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Comparative evaluation of integrin α- and β-chain expression in colorectal carcinoma cell lines and in their tumours of origin

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Abstract The integrin family consists of broadly expressed cell surface adhesion receptors, each member of which is composed of a non-covalently linked α/β heterodimer. Integrin receptors are involved in the interaction with matrix proteins and may contribute to invasion and metastasis of carcinomas. To examine the biological role integrins play in colorectal carcinoma we compared the expression of integrin α- and β-subunits in situ and in vitro. Eight newly established cell lines derived from immunohistochemically characterized colorectal carcinomas together with two sublines obtained after nude mouse passage and the commonly used colon carcinoma lines HT-29, SW480, SW620, and COLO 205 were investigated by immunocytochemistry and flow cytometry. The carcinomas in situ expressed α1-, α2-, α3-, α6-, αv- and β1-subunits in variable amounts while being devoid of α4, α5 and β3. The individual integrin profile of the tumour in tissue was essentially maintained in vitro. However, a neo-expression of the α5 chain was found, together with an induction or increase in α1, α2, α3, αv and β1 levels. No decrease in integrin subunit expression was observed. Standard-serum and serum-free medium revealed no striking differences in α- and β-chain expression in the cell lines HT-29 and COLO 205. In serum-free medium, SW480 showed a slight increase of α1 and α5 and a decrease of α3 and αv while SW620 expressed more α1. We conclude that the great variability of adhesion receptor expression of the integrin family in colorectal carcinomas in situ is essentially maintained in vitro, although culture conditions which are only marginally influenced by serum factors unpredictably lead to some increase in expression or even induction of several integrin subunits.

Key words Colorectal carcinoma · Integrin receptors

This work is dedicated to Prof. Wilhelm Doerr on the occasion of this 80th birthday

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Introduction

Integrin α/β heterodimers are widely expressed cell surface adhesion receptors [14] and were found to play an important role in the cell-cell and cell-matrix interaction during embryogenesis [5, 7], tissue remodelling and repair [10], and also in tumour invasion and metastasis [2, 13, 19]. Integrin receptors that are primarily involved in binding to matrix proteins are $\alpha1\beta1$ (laminin/collagen), $\alpha2\beta1$ (laminin/collagen), $\alpha3\beta1$ (laminin/collagen/fibronectin/entactin), $\alpha4\beta1$ (fibronectin), $\alpha5\beta1$ (fibronectin), $\alpha6\beta1$ (laminin), $\alpha6\beta4$ (laminin), $\alpha v\beta1$ (fibronectin), $\alpha v\beta3$ (vitronectin) and $\alpha v\beta5$ (vitronectin) [14]. Some of these receptors are broadly expressed in various tissues and cultured cells [2] and some of these receptors recognize the arginine-glycine-asparagine (RGD) sequence in their ligands [20]. The expression of integrin receptors in epithelial tumours in situ is rather complex, although there is a tendency for gastrointestinal tract carcinomas to express abnormally low levels of integrin α- and β-chains when compared with their non-neoplastic cellular counterparts [12, 15, 18, 22, 24]. This down-regulation was found to be correlated with low degree of differentiation or with a metastasizing phenotype [15, 18].

Tumour cell lines are useful models to elucidate functional properties of a tumour type. Cell-matrix binding studies and cell-matrix invasion studies on gastrointestinal cancer cell lines have already shed some light on the role of integrin receptors in spread and invasion [6, 21, 24]. However, the mere fact that a neoplastic epithelial cell gives rise to a tumour cell line might be the consequence of severe alterations in its functional repertoire. It has actually been observed that cell lines exhibit properties that are undetectable in the tumour type they are derivatives of [16]. Up to now no comparative evaluation of in situ integrin expression in individual carcinomas and of derived carcinoma cell lines has been conducted.

To examine the role of integrins in the biology of colorectal carcinoma, we undertook the task of investigating a battery of new colon carcinoma cell lines derived from tumour tissue still available as fresh frozen

material; thus the original in situ situation could be examined. Here we present data on ten colon carcinoma cell lines together with phenotypic data from the parent tumour tissue. Commonly used colon carcinoma cell lines were analysed for comparison.

Materials and methods

Tumour tissues were obtained from patients with primary and metastasizing colorectal tumours immediately after surgical removal. Tumour specimens of at least 1 cm^2 were collected for immunohistochemistry while adjacent tissue was used for cell culture. For immunohistochemistry tumour tissues were quick frozen in liquid nitrogen and stored at −70° C until sectioning. The procedure of dissociation and tumour cell harvesting has been described in detail [4]. Briefly, tumour tissues were washed, minced and centrifuged, erythrocytes were lysed in 0.15 M amonium chloride (plus 0.01 M potassium bicarbonate). After trypsin treatment (0.05% trypsin, 0.01% ethylene-diamine-tetraacetate (EDTA); Seromed, Biochrom, Berlin, Germany) cells were cultured in MEM Iscove medium (Seromed) which contained 10% fetal calf serum (FCS) (CCPro, Karlsruhe, Germany), penicillin and streptomycin (100 U/100 µg/ml; Seromed) and amphotericin (1.5 µg/ml; Seromed). Twenty-eight cell lines were established [4], 8 of these were used in this study together with 2 sublines obtained after nude mouse passage (HDC-8NMIIK1, HDC-9NMI; [4]; Table 1). The histopathological data from the original tumours are listed in Table 1. They were obtained from seven males and one female, 56–81 years of age. HT-29, SW480, SW620 and COLO 205 were obtained from ATCC (Rockville, Md., USA). To evaluate the influence of cell culture conditions on integrin α- and β-chain expression ATCC cell lines were cultured with and without FCS. Adaptation to the serum-free condition was achieved according to Zirvi et al. [25] and Fantini et al. [8] with slight modifications. Briefly, ATCC cell lines were accommodated to Dulbecco's modified Eagle's medium (DMEM)/HAM'S F-12 medium (Seromed) with 10% FCS and then reduced to 1% FCS supplemented with ITS (5 µ/ml insulin, 5 µg/ml transferrin, 5 ng/ml sodium selenite; Boehringer, Mannheim, Germany). After accommodation FCS was completely omitted and cells were cultured serum-free for at least 2 weeks before the first assay was done. Control cultures with DMEM/HAM'S F-12 with 10% FCS were run in parallel.

Chromosome preparation of HDC cell lines has been described elsewhere [4]. Briefly, cells were treated with colcemid (0.5 µg/ml), detached with trypsin/EDTA, suspended in hypotonic potassium chloride (0.075 M), fixed with methanol:acetic acid (3:1) and spread on dry slides. Slides were stained after trypsin-treatment (0.05% in phosphate buffered saline) with Giemsa (10% in phosphate buffer, pH 6.8). Methaphases were photographed immediately.

For immunohistochemical staining 4–6 µm thick frozen sections were air dried overnight and fixed in acetone for 10 min. For immunocytochemistry cells were detached with trypsin/EDTA (0.05/0.02%) centrifuged and washed in culture medium. As evidenced in a pilot study (unpublished data) this detachment procedure did not alter the concentration of surface antigen as detected by flow cytometry. Cytospin preparations were made with a cytocentrifuge (Shandon, Pittsburgh, Pa., USA), air-dried, and fixed in acetone. Fixed sections and cytospins were stained immediately or stored at −20° C.

In order to determine the epithelial nature of the cell lines, the following monoclonal antibodies (mAbs) were used: anti-cytokeratin gp56 (clone KL1; IgG1; Dianova-Immunotech, Hamburg, Germany), anti-vimentin (clone V9; IgG1; Dako, Copenhagen, Denmark), anti-Egp34 (clone HEA125; own laboratory in cooperation with Dr. G. Moldenhauer, German Cancer Research Center, Heidelberg, Germany), anti-epithelial membrane antigen (EMA; clone E29; IgG2a; Dako), anti-carcinoembryonic antigen; clone HEA19; IgG1; own laboratory in cooperation with Dr. G. Moldenhauer), anti-CA19-9 (clone NS19-9; CIS Bio International, Gif-

Table 1 Clinical data and histopathology of eight colorectal adenocarcinomas and cytogenetic findings in their cell lines

Tumour number	Tumour location	Tumour stage	Tumour type	Degree of differentiation	Cell line	Number of chromosomes	Karyotype abnormalities
1	Rectum	Dukes C	Non-mucinous	Poor	HDC-8	37–68	del(1)(qter→p11:), del(1)pter→q21:), t(3;9)(qter→p25::q12→qter) i(10)(qter-cen-qter), t(12;?)(qter→q13::?), i(14)(qter-cen-qter) del(17)(qter-cen:)
2	Ascending colon	Dukes B	Mucinous	Well	HDC-8NMIIK1[a] HDC-9 HDC-9NMI[a]	46	del(7)(pter→q34?:), del(11)(pter→q23:)
3	Ascending colon	Dukes B	Non-mucinous	Poor	HDC-57	72	del(1)(qter→p12:), i(8)(qter-cen-qter), t(11;11)(qter→p15::q13→qter)
4	Caecum	Dukes C	Non-mucinous	Intermediate	HDC-63	73	t(1:?)(qter→p12::?), t(13;?)(qter→q12::?)
5	Sigmoid colon	Dukes C	Non-mucinous	Intermediate	HDC-101	70	del(17)(qter→p11:)
6	Lung metastasis	Dukes D	Non-mucinous	Poor	HDC-102	50	t(1:?)(qter→p36::?), i(8)(qter-cen-qter), t(12;?)(pter→q24::?)
7	Sigmoid colon	Dukes B	Non-mucinous	Intermediate	HDC-114	90	t(14;15)(qter-cen-qter)
8	Ascending colon	Dukes C	Mucinous	Poor	HDC-117	64	t(3:?)(pter-cen::?), rcp(6;8)(6qter-cen-8pter;8qter-cen-6pter), i(7)(ptr-cen-pter), t(13;14)(qter-cen-qter), t(20:?)(pter→q13::?)

[a] "N" symbolizes a nude mouse passage. These two sublines had essentially the same karyotype as their parental lines

Table 2 Expression of integrin α- and β-chains in eight colorectal carcinomas and their cell lines, as determined by immunohisto- and cytochemistry

Tumour/ cell line	α1	α2	α3	α4	α5	α6	αv	β1	β3
Tumour	+/–	+>>–	+	–	–	+	+	+	–
HDC-8	+/–	+	+	–	+>–	+	+	+	–
HDC-8NMIIK1	+/–	+	+	–	–>>+	+	+	+	–
Tumour 2	–>>+	–>>+	+	–	–	+	–	+	–
HDC-9	+/–	+>>–	+	–	–>+	+	+	+	–
HDC-9NMI	+>–	+	+	–	–	+	+>–	+	–
Tumour 3	–	+	+	–	–	+	+>–	+	–
HDC-57	–>>+	+	+	–	+/–	+	+	+	–
Tumour 4	–>>+	+	+	–	–	+	+	+>–	–
HDC-63	+/–	+	+	–	–	+	+	+	–
Tumour 5	–	+	+/–	–	–	+	+	+	–
HDC-101	+>>–	+	+	–	–	+	+	+	–
Tumour 6	–	+	+	–	–	+	+	+	–
HDC-102	–>+	+	+	–	–	+	+	+	–
Tumour 7	–	–>>+	+	–	–	+	–	+	–
HDC-114	–>>+	+	+	–	+>–	+	+	+	–
Tumour 8	–	–>+	–>>+	–	–	+	–	+	–
HDC-117	–>>+	+>–	+	–	–	+	+>–	+	–

Sur-Yvette, France), anti-secretory component (SC; clone GA-1; IgG1; Sigma, St. Louis, Mo.) and CD53 (clone HD77; IgG1; own laboratory in cooperation with Dr. G. Moldenhauer). The mAb anti-α1-chain (CD49a; clone TS/7; IgG1) was obtained from T cell Sciences, Cambridge, Mass., USA. The mouse mAbs anti-α2-chain (CD49b; clone Gi9; IgG1), anti-α4-chain (CD49d; clone HP2/1; IgG1), anti-α5-chain (CD49e; clone SAM1; IgG2b), anti-αv-chain (CD51; clone AMF7; IgG1), anti-β1-chain (CD29; clone K20; IgG2a), anti-β3-chain (CD61; clone SZ.21; IgG1) and the rat mAb anti-α6-chain (CD49f; clone GOH3; IgG2a) were obtained from Dianova-Immunoech. The mouse mAb anti-α3-chain (CD49c; clone P1B5; IgG1) was obtained from Telios Pharmaceuticals, San Diego, Calif., USA. Purified reagents were used in a protein concentration of 10–20 µg/ml or were used according to the manufacturers' instructions. Ascites preparation was diluted 1:3000 (P1B5). A polyclonal biotinylated sheep antibody to mouse or rat immunoglobulins (Amersham, High Wycombe, UK) was used as second step reagents at a dilution of 1:50. A streptavidin-biotinylated peroxidase complex (Amersham) was used as a third step reagent at a dilution of 1:100. All dilutions and washing steps were carried out in PBS. Incubation times of the mAbs were 1 h, of the second and third step reagents 30 min.

A semiquantitative evaluation system was established to determine antigen expression and to evaluate the amount of stained cells for immunohistochemical and immunocytochemical stainings. Antigen expression was scored + whenever specific staining was detectable and – when no antigen was detectable. A>>B indicates that only a minority of cells revealed the modality B; A>B means that cells with staining modality A clearly outnumbered those with modality B; +/– means that cells were positive and negative in about equal proportions.

Integrin receptor α- and β-chains were screened by flow cytometry on the colorectal carcinoma cell lines using 1×10^6 cells per sample. Cells were suspended in Hanks' salt solution with 0.35 g/l sodium bicarbonate (Seromed) supplemented with 10 mM HEPES, 0.1% sodium nitrite and 1% FCS referred to as FACS-medium. The mAb CD21 (clone BU-36; [3]) was used as negative control. The cells were incubated with the purified antibodies at a concentration of 50–100 µg/ml, ascites preparation at a dilution of 1:300. After three washing steps, cells were incubated with a polyclonal fluorescein isothiocyanate-coupled $F(ab)_2$ goat-anti-mouse IgG and IgM (Dianova-Immunotech), diluted 1:50. The incubation time for each antibody was 1 h. After three washing steps propidium iodide (1 µg/ml diluted in FACS-medium) was used to exclude non-viable cells. Flow cytometry was performed on a FACScan (Becton Dickinson, Mountain View, Calif., USA) with the LYSYS II software.

Results

The histopathological data of the eight colorectal carcinomas and the consistently detectable cytogenetic abnormalities of the derived cell lines are listed in Table 1. All but one tumour cell line originated from colorectal primaries, tumour number 6 (cell line HDC-102) was a lung metastasis. The cell line HDC-9 and its derivative, HDC-9NMI, obtained after nude mouse passage, retained the ability of the originating tumour to produce mucus. In contrast, HDC-117 which was also established from a mucinous carcinoma, lost this feature under culture conditions. Both cell lines obtained after nude mouse passage, HDC-8NMIIK1 and HDC-9NMI, were morphologically identical with their parental cell lines.

Immunohistochemical and cytochemical data

The immunoprofile of the colorectal carcinoma lines of the HDC-series demonstrated their epithelial nature. All lines were $Egp34^+$, $CD53^-$ and co-expressed secretory component, carcinoembryonic antigen, epithelial membrane antigen, CA19-9 antigen and cytokeratin gp56 in different amounts and in different percentages of cells in culture (data not shown).

The expression of integrin α- and β-chains in the eight colorectal carcinomas and the respective cell lines

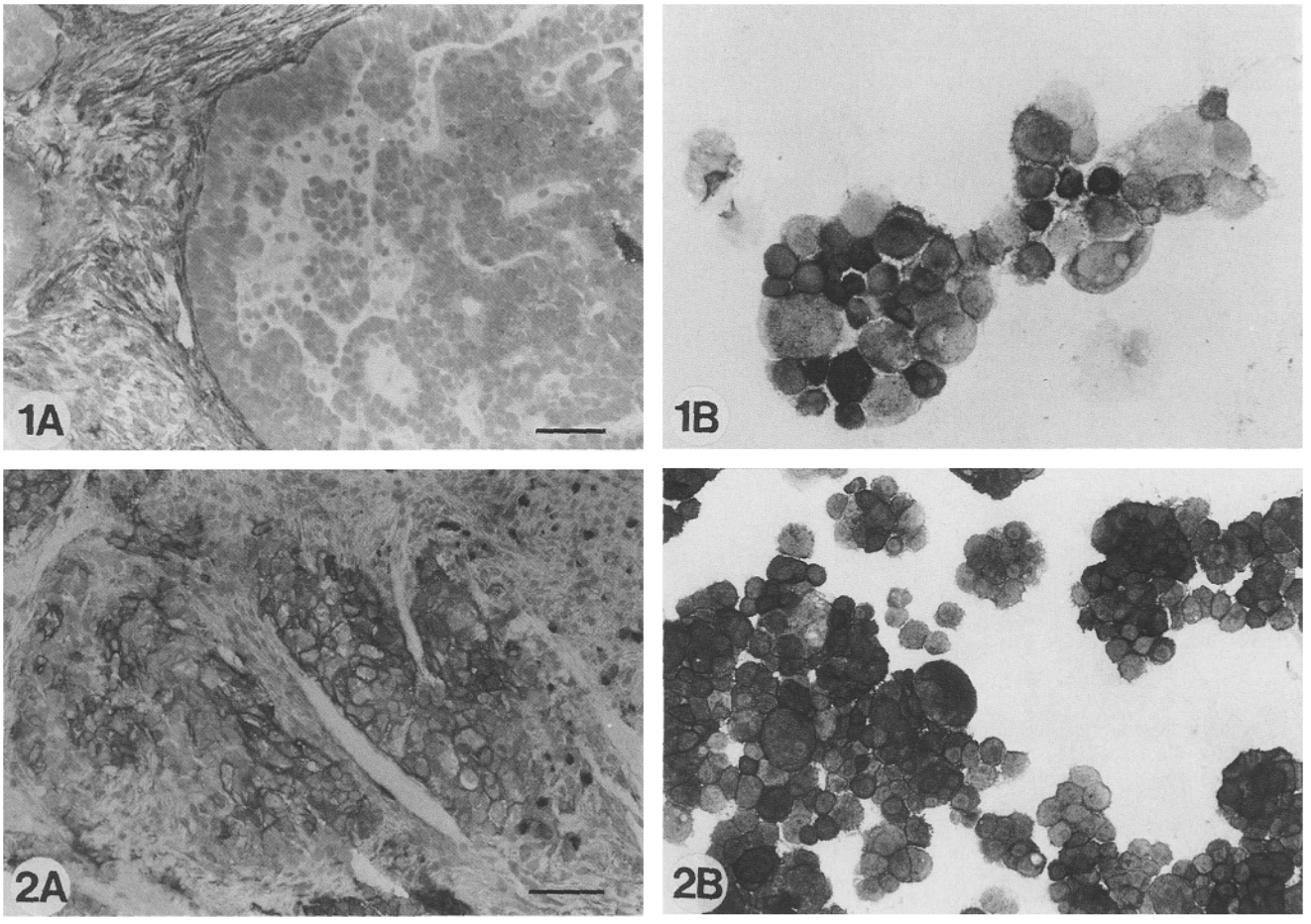

Fig. 1A, B **A** Immunohistochemical demonstration of α1-chain expression in tumour number 5. The α1-chain is lacking in the tumour cells but is present in peritumorous fibroblasts and lymphocytes (*bar* 80 μm). **B** Immunocytochemical detection of α1-chain expression in HDC-101. The cell line originating from tumour number 6 expresses the α1-chain in the majority of cells in the cytoplasm and on the cell surface (original magnification ×200)

Fig. 2A, B **A** α2-chain expression in tumour number 1. The carcinoma expresses the α2-chain in most tumour cells. Depicted is a focal lack of α2 within the neoplastic population (*bar* 80 μm). Fibroblasts are essentially α2-negative. **B** α2-chain expression in HDC-8NMIIK1. This cell line originating from tumour number 1 was re-established after nude mouse passage. This line shows high expression of the α2-chain in almost all cells (original magnification ×125)

are shown in Table 2. The integrin α1-chain was inconsistently expressed in three of the eight original carcinomas, one carcinoma showing equal amounts of positive and negative tumour cells (tumour 1), two tumours (numbers 2 and 4) had α1 only in a minority of tumour cells, and in five tumours the α1-chain was completely lacking (Fig. 1A). Apart from expression or non-expression of α1 in neoplastic population the α1-chain was expressed consistently in blood vessel endothelium, smooth muscle cells, some peritumour fibroblasts and interstitial lymphocytes. HDC-57, HDC-101, HDC-102, HDC-114 and HDC-117 showed an induction of the α1-chain, the other cell lines showed a comparable or an increased antigen expression (Fig. 1B). Four of eight carcinomas were entirely α2-positive, four tumours showed a mixed pattern of α2 expression (Fig. 2A) with two tumours (numbers 2 and 7) showing α2 just in a minority of tumour cells. However, no carcinoma was completely negative. Stromal cells expressed the α2-chain as previously described [15]. All lines whose original tumours showed a mixed pattern of α2-chain expression, expressed α2 in a majority of cells or in almost all cells (Fig. 2B). In situ, the α3-chain was expressed in the entire neoplastic compartment in six of the eight tumours. Only tumour number 5 showed α3-positive and -negative tumour cells in about equal parts (Fig. 3A). Tumour number 8 showed α3-positive cells in but a minority of neoplastic cells. The expression of α3 in stromal cells was broad. All cells of each line expressed the α3-chain (Fig. 3B). The α4-chain was found neither in the original carcinomas nor in the cell lines. Immunohistochemically, α4 expression was essentially restricted to lymphocytes. The α5-chain was not expressed in the epithelial compartment of carcinomas, however, broadly present in stromal cell types (Fig. 4A). By contrast, in five of the newly established ten cell lines, HDC-8, HDC-8NMIIK1, HDC-9, HDC-57 and HDC-114, an α5-chain neo-expression was found in a subpopulation of tumour cells (Fig. 4B). Immunocytochemically, α5 was prominently found in the cytoplasm. Original tumours and cor-

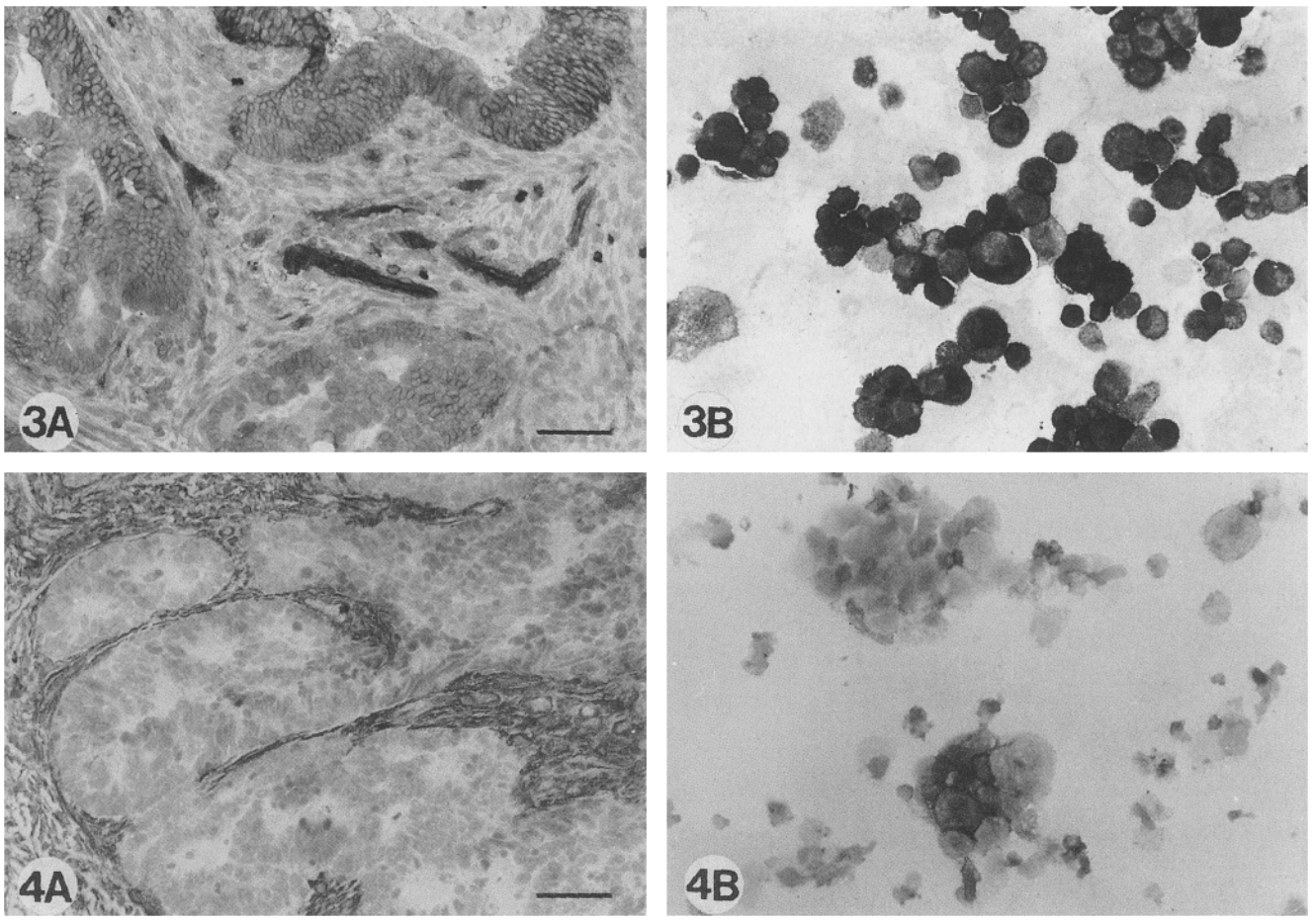

Fig. 3A, B **A** α3-chain expression in tumour number 5. The carcinoma expresses the α3-chain in various intensities in parts of the neoplastic population. The antigen is mostly confined to the cell surface. Endothelial cells of stromal collapsed vessels are strongly positive; peritumour fibroblasts express α3 at very low levels (*bar* 80 µm). **B** α3-chain expression in HDC-101. The cell line originating from tumour number 5 expresses the α3-chain in almost all tumour cells (original magnification ×125)

Fig. 4A, B **A** α5-chain expression in tumour number 3. The α5-chain is lacking in the tumour cells but is present in peritumorous fibroblasts (*bar* 80 µm). **B** α5-chain expression in HDC-57. The cell line originating from tumour number 3 cytoplasmically and surface expresses the α5-chain in fraction of tumour cells (original magnification ×200)

responding cell lines expressed the α6-chain in high amounts. The αv-chain was differentially expressed in carcinomas in situ and their cell lines in vitro. In the original carcinomas αv was expressed in the whole neoplastic compartment of four tumours. In one of the eight tumours (number 3) more positive than negative tumour cells were found and in three of the eight carcinomas the tumour cells were completely negative (numbers 2, 7 and 8). In addition, the αv molecule was sometime rather weakly expressed, although many types of stromal cells strongly expressed the αv-chain. In contrast, eight of the ten tumour cell lines expressed αv in almost all tumour cells. The αv-chain was prominently found in the cytoplasm. In HDC-9, HDC-9NMI, HDC-114, and HDC-117 an induction of the αv-chain was found. The β1-chain was expressed in all carcinomas but one tumour (number 4) showed a small fraction of negative tumour cells. The expression of the β1-chain among stromal cells was broad as previously described [15]. All cell lines showed a comparably strong expression of the β1-chain. The β3-chain was neither expressed in the original carcinomas nor in the corresponding cell lines, but was regularly detectable in the endothelium of blood vessels and in smooth musculature.

The sub-lines HDC-8NMIIK1 and HDC-9NMI established after a nude mouse passage showed no striking differences in their integrin profile when compared with their parental lines HDC-8 and HDC-9, respectively.

The comparative evaluation of integrin α- and β-chain expression in colorectal carcinomas and their cell lines showed a slight increase of the α2-, α3- and β1-chain expression under standard culture conditions and a moderate increase or an induction of the α1- and αv-chain. An apparent neo-expression of the α5-chain was found in cultured cells.

In flow cytometry the cell lines were measured under standard culture conditions (Table 3). The results of flow cytometry and immunohistochemistry were essentially the same, although in cytospin preparations, a higher antigen expression was sometimes found which was due to cytoplasmic antigen deposits (Tables 2 and 3). The phe-

Table 3 Flow cytometry data on surface expression of integrin α- and β-chains in the ten colon carcinoma cell lines (*mF* mean fluorescence)

Cell line	α1		α2		α3		α4		α5		α6		αv		β1		β3	
	mF[a]	%[b]	mF[a]	%[b]	mF[a]	%[b]	mF[a]	%[b]	mF[a]	%[b]	mF[a]	%[b]	mF[a]	%[b]	mF[a]	%[b]	mF[a]	%[b]
HDC-8	+	38	++	99	++	98	–		–		++	99	+	89	+++	100	–	
HDC-8NMIIK1	+	49	++	99	++	98	–		–		++	98	+	63	+++	100	–	
HDC-9	+	3	+	33	–		–		–		+	62	n.d.		+	55	n.d.	
HDC-9NMI	+	31	+	84	+	16	–		–		++	91	–		+	63	–	
HDC-57	–		+	95	+	72	–		+	24	++	99	+	75	++	99	–	
HDC-63	–		++	97	++	95	–		–		++	96	n.d.		+++	99	n.d.	
HDC-101	++	88	++	90	+80	–	–		–		++	90	+	70	+++	98	–	
HDC-102	+	31	++	87	+	13	–		–		++	97	+	45	++	90	–	
HDC-114	+	17	++	99	+	94	–		+	78	+++	100	n.d.		+++	100	n.d.	
HDC-117	+	21	++	97	+	87	–		–		+++	100	n.d.		++	99	n.d.	

[a] – negative, negligible; + dim; ++ intermediate; +++ bright
[b] Percent positive cells

Table 4 Flow cytometry data on surface expression of integrin α- and β-chains in ATCC colon carcinoma cell lines grown with (*w*) or without (*w/o*) serum in culture medium

Cell line	α1		α2		α3		α4		α5		α6		αv		β1		β3	
	mF	%	mF	%	mF	%	mF	%	mF	%	mF	%	mF	%	mF	%	mF	%
HT-29 w	++	92	+++	100	+++	96	–		–		+++	100	++	99	+++	100	–	
HT-29 w/o	++	96	++	100	+++	100	–		–		+++	100	++	100	+++	100	–	
SW480 w	+	47	+	81	+++	95	–		+	10	+++	100	++	91	+++	100	–	
SW480 w/o	++	88	++	81	++	74	–		+	67	+++	100	+	62	+++	93	–	
SW620 w	–		+	39	+	6	–		+	52	+++	100	+	11	++	100	–	
SW620 w/o	+	56	+	53	+	40	–		+	67	+++	100	+	26	+++	99	–	
COLO 205 w	++	100	+++	100	++	97	–		+	29	+++	100	++	98	+++	100	–	
COLO 205 w/o	++	100	+++	100	++	100	–		+	33	+++	100	+	98	+++	100	–	

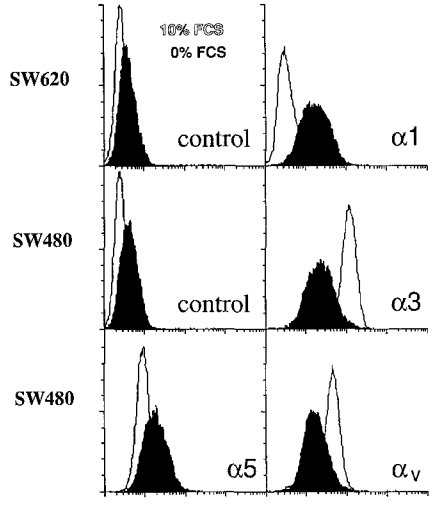

Fig. 5 Flow cytometric analysis of integrin α- and β-chains in SW620 and SW480 grown with (white histogram) and without (black histogram) fetal calf serum shows some modifications in integrin α-chain expression. The logarithmic x-axis represents fluorescence in arbitrary units reflecting antigen density on the cell surface. The y-axis represents number of cells. For each experiment about 10^6 cells were analysed. Paradigmatically, SW620 neo-expresses α1 under serum-free conditions. In SW480 a minor increase of α5 but a decrease of α3 and αv expression is notable

nomenon of cytoplasmic antigen accumulation was found in particular for α1 and αv, and, to a lesser extent, for α5. In order to elucidate whether neo-expression induction or increased expression of integrin α- and β-chains under culture conditions is influenced by factors in FCS, ATCC cell lines HT-29, SW480, SW620 and COLO 205 were cultured in parallel in 10% FCS-medium and in serum-free medium containing insulin, transferrin, and sodium selenite. HT-29 and COLO 205 showed no striking differences in expression of integrin α-chains (Table 4). In SW480, a minor increase in levels of α1- and α5-chain and a decrease in the α3- and αv-chain levels were found under serum-free condition. In serum-free medium SW620 showed an increase in the level of α1-chain expression (Table 4; Fig. 5). In conclusion, effects of FCS on integrin expression in colon carcinoma cell lines, especially with respect to the neo-expression of the α5-chain in vitro, were marginal.

Discussion

The immunohistological analysis of normal colorectal epithelium revealed considerable diversity in expression

of integrin α- and β-subunits. The subunits of adhesion receptors found highly expressed in normal colorectal epithelium were those which heterodimerize to form receptors recognizing collagen or laminin either alone or as one of their ligands, that is α2- α3-, α6-, β1-, and β4-chain [15, 17, 18, 22, 26]. In contrast, those α- and β-subunits which recognize fibronectin alone or as one of their ligands were either inconsistently expressed, for example the αv-chain [Koretz et al. (submitted)] or were undetectable, (the α4-, α5- and β3-chain). In colorectal carcinomas sporadic loss of some collagen and laminin receptor subunits in particular was found. This loss was more frequent in poorly differentiated carcinomas [18] and in tumours which had already metastasized [15].

As integrin receptors are involved in cell-cell and cell-matrix interaction the absence of the natural microenvironment under cell culture conditions might change the integrin adhesion receptor profile. Indeed, integrin receptor expression on the cell surface was shown to be induced by the presence of extracellular matrix proteins [11] or reduced by the loss of direct interaction with extracellular matrix molecules [1]. It was of interest then, to examine whether the expression of integrin receptor subunits in colorectal carcinomas is a constitutive trait of malignant clone derived from a distinct cell type or the result of environmental adoptive changes. Therefore, the integrin expression of a large series of colorectal carcinoma cells lines in vitro was compared with the immunoprofile of their original carcinomas in situ. We showed that the sets of integrin subunits expressed in cells giving rise to our new colon carcinoma lines were essentially the same as those detectable in situ in the neoplastic populations. If there was a change, it always went in the direction of increase, induction or neo-expression. This finding is at variance with other molecular characteristics of colon carcinoma cells such as the secretory component and EMA which tended to be down-modulated in the cell lines (data not shown). An induction of the α1-chain was found in five cell lines and an induction of the αv-chain in four cell lines. The most striking phenomenon was the apparently neo-expression of the so-called "classic" fibronectin receptor α5-chain which was found in five cell lines (Table 2).

A comparable change in the expression of the fibronectin receptor was also found in keratinocytes which under cell culture conditions neo-expressed the fibronectin receptor, which was absent in freshly isolated keratinocytes [23]. A neo-expression of the fibronectin receptor was also found in keratinocytes which had been isolated from healing wounds. Grinnell [10] suggested that the $\alpha5\beta1$ inducing signal might be the same in both conditions. This neo-expression of $\alpha5\beta1$ in keratinocytes was functional, resulting in adhesiveness and migration of fibronectin not found in keratinocytes of unwounded skin or from freshly prepared cell culture [23]. It has been suggested that this modulation might be a result of changes in extracellular matrix proteins, and a local release of cytokines and growth factors [10]. However, as far as colon carcinoma cells are concerned, environmental factors of that kind might well exist, but their effects are likely to be of minor importance. To further elucidate the influence serum factors have, four long-lived standard carcinoma cell lines which were adapted in serum-free medium were examined. This question had already been addressed in a study of Fingerman and Hemler [9] who found that quiescence of normal human fibroblasts, induced by density arrest of cells at confluence or serum deprivation, resulted in an increase of α1 expression and a decrease in α2 and α3. In our system, however, integrin α- and β-chain expression was only slightly modified under serum-free conditions in the sense of an increase of the α1- and α5-chain and a decrease of the α3- and αv-chain in some of the cell lines.

In conclusion, our results indicate that the expression of integrin collagen or laminin receptors in colorectal carcinomas is likely be a constitutive feature of the tumour since the expression of the respective α- and β-chains were essentially maintained or, at most, slightly increased in vitro. Serum factor(s) only marginally influenced the α- and β-chain expression. The neo-expression of the α5-chain, however, might be an adoptive feature of the in vitro condition since α5 was not found in the primary tumours in situ. We conclude that aberrations in integrin receptor expression in colon carcinomas are abnormalities probably acquired during the process of malignant transformation. The relative stability of the integrin receptor profile in colon carcinoma might be applied to predict the biological consequences of a given pattern of adhesion receptors expressed in vivo by using colon carcinoma cell lines as functional models.

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