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Paracrine regulation of fetal lung morphogenesis using human placenta-derived mesenchymal stromal cells

Julie Di Bernardo, PhD,[a] *Michael M. Maiden, BS,*[a] *Guihua Jiang, MS,*[a] *Marc B. Hershenson, MD,*[b] *and Shaun M. Kunisaki, MD, MSc*[a,*]

[a] *Department of Surgery, University of Michigan Health System, Ann Arbor, Michigan*
[b] *Department of Pediatrics and Communicable Diseases, University of Michigan Health System, Ann Arbor, Michigan*

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ABSTRACT

Background: Recent experimental work suggests the therapeutic role of mesenchymal stromal cells (MSC) during perinatal lung morphogenesis. The purpose of this study was to investigate the potential paracrine effects of human placenta-derived mesenchymal stromal cells (PL-MSCs) on pulmonary development.

Methods: Human MSCs were isolated from preterm placental chorion. Normal E14.5–15.5 fetal rat lungs were subsequently harvested and cultured *ex vivo* in the presence of conditioned media from PL-MSCs for 72 h. The lungs were analyzed morphometrically and by quantitative DNA, protein, and gene expression. Postnatal human bone marrow-derived mesenchymal stromal cells and neonatal foreskin fibroblasts (FF) were used as controls.

Results: The MSC phenotype of the isolated placental cells was confirmed. Compared with lungs cultured in the absence of PL-MSCs, fetal lung growth was markedly accelerated on exposure to PL-MSC conditioned media as demonstrated by increases in Δlung surface area, terminal bud formation, and Δterminal bud formation. Pulmonary growth was predominantly impacted by enhanced branching morphogenesis, as shown by 73.5 ± 6.1 terminal buds after stimulation with PL-MSCs compared with 46.7 ± 5.7 terminal buds in control unconditioned media ($P < 0.05$). Significant differences were noted favoring PL-MSCs over FFs based on terminal bud formation and Δterminal bud formation ($P < 0.05$). There was significant upregulation of club cell secretory protein in lungs exposed to PL-MSCs compared with all other groups.

Conclusions: These data suggest that human PL-MSCs are potent paracrine stimulators of pulmonary morphogenesis in a fetal organ culture model. Cell therapies based on autologous or donor-derived PL-MSCs may represent a novel strategy for enhancing perinatal lung growth.

* *Corresponding author*. Department of Surgery, C.S. Mott Children's Hospital, University of Michigan Health System, 1540 E. Hospital Drive, SPC 4211, Ann Arbor, MI 48109. Tel.: +1 734 936 8464; fax: +1 734 936 9784.
E-mail address: shaunkun@umich.edu (S.M. Kunisaki).
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1. Introduction

Respiratory failure secondary to pulmonary hypoplasia is a major cause of death and morbidity in neonatal intensive care units worldwide. In the United States alone, the disorder affects over 10,000 infants each year. Many infants develop bronchopulmonary dysplasia (BPD), a premature lung disease characterized by a stereotypical pattern of chronic inflammation and alveolar arrest. Another important cause of lung hypoplasia, congenital diaphragmatic hernia, is associated with impaired pulmonary branching morphogenesis, which eventually leads to profound pulmonary hypertension within the first several days of life. Unfortunately, state-of-the-art medical care, including pharmacotherapy, novel ventilator strategies, and extracorporeal membrane oxygenation has failed to make a substantial impact in altering survival and morbidity in these diseases [1–5]. Among survivors of lung hypoplasia, substantial pulmonary disease can persist well into adulthood, and permanent neurodevelopmental delays are not uncommon [6]. Thus, there remains a vital need for neonatal pulmonary research aimed at improving clinical outcomes.

Recently, stem cell-based approaches have been investigated experimentally as an innovative means toward understanding pulmonary morphogenesis and repair after injury [7–9]. In particular, animal models of perinatal lung disease have suggested a therapeutic benefit of postnatal bone marrow-derived mesenchymal stromal cells (BM-MSCs) on lung development [8,10–12]. The mechanisms involved in the observed lung repair by mesenchymal stromal cells (MSCs) have not been well elucidated but may involve paracrine modulation of inflammatory responses by growth factors, possibly with activation of adjacent lung epithelial progenitors and endothelial cells [13]. However, although the administration of BM-MSCs before BPD lung injury has been shown to prevent hypoalveolarization, most investigators have not shown a dramatic reversal in lung developmental arrest that has already occurred.

Our laboratory, among others, has focused on the potential role of cells derived from extraembryonic tissues during organogenesis [14–16]. In particular, the placenta is known to contain a heterogeneous population of stromal cells of fetal origin, herein referred to as placenta-derived mesenchymal stromal cells (PL-MSCs) [17]. By definition, MSCs are adherent to plastic in culture, have defined immunophenotypic characteristics, possess limited differentiation capabilities, and are susceptible to cell senescence over time [18]. PL-MSCs are predominantly CD117-negative and have been shown to uniquely express higher levels of stimulatory pulmonary morphogens, including hepatocyte growth factor (HGF), a heterodimeric heparin-binding growth factor regarded as a major initiator of normal lung organogenesis [17,19]. Given the apparent profetal lung growth paracrine characteristics of PL-MSCs and their potential role during normal fetal development, we proposed a novel treatment strategy to accelerate pulmonary morphogenesis through the delivery of preterm human PL-MSCs in a fetal rat lung organ culture model.

2. Methods

2.1. Isolation of human placental MSCs

This study was approved by the Institutional Review Board under protocol #38565 at the University of Michigan. Human placentae ($n = 3$) were retrieved fresh from uncomplicated 34–39 wk gestation pregnancies after elective cesarean section at Von Voigtlander Women's Hospital, Ann Arbor, Michigan. Informed consent was obtained from each pregnant mother. The specimens came from one male and two female patients. The chorion was mechanically separated from the overlying amnion and from the underlying chorionic villi and decidua under ×2.5 loupe magnification on the operating room back table. Specimens were immediately transported to the laboratory at 4°C where they were washed extensively in sterile phosphate-buffered saline (PBS; Gibco, Carlsbad, CA). One cm^2 samples were cut into 1 × 1 mm pieces and enzymatically digested in a 10 mL solution of collagenase (1.25 mg/mL, Worthington Biochemical Corp, Lakewood, NJ) and dispase (1.2 U/mL, Worthington Biochemical) for 1–2 h at 37°C with agitation. The solution was filtered through a 40-μm nylon strainer and centrifuged at 700*g* for 15 min. Cells were washed twice in PBS before resuspension in mesenchymal basal medium (DMEM, 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin, and 2 mM L-glutamine; Gibco) and cultured in a humidified incubator, at 37°C with 5% CO_2, until 75%–90% confluent. For controls, human dermal fibroblasts derived from neonatal foreskin (FF) were processed in parallel fashion based on methods as previously described [20]. Human postnatal BM-MSCs were kindly provided by the Prockop laboratory, Texas A&M University, Temple, TX.

Plastic adherent, spindle-shaped cells between passage 3 and 5 were evaluated based on criteria as described elsewhere [21]. For flow cytometry analyses, 5×10^5 PL-MSCs were incubated in a PBS solution with 5% fetal bovine serum, and primary antibody at 4°C for 30 min. Fluorophore-conjugated antibodies against CD11 (BD Biosciences, San Jose, CA), CD34 (Santa Cruz, Dallas, TX), CD44, CD45, CD73, CD90, CD117 (BD Biosciences), CD79, and CD105 (Novus Biologicals, Littleton, CO) were used as well as same isotype antibodies as negative controls. Primary antibodies were used at a dilution of 1:200 in an incubation volume of 200 μL. Evaluation of staining was performed using the LSRII flow cytometer (BD Biosciences), and data were analyzed using FloJo software (Tree Star, Ashland, OR).

For reverse transcription polymerase chain reaction (RT-PCR) experiments, total RNA was extracted using TRIzol (Invitrogen, Carlsbad, VA) and reverse transcribed into cDNA before amplification using primers for alpha smooth muscle actin (αSMA) and runt related transcription factor 1(RUNX1) with the appropriate positive and negative controls. Actin beta (ACTB) was used as the housekeeping gene. Primer sequences: αSMA forw: 5′GATCACCATCGGAAATGAACGT3′, αSMA rev: 5′ TTTA GAAGCATTTGCGGTGGAC3′, RUNX1 forw: 5′GCAAGCTGAGGA GCGGCG3′, RUNX1 rev: 5′GACCGACAAACCTGAGGTC3′. ACTB forw: 5′ GCCGAGGACTTTGATTGC3′, ACTB rev: 5′GTGTGGA CTTGGGAGAGG3′.