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Mucin gene cDNA sequence characterization in chinchilla middle ear epithelium☆

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Summary

Objectives: To identify mucin genes in chinchilla middle ear epithelium and characterize complimentary deoxyribonucleic acid (cDNA) sequences to facilitate further investigations into mucin physiology and pathophysiology on a molecular level using the chinchilla model.
Methods: Chinchilla mucin gene exploration and cDNA characterization was accomplished using reverse transcriptase-polymerase chain reactions (RT-PCR). Forward and reverse primer pairs were designed using consensus sequences available for human and rodent species. Chinchilla middle ear epithelium was harvested and primary cell cultures (CMEEC) were established. The CMEEC were explored for the expression of chinchilla mucin genes 1, 2, 4 and 5AC (cMuc1, cMuc2, cMuc4 and cMuc5AC). Identified cDNA amplicons for each of these genes was sequenced and homology compared to previously published human and rodent sequences.
Results: CMEEC express all four of the mucin genes cMuc1, cMuc2, cMuc4 and cMuc5AC. cDNA amplicons for each of the genes were able to be sequenced with lengths ranging from 66 to 362 base pairs. Each of the chinchilla cDNA sequences expressed significant homology with published human and rodent cDNA for these

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mucin genes. A cDNA sequence for the housekeeping gene, β-actin, was also identified.
Conclusions: Chinchilla middle ear epithelium grown in culture expresses the mucin genes 1, 2, 4 and 5AC, which have been identified as important in mucin regulation in the middle ear. cDNA sequences corresponding to these mucin genes were identified and may serve as important molecular tools in future studies of otitis media using the chinchilla model.

1. Introduction

Otitis media is the most common diagnosis in pediatric patients who visit physicians for illness in the United States [1], causing an estimated 5 million annual episodes at a national cost of $3—$6 billion [2]. Approximately 5—10% of acute otitis media progresses to chronic otitis media with effusion (COME), which is a leading cause of hearing loss in children.

Despite the significant potential for morbidity from otitis media and increasing therapeutic challenges imposed by antimicrobial resistance, much is still unknown about the cellular and molecular events in this disease process and few good models exist to further our study of otitis. Mucins are high molecular weight glycoproteins produced in a variety of conditions but are particularly important in respiratory epithelium such as that found in the nasal cavity, trachea and middle ear. Variation in the quantity and character of middle ear secretions and specifically mucin secretion is known to be important in the pathophysiologic mechanisms of otitis media [3,4]. Mucins are the only component of middle ear effusions responsible for its rheologic properties and are responsible for creating a high viscosity fluid that can prevent normal mucociliary clearance [5—7], which, in turn, causes pathology such as chronic otitis media and hearing loss. However, mucins are also known to be important in normal host defenses through participating in mucociliary clearance of pathogens, providing protective barriers to underlying epithelium and interacting with the host's innate immune mechanisms [8—10]. In addition, there is evidence that epithelial mucins interact with biofilms [11—13], which have recently been implicated as central to the pathogenesis of chronic otitis media, further supporting the importance of these glycoproteins in regulating middle ear epithelial physiology. The mounting evidence of the significance of mucin in middle ear pathophysiology, the importance of the chinchilla as an animal model in the study of otitis media and the complete lack of molecular tools to allow for mucin experimentation using this animal model prompted this study.

2. Methods

2.1. Animal donor

Tissues used in these experiments were obtained from mature (6—10 month old), 400—600 g mixed breed chinchillas (Moulton, Rochester, MN). Animals were treated in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act; the animal use protocol was approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin. A total of six chinchillas were required to perform the experiments described. Prior to tissue harvest, chinchillas were anesthetized with 50 mg/kg ketamine hydrochloride (Phoenix, St. Joseph, MO, USA) and then euthanized with an intracardiac injection of 2 mg pentobarbital (Abbott Laboratories, North Chicago, IL, USA) as approved by the Panel on Euthanasia of the American Veterinary Medical Association. The temporal bone, including tympanic membrane and middle ear cavity, was removed bilaterally. The tympanic membrane and middle ear cavity was examined closely to identify any evidence of infection. Following harvest of the temporal bones and preparation the entire trachea and small bowel were also harvested. Immediately after harvest, total ribonucleic acid (RNA) was extracted from the trachea and small bowel using RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. A dounce homogenizer was used to homogenize the tissue. DNase digestion was performed using the RQ1 RNase-Free DNase (Promega Madison, WI, USA). The yield and purity was determined by spectrophotometric determination. Purified RNA was stored at −70 °C until RT-PCR analysis.

2.2. Creating primers for RT-PCR

In order to design primers for RT-PCR in an animal model with no previous publications of mucin genomic sequences, previously published sequences of other rodent species (rat and mouse) and human species were analyzed to assess for potential areas