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Immunohistochemical demonstration of airway epithelial cell markers of Guinea pig

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ABSTRACT

The guinea pig (*Cavea porcellus*) is a mammalian non-rodent species in the Caviidae family. The sensitivity of the respiratory system and the susceptibility to infectious diseases allows the guinea pig to be a useful model for both infectious and non-infectious lung diseases such as asthma and tuberculosis. In this report, we demonstrated for the first time, the major cell types and composition in the guinea pig airway epithelium, using cell type-specific markers by immunohistochemical staining using the commercial available immunological reagents that cross-react with guinea pig. Our results revealed the availability of antibodies cross-reacting with airway epithelial cell types of basal, non-ciliated columnar, ciliated, Clara, goblet and alveolar type II cells, as well as those cells expressing Mucin 5AC, Mucin 2, Aquaporin 4 and Calcitonin Gene Related Peptide. The distribution of these various cell types were quantified in the guinea pig airway by immunohistochemical staining and were comparable with morphometric studies using an electron microscopy assay. Moreover, this study also demonstrated that goblet cells are the main secretory cell type in the guinea pig's airway, distinguishing this species from rats and mice. These results provide useful information for the understanding of airway epithelial cell biology and mechanisms of epithelial–immune integration in guinea pig models.

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1. Introduction

Guinea pigs (*Cavea porcellus*) are mammals in the Caviidae family, which are currently designated as a nonrodent species (D'Erchia et al., 1996; Graur et al., 1991). They share many similarities with humans, including hormonal and immunologic responses, pulmonary physiology, exogenous vitamin C requirement and delayed-type hypersensitivity (DTH) reaction to infections such as tuberculosis (Padilla-Carlin et al., 2008). These biological characteristics make guinea pigs valuable animal models for studying developmental biology and the pathogenesis of numbers of diseases (Mess, 2007; Padilla-Carlin et al., 2008; Soliman, 1990). Of the similarities, the sensitivity of the respiratory system and susceptibility to infectious diseases lead guinea pigs to be broadly used as models of respiratory diseases such as asthma and tuberculosis (Kashino et al., 2008; Williams et al., 2009; Wright et al., 2007). With respect to the pathogenesis and immune response to these diseases, guinea pigs were more representative of a human than models using a rodent species such as mice.

The lung is an organ directly open to the environment, which is lined by many distinct types of epithelial cells in different anatomical regions. The respiratory epithelium constructs a large surface area in contact with particles of pollutants, microorganisms, and antigens in the environment. The respiratory epithelium and its antimicrobial products (such as lysozyme and lactoferrin), together inflammatory cells including macrophages, dendritic cells, neutrophils, natural killer cells and cytotoxic T cells—compose the main cellular components of innate immunity in the airway to deactivate or clear inhaled pathogens (Bartlett et al., 2008; Opitz et al., 2010). The respiratory epithelial cell biology in humans, rodents (rats and mice) and other laboratory animals such as ferrets, has been extensively investigated (Boers et al., 1996, 1998, 1999; Liu et al., 2006a; Mercer et al., 1994; Plopper et al., 1980a; Rogers, 2003; Wang et al., 2001). As an important animal model in the studies of

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Table 1
Primary antibodies used for immunohistochemical staining in guinea pig tissues.

Antibody	Host	Dilution	Company	Catalog number	Works for IHC
Keratin 14	Rabbit	1:500	Thermo Sci	RB-9020	Yes
Keratin 14	Mouse	1:1000	Thermo Sci	MS-115	Yes
Keratin 18	Rabbit	1:200	Abcam	Ab32118	Yes
Keratin 18	Mouse	1:200	Millipore	MAB1600	No
Keratin 18	Mouse	1:200	Lab vision	MS-1242	No
Tubulin IV	Mouse	1:2000	BioGenex	MU178-UC	Yes
Mucin 5AC	Rabbit	1:500	Santa Cruz	sc-20118	Yes
Mucin 5AC	Mouse	1:200	Thermo Sci	MS-145	No
Mucin 2	Rabbit	1:500	Santa Cruz	sc-15334	Yes
Aquaporin 4	Rabbit	1:500	Millipore	MAB3068	Yes
CGRP	Rabbit	1:5000	Sigma	C8198	Yes
CGRP	Rabbit	1:200	Abcam	Ab8056	No
CGRP	Goat	1:500	Abcam	Ab36001	No
pro-SPC	Rabbit	1:5000	Millipore	AB3786	Yes
CCSP	Rabbit	1:5000	USBiological	C5828-03	Yes
CCSP	Rabbit	1:1000	Millipore	07-623	No
CCSP	Goat	1:2000	Santa Cruz	Sc-9773	No

both pulmonary allergic and infectious diseases (such as asthma and tuberculosis, respectively), little information on the airway epithelial cell biology is available for guinea pigs, mainly due to the lack of appropriate immunological reagents in comparison with other species. Using electron microscopy and morphological analysis, the morphology and ultrastructure of distal airway epithelium (Davis et al., 1984; Tyler, 1983) and non-ciliated epithelial (Clara) cells (Plopper et al., 1980a,b) of guinea pigs have been well documented. The morphometry of the developing lungs of fetal guinea pigs have also been investigated (Collins et al., 1986).

Markers for a diversity of airway epithelial cell types have been identified for humans and mice. This has made possible numerous studies on airway epithelial cell biology, stem cell biology, and immunology of specific epithelial cell populations in these species (Boers et al., 1998, 1999; Crosby and Waters, 2010; Liu et al., 2006a, 2009; Senju et al., 2000). However, unlike that demonstrated in other species of laboratory animals and humans, there is no report concerning the availability of epithelial cell type-specific markers for the epithelial cell types in the airway of guinea pigs. To this end, we have investigated the epithelial cell types of guinea pig airways using commercially available antibodies against epithelial cell type-specific markers of other species. Our results clarify that few of the available immunological reagents cross-reacting with guinea pigs and can be employed in the studies of guinea pig airway epithelial cell biology. However, several useful cross-reactive antibodies were identified that will facilitate future investigations in this species.

2. Materials and methods

2.1. Animals and tissue processing

The animal care and all experimental procedures were carried out according to ethical guidelines established by the Ningxia University. Three month-old healthy outbred Kunming White mice (23 ± 5 g) and outbred Hartley–Duncan guinea pigs of both sexes (300 ± 50 g) obtained from the Animal facility of Ningxia Medical University (Yinchuan, China). They were housed in the animal facility under clean condition (not specific-pathogen free, non-SPF) according to the Housing and Husbandry Guidelines for Laboratory Animals of Ningxia Medical University. The animal was euthanized with an overdose of intraperitoneal injection of sodium pentobarbital (50 mg/kg) in the facility, and the lungs were perfused with PBS prior to harvesting. The trachea and lungs were removed and fixed in 10% neutral buffered formalin or 2.5% glutaraldehyde (for electronic microscopy) immediately following the perfusion, immediately followed by being fixed and processed for embedding in a RMC Paraffin Tissue Processor (Model 1530, Research & Manufacturing Inc.). Nine animals from each species were evaluated in this study.

2.2. Electron microscopy

Glutaraldehyde-fixed guinea pig and mouse tracheas were stained with 1.25% osmium tetroxide in PBS, dehydrated, and sputter coated prior to visualization on a Hitachi S-450 microscope (Tokyo, Japan) for scanning electron microscopy (SEM). For transmission electron microscopy (TEM), tissues were fixed as for SEM, followed by infiltration with Spurr resin following dehydration. 80 nm serial sections were then viewed on a Hitachi H-7000 Electron Microscope (Tokyo, Japan).

2.3. Immunohistochemical staining

The immunohistochemistry (IHC) staining was performed on 6 μm thick paraffin sections. Paraffin sections were dewaxed in xylene, followed by incubation in methanol containing 0.3% H_2O_2 for 30 min to inactivate endogenous peroxidase. Sections were then rehydrated in a series of graded ethanol according to histological standards. The antigen was retrieved by boiling in citrate buffer (pH 6.0) for 20 min, followed by to slow cooling down for 2 h at room temperature (RT). The sections were then blocked with blocking buffer (5% horse serum in PBS) at room temperature for 2 h before incubating with a primary antibody (diluted in blocking buffer) in a humid chamber at 4 °C overnight. Following washing three times for 5 min in PBS, a mixture of biotinylated horse anti-rabbit or mouse (Vector Laboratories, Burlingame, CA, USA) secondary antibodies was applied at room temperature for 2 h. The antigen was detected with an elite ABC kits and developed with a DAB Peroxidase substrate kit (Vector Laboratories, Burlingame, CA, USA). The antibodies and working dilutions used in this study were listed in Table 1. Finally, the stained sections were briefly (10 s) counterstained with haematoxylin (Gill's formula) (Vector Laboratories, Burlingame, CA, USA). Followed by rinsing in running tap water for 5 min, dehydrated, cleared with xylene and mounted in Permount (Fisher Scientific, Pittsburgh, PA, USA). Periodic Acid Schiff's (PAS) staining was conducted using Schiff's reagents from Sigma (St. Louis, MO, USA). Synthesized Calcitonin Gene Related Peptide (CGRP) was product of Phoenix Pharmaceuticals, Inc. (Burlingame, CA, USA). The staining was visualized under a light microscope and the picture was captured using a Leica DFC300 F camera. For all antibodies tested, mouse positive tissues and negative tissues were utilized as positive and negative controls, respectively.

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