Type IV collagen immunoreactivity of basement membrane in inflammatory-regenerative and dysplastic lesions of the flat colonic mucosa

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Abstract

The aim of this research is to establish by immunohistochemistry if there is a change in the expression of collagen type IV, as a substitute of basement membrane, in development of epithelial dysplasia in chronically inflamed colon mucosa.

Methods. Biopsy specimens from 270 patients were examined: 74 were classified as inflammatory-regenerative and 196 as dysplastic lesions. There were 108 cases of mild dysplasia, 58 cases of moderate and 30 cases severe dysplasia, respectively. Visualisation of collagen IV and its way of expression within basement membrane of glandular crypts was performed by immunohistochemistry and then compared with findings in normal colon mucosa and colon adenocarcinoma tissue.

Results. Changes in the expression of collagen IV comprised of its focal irregularities, diffuse thinning and/or thickening, focal interruptions or its complete absence. Significant changes in the expression of collagen IV in relation to normal mucosa already occur in inflammatory -regenerative mucosa. In mild dysplasia, these changes are more intensive in relation to those in inflammatory altered mucosa as well as at severe dysplasia in relation to moderate dysplasia. Changes in the expression of collagen IV in severe dysplasia are significantly more serious than in moderate dysplasia but are identical to those in colon adenocarcinoma tissue.

Conclusion. These findings suggest that change in the expression of collagen IV is in correlation to a degree of epithelial dysplasia that developed in flat chronically inflamed colon mucosa.

Key words: collagen IV, epithelial dysplasia, colon mucosa

Introduction

Basement membrane is a structure that separates connective tissue from parenchymatous cells, endothelium, nerve trunks and myocytes. Five types of proteins, as components of basement membranes (BM) are identified by immunohistochemistry: proteoglycane heparin sulphate, glycoprotein laminin, collagen type IV, entactin and fibronectin. Presence and distribution of entactin and fibronectin are variable. BM in all tissues develops as a result of mutual activity of epithelium and extracellular matrix.. Preservation of its integrity is a reflection of preservation of structural and functional relation between epithelium and stroma.
In colon mucosa BM is primarily product of epithelial cells that lay on it but also of pericryptal fibroblasts that surround glandular crypts. Heparin sulphate is exclusively produced by epithelial cells, collagen IV by mesenchymal cells while laminin is produced by both types of cells. Close contact of epithelial cells and pericryptal fibroblasts is essential for production of aforementioned components as achieved only in normal mucosa.

Abnormalities in biosynthesis and/or in metabolism in BM occur within numerous pathologic processes (1), in which either hyper-production or reduction of extracellular matrix develops as a final outcome (2). Thickening, thinning or absence of BM might occur in reflection to these processes and was observed by microscopy. Thinning of BM is mostly present at different malignant tumours (3, 4, 5, 6), inside of which thickening of BM sometimes might be present as well (5,7). Irregularities in the expression of BM are observed at hyperplastic and mild dysplastic changes of larynx mucosa (8, 9, 2), as well as at paraneoplastic lesion of bronchial mucosa (4,2).

In this paper we tried to determine a relation between the intensity of changes in collagen IV production and grade of epithelial dysplasia in flat chronically inflamed colon mucosa.

Material and methods

During the routine endoscopic examination, 2-3 specimens of colonic mucosa (always at 30 cm from the anus) were taken with biopsy forceps from each patient after a clinician established the diagnosis of inflammatory process. Total number of patients was 270, out of which 208 males and 62 females. All patients were older than 45 years (median age 65 years, range 46-82).

As a control group we used biopsy specimens of normal

colonic mucosa of 40 deceased patients, between 30 and 70 years old. There were 26 males and 14 females. These specimens were taken during the autopsy and only when colonic mucosa showed no sign of inflammatory disease.

From the regular autopsy material also the specimens of colon adenocarcinoma from cases with "de novo" carcinoma have been taken. Carcinoma samples were taken from 40 deceased patients, between 38 and 76 years old (median age 65), 27 males and 13 females. Carcinomas "de novo" are small carcinomas in colonic flat mucosa ranging from 7+/-10 mm in diameter. In 29 cases the surface was slightly sagged, in 8 cases slightly elevated and in 3 cases a discrete swelling in the level of mucosa surface appeared. In "de novo" carcinoma tissue on serial sections, neither by macroscopic nor by microscopic method, the existence of resident adenoma could be established.

The specimens were fixed in 10% buffered formalin, embedded in paraffin, cut into 3-5 micrometer sections and stained by standard haematoxylin-eosin (HE) and analysed immunohistochemically by mouse anti human collagen IV (CIV 22, prediluted, code No: L 1863, DAKO).

Histological criteria were defined to ease differentiation of inflammatory-regenerative and dysplastic changes and grading of dysplasia intensity (10). According to these criteria, dysplastic changes are classified into three groups (mild, moderate and severe dysplasia). The classification was based on 19 criteria regarding morphology of lesions, graded on a 1-4 scale with respect to the intensity of change. The scores were summed up and their mean marked as index (I). Numerical values of index I for individual categories of changes are:

$1.3<I<1.8$ for inflammatory-regenerative changes;
$1.9<I<2.3$ for mild dysplasia;
$2.4<I<2.9$ for moderate dysplasia and
$3.0<I<3.7$ for severe dysplasia.

Upon immunohistochemical staining, the evaluation of biopsy specimens was performed regarding the expression of collagen fibres. We notified intact fibres (prominent and continuous distribution around glands that is present in normal mucosa), focal irregularities like reduplication, thinning and/or thickening of collagen, diffuse thinning, diffuse thickening, focal interruptions or complete absence. Eventual presence of intracytoplasmatic staining of epithelium for collagen was also notified.
BM under skin epidermis served as a positive control. Deposition of collagen IV in BM of capillary blood vessels, nerve fibres and smooth muscles fibres of colon mucosa served as a positive internal control in each specimen.

Results

Among biopsy specimens of 270 examined patients, chronic ulcerative colitis was found in 105 patients, lymphocytic colitis in 40 patients and eosinophilic colitis in 25 patients. In 74 cases the changes have been defined as inflammatory-regenerative and in 196 cases as dysplastic. Mild dysplasia was found in 108 cases, moderate in 58 while severe dysplasia was found in 30 cases (Table 1).

Table 1. Classification of morphological changes in colon mucosa of 270 patients with colonic inflammatory-regenerative and dysplastic epithelial lesions

Morphological changes	Index*	Number of patients
Inflammatory-regenerative changes	1.3	7
	1.4	10
	1.5	16
	1.6	9
	1.7	20
	1.8	12
Total		**74**
Mild dysplasia	1.9	21
	2.0	10
	2.1	29
	2.2	30
	2.3	18
Total		**108**
Moderate dysplasia	2.4	14
	2.5	15
	2.6	8
	2.7	6
	2.8	11
	2.9	4
Total		**58**
Severe dysplasia	3.0	5
	3.1	9
	3.2	4
	3.3	6
	3.4	2
	3.5	2
	3.6	2
	3.7	0
Total		**30**

* Index (I) is a numerical estimate of the extent of morphological changes

In normal colon mucosa collagen IV is expressed by imunohistochemistry as a linear, intact, prominent structure located immediately between epithelial cells of glands and lamina propria. Immunoreactivity of epithelial cell cytoplasm was not observed in normal mucosa.

Variations in immunohistochmical staining of collagen IV were perceived in the category of inflammatory-regenerative and dysplastic changes as well as in colon adenocarcinoma tissue (Table 2.)

Table 2. Immunohistochemical status of collagen type IV in basement membrane of normal mucosa in 40 patients, inflammatory-regenerative and dysplastic flat colon mucosa in 270 patients, and in colon adenocarcinoma in 40 patients

	Morphological changes					
Distribution of collagen IV in BM	**Normal mucosa N=40**	**Inflammatory-regenerative changes N=74**	**Mild dysplasia N=108**	**Moderate Dysplasia N=58**	**Severe dysplasia N=30**	**Colon Adeno-carcinoma N=40**
Prominent and continuous	40 (100%)	12 (19.2%)	95 (87.9%)	0	0	0
Focal irregularities	0	6 (9.5%)	8 (7.4%)	2 (3.4%)	0	2 (5.0%)
Diffuse thinning	0	0	1 (0.9%)	6 (10.3%)	2 (6.6%)	0
Diffuse thickening	0	0	4 (3.7%)	2 (3.4%)	0	0
Focal interruptions	0	40 (54.0%)	0	47 (81.0%)	7 (23.3%)	2 (5.0%)
Complete absence	0	16 (21.6%)	0	1 (1.7%)	21 (70.0%)	36 (90.0%)

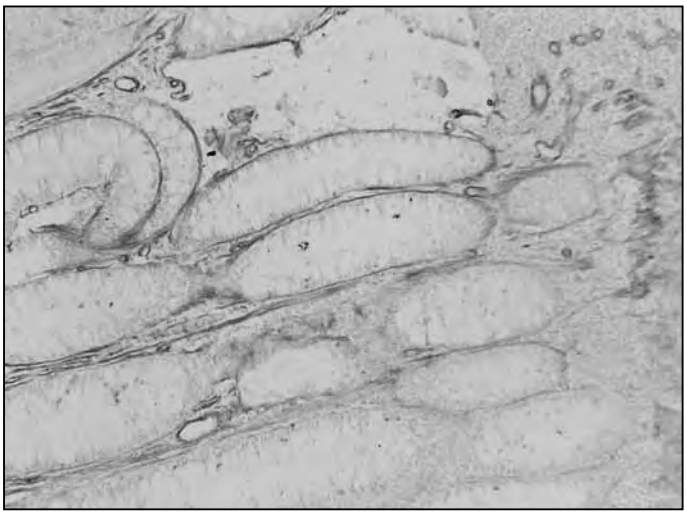
In 40 cases of inflammatory-regenerative changes (54%), (Figure 1.) there were numerous and clearly noticeable focal interruptions of collagen fibres. In 16 cases (21.6%) they were completely absent and in 6 cases (9.4%) there were focal irregularities like reduplication, thickening and/or thinning of fibres. In the rest 12 cases (19.2%) the changes in BM were not observed. All changes were always limited to those places in which epithelium was pervaded and disintegrated with inflammatory cell infiltrates where the polymorphs were the dominant cells.

In 95 cases of mild dysplasia (87.9%) (Figure 2.) the changes in BM were not found. It looked the same way as BM in normal mucosa. In 8 cases (7.4%) there were

Table 2a. Results of Table 2 testing

Morphological groups tested for significance	Number of free degrees	Value of Hi-quadrate test	Conclusion on significance of differences between morphological groups and level of significance
1. Normal mucosa -inflammatory-regenerative changes	2	70.174	Significant, $p<0.005$
2. Normal mucosa - mild dysplasia	1	3.883	Significant, $p<0.005$
3. Normal mucosa - moderate dysplasia	2	96.893	Significant, $p<0.005$
4. Normal mucosa - severe dysplasia	2	68.815	Significant, $p<0.005$
5. Normal mucosa - adenocarcinoma	1	72.042	Significant, $p<0.005$
6. Inflammatory-regenerative changes - mild dysplasia	4	123.431	Significant, $p<0.005$
7. Mild dysplasia - moderate dysplasia	3	139.259	Significant, $p<0.005$
8. Moderate dysplasia - severe dysplasia	2	53.529	Significant, $p<0.005$
9. Severe dysplasia - adenocarcinoma	1	3.788	not significant for $p<0.05$, but significant for $p<0.01$

Figure I. Focal attenuation and focal interruption of type IV collagen of the basement membrane of glands of the colon mucosa with inflammatory-regenerative changes (X 250).



irregularities like thickening and/or thinning of collagen IV fibres that preserved their continuity. Diffuse thickening was observed in 4 cases (3.7%), while diffuse thinning was observed in only one case (0.9%).

In mild dysplasia (Figure 3.) there was a wide range of different expressions of collagen IV fibres. Focal interruptions in BM were found in 47 cases (81%), diffuse thinning in 6 cases (10.3%) and diffuse thickening and focal irregularities in 2 cases each (3.4%). In one cases these fibres in BM were completely missing.

In 21 case of severe dysplasia (70.0%) collagen IV type fibres were completely missing and in 7 cases (23.3%) there were focal interruptions while in 2 cases (6.6%) the diffuse thinning of these fibres within BM was notified.

In 36 cases of colon adenocarcinoma (90.0%), collagen IV fibres were totally missing from BM of atypical glands. Focal interruptions and focal irregularities in the expression of these fibres were found in two cases each (5.0%).

Differences in the expression of collagen IV fibres in BM were tested for significance and these results showed high significance ($p<0.005$) regarding severity collagen fibres damage for each following category in comparison to the previous category of morphological changes (Table 2a.). Only the differences between severe dysplasia and adenocarcinoma are lees significant ($p<0.10$) in relation to the differences between other categories.

Figure 2. Type IV collagen of the basement membrane of glands of the colon mucosa with mild dysplasia (X 100).

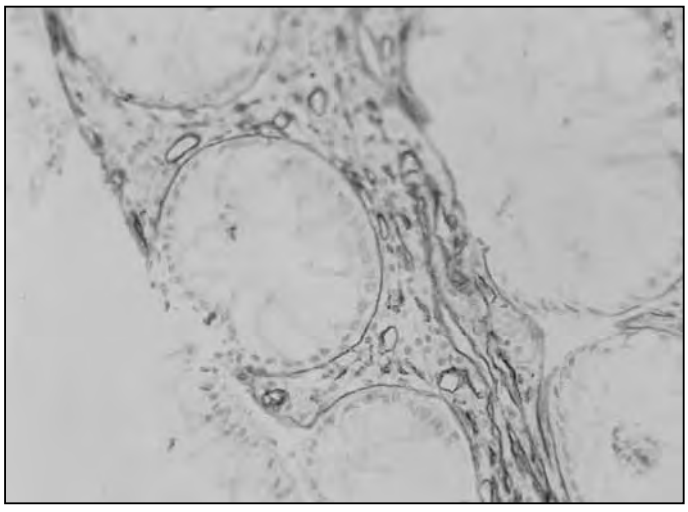
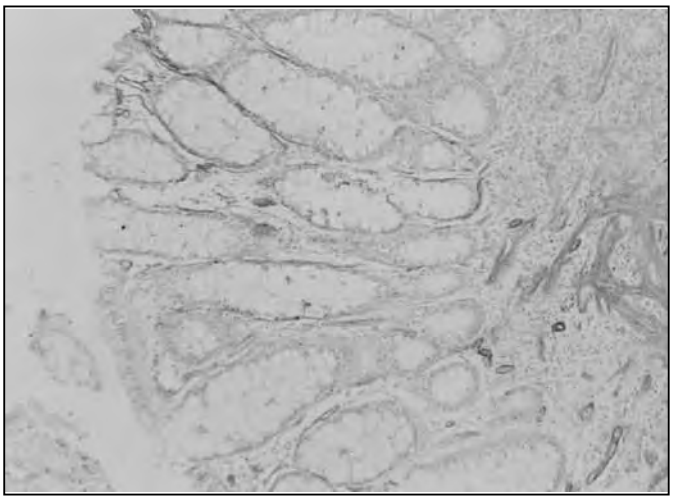


Figure 3. Well defined type IV collagen basement bembrane interruption of glands of the colonic mucosa with moderate dysplasia (X 100).



Discussion

There is a small number of studies related to changes of extracellular matrix (ECM) in pre-neoplastic lesions of different tissues (2, 4, 9). The majority of these studies is focused on the changes of ECM in tumour tissue. It is well known that modifications in the expression of extracellular matrix are the reflection of disturbed interaction of epithelium and matrix and have a big role in the growth and spreading of tumour (1, 3). The biggest number of observations is mostly related to malignant tumours. Findings regarding changes in extracellular matrix are rather controversial. These studies deal with destruction and degrading of BM but also with the new synthesis of BM (9,2). Former findings suggest that neoplastic cells of well-differentiated carcinomas preserve ability of BM components' synthesis, like normal cells (9), which can be induced by mesenchymal cells (4).

This paper analysed the ways of expression of collagen IV fibres within BM of inflammatory-regenerative and dysplastically changed flat colonic mucosa. The findings are then compared with those in normal mucosa and colon adenocarcinoma tissue.

There are significant differences in the expression of collagen IV in inflammatory-regenerative mucosa in relation to normal mucosa, reflected in the occurrence of focal irregularities, interruptions and complete lack of fibres. These lesions of BM were at the sites with a lot of inflammatory cell infiltrates dominated with the polymorphs that were not only close to epithelium but also tuck into it. In mild dysplasia, the significance of changes compared with aforementioned category was even higher. With an increase in ED intensity, changes in BM were more prominent. In moderate dysplasia, in the biggest number of cases, we notified focal interruptions, diffuse thinning and focal irregularities. In one case collagen IV fibres were completely missing. In severe dysplasia, in the biggest number of cases these fibres were completely missing while in a few cases we notified focal interruptions of their continuity and diffuse thinning. There were no differences in the expression of collagen IV fibres between cases of severe dysplasia and adenocarcinoma tissue. These findings are in correlation with Fosseler-Eckhoffov findings (4), that notified degradation of BM components (fibronectin, collagen III and laminin) in the areas of dysplastic epithelium of bronchial mucosa which severity depended on ED degree. In dysplastic lesions, with an increase of their intensity, a quantity of mononuclear cellular infiltrate also enlarged (macrophages were the dominant cells), but direct lytical activity of inflammatory cells on the epithelium was not observed.

The findings of collagen IV lesions in inflammatory-regenerative and dysplastic colon mucosa can be the result of different cellular mechanisms but are still not completely solved. Inflammatory cells contain protease enzymes (elastases, collagenases and plasminogen activator) that are capable to cause severe damage of extracellular matrix (ECM) (11). Collagenasa, enzyme of neutrophil leukocytes has a direct effect on collagen IV fibres. Collagenases are excreted in inactive zymogen form (as proenzyme), which only later acquire catalytic activity but the exact mechanism of activation is still unknown (12). It is the most probable that these injuries and interruptions of collagen fibres in the category of inflammatory-regenerative changes are the result of neutrophils' collagenases activity since this type of cell dominated in inflammatory infiltrate. Range of anomalies of collagen IV fibres is in correlation with ED intensity.

Macrophages were the most numerous inflammatory cells in dysplastic lesions and in stroma of colon adenocarcinoma but had no direct contact with the epithelium. Since the macrophages are capable of excreting proteolytic enzymes, which are responsible for digestion of BM substrate, their possible action on collagen fibres is not excluded. It is also notified that dysplastic epithelial cells are capable of production and release of collagenas-

es (13,14), that destroy ECM and in that way they trace a path for tumour cells of their own origin to invade. Dysplastic cells are capable to activate inflammatory cells i.e. macrophages and to stimulate them to release lysosomal enzymes (15).

At chemically induced carcinomas of murine colon, preservation of collagen IV fibres in BM was notified at well-differentiated adenocarcinomas but only in those areas where the pericriptal fibroblasts were in close contact with carcinoma cells (16). The areas where there was no such close contact between these two types of cells were deprived of collagen IV. Since mesenchymal cells primarily produce collagen IV, lack of these fibres in these cases is interpreted through a loss of close contact between these two types of cells (16,17). It is found that stroma of myofibroblasts around atypical glands of colon adenocarcinoma produce lytic enzymes that lead to BM components' degradation and collagen IV fibres in particular (17). In a recent research of pericryptal fibroblasts (PCF) in the dysplastic lesions of flat colonic mucosa (18) we notified that a decrease in the number of PCF correlated with the increase of ED grade. Therefore a reduction in the number of these cells could serve as a risk marker for pre-neoplastic and neoplastic progression of the lesion.

We cannot be certain about the mechanism of observed changes only on the basis of morphologic indicators and without experimental controls. It is not clear whether the abnormalities of BM in ED are the result of cessation in collagen IV production because of pre-neoplastically changed epithelium, changes in PCF number or that is because increased degradation by specific enzymes originated from mononuclear inflammatory cells. It is also possible that all these aforementioned mechanisms are involved in these events at the same time.

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