

Developing bronchial submucosal glands of human foetus –epithelial mucin histochemical study

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Abstract

Background: Mucins are the principle organic constituents of mucus. These serve as first line of protection and act as protective barrier against pathogens and toxins which contributes to innate defense system in mucosal immunity. Mucin proteins are known for providing protection and lubrication to epithelial surface. The aim of the present study was to know the mucin pattern in submucosal bronchial glands of human foetus.

Methods: Foeti were divided into 3 groups according to gestational age (GA): Group 1- GA between 16weeks-26weeks, Group 2 -GA between 27 to 32 weeks, Group3- GA between 33weeks to full term. The tissues were processed and embedded in paraffin wax and sectioned at 6 microns. The sections were stained with the different stains to demonstrate the epithelial mucins . Haematoxylin and Eosin, Periodic acid Schiff (PAS), PAS-Diastase , PAS-Phenyl hydrazine, Alcian Blue (ph-2.5), Alcian Blue (ph-1.0)- Aldehyde Fuschin, Alcian Blue-PAS, Aldehyde Fuschin with Alcian Blue.

Results: In group 1(16-26 weeks)—all three types of mucins were present. Sialo mucins and neutral mucin concentration was more compared to sulpho mucins. In group 2(27-32weeks) – all three types of mucins were present. There was an increase in sulpho mucin concentration compared to sialo and neutral mucins. In group 3 (33- full term) – all three types of mucins were present. There was an increase in sulpho mucins and neutral mucins compared to sialo mucins.

Conclusion: There is change in functional collaboration of the cell before change in structure and hence affects the formation of mucins. Early detection of mucinal changes may be the first step before structural changes occur in many diseases.

Key words: Acidic mucins; carboxylated mucin; sialo mucins; neutral mucins; sulphated mucins; bronchial submucosal glands.

Introduction

Mucins are secreted by different types of cells and glandular tissues. This is a high- molecular weight glycoprotein with mixture of water, ions, proteins and lipids that plays important roles at several mucosal surfaces of the body including respiratory, gastrointestinal and uro-genital tracts. Mucins are the principle organic constituents of mucus. They serve as first line of defence and act as protective barrier against pathogens and toxins which contributes to the innate defence system in mucosal immunity¹. They may be present as mixtures of different types of epithelial mucins. They have been classified into neutral and acidic type; acidic mucins are again

divided into sialo mucins and sulfo mucins². These are secreted by different cell types in the airway: goblet cells of the surface epithelium and mucous cells of the sub mucosal glands. Therefore, mucins present in the airway lumen are a mixture secreted from the two different cell types.

Mucin proteins are known for providing protection and lubrication to epithelial surfaces; in addition, their roles in cell signalling are beginning to be elucidated .The aberrant expression of mucins, as found in many cancers, is likely associated with cancer biology as alterations in the expression and/or glycosylation patterns of various mucins influence cellular growth, differentiation, transformation, adhesion, invasion,

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and immune surveillance³.

Mucin histochemistry is one of the important techniques, which can be done with ease in routine laboratories not demanding much technical expertise and moreover it is cost effective technique.

Present study was under taken to study the mucin normal distribution and histochemical characteristics of human foetal submucosal glands of bronchus in different weeks of gestational age.

Materials and Methods

After obtaining Institutional ethical committee clearance, 50 normal bronchus samples from human foetus were collected from S. N. Medical College and HSK & RC Bagalkot. **Inclusion criteria:** Normal aborted foetus; **Exclusion criteria:** Foetus with congenital anomalies.

Foeti were divided into 3 groups according to gestational age (GA): Group 1- GA between 16weeks-26weeks; Group 2 -GA between 27 to 32 weeks; Group 3- GA between 33weeks to full term.

The tissues obtained were fixed in 2% calcium acetate in 10% formalin and pinch of phosphotungstic acid to preserve mucin. They were kept for 3 to 4 days at room temperature. Later, tissues were processed and embedded in paraffin wax and sectioned at 6 microns. The sections were stained with the following standard stains to demonstrate the different epithelial mucins.

1. Haematoxylin and Eosin- To know the normal

architecture of the tissue

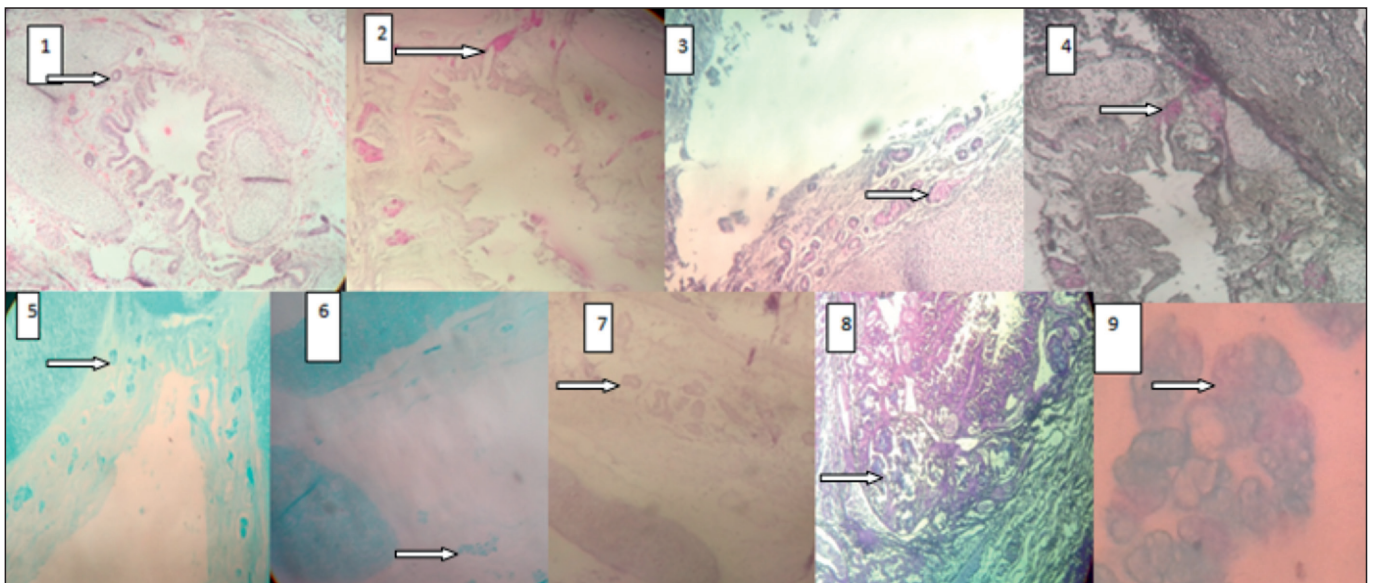
2. Periodic acid Schiff (PAS) - PAS positive substance
3. PAS-Diastase - Glycogen and confirmation of neutral mucin
4. PAS-Phenyl hydrazine- Confirmation of neutral mucin
5. Alcian Blue (pH 2.5)- Acidic mucin contains both sulphated and sialo mucin
6. Alcian Blue (pH-1.0)- Demonstration of sialo mucins
7. Aldehyde Fuschin Stain- Confirmation of sulphated mucin
8. Alcian Blue-PAS-To distinguish between acidic mucins and neutral mucins
9. Aldehyde Fuschin with Alcian Blue- Distinguish between sulphated mucin and carboxylated mucin (sialo mucin).

All quality control measures were followed carefully.

Results

In all 3 groups, the foetal bronchial glands contained glycogen, neutral mucins, sulphomucins and carboxylated mucins (sialomucins) in different concentrations (Figure 1).

- **In group 1 (16-26 weeks)**—All three types of mucins were present. Sialo mucins and neutral mucins concentration was more compared to sulpho mucins.



1 .Haematoxylin and Eosin (H&E) 4X .2. Periodic acid Schiff (PAS) 10X. 3. PAS-Diastase(PAS-D) 10X. 4. PAS-Phenyl hydrazine (PAS-PH) 10X .5 .Alcian Blue (ph-2.5)- (AB) 10X 6. Alcian Blue (ph-1.0) 10X 7 . Aldehyde Fuschin Stain (AF) 10X .8. Aldehyde Fuschin with Alcian Blue (AF-AB 2.5) 40X .9. Alcian Blue 2.5 -PAS- (PAS-AB 2.5) 40X

Figure 1. Bronchial submucosal glands of human foetus

- **In group 2 (27-32 weeks)** – All three types of mucins were present. There was an increase in sulpho mucin concentration compared to sialo and neutral mucins
 - **In group 3 (33 weeks - full term)** – All three types of mucins were present. There was an increase in sulpho mucins and neutral mucins compared to sialo mucins.
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Discussion

There is predominance of epithelial glycogen in first half of foetal life prior to the appearance of hepatic glycogen. Glycogen synthesizing enzymes were more prominent in foetal than in adult specimens including man. Synthesis of mucin is initiated in rough endoplasmic reticulum of the producing cells and in the Golgi apparatus. Role of glycogen or its breakdown products involved in synthesis of glycoprotein in different species⁴. In general sulphated; sialo mucins are more predominant in all parts of the respiratory tract⁵.

Mucin secreted by tracheobronchial glands in the foetus, newborn and child under four years of age is different from that of adults. Secretary granules of the serous cells contain an acid glycoprotein including sialic acid, sulpho mucin and mucosubstances⁶.

Conclusion: In all 3 groups, foetal bronchial glands contain glycogen, neutral mucins, sulpho mucins and carboxylated mucins (sialo mucins) in different concentrations.

There was an increase in sialo mucins compared to neutral and sulfomucins in 2nd trimester and increase in sulpho mucins in 3rd trimester of foetal life.

There is change in functional collaboration of the cell before change in structure and hence affects the formation of mucins. Hence, early detection of mucinal changes may be the first step before structural changes occur in many diseases.

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