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Changes in the expression of syndecan-1 in the colorectal adenoma–carcinoma sequence

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Abstract Syndecan-1, a transmembrane heparan sulphate proteoglycan (HSPG), functions as a matrix receptor on the basal surface of epithelial cells. It also co-localizes with E-cadherin at the lateral cell surface where its function is uncertain. Tumour development in the large bowel is associated with loss of normal epithelial adhesion and altered patterns of expression of cell adhesion molecules, possibly including syndecan-1. To evaluate changes in syndecan-1 expression during the development of colorectal neoplasia, 59 adenomas and 20 carcinomas arising from adenomas were investigated by immunohistochemistry. The staining intensity and distribution of syndecan-1 and E-cadherin in sequential sections was examined, semi-quantified and compared. Staining of syndecan-1 and E-cadherin was uniform in normal colorectal epithelial cells, and located at the basolateral surface. No significant change was seen in either molecule in mildly or moderately dysplastic adenomas. A significant reduction in expression of both syndecan-1 and E-cadherin was seen in severely dysplastic epithelium as compared to moderate dysplasia (P=0.001 and P=0.004 respectively). Similarly, there was a significant reduction of both molecules in carcinomas compared with associated adenomas (syndecan-1 P=0.00003; E-cadherin P=0.002). In both cases the loss of syndecan-1 expression was more striking than that of E-cadherin. Previous in vitro studies have shown that epithelial cells made deficient in syndecan-1 cease to express E-cadherin, suggesting a causal association. Our results support these findings and indicate that disruption of cell-matrix adhesion is critical in colorectal carcinogenesis, probably preceding changes in the purely homotypic cell-cell adhesion mediated by E-cadherin.

Key words Syndecan-1 · E-cadherin · Colorectal carcinogenesis

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Introduction

The development of malignant epithelial neoplasms is associated with disruption of cell–cell and cell-matrix adhesion [1, 2]. This is thought to occur as a result of altered expression of certain cell adhesion molecules (CAMs) [3–6].

Epithelial cell–cell adhesion is mediated predominantly by E-cadherin at the adherens junctions. E-cadherin is a calcium-dependent transmembrane glycoprotein, the cytoplasmic domain of which is associated with the catenins (α, β and γ) to form the cadherin–catenin complex that associates with the actin cytoskeleton of the cell.

The binding of cells to the extracellular matrix (ECM) involves a number of different adhesion receptors including the integrins [7] and syndecans [8]. Syndecans are a family of heparan sulphate proteoglycan (HSPG) receptors that are thought to participate in both cell–cell and cell–matrix adhesion. In addition, they may act as receptors for growth factors and thereby may be involved in control of cell proliferation [8–10]. All syndecans are transmembrane proteins with functional cytoplasmic and extracellular domains. Heparan sulphate chains are covalently attached to the extracellular domain and mediate binding to components of the ECM. Syndecan-1 is a hybrid HSPG with both heparan sulphate and chondroitin sulphate chains attached to the extracellular domain [8].

In adult life, syndecan-1 is expressed predominantly in epithelial tissues [11], but is also found on fibroblasts [12] and plasma cells [13]. It has been shown to co-localize with the actin cytoskeleton in mouse mammary epithelial cells [14], an association similar to that of E-cadherin (via the catenins) at the zonula adherens. Thus, an adhesion complex is thought to exist at the zonula adherens involving E-cadherin, syndecan-1 and the actin cytoskeleton [8]. The expression of syndecan-1 has been shown to be altered in malignant epithelial tumours in animal models [15] and in human cervical carcinomas [4] and squamous carcinomas of the head and neck [5]. Since syndecan-1 is thought to participate in cell–cell adhesion and to associate with E-cadherin, we studied changes in the expression of syndecan-1 in comparison to that of E-cadherin in human colorectal tumours.

Materials and methods

Fifty-nine adenomas and 20 carcinomas (Table 1) arising from adenomas were selected from the archive of the Academic Department of Pathology, St Mark's Hospital, UK, according to the following criteria: (1) patients had no known personal history of malignancies or of familial adenomatous polyposis, (2) all polyps were completely excised by polypectomy or surgical resection; and (3) if multiple adenomas were present, the largest and that with the most severe dysplasia was chosen. All samples also contained some histologically normal epithelium in addition to tumour tissue. Fresh 4-µm-thick serial sections were cut from routinely fixed, paraffin-embedded blocks and placed on Poly-L-lysine-coated slides (Sigma Chemical, Poole, UK). One slide of each specimen was stained with haematoxylin and eosin and used to confirm the recorded histological classification (ICT/XPH). These tumours have previously been studied for changes in E-cadherin expression [3].

Immunohistochemical staining of syndecan-1 was performed in accordance with standard procedures on 4-µm-thick sections of formalin-fixed, paraffin-embedded sequential tissue sections to those previously stained for E-cadherin [3]. Antigen retrieval was performed by boiling for 12 min in an aluminium pressure cooker (Prestige, UK) at 103 kPa in pre-heated 10 mM sodium citrate buffer (pH 6.0). After cooling in running tap water, the slides were rinsed in 0.1 M phosphate-buffered saline (PBS; pH 7.4). Nonspecific staining was blocked by incubation of the sections in normal horse serum for 30 min, prior to application of the primary monoclonal antibody to syndecan-1 (MCA681, clone B-B4; Serotec, Kidlington, UK) at a concentration of 0.001 mg/ml. This is an IgG_1 (k) antibody which reacts specifically with syndecan-1, as revealed by molecular cloning [16]. After incubation in a moist chamber overnight at room temperature, the slides were washed in PBS and incubated for 30 min with biotinylated horse anti-mouse IgG (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, Calif.). Slides were washed and then incubated for 30 min with avidin-biotin complex (Vectastain Elite ABC kit), according to the manufacturer's recommendations. Staining was performed by incubation with 3–3′diaminobenzidine (DAB; Sigma) activated with hydrogen peroxide. Slides were counter stained with Mayer's haematoxylin. Negative controls were obtained by omitting the primary antibody, replacing it with PBS.

Immunohistochemical staining was read independently by two observers (RD and XPH), using a previously described scoring system [3, 17] as follows: slides were assessed for the proportion of cells stained and/or their intensity. A semi-quantitative method was used and the figures quoted are approximations rather than the product of a formal counting method. The intensity of membranous staining in adenomas and carcinomas was graded as negative (0, no staining), weak (+), moderate (++) and intense (+++, as strong as in normal mucosal epithelium). As all epithelial cells in the adenomas were positive for both syndecan-1 and E-cadherin, only the intensity of immunoreactivity was scored. For carcinomas arising in adenomas and their associated adenomas the percentage of cells showing membranous positive staining was graded as follows: 0 (<5%), 1 (5–25%), 2 (26–50%), 3 (51–75%) and 4 (more than 75%). A cumulative total was obtained by multiplying the values of the intensity by the percentage of cells showing membranous staining, producing a score from 0 to 12. The scores were defined as: strong staining (12–9), moderate staining (8–6), weak staining (4–1), and negative (0). Cases in which there was a discrepancy in the scoring were jointly re-evaluated by the two observers and an agreement was reached. As staining with syndecan-1 and E-cadherin was performed on sequential sections from each tumour, the scores and distribution of staining obtained with each antibody were compared.

Statistical analyses were performed using the Fisher exact probability test, and *P* values equal to or smaller than 0.05 were

Table 1 Staining scores in the 20 carcinomas studied together with Dukes' staging and grade of dysplasia in associated adenomas

Case	Dysplasia grade	Syndecan-1 score[a]	E-cadherin score[a]	Tumour stage	Syndecan-1 score[b]	E-cadherin score[b]
1	Severe	4	12	Dukes' stage B	9	12
2	Severe	8	8	Dukes' stage A	0	4
3	Severe	4	8	Dukes' stage B	0	8
4	Severe	8	8	Dukes' stage C1	2	2
5	Severe	8	8	Dukes' stage C1	1	8
6	Mild	12	12	Dukes' stage C1	6	3
7	Moderate	8	8	Dukes' stage A	1	3
8	Severe	8	8	Dukes' stage A	6	3
9	Moderate	8	12	Dukes' stage A	0	12
10	Severe	12	12	Dukes' stage A	0	12
11	Severe	8	12	Dukes' stage A	0	12
12	Severe	12	12	Dukes' stage A	0	8
13	Severe	8	8	Dukes' stage A	9	8
14	Severe	8	8	Dukes' stage A	4	4
15	Moderate	12	12	Dukes' stage A	0	12
16	Severe	12	8	Dukes' stage A	0	3
17	Severe	12	4	Dukes' stage A	9	4
18	Severe	12	8	Dukes' Stage A	3	4
19	Severe	12	8	Dukes' stage A	0	3
20	Severe	8	12	Dukes' stage A	2	12

[a] Scores in adenoma
[b] Scores in carcinoma

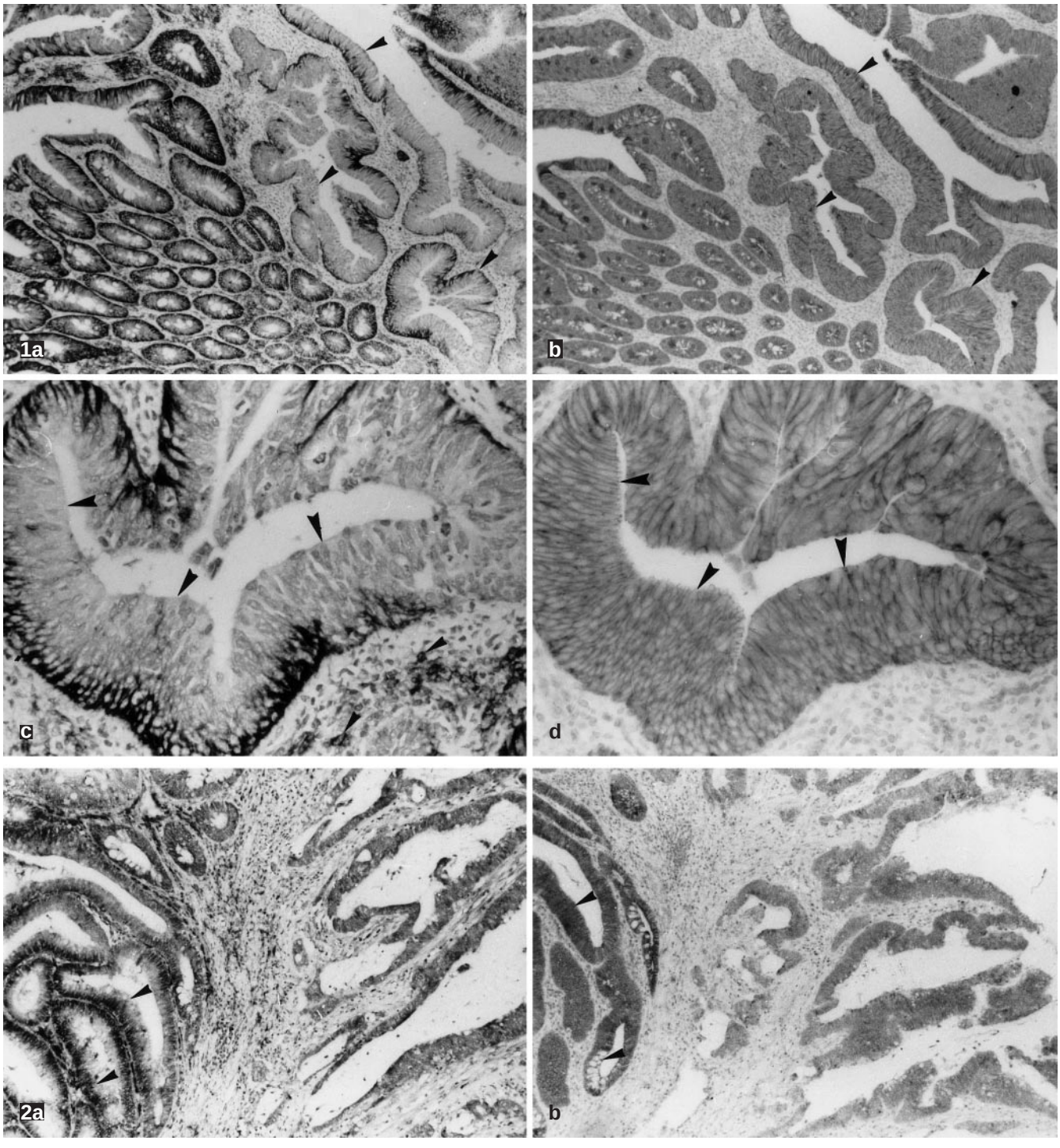

Fig. 1 **a** Syndecan-1 and **b** E-cadherin immunoreactivity in large bowel mucosa. Histologically normal mucosa shows strong uniform membranous staining of syndecan-1 and E-cadherin. Severely dysplastic epithelium (*arrowheads*) shows a significantly reduced expression of syndecan-1 compared with adjacent normal mucosa. Loss of E-cadherin expression is less apparent in the same areas of dysplastic epithelium. ×100. **c**, **d** Higher magnification of the dysplastic epithelium clearly shows the greater reduction of syndecan-1 (**c**) than of E-cadherin membrane staining (**d**: *large arrowheads* in both). Plasma cells in the lamina propria are also positively stained for syndecan-1 (*small arrowheads*). ×300

Fig. 2 **a** Syndecan-1 and **b** E-cadherin immunoreactivity in moderately differentiated carcinoma arising in a villous adenoma. The area of tissue containing the moderately differentiated carcinoma shows a significant reduction in the expression of both syndecan-1 and E-cadherin compared with the associated adenoma (*arrowheads*). ×100

considered significant for differences between: (1) the staining intensity and grade of dysplasia in sporadic adenomas, and (2) the staining score in carcinomas and their associated adenomas.

Results

Histologically normal epithelium adjacent to adenomas showed uniform membranous staining of syndecan-1 and E-cadherin in surface epithelium and along the whole length of the crypt, thus serving as an internal positive control for each section. Staining was located at the basolateral surface of columnar epithelium (Fig. 1a, b). No cytoplasmic staining or background stromal staining was evident with either antibody. However, plasma cells in the lamina propria were intensely stained by syndecan-1.

Membranous staining of syndecan-1 and E-cadherin was displayed in all adenomas examined, although differences in intensity were seen. Reduced expression of both syndecan-1 and E-cadherin was associated with the change from moderate to severe dysplasia. There was some reduction of staining of both molecules in both mildly and moderately dysplastic tissue as compared to normal tissue, although in neither case was this significant (syndecan-1 P=0.2 and E-cadherin P=0.2). There was, however, a marked reduction in severely dysplastic as against moderately dysplastic tissue for both molecules (syndecan-1 P=0.001 and E-cadherin P=0.004) and the reduction of syndecan-1 appeared to be greater than that of E-cadherin (Fig. 1a–d, Table 2).

In carcinomas, changes in the proportion of cells with positive staining were seen as well as changes in the intensity of the membranous staining. Analysis of the weighted scores showed a significant reduction in staining for both syndecan-1 and E-cadherin in carcinomas compared with their associated adenomas (syndecan-1 P=0.00003 and E-cadherin P=0.002). As with the severely dysplastic adenomas, there was an apparently greater reduction in the staining of syndecan-1 than in that of E-cadherin (Fig. 2a, b, Table 3).

Discussion

We have shown that a major change in the expression of syndecan-1 occurs during the development of colorectal tumours. In the mildly and moderately dysplastic adenomas the pattern of expression was similar to that in adjacent nonneoplastic epithelium. There was, however, a significant decrease in the level of syndecan-1 expression, firstly in severely dysplastic adenomas compared with mildly or moderately dysplastic adenomas, and secondly, in invasive carcinomas compared with the adjacent associated adenomatous component. These changes were mirrored by decreases in the expression of E-cadherin. The changes in E-cadherin, however, were less dramatic than that in syndecan-1, especially the change from mild/moderate dysplasia to severe dysplasia. In invasive tumours the change in syndecan-1 expression was also visually more marked than that in E-cadherin. The fact that these changes occurred at specific points during tumour development suggests that there is possibly selection for these changes and that they are not epi-phenomenal to tumour progression.

The mechanism behind the reduction of membrane syndecan-1 remains unclear. Proteases which normally participate in the lysis and physiological turnover of the basement membrane may also facilitate tumour invasion [2]. These enzymes include heparanase (endoglucoronidase), which is capable of cleaving heparan sulphate [18]. If, as might be expected, increased amounts of protease enzymes are released in the extracellular compartment by neoplastic cells and/or stromal cells in the later stages of large bowel tumour development, heparan sulphate could be cleaved from syndecan-1, perhaps resulting in degradation of the nonfunctional molecule. This could explain the dramatic decrease in the expression of membrane syndecan-1 seen between moderate and severe dysplasia.

Heparin-binding growth factors regulate cell growth and differentiation and bind to their high affinity signal transducing receptors via HSPGs, thought to include syndecan-1 [8, 9]. Syndecan-1 expression is transiently

Table 2 Correlation between staining intensity and grade of dysplasia in sporadic adenomas for each antibody. The Fisher exact probability test was used to analyse differences between the groups. *P* values equal to or smaller than 0.05 were considered significant

	Syndecan-1				E-cadherin			
	+++	++	+	–	+++	++	+	–
Mild	20	2	0	0	21	1	0	0
Moderate	23	3	3	0	24	5	0	0
Severe	1	3	4	0	2	2	4	0
Mild → moderate	P=0.23				P=0.2			
Moderate → severe	P=0.001				P=0.004			

Table 3 Correlation between staining score in carcinomas and their associated adenomas for each antibody. The Fisher exact probability test was used to analyse differences between the groups. *P* values equal to or smaller than 0.05 were considered significant

	Syndecan-1				E-cadherin			
	12–9	8–6	4–1	0	12–9	8–6	4–1	0
Adenomas	8	10	2	0	8	11	1	0
Carcinomas	3	2	6	9	6	4	10	0
	P=0.00003				P=0.002			

induced in proliferating keratinocytes and endothelial cells during wound healing [19]. Transfection studies have revealed that overexpression of syndecan-1 results in down-regulation of growth factor responses [20], suggesting that enhanced expression is associated with non-malignant proliferation and restriction of excessive growth. Transformed cells with reduced syndecan-1 expression may have lost this restrictive mechanism important for regulating cell proliferation [4].

The greater reduction of syndecan-1 expression than of E-cadherin expression seen in the transition from moderate to severe dysplasia demonstrates that changes in the expression of syndecan-1 probably occur before those in E-cadherin, perhaps influencing the expression of the latter adhesion molecule. Similar observations have been made in vitro using NMuMG mouse mammary epithelial cells transfected with antisense cDNA encoded for the core protein of syndecan [21]. Cells expressing less than 10% normal cell surface syndecan grew as individual fusiform cells, showing a marked reduction in their expression of E-cadherin.

Syndecan-1 is thought to be an important prognostic factor of SCC of the head and neck [5]. Syndecan-1-positive tumours were associated with higher overall, recurrence-free survival compared with tumours with reduced expression of this molecule. The prognostic significance of syndecan-1 in colorectal carcinoma remains to be evaluated.

In summary, our results demonstrate that the expression of syndecan-1 is significantly reduced during the late stages of tumour development, especially in the transition from moderate to severe dysplasia and noninvasive to invasive tumour. The apparently greater loss of syndecan-1 relative to E-cadherin also suggests that a sequential process is pertinent to this aspect of tumour progression and that syndecan-1 changes occur before, and may influence, E-cadherin changes. Further studies on the prognostic significance of syndecan-1 expression in colorectal carcinomas are warranted.

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