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Analysis of lectin receptors in normal nasal mucosa, nasal polyp, inverted papilloma and papillary adenocarcinoma

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Abstract

In order to investigate the changes in glycoprotein structure in the process of cellular differentiation of the nasal mucosa, formalin-fixed, paraffin-embedded biopsy specimens of normal nasal mucosae, nasal polyps, inverted papillomas and papillary adenocarcinomas were analysed by the Avidin Biotin-Peroxidase Complex technique for the demonstration of peanut agglutinin (PNA) receptors, concanavalin ensifomis agglutinin (ConA) receptors, ulex europeaus agglutinin (UEA-I) receptors, wheat germ agglutinin (WGA) receptors, carcino-embryonic antigen (CEA) and keratin. The quantity and distribution of PNA receptors, ConA receptors, UEA-I receptors and CEA were different, in relation to the varying pathological changes. The results suggest that the glycoprotein structure in the cells of the nasal mucosa will change following their differentiation and malignant transformation, which may be helpful in establishing the diagnosis.

Key words: Receptors, mitogen; Nasal neoplasms

Introduction

Lectins are sugar-binding proteins or glycoproteins of non-immune origin. Their most important characters are that they can combine with specific carbohydrate residues. Every kind of lectin has its differing carbohydrate specificity.

There are glycoconjugates in all kinds of cellular membranes. Changes in lectin-binding patterns have been related to cellular differentiation and malignant transformation. Therefore, lectins have been used extensively to investigate the quality and distribution of glycoconjugates in normal and neoplastic cells such as those in the lung, uterus, colon and other organs (Rhodes *et al.*, 1986; Lee, 1988; Feinmesser *et al.*, 1989).

The authors have studied PNA receptors, ConA receptors, UEA-I receptors, WGA receptors, CEA and keratin in normal nasal mucosa, nasal polyp, nasal inverted papilloma and nasal papillary adenocarcinoma to demonstrate the changes of the type, quantity and distribution of lectin receptors and neoplasm-related antigens.

TABLE I

LECTINS USED IN THIS STUDY

Agglutinin	Abbreviation	Specificity
Peanut	PNA	D-Gal B(1–3) Gal NAc
Concanavalin	ConA	α-D-Man, α-D-Glc
Ulex europeaus	UEA-I	α-L-Fucose
Wheat germ	WGA	NeuNAc, GlcNAc

Materials and methods

Normal nasal mucosae (n = 20) were obtained from biopsy specimens which showed no abnormality. Surgically resected nasal polyps (n = 80), inverted papillomas (n = 60) and papillary adenocarcinomas (n = 40) were obtained. All tissues were fixed in formalin, embedded in paraffin, and cut into serial sections (5 μm thick). One of them was stained with haematoxylin and eosin. Biotinylated lectins were purchased from Vector Laboratories Inc., USA; CEA from Dako Inc., USA; keratin from Biosynthetic Laboratories, Beijing, China. The entire procedure was carried out at room temperature.

Sections were deparaffinized and rehydrated in the standard fashion and washed 3 times in PBS (0.1M phosphate 1.5 NaCl, pH 7.4). Then incubated in one of the lectins (10 μg/ml) for 60 minutes and PBS rinsed as above; incubated with 1:100 ABC reagent for 60 minutes and PBS rinsed; counterstained with 1 per cent methyl green in methanol, washed, dehydrated, cleared and mounted. Parallel experiments, in which lectin binding was

TABLE II

POSITIVE REACTION OF LECTINS IN THE TEST

Tissue	No.	PNA	UEA-I	ConA	WGA
NM	20	0	6	12	20
NP	80	0	34	63	80
IP	60	47	31	50	60
PA	40	37	0	36	40

NM = Normal nasal mucosae; NP = nasal polyps; IP = inverted papillomas; PA = papillary adenocarcinomas.

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TABLE III

THE REACTIVE INTENSITY OF PNA IN THE CELLS OF INVERTED PAPILLOMAS WITH DIFFERENT DEGREES OF DYSPLASIA

Degree of dysplasia	No.	+++	++	+	–
Active	18	8	4	1	5
Inactive	42	4	12	18	8

inhibited by pre-incubation of each lectin with its appropriate binding sugar (Table I), confirmed the specificity of lectin binding. For the CEA and keratin tests sections were reacted routinely with carcino-embryonic antibody and keratin antibody.

The appropriate controls were negative: if tissues were not stained the result was (–). Other results: slight yellow, (+); yellow, (++); brown, (+++).

Results

PNA reaction was positive in the cells of nasal neoplasms ($p<0.01$). In inverted papillomas, positive intensity of PNA reaction was related to the degree of dysplasia, with stronger staining on the active cells ($p<0.01$) (Table III). UEA-I receptors were negative in the cells of papillary adenocarcinomas (Table II). From normal nasal mucosae to papillary adenocarcinomas, ConA showed a progressively increasing reactivity ($p<0.01$) (Table IV). WGA reaction was intense in all specimens. CEA only existed in the cells of inverted papillomas and papillary adenocarcinomas (Table V).

Discussion

PNA has a high specificity for D-Gal B(1→3) Gal NAc. The lack of PNA binding in normal nasal mucosae and nasal polyps suggests that Gal group, masked by other carbohydrate, does not expose itself to the terminal residues of the oligosaccharide chain (Elias *et al.*, 1988). PNA receptors found in inverted papillomas and papillary adenocarcinomas imply the presence of exposed galactose at the end of the glycoprotein side chain, which would suggest some degree of desialylation or lack of sialylation. In inverted papillomas, PNA reaction was related to the degree of dysplasia, with stronger staining in the active cells. It may show the relationship between the carbohydrate metabolism of the cells and the degree of cellular differentiation (Altavilla *et al.*, 1984; Yuan *et al.*, 1986).

UEA-I binds most specifically to α-L-fucose. In papillary adenocarcinomas, the lack of UEA-I receptors suggests that the loss of α-L-fucose in the process of cellular malignant transformation of nasal mucosa reflects a change in cellular function.

TABLE IV

THE RESULT OF CONA REACTION IN THE TEST

Tissue	No.	+++	++	+	–
NM	20	0	3	9	8
NP	80	8	38	17	17
IP	60	19	17	14	10
PA	40	20	9	7	4

NM = normal nasal mucosae; NP = nasal polyps; IP = inverted papillomas; PA = papillary adenocarcinomas.

TABLE V

THE POSITIVE RESULTS OF CEA AND KERATIN IN THE TEST

Tissue	No.	CEA	Keratin
NM	10	0	10
NP	10	0	10
IP	10	10	10
PA	10	10	10

NM = normal nasal mucosae; NP = nasal polyps; IP = inverted papillomas; PA = papillary adenocarcinomas.

ConA binds to both α-D-Man and α-D-Glc. From normal nasal mucosae to papillary adenocarcinomas, their ConA receptors increased progressively. Some authors considered that trans-glycosylases in the cells reduced in the process of cellular malignant transformation, which resulted in the changes in structural glycoproteins on the cellular surface in this course.

CEA is a kind of glycoprotein, with 45 per cent of carbohydrate, mainly including D-Gal-B(1→3) Gal NAc, α-D-Glc, α-L-fucose, D-GalcNAc and sialic acid (Darcy *et al.*, 1973; Zamcheck, 1975). Under observation by electron-microscopy, CEA adhered to the cellular membrane. CEA was negative on the cells of normal nasal mucosae and nasal polyps, but positive on the cells of inverted papillomas and papillary adenocarcinomas. It suggested that the antigen on the surface of the cells changed in the process of malignant transformation.

Conclusion

This study reveals a relationship between the different lectin-binding patterns and the degree of cellular differentiation in normal and neoplastic nasal tissues. The results suggest that the changes in glycoprotein structure on the surface of the cells of nasal mucosa are followed in the process of cellular differentiation and malignant transformation. Therefore, lectins may be used as probes for specific carbohydrate residues in histological sections to help make the diagnosis of nasal neoplasms.

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