Relationship between immunoexpression of mucin peptide cores MUC1 and MUC2 and Lauren's histologic subtypes of gastric carcinomas

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Laurèn's system subdivides gastric cancers into an intestinal type and a diffuse type. This histological classification mirrors histogenetic hypotheses according to which the intestinal-type cancer derives from intestinal metaplasia and dysplasia, while the diffuse-type originates directly from gastric mucosa, with or without a preceding non-metaplastic dysplasia. Studies concerning mucins expression in gastric neoplastic and preneoplastic lesions have provided contradictory data concerning such histogenetic relationships. The aim of the present study was to verify whether a correlation between mucins phenotype and Lauren's classification subsists. 40 gastric adenocarcinomas, subdivided, according to Laurèn's classification, into 27 intestinal-type, 10 diffuse-type and 3 unclassified cases, were examined for MUC1 and MUC2 immunohistochemical expression. Intestinal-type carcinomas displayed a MUC1-positive staining in 23/27 cases and a MUC2-positive immunoreaction in 10/27 cases. Diffuse-type carcinomas expressed MUC1 in 3/10 and MUC2 in 8/10 cases, respectively. According to the mucins expression pattern, three phenotypes were identified: the gastric phenotype ($MUC1^+/MUC2^-$); the gastro-intestinal phenotype ($MUC1^+/MUC2^+$) and the intestinal phenotype ($MUC1^-/MUC2^+$). The gastric phenotype was significantly higherin intestinal-type adenocarcinomas, whereas cases showing an intestinal phenotype were significantly more frequent in diffuse-type adenocarcinomas. These findings provide evidence for a lack of correlation between Lauren's classification and MUC1 and MUC2 phenotypes. In particular, the term intestinal-type tumour as referred to gland-forming gastric cancer does not seem to reflect an immunohistochemical phenotype.

Key words: MUC1, MUC2, gastric carcinoma, Lauren's classification.

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In Western countries, Lauren's histologic classification system for gastric carcinomas is largely used (Lauren, 1965). It classifies human gastric carcinomas from a standard haematoxylin and eosin stain, into two main groups, the intestinal and diffuse types, corresponding to differentiated and undifferentiated types (Nakamura *et al.,* 1968; Sugano *et al.,* 1982), respectively. In terms of histogenesis, it is generally accepted that the Lauren's intestinal-type gastric carcinoma is preceded by the sequential steps of chronic gastritis, intestinal metaplasia, dysplasia, and intramucosal carcinoma (Uchino *et al.,* 1993), whereas diffuse-type gastric carcinoma seems to originate via hyperplastic or *de novo* changes, with or without concurrent non-metaplastic dysplasia (Correa *et al.,* 1992). Nonetheless, conflicting data have recently emerged regarding such histogenetic relationships from studies concerning mucins expression in gastric cancerous and pre-cancerous lesions (Baldus *et al.,* 1998; Machado *et al.,* 2000; Tsukashita *et al.,* 2001; Gurbuz *et al.,* 2002; Wada *et al.,* 2005). Mucins are high-molecular-weight glycoproteins consisting of a central polypeptidic structure (apomucin) to which numerous carbohydrate chains are attached by an O-glycosidic linkage. They can be divided into two types, secretory and membrane-bound mucins, respectively. At present, 20 different human genes encoding for apomucins have been identified (MUC1, 2, 3A, 3B, 4, 5AC, 5B, 6, 7, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20) (Swallow *et al.,* 1987a; Swallow *et al.,* 1987b; Gum *et al.,* 1989; Gum *et al.,* 1990; Porchet *et al.,* 1991; Toribara *et al.,* 1991; Fox *et al.,* 1992; Bobek *et al.,* 1993; Gross *et al.,* 1993; Shankar *et al.,* 1994; Guyonnet-Duperat *et al.,* 1995; Ho *et al.,* 1995a; Lapensèe *et al.,* 1997; Williams *et al.,* 1999; Wu *et al.,* 2001; Williams *et al.,* 2001; Yin *et al.,* 2001; Pallesen *et al.,* 2002; Gum *et al.,* 2002; Chen *et al.,* 2004; Higuchi *et al.,* 2004). MUC1 and MUC2 are the most widely investigated mucins in gastric carcinomas. MUC1 has been detected in mucous cells of the

Table 1. Clinico-pathological characteristics and mucins expression profile in 40 gastric adenocarcinomas.

Case	*Age*	*Gender*	*Localization*	*WHO Classif.*	*Laurèn Classif.*	*WHO Grade*	*pTNM*	*MUC1*	*MUC2*
1	74	F	Cardias	SCR	Diffuse	MD	T2N1Mx	0	2
2	73	M	Cardias	SCR	Diffuse	WD	T3N1Mx	0	1
3	63	F	Corpus	SCR	Diffuse	WD	T3N3Mx	0	2
4	60	F	Corpus	SCR	Diffuse	MD	T2N1Mx	0	1
5	73	M	Corpus	SRC	Diffuse	MD	T2N1Mx	0	2
6	76	M	Corpus	Mucinous	Unclassified	WD	T2N1Mx	2	2
7	69	F	Corpus	Tubular	Intest	WD	T3N1Mx	1	0
8	77	M	Corpus	Tubular	Intest	WD	T2N0Mx	2	0
9	66	M	Corpus	SCR	Diffuse	WD	T2N0Mx	0	1
10	71	F	Antrum	SCR	Diffuse	MD	T2N1Mx	0	2
11	72	F	Antrum	Tubular	Intest	WD	T2N0Mx	0	2
12	69	F	Corpus	Tubular	Intest	MD	T1N0Mx	1	0
13	78	M	Cardias	SCR	Diffuse	WD	T2N1Mx	2	0
14	54	F	Antrum	SCR	Diffuse	MD	T2N3Mx	1	0
15	66	M	Corpus	Mucinous	Unclassified	WD	T2N0Mx	0	2
16	74	F	Corpus	Tubular	Intest	WD	T1N2Mx	1	0
17	70	M	Antrum	SCR	Diffuse	MD	T1N0Mx	1	2
18	66	F	Corpus	Tubular	Intest	WD	T2N1Mx	1	0
19	74	F	Corpus	Tubular	Intest	MD	T2N1Mx	2	0
20	77	M	Corpus	Tubular	Intest	WD	T1N0Mx	2	0
21	73	M	Corpus	Tubular	Intest	WD	T2N1Mx	1	0
22	77	M	Antrum	Tubular	Intest	MD	T1N0Mx	1	0
23	71	M	Corpus	Tubular	Intest	WD	T2N1Mx	2	0
24	57	M	Antrum	Tubular	Intest	WD	T1N0Mx	2	0
25	61	M	Corpus	Tubular	Intest	MD	T2N1Mx	2	0
26	66	F	Antrum	Tubular	Intest	WD	T2N0Mx	1	2
27	71	M	Corpus	Tubular	Intest	MD	T2N1Mx	1	0
28	74	F	Corpus	Papillar	Intest	WD	T2n1Mx	1	0
29	77	M	Corpus	Mucinous	Unclassified	WD	T1N0Mx	2	0
30	71	M	Corpus	Tubular	Intest	MD	T2N1Mx	1	2
31	59	M	Antrum	Tubular	Intest	WD	T2N1Mx	2	2
32	70	F	Corpus	Tubular	Intest	WD	T1N0Mx	0	2
33	64	F	Antrum	Tubular	Intest	WD	T2N1Mx	2	0
34	66	M	Corpus	Tubular	Intest	MD	T2N1Mx	2	1
35	59	F	Corpus	Tubular	Intest	WD	T3N1Mx	1	2
36	74	F	Antrum	Tubular	Intest	WD	T2N1Mx	1	0
37	77	M	Corpus	Tubular	Intest	MD	T1N0Mx	0	1
38	65	M	Corpus	Tubular	Intest	WD	T3N1Mx	1	1
39	66	M	Corpus	Tubular	Intest	MD	T2N1Mx	1	0
40	77	M	Antrum	Tubular	Intest	WD	T2N1Mx	0	2

surface epithelium and neck region of the antrum, as well as in pyloric glands and in oxynthic glands of the body region in normal gastric mucosa (Ho *et al.,* 1993; 1995b; Lesuffleur *et al.,* 1994). During gastric carcinogenesis, expression of underglycosylated forms of MUC1 mucin has been demonstrated (Baldus *et al.,* 1998; Reis *et al.,* 1998) and their presence seems to be closely tied to a poor prognosis (Akyurek N *et al.,* 2002; Kocer *et al.,* 2004). MUC2 mucin, expressed in the colon, small intestine and airways, and not found in normal gastric mucosa, is detected in intestinal metaplasia and in gastric carcinoma (Filipe *et al.,* 1996); it is considered a prognostic factor associated with an unfavourable outcome in patients with gastric cancer (Zhang *et al.,* 2004; Leteurtre *et al.,* 2006). Despite the many studies that have been performed, conflicting data have emerged as to correlations between mucins phenotype and gastric carcinoma classification systems (Machado *et al.,* 2000; Tsukashita *et al.,* 2001; Gurbuz *et al.,* 2002; Roessler *et al.,* 2005; Kabashima *et al.,* 2005; Leturtre *et al.,* 2006). Lauren's hypothesis, according to which gland-forming gastric cancer is related to intestinal differentiation, remains still to be validated. Indeed, whereas some authors find a higher rate of MUC2 expression in intestinal-type cancer compared to diffuse-type (Baldus *et al.,* 1998; Kocer *et al.,* 2004), others report a low prevalence of this intestinal marker of differentiation in intestinal-type gastric adenocarcinomas (Machado *et al.,* 2000; Tsukashita *et al.,* 2001), in contrast with histogenetic theory according to which gastric intestinal-type cancer would derive via chronic gastritis, intestinal metaplasia and dysplasia. In the present study we determined MUC1 and MUC2 immunohistochemical expression profiles in a series of gastric cancers preliminarily classified according to Laurèn's system,

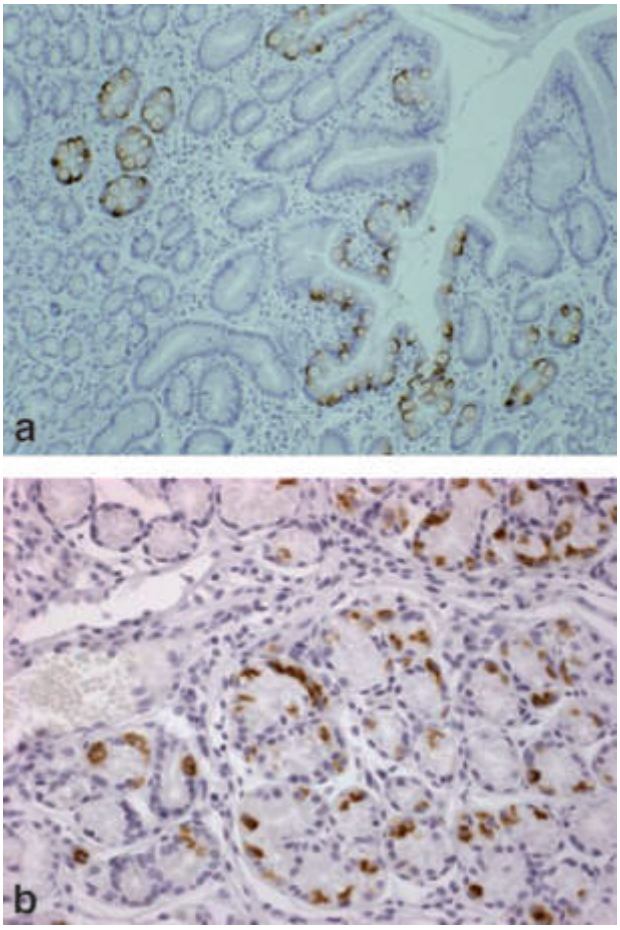

Figure 1. a. Normal gastric glands are not labelled by MUC2 antibody, whereas MUC2 staining is clearly evident in intestinal metaplasia (MUC2 staining; original magnification x100). b. MUC1 intense immunoreaction in mucous neck cells of gastric normal epithelium (MUC1 staining, original magnification x200).

and aimed to investigate whether any correlation exists between MUC1 and MUC2 mucin phenotypes and Lauren's classification system.

Materials and Methods

40 cases of gastric adenocarcinomas were selected from the files of the Department of Human Pathology, University of Messina, Italy. Tissue samples were obtained from surgical resection; for each case, age and gender of the patient, tumour location, grading and pTNM stage were evaluated by reviewing clinical charts and pathological records. Patients that had undergone pre-operative chemotherapy were excluded from the study. According to the Lauren's system, cases were subdivided into 27 intestinal-type, 10 diffuse-type, and 3 unclassified adenocarcinomas. Helicobacter Pylori infection was not evidenced in any sample. Immunohistochemical procedures were performed on 4 μm formalin fixed, paraffin embedded, tissue sections obtained from each representative paraffin block. Briefly, endogenous peroxidase activity was preliminarily blocked with 3% H_2O_2 in PBS for 30 min at room temperature. Sections were successively incubated at 4°C overnight with the following primary monoclonal antibodies: NCL-MUC1 (Ma695; 1:100, Novocastra, Newcastle, UK) and NCL-MUC2 (Ccp58; 1:100, Novocastra Newcastle, UK). Microwave pre-treatment using 0.01M sodium citrate buffer was employed for each immunoreaction. The bound primary antibody was visualized by the avidin-biotin-peroxidase detection complex (ABC) method with a commercial kit (Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, Calif.,USA), according to the manufacturer's instructions. 3-3' diaminobenzidine (DAB) was used as the chromogen. Sections of breast carcinoma for MUC1 and normal small bowel mucosa for MUC2 were used as positive controls. Negative control slides were also made by omitting the primary antibody. The extent of positivity for MUC1 and MUC2 was scored according to the percentage of stained neoplastic cells: 0= <5% positive cells; 1= 5-50% positive cells; 2= 51%-100% positive cells. The percentages of positive cells were estimated in several optical fields (10x objective lens). Scoring was performed blindly by two pathologists (G.B., V.B.) with an inter-observer concordance of nearly 100%. When disagreement was present, the mean value was considered. According to MUC1 and MUC2 immunohistochemical expression patterns, gastric carcinomas of our series were then subdivided into: gastric (G) ($MUC1^+/MUC2^-$), gastro-intestinal (GI) ($MUC1^+/MUC2^+$) and intestinal (I) ($MUC1^-/MUC2^+$). Statistical correlations between clinico-pathological characteristics and mucin phenotype as well as extent of positive immunoreaction were peformed by the Chi-square test. *P* values less than 0.05 were considered to be statistically significant.

Results

Clinico-pathological characteristics and MUC1 and MUC2 immunohistochemical expression patterns are shown in Table 1. Staining for MUC1 mucin, but not for MUC2, was evidenced in normal gastric epithelium present in analyzed samples, while MUC2-positive staining was always identified in intestinal metaplasia areas in proximity to some intestinal-type cancers (Figure 1).

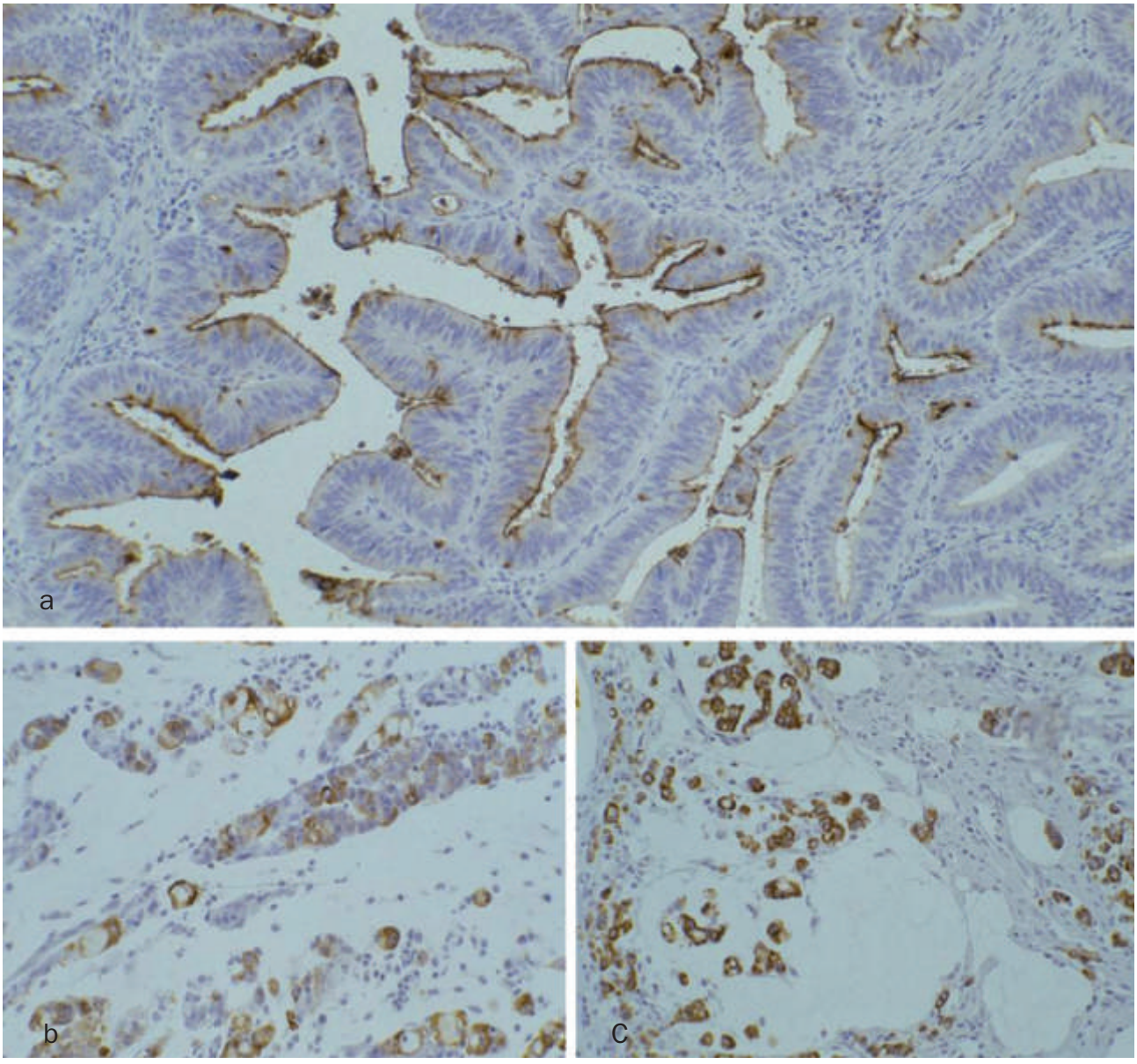

Figure 2. a. Intestinal-type adenocarcinoma showing a MUC1 positive immnunoreaction mainly localized at the apical membrane of the cells (MUC1 staining; original magnification x100). b. Cells of diffuse-type adenocarcinoma labelled by MUC1 antibody (MUC 1 staining; original magnification x100). c. Gastric mucinous adenocarcinoma displaying a MUC1positive staining (MUC1 staining; original magnification x100).

Immunoreactivity for MUC1 and MUC2 was observed in 28 cases (70%) and in 20 cases (50%), respectively, out of the 40 analyzed gastric adenocarcinomas. In particular, MUC1 was expressed in 23/27 intestinal-type adenocarcinomas and in 3/10 diffuse-type adenocarcinomas, whereas 10/27 intestinal-type and 8/10 diffuse-type adenocarcinomas exhibited an MUC2-positive immunoreaction. The three analyzed unclassified adenocarcinomas, corresponding to mucinous histopathological subtypes of WHO classification, expressed MUC1 in 2/3 and MUC2 in 2/3 cases, respectively. MUC1 staining was mainly localized at the apical membrane in the intestinal-type carcinoma and in the cytoplasm in the diffuse-type (Figure 2 a, b, c). The immunostaining for MUC2 was detected in the cytoplasm of both examined histopathological subtypes (Figure 3 a, b, c). Classifying analyzed samples according to mucin phenotypes, we found that the rate of cases with a G phenotype ($MUC1^+/MUC2^-$) was significantly (p=0.025) higher in intestinal-type carcinomas than in diffuse-type, while the opposite happened for cases displaying an I phenotype, whose rate was significantly

Table 2. Correlation between mucin phenotype and clinico-pathological parameters.

	N	*Gastric Phenotype (MUC1⁻/MUC2⁻)*	*Gastro-intestinal Phenotype (MUC1⁻/MUC2+)*	*Intestinal Phenotype (MUC1⁻/MUC2⁺)*
Total number				
Age	40	20	8	12
<70	17	8	5	4
≥70				
Gender	23	12	3	8
Male	23	11	6	6
Female	17	9	2	6
Histotype (Laurèn Classification)*				
Intestinal	27	17	6	4
Diffuse	10	2	1	7
Unclassified	3	1	1	1
Localization				
Cardias	3	1	0	2
Corpus	26	14	5	7
Antrum	11	5	3	3
Grade (Intestinal histotype)				
1 (WD)	18	11	4	3
2 (MD)	9	6	3	0
pTNM				
T1	9	6	1	2
T2	26	13	5	8
T3	5	1	2	2
N0	13	6	2	5
N1	24	12	6	6
N2	1	1	0	0
N3	2	1	0	1

** p =0.025.*

(*p*=0.025) higher in diffuse-type carcinomas (Figures 4 and 5). Taking into consideration the three analyzed unclassified carcinomas, one exhibited a G phenotype, another a GI phenotype, and the third an I phenotype. No statistically significant correlations were found between mucin phenotypes and other clinico-pathological parameters (Table 2). Pertaining to staining extent, a statistically significant difference was only found between MUC1 percentage of positive cells and cancer histopathological subtypes as shown in Table 3.

Discussion

In the present study, we analyzed MUC1 and MUC2 immunohistochemical expression in a series of 40 gastric adenocarcinomas, classified according to Laurèn's system. In agreement with other authors (Baldus *et al.,*1998; Akyurek *et al.,* 2002; Gurbuz *et al.,* 2002; Reis *et al.,* 1998; Lee *et al.,* 2001; Pinto-de-Sousa *et al.,* 2002, Roessler *et al.,* 2005; Leteurtre *et al.,* 2006), who demonstrated a lack of correlation between mucin phenotypes and Lauren's histopathological classification, we found that MUC1 expression was significantly higher in

Table 3. Correlation between mucins staining extent and clinicopathological parameters.

	N	*MUC1 Staining area*			*MUC2 Staining area*		
		0 (<5% Positive cells)	*1 (5-50% positive cells)*	*2 (51-100% positive cells)*	*0 (<5% Positive cells)*	*1 (5-50% positive cells)*	*2 (51-100% positive cells)*
Total number	40	12	16	12	20	6	14
Age							
<70	17	4	8	5	8	4	5
?70	23	8	8	7	12	2	9
Gender							
Male	23	6	7	10	11	5	7
Female	17	6	9	2	9	1	7
Histotype (Laurèn Class.)							
Intestinal	27	* 4	14	9	17	3	7
Diffuse	10	7	2	1	2	3	5
Unclassified	3	1	0	2	1	0	2
Location							
Cardias	3	2	0	1	0	0	0
Corpus	26	7	11	8	14	5	7
Antrum	11	3	5	3	5	0	6
Grade (Intestinal Histotype)							
1 WD	18	3	9	6	11	1	2
2 MD	9	1	5	3	6	2	1
TNM							
T1	9	2	4	3	6	1	2
T2	26	8	9	9	13	3	10
T3	5	2	3	0	1	2	2
N0	13	5	4	4	6	2	5
N1	24	6	10	8	12	4	8
N2	1	0	1	0	1	0	0
N3	2	1	1	0	1	0	1

** p= 0.010.*

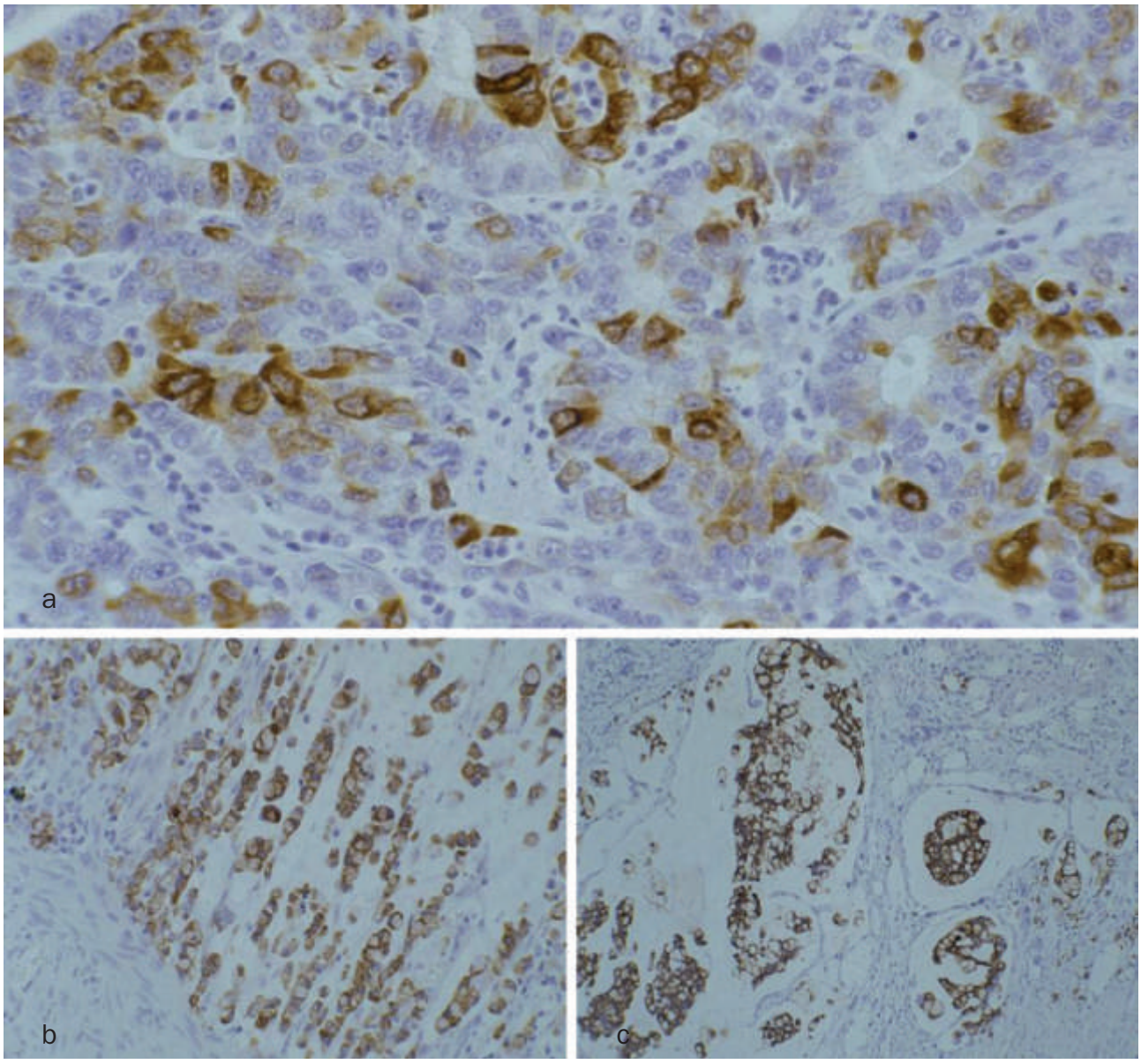

Figure 3. a. Cytoplasmic staining for MUC2 is evident in intestinal-type adenocarcinoma (MUC2 staining; original magnification x200). b. Signet ring cell carcinoma showing a diffuse, intense labelling for MUC2 (MUC2 staining; original magnification x100). c. Neoplastic elements floating in extracellular mucin in the context of a mucinous carcinoma display a positive immunoreaction for MUC2 (MUC2 staining; original magnification x100).

gland-forming (intestinal-type) tumours than in diffuse ones, whereas MUC2 stained positive in a significantly greater proportion of diffuse-type carcinomas than of intestinal-type.

Moreover, when cases were stratified according to mucin phenotypes, we found that, among intestinal-type considered adenocarcinomas, only 4/27 exhibited an I phenotype ($MUC1^-/MUC2^+$), while the majority of cases (17/27) displayed a G phenotype ($MUC1^+/MUC2^-$) and 6/27 cases co-expressed both mucins. These findings seem to be in contrast with an absolute origin of intestinal-type adenocarcinomas via intestinal metaplasia. Indeed, the hypothesis of a direct derivation from native gastric cells seems to be more suitable for the majority of intestinal-type tumours of our series, which, as mentioned above, mostly presented a G phenotype, with an absence of expression of the intestinal mucin MUC2. Thus, intestinal metaplasia would not be an indispensable factor in the development of this gastric carcinoma histopathological subtype. Nevertheless, the presence of a mucin GI phenotype

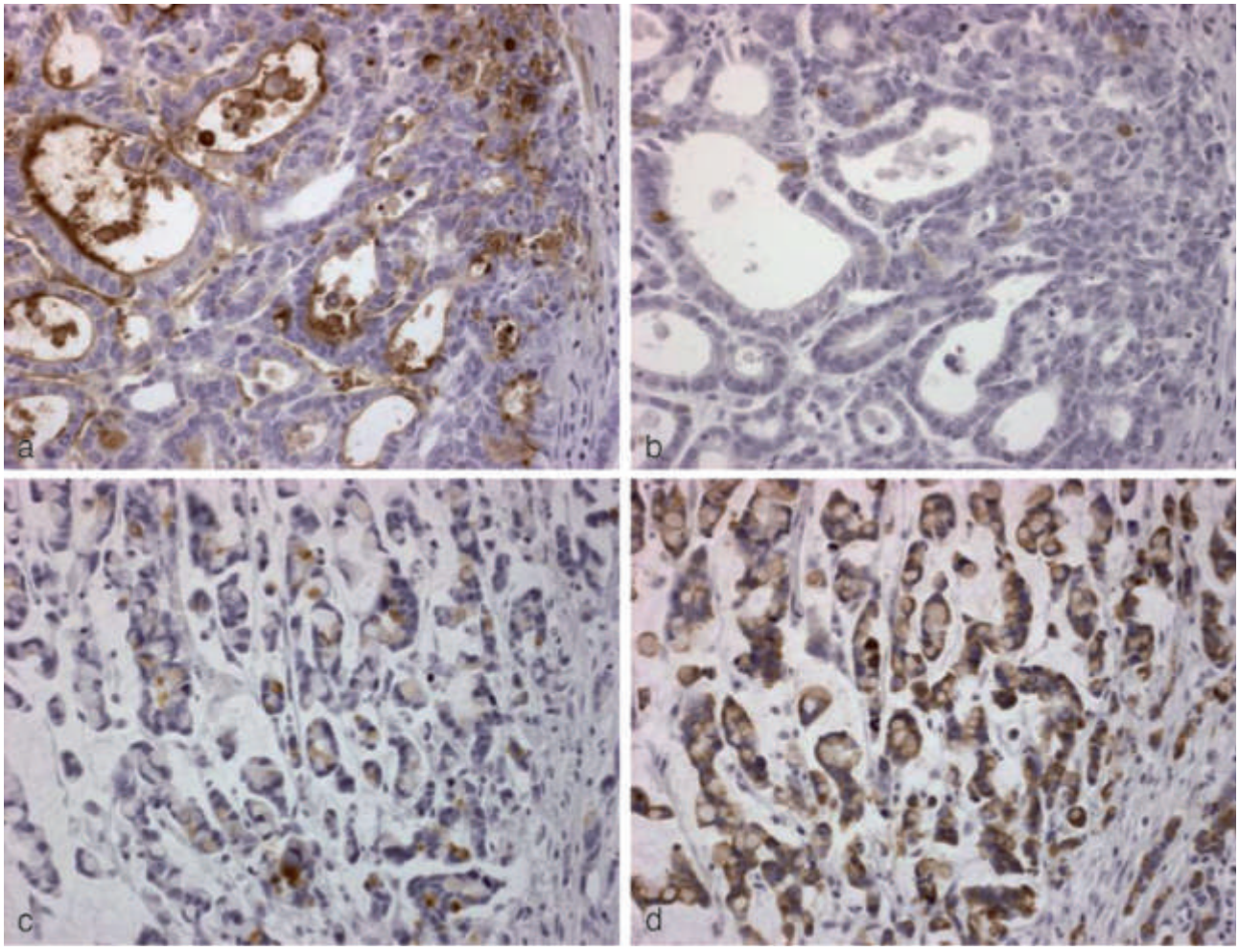

Figure 4. a, b. Consecutive sections of a gastric intestinal-type adenocarcinoma. MUC1-positive immunoreaction was evidenced in most cells (a. MUC1 staining, original magnification x200), whereas only scattered elements were labelled by MUC2 antibody (b. MUC2 staining, original magnification x200). c, d. Consecutive sections of a diffuse-type adenocarcinoma displaying a slight immunoreaction for MUC1 (c. MUC1 staining, original magnification x200) and a strong diffuse labelling for MUC2 (d. MUC2 staining, original magnification x200).

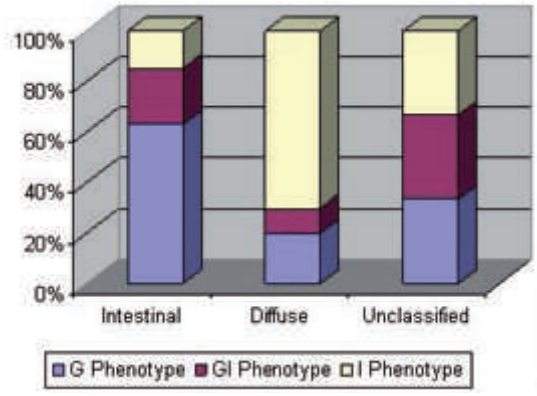

Figure 5. The graph illustrates the distribution of the three mucin phenotypic groups in intestinal-type, diffuse-type and unclassified type of Lauren's classification. The percentage of cases displaying a G phenotype was significantly higher in intestinal-type tumours, whereas a significantly higher proportion of cases showing an I phenotype was evidenced in diffuse-type tumours.

in 6/27 intestinal-type adenocarcinomas of our series might reflect a histogenetic origin of such cases from incomplete intestinal metaplasia. Indeed, as is known, this latter is an unstable lesion, associated with the development of intestinal type gastric cancer (Filipe *et al.,* 1994; Cassaro *et al.,* 2000) which shows both gastric and intestinal phenotypes (Silva *et al.,* 2002). Incomplete intestinal metaplasia gradually shifts towards the complete type, with loss of the gastric phenotype and permanence of the intestinal phenotype (Inada *et al.,* 1997; Tatematsu *et al.,* 2003). A small percentage of cases, only expressing MUC2, might, therefore, originate from complete intestinal metaplasia.

On the other hand, diffuse-type carcinomas of our series mostly tended to exhibit an I phenotype (7/10 cases), showing a positive immunoreaction

for only MUC2 in the majority of cases. High frequency of an I phenotype in undifferentiated (diffuse-type) gastric adenocarcinomas has already been found by other authors (Kabashima *et al.,* 2005). Diffuse-type carcinomas are commonly thought to arise from gastric mucosa without preceding intestinal metaplasia (Correa *et al.,* 1992). Intestinal phenotypes in signet ring cell carcinomas seems to be successively acquired during tumour progression together with gastric wall infiltration (Yamachika *et al.,* 1997; Bamba *et al.,* 2001). In our casuistry, the predominance of an I phenotype among diffuse type adenocarcinomas might therefore be related to the prevalence of deeply infiltrating tumours. 9/10 cases, indeed, invaded muscolaris propria, subserosa or serosa (T2-T3). Moreover, the only T1 case displayed a GI phenotype. Throughout the use of CK7 as marker of de-differentiation, Kirchner *et al.,* (2001) demonstrated the presence of un-differentiated epithelial cells in the early stages of development of gastric carcinoma, especially of the diffuse-type, and within areas of intestinal metaplasia and non-atrophic chronic gastritis. In particular, these workers speculated that inflammatory chronic stimulus could trigger a repetitive process of de-differentiation and re-differentiation, with a subsequent accumulation of genetic alterations leading to cancer development. Therefore, both diffuse and intestinal histo-pathological subtypes of gastric cancer may derive from such un-differentiated elements, with preceding loss of the original phenotype and subsequent acquirement of a different phenotype. The putative origin of gastric malignancies from undifferentiated elements is corroborated by the results achieved by Kawachi *et al.,* (2003), showing an absence of either gastric or intestinal phenotypes in microscopic differentiated gastric carcinomas. Subsequently, we may also speculate that diffuse cancers in our series might have arisen from such de-differentiated cells with a further acquisition, during cancer progression, of an intestinal phenotype.

On the contrary, a correlation between chronic gastritis-metaplasia and dedifferentiation might explain our finding of a low proportion of a mucin I phenotype and a prevalence of a mucin G phenotype among analyzed intestinal-type cancer. Such types of gastric adenocarcinomas, in fact, as previously mentioned, are thought originatefrom chronic gastritis-intestinal metaplasia-displasia. Loss of an intestinal phenotype, and subsequent acquirement of a gastric one might be due to de-differentiation at the stage of metaplasia.

In conclusion, our observations demonstrate that Laurèn's Classification of gastric cancer does not fit mucin phenotypes of these neoplasias. In fact, Laurèn's intestinal type adenocarcinomas often display a gastric mucin phenotype; furthermore, so-called diffuse carcinomas frequently exhibit differentiation towards an intestinal mucin phenotype; hence, in particular, in our opinion, the notion of intestinal-type carcinoma seems not to be fully appropriate for a lesion mostly exhibiting a gastric rather than an intestinal phenotype upon immunohistochemical analysis.

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