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Expression of Type 1 Blood Group Precursor in Human Gastric Carcinoma

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The surface epithelium of normal gastric mucosa from patients with gastric adenocarcinoma expressed the type 1 blood group precursor only in Lewis (Le) non-secretor individuals, Le a+b− (se/se, Le/−) and Le a−b− (se/se, le/le). In secretors, the superficial mucosa was negative. Deep areas of the mucosa showed no type 1 precursor regardless of secretor status. Expression of type 1 precursor was anomalously found in neoplastic cells in 14 of 16 Le a−b+ (secretors) patients and in 4 of 5 Le a−b− (secretors) patients. The 1 Le a−b− non-secretor carcinoma expressed type 1 precursor strongly. 6 of 8 Le a+b− non-secretor carcinomas showed positivity for the monoclonal antibody K-21. Thus the type 1 precursor reacted with the non-neoplastic gastric surface of non-secretors but not with those of secretors, and also with most gastric adenocarcinoma regardless of secretor status and Lewis phenotype.
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INTRODUCTION

THE POSITION of blood group antigens in the cell surface suggests that they have an important role as recognition targets in cell–cell interactions. Many mediators, such as activation factors, chemotactical and cell growth factors, bind to carbohydrates in these structures. These antigens form a complex signal code, genetically regulated, which intervenes in cell differentiation, growth and recognition, and which may undergo important modification during malignant transformation [1, 2]. Their structure differs only in the type and location of some residual sugars. Lewis (Le) a and b antigens are formed by sequential addition of two fucoses to the type 1 precursor disaccharide, Gal B1-3 GlcNac [3, 4]. Anomalous Le a antigen in gastric carcinomas from patients with Le a−b+ phenotype has been reported at a high frequency [5, 6]. This alteration might be a significant index of the severity of histological premalignant processes [7], in agreement with the commonly accepted model of gastric carcinogenesis [8], and help to identify high-risk individuals.

We have analysed the presence of type 1 precursor in normal mucosa and gastric tumours obtained from 30 gastric adenocarcinoma patients. We correlated our results with the patients' Le phenotypes and secretor status.

MATERIAL AND METHODS

Tissue samples were obtained from 30 surgical specimens from gastrectomised patients with gastric adenocarcinoma. Representative samples were taken from the tumour and from adjacent and distant areas of the non-tumoral mucosa. Tissues were fixed in neutral buffered formalin and embedded in paraffin. Sections were stained with haematoxilin–eosin.

Eight mouse monoclonal antibodies (Mabs) were used for blood groups A, B, H type 2 and Le a and b and type 1 precursor specificities. Mabs A581 (DAKO) and 2521 B8 (DIAGAST) [9] are anti-A. A582 (DAKO) and 164B5G10 (DIAGAST) are anti-B [10]. A583 (DAKO) has an H type 2 specificity. 2.25LE (J. Bara) reacts strongly with Le b and very weakly with Le a antigen [11]. 7LE (J. Bara) reacts with Le a and does not react with Le b antigen [12, 13]. K-21 (SIGNET) was used to recognise type 1 blood group chain precursor [14, 15].

An indirect immunoperoxidase method with a double bridge was used. Sections were deparaffined, rehydrated, washed in Tris buffer and immersed in methanol peroxide. After washing, the sections were incubated for 1 h at room temperature in a solution with each of the Mabs, washed again and exposed to peroxidase-labelled rabbit anti-mouse immunoglobulins (DAKO P-161). After further washing, the sections were incubated with peroxidase-labelled goat anti-rabbit immunoglobulins (NORDIK GAR-IgG Po). The peroxidase staining was revealed with diaminobenzidine/peroxide solution, and the sections were weakly counterstained with Mayer's haematoxilin.

The Le phenotype was assessed by conventional haemagglutination techniques. Patients' ABH secretor status was measured in saliva by haemagglutination inhibition. We found 8 Le a+b−, 16 Le a−b+, 5 Le a−b− (secretors) and 1 Le a−b− (non secretor).

RESULTS

In normal gastric mucosa of non-secretor individuals (Le a+b− [se/se, Le/−] and Le a−b− [se/se, le/le]) type 1 precursor was always positive in the foveolar epithelium (Table 1). Antigen was not expressed in deep areas in any cases. In secretors (Le a−b+ [Se/−, Le/−] and Le a−b− [Se/−, le/le]) both superficial and deep gastric mucosa were negative with K-21. Thus, in the gastric superficial mucosa, expression of type 1 precursor antigens was under the control of the Se gene who code for one α1-2 fucosyltransferase.

We found anomalous expression of type 1 precursor in 14 carcinomas from 16 patients with the Le a−b+ phenotype and in 4 of 5 Le a−b− secretors. Normal mucosa was negative in all cases but in some adjacent tumoral areas with intestinal

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Table 1. Le antigen expression in normal superficial gastric mucosa and in gastric adenocarcinomas

Phenotype	Mab*					
	2.25LE		7LE		K-21	
	Normal	Adenoca.	Normal	Adenoca.	Normal	Adenoca.
Le a−b+ (secretor)						
1	+	+	−	−	−	−
2	+	+	−	+	−	+
3	+	+	−	+	−	+
4	+	+	−	+	−	+
5	+	+	−	+	−	+
6	+	+	−	+	−	+
7	+	+	−	+	−	+
8	+	−	−	+	−	+
9	+	−	−	+	−	+
10	+	−	−	+	−	+
11	+	−	−	+	−	+
12	+	−	−	+	−	+
13	+	−	−	+	−	+
14	+	−	−	−	−	+
15	+	−	−	−	−	+
16	+	−	−	−	−	−
Le a+b− (non-secretor)						
17	−	−	+	−	+	−
18	−	−	+	−	+	+
19	−	−	+	+	+	−
20	−	−	+	+	+	+
21	−	−	+	+	+	+
22	−	−	+	+	+	+
23	−	−	+	+	+	+
24	−	−	+	+	+	+
Le a−b−, Se/− (secretor)						
25	−	−	−	−	−	−
26	−	−	−	−	−	+
27	−	−	−	−	−	+
28	−	−	−	−	−	+
29	−	−	−	−	−	+
					−	
Le a−b−, se/se (non-secretor)						
30	−	−	−	−	+	+

*Against Le b, Le a and type 1 precursor, respectively.
Adenoca. = adenocarcinoma.

metaplasia and dysplasia were positive (Table 1 and Figs 1 and 2).

In non-secretor patients most gastric carcinomas showed the type 1 precursor chain (7 of 9 cases). Nevertheless, we found a focal loss of expression of type 1 precursor in some of the Le a+b− adenocarcinomas. The 1 Le a−b− non-secretor carcinoma showed diffuse expression of this blood group antigen (Table 1).

DISCUSSION

Blood group structures are regarded as differentiation antigens. They are expressed especially in epithelial cells and undergo important modifications during malignant transformation [16, 17]. Le antigens are formed by the sequential addition of two fucoses from the Type 1 chain precursor. Adding a α1-4 fucose to the GlcNac unit produces the Le a antigen, which is

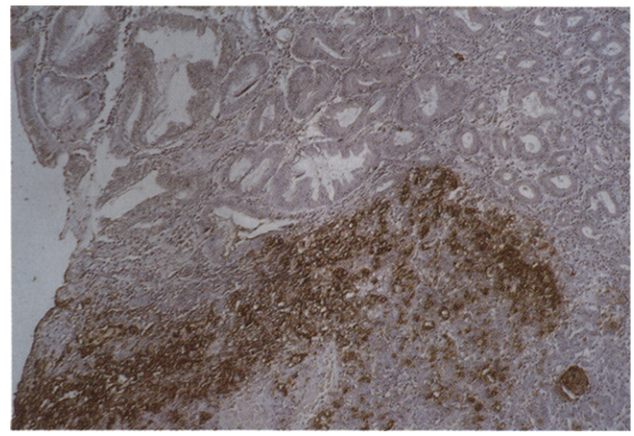

Fig. 1. Gastric adenocarcinoma from patient with Le a−b+ phenotype showing diffuse expression of type 1 precursor antigen. Normal mucosa was negative.

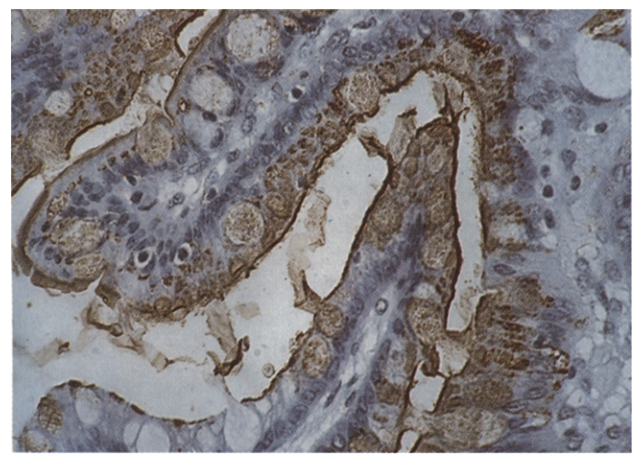

Fig. 2. Positive reaction to anti-type 1 precursor Mab in tumour-adjacent gastric mucosa with intestinal metaplasia in Le a−b+ patient.

determined by Le gene. The action of this gene together with a α1-2 fucosyltransferase produces the Le b epitope. There are at least two α1-2 fucosyltranferases, one coded for by the H gene and other coded for by the Se gene [18]. The fucosyltranferase coded by the Se allele could be expected to act on all precursors, but this enzyme strongly favours the type 1 acceptor [19]. The α1-4 fucosyltranferase is coded for by the Le gene.

Gastric mucosa expresses Le antigens but the products of each enzyme are found in different areas. There are two patterns. The upward differentiation process would favour the expression of the α12 fucosyltranferase coded for by the Se gene, and downward differentiation would favour the expression of the α12 fucosyltranferase coded for by the H gene [11–20]. Thus, in normal gastric mucosa, the type 1 Le antigen reaction pattern is related to Le phenotype and secretor status. Le a antigen is limited to the upper mucosa from Le a+b− individuals and Le b antigen is only present in the superficial mucosa from Le a−b+ individuals.

The anomalous appearance of Le a antigens has been described in patients of Le b phenotype with gastric adenocarcinomas [5, 6]. This could result from blocked synthesis of Le b antigen with accumulation of its precursors.

Because of these findings we are interested in the expression

of the type 1 precursor in gastric mucosa and its alterations in the neoplasic process. This precursor is a marker of human embryonal carcinoma [21]. A human Mab against this structure has now been described in uterine endometrial cancer [22]. Retting *et al.* produced Mab K-21 against type 1 precursor with a Tera 1 teratocarcinoma cell line as immunogen [14]. All embryonal carcinoma and yolk salk tumours exhibit strong positivity for this antigen as detected by K-21 [23].

In our study in the stomach, type 1 precursor were exclusively detected in the superficial epithelium of the normal gastric mucosa from non-secretor individuals. Secretor patients did not express this antigenic structure. Deep areas were constantly negative. We found very strong expression of this antigen in gastric adenocarcinomas, especially in patients with the Le a−b+ phenotype who showed a total loss of Le b isoantigen and who also had negative results for Le a expression. In some of these gastric carcinomas, Le precursor expression was anomalous in adjacent areas with intestinal metaplasia and/or displasia. Moreover, tumours from Le a+b− patients, who showed a focal loss of Le a antigens, expressed type 1 precursor.

Thus, during malignant transformation in gastric carcinoma, neoplastic cells undergo a defect of fucosylation. Sequentially, there is first a loss of α1-2 fucose and then a loss of α1-4 fucose. One of our more interesting findings was the presence of this structure in gastric carcinomas from Le a−b− patients. Thus the type 1 precursor reacted with most gastric adenocarcinomas regardless of secretor status and Le phenotype. These findings confirm the importance of Le antigens as tumour markers and that these antigens might be useful for the study of gastric carcinogenesis.

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