cord mesenchymal stromal cells (hUCMSCs), human (h)iPSCs, hiPSC-derived LPCs and hiPSC-derived AECs were delivered intratracheally to hyperoxia-exposed newborn mice. Cells were pre-labeled with a red fluorescent dye for *in vivo* tracking. *Results.* Airway delivery of primary mAECs and undifferentiated murine pluripotent cells prevented hyperoxia-induced impairment in lung function and alveolar growth in neonatal mice. Similar to hUCMSC therapy, undifferentiated hiPSCs also preserved lung function and alveolar growth in hyperoxia-exposed neonatal NOD/SCID mice. Long-term assessment of hiPSC administration revealed local teratoma formation and cellular infiltration in various organs. To develop a clinically relevant cell therapy, we used a highly efficient method to differentiate hiPSCs into a homogenous population of AEC2s. Airway delivery of hiPSC-derived AEC2s and hiPSC-derived LPCs, improved lung function and structure and resulted in long-term engraftment without evidence of tumor formation. *Conclusions.* hiPSC-derived AEC2 therapy appears effective and safe in this model and warrants further exploration as a therapeutic option for BPD and other lung diseases characterized by AEC injury.

Key Words: *alveolar epithelial cells, bronchopulmonary dysplasia, induced pluripotent stem cells, lung injury, newborn, oxygen, regenerative medicine*

Introduction

Preterm delivery is a major and growing health care problem, affecting 10% of all births and accounting for more than 85% of all perinatal complications and death [1]. Recent advances in perinatal care enable the survival of premature infants born at increasingly earlier stages of gestation, which is synonymous with earlier stages in lung development [2,3]. Therefore, the prevention of lung injury in this patient population becomes gradually more challenging. Bronchopulmonary dysplasia (BPD), the chronic lung disease that follows ventilation and oxygen therapy for acute respiratory failure after premature birth, remains the most common complication [3]. BPD is characterized by impaired lung development, including simplified alveolar structure and blunted lung capillary growth [4]. BPD has long-term respiratory [5]

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and neurodevelopmental [6,7] consequences, leading to chronic diseases in adulthood [8] and increased health care costs [9]. Currently, there is no effective treatment for BPD.

A subset of alveolar epithelial type (AEC) 2 cells are distal lung progenitor cells (LPCs) that have the ability to self-renew and differentiate *in vivo* and form alveolospheres *in vitro*, harboring both AEC1s and AEC2s [10]. After postnatal lung injury AEC2s undergo proliferation and display regenerative potential [11–14]. Neonatal exposure to hyperoxia in rodents, a well-established model mimicking some of the features observed in BPD [15,16], causes AEC2 depletion [17], apoptosis [18] and necrosis due to high levels of reactive oxygen species [19], inhibition of AEC2 proliferation [20] and AEC2 DNA damage [21,22]. This may contribute to incomplete lung repair and persistent lung disease.

Several studies have reported the generation of AEC2s from induced pluripotent stem cells (iPSCs) [23,24], but the therapeutic potential of human (h)iPSC-derived AEC2s has not been investigated. In the present study, we tested the feasibility, safety and efficacy of therapeutically administering freshly isolated mAECs, undifferentiated murine (m) and hiPSCs as well as hiPSC-derived LPCs and AEC2s in a mouse model of neonatal oxygen-induced injury mimicking features of BPD.

Methods

All procedures were approved by the Animal Welfare Committee of the University of Alberta and Animal Care and Veterinary Service Committee of the University of Ottawa.

Mouse AEC2 isolation and culture

AECs were isolated from the lungs of 6- to 8-week-old CD1 mice. Briefly, the mice were euthanized with intraperitoneal (IP) injection of sodium pentobarbital (Euthanyl; Bimeda-MTC; 0.05–0.1 mL, 65 mg/kg). Cold phosphate-buffered saline (PBS; 10 mL) was injected into the right ventricle to perfuse and flush blood from lung and the heart was removed. The trachea was cannulated with a 22- to 24-gauge catheter, and the lung was instilled with an enzyme mix (30 U Neutral Protease, 2500 U Collagenase I and 500U DNAse-I in 10 mL Dulbecco's PBS with $Mg2^{+}/Ca2^{+}/Na^{-}$pyruvate/glucose) followed immediately by a 1.3% low melting agarose. The lung was dissected, placed into 10 mL of enzyme mixture and incubated at 37°C for 1 h at 300 rpm. Trachea and large bronchi were separated from lungs. In a dish containing 10 mL of 1% AA alpha minimum essential medium, the lungs were dissociated into small pieces using forceps and further with passing through 18- and 21-gauge needles three times. The tissue/cell suspension was filtered through 40-μm falcon strainers into a 50-mL tube and centrifuged for 5 min at 500*g* at 4°C. The cell pellet was washed twice with 20 mL PBS/2% fetal bovine serum (FBS) at 500*g* at 4°C for 5 min and resuspended into 5 mL PBS/2%FBS. The cell suspension was added very slowly to the top of 3 mL Ficoll (1.077 g/m^3) in a 15-mL tube. The cells were spun at 600*g* at room temperature for 20 min with breakoff. The cells were harvested from the interface of PBS and Ficoll and washed three times with 10 mL PBS/2% bovine serum albumin (BSA). The cells were plated onto two or three wells of six-well tissue culture plates for 1 h using Small Airways Growth Media (SAGM, Lonza, Walkersville, MD, USA). The non-attached cells were collected and transferred to two wells of an ultra-low attachment plate using SAGM supplemented with FBS 1% + keratinocyte growth factor (KGF; 10 ng/mL), cyclic adenosine monophosphate (100 μmol/L), IBMX (100 μmol/L) and dexamethasone (50 nmol/L) to enable AEC2 maturation and expansion.

Mouse embryonic stem cells and miPSC culture maintenance

Mouse iPSCs (miPSCs; EOS3F-29) were generated from CD-1 mouse embryonic fibroblasts using the retroviral reprogramming system (Oct4, Sox2 and Klf4) [25,26]. Mouse embryonic fibroblasts (MEFs) were also infected with EOS (early transposon promoter and Oct-4 and Sox2 enhancers) lentiviral vector selection system. This bicistronic EOS vector marked undifferentiated iPSCs with enhanced green fluorescent protein and the puromycin-resistance gene that allowed for puromycin selection of iPSCs expressing endogenous pluripotency markers.

Mouse embryonic stem cells (mESCs; J1 line, Jackson Laboratory) and miPSCs were initially cultured on a feeder layer of irradiated MEFs (Jackson Laboratory) and gradually adapted to feeder-free conditions in six-well plates coated with a solution of 0.1% gelatin in deionized water (Millipore). Cells were grown in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 15% ESC-qualified FBS, 4 mmol/L L-glutamine, 0.1 mmol/L non-essential amino acids, 1 mmol/L sodium pyruvate, 130 μmol/L β-mercaptoethanol (all Invitrogen) and 10^6 U/L leukemia inhibitory factor (LIF, Millipore). Culture medium was replaced daily. For iPSC selection, puromycin (1 μg/mL, Invitrogen) was added to the culture medium. To ensure the absence of inactivated MEFs, ESCs and iPSCs were grown on gelatin-coated plates for at least 4 weeks before *in vivo* administration.