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Glycopathological Study of Eyelid Tumors and Pseudotumors

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Abstract: The expression of MUC1, Tn (GalNAc α 1-O-Ser/Thr) and sialosyl Tn (STn) (NeuAc α 2,6 GalNAc α 1-O-Ser/Thr) antigens, which are useful markers for the prognosis of cancer in other organs, was examined immunohistochemically in a series of 45 eyelid tumors and 5 pseudotumors: basal cell carcinoma, 18; squamous cell carcinoma, 11; sebaceous gland carcinoma, 6; seborrheic keratosis, 4; papilloma, 3; verruca vulgaris, 2; nevus, 1; and granuloma, 5. The MUC1 antigen was identified in all squamous cell and sebaceous gland carcinomas, but not in basal cell carcinoma or the benign tumors. The Tn antigen was expressed in all the sebaceous gland, half of the squamous cell, and only rarely in the basal cell carcinomas. The STn antigen was expressed in all seborrheic keratosis and in the majority of squamous cell carcinomas, but only rarely in sebaceous gland and basal cell carcinomas. Eyelid tumors are frequently associated with apomucin and mucin-carbohydrate antigens: the MUC1 glycoprotein appears to be related to the malignant potential of eyelid tumors, and may be a useful marker for the differential diagnosis of invasive tumors, including sebaceous gland and squamous cell carcinomas. **Jpn J Ophthalmol 1997;41:362–369** © 1997 Japanese Ophthalmological Society

Key Words: Eyelid tumor, invasion, MUC1 antigen, sialosyl Tn antigen, Tn antigen.

Introduction

Oncogenic transformation is often associated with changes in glycosylation of glycolipids or glycoproteins in the cell membranes. Mucins are high molecular weight glycoproteins with O-glycoside linked glycans.[1] Incomplete synthesis associated with precursor accumulation is found in O-linked (mucin type) glycosylation in which the core structure, Tn or sialosyl Tn (STn) is exposed, probably due to incomplete synthesis. These structures are normally encoded in human tissue mucin and secretions by sialylation and/or chain elongation and branching, by addition of other sugar residues. Many cancer-associated antigens are mucin-type carbohydrates which show alterations in the earliest phases of glycosylation. These include the Tn antigen, with an accumulation of a core oligosaccharide from incomplete glycosylation, and the STn antigen with its aberrant disaccharide.[1,2,3,4] Mucin core protein antigens such as MUC1 gene product and mammary apomucin[5] are also correlated with the biologic and clinical behavior of cancers.[6,7,8,9] Immunocytochemical examination of the cancer-associated antigens has proven useful in the prognosis of neoplasms, including colon and breast cancer, because expression of altered antigens correlates with prognosis.[6,7,9]

We examined, histochemically, tumor-associated mucin-type carbohydrate antigens in eyelid tumors to determine if their expression also was correlated with the malignancy of the tumor cells.

Materials and Methods

Antibodies

Monoclonal antibodies to three mucin carbohydrate antigens were obtained commercially and used according to the manufacturer's protocols: NCL-MUC1 (mouse IgG, Novocastra Laboratories, Newcastle, UK) to detect MUC1 mucin core protein with a carbohydrate epitope; DAKO-HB-Tn1 (DAKO,

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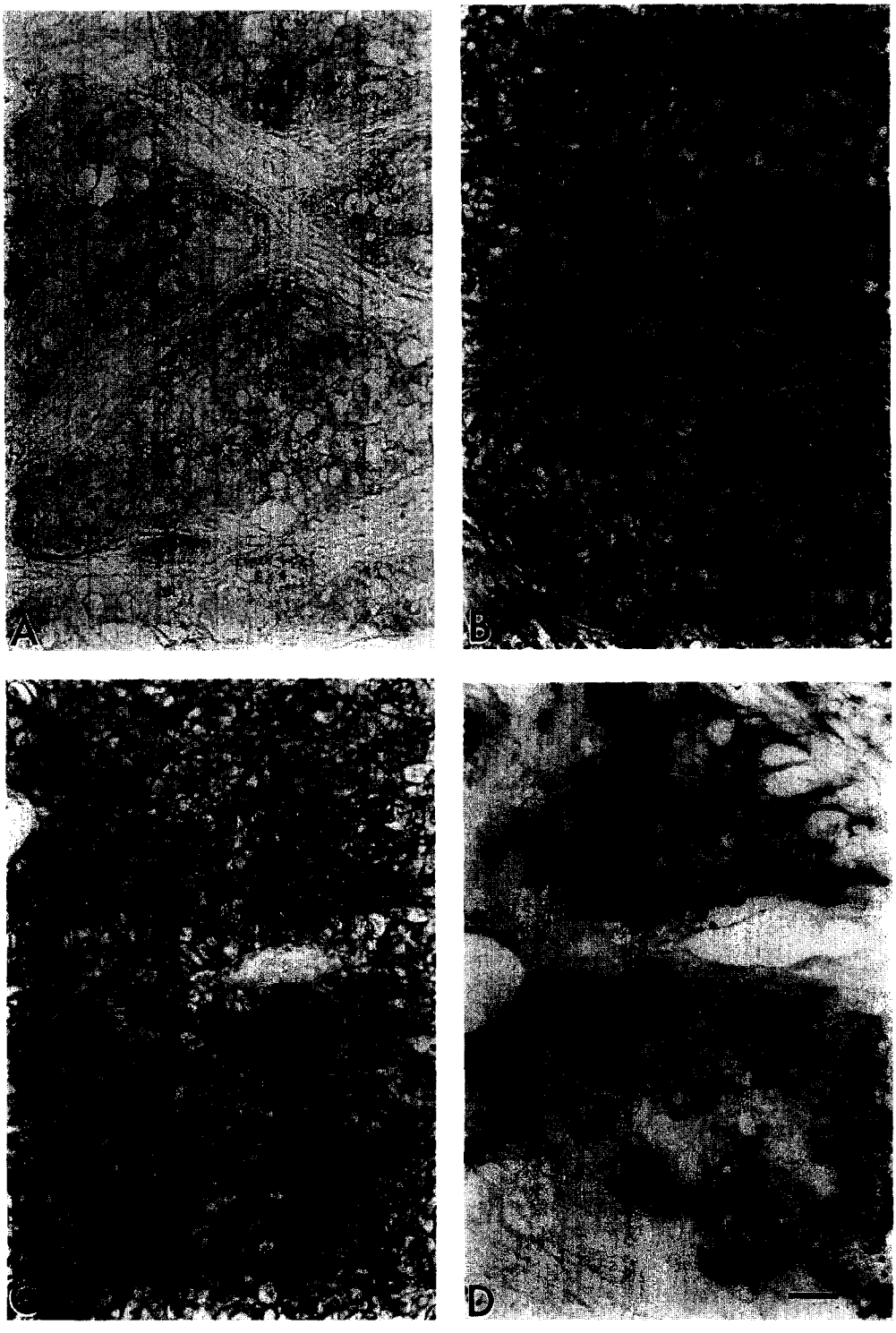

Figure 1. Sebaceous gland carcinoma (**A–C**: Case 1 and **D**: Case 2, in Table 1). (**A**) Control section incubated with nonimmune serum: no staining. (**B**) Stained with hematoxylin and eosin. (**C**) Reaction with MUC1: staining in cell membranes of most tumor cells. (**D**) Reaction with MUC1: staining of cell membranes, and occasionally cytoplasm, of 5%–50% of cells. Bar = 30 μm.

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Table 1. Staining Results of Mucin Carbohydrate Antigens in Patients With Eyelid Tumor

Case	Age	Sex	Pathologic Diagnosis	MUC1[a]	Tn[a]	STn[a]	Prognosis[b]
1	79	M	Sebaceous gland carcinoma	++	+	+	met
2	83	F	Sebaceous gland carcinoma	+	+	−	met rec
3	47	M	Sebaceous gland carcinoma	+	++	−	met
4	30	F	Sebaceous gland carcinoma	++	+	−	−
5	85	F	Sebaceous gland carcinoma	+	+	−	−
6	53	M	Sebaceous gland carcinoma	+	+	+	met
7	85	F	Squamous cell carcinoma	++	−	+	met rec
8	86	F	Squamous cell carcinoma	+	−	−	met
9	89	M	Squamous cell carcinoma	++	−	+	rec
10	78	F	Squamous cell carcinoma	+	+	−	−
11	54	F	Squamous cell carcinoma	+	+	+	met
12	61	F	Squamous cell carcinoma	+	+	+	−
13	78	M	Squamous cell carcinoma	+	−	+	met
14	87	M	Squamous cell carcinoma	+	+	+	met
15	92	M	Squamous cell carcinoma	+	+	+	−
16	70	M	Squamous cell carcinoma	+	−	+	−
17	78	F	Squamous cell carcinoma	+	+	−	−
18	87	F	Basal cell carcinoma	−	−	−	−
19	83	F	Basal cell carcinoma	−	+	−	−
20	81	F	Basal cell carcinoma	−	−	−	−
21	66	F	Basal cell carcinoma	−	−	−	−
22	78	F	Basal cell carcinoma	−	−	−	−
23	70	F	Basal cell carcinoma	−	−	−	−
24	83	F	Basal cell carcinoma	−	−	−	−
25	71	M	Basal cell carcinoma	−	−	−	−
26	78	F	Basal cell carcinoma	−	−	−	−
27	86	M	Basal cell carcinoma	−	−	−	−
28	65	M	Basal cell carcinoma	−	−	−	−
29	41	F	Basal cell carcinoma	−	−	−	−
30	75	F	Basal cell carcinoma	−	−	−	−
31	61	M	Basal cell carcinoma	−	−	−	−
32	79	F	Basal cell carcinoma	−	−	−	−
33	85	F	Basal cell carcinoma	−	+	+	−
34	86	M	Basal cell carcinoma	−	−	−	−
35	86	F	Basal cell carcinoma	−	−	−	−
36	61	M	Seborrheic keratosis	−	−	++	−
37	81	F	Seborrheic keratosis	−	−	+	−
38	60	M	Seborrheic keratosis	−	−	+	−
39	72	M	Seborrheic keratosis	−	−	++	−
40	55	F	Papilloma	−	−	+	−
43	37	M	Papilloma	−	−	−	−
44	49	F	Papilloma	−	−	−	−
41	60	F	Verruca vulgaris	−	−	−	−
42	85	M	Verruca vulgaris	−	−	−	−
45	66	M	Intradermal nevus	−	−	−	−
46	85	M	Epitheloid granuloma	−	−	−	−
47	75	M	Chalazion	−	−	−	−
48	63	F	Chalazion	−	−	−	−
49	64	M	Chalazion	−	−	−	−
50	59	M	Chalazion	−	−	−	−

[a] −: <5% stained tumor cell. +: 5–50 stained tumor cell. ++: >50% stained tumor cell.

[b] met: lymph node metastasis positive. rec: recurrence positive. −: no metastasis and recurrence during observation.

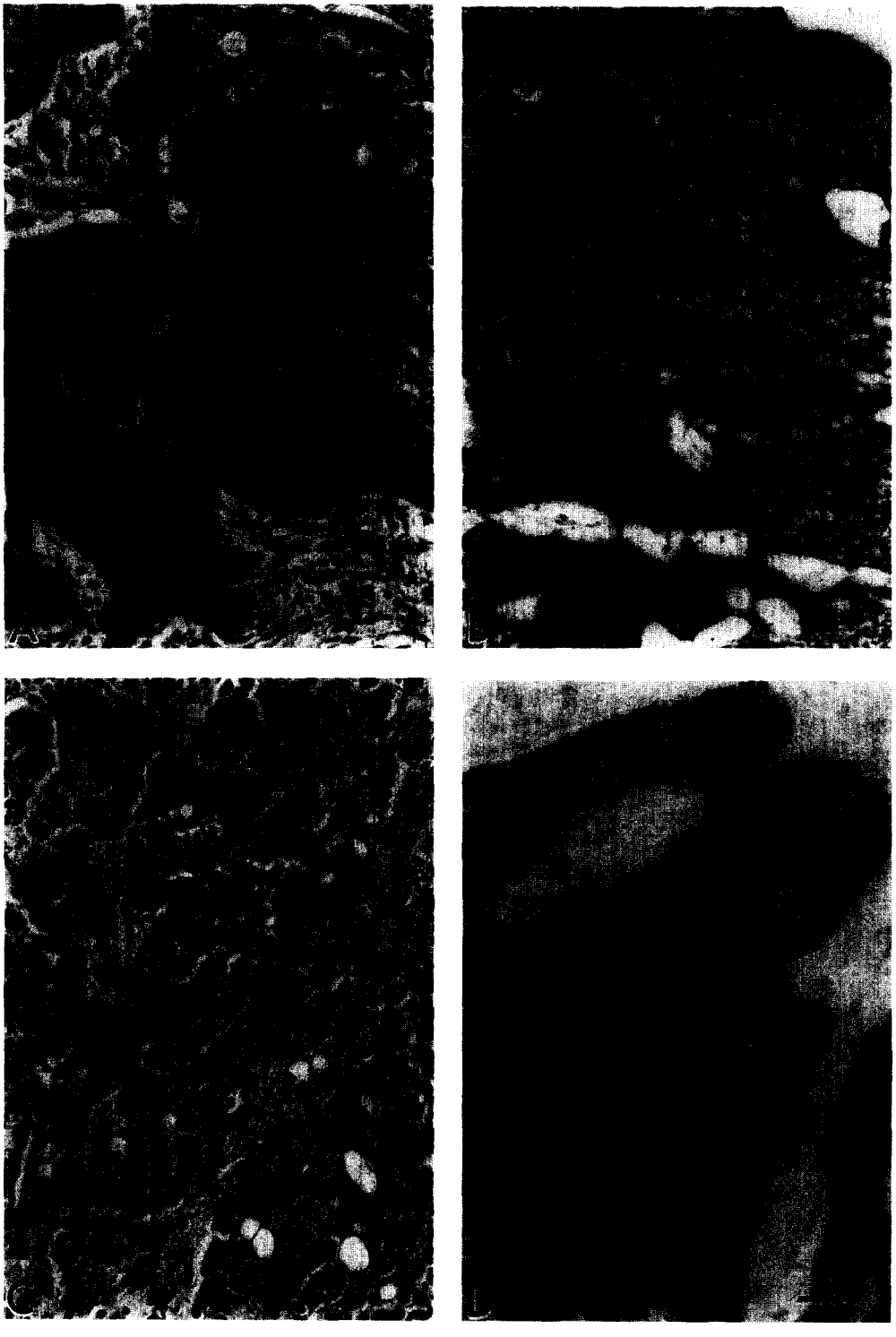

Figure 2. Squamous cell carcinoma (**A**, **B**: Case 7; **C**, **D**: Case 8). (**A**) Hematoxylin and eosin staining. (**B**) Reaction with MUC1: intense staining in cell membranes of most tumor cells. (**C**) Hematoxylin and eosin staining. (**D**) Reaction with MUC1: staining of cell membranes, and occasionally cytoplasm, of 5%–50% of cells. Bar = 30 μm.

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Glostrup, Denmark) to detect Tn antigen and DAKO-HB-STnl (DAKO) to detect sialosyl-Tn (STn) antigen.

Tissue Samples

Specimens of 45 eyelid tumors and 5 pseudotumors were used for a histochemical study of tumor-associated mucin antigens. Patients were treated at the Kagoshima University Hospital from 1986 to 1995: sex, age, diagnosis and prognosis are shown in Table 1.

Tissue Preparation and Staining

Resected tissues were immersion-fixed in buffered formalin (3.7%); embedded in paraffin; deparaffinized in xylene; hydrated in a graded ethanol series; immersed in 0.01 M sodium citrate buffer, pH 6.0; boiled for 4 minutes in a pressure cooker to unmask antigens; and treated with 0.2% H_2O_2 in methanol for 30 minutes to block endogenous peroxidase activity. The slides were then immersed in Dulbecco's phosphate-buffered saline 0.01 M, pH 7.4 (PBS; Sigma, St Louis, MO, USA) for 30 minutes; incubated with 3% horse serum (diluted with PBS) at room temperature for 30 minutes to block nonspecific binding; and overlaid with primary antibodies (MUC1, 1:100; Tn and STn, 1:50) in PBS with 2% horse serum at room temperature for 2 hours. Serial control sections were treated the same way but without incubation with nonimmune C57BL mouse serum (1:100) in PBS with 2% horse serum. The slides were washed in PBS for 30 minutes; incubated at room temperature for 1 hour with biotinylated anti-mouse IgG (1:100 in PBS, Vector Laboratories, Burlingame, CA, USA) washed in PBS for 30 minutes, and stained with reagents of the Vectastain Elite ABC kit and diaminobenzidine as the peroxidase substrates, following the manufacturer's protocol.

After the slides were dehydrated and coverslipped, brown reaction products were examined with a light microscope. Antigen expression was classified by approximate percentages of positively stained tumor cells: <5%, −; 5%–50%, +; >50%, ++.

Results

Tumor tissues incubated with nonimmune mouse serum showed almost no reaction (Figure 1A). Positive staining was regarded as specific expression of mucin antigens. Staining was primarily seen in the tumor cell membranes; the degree of staining varied with the tumor type (Table 1).

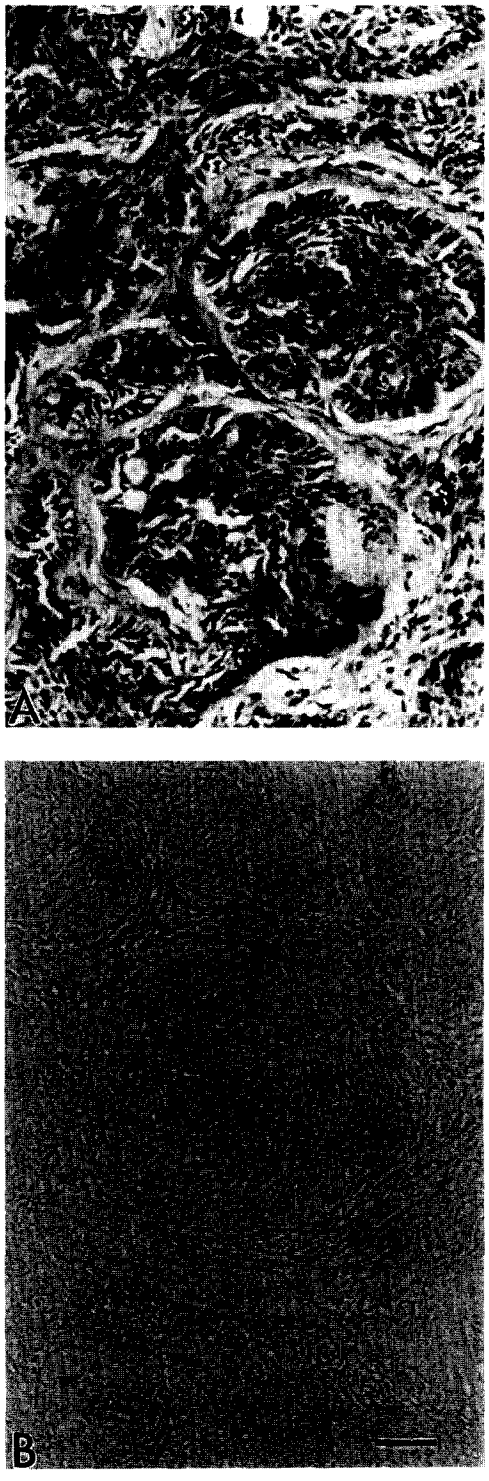

Figure 3. Basal cell carcinoma (**A**, **B**: Case 18). (**A**) Hematoxylin and eosin staining. (**B**) Incubation with MUC1: almost no staining. Bar = 30 μm.

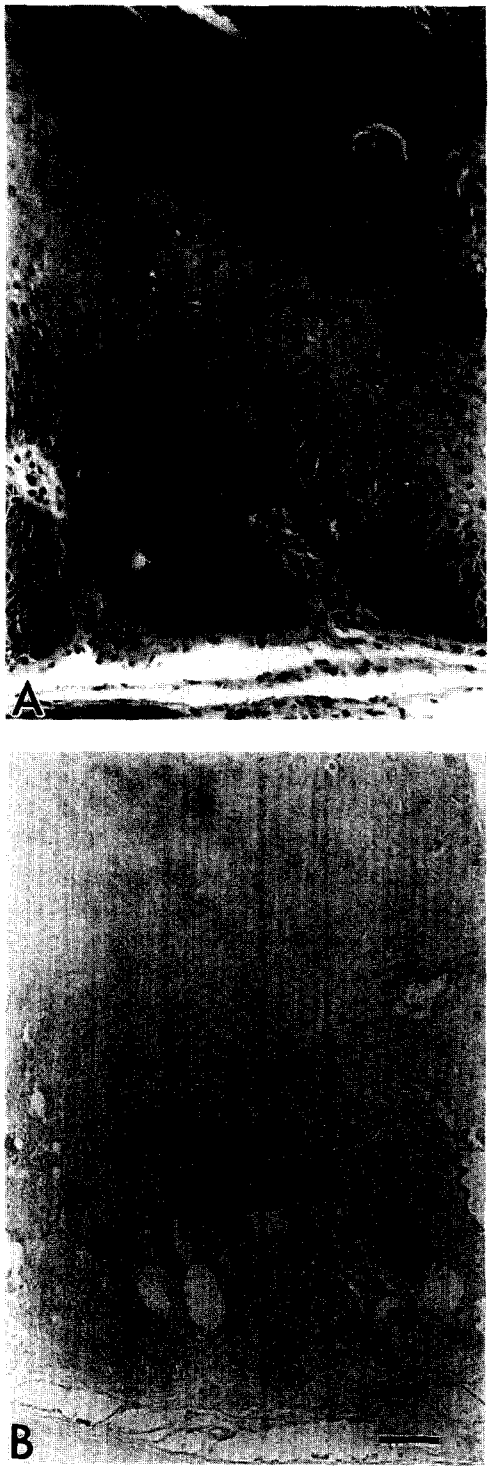

Figure 4. Seborrheic keratosis (**A**, **B**: Case 36). (**A**) Hematoxylin and eosin staining. (**B**) incubation with MUC1: showing no staining. Bar = 30 μm.

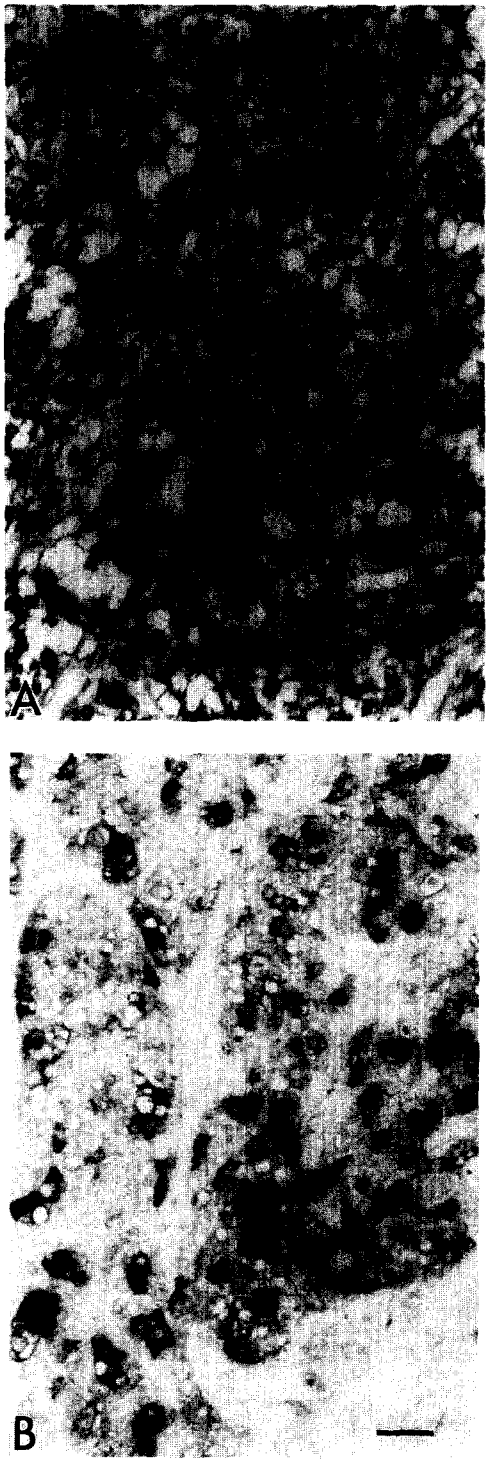

Figure 5. The antigen expression in sebaceous gland carcinoma: staining of cell membranes, and occasionally cytoplasm. (**A**) Case 3, staining of most cells. (**B**) Case 1: positive for Tn antigen. Bar = 30 μm.

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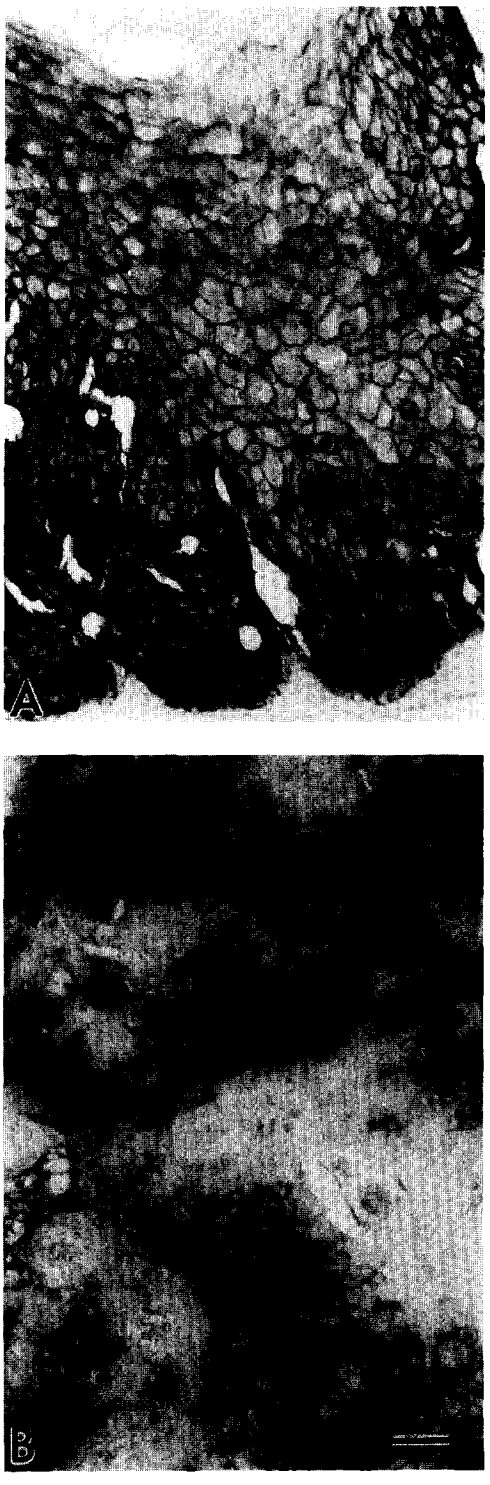

Figure 6. STn antigen expression: predominant staining at cell membranes (**A**) Seborrheic keratosis (Case 36). (**B**) Squamous cell carcinoma (Case 9). Bar = 30 μm.

MUC1 antigen was expressed in all sebaceous gland and squamous cell carcinomas (Figures 1 and 2) but not in basal cell carcinoma or benign tumors (Figures 3,4).

Tn antigen was identified in all sebaceous gland (Figure 5) and six (54.5%) of the squamous cell carcinomas; only two (11.1%) of the basal cell carcinomas and none of the benign tumors stained positively for the Tn antigen.

STn antigen was expressed in 8 (72.7%) of the squamous cell carcinomas but only occasionally in other eyelid cancers. It was, unlike the MUC1 and Tn antigens, found in 5 of the 15 benign tumors, and in all of the seborrheic keratosis (Figure 6).

Discussion

The results of this study show that MUC1 glycoprotein is universally expressed in the cell membranes of sebaceous gland and squamous cell carcinomas, but not in basal cell carcinoma and the benign eyelid conditions. These findings are consistent with reports on tumors of other organs. Pancreatic and intrahepatic-duct carcinomas express MUC1 when they have advanced to the invasive stage and the prognosis is poor, but not in the preinvasive stage when the prognosis is more favorable.[6,7,9] MUC1, therefore, is useful for differentiating invasive from noninvasive eyelid tumors, including basal cell carcinoma and other benign lesions. Tn antigen, also linked to the invasive carcinomas of other organs,[6,7,9] and present in all sebaceous and about half the squamous cell carcinomas, but not the basal cell and benign tumors, can be similarly useful although the pattern of expression is less definitive than MUC1.

STn antigen expression was not significantly correlated with eyelid tumor growth, and would not be helpful in their evaluation, although it is known to correlate with epithelial differentiation of head and neck mucous epithelium.[10]

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