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ORIGINAL ARTICLE

Expression and Prognostic Value of Oestrogen Receptor Beta in Colorectal Cancer

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Abstract Differences between men and women in the incidence and biological mechanisms of colorectal cancer (CRC) suggest that estrogens may play a role in the pathogenesis of this disease. The identification of the human estrogen receptor beta (ERβ) and its expression in the intestinal mucosa led to further studies that revealed that estrogens have a protective function against CRC mediated by the activation of ERβ. However, ERβ expression and its role in CRC is controversial. The purpose of this study was to determine the distribution and prognostic value of ERβ expression in the intestinal mucosa of patients diagnosed and surgically treated for CRC, and its association with other known prognostic factors. A total of 109 paraffin-embedded samples of the wild-type ERβ isoform were analyzed by immunohistochemical nuclear staining in patients with colorectal adenocarcinoma. Clinical/pathological and survival data were collected. Immunohistochemical quantification was performed using the category scoring system, which has been validated for assessing estrogen receptor alfa. The wild-type ERβ isoform –also called ERβ1– was positive in 101 patients (92.7%) and negative in nine patients (7.3%). Univariate analysis revealed that the absence of expression of the ERβ1 gene was correlated with mucinous adenocarcinoma ($p < 0.05$). Also, a non-significant tendency was observed for ERβ expression to be down-regulated in advanced tumors. With a median follow-up of 47 months, the overall survival and progression-free survival were not found to be associated with ERβ1 expression ($p = 0.2$). Although the wild-type ERβ isoform was expressed in most study patients with colorectal cancer, it does not seem to have any prognostic value for the course of the disease. Further studies should be conducted to investigate whether the down-regulation of ERβ expression has any biological function in mucinous colorectal cancer.

Keywords Colorectal cancer · Prognostic factor · Estrogen receptor beta · Wild-type isoform ERβ1

Background

Colorectal cancer (CRC) is the third most common type of cancer and the second leading cause of cancer-related death in western countries both in men and women [1, 2]. In the recent years, a slight increase has been observed in the incidence of CRC in women [3], although it is still more prevalent in men (1.5:1 ratio). The oncogenic effect of estrogens has been associated with differences between sexes in the incidence of a large number of neoplasms such as breast cancer, where estrogen receptor alfa modulators are essential for the management of this type of tumors. The role that estrogens may play in the pathogenesis of CRC is mediated by their binding an estrogen receptor identified in the 60s and known as "estrogen receptor beta" (ERβ) [4, 5]. Some studies performed in the past revealed that it is the ERβ gene rather than the ER alfa gene which is more highly expressed in the intestinal mucosa.

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Also, it has been demonstrated that, unlike in breast cancer, ERβ expression has a protective effect [6]. This evidence led to further studies that investigated the role of ERβ in intestinal carcinogenesis.

Some studies in vitro have demonstrated that the presence of ERβ in intestinal crypts seems to have a protective effect against the development of cancer. Thus, the down-regulation of ERβ expression in the intestinal mucosa is associated with the development of CRC [7]. Barone M. et al. performed a study where male ApcMin/+ mice received a combination of the ERβ-selective agonist silymarin and/or lignin and observed that the resulting up-regulation of ERβ expression counteracted the development of CRC [8]. Additionally, prospective epidemiological studies have demonstrated that estrogen replacement therapy plays a protective role against the development of CRC in postmenopausal women [9, 10]; specifically, the risk for CRC was observed to decrease when ERβ was expressed in the tumor [11].

There is evidence that a relationship exists between low expression of ERβ, advanced stages of the tumor and lower survival rates [12, 13]. Therefore, it has been suggested that ERβ may have a potential prognostic value for CRC. However, the only prognostic tool currently available is based on the TNM classification proposed by the American Joint Committee of Cancer [14]. Nevertheless, this staging system does not reveal either the biological profile of the underlying tumor or the molecular heterogeneity associated with CRC. This added to the scarce evidence available on the prognostic value of ERβ support the performance of our study.

The purpose of this study was to determine the distribution and prognostic value of ER beta expression in the intestinal mucosa of patients diagnosed with and surgically treated for CRC and investigate its association with other known prognostic factors.

Methods

Study Design and Patient Selection

This is a retrospective cohort study involving patients who received the same treatment and were monitored for a minimum follow-up of at least three years. This study was performed in compliance with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants. This study was approved by the Ethics Committee of the Virgen de la Victoria Hospital, Málaga, Spain, where the study was performed.

The study involved patients >18 years-old diagnosed with colon or rectal carcinoma of any stage undergoing radical surgery of the primary tumor *(en bloc* resection plus lymphadenectomy). We excluded patients who had undergone other histological tests, had received neoadjuvant therapy such as chemotherapy –or radiotherapy in the case of rectal neoplasms– or had a history of other tumors (except for non-melanoma skin cancer).

All patients with high-risk stage II or III cancer received adjuvant chemotherapy with fluoropyrimidines in combination with oxaliplatin. Patients with rectal tumors received adjuvant radiotherapy with fluoropyrimidines. Patients were considered to have high-risk stage II colon cancer if they met some of the following criteria: having less than 12 lymph nodes analyzed; poorly differentiated; lymph node, vascular or perineural involvement; tumor presentation with perforation or obstruction; or stage T4 cancer.

Variables Analyzed

ERβ expression in the primary tumor was measured by immunohistochemistry (IHC). The following demographic and clinical data were collected: age, sex, tumor site, histology, cancer stage at diagnosis (as determined by the 6th Edition of the AJCC) [14], date of diagnosis and treatment administered for local and metastatic disease. Other data were gathered such as whether or not the patient had undergone surgery for their metastatic disease and the antiangiogenic or anti-EFGR treatment received (epidermal growth factor receptor). Overall survival (OS) –defined as the time from diagnosis to death– was calculated according to the patient status and date of last follow-up visit. As to patients with a no metastatic disease, other data–such as the date when disease progression was observed– was collected in order to calculate disease-free survival (DFS)–defined as the time from diagnosis to first relapse.

ERβ Analysis

ERβ expression levels were measured by immunohistochemistry, since it is the technique most widely employed in recent studies as compared to RT-PCR [12, 13, 15, 16].

For IHC, we used an antibody from Serotec®, clona PPG5/10 which recognizes the isoform 1 or wild-type of ERβ (ERβ1). Nuclear expression was assessed because no cytoplasmatic immunostaining was seen for the isoforms in other study [14–20].

The procedure was as follows:

Formalin-fixed, paraffin-embedded tissue sections (3 micres) were obtained from 109 primary colorectal cancer specimens (all samples submitted for the assays were obtained from the primary lesions). After routine deparaffinization in xylene, the sections were hydrated through a series of graded alcohols, distilled water, and phosphate-buffered saline (PBS) at pH 7.2–7.4. Antigen retrieval was performed using Tris–EDTA (pH 9). The slides were put in DAKO autostainer PLUS which performed the following steps:

- Incubation in 3% H2O2 for 5 min.
- Incubation over night with monoclonal rabbit anti-human estrogen receptor ß diluted 1:5.
- Applying the EnVision PT-Link optimized for DAKO cytomation automated systems for 20 min.
- Applying 3,3′-di-amino-benzidine tetrahydrochloride as chromogen for 5 min
- Rinsing well in distilled water for 5 min.

The slides in the autostainer were removed and hematoxylin counterstaining was performed. Slides were dehydrated in ascending grades of alcohol and were cleared in xylene for three changes and cover slips were applied. Sections from normal ovarian tissue were used as positive controls. Negative controls were processed by substituting the primary antibody with non-immune mouse serum.

The REβ stained sections were assessed by two observers each using the category scoring system, which has been well validated for assessing estrogen receptor alfa IHQ [17] and takes into account intensity of staining (1 = weak; 2 = moderate; 3 = strong) and proportion of positively stained tumor cells (1 = 0–1%; 2 = 2–10%; 3 = 11–33%; 4 = 34–67%; 5 = 68–100%). Addition of the intensity and proportion scores provided the final category score used for further analysis**.** To allow comparisons with previous immunohistochemical studies of REβ in CRC negative expression of ERβ1 was defined as final score less than or equal to 3 and positive if it was 4 or more.

Given that the objective of our study was to determine ERβ1 expression in the mucosa of CRC and its prognostic value, ERβ1 expression was not analyzed in normal tissue. In addition, ERβ1 is known to be highly expressed in normal tissue of the intestinal mucosa [15, 18].

Statistical Analysis

Descriptive analysis of study variables was performed. Qualitative variables are grouped by frequency distribution. Quantitative variables are expressed as central tendency, position and dispersion. Univariate analysis was performed using ERβ1 and mortality from disease as dependent variables. Qualitative variables were compared by Chi-squared test or Fisher's exact test (for observations expected to be <5), and quantitative variables were compared using U Mann-Whitney test (two categories) or Kruskal-Wallis test (three or more categories). Finally, disease-free survival and overall survival were calculated by the Kaplan-Meier test. Differences between groups were evaluated by the long-rank test. Multivariate analysis of the influence of different factors on survival was performed using the Cox regression model (Hazard Ratio was included with a 95% confidence interval). Survival functions were represented for both analyses. A $p < 0.05$ difference was considered statistically significant. Statistical analysis was performed using SPSS v.15 software.

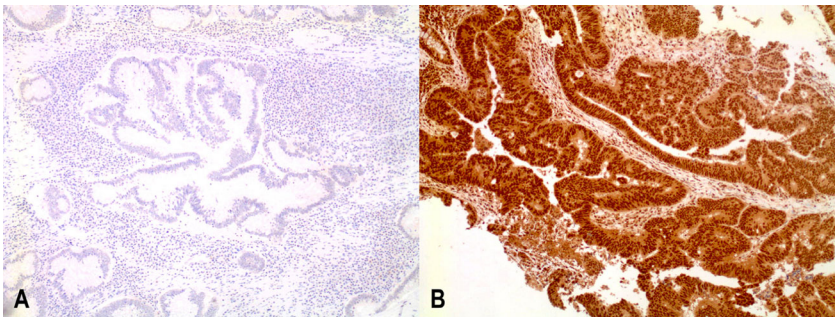
Results and Discussion

Results

Of the 120 patients initially recruited, four refused to participate and seven did not meet the inclusion criteria.

Table 1 Clinico-pathological characteristics of the study cohort ($n = 109$)

Age	Median: 66 years (R: 57–73)
Sex	
-Man	60 (55%)
-Woman	49 (45%)
Stage	
-Stage I	12 (11%)
-Stage IIA	26 (23.9%)
-Stage IIB	7 (6.4%)
-Stage IIIA	4 (3.7%)
-Stage IIIB	24 (22%)
-Stage IIIC	12 (11%)
-Stage IV	24 (22%)
Histology	
-Adenocarcinomas	98 (89.9%)
-Mucinous adenocarcinoma	11 (10.1%)
Stage–T-	
-Tx	1 (0.9%)
-T1	3 (2.8%)
-T2	15 (13.7%)
-T3	64 (58.7%)
-T4	26 (23.9%)
Stage–N-	
-Nx	3 (2.8%)
-N0	52 (47.6%)
-N1	32 (29.4%)
-N2	22 (20.2%)
Stage–M-	
-M0	85 (78%)
-M1	24 (22%)
Histological Grade	
-Grade I	75 (68.8%)
-Grade II	26 (23.9%)
-Grade III	7 (6.4%)
-Unknown	1 (0.9%)
Site	
-Right (ascending) colon	32 (29.3%)
-Left (descending) colon	55 (50. 5%)
-Rectum	22 (20.2%)



A Negative expression of ERβ1
B Positive expression of ERβ1

Fig. 1 **a**. Negative expression of ERβ1. **b**. Positive expression of ERβ1

Therefore, 109 patients with stage I-IV CRC who were initially treated with radical surgery of the primary tumor between 2004 and 2008 were finally included in the study. The number of male patients included was slightly higher than that of women and the mean age was 66 years. Most tumors (78%) were diagnosed at initial stage of development. The most frequent histology was non-mucinous adenocarcinoma. Up to 58.7% of patients had T3 cancer and 48% had not lymph node involvement (N0). In total, 70% of patients had left-sided lesions (left colon/rectum), and the most frequent histological grade was grade I (68.8%) followed by grade II (23.9%) (see Table 1). ERβ1 was expressed in 92.1% (n = 101) of tumors, whereas 7.9% of cases were negative (n = 8) (Fig. 1).

The distribution of ERβ1 expression according to the clinical variables was similar in both sexes. ERβ1 was not expressed mostly in advanced-stage tumors (IIIB-IV), although differences were not statistically significant. Similarly, ERβ1 expression was not found to be associated with extent of tumor differentiation or tumor site (Table 2). Conversely, a statistically significant relationship was observed between loss of ERβ1 expression and histological type (p = 0.03).

During a median follow-up of 47 months (range: 25–61), 24 (28.3%) local and/or distant relapses were observed. Also, 30 patients died (27.5%) because of metastatic disease.

Disease-free survival (DFS) was significantly associated with the depth of bowel wall tumor infiltration (Stage T, p = 0.05) and regional lymph node involvement (stage N, p = 0.013). Conversely, DFS was not found to be correlated with other clinical variables or with ERβ1 expression (Table 3).

A statistically significant relationship was observed between overall survival (OS) and stage (p = 0.0001), stage T (0.001), stage N (p = 0.0001) and the presence or absence of metastasis (p = 0.0001). Also, OS was poorer in patients with mucinous adenocarcinoma, as compared to patients with non-mucinous adenocarcinomas, with differences almost reaching statistical significance (p = 0.07).

OS was higher in ERβ1-positive patients as compared to ERβ1-negative patients. Differences in survival between ERβ1-positive and ERβ1-negative patients ranged from 76 to 40 months for DFS (p = 0.3) and from 73 to 63 months for OS (p = 0.2) (Fig. 2) (Table 3) .

Multivariate analysis revealed that only tumor stage was significantly related to DFS and OS (Table 4).

Discussion

This study sheds light on the prognostic value of ERβ1 in CRC. The main findings of this study are: A) ERβ1 is expressed in most patients with CRC, which is consistent with the literature. B) ERβ1 is less frequently expressed in patients with mucinous adenocarcinoma. C) ERβ1 is less frequently expressed in patients with advanced tumors (differences being not statistically significant). D) ERβ expression seems to have a limited prognostic value in CRC.

The ERβ1 levels observed in our study are consistent with the maximum levels (the interval ranging from 57.5% to 89.4%) reported in other case-series studies using the same score for ERβ1 quantification but different antibodies. Thus, on the one hand, Fang et al. [13] and Xie et al. –who employed antibodies against all ERβ isoforms– [18] found that ERβ was expressed in 67.7% and 57.5% of patients, respectively. On the other hand, Elbanna et al. [16] and Grivas et al. –who, as in our study, used antibodies against the ERβ isoform 1– [15] observed that ERβ was expressed in 65% and 84.9% of patients, respectively. Although the evidence available is not conclusive, the five ERβ isoforms identified so far in CRC seem not to have the same mechanism of action. Thus, the ERβ isoform 1 is the most frequent ERβ

Table 2 Relationship between ERβ1 expression and the clinico-pathological characteristics of the cohort

Total	ERβ1-Positive 101 (92.7%)	ERβ1-Negative 8 (7.2%)	*P*
Sex			
-Man	56 (93.4%)	4 (6.6%)	0.28
-Woman	45 (91.8%)	4 (8.2%)	
Stage			
-Stage I	12 (100%)	0 (0%)	0.57
-Stage IIA	25(96.2%)	1 (3.8%)	
-Stage IIB	7 (100%)	0 (0%)	
-Stage IIIA	4 (100%)	0(0%)	
-Stage IIIB	22 (91.7%)	2 (8.3%)	
-Stage IIIC	10 (83.3%)	2 (16.7%)	
-Stage IV	21 (87.5%)	3 (12.5%)	
Histology			
-Adenocarcinoma	93 (94.9%)	5 (5.1%)	0.03
-Mucinous adenocarcinoma	8 (72.7%)	3 (27.3%)	
Stage–T-			
-Tx	1 (100%)	0 (0%)	0.8
-T1	3 (100%)	0(0%)	
-T2	14 (93.3%)	1 (6.7%)	
-T3	58 (90.6%)	6 (9.4%)	
-T4	25 (96.2%)	1(3.8%)	
Stage–N-			
-Nx	3 (100%)	0 (0%)	0.4
-N0	50 (96.2%)	2 (3.8%)	
-N1	29 (90.6%)	3 (9.4%)	
-N2	19 (86.4%)	3 (13.6%)	
Stage–M-			
-M0	79 (92.9%)	6 (7.1%)	0.3
-M1	22 (91.7%)	2 (8.3%)	
Histological Grade			
-Grade I	68(90.7%)	7 (9.3%)	0.2
-Grade II	26(100%)	0(0%)	
-Grade III	6 (85.7%)	1 (14.3%)	
-Unknown	1 (100%)	0 (0%)	
Site			
-Right (ascending) colon	28 (87.5%)	4 (12.5%)	0.92
-Left (descending) colon	53 (96.4%)	2 (3.6%)	
-Rectum	20 (90.9%)	2 (9.1%)	

Table 3 Relationship between ERβ1 and SLE and OS

	ERβ-Positive	ERβ-Negative
n (%)	101 (92.7)	8 (7.3)
95%CI	*87.3–98*	*2–12.7*
n (%) relapse*	22 (27.5)	2 (40.0)
DFS** [median]	76	40
n (%) deaths	27 (26.7)	3 (37.5)
OS*** [median]	73	63

*85 patient values for relapse

**DFS: Disease-Free Survival

***OS: overall survival (months)

isoform in CRC and might have a protective role in the intestinal mucosa [19].

In other studies where a different quantification score was used, ERβ was not expressed in a higher proportion of patients, as compared to our study. Such is the case of the study by Jassam et al. (12), where 21% of patients of their series of 91 patients were negative for ERβ. Conversely, Taggarshe D. et al.(20), Rudolph A et al. [21] and Rath-Wolfon L et al. [22] used the score proposed by Konstantinopoulos in 2003 [23], which establishes three levels of ERβ expression: negative, moderate and high. Thus, ERβ expression was found to be moderate to high in 52.4% of the 1101 CRC patients included in the study conducted by Rudolph A. et al. [21] vs. 63.4% of the 72 patients included in the study by Taggarshe D. et al. [20]. In the latter, ERβ was detected by IHC using an antibody that only detected ERβ isoform 1.

In our study, we also assessed the potential association between ERβ expression and the prognostic factors traditionally used. As to ERβ expression by stage –T or N–, we observed that most ERβ-negative patients had T3–4 or N+ cancer. Multivariate analysis, however, revealed that ERβ expression and tumor stage were not correlated. No correlation was found either in our study between ERβ1 expression and metastasis. As to the literature, ERβ1 expression was determined by IHC by Grivas et al. [15] in a cohort of 113 patients, and no significant differences were found between ERβ1 expression and the prognostic factors T, N or M. As mentioned above, although these authors used the same score to quantify ERβ1 levels, they used an antibody that was sensitive to all ERβ1 isoforms. Fang et al. [13] determined the expression of all ERβ isoforms using the same score as in our study. All 423 patients included in this study had stage I-III tumors (IV-stage tumors were excluded). A statistically significant difference was found between ERβ expression and lymph node involvement, but not between ERβ expression and TNM. Conversely, Castiglione et al. [24] demonstrated that the expression levels of the ERβ isoforms 1, 2 and 5 was down-regulated in advanced tumors as assessed by RT-PCR, and differences were just below significance. Rudolph A et al. [21] observed that the loss of ERβ expression was associated with increased tumor extention (stage T) and advanced stages, along with a higher risk of

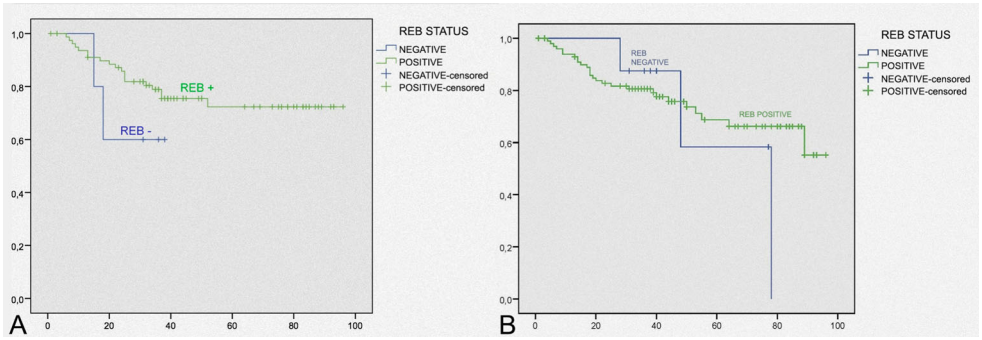


Fig. 2 **a**. Expression of ERβ1 and probability of relapse. **b**. Expression of ERβ1 and probability of survival

A Expression of ERβ1 and probability of relapse
B Expression of ERβ1 and probability of survival

recurrence and death, which is not supported by the results obtained by Taggarshe D. et al. [20] (Table 5).

On univariate analysis, differences in ERβ expression by sex were slight and non-significant. Gender and ERβ expression were not found to be correlated in previous studies [13, 15, 18], except for the study by Jassam et al. [12], who observed a significant loss of ERβ expression in female patients with rectal cancer. Also, Taggarshe D et al. [20] and Campbell-Thompson et al. [19] found that female patients presented lower ERβ expression levels as compared to males. Concerning the remaining pathological variables analyzed in our study (degree of differentiation, tumor site and histological type), we only found significant differences between loss of ERβ expression and the mucinous histological type. Among the studies mentioned above, the one conducted by Elbanna et al. [16] revealed that the down-regulation of ERβ expression was associated with a loss of tumor differentiation, which is not supported by the results obtained in our study. In their study, Wong et al. [25] demonstrated that ERβ expression levels were higher in low-grade non-mucinous tumors, which is not consistent with the results obtained in our study.

For the primary endpoint of the study –the prognostic value of ERβ1 in CRC– we selected an homogenous sample of patients, as follows: patients treated from 2004 with combination therapies with fluoropyrimidines and oxaliplatin monitored for a minimum follow-up of 3 years. DFS and OS were higher in ERβ1-positive patients, as compared to ERβ1-negative patients, although differences were not statistically significant.

Regarding other studies, Fang et al. [13] found that OS and DFS were higher in patients with higher ERβ1 expression levels. Although the type of treatment administered (either adjuvant or for disseminated disease) was not specified in the study, the proportion of patients with T1–3 N0 cancer with a good prognosis was very high. Consistently, Elbanna et al. [16] reported higher OS rates for ERβ1-positive patients, although differences were not statistically significant. It should be noted that this study involved only 40 patients with all-stage tumors (30% were IV-stage tumors) and the duration of follow-up was only 2 years. Conversely, Grivas et al. [15] quantified ERβ1 levels in a cohort of 113 patients, but their results do not match ours. Thus, they report lower progression-free survival rates for ERβ1-positive patients. Nevertheless, it is worth mentioning that patients prevailingly had stage III cancer and 6% had stage VI cancer. Finally, the study conducted by Rudolph A et al. [21] –which involved all-stage cancer patients– reported that a correlation exists between down-regulated ERβ1 expression and lower OS and DFS rates.

Table 4 Cox Regression

Cox Regression - Dependent variable: disease-free survival				
	p	HR	CI95% lower	CI95% upper
Stage				
I - IIA	0.03	1.00		
IIB - IIIC		2.78	1.10	7.04
Cox Regression - Dependent variable: overall survival				
	p	HR	Lower CI95%	Upper CI95%
Stage				
I - IIA	<0.01	1.00		
IIB - IIIC		3.21	0.86	12.01
IV		17.28	5.03	59.38

Conclusions

In light of the encouraging results obtained in our study and supported by the literature, further studies should be conducted to assess how ERβ expression influences the

Table 5 Review of studies

Study And Year	n	Stage	Score IHC	ERβ Antibody	Relationship With Clinicopathological Variables	Correlation With OS Or DFS
Konstantinopoulos et al.(2003) [23]	90	No date	**0** < 10% tumor cell was 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	Isoforms 1,2 and 3	↑ REβ in well differenciated tumors	No date
Xie et al. (2004) [18]	40	Duke A-B (62.5-%) Duke C (17.5-%) Duke D (7.5%) Unknown (12.5-%)	Positive >10% of cancer cells stained for REβ	All ERβ iso-forms	Age, sex, N, Duke's type, histological grading were not significant.	No date
Wong et al.(2005) [25]	91	pT1–3 (85.7-%) pT4 (14.3-%) N0 (33%) N+ (67%) M+ (0%)	Positive >10% of cancer cells stained for REβ	IsoformEs 1,2 and 5	↑ ERβ1 and lower pT and mucinous ↑ REβ2 in right-sided carci-nomas and N+ No relation with age.	No date
Jassam et al.(2005) [12]	91	Duke A-B (45%) Duke C (47.3-%) Duke D (7.7%)	Score = Staining intensity (0–3) + Proportion of positively stained (0–5) Positive = Score 4 o more	All ERβ iso-forms	↓ REβ if increased Duke's stage ↓ REβ in left site ↓ REβ in female No relationship with age.	No date
Castiglione et al.(2008) [24]	40	Duke A-B (55%) Duke C (32.5-%) Duke D (12.5-%)	No IHC. It was used PCR	Isoforms 1,2 and 5	> REβ in Duke A-B than Duke C-D but $p = 0.06$	No date
Grivas et al.(2009) [15]	113	Stage I-II (38.9-%) Stage III (55.8-%) Stage IV (5.3%)	Positive >10% of cancer cells strong stained for REβ	ERβ1	No correlation with age, stage, grade, gender, site, histological type or T,N,M	No correlaTion with OS ↑ ERβ1 correlation with ↓DFS
Fang et al.(2010) [13]	423	Stage I-III	Score = Staining intensity (0–3) + Proportion of positively stained (0–3)	All ERβ iso-forms	Correlation with N stage	↓ REβ in patient with ↓ OS

Table 5 (continued)

Study And Year	n	Stage	Score IHC	ERβ Antibody	Relationship With Clinicopathological Variables	Correlation With OS Or DFS
Elbanna et al.(2012) [16]	40	Stage I-II (40%) Stage III (30%) Stage IV (30%)	Positive >10% of cancer cells stained for REβ	ERβ1	↓ ERβ1 in vascular invasiOn and high-grade tumors	↓ ERβ1 in patient with ↓ OS but the correlation was no significant
Rudolph et al. (2012) [21]	1101	Stage I-II (52.2-%) Stage III (33.8-%) Stage IV (14%)	Equal that Konstantinopoulos et al.	All ERβ iso-forms	↓ REβ in higher stage and greater pT	↓ REβ associated with ↓ OS and DFS
Taggarshe et al.(2012) [20]	72	Stage I-II (27.8-%) Stage III (38.9-%) Stage IV (13.9-%) Unknown (19.4-%)	Score = Staining intensity (1–3) x Percentage of positively stained	All ERβ iso-forms	↓ REβ in women	No correlation with OS and DFS
Rath-Wolfson et al. (2012) [22]	55	Stage II-III (44%) Stage IV (55%)	Score = Staining intensity (1–3) x Percentage of positively stained	ERβ1	↑ ERβ1 in patient with M+ than M-	↑ ERβ1 in patients dead than alive
Pérez-Ruiz et al.(2015)	109	Stage I-II (41.3-%) Stage III (36.7-%) Stage IV (22%)	Score = Staining intensity (0–3) + Proportion of positively stained (0–5) Positive = Score 3 o more	ERβ1	↓ ERβ1 in mucinous tumors No correlation with age, stage, grade, gender, site, or pT,N,M	↓ ERβ1 in patient with ↓ OS and DFS but the correlation was no significant

n: number of patients**; IHC:** immunohistochemical analysis**; REβ:** estrogen receptor beta**; OS:** overall survival**; DFS:** disease free-survival**; PCR:** polimerase chain reaction**; pT:** tumor extent**; N:** lymph node**; M:** metastasis**;**

biology and natural course of colorectal cancer. The first step should be to validate the different antibodies used to quantify ERβ expression and establish the appropriate cut-off point for determining that a patient is positive or negative to ERβ.

In agreement with other studies, we observed that the wild-type ERβ isoform was expressed in most CRC patients. Although we observed that OS was higher in ERβ-positive patients, differences were not significant, which suggests that ERβ has a limited prognostic value in CRC. It could be interesting to study the relationshipt of ERβ and others drive-mutation as RAS in colorectal cancer because our study and other demostrated that ERβ is lossed in advanced cancer and it could indicated that ERβ is a step in a signaling pathway. Their presence in normal mucose could have taken into account in a preventive options.

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Compliance with Ethical Standards This study was performed in compliance with the principles of the Declaration of Helsinki (Seul 2008). Informed consent was obtained from all participants. This study

was approved by the Ethics Committee of the Virgen de la Victoria Hospital, Malaga, Spain, where the study was performed.

Conflict of Interests The authors have no conflict of interest.

References

1. Siegel R, Naishadham D, Jemal A (2013) Cancer statistics. CA Cancer J Clin 63:11–30
2. Sánchez MJ, Payer T, de Angelis R, Larrañaga R, Capocaccia R, Martinez C, CIBERESP working group (2010) Cancer incidence and mortality in Spain: estimates and projections for the period 1981–2012. Ann Oncol 21:30–36
3. Howe HL, Wu X, Ries LA, Cokkinides V, Ahmed F, Jemal A et al (2006) Annual report to the nation on the status of cancer, 1975–2003, featuring cancer among U.S. Hispanic/Latino populations. Cancer 107:1711
4. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA (1996) Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci U S A 93:5925–5930
5. Mosselman S, Polman J, Dijkema R (1996) ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett 392:49–53
6. Moore JT, Mckee DD, Slentz-Kesler K, Moore LB, Jones SA, Horne EL et al (1998) Cloning and characterization of human estrogen receptor beta isoforms. Biochem Biophys Res Commun 247:75–78
7. Hasson RM, Briggs A, Carothers AM, Davids JS, Wang J, Javid SH et al (2014) Estrogen receptor alfa or beta loss in the colon of min/+ mice promotes crypt expansion and impairs TGFbeta and HNF3beta signaling. Carinogenesis 35:96–102
8. Barone M, Tanzi S, Lofano K, Scavo MP, Pricci M, Demarinis L et al (2010) Dietaryy-induced ERbeta upregulation counteracts intestinal neoplasia development in intact male $Apc^{Min/+}$ mice. Carcinogenesis 31:269–274
9. Calle EE, Miracle-Mahill HL, Thun MJ, Heath CW Jr (1995) Estrogen replacement therapy and risk of fatal colon cancer in a prospective cohort of postmenopausical women. J Nat Cancer Inst 87:517–523
10. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML et al (2002) Risk and benefits of estrogen plus progestin in healthy postmenopausal women: principal result from the Women's health initiative randomized controlled trial. JAMA 288:321–333
11. Rudolph A, Toth C, Hoffmeister M, Roth W, Herpel E, Schirmacher P et al (2013) Colorectal cancer risk associated with hormone use varies by expression of estrogen receptor-beta. Cancer Res 73: 3306–3315
12. Jassam N, Bell SM, Speirs V, Quirke P (2005) Loss of expression of oestrogen receptor β in colon cancer and its association with dukes` staging. Oncol Rep 14:17–21
13. Fang YJ, Lu ZH, Wang F, Wu XJ, Li LR, Zhang LY et al (2010) Prognostic impact of ERβ and MMP7 expression on overall survival in colon cancer. Tumor Biol 31:651–658
14. Reproducido con permiso del AJCC: Greene FL, Page PL, Fleming ID, et al. AJCC Cancer Staging Manual 6th ed. New York: Springer-Verlag, 2002
15. Grivas PD, Tzelepi V, Sotiropoulou-Bonikou G, Kefalopoulou Z, Papavassiliou AG, Kalofonos H (2009) Expression of ERβ, ER alpha and co-regulator PELP1/MNAR in colorectal cancer: prognosis significance and clinopathologic correlations. Cell Oncol 31: 235–247
16. Elbanna HG, Ebrahim MA, Abbas AM, Zalata K, Hashim MA (2012) Potential value of estrogen receptor beta expression in colorectal carcinoma: interaction with apoptotic index. J Gastrointest Cancer 43:56–62
17. Harvey JM, Clark GM, Osborne CK, Allred DC (1999) Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 17:1474–1481
18. Xie LQ, Yu JP, Luo HS (2004) Expression of estrogen receptor β in human colorectal cancer. Word J Gastroenterol 10:214–217
19. Campbell-Thompson M, Lynch J, Bhardwaj B (2001) Expression of estrogen receptor subtypes and Erβ isoforms in colon cancer. Cancer Res 61:632–640
20. Taggarshe D, Lobocki C, Silberberg B, McKendrick A, Mittal VK (2012) Clinicopathological significance of the expression of estrogen receptor-beta and vascular endotelial growth factor-a in colorectal cancer. Am Surg 78:1376–1382
21. Rudolph A, Toth C, Hoffmeister M, Roth W, Herpel E, Jansen L et al (2012) Expresion of oestrogen receptor β and prognosis of colorectal cancer. Br J Cancer 107:831–839
22. Rath-Wolfson L, Purim O, Ram E, Morgenstern S, Koren R, Brenner B. Expression of estrogen receptor β1 in colorectal cancer: correlation with clinicopathological variables. Oncology Reports 27:2017–2022
23. Konstantinopoulos PA, Kominea A, Vandoros G, Sykiotis GP, Andricopoulos P, Varakis I et al (2003) Oestrogen receptor beta is abundantly expressed in normal colonic mucosa, but declines in colon adenocarcinoma paralleling the tumour's dedifferentiation. Eur J Cancer 39:1251–1258
24. Castiglione F, Taddei A, DeglÍnnocenti R, Buccoliero AM, Bechi P, Garbini F et al (2008) Expression of estrogen receptor β in colon cancer progression. Diagn Mol Pathol 17:231–236
25. Wong NA, Malcomson RD, Jodrell DI, Groome NP, Harrison DJ (2005) Saunders PT.ERbeta isoform expression in colorectal carcinomas:an in vivo and in vitro study of clinicapathological and molecular correlates. J Pathol 207:53–60