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Mucoepidermoid carcinoma-associated expression of MUC5AC, MUC5B and mucin-type carbohydrate antigen sialyl-Tn in the parotid gland

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ABSTRACT

Objectives: The aberrant expression of mucins and mucin-type carbohydrates has been described in many types of cancer, including mucoepidermoid carcinoma (MEC), a malignant salivary gland tumor. In this study, we examined the aberrant expression patterns of mucins (MUC1, MUC4, MUC5AC and MUC5B), simple mucin-type carbohydrate antigens (Tn, sialyl-Tn and T) and mature carbohydrate antigens (Lewis[a] and sulfo-Lewis[a] antigens) in MEC originating from the parotid gland, which normally does not secrete mucins.
Design: We conducted an immunohistochemical study to investigate the presence of mucins and carbohydrates in 24 MEC samples originating from the parotid gland and in surrounding normal tissue of the same gland in comparison 6 samples of normal salivary glands. The expression levels were compared with respect to the histological grading. Furthermore, 24 MEC samples from non-parotid salivary glands were included.
Results: We observed loss of topology of membrane-bound MUC1 and MUC4, and *de novo* expression of MUC5AC, MUC5B and sialyl-Tn in MEC that originated in the parotid gland. Furthermore, mucins MUC1, MUC4 and carbohydrate antigens Tn, sialyl-Tn, T, Lewis[a] and sulfo-Lewis[a] were overexpressed in MEC samples compared to surrounding normal salivary gland tissues. MUC1 was expressed in both low- and high grade MECs, whereas MUC4 was not expressed in high grade MECs of the parotid gland.
Conclusion: During the development of MEC in the parotid gland, the genes for gel-forming secretory mucins are switched on. Besides these MEC tissues overexpress short oligosaccharides, suggesting that the glycosylation machinery is altered.

1. Introduction

Mucins are a heterogeneous family of large glycoproteins with a great diversity of O-linked carbohydrate side-chains which constitute a major part of the mature mucins (Varki et al., 2009, Chap. 9). They are expressed by epithelial cells and are either membrane-associated or secreted (Corfield, 2015; Hollingsworth & Swanson, 2004; Moniaux, Escande, Porchet, Aubert, & Batra, 2001; Varki et al., 2009). Mucins are the main component of the mucous layer that protects epithelial tissue throughout the body (Corfield, 2015; Hollingsworth & Swanson, 2004; Moniaux et al., 2001). In addition, mucins are involved in the differentiation and renewal of the epithelium, modulation of cell adhesion, immune response and cell signaling (Hollingsworth & Swanson, 2004; Moniaux et al., 2001).

Membrane-associated mucins are expressed on the apical borders of normal epithelial cells. In malignant cells of epithelial origin, there is a loss of cell membrane polarity, which is associated with the activity of a proliferation and survival program, resulting in the transient repositioning of membrane-bound mucins over the entire cell membrane (Kufe, 2009). Most studies that have reported aberrant expression of mucins in cancer are based on the expression of these membrane-bound mucins, such as MUC1 and MUC4. Overexpression of MUC1 and MUC4 has been observed in gastric, pancreatic, prostate, breast, lung, and salivary gland tumors (Alos et al., 2005; Giuntoli, Rodriguez, & Whitaker, 1998; Handra-Luca et al., 2005; Liu et al., 2002; Llinares et al., 2004; Saitou et al., 2005; Singh et al., 2006; Senapati, Sharma, Bafna, Roy, & Batra, 2008; Khodarev et al., 2009).

Secreted gel-forming mucins, such as MUC2, MUC5AC, MUC5B, and MUC6, are the main components of the protective mucous layer covering the sensitive epithelial cells and form a physical barrier between

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the underlying tissue and the environment, each with its own expression preference for certain organ, tissue and cell type. In neoplastic conditions, however, these secretory mucins are highly expressed in carcinomas of different organs, including gastrointestinal, pancreatic, ovary, breast, lung, and salivary glands (Alos et al., 2005; Dijkema et al., 2012; Handra-Luca et al., 2005; Hanski et al., 2003; Li et al., 2013; Park et al., 2009; Perrais et al., 2001; Reis et al., 2000; Sóñora et al., 2006).

Carbohydrates comprise on weight basis > 90% of the mucins. These are attached to serine or threonine residues in the protein backbone and are synthesized by concerted action of glycosyltransferases (Varki et al., 2009). In cancer cells, there are dramatic alterations in the glycosylation machinery, at least partially due to the misfolding of glycosyltransferases (Stowell, Ju, & Cummings, 2015). This can lead to mal-functional glycosyltransferases resulting in the expression of short carbohydrates antigens Tn (GalNAcα1-O-Ser/Thr), sialyl-Tn (Sialylα2-6GalNAcα1-O-Ser/Thr) and T (Galβ1-3GalNAcα1-O-Ser/Thr). These so called simple mucin-type carbohydrates, which have a restricted expression pattern in normal salivary glands, are increased in several cancers including breast, prostate, colon and salivary gland tumors (Brockhausen, 2006; Carneiro et al., 1994; Colpitts et al., 2002; Nakagoe et al., 2002; Kirkeby, Moe, & Bardow, 2010; Therkildsen, Mandel, Christensen, & Dabelsteen, 1993, Therkildsen, Mandel, Thorn, Christensen, & Dabelsteen, 1994).

One of the most common and most studied malignant salivary gland tumor is the mucoepidermoid carcinoma (MEC). MEC is characterized by mucous, intermediate, epidermoid, clear and/or columnar cells and can be classified as low-, intermediate- or high-grade tumors based on histological parameters such as necrosis, anaplasia, neural invasion, mitoses and cystic growth (Brandwein et al., 2001; Goode & El-Naggar, 2005). Low grade MEC has well-formed glandular structures or (micro-) cysts lined by a single layer of mucous cells characterized by a swollen appearance due to the presence of high-molecular weight mucins in their cytoplasm. In high grade tumors, the epidermoid cells predominate and the tumor has a more solid architecture (Goode, Auclair, & Ellis, 1998). Aberrant expression of mucins (Alos et al., 2005; Handra-Luca et al., 2005) as well as mucin-type carbohydrates (Therkildsen et al., 1993) have been analyzed in salivary gland MECs in relation to diagnostic and prognostic implications.

However, there has been minor attention given to the different characteristics of the individual salivary glands with respect to mucin expression. While there are no large differences in the expression of membrane bound mucins in all normal salivary glands there is a striking difference between the normal parotid glands and the other salivary glands when comparing the secretory gel-forming mucins. All salivary glands except the parotid gland are made up of serous and mucous acini, the latter being responsible for the secretion of mucins, mainly MUC5B (Bolscher, Veerman, Van Nieuw Amerongen, Tulp, & Verwoerd, 1995; Veerman et al., 1997). Notably, the parotid gland is a purely serous gland which inherently does not express any secretory gel-forming mucin (Veerman et al., 2003). Therefore, we have chosen for the present study MEC that originate specifically from the parotid gland to analyze the expression of the membrane-associated and secretory gel-forming mucins as well as some mucin-type carbohydrates. We conducted an immunohistochemical survey to examine the aberrant expression patterns of the membrane-associated MUC1 and MUC4 and the secretory gel-forming mucins MUC5AC and MUC5B. Furthermore the expression levels of the simple mucin-type carbohydrate antigens Tn, sialyl-Tn and T, and the mature carbohydrate antigens Lewis^a (Galβ1-3GlcNAc[Fucα1-4]β1-O-Ser/Thr) and sulfo-Lewis^a (Sulfo-3Galβ1-3GlcNAc[Fucα1-4]β1-O-Ser/Thr) were determined. For comparison reasons minor salivary glands were included in the survey.

We observed a MEC-associated loss of topology intimated by aberrant expression of MUC1, MUC4 and altered glycosylation of mucin-associated carbohydrate. Strikingly, we observed *de novo* expression of MUC5AC and MUC5B, as well as the sialyl-Tn antigen in the MEC samples originating from the parotid gland.

Table 1
Patients Clinicopathological Characteristics.

Clinicopathological Characteristics	Low grade MEC (n = 30)	Intermediate grade MEC (n = 7)	High grade MEC (n = 11)
Mean age in years (range)	48 (9–82)	42 (14–58)	68 (43–82)
Gender			
Male	16	4	7
Female	14	3	4
Tumor location			
Parotid gland	15	5	4
Minor salivary gland	15	2	7
Lymph node metastasis	4	1	3
Resection type			
Radical	18	5	7
Irradical	3	0	3
Unknown	9	2	1
Recurrence	3	1	1
Died of the disease	0	0	1
Mean follow-up in months (range)	35 (1–164)	115 (44–234)	33 (1–84)

2. Methods and materials

2.1. Normal and MEC samples

Forty-eight formalin-fixed-paraffin-embedded (FFPE) MEC samples (24 from the parotid gland and 24 from minor salivary glands; 11 palate and 13 elsewhere in oral cavity) and 6 normal salivary glands samples (2 parotid, 2 submandibular, and 2 sublingual glands) were retrieved from the archives of the Department of Pathology, VU University medical center (Amsterdam, The Netherlands). All tumors had been surgically removed between 1984 and 2011; in 2 cases only incisional biopsies were taken. HE slides were reviewed by an experienced pathologist (EB). Diagnoses were confirmed, and tumors were graded according to the WHO criteria (Barnes, Eveson, Reichart, & Sidransky, 2005, Chap. 1): 30 low grade, 7 intermediate grade and 11 high grade MEC (Table 1).

The design of this study adheres to the code for proper secondary use of human tissue established by the Dutch Federation of Biomedical Scientific Societies (Oosterhuis, Coebergh, & van Veen, 2003).

2.2. Immunohistochemistry of mucins and mucin associated carbohydrate antigens

All tumor and normal samples were fixed in 4% buffered formalin, processed and embedded in paraffin according to routine procedures. From each tissue block, multiple 4 μm thick sections were cut on coated slides and dried overnight at 37° C. The sections were deparaffinized in xylene and rehydrated through graded concentrations of ethanol. Endogenous peroxidase was blocked by 0.3% H_2O_2 in methanol for 30 min at room temperature. Immunohistochemistry was subsequently performed using a panel of antibodies and or lectins directed against protein and carbohydrate epitopes on mucins (Table 2). 3,3-Diaminobezidine (DAB) was used as substrate for the Powervision method (Immunologic, Klinipath). Sections were counterstained with Mayer's hematoxylin, dehydrated and mounted. In this way 24 MEC samples originating parotid, 24 MEC samples from minor glands, and 6 normal tissue samples were probed with nine antigens, resulting in 486 sections.

The results of the immunohistochemistry were evaluated by semi-quantitatively scoring the percentage of positive neoplastic cells by two investigators (JHM and WB) and included the overall tumor slide. Tumors were considered positive when the tumor section of the slide contained 5% or more positive neoplastic cells. Distinction was made