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Oestrogen receptor beta (ERβ) is abundantly expressed in normal colonic mucosa, but declines in colon adenocarcinoma paralleling the tumour's dedifferentiation

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Abstract

Oestrogen Receptor β (ERβ) may protect against prostate and mammary cell proliferation and malignant transformation. Epidemiological studies indicate that oestrogens may reduce colon cancer risk. Since ERα is minimally expressed in normal and malignant colon, the aim of this study was to investigate the expression of ERβ in both normal colonic wall and colon cancer. ERβ expression was evaluated by immunohistochemistry in 90 cases of colon adenocarcinoma and nearby (>30-cm away) normal colonic wall, using a monoclonal antibody. Moderate or strong nuclear immunostaining was detected in superficial and crypt epithelium, endothelial cells, vascular smooth muscle cells, lymphocytes, enteric neurons and smooth muscular cells of the normal colonic wall. Superficial epithelial cells in normal colon demonstrated a significantly higher ERβ expression than colon adenocarcinoma cells in both genders. The decline in ERβ expression paralleled the loss of differentiation of malignant colon cells, regardless of the tumour's localisation. These findings suggest a protective role for ERβ against colon carcinogenesis.

Keywords: Oestrogen receptor beta; Normal colonic wall; Colon adenocarcinoma; Carcinogenesis; Cell differentiation; Tumour localisation

1. Introduction

Oestrogen receptors (ERs) are members of the evolutionary conserved nuclear receptor superfamily of ligand-inducible transcription factors. ERα and the recently cloned ERβ exhibit a modular structure consisting of six well-defined functional domains (A–F) [1]. Unliganded ERs reside in multiprotein complexes located in the nucleus. Oestrogen binding to the ligand-binding domain (LBD) induces a conformational change that facilitates receptor homodimerisation (ERα/ERα or ERβ/ERβ) or heterodimerisation (ERα/ERβ) and high-affinity binding to specific DNA recognition sequences (oestrogen response elements, EREs) in the regulatory regions of oestrogen target genes. In this 'classical' mode of ER action, ERα and ERβ homodimers promote ERE-regulated transcription in response to 17β-oestradiol, with ERβ being approximately 30% as efficient as ERα in most cell systems. Tamoxifen is a mixed agonist/antagonist of ERα, but is a pure antagonist of ERβ. Notably, in ERα/ERβ heterodimers, at low concentrations of oestradiol and in the presence of tamoxifen, the suppressive effect of ERβ on gene transcription predominates [2].

Oestrogens and their cognate receptors also regulate target genes via a 'non-classical' mode of action. These effects are mediated through promoter elements that bind heterologous transcription factors, including activating protein-1 (AP-1)-binding sites, cyclic AMP-response elements (CREs), antioxidant elements and SP-1-binding sites. Interestingly, ERα and ERβ can exert opposite actions at AP-1 sites in the presence of

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different ligands. 17β-oestradiol potentiates ERα/AP-1-mediated transcription, but represses ERβ/AP-1 effects, while anti-oestrogens like tamoxifen enhance AP-1-induced transcription through both ERs [3,4].

It is evident that in both the 'classical' and 'non-classical' mode of ER action, ERβ, in the presence of oestrogen, modulates the proliferating effects of ERα by suppressing transcriptional activation. Thus, ERβ may protect the cell from uncontrolled proliferation and malignant transformation. Consistent with this notion, a progressive decline of ERβ expression has been reported in multistage mammary carcinogenesis [5] and in prostate cancer [6]. Epidemiological studies have demonstrated that colorectal cancer incidence and mortality rates are lower in women than men [7]. Many studies indicate that oestrogen replacement therapy (ERT) exerts a protective role against colon cancer in postmenopausal women [8]. According to a meta-analysis, recent use of ERT is associated with a 33% reduction of colon cancer risk and the relative risk for death from colon cancer in ERT users is 0.72, whereas rectal cancer incidence is not associated with ERT [9].

ERα, previously regarded as the sole ER, is minimally expressed in normal and cancerous colon [10,11]. ERβ mRNA has been detected in normal colonic mucosa by the reverse transcriptase-polymerase chain reaction (RT-PCR) and subsequent Southern analysis [11], while ERβ protein expression has been documented in the normal colon tissue of 5 male and 6 female patients by western immunoblotting [12]. No expression of ERβ in colon adenocarcinoma was reported in this western-blot analysis [12], while an immunohistochemical study in 55 colon cancer patients has demonstrated ERβ expression [13].

The primary aim of the present study was to investigate and compare the expression of ERβ not only in the normal colonic epithelium, but also in the entire normal colonic wall, as well as in colon adenocarcinoma, and to correlate its expression with the degree of cancer cell differentiation.

2. Patients and methods

2.1. Specimens and clinicopathological data

We studied 90 colorectal adenocarcinomas that were surgically resected from 50 men and 40 women at the Department of Surgery of the Aegion General Hospital (Aegion, Greece). The patients' age ranged from 43 to 95 years (mean age: 70±11 years). Twenty-eight adenocarcinomas were located proximally to the mid-transverse colon and 62 tumours were located distally. Tissue specimens were taken from the tumours and from normal colon located more than 30 cm away from the tumour, but at the same region of the colon as the tumour. Samples were fixed in 10% (v/v) buffered formalin and embedded in paraffin. Colon adenocarcinomas were classified as well, moderately and poorly differentiated, according to standard pathological criteria [14]. Serial 5-μm sections were obtained for staining with haematoxylin and eosin and for immunohistochemistry.

2.2. Immunohistochemistry and evaluation of ERβ expression

ERβ expression was detected by immunohistochemistry using the biotin-streptavidin peroxidase (B-SA) method (Biogenex kit; Biogenex Laboratories, San Ramon, CA, USA). Enzymatic pretreatment with trypsin followed by microwave irradiation in 0.01 M citric buffer (pH 6.0) was employed as the antigen retrieval method. A primary mouse monoclonal anti-ERβ antibody (anti-ERβ 14c8, Abcam, Cambridge, UK) that recognises residues 1–153 of the human ERβ N-terminus was used. At least six different ERβ splice variants exist [15], and the above antibody recognises receptor isoforms 1–3, but not ERβ4 and ERβ5. The primary antibody was diluted 1:100 with phosphate-buffered saline (PBS) and applied on tissue sections overnight at 4 °C. Sections from normal prostatic tissue were used as positive controls. Negative controls were processed by substituting the primary antibody with non-immune mouse serum.

Immunostained sections were assessed by a semi-quantitative method based on a four-level scale (Table 1). Immunopositivity was graded as negative (0), weak (+), moderate (++) or strong (+++). This scale was used by two independent pathologists to evaluate and score all sections. Specimens with inter-observer disagreement were reassessed by simultaneous examination by the two pathologists in a double-headed light microscope. In normal colon specimens, nuclear staining was assessed in the superficial epithelial cells, the epithelial cells of the crypts, the lymphocytes, the smooth muscle cells of the *muscularis mucosa* and *muscularis propria*, the vascular smooth muscle cells, the endothelial cells and the enteric neurons.

2.3. Statistical analyses

Mann–Whitney tests were employed to compare the level of ERβ expression between genders and between samples from the proximal and distal colon. ERβ expression was compared among the three tumour differentiation degrees by Kruskal–Wallis ANOVA. The association of gender with tumour differentiation and with tumour localisation was evaluated by Chi-square tests. The correlation of ERβ expression among the cell types was assessed by Kendall's t coefficient. Wilcoxon signed-rank tests were used to compare the level of ERβ

Table 1
Immunohistochemical scoring scale[a]

Score	Criterion
0	<10% of cells with nuclear staining
(+)	Weak nuclear staining intensity *OR* 10–50% of cells with nuclear staining
(++)	Moderate nuclear staining intensity *AND* >50% of cells with nuclear staining
(+++)	Strong nuclear staining intensity *AND* >50% of cells with nuclear staining

ERβ, oestrogen receptor beta.

[a] Since the primary aim of the study was to assess the level of ERβ expression, the scale was mainly based on the intensity of nuclear staining, although the percentage of ERβ-positive cells was also taken into consideration. Due to the relative shortage of data concerning ERβ expression in colon cancer, there is no consensus cut-off level to distinguish positive from negative cases. A cut-off level of 10% was employed that has also been used in the evaluation of ERβ expression in breast cancer [30] and of cyclin D1 and other markers in colon cancer [24]. Because most samples of the normal colonic wall abundantly expressed ERβ, only specimens with more than 50% of ERβ-positive cells were considered as (++) or (+++) according to the intensity of the staining. Specimens with only 10–50% of ERβ-positive cells were scored as (+).

expression among the cell types. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) 9.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Normal colonic wall

Immunohistochemical findings are summarised in Tables 2–4. ERβ immunostaining was observed in both superficial (Fig. 1b) and crypt epithelial cells of normal colonic mucosa (Fig. 1c), and was exclusively nuclear. Superficial epithelial cells were moderately or strongly positive for ERβ in 65% of women and 60% of men, and negative for ERβ in only 10% of women and 24% of men. Crypt epithelium was also immunoreactive to ERβ, showing moderate or strong positivity in 82.5% of women and 70% of men. ERβ expression was more pronounced at the base of the crypts, which harbours the proliferating zone, and lower at the maturation zone —located closer to the lumen—and at the superficial epithelium (Fig. 1c). In both genders, the intensity of nuclear ERβ immunostaining was statistically significantly higher in the crypt than in superficial epithelium ($P<0.001$).

ERβ immunoreactivity was also detected in the nuclei of the smooth muscle cells of the *muscularis mucosa* and *muscularis propria*. Although they were independently evaluated, smooth muscle cells of the *muscularis mucosa* and *propria* almost always exhibited very similar immunopositivity. By contrast, in both genders, a statistically significant difference in the intensity of staining was observed between vascular smooth muscle cells (Fig. 1d) and smooth muscle cells of *muscularis mucosa* and *propria* ($P<0.001$). Vascular smooth muscle cells were significantly more immunoreactive to ERβ, showing moderate or strong positivity in 97.5% of women and 92% of men.

Lymphocytes within the lamina propria were highly immunoreactive to ERβ (Fig. 1b). In all specimens, lymphocytes were ERβ-positive, and in 90% of women and 86% of men strong immunostaining was observed. ERβ expression was also detected in the nuclei of endothelial cells (Fig. 1e). In 87.5% of women and 84% of men, endothelial cells displayed moderate or strong positivity. Neurons of the submucosal and myenteric plexuses were also ERβ immunoreactive (Fig. 1f). Moderate or strong positivity was detected in 80% of women and 72% of men.

ERβ expression levels were not statistically significantly different between male and female subjects in any cell type. Moreover, ERβ expression levels were not different between cells from the proximal and distal colon. However, in both genders, ERβ expression was highly correlated among all cell types, and this correlation was observed regardless of the samples' localisation. The strongest correlation was observed between surface and crypt ERβ expression (Kendall's $t=0.9$, $P<0.001$). The weakest correlation was observed

Table 2
Oestrogen receptor beta (ERβ) expression in normal colonic wall in female subjects

Cell type	ERβ immunopositivity				
	Negative	Weak	Moderate	Strong	Total
Superficial epithelium	4 (10%)	10 (25%)	10 (25%)	16 (40%)	40 (100%)
Crypt epithelium	–	7 (18%)	12 (30%)	21 (53%)	40 (100%)
Lymphocytes	–	1 (3%)	3 (8%)	36 (90%)	40 (100%)
Muscularis mucosa smooth muscle cells	–	9 (23%)	17 (43%)	14 (35%)	40 (100%)
Endothelial cells	–	5 (13%)	18 (45%)	17 (43%)	40 (100%)
Vascular smooth muscle cells	–	1 (3%)	10 (25%)	29 (73%)	40 (100%)
Enteric neurons	–	8 (20%)	17 (43%)	15 (38%)	40 (100%)
Muscularis propria smooth muscle cells	1 (3%)	8 (20%)	16 (40%)	15 (38%)	40 (100%)

Table 3
Oestrogen receptor beta (ERβ) expression in normal colonic wall in male subjects

Cell type	ERβ immunopositivity				
	Negative	Weak	Moderate	Strong	Total
Superficial epithelium	12 (24%)	8 (16%)	13 (26%)	17 (34%)	50 (100%)
Crypt epithelium	5 (10%)	10 (20%)	9 (18%)	26 (52%)	50 (100%)
Lymphocytes	–	2 (4%)	5 (10%)	43 (86%)	50 (100%)
Muscularis mucosa smooth muscle cells	1 (2%)	10 (20%)	22 (44%)	17 (34%)	50 (100%)
Endothelial cells	2 (4%)	6 (12%)	15 (30%)	27 (54%)	50 (100%)
Vascular smooth muscle cells	–	4 (8%)	12 (24%)	34 (68%)	50 (100%)
Enteric neurons	–	14 (28%)	22 (44%)	14 (28%)	50 (100%)
Muscularis propria smooth muscle cells	3 (6%)	10 (20%)	21 (42%)	16 (32%)	50 (100%)

Table 4
Oestrogen receptor beta (ERβ) expression in normal superficial epithelium of proximal and distal colon in female and male subjects

Localisation of normal colon sample	ERβ immunopositivity			
	Negative	Weak	Moderate	Strong
Female patients				
Proximal colon ($n=12$)	2 (17%)	1 (8%)	4 (33%)	5 (42%)
Distal colon ($n=28$)	2 (7%)	9 (32%)	6 (21%)	11 (39%)
Male patients				
Proximal colon ($n=16$)	2 (13%)	4 (25%)	5 (31%)	5 (31%)
Distal Colon ($n=34$)	10 (29%)	4 (12%)	8 (24%)	12 (35%)

between ERβ expression in the enteric neurons and lymphocytes (Kendall's $t=0.38$, $P<0.012$).

3.2. Colon adenocarcinoma

Immunohistochemical findings are summarised in Tables 5–7 and depicted in Fig. 2. In male patients, 2% of all carcinomas exhibited strong ERβ positivity, 22% moderate positivity, 38% weak positivity and 38% were negative for ERβ. When the level of differentiation of male subjects' tumours was examined, 8% of the well differentiated carcinomas showed strong nuclear immunopositivity, 33% showed moderate positivity and 25% were negative. None of the moderately differentiated carcinomas displayed strong positivity, while 21% showed moderate positivity and 39% were negative. Moderate positivity was obtained in 10% of the poorly differentiated carcinomas, whereas 50% of them were immunonegative.

Similarly, in female patients, 3% of the colon adenocarcinomas exhibited strong ERβ positivity, 25% moderate positivity, 35% weak positivity and 38% were ERβ-negative. Among the well differentiated carcinomas, 9% demonstrated high nuclear ERβ expression, 36% displayed moderate expression and 18% were negative. 25% of the moderately differentiated carcinomas showed moderate positivity and 35% were negative. Moderate positivity was observed in 11% of the poorly differentiated carcinomas, whereas 67% of them were immunonegative.

ERβ expression level in the cancer cells was not statistically significantly different between male and female subjects ($P=0.82$). Moreover, the two genders did not differ in the frequency distribution of well, moderately and poorly differentiated adenocarcinomas ($P=0.85$). However, there was an overall difference in ERβ expression between the three differentiation levels ($P=0.014$). Well differentiated tumours displayed significantly higher ERβ immunopositivity than poorly differentiated ones ($P=0.006$). ERβ expression in moderately differentiated adenocarcinomas was intermediate between the poorly and well differentiated tumours.

In both genders, ERβ expression was significantly higher in superficial epithelial cells of the normal colon than in cancer cells ($P<0.001$). The decline in ERβ levels from normal superficial epithelium to colon cancer cells was not different between male and female patients ($P=0.2$). However, this reduction in ERβ expression was significantly correlated with the degree of tumour dedifferentiation ($P=0.001$). Specifically, poorly differentiated cancers exhibited greater loss of ERβ expression (compared with normal superficial epithelium) than moderately differentiated tumours ($P=0.008$), and moderately differentiated adenocarcinomas displayed greater reduction of ERβ levels than well differentiated tumours ($P=0.007$). The decline in ERβ expression was confirmed in sub-group comparisons that analysed tumours of the proximal and distal colon separately. The localisation of the tumours was similar in men and women ($P=1.0$). The reduction of

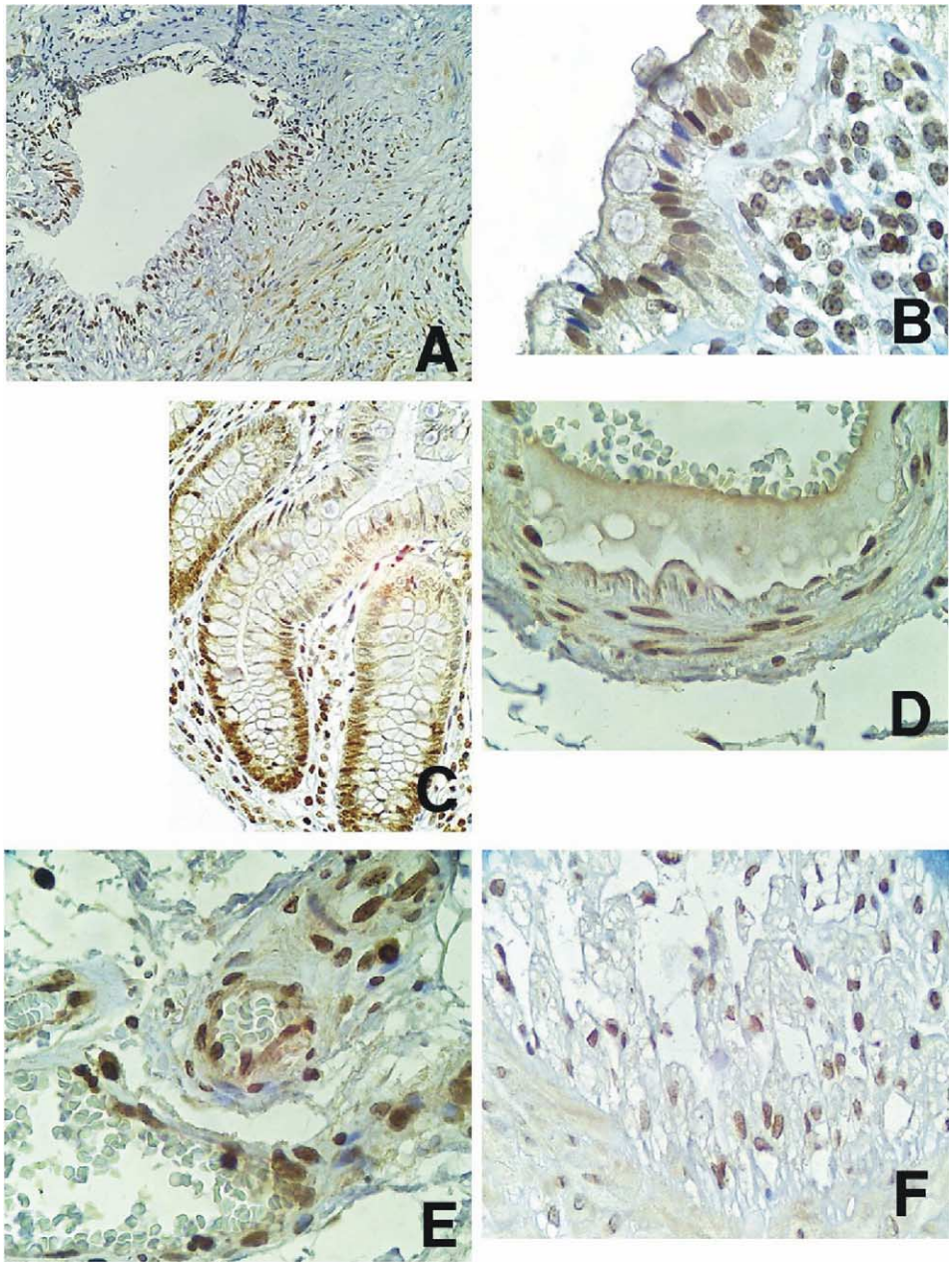

Fig. 1. Evaluation of oestrogen receptor beta (ERβ) expression in normal colonic wall (DAB, original magnification ×40): (a) positive control: nuclear ERβ expression in normal prostatic epithelium (original magnification ×10); (b) superficial epithelium and lymphocytes displaying strong immunostaining positivity (+++); (c) strong immunostaining positivity in the base of the crypts (+++) shifting to weak positivity (+) in superficial epithelium; (d) vascular smooth muscle cells exhibiting strong immunopositivity (+++); (e) endothelial cells showing strong immunopositivity (+++); (f) Auerbach's plexus showing neurons with strong immunostaining positivity (+++).

Table 5
Oestrogen receptor beta (ERβ) expression in colon adenocarcinomas in female subjects

Adenocarcinomas	ERβ immunopositivity				
	Negative	Weak	Moderate	Strong	Total
Well differentiated	2 (18%)	4 (36%)	4 (36%)	1 (9%)	11 (100%)
Moderately differentiated	7 (35%)	8 (40%)	5 (25)	–	20 (100%)
Poorly differentiated	6 (67%)	2 (22%)	1 (11%)	–	9 (100%)
Total	15 (38%)	14 (35%)	10 (25%)	1 (3%)	40 (100%)

Table 6
Oestrogen receptor beta (ERβ) expression in colon adenocarcinomas in male subjects

Adenocarcinomas	ERβ immunopositivity				
	Negative	Weak	Moderate	Strong	Total
Well differentiated	3 (25%)	4 (33%)	4 (33%)	1 (8%)	12 (100%)
Moderately differentiated	11 (39%)	11 (39%)	6 (21%)	–	28 (100%)
Poorly differentiated	5 (50%)	4 (40%)	1 (10%)	–	10 (100%)
Total	19 (38%)	19 (38%)	11 (22%)	1 (2%)	50 (100%)

Table 7
Oestrogen receptor beta (ERβ) expression in adenocarcinomas of proximal and distal colon in female and male subjects

Tumour localisation	ERβ immunopositivity			
	Negative	Weak	Moderate	Strong
Female patients				
Proximal colon ($n=12$)	4 (33%)	5 (42%)	3 (25%)	–
Distal colon ($n=28$)	11 (39%)	9 (32%)	7 (25%)	1 (4%)
Male patients				
Proximal colon ($n=16$)	4 (25%)	7 (44%)	5 (31%)	–
Distal colon ($n=34$)	15 (44%)	12 (35%)	6 (18%)	1 (3%)

ERβ expression in the tumour cells compared with normal epithelium was significantly correlated with the degree of tumour dedifferentiation in cancers of the proximal colon ($n=28$, $P=0.026$), as well as in cancers of the distal colon ($n=62$, $P=0.001$).

In addition to nuclear immunostaining—wherever it was present—colon adenocarcinomas almost invariably demonstrated cytoplasmic ERβ immunostaining, regardless of the subject's gender and the degree of tumour differentiation. In contrast, dysplastic cells located at the transition zone between the normal epithelium and the adenocarcinoma, showed exclusively nuclear ERβ immunoexpression (Fig. 2a).

4. Discussion

Accumulated epidemiological data suggest a protective effect of oestrogen against colon cancer. Age-adjusted colon cancer incidence rates are lower in women than men [7], ERT and parity are inversely associated with the disease [8,9] and the lifetime risk of colon cancer is significantly lower in female than male members of families with hereditary non-polyposis colorectal cancer (HNPCC) [16]. Since ERα is reported to be minimally expressed in normal colon mucosa and colon cancer cells [10,11], the effects of oestrogen on colon cancer susceptibility may be mediated by ERβ. The present retrospective immunohistochemical study demonstrates that ERβ is highly expressed in superficial and crypt epithelium of the normal colon in both genders. ERβ expression was highly correlated among all cell types in both genders, and the strongest correlation was observed between surface and crypt ERβ expression. This finding suggests that there may be an inter-subject difference in ERβ expression that is manifested in all cell types.

ERβ expression was significantly lower in colon cancer cells compared with normal colonic epithelium and there was a progressive decline in ERβ expression that paralleled the loss of cancer cell differentiation. The present findings are consonant with previous results

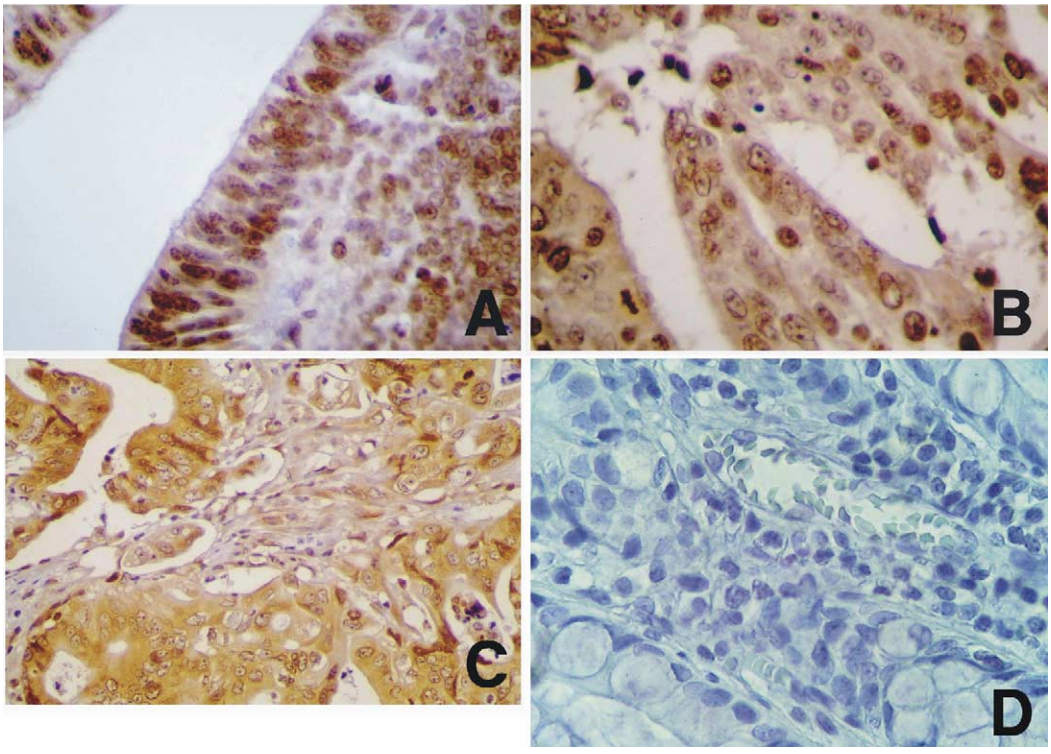

Fig. 2. Evaluation of oestrogen receptor beta (ERβ) expression in colon adenocarcinomas (DAB, original magnification ×40): (a) transition zone between normal colonic epithelium and well differentiated adenocarcinoma exhibiting moderate nuclear immunopositivity (++); (b) moderately differentiated adenocarcinoma displaying weak nuclear immunopositivity (+) and cytoplasmic staining; (c) poorly differentiated adenocarcinoma demonstrating solely cytoplasmic ERβ immunostaining; (d) negative control (no anti-ERβ antibody added).

reported by Foley and colleagues [12], who also detected a loss of ERβ protein expression in malignant colon tissue by western immunoblotting. Campbell-Thompson and colleagues [11] demonstrated a loss of ERβ1 and ERβ2 mRNA expression in adenocarcinomas of female subjects. Another immunohistochemical study of ERβ in 55 patients with colorectal adenocarcinomas showed that 32% of all tumours in both genders were ERβ-negative; the 10% cut-off threshold was used to distinguish ERβ-positive from negative tumours [13]. The present study, utilising the same cut-off value, showed that 38% of adenocarcinomas in male and female patients were ERβ-negative.

The cytoplasmic ERβ immunostaining that was almost invariably detected in colon adenocarcinomas may reflect either alternative processing of ERβ or expression of a different receptor isoform. At least six different ERβ isoforms exist [15] and the monoclonal antibody employed in this study recognises isoforms 1–3. Thus far, no immunohistochemical study of ERβ has examined the remaining isoforms, whose role remains unknown, not only in colon, but also in other tissues. One study reported that only ERβ1, ERβ2 and very low levels of ERβ5 are detectable in normal colon by comparative RT-PCR [11]. Nevertheless, the use of additional, isoform-specific antibodies in future studies would allow more detailed conclusions to be drawn about the role of ERβ isoforms in colon carcinogenesis.

A progressive loss of ERβ expression during the process of carcinogenesis has also been documented in prostate [6] and breast cancers [5], suggesting a role for ERβ as a potential inhibitor of cellular proliferation and/or transformation. However, the mechanisms whereby ERβ protects against colon cancer are only just beginning to be deciphered. At the breast, ERβ protects against the mitogenic activity of oestrogens by modulating the proliferating effects of ERα. In normal colon mucosa, where ERα expression is low, other mechanisms must be involved.

Several studies have implicated the AP-1 transcription factor in colorectal carcinogenesis. Bile acids, which are known to promote colon cancer, trigger AP-1 activation [17] and inhibitors of Jun–Fos dimer action reportedly suppress colon cancer cell growth *in vitro* [18]. Induction of the activity of the AP-1 upstream extracellular signal-regulated kinases (ERKs) and c-Jun N-terminal kinases (JNKs) has also been documented in human colorectal carcinomas [19]. When ERβ binds 17β-oestradiol, AP-1-mediated transcription is repressed, whereas ERα in the presence of oestrogen induces target gene expression [3]. These data, taken together with the progressive loss of ERβ expression in colon cancer, suggest that ERβ may protect against carcinogenesis by suppressing AP-1-mediated gene transcription.

It is well documented that different genetic pathways are implicated in the carcinogenic process of colon tumours that are proximal or distal to the mid-transverse colon. Mutations of the tumour suppressor gene *p53* are predominantly found in cancers of the distal colon, whereas proximal tumours display a much higher frequency of microsatellite instability (MSI)-positive phenotype [20]. Our finding that the decline in ERβ expression parallels the tumour's dedifferentiation was valid for tumours of both the proximal and distal colon, but with varying statistical significance. However, at each level of tumour differentiation, cancers of the distal and proximal colon displayed a similar degree of loss of ERβ expression. Therefore, the difference in the magnitude of statistical significance should be attributed to the corresponding difference in the sample sizes of the proximal and distal colon cancers, and should not be regarded as evidence for a different role for the loss of ERβ expression in the carcinogenesis of proximal and distal colon tumours. Thus, the results of the present study suggest a protective role for ERβ in colon carcinogenesis that may be pertinent to both tumour localisations.

Available data strongly implicate ERβ in the p53/$p21^{WAF1/CIP1}$ cascade that commonly mediates carcinogenesis of the distal colon. The cytostatic effect of p53 is funnelled by transcriptional activation of the cyclin-dependent kinase (CDK) inhibitor, $p21^{WAF1/CIP1}$ [21]. $p21^{WAF1/CIP1}$ inhibits cyclin/CDK complexes and thus abrogates the phosphorylation of pRb (retinoblastoma protein) and prevents the cell-cycle transition from G_1 into S phase [22]. Reduction of $p21^{WAF1/CIP1}$ expression has been documented in colon adenocarcinomas, especially in tumours bearing *p53* mutations [23,24]. Genistein, a soy metabolite that is a selective agonist of ERβ, has been shown to induce $p21^{WAF1/CIP1}$ expression in various human cancers including colorectal carcinoma, thus leading to cell-cycle arrest [25,26]. In addition, ERβ interferes with this pathway of carcinogenesis by inhibiting *cyclin D1* gene transcription. Oestrogens activate *cyclin D1* gene expression through ERα, but inhibit *cyclin D1* gene transcription through ERβ [27]. The *cyclin D1* promoter does not harbour an ERE and its transcriptional repression is mediated through AP-1 and CRE sites. *Cyclin D1* is not expressed in normal colorectal tissue, but deregulated expression of *cyclin D1* is well documented in colorectal cancers [24,28]. Up to 55% of colon tumours exhibit nuclear expression of cyclin D1, and this finding is associated with a poor tumour differentiation.

However, there is a lack of evidence directly linking ERβ with the MSI-positive tumour phenotype. Strong epidemiological data suggest that oestrogens, whether endogenous or exogenous, prevent MSI-positive colon tumours [29]. A large population-based case–control study of patients with colon cancer revealed that women were less likely than men to have MSI-positive tumours at a younger age, and that ERT, oral contraceptives and pregnancy were associated with a reduced risk of MSI-

positive tumours [29]. In HNPCC nearly all colon cancers are MSI-positive; female patients have a significantly lower risk of developing colonic adenomas and adenocarcinomas than their male relatives [16]. The exact mechanism of this oestrogenic effect is presently unknown. Because the expression of ERα in normal and malignant colon is minimal, our data regarding the progressive loss of ERβ in tumours of the proximal colon suggest that the decline of ERβ expression may also underlie the pathogenesis and progression of these tumours.

In conclusion, ERβ is highly expressed in normal colonic mucosa, in vascular smooth muscle and endothelial cells, in enteric neurons, in lymphocytes and in smooth muscle cells of the *muscularis mucosa* and *propria*. Colon adenocarcinoma cells display significantly lower ERβ expression, which parallels the loss of their differentiation. Cancers of the proximal and distal colon may have different underlying mechanisms, but the decline in ERβ expression is independent of the tumour's localisation. Because ERβ is the dominant ER in normal colonic mucosa, these data suggest an instrumental role for ERβ in preventing the malignant transformation of colonic epithelial cells and selective ERβ agonists may become important in colon cancer chemoprevention.

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