ARCHIVES OF ORAL BIOLOGY 55 (2010) 830–841

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journal homepage: http://www.elsevier.com/locate/aob

MUC1 and the simple mucin-type antigens: Tn and Sialyl-Tn are differently expressed in salivary gland acini and ducts from the submandibular gland, the vestibular folds, and the soft palate

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ARTICLE INFO

Article history:
Accepted 27 July 2010

Keywords:
MUC1
Tn antigen
Sialyl-Tn antigen
Submandibular gland
Larynx
Vestibular folds
Soft palate
Cancer

ABSTRACT

Objective and design: Autopsies of the submandibular gland, the vestibular folds and the soft palate from 65–87 old humans were examined to record the immunohistochemical expression of MUC1 and the simple mucin-type antigens Tn and Sialyl-Tn.

Results: (1) The serous acini in the submucosal glands from the larynx and the soft palate expressed MUC1-associated glycans that were not detectable in the serous acini from the submandibular gland. (2) Virtually all the submucosal acini at oral site of the soft palate are mucous, and in contrast to mucous acini in the vestibular folds and submandibular gland, the palatinal acini in the submucosa underneath the oral mucosa showed a well-defined cytoplasmic reaction with anti-MUC1 antibodies as wells as with anti-Tn. (3) Both the mucous acini and the ducts at the oral site of the soft palate showed reaction for Sialyl-Tn while in the vestibular folds and in the submandibular gland expression for this carbohydrate was observed only in the acini. (4) The staining obtained after incubation with the Tn antibodies showed no cross localization with the staining obtained after incubation with an anti-A blood group antibody. (5) All the autopsies showed reaction in the glands after incubation with the MUC1 antibodies while some autopsies reacted with the anti-Tn antibodies and/or with the anti-Sialyl-Tn antibodies and others did not.

Conclusion: The mucin expression in the acini and ducts from the upper human aerodigestive tract strongly depended on the location of the glandular tissue.

1. Introduction

Mucins are high-molecular weight glycoproteins rich in carbohydrates attached via O-glycosidic linkages to serine or threonine. In the human alimentary and airway tracts they are produced by goblet cells and by serous, mucous or mixed exocrine glands and in neoplastic epithelial cells the mucins may be overexpressed and change their glycosylation[1,2] often leading to a reduction in the length of the carbohydrate chains and to demasking of normally cryptic peptidic and carbohydrate structures.[3] Until now at least 20 distinct epithelial mucins have been identified. Among them MUC1 is present in many simple and glandular epithelia and tend to be overexpressed and change glycosylation in head and neck tumours (HNSCC).[4–7]

Mucins are considered to play a protective and regulatory role in normal epithelial tissues. Being secreted to the surface of the epithelia MUC1 mucin binds bacterial and viral pathogens. An anti-inflammatory role that may be mediated through

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0003–9969/$ – see front matter
doi:10.1016/j.archoralbio.2010.07.007

inhibition of toll-like receptor signalling has been proposed together with cell–cell and cell–matrix interactions.[8–10] Mucins contribute to the defence of the oral cavity and in unstimulated whole saliva they make up to 20–30% of the total protein amount. MUC1 which is a membrane associated mucin may, like the gel forming mucins, adds to the protection of epithelial surfaces. It has a rigid extended conformation due to the presence of many O-linked glycans and proline residues in the tandem repeat region. Based on its size MUC1 molecules could be expected to extend up to 0.5 μm from the cell surface, and the tooth surface is thus continuously protected against wear by a film of salivary mucins and proline-rich glycoprotein that may prevent the adherence to the enamel pellicle.[11,12]

The simple mucin-type carbohydrate antigens, the Tn antigen (GaNAcα1-O-Ser/Thr) and Sialyl-Tn (Sialylα2-6GalNacα1-O-Ser/Thr) are normally masked in human cells and secretions partly due to chain elongation and/or branching by addition of other sugar residues.[13] These core structures of mucins are known to be cancer associated[14] and have been demonstrated in oral carcinoma[15] although they also show restricted expression in normal and hyperplastic tissues.[16]

MUC1 expression, determined by immunohistochemistry and in situ hydridization, has previously been described in the ducts and some acini in the major and minor (only reaction in ducts) salivary glands.[17–20] Reports concerning MUC1 localization in the upper respiratory system the results are conflicting. Some authors observed no MUC1mRNA in the submucosal glands using in situ hybridization[21,22] while others employing immunohistochemistry showed antibody localization in the submucosal glands.[18,23] In spite of the many papers on mucin localization in the oral cavity there seems be no studies that compare the distribution of MUC1 mucin and the simple mucin-type antigens Tn and Sialyl-Tn in the upper aerodigestive tract and accessory glands from normal individuals. In this study we have used immunohistochemistry to determine the in situ localization of MUC1, Tn and Sialyl-Tn antigens in autopsies samples from the submandibular gland, the soft palate and the vestibular folds of the larynx.

2. Materials and methods

2.1. Tissue samples

From 15 deceased men and women (age 65–87 years) tissue samples of the submandibular gland, the soft palate and the vestibular folds of the larynx were dissected less than 36 h postmortem. All the autopsies were achieved from humans that had no known history of HNSCC and which, postmortem, showed no lesions in the upper aerodigestive tract as evaluated by both gross anatomical and histological examinations. Although fresh material should be preferred it is impossible and unethical to obtain biopsies from the above mentioned areas from a healthy individual and therefore autopsies had to be used in the present study. Further, Therkildsen et al.[24] have previously used human autopsies to evaluate the immunohistochemical localization of mucin-type carbohydrate antigens and concluded that the staining of salivary glands from the autopsy material showed no differences compared with salivary glands from their surgical material.

The tissue blocks were fixed with a freshly prepared mixture of alcohol (three parts) and glacial acetic acid (one part) as this fixative has proven suitable for preserving glycan antigens in tissues.[25] The autopsies immersed in the fixative for 24 h, stored in Tris buffered saline for 48 h, were embedded in paraffin and cut in 4 μm sections.

2.2. Antibodies

The following monoclonal antibodies (supernatants) were used: Tn antigen (GalNAc-O-Thr/Ser) Mab 5F4[26] and Mab 1E3.[13] Sialyl-Tn antigen (Sialylα2-6GalNAc-O-Thr/ser) Mab 3F1 (H. Clausen, unpublished). Two antibodies directed against MUC1 were used: Mab 5E5 recognizes Tn-/STn MUC1 and 5E10 reacts with all glycoforms of MUC1.[27,28] To decide coexpression of mucin-type antigens and blood type A antigen we used a Mab (HH6) that reacts to all variants of blood group A (H. Clausen, unpublished).

2.3. Incubation

Incubation was performed for 24 h at 4°. To ensure the specificity of antibody binding, a number of control measures were performed: (1) to prevent non-specific staining the deparaffinized sections were preincubated with 1% BSA in TBS pH 7.4 for 1 h. (2) Sections were incubated without primary antibody. (3) Sections were incubated in medium with irrelevant IgG or IgM antibodies. To demonstrate antibody binding, the supernatants were diluted 1:3 in TBS and we used the DAB-Envision system (DAKO, Denmark) for visualizing the reaction product. To test the binding specificity of 5E5, the antibody was titrated down to a dilution 1:3000.

The combined alcian blue and PAS method[29] was used to demonstrate both neutral and acid mucosubstances in the sections. For this purpose the alcian blue stain was performed at pH 2.5. The alcian blue–PAS method stains acid mucins blue; neutral mucins magenta and mixtures of above blue/purple.

3. Results

3.1. Morphology and blood type

The autopsies used in this study were obtained from elderly people, and some minor age-related changes were noticed and compared to what is described in textbooks on normal oral histology.

Soft palate: At the nasopharyngeal site, the aging palate possessed pseudostratified columnar respiratory epithelium showing a normal appearance that could alternate with atrophic stratified epithelium, and the squamous epithelium at the oral site was often lightly keratinized. At the respiratory site, the small submucosal glands were of mixed type with many mucous cells and few serous cells appearing as demilunes surrounding the ends of the mucous cell tubules or as isolated serous acini. At the oral site, the numerous lobuli contained small submucosal glands that all were of the mucous type. The submucosa also possessed large amount of adipocytes and infiltrates of mononuclear cells.

The *vestibular folds* are a pair of sagittally orientated duplications of mucous membranes of the sidewalls of the