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Expression of Epithelial Antigens EPM-1 and EXO-1 in Normal, Transitional, Inflammatory and Neoplastic Colorectal Mucosa

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EPM-1 (a high molecular weight glycoprotein) and EXO-1 (a carbohydrate epitope expressed on polar neutral glycolipids and mucins) are two developmental antigens of normal and neoplastic human epithelia and were characterised by monoclonal antibodies. Their distribution was investigated in normal and pathological human colorectal mucosa. In normal mucosa, EPM-1 and EXO-1 showed characteristic expression patterns. EPM-1 was differentially expressed along the crypt villus axis with maximum at the crypt basis. EXO-1 was present throughout the whole mucosa. The characteristic gradient of EPM-1 expression along the crypt axis in normal mucosa was no longer detectable in benign polyps. Intact gradient of EPM-1 staining discriminated between neoplastic changes of the benign adenomatous polyp and mucosal inflammation. Neoplastic mucosa in benign polyps and especially atypical glands in highly differentiated tumours showed essentially identical expression patterns. In colorectal carcinomas the overall reactivities for EPM-1 and EXO-1 were independently associated with the histopathological grade of tumour differentiation.
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INTRODUCTION

COLORECTAL TUMOURS provide an excellent system to study the events that underlie the initiation and progression of the multistep process of tumorigenesis in this epithelial tissue. Self renewal of normal colonic epithelium is based on polyclonal proliferation of cells in crypts and characterised by a balanced state of proliferation and differentiation. The initiated process of neoplasia results in colonal expansion which morphologically corresponds to the development of aberrant crypts representing putative preneoplastic foci [1]. This observation is paralleled by the appearance of crypt restricted heterogeneity of mucus glycoproteins [2]. In general, tissue development and malignant transformation are reflected by significant changes in the expression of glycolipids and carbohydrate structures of cell membrane glycoproteins [3]. Also, tissue-specific expression of carbohydrate carrier chains has been well documented [4]. Functional properties of carbohydrate determinants of gastrointestinal epithelium are still not well defined. Structural and antigenic similarities between glycoproteins and glycolipids emphasise the biological significance of the carbohydrate determinants themselves. During the adenoma-carcinoma sequence alterations of cellular functions take place such as synthesis, transport and secretion of mucin components. Pronounced cytoplasmic vs. surface carcinoembryonic antigen (CEA) expression and changes in the composition of mucin secretions have been described in association with neoplastic growth [5]. CEA also constitutes an important model for modifications of the glycosylation pattern that are induced during cancer biogenesis [6].

EPM-1 (a high molecular weight glycoprotein) and EXO-1 (a carbohydrate epitope detected on polar neutral glycolipids and mucin molecules) are two developmental antigens of normal and neoplastic human epithelia and were identified by monoclonal antibodies [7–9]. In human keratinocytes alterations in growth and differentiation occurring during hyperproliferation and different stages of neoplastic transformation of the human epidermis were associated with significant changes of EPM-1 and EXO-1 expression [10]. EXO-1 was correlated to an early embryonic differentiation pathway, which can reemerge abnormally in the adult in case of hyperproliferation. EPM-1 expression was part of developmental programs and indicated a steady state of proliferation and differentation in the completely developed adult tissue, i.e. tissue homeostasis. The regulation of EPM-1 expression was substantially different and independent of EXO-1 but in combination provided new insights into regulation and alterations of growth and differentiation in human epidermis. To assess the biological significance of the EPM-1/EXO-1 antigenic system in human colorectal mucosa at cellular levels and in the tissue architecture, the expression of these was investigated in normal, transitional, inflammatory and neoplastic colon mucosa by immunohistochemistry.

MATERIALS AND METHODS

Monoclonal antibodies

Epithelial antigen EXO-1 was detected by monoclonal antibody (MoAb) PaG-14 (IgM), EPM-1 antigen by MoAb Pa25 (IgM) [7, 8, 10]. Pools of (5×) concentrated hybridoma supernatant (immunoglobulin concentration approximately 200 μg/ml) were used throughout the whole study. A monoclonal antibody to the mouse lymphocyte antigen Lyt.1.1 (IgM) served as a negative control and the anti-HLA-ABC MoAb W6/32 as a positive control for immunostainings.

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Tissue samples

Tissue samples of histologically normal colon mucosa distant from colorectal cancer (n = 15), colorectal mucosa from patients with histologically proven long-standing ulcerative colitis (n = 7) or Crohn's disease (n = 7), histologically normal non-neoplastic transitional mucosa adjacent to colorectal tumours characterised by crypt elongation and an increased number of mucin secreting cells (n = 7) [6], benign colorectal polyps (n = 9) and malignant colorectal tumours (n = 38) were obtained from surgical material and endoscopic biopsies or polypectomies. The specimens were snap-frozen and stored in liquid nitrogen or at −70°C. Cryostat sections (4–6 μm) were obtained according to standard procedures.

Each of the biopsies was evaluated for presence of dysplasia or grading in case of malignant tumours by reviewing haematoxylin and eosin-stained tissue sections with a pathologist. This diagnosis was then compared to the original pathology report. Specimens, which were representative for the diagnosis of the patient studied, were included in the analysis.

Immunohistochemistry

Fresh frozen, non-fixed tissue sections (4–6 μm) were analysed by an indirect immunoperoxidase method, essentially as described previously [10, 11]. Briefly, MoAb and subsequently peroxidase-conjugated polyclonal rabbit anti-mouse antibodies were incubated for 1 h at room temperature. The immunohistochemical reaction was developed with the red dye 3-amino-9-ethyl-carbazole or diaminobenzidine and counterstained with blue Meyer's Haemalum solution (Merck, Darmstadt, FRG). For analysis of normal colon mucosa and colorectal polyps, indirect immunofluorescence with a rhodamine-labelled goat anti-mouse Ig antiserum was additionally used. Results were essentially identical for both techniques. Results were assessed by the following three-point scale: + : strong positive reaction; (+): weak but specific positive reaction; −: negative reaction. Percentage of positive tumour cells was estimated by percentage of positive reactions of three different, randomly selected tissue specimens of each colorectal carcinoma, which were representative of the overall histomorphological grading of the tumour. The percentage was estimated in 10 optical fields.

RESULTS

The immunohistochemical distribution of the epithelial antigens EPM-1 and EXO-1 was examined immunohistochemically in 83 human colonic tissue specimens originating from benign and malignant disease entities of the colorectum.

Normal colorectal mucosa

Fifteen tissue samples of normal colorectal mucosa were analysed. No difference in the staining for EPM-1 and EXO-1 between distal or proximal parts of the colon was observed. Normal mucosa from ascending, transverse and descending colon was obtained from surgically resected tumours in respective locations. Also, the patients' blood group did not influence the immunohistochemical reactivity. In normal colorectal mucosa expression of the EPM-1 antigen was detected in the entire crypt cell population (Fig. 1a). At the base of the crypt, homogeneous cytoplasmic staining was observed (Fig. 2a,b). Towards the surface epithelium, columnar epithelial cells showed a decreasing intensity of EPM-1 expression, accentuating the apical cell pole. In goblet cells the staining was seen in the periplasmic region of goblet cells including the subvacular cytoplasm. Besides some reactions in the lower half of crypts, goblet cell mucin in vacuoles remained negative.

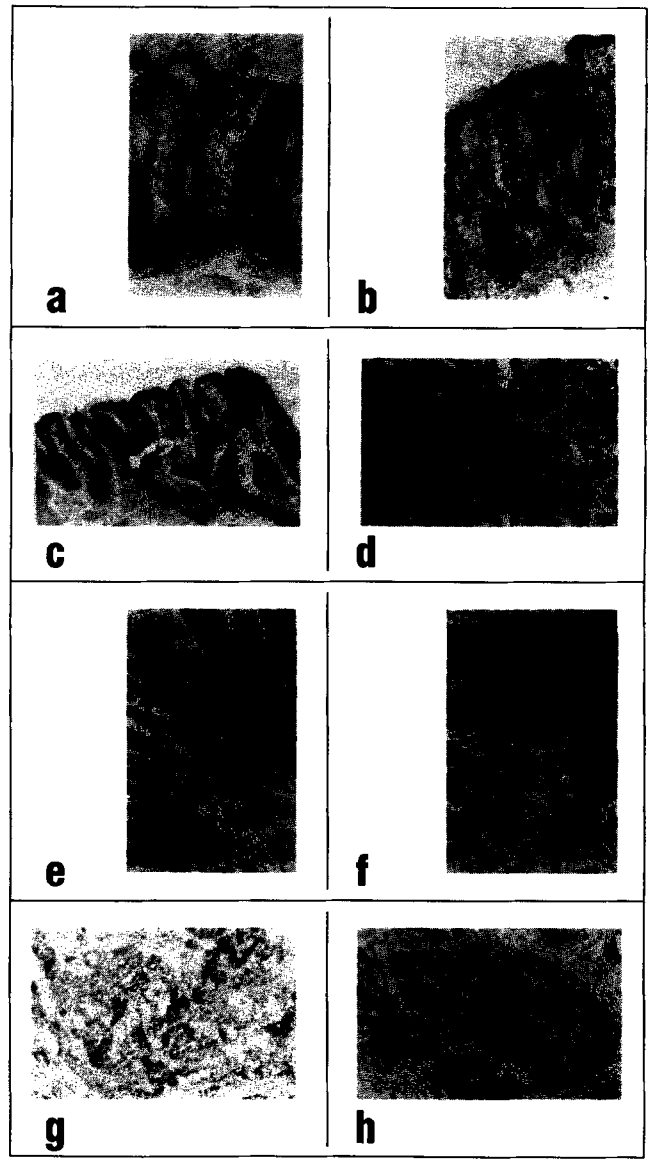

Fig. 1. Immunohistochemical distribution of EPM-1 and EXO-1: normal colon mucosa (a) EPM-1, (b) EXO-1. Benign adenomatous polyp (c) EPM-1, (d) EXO-1. Colorectal carcinomas (grading of tumour differentiation G1–G4 according to TNM classification of malignant tumours): (e) G1 tumour EPM-1, (f) G2 tumour EXO-1, (g) G3 tumour EPM-1, (h) G3 tumour EXO-1. (Original magnifications ×100: a,b,g,h; ×50: c–f.)

For EXO-1 the complete colorectal mucosa showed cytoplasmic staining (Fig. 1b). Particularly in the surface epithelium, the apical cell pole showed marked reactivity. At the base of the crypt subvacuolar cytoplasm of goblet cells was positive. Contents of goblet cell vacuoles were not stained. Secretions on top of surface epithelium showed specific immunohistochemical reactivity.

Inflammatory large bowel disease

Fourteen samples of colonic mucosa from patients with inflammatory large bowel disease obtained from surgical resections and endoscopic biopsies showed chronic active disease with severe active inflammation. Included were 7 patients with histologically proven ulcerative colitis and 7 with Crohn's disease. EPM-1 and EXO-1 showed an identical expression pattern as it was described above for normal mucosa. EPM-1 was

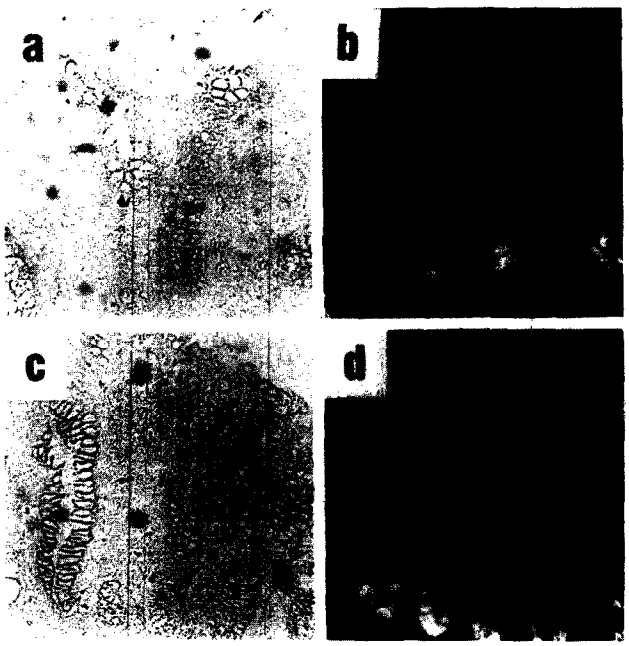

Fig. 2. Gradient of EPM-1 expression demonstrated by immunofluorescence with monoclonal antibodies in normal (a,b) and transitional colon mucosa (c,d). Phase contrast photomicrographs (a,c) and coincident immunofluorescence fields (b,d). (Original magnifications ×100.)

predominantly detected in crypts with decreasing reactivity towards the superficial mucosa. The gradient was not altered by inflammatory cell infiltrates. Reactivity with monoclonal antibody against EXO-1 was observed consistently in epithelial cells both at the mucosal surface and in the crypts as well as associated with secretions. Cytoplasmic staining was predominantly located at the apical cell pole but in goblet cells also in subvacular cytoplasm.

Transitional mucosa

Transitional mucosa was investigated in seven specimens with 1 cm of the colorectal tumours. Morphologically, this tissue did not show any evidence of malignant changes. As it was observed in mucosa affected by inflammatory disease, the expression of EPM-1 and EXO-1 did not show a significant difference compared with normal colorectal mucosa. The EPM-1 gradient was present (Fig. 2c, d).

Benign adenomatous colorectal polyps

The nine benign adenomatous polyps investigated were obtained from tubular (n = 5) and tubulovillous adenomas (n = 4) and were classified histopathologically as having no significant epithelial atypia or only mild dysplasia and were free of malignant foci. In contrast to normal, inflammatory and transitional colorectal mucosa, the entire crypt length including the surface epithelium showed reactivity for EPM-1 (Fig. 1c). The neoplastic epithelium showed marked intensity of staining at the apical cell pole, sometimes staining seemed to be restricted to this cell compartment. Staining of secretions was also observed. Loss of gradient of the EPM-1 antigen did not correlate with lack of structural enterocyte differentiation, for example, content of goblet cells in the polyp mucosa.

EXO-1 was detected throughout the whole neoplastic mucosa essentially identical to the normal situation (Fig. 1d). The complete epithelium was stained. Reactivity for EXO-1 was predominantly located in the cytoplasm of the cell apex and strong staining was visible as luminal layer.

Colorectal carcinomas

Tissue specimens from 38 patients with colorectal carcinomas were obtained from 32 primary tumour lesions and six liver metastases. Tumours were divided into three groups regarding morphological differentiation. Standard histopathological grading according to the TNM classification of malignant tumours was used: 11 well (G1), 16 moderately (G2) and 11 poorly to non- (G3+G4) differentiated colorectal tumours were included. For the expression of both EPM-1 and EXO-1, considerable heterogeneity was observed (Fig. 1e–h). In some tumours, all malignant cells in the specimen were stained, whereas in others areas with focal immunoreactivity of stained cells alternated with unstained. Staining included strong cytoplasmic reactivity and extracellular mucins. Accentuation in the supranuclear Golgi zone, the cell apex, or along the luminal border, often creating a rim over the surface epithelium as well as a diffuse distribution throughout the cell cytoplasm, were observed.

All tumour cells showed expression of EPM-1 in seven of 11 well differentiated colorectal carcinomas, eight of 16 moderately differentiated tumours and two of 11 poorly differentiated. In contrast, less than 20% of tumour cells were EPM-1 positive in one of 11 well, three of 16 moderately and four of 11 poorly differentiated tumours. Atypical glands, especially of highly differentiated colorectal carcinomas, showed staining at the apical cell pole and luminal surface very similar to the pattern observed in benign neoplastic polyps. One G1 case which was 100% positive for EPM-1 and EXO-1 derived from a malignant focus of a large villous rectal polyp.

Regarding expression of EXO-1, 100% positive tumour cells were observed in nine of 11 well differentiated colorectal carcinomas, 10 of 16 moderately differentiated tumours and five of 11 poorly differentiated tumours. EXO-1 was detectable in 50% or less of tumour cells in one of 11 well, five of 16 moderately and six of 11 poorly differentiated tumours. Prominent staining of luminal contents was noted especially in atypical glandular structures of well and moderately differentiated carcinomas. In tumours expressing EXO-1 in the minority of cells these were observed as single cells or small clusters of positive cells.

The expression of both antigens was correlated with morphological tumour grading. The percentage of tumours which were completely stained for EPM-1 or EXO-1 decreased associated with a morphologically lower degree of differentiation. This overall correlation was not observed with respect to the percentage of positive tumour cells in all individual tumours. Well and poorly differentiated tumours were found with 100% positive tumour cells, but associated to the morphological grading the number of tumours which homogeneously expressed EPM-1 and EXO-1 decreased and tumours with an EPM-1 or EXO-1 negative portion were more frequent. Tumours were divided into five groups according to the percentage of EPM-1 or EXO-1 positive cells: 100%, approximately 80%, approximately 50%, approximately 30%, < 20% of tumour cells stained. Reduced expression of EPM-1 or EXO-1 in summary seemed to occur independently in a single tumour. Also, the cellular pattern of EPM-1 and EXO-1 distribution showed a certain association to tumour grading. A mucin-like pattern of staining was observed, with apical cytoplasmic staining as well as staining of luminal material in the well and moderately differentiated tumours. Poorly differentiated tumours predominantly showed cyto-

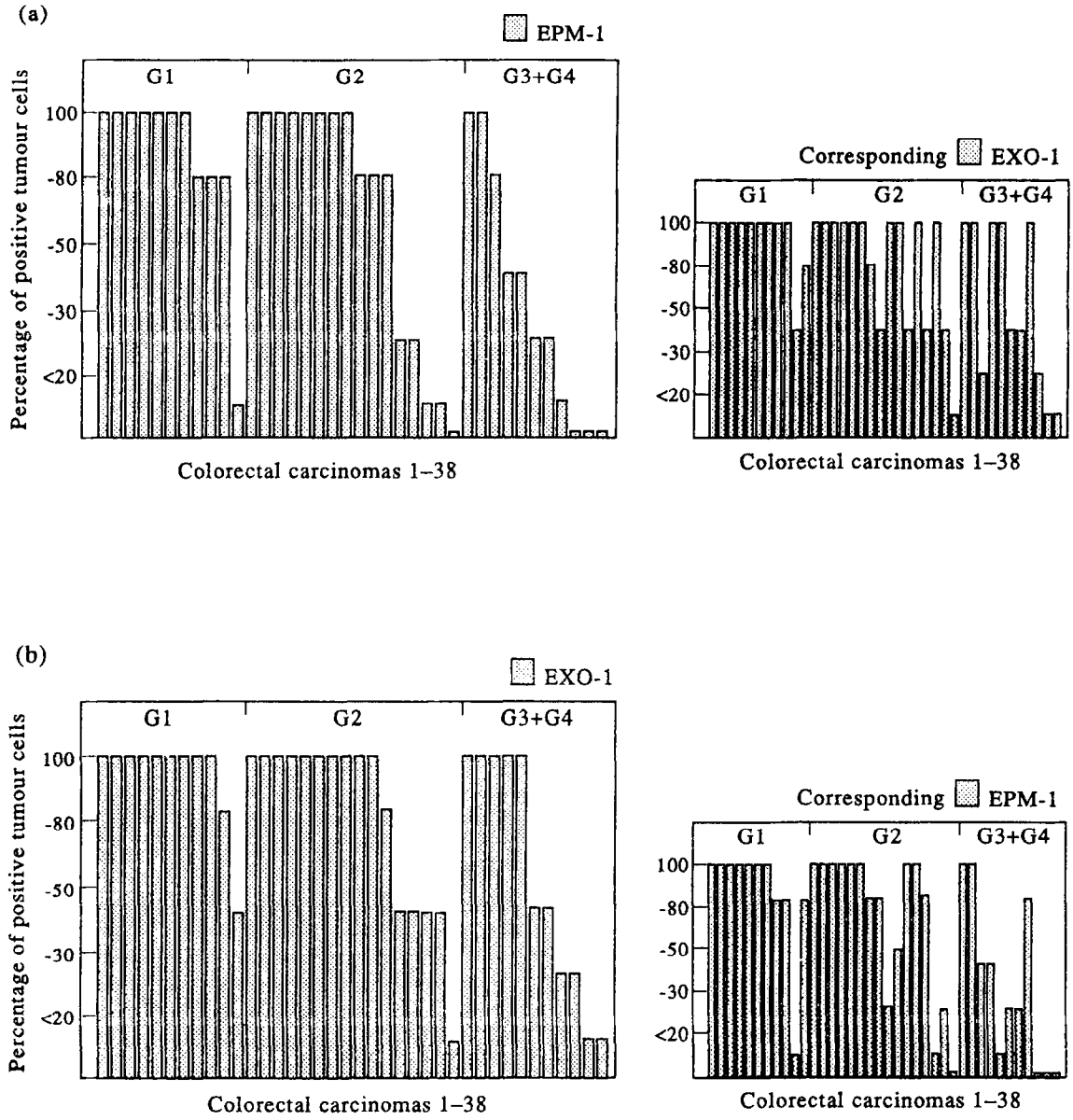

Fig. 3. Percent cellular immunohistochemical reactivity of EPM-1 and EXO-1 in colorectal carcinomas arranged by grading of morphological differentiation G1–G4, according to the TNM classification of malignant tumours: (a) tumours in fixed order of decreasing EPM-1 expression and corresponding EXO-1 expression; (b) tumours in order of decreasing EXO-1 expression with corresponding EPM-1 expression.

plasmic staining. Luminal material in atypical glands was not frequently seen.

DISCUSSION

Carbohydrate epitopes of EPM-1 and EXO-1 are epithelial developmental antigens initially characterised by monoclonal antibodies as high molecular weight glycoprotein (EPM-1) and polar neutral glycolipid (EXO-1) [7, 8]. The EXO-1 carbohydrate epitope was also detected on mucin molecules [9]. In human epidermis, expression of EPM-1 was associated with a balanced state of differentiation and proliferation and EXO-1 expression indicated a differentiation pathway of fetal development and re-emerged in hyperproliferative changes in the adult [10]. In order to assess the biological significance of the expression of EPM-1 and EXO-1 carbohydrate epitopes for the human colorectal mucosa, their distribution was analysed by immunohistochemistry. Tissue samples of normal colorectal mucosa, inflammatory colorectal mucosa from patients with histologically proven long-standing ulcerative colitis or Crohn's disease, transitional mucosa adjacent to colorectal tumours and benign and malignant neoplastic colorectal mucosa from polyps and carcinomas were investigated.

In normal colorectal mucosa EPM-1 and EXO-1 showed individual expression patterns which did not differ in proximal or distal parts of the colorectum. In contrast, the Lewis Y antigen (Le Y) also shows changes associated to neoplastic colorectal mucosa but the expression is mostly located in the proximal colon [12]. EPM-1 was differentially distributed along the crypt–villus axis with maximum at the crypt basis in normal colorectal mucosa as well as transitional mucosa. Transitional mucosa adjacent to a colorectal cancer is characterised by crypt elongation and an increased number of proliferating cells of the crypt but the overall tissue architecture remains intact [13]. EXO-1 was present throughout the whole mucosa. For both antigens, luminal staining was observed and goblet cell vacuoles remained mainly negative.

Table 1. Presence of EPM-1 gradient of expression in the tissue architecture of normal, inflammatory, transitional and neoplastic colorectal mucosa

	Number tested	EPM-1 gradient
Normal mucosa	15	Present
Inflammatory mucosa		
Ulcerative colitis	7	Present
Crohn's disease	7	Present
Transitional mucosa	7	Present
Neoplastic mucosa		
Benign polyp	9	Absent
Colorectal carcinoma	38	Absent

Gradient of EPM-1 staining discriminated between neoplastic changes of the benign adenomatous polyp and mucosal inflammation (Table 1). The gradient observed for EPM-1 distribution was present in inflammatory mucosa but not in polyps. Alterations of the balanced steady state of crypt tissue architecture leading to hyperproliferation have to be regarded as an early step of colorectal carcinogenesis. This event seemed to be reflected by changes of EPM-1 expression pattern along the axis from surface epithelium to crypt mucosa. The characteristic EPM-1 staining was lost in all benign polyps tested irrespective of structural enterocyte differentiation within the polyps. This observation parallels changes of EPM-1 expression in the human epidermis. In this epithelial tissue compartment EPM-1 expression was altered when tissue homeostasis was disturbed by benign or malignant hyperproliferative changes [10]. EPM-1 and EXO-1 both did not differentiate between the benign or malignant state of colorectal epithelium. Neoplastic mucosa in benign polyps and atypical glands in highly differentiated tumours showed essentially identical expression patterns.

Differential expression of tissue antigens along the crypt–villus axis is not frequently reported. The monoclonal antibody MT334 detects a mucin-like differentiation antigen only secreted by goblet cells located at the base of colonic crypts [14]. But even brush border-associated enzymes (peptidases and disaccharidases) are expressed without a consistent gradient along the crypt–villus axis [15]. Neither monoclonal antibodies obtained by immunisation of mice with normal colorectal epithelial cells nor a monoclonal antibody specifically reactive to a mucin antigen present in gastrointestinal goblet cell vacuoles detected a gradient of staining within the normal colon mucosa similar to EPM-1 [16, 17]. Oncodevelopmental antigens of gastrointestinal mucosa: human intestinal mucin, CEA, CA 19-9 (monosialosyl Le a), TAG-72 and colon-specific antigen p (CSAp) do not exhibit similar expression patterns [18–22].

Biochemically, the tumour-associated antigen CA 19-9 parallels characteristics of EXO-1. The carbohydrate epitope detected by monoclonal antibody 19-9 occurs in a monosialoganglioside but is also associated to a mucin [23]. Unlike EXO-1, CA 19-9 is in the normal colon, predominantly present in goblet cells of the upper part of crypts of Lieberkühn and the expression is influenced by the Lewis a blood group status of the patient [24, 25]. The expression of the CA 19-9 carbohydrate epitope is associated with dysplasia but not with inflammation in colorectal mucosa [26]. 59–82% of colon adenocarcinomas are positive for CA-19-9 by immunohistochemistry [27]. TAG-72, initially recognised by the monoclonal antibody B72.3, is a mucin expressed at high levels in several types of carcinoma and in fetal gut epithelium, but is also detectable in normal adult colonic mucosa. The monoclonal antibody B72.3 detecting the sialyl-Tn epitope of the high molecular weight mucin glycoprotein TAG72 was strongly and homogeneously reactive with transitional mucosa in contrast to normal mucosa [28]. The same phenomenon was observed in colonic mucosa overlying non-epithelial tumours like gastrointestinal lymphoma [28]. So in combination, EPM-1 and TAG-72 differentiate between normal, transitional and neoplastic mucosa. For CSAp, a gradient of expression was observed with reversed orientation compared with EPM-1. Maximum of reactivity is found in the mid and upper crypt [18]. CSAp is not expressed in poorly differentiated colorectal carcinomas [18]. Differential activation of carbohydrate modifying enzymes could be the functional basis.

Heterogeneous expression of EPM-1 and EXO-1 was observed in colorectal tumours, referring to the total number of tumours as well as to the amount of tumour cells in an individual tumour. The distribution of EPM-1 and EXO-1 correlated with the histopathological grading of the tumour differentiation. Metastatic tumour lesions showed a similar heterogeneity of staining as primary tumours, but the number of metastases tested as autologous pairs with primary tumours was too small to draw a final conclusion. It remains to be elucidated whether differentially stained tumour cells in an individual tumour represent subpopulations. These findings are in accordance with observations of common staining patterns reflecting intratumoral heterogeneity in the glycosylation pattern not only among different tumours but also in morphologically distinct areas of a single tumour [21, 29]. Polarity of epithelial cells is characterised by the development of a basolateral and apical surface [30]. Loss of differentiation in epithelial cells is paralleled by loss of cell polarity. EPM-1 and EXO-1 were immunohistochemically detected in the apical cell pole in high grade tumours whereas low grade tumours frequently showed more diffuse cytoplasmic staining. Regarding CEA associations between increasing degree of dysplasia and CEA expression on the mucosal surface and in cytoplasm were found [5].

In conclusion, epithelial antigens EPM-1 and EXO-1 both showed specific expression patterns in normal human colorectal mucosa. Inflammatory changes did not influence the distribution of both carbohydrate antigens. The characteristic gradient of EPM-1 expression along the crypt–villus axis in normal mucosa was no longer detectable in benign polyps and highly differentiated colorectal carcinomas and, therefore, discriminated the neoplastic state in these lesions. In colorectal carcinomas the expression of EPM-1 and EXO-1 varied independently but in summary correlated with morphological differentiation. Further prospective studies will address the question of whether EPM-1 or EXO-1 expression in colorectal tumour tissue could be related to Dukes' stage and the clinical course of an individual patient and so define subgroups of tumours besides morphological criteria, which could lead to therapeutic implications.

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