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Diminished expression of integrin adhesion molecules on human colonic epithelial cells during the benign to malign tumour transformation

A Stallmach, B v Lampe, H Matthes, G Bornhöft, E O Riecken

Abstract

Integrins are transmembrane molecules that mediate cell-cell and cell-substratum adhesion. Because alterations in the adhesive properties of tumour cells are thought to influence tumour cell invasion, the expression of integrin α and β chains in 19 human colorectal carcinomas, eight adenomas, and eight normal colon tissues was examined immunohistochemically using an indirect immunofluorescent technique. Normal colonic epithelial cells were found to express the integrin α_3, α_5, α_6, β_1, and β_4 chains, whereas the α_2 chain was expressed only on epithelial cells lining the base of the crypts and was absent from cells lining the mouth of the crypts or the surface epithelium. No epithelial staining of the α_1, α_4, β_2, and β_3 chains was observed. A progressive reduction of all normally expressed α and β chains was associated with increasing neoplastic transformation. The expression of the α_3 and α_5 chains was already noticeably reduced in adenomas, and was completely absent in most colonic carcinomas. In contrast, α_6, β_1, and β_4 expression was maintained in adenomas, whereas the transformation from benign to malignant neoplasms associated with infiltrative growth was characterised by diminished or lost expression of α_6, β_1, and β_4 chains. Thus, the decreased expression of integrins in human colon carcinomas may contribute to the altered adhesion and migration properties of these tumour cells.

Cancer of the large bowel, the second most common site for carcinomas in both men and women, accounts for about 20% of all deaths from malignant disease in western Europe. Unfortunately, the death rate for this disease has not changed significantly for the past 40 years.[1] The propensity of malignant tumour cells to spread from their site of origin to other parts of the body is one of the principal reasons for the high rate of cancer mortality. If oncologists were able to treat metastatic disease or prevent the formation of metastases, the incidence of death would be substantially reduced. The ability of tumour cells to adhere to basement membranes is known to play a crucial role in the complex process of metastasis formation,[2] and tumour cell adhesion to basement membranes has been shown to correlate with their ability to metastasise.[3] The interaction of tumour cells and the extracellular matrix is mainly mediated through binding of extracellular matrix components to specific tumour cell surface adhesion receptors.

Integrins are transmembrane protein complexes consisting of non-covalently associated α and β subunits.[4] They mediate both cell-substratum and cell-cell adhesion.[5] As originally described,[6] integrins were divided into three subfamilies, each with a common β subunit capable of associating with a specific group of α subunits. More recent works have shown that there are at least 11 different α subunits and eight β subunits, and that certain α subunits can combine with more than one β subunit.[7] The β_1, β_2, and β_3 subunits can associate with several distinct α subunits, and define the three integrin subfamilies – the VLA (very late activation antigens) protein family, the LEU-CAM (leucocyte cell adhesion molecule) family, and the cytoadhesions, respectively.[8] The first subfamily comprises at least six related complexes, each consisting of a β_1 chain with a distinct α chain companion. Most of these integrins are promiscuous receptors because they bind to various matrix proteins.[7] Members of the VLA subfamily include receptors for fibronectin (VLA-3, VLA-4, and VLA-5), laminin (VLA-1, VLA-2, VLA-3, and VLA-6) and collagen types I and IV (VLA-1, VLA-2, and VLA-3).[9,10] However, VLA-5 and VLA-6 seem to be specific for fibronectin and laminin, respectively.[7,11]

The β_2 subfamily of integrins, also termed LEU-CAMs or the CD18 antigens, consists of three leukocyte adhesion receptors.[7] The β_3 subfamily of integrins, also known as cytoadhesions, consists of vitronectin receptor ($\alpha v/\beta_3$) and the platelet glycoprotein IIb/IIIA (α_{IIb}/β_3) complex (reviewed in [7]).

It has previously been shown that epithelial cells express VLA-1, -2, -3, and -6 molecules in normal human small intestine.[12] Recently, there has been increasing evidence for a pathological distribution of cell adhesion receptors, including members of the integrin family, in colonic carcinomas.[13,14] Since little is known about the molecular interaction of epithelial colonic cells and the extracellular matrix via integrins during the adenoma-carcinoma sequence in the large intestine, we have performed immunohistological staining for the integrins in human colorectal carcinomas in an attempt to compare their expression with that of precancerous adenomas and normal colonic mucosa.

Methods

TISSUES

Intestinal tissues were obtained from two different groups of patients. The first group comprised 19 patients, 12 men and seven women ranging from 37 to 84 years of age, with colo-

Departments of Medicine and Pathology, Klinikum Steglitz, Free University of Berlin, Berlin, Germany
A Stallmach
B v Lampe
H Matthes
G Bornhöft
E O Riecken

Correspondence to:
Dr A Stallmach, Freie Universität Berlin, Klinikum Steglitz, Medizinische Klinik und Poliklinik, Abteilung für Innere Medizin mit Schwerpunkt Gastroenterologie, Hindenburgdamm 30, 1000 Berlin 45, Germany.

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rectal adenocarcinomas. Samples were obtained from patients with colorectal carcinomas that displayed differentiation of a high degree in four, of a moderate degree in 10, and of a low degree in 5 cases according to the classification of Morson and Dawson.[15] In eight cases, macroscopically and histologically normal adjacent mucosa (10 cm distant from the primary tumour) as well as tumour tissues were examined. The second group included eight patients with adenomas of the colon. Resection specimens were obtained immediately after surgery and tissue samples were snap frozen and stored in liquid nitrogen.

IMMUNOHISTOLOGICAL TECHNIQUE

The expression of VLA antigens in these tissues was examined by an indirect immunofluorescent technique. Cryostat sections (6 μm) were air dried overnight and stored at −20°C. Sections were incubated with either 20 μl of each primary antibody or 0·2 mol/l phosphate buffered saline (PBS), serving as a control, for 60 minutes in a humidified chamber. They were washed three times with 0·1 mol/l PBS containing 0·2% bovine serum albumin, incubated for 30 minutes with the Rhodamine conjugated secondary antibody and washed three times with PBS. The monoclonal antibodies used are listed in the Table. Antibody staining was examined with a Zeiss immunofluorescent microscope, and pictures were taken using an Agfa-Chrome 1000 ASA film. Staining of epithelial cells was graded semiquantitatively by a four point scale (−=no or weak staining, +=moderate, ++=strong, +/−=moderate and unstained epithelial cells on the same section).

Receptor, structure, suggest ligands (collagen (col), laminin (lam), fibronectin (fn)) and specificity of the antibodies used

Receptor	*Structure*	*Suggested ligands*	*Monoclonal antibodies*	*References*
VLA-1	$\alpha_1\beta_1$	col I, col IV (lam)	Anti-α_1 TS2/7	[16]
VLA-2	$\alpha_2\beta_1$	lam (collagens) cell-cell-contact	Anti-α_2 P1E6	[17]
VLA-3	$\alpha_3\beta_1$	lam, fn, col I, col V cell-cell-contact	Anti-α_3 VM2	[18]
VLA-4	$\alpha_4\beta_1$	fn (alternative spliced)	Anti-α_4 P4G9	[19]
VLA-5	$\alpha_5\beta_1$	fn	Anti-α_5 P1D6	[20]
VLA-6	$\alpha_6\beta_1$	lam	Anti-α_6 GoH3	[21]
			Anti-β_1 4B4	[22]
			Anti-β_2 MHM23	[23]
			Anti-β_3 SZ.21	[24]
	$\alpha_6\beta_4$	not known	Anti-β_4 3E1	[25]

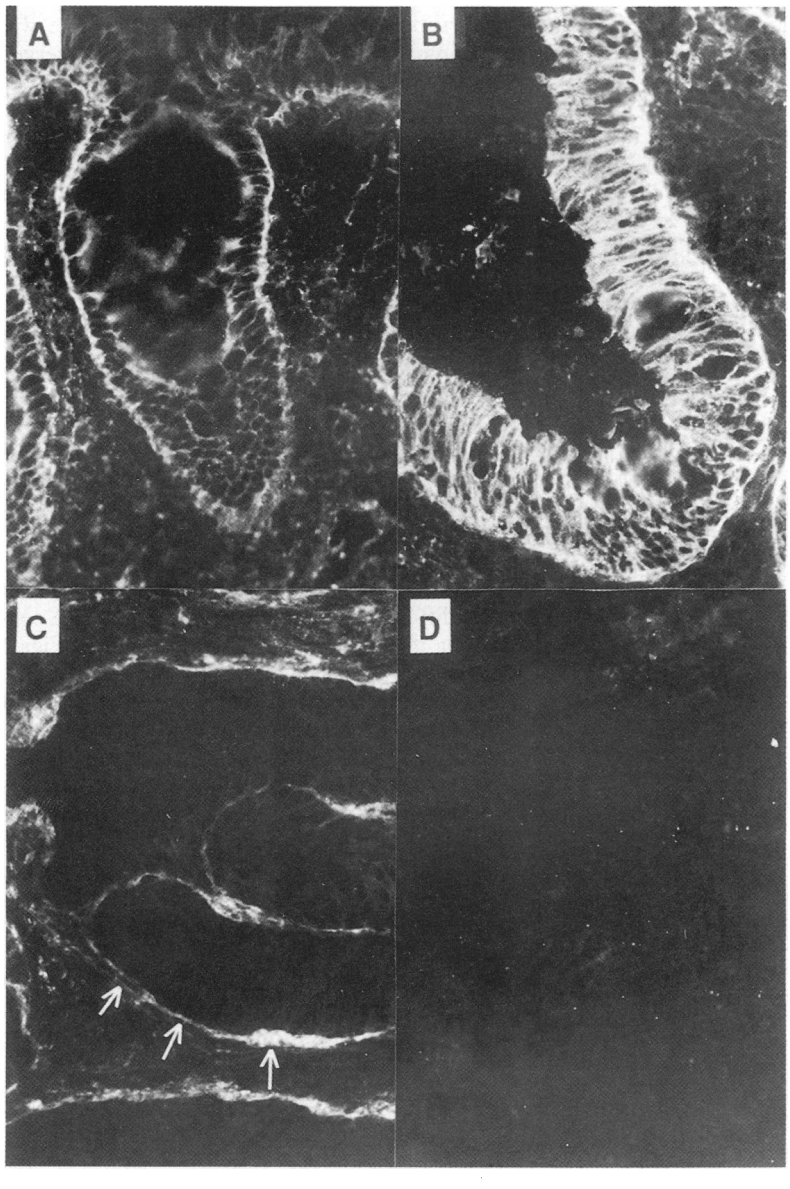


Figure 1: Immunohistological staining of α_6 chain in epithelial cells. (A) Normal colonic mucosa. (B) Tubulovillous adenoma. (C) Well differentiated carcinoma. Arrows indicate α_6 chain expression at the epithelial-mesenchymal interface. (D) Poorly differentiated carcinoma. (Magnification ×200.)

STATISTICAL ANALYSIS

For statistical analysis the grades of antigen expression were converted to numbers (−=0, +/−=0·5, +=1, ++=2) as described by MacDonald *et al.*[26] Statistical significance was calculated using the Wilcoxon rank test and considered to be significant at the $p<0·05$ level.

Results

IMMUNOFLUORESCENCE ANALYSIS OF INTEGRIN EXPRESSION IN NORMAL COLONIC MUCOSA

A comparable pattern of staining was seen in all eight specimens. Antibodies against α_3, α_5, α_6, β_1, and β_4 showed brilliant staining of the epithelium in normal colonic mucosa. The α_6 and β_4 expression were strongly accentuated on the basal epithelial cell membrane, while α_3, α_5, and β_1 were expressed more evenly on the cell surface. The α_2 chain was expressed only on epithelial cells lining the base of the crypts, but was absent from cells lining the mouth of the crypts or the surface epithelium. Expression of α_1, α_4, β_2, and β_3 chains on epithelial cells was not detected by the used antibodies.

ADENOMAS

The expression of the α_2, α_6, and β_4 chains in colorectal adenomas was similar to that in normal mucosa. Moderate or strong expression of the α_6 and β_4 chains was observed at the contact sites of the epithelial cells and basement membrane in all cases. Increased cytoplasmatic staining in epithelial cells of adenomas was observed when compared with the immunofluorescence pattern of controls (Fig 1). No statistical differences were observed when α_6 and β_4 chain cell mem-

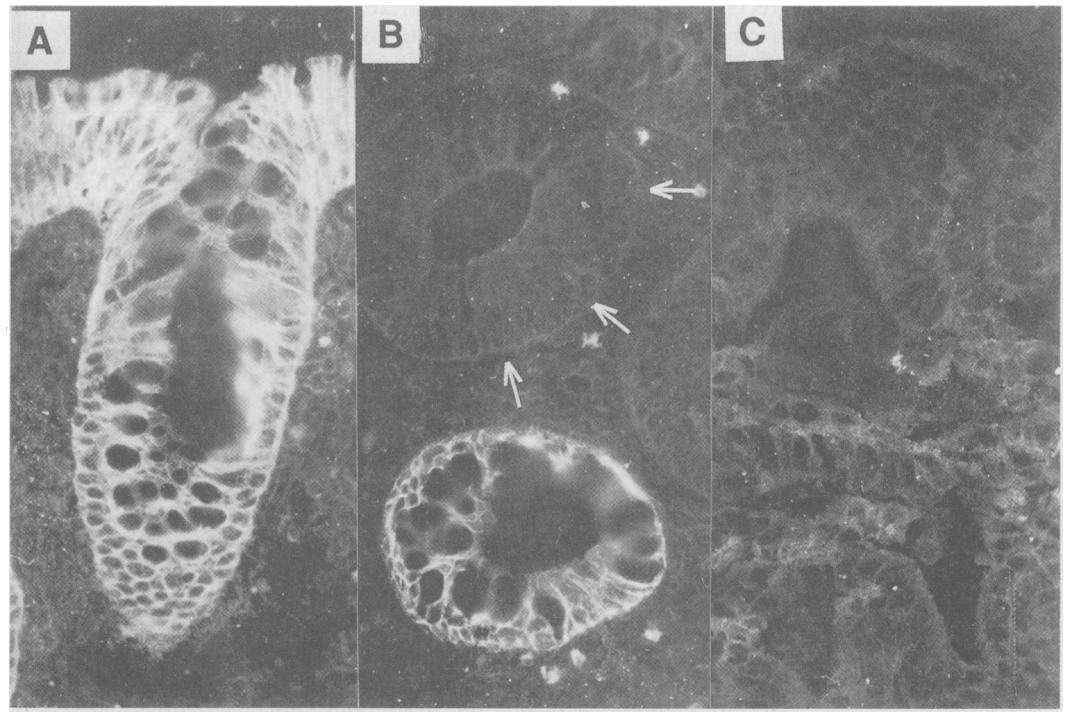
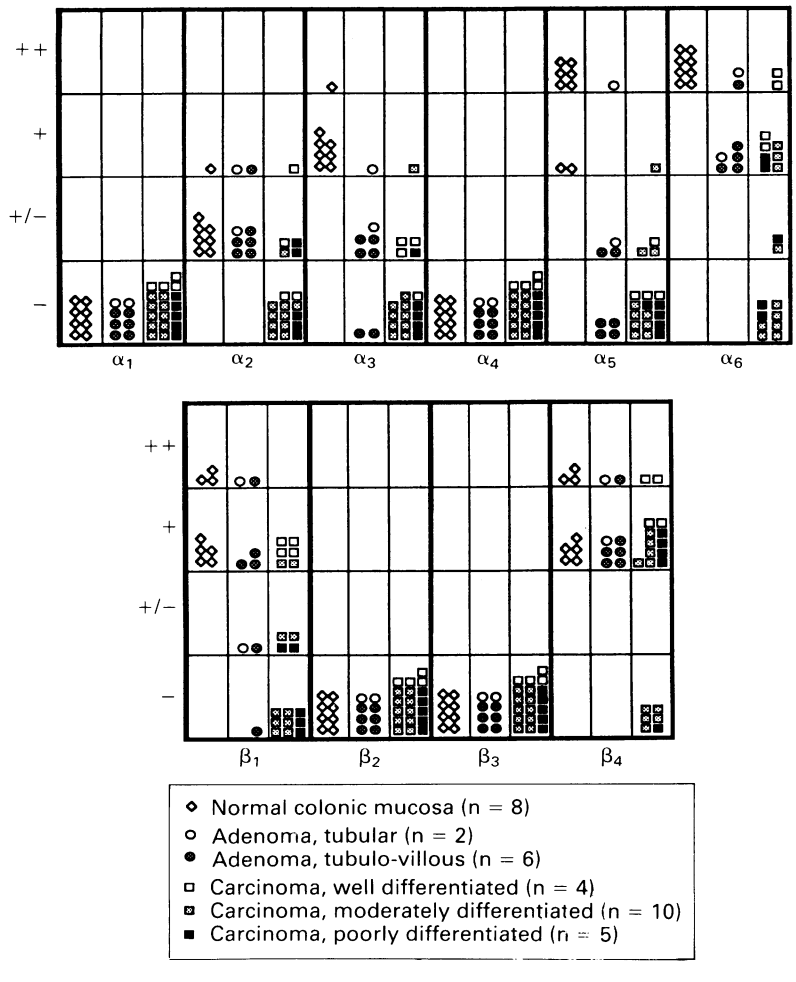


Figure 2: Immunohistological staining of the α_5 chain in epithelial cells. (A) Normal colonic mucosa. (B) Tubulovillous adenoma. Arrows indicate negative epithelial cells. (C) Moderately differentiated carcinoma. No positive staining of epithelial cells was seen using the monoclonal antibody against the α_5 chain. (Magnification ×200.)

brane expression of epithelial cells of adenomas was compared with expression in normal colonic mucosa. In contrast, α_3 and α_5 chain expression in adenomas showed a noticeably altered pattern. Only in one case were the α_3 and α_5 chains expressed by all epithelial cells. In five cases, α_3 expression showed a pattern of areas of stained epithelial cells mixed with areas of complete negative cells on the same section (Fig 2). A similar pattern for α_5 chain expression was observed in three cases. In no other case was staining for α_3 or α_5 chain expression observed (see also Fig 3).

Figure 3: Integrin adhesion molecule expression in various colon disease states. Each symbol represents tissue from one patient. Intensity of α or β chain expression on epithelial cells was graded as indicated in Methods.



COLORECTAL CARCINOMAS

We found an obviously diminished expression of α_2, α_3, α_5, α_6, β_1, and β_4 chains in the 19 colorectal carcinomas tested. Epithelial staining for α_3 and α_5 chains similar to that seen in normal tissues was observed in only 1/19 of the adenocarcinomas (see also Fig 3). Fourteen of them were completely negative for α_3 chain antibodies and 15 for α_5 chain antibodies (Fig 2). Diminished expression of α_2 chain antibodies was also seen. In 14/19 cases no staining was observed, while at least 50% of epithelial cells showed moderate staining of VLA-2 in all adenomas and normal tissue samples.

The α_6, β_1, and β_4 chains were moderately expressed by carcinoma cells in all (4/4) cases of well differentiated adenocarcinomas at the cell-stroma interface as well as in adenomas (Figs 1 and 4). Upper cell layers were only weakly stained. Statistical analysis showed a significant reduction in the intensity of α_6 chain expression in comparison with normal colonic mucosa ($p<0{\cdot}05$), whereas expression of β_1 and β_4 chains was not statistically different. In contrast, in 9/15 cases of moderately or poorly differentiated carcinomas there was no detectable expression of the α_6 chain (stained with GoH3), in 9/15 cases there was no detectable expression of the β_1 chain (stained with 4B4), and in 6/15 cases there was no detectable expression of the β_4 chain (stained with 3E1). Staining was seen in the other cases at the basal cell margin only and this was weaker than in normal tissue samples.

Discussion

The interaction of malignant cells with the

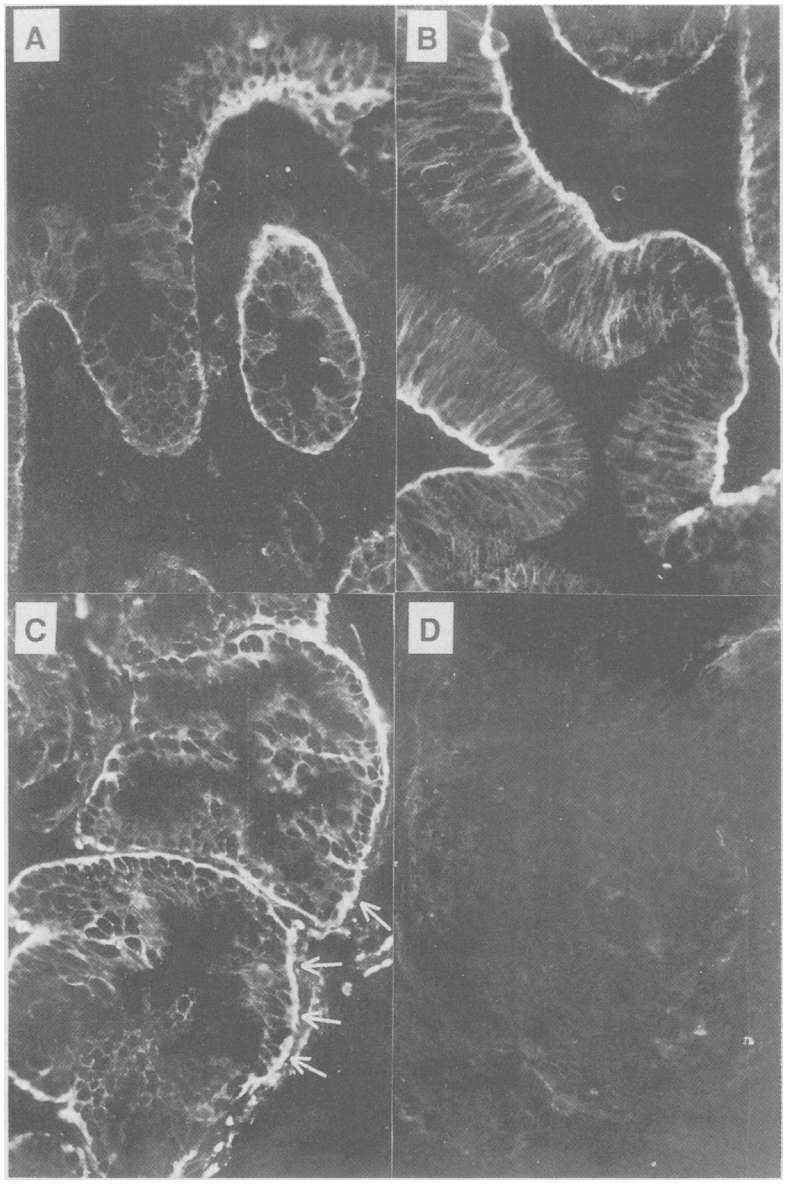


Figure 4: Immunohistological staining of β_4 chain in epithelial cells. (A) Normal colonic mucosa. (B) Tubulovillous adenoma. (C) Well differentiated carcinoma. Arrows indicate β_4 chain expression at the epithelial-mesenchymal interface. (D) Poorly differentiated carcinoma. (Magnification ×200.)

surrounding extracellular matrix, especially with basement membranes, is known to play a crucial role in the complex mechanisms of tumour invasion and metastasis.[2] Integrin molecules are involved in these interactions as they mediate cell adhesion to various components of the extracellular matrix.

In this study, we have analysed the expression of different α and β chains of the integrins during the transformation of normal colonic mucosa via adenomas to invasive colorectal carcinomas. Our data show a significant loss of α_3 and α_5 chains of the VLA complexes in tubulovillous adenomas and colorectal carcinomas in comparison with normal colonic mucosa. The observation of a mixed pattern of α_3 and α_5 chain expression in adenomas is of particular interest. In some crypts, epithelial cells were positive, whereas in others were completely negative. It is possible that this immunohistological difference in the expression of cell adhesion components reflects different types of preneoplastic cells as well as neoplastic cells in tubulovillous adenomas. These findings confirm previous results of Pignatelli *et al*, who observed diminished expression of the α_2 and α_3 chains of the known collagen receptors VLA-2 and VLA-3 in moderately or poorly differentiated human colorectal cancers.[14] However, in this study, the expression of the α_2 and α_3 chains, and, in agreement with our results, of the β_1 chain, on epithelial cells of adenomas was unchanged in comparison with controls.

Furthermore, there was a significant correlation between reduced expression of the α_6 chain of VLA-6, one of the known laminin receptors of the β_1 integrin family, and the transformation of adenomas to invasive carcinomas. The addition of the mAB GoH3 against the α_6 chain of VLA-6 inhibited binding of colon cancer derived HT 29 cells to laminin in cell adhesion assays completely (own unpublished results). Therefore, reduction of VLA-6 expression may allow the colonic carcinoma cells to detach from the basement membrane. Thus, the carcinoma cells and not the α_6 positive adenoma cells would be able to infiltrate the underlying mesenchyme. It is possible that a diminished expression of VLA adhesion molecules as described for neoplastic colonic epithelial cells is a general phenomenon for cells attaining a malignant phenotype. A similar diminished expression of α_2, α_5, and β_1 chains in carcinomas of the breast and of α_6 and β_4 chains in small cell lung cancers was reported recently.[16 27] An altered expression of these adhesion molecules may influence the aggressiveness of local infiltrative growth and metastasis in human cancers. This hypothesis is corroborated by in vitro studies. Giancotti *et al*[28] observed that after transfection of the genetic information for $\alpha_5\beta_1$, the known fibronectin receptor of the VLA family, into malignant Chinese hamster ovary cells, these cells regained a normal phenotype and were non-tumourigenic when injected into nude mice. However, contrary results of an increased or unaltered expression of integrins in transformed malignant cells in in vitro studies have been reported. For example, the malignant transformation by N-methyl-N-nitrosoguanidin in human osteosarcoma cells results in an increased expression of the α_1, α_2, and α_6 chains.[29] Thus, it seems clear that malignant transformation can affect the expression, distribution, and probably the functioning of cell adhesion components of the integrin class, but the pattern of changes may be complex.

Our results indicate a sequence of alterations in integrin expression during the transformation of normal colonic mucosa via adenomas into colorectal carcinomas. Firstly, the transformation to adenomas was characterised by a diminished expression of α_3, and α_5 chains of the VLA complexes (Fig 2), and secondly, the transformation from adenomas to carcinomas was characterised by a diminished expression of α_6 chain (Fig 1). These changes could be the result to a series of genetic events which have been recently described in colorectal cancer.[30] An activation of ras oncogenes is observed at an early stage in the course of the adenoma-carcinoma sequence in the human intestine. Approximately 50% of adenomas larger than 1 cm in size have been found to have ras gene mutations.[31] Oncogenic transformation of rodent fibroblasts with Rous-sarcoma virus or murine sarcoma viruses encoding ras oncogens led to a reduction of the expression of $\alpha_5\beta_1$ in these cells,

whereas the $\alpha_3\beta_1$ expression was retained by the transformed cells.[32]

The second most common region of genetic alteration in colorectal tumours is in chromosome 18q, which is changed in more than 70% of carcinomas. Using the polymerase chain reaction in an exon connection strategy, Fearon *et al* identified the altered sequence mapping as 18q.[33] This gene, termed the DCC gene (deleted in colorectal carcinomas), encodes a protein with significant homologies to members of the family of cell adhesion molecules.[34] Therefore, the DCC gene might play a role in the development of colorectal tumours, perhaps through changes in physiological cell-cell and cell-matrix interactions. However, this speculation as well as the relation between the genetic changes and the diminished expression of integrins in human colorectal adenomas and cancers remain to be investigated.

Addendum

After submission of this manuscript, Koretz *et al*, reported that VLA-α_6 expression in adenomas and carcinomas resembled that of normal mucosa using the immunoperoxidase technique (Koretz K, Schlag P, Boumsell L, Möller P. Expression of VLA-α_2, VLA-α_6 and VLA-β_1 chains in normal mucosa and adenomas of the colon, and in colon carcinomas and their liver metastases. *Am J Pathol* 1991; **138**: 741–50). The fact that most malign lesions were not stained using the immunofluorescence technique does not mean that cancerous cells did not contain VLA-6. The negative cells may have had levels of receptor below the sensity of the technique we used. However, our data demonstrated a significant decrease in α_6 expression during the adenoma-carcinoma sequence.

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