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Submucosal gland development in the human fetal trachea xenograft model: implications for fetal gene therapy☆

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Abstract

Background/Purpose: Our previous work in a human-fetal trachea xenograft model suggests potential benefits of treating cystic fibrosis in utero. The target for postnatal gene therapy in cystic fibrosis is tracheal submucosal glands (SMGs). The aim of this study was to determine if SMG development in our model recapitulates normal trachea development and its validity for studying fetal gene transfer.

Methods: Fetal tracheas were divided into developmental phases: early, mid, and late. Fetal tracheas were xenografted onto immunocompromised mice and analyzed for SMG developmental staging and mucopolysaccharide production.

Results: There were no significant differences in gland number, size, or density from early through late phase between groups. Xenografted tracheas demonstrated a similar progression through the stages of SMG development as controls after an initial phase shift. Control and xenografted tracheas demonstrated characteristic patterns of acidic mucin production at the base of the SMGs.

Conclusions: Fetal trachea xenograft SMG recapitulates normal development and is a valid model for studying human fetal gene transfer. The accessibility of SMG stem cells in early tracheal development may afford a unique window of opportunity for gene transfer. This model has the benefit of providing access to human fetal tracheas in vivo and permits the study of novel fetal gene therapy strategies.

Cystic fibrosis (CF) is the most common lethal monogenetic disease, which is because of the absence of a functional cystic fibrosis transmembrane conductance regulator (CFTR) protein [1]. Replacement of a functionally active CFTR gene has been limited by physical and immunologic barriers to gene transfer vectors in postnatal, preclinical studies [1]. Postnatal gene transfer has been especially ineffective in reaching submucosal gland (SMG) cells, the presumed target cells for CFTR gene transfer. This may be because of physical barriers, such as mucous production, or

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the relative distance to the tracheal lumen of the SMGs. The SMG cells express the highest levels of CFTR in the entire tracheobronchial epithelium [2] and are postulated to be the location of probable airway stem cells. These factors support the concept that the SMG cells are the biologic target cells of tracheobronchial epithelial gene transfer [3]. Clinical trials for CFTR gene transfer have failed to achieve efficient gene transfer in the surface epithelium, let alone the SMG cells [1]. The discrepancy between gene transfer efficiencies in animal models and human clinical trials may be because of species-specific differences in the SMG cells between humans and lower species [4].

Inefficient postnatal gene transfer has led us to hypothesize that the fetal environment may be more receptive to gene transfer because of decreased physical barriers, an immunologically permissive environment, and more readily accessible developing SMG cell population and respiratory epithelial stem cell population. We have tested this hypothesis by using a human fetal trachea xenograft model where we take whole human fetal tracheas and implant them into subcutaneous pockets in severe combined immunodeficient (SCID) mice [5,6]. We have previously reported efficient transduction in this xenograft model using adenoviral- and pseudotyped lentiviral-based vectors [5,6], suggesting that the fetal trachea may be a permissive environment for gene transfer. This model has the benefit of representing a human fetal tracheobronchial tree in an adult environment that readily permits the study of viral vector-cellular interactions and evaluation of transduction efficiencies in an in vivo setting. Previously, we have qualitatively observed that these xenografted tracheas demonstrate progressive gland development [7]; but we have not determined if this model is developmentally representative of the normal human fetal trachea. To assess gland development, we have used a staging system of SMG development described by Thurlbeck et al. [8] (Fig. 1). In this classification, SMGs progress from cords of immature SMG cells (stage I) to the complex acinar structures found in postnatal glands (stage IV).

In the present study, we aim to determine if the human fetal trachea-SCID mouse xenograft model is developmentally representative of normal human tracheal development. To accomplish this goal, we morphologically and functionally analyzed the development of the SMG, the target cell for CF, in the xenograft and compared it with gestational age (GA) matched normal human fetal trachea SMG development.

1. Methods and materials

1.1. Human fetal tracheas

Legally aborted human fetal tracheas were obtained from Advanced Bioscience Resources (Alameda, CA). Gestational age of aborted fetuses was based on prenatal ultrasounds.

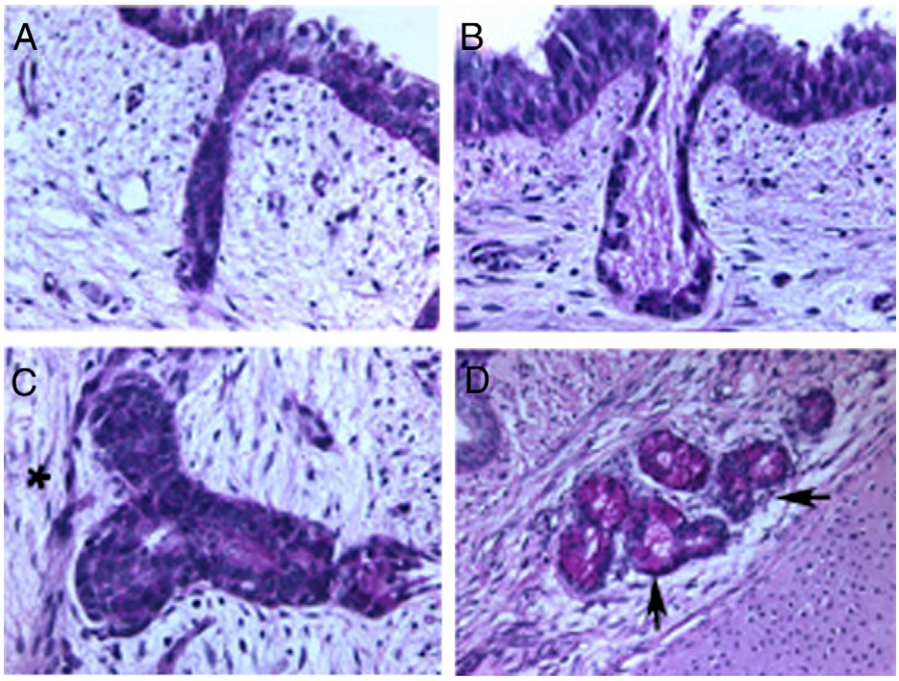

Fig. 1 Submucosal gland staging in human fetal xenografts and normal fetal tracheas. A tracheal SMG development staging system described by Thurlbeck et al. [8] was used to assess gland development in xenografts and controls. In stage I, epithelial buds grow as cords in to the submucosal matrix (A); these cords then develop lumens in stage II (B) and then divide laterally into 2 ducts as the gland approaches the cartilaginous ring (* denotes cartilage border) in stage III (C). Stage IV is characterized by maturation into the complex acinar structure of the postnatal SMG (D) (PAS stain).