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Differentiation of xenografted human fetal lung parenchyma

Jelena Pavlovic [a], Joanna Floros [a,b,c,*], David S. Phelps [b], Brian Wigdahl [d,1], Patricia Welsh [d], Judith Weisz [c], Debra A. Shearer [c], Alphonse Leure du Pree [e], Roland Myers [e], Mary K. Howett [c,d,*,2]

[a] *Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, PA 17033, USA*
[b] *Department of Pediatrics, The Pennsylvania State University College of Medicine, Hershey, PA 17033, USA*
[c] *Department of Obstetrics and Gynecology, The Pennsylvania State University College of Medicine, Hershey, PA 17033, USA*
[d] *Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA 17033, USA*
[e] *Department of Neuroscience and Anatomy, The Pennsylvania State University College of Medicine, Hershey, PA 17033, USA*

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Abstract

The goal of this study was to characterize xenografted human fetal lung tissue with respect to developmental stage-specific cytodifferentiation. Human fetal lung tissue (pseudoglandular stage) was grafted either beneath the renal capsule or the skin of athymic mice (NCr-nu). Tissues were analyzed from 3 to 42 days post-engraftment for morphological alterations by light and electron microscopy (EM), and for surfactant protein mRNA and protein by reverse transcription-polymerase chain reaction (RT-PCR) and immunocytochemistry (ICC), respectively. The changes observed resemble those seen in human lung development *in utero* in many respects, including the differentiation of epithelium to the saccular stage. Each stage occurred over approximately one week in the graft in contrast to the eight weeks of normal *in utero* development. At all time points examined, all four surfactant proteins (SP-A, SP-B, SP-C, and SP-D) were detected in the epithelium by ICC. Lamellar bodies were first identified by EM in 14 day xenografts. By day 21, a significant increase in lamellar body expression was observed. Cellular proliferation, as marked by PCNA ICC and elastic fiber deposition resembled those of canalicular and saccular *in utero* development. This model in which

* Corresponding authors. For xenograft information, contact Dr. Mary K. Howett, Professor and Department Head of Bioscience and Biotechnology, Drexel University, Philadelphia, PA, USA. For information on lung differentiation, contact Dr. Joanna Floros, Evan Pugh Professor of Cellular and Molecular Physiology, Penn State College of Medicine, Hershey, PA, USA.
E-mail addresses: jfloros@psu.edu (J. Floros), bw45@drexel.edu (B. Wigdahl), mkh28@drexel.edu (M.K. Howett).
[1] Current address: Department of Microbiology and Immunology, and Center for Molecular Virology and Neuroimmunology, Center for Molecular Therapeutics, Institute for Molecular Medicine and Infectious Disease, 15 North Vine Street, Philadelphia, PA 19102, USA.
[2] Current address: Bioscience and Biotechnology, Drexel University, Philadelphia, PA 19104, USA.

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xenografted lung tissue in different stages of development is available may facilitate the study of human fetal lung development and the impact of various pharmacological agents on this process.

1. Introduction

Development of a functional alveolar epithelium capable of gas exchange and surfactant secretion is essential for successful adaptation of the fetus to extra-uterine life. Because lung maturation is a late gestational event, respiratory distress is frequent in babies born prematurely. Premature infants are most commonly born during the saccular stage of lung development (26–34 week of gestation) when lung pneumocytes are not yet completely differentiated. These infants often exhibit a deficiency of surfactant production that leads to insufficient gas exchange. Due primarily to a higher rate of multiple gestations, the overall number of premature live births [1] has increased by 26% during the last three decades. This has lead to a significant increase in neonatal respiratory disease, including Respiratory Distress Syndrome (RDS) and Bronchopulmonary Dysplasia (BPD) [2,3].

Premature birth is commonly marked by immaturity of the gas exchange regions, poorly developed blood supply, and a deficiency and perturbation of the surfactant system. Pulmonary surfactant is a lipoprotein complex produced by type II pneumocytes that acts to reduce surface tension at the air–liquid interface in the alveolus and thereby, prevent atelectasis [4,5]. Surfactant proteins (SPs) may contribute to surfactant function (SP-A, SP-B, SP-C) or associate with the surfactant complex but not contribute to its function (SP-D). Each SP has multiple important roles within the alveolus [6,5,7] and is subject to developmentally-and hormonally-regulated expression [8–17]. SP production starts early in development (pseudoglandular stage), but active secretion of functional surfactant (predominantly in the form of lamellar bodies) is initiated during the saccular stage of development, after almost 75% of gestation is completed [8].

While lung development has been extensively studied in multiple models involving different animal species, studies of human lung development have primarily been limited to fetal lung explants [10,12–16]. Although the importance of the fetal lung explant model is undisputed, the highly accelerated morphological development and limited viability of explants in cell culture (on the order of days) limits their use in studies of cellular and molecular changes specific for particular stages of lung development and studies over longer periods of time.

Models of organ development have been developed using immunocompromised mice as hosts for grafted fetal tissue [18,19]. Various xenograft models have been used extensively in cancer research to study mechanisms of carcinogenesis and the effects of different pharmacological agents on human tumor growth [20,21], as well as in studies of transmission of infection in various tissues by pathogens [22–26]. With respect to lung embryogenesis, immunocompromised mice have been used to study whole organ lung development in mice [27,28], as well as lower [29,30] and upper airway development in humans [31–36]. Allograft models have been used where whole embryonic mouse lungs were grafted into immunodeficient mice [27,28]. Progression of structural development as well as appearance of mature alveoli was observed 14 days after grafting. It has also been observed that [19] human fetal lung tissues grafted into a mouse treated with antilymphocytic serum (ALS, lymphocyte suppressing antibodies) could develop and grow in the host for up to 90 days. Others observed differentiation of lung-specific cell types in two, five, and eight week old transplant grafts [29]. More recently, a number of studies involving xenograft models of human upper airway development have been reported [31,36]. The focus in these studies was on the development of bronchial xenografts to study the pathogenesis of cystic fibrosis [33,34]. Although these studies have clearly shown that human embryonic lung tissue can be successfully differentiated in immunocompromised mice, the focus was on the endpoint of tissue development, rather than on the characterization of developmental processes.

Our goal was to characterize a model for the study of successive stages of development of the human lower airways, with particular emphasis on the expression of surfactant components. Based on the knowledge available from a number of lung xenograft models [18,19,27–36], we hypothesized that human fetal lung parenchyma grafted into immunocompromised mice will undergo changes that mimic the stages of *in utero* lung development in many respects. The assumption was that although grafted tissue may undergo accelerated maturation, this acceleration would not be as rapid as that seen in fetal lung explants, enabling one to study stage-specific processes of lung development. In the present study, we characterized these stage-specific processes with respect to the progressive cytodifferentiation of a model of human fetal lower airway development.

2. Materials and methods

2.1. Fetal lung xenografts

Six to fourteen week-old female NCr-nu (nude) (Taconic Farms, Germantown, NY) were used for both renal subcapsular and subcutaneous grafts. Animals were maintained under pathogen-free conditions and were fed sterilized rodent Purina 5K52 diet. Research involving all animals followed the "Guiding Principles in the Care and Use of Animals" by the Council of the American Physiological Society and was approved by The Penn State College of Medicine Institutional Animal Care and Use Committee. Human fetal lung tissues ($n=5$), ranging from 13 to 17 weeks of gestation, were obtained from legal abortions where written informed consent was obtained according to a protocol approved by the Penn State College of Medicine Institutional Review Board. Fetal age was obtained from clinical information and confirmed by fetal foot-length measurements. To ensure that graft tissue was derived from fetal lung, only whole lung lobes were used for preparation of xenograft tissue. Fresh lower airways were cut under sterile conditions into 1–3 mm^3 pieces. Surgery was performed on nude mice anesthetized by intraperitoneal injection of