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Oestrogen receptor-related receptor alpha (ERRα) and oestrogen receptors (ERα and ERβ) exhibit different gene expression in human colorectal tumour progression

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Abstract

We have investigated the presence of oestrogen receptor-related (ERR) mRNA in human colorectal tumour tissues and adjacent normal mucosa by reverse transcriptase and nested-polymerase chain reaction. ERRα was found in 100% of the patients and ERRγ in approximately 30% while ERRβ was not detected at all. The multiplex PCR analysis showed elevated levels of ERRα mRNA in tumour tissue compartment as compared to normal mucosa, whereas ERRγ mRNA was found in lower levels but in both tissue compartments. In contrast, oestrogen receptor (ERα and ERβ) mRNA levels were shown to be decreased in tumour tissues. A positive correlation was observed between ERα and ERβ and between ERα and ERRα, respectively, in normal mucosa but not in tumour tissue. ERRα expression in tumour tissues significantly increased from TNM stages II to IV, whereas both ERs progressively declined. These findings suggest that ERRα, as well as the two ERs, might play a critical role in the progression of the colorectal cancer.

Keywords: Oestrogen receptor-related receptor; Oestrogen receptor alpha; Oestrogen receptor beta; Colorectal carcinoma; Tumour progression; Peroxisome proliferator-activated receptor γ coactivator-1; Peroxisome proliferator-activated receptor γ

1. Introduction

Oestrogen receptors (ERα and ERβ) mediate the effects of the natural oestrogens on growth, development, and homeostasis maintenance in different human tissues and organs [1]. Knowledge of the biological action of sex steroids resulted in the application of oestrogen hormone replacement therapy (HRT) as a supplement to alleviate the climacteric symptoms associated with menopause [2]. Despite these benefits, steroid hormones may solicit the development of breast and uterus tumours, whereas epidemiological studies have reported that the risk of colorectal cancer among HRT users is reduced [3,4]. Between the two ERs present in colorectal tissue [5–8], ERβ is the predominant subtype and could exert this protective role against colorectal cancer [9], even if its mechanism of action is not fully known [10].

Other nuclear receptors, linked to the activity of the ERs, are the oestrogen receptor-related receptors (ERRs). ERRα and ERRβ were isolated in 1988, while ERRγ, the third member of this family, has been

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identified only recently [11,12]. Although the ERRs share structural homology with ERs and show interactions with some of the ER co-regulators they differ in key aspects from them [13]. The ERR proteins are structurally related to ERα protein in the ligand binding domain (LDB), but their physiological ligands have not been identified so far [14]. The ERRs in their activated state bind to a specific DNA nucleotide sequence (oestrogen responsive element or ERE) present in some genes that is also recognised by the two ERs. However, it also binds to another DNA response element referred to as ERRE, that is recognised by ERα but not by ERβ [15,16].

In summary, all these data suggest a possible cross-talk among members of these two different nuclear receptor families.

Another possible candidate that might exert a control on transcriptional activity of both ERRα and ERα is the peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1) [17,18].

In addition to PGC-1, its target gene peroxisome proliferator-activated receptor gamma (PPARγ) also seems to interact *in vitro* with ERs [19]. PPARγ in particular plays a special role in the development of human colorectal diseases since it participates in biological pathways involved in intestinal cell differentiation, inflammatory bowel disease (IBD) and carcinogenesis [20].

Little is known about the mechanism that regulates the expression and the activity of ERRs in human tissues and available information is derived from *in vitro* or animal model studies [21,22]. Recently, Ariazi [23] proposed that ERRα and ERRγ are negative and positive biomarkers respectively, for the clinical course and in selection of appropriate therapies for human breast cancer. Due to the lack of information regarding ERR expression in human colorectal tract, the present study was performed to investigate the presence of these nuclear receptors in samples of colorectal adenocarcinoma matched with surrounding normal mucosa of eighty patients. The profiles of the ERR and ER mRNAs were determined and possible correlations among their mRNA levels, as well as possible relationships with clinicopathological parameters, were investigated.

Since the recent advances in the knowledge of the ER and ERR activities have identified a number of nuclear receptors that directly interact with them, we also compared PGC-1 and PPARγ gene expressions to levels of ERs and ERRs.

2. Materials and methods

2.1. Patients and tissue samples

Eighty patients from a large primary list of patients operated consecutively in two Units of Digestive Surgery from January 2001 to October 2002 were enrolled. The inclusion criteria were: (a) a negative anamnesis for familial colorectal carcinoma, (b) females patients >59 years old (women in pre- and perimenopausal period were excluded), (c) no chemo or radiotherapy before tissue collection, and (d) no hormonal therapy in female patients.

Information concerning age, sex, tumour site and TNM grade was retrieved by reviewing the pathology surgical reports. The tumour characteristics and salient clinical features of the 80 patients are illustrated in Table 1. The tumour and surrounding normal tissue specimens were stored at −80 °C until evaluation of mRNA.

3. RT-multiplex PCR

3.1. Multiplex PCR validation

Previously, we have described a nested PCR (n-PCR) method [8]. In this study, the same method was used as qualitative PCR, to determine levels of gene expression. A multiplex PCR assay (m-PCR) was performed to determine the relative mRNA levels of the same genes.

The m-PCR method was performed after modifying the number of cycles form the first round (PCR-1) of the n-PCR (from 30 to 20 cycles) and co-amplifying in the second round (PCR-2) the cDNA of each gene with the one of the β-actin according to Henegariu and colleagues [24]. The parameters modified for PCR-2 were: (a) the two primer sets (target gene and β-actin) were used in equimolar mixture (40 pmol for each pri-

Table 1
Clinical parameters of patients with sporadic colorectal adenocarcinoma

No. of patients	80
Sex	
Females	36
Males	44
Age (mean)	
Females	71 years (range 60–82)
Males	66 years (range 56–76)
Colorectal tumour site	
Right site	26
Left site	29
Rectum	25
TNM stage	
II	37
III	28
IV	15
Differentiation grade	
Well differentiated	15
Moderately differentiated	34
Poorly differentiated	32

mer); (b) the annealing temperature for all primers was fixed at 55 °C to prevent mismatches and primer dimerisation; (c) the reaction of amplification was fixed at 45 cycles, so that all reactions occurred within the linear range of amplification; (d) the optimal length of the PCR products was estimated at approximately 250–330 bp for target genes and 479 bp for β-actin, so all PCR products had a magnitude of fluorescent signal that was comparable amongst them. Only the length of the ERα PCR product was 149 bp because it gave comparable levels with other PCR products.

In the first series of experiments, aliquots of total RNA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 μg) were retrotranscribed and amplified (PCR-1) by specific primers for each target gene using RNA prepared from a pool of normal tissue samples, to validate the m-PCR. Then, 2 μl of each PCR-1 was co-amplified with a fixed amount of β-actin cDNA (corresponding to 0.5 μg of RNA retrotranscribed). In a second series of experiments, a constant amount of a target gene cDNA (corresponding to 2 μg of RNA) was co-amplified with increasing amount of β-actin cDNA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 μg of RNA). In parallel, the same solutions of the target genes and β-actin were also amplified independently and the results were compared to those obtained by co-amplification. The PCR bands of the target gene and β-actin showed a similar intensity when 2 μg of total RNA of a target gene were used for RT and PCR-1, and when an aliquot of 2 μl of the PCR-1 was co-amplified with 5 μl of the β-actin cDNA (corresponding to 0.5 μg of total RNA) in m-PCR (data not shown). The β-actin was used as control gene because we have previously observed that the levels of its transcript were similar either in colorectal tumour or adjacent normal mucosa samples [8].

3.2. RNA extraction, RT and multiplex PCR method

RNA extraction, reverse transcriptase and n-PCR have been described previously [8].

In brief, total RNA was extracted from tissue specimens by acid–phenol guanidinium method and a precise amount (2 μg) of the total RNA was retro-transcribed (20 μl final volume). Then, cDNA aliquots (5 μl) were amplified in parallel for 20 cycles (PCR-1). The m-PCR was then performed co-amplifying 2 μl of each PCR-1 of each gene with 5 μl of β-actin cDNA belonging to the same tissue sample in 50 μl of final volume for 45 cycles (m-PCR). For each PCR sample the amplification was conducted in duplicate. The PCR products were then electrophoresed in 2% agarose gel w/v, stained with ethidium bromide, and the PCR bands were analysed by Gel Doc 1000 instrument and by Image Analysis System software (BioRad, Monza, Italy).

The identity of each PCR product was confirmed by complete sequencing of the purified product.

The value of the internal standard (β-actin) in each test tube was used as the baseline gene expression for the target gene amplified in the same test tube to evaluate the relative levels of target gene expression in m-PCR. These values were then used to compare mRNA expression across the tested samples. The sequences of the primers are shown in Table 2. The primers for ERR, PGC-1, PPARγ were using Primer Premier software (Primer Biosoft International, Palo Alto, CA,

Table 2
Primer sequences and PCR products

Target genes[a,b]	Primer sequences forward/reverse (f/r)	PCR product
ERR-α (f) outer	5′-AGCGCCTCTGCCTGGT-CTGT-3′	
ERR-α (r) outer	5′-GATGAGAAGCCTGGGAT-GCTCTTGG-3′	
ERR-α (f) inner	5′-TCACCAAGCGGAGACGC-AAGGC-3′	
ERR-α (r) inner	5′-CGGTCAAAGAGGTCACAG-AGGGTAG-3′	332 bp
ERR-β (f) outer	5′-CAGAGGGCTGCTGAACA-GGATG-3′	
ERR-β (r) outer	5′-TGATCTCGCACTCGTTG-GTGGC-3′	
ERR-β (f) inner	5′-CAGAGGGCTGCTGAAC-AGGATG-3′	
ERR-β (r) inner	5′-AGCATGTACTCGCACT-TGATGG-3′	302 bp
ERR-γ (f) outer	5′-CATCAAACTAGAAGGC-AAAGTCCC-3′	
ERR-γ (r) outer	5′-GAAGGCAGGCAGAC-GGGAAG-3′	
ERR-γ (f) inner	5′-GTTGGAGGCCAAGGT-CTGCTAAA-3′	
ERR-γ (r) inner	5′-AGCAAAGAAATAAGG-GAGGTGAAAG-3′	298 bp
PGC-1 (f) outer	5′-GACGGATTGCCCTCA-TTTGAT-3′	
PGC-1 (r) outer	5′-TGGTTTGGCTTGTAA-GTGTTGTG-3′	
PGC-1 (f) inner	5′-TTTGATGCGCTGA-CAGATGG-3′	
PGC-1 (r) inner	5′-GAGAAGCTCCGAG-CAGGGAC-3′	309 bp
PPARγ (f) outer	5′-GCCATCCGCATCTT-TCAGGG-3′	
PPARγ (r) outer	5′-CTCCAGGGCTTGTAG-CAGGTTGTCT-3′	
PPARγ (f) inner	5′-TTCGCTCCGTGGAG-GCTGTG-3′	
PPARγ (r) inner	5′-GGCTTGTAGCAGGTT-GTCTTGAATG-3′	329 bp
β-Actin mRNA		
β-Actin (f)	5′-GCGGGAAATCGTGCGTG-ACATTAAGGAGA-3′	
β-Actin (r)	5′-CGTCATACTCCTGCTTG-CTGATCCACATCTGC-3′	479 bp

[a] The primer sequences of oestrogen receptor alpha and beta (ERα and ERβ) have been described previously [8].

[b] The inner primers of each target gene and the primers of the β-actin were used in same tube for multiplex PCR (m-PCR) analysis.

USA) on published gene sequences. ERRα (GenBank Entry NM-004451), ERRβ (GenBank NM-004452); ERRγ (GenBank NM-001438); PGC-1 (GenBank NM-013261); PPARγ (GenBank NM-138712) and β-actin (GenBank NM-001101). A representative multiplex PCR gel is shown in Fig. 1.

3.3. Western blotting analysis

Western immunoblot analysis was conducted to determine whether the ERα and ERβ mRNA levels agreed with their protein levels in pathological and surrounding normal compartments. The analysis was carried out in 20 patients: 6 patients with tumour grade II, 7 with tumour grade III and 7 with tumour grade IV. The protein extraction and Western blot assay have been described previously [8].

In brief, 30 μg of tissue homogenate was resolved by 10% SDS–PAGE w/v. The resolved proteins were transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was incubated with specific antibodies against ERα, ERβ and β-actin and protein was detected using the enhanced chemiluminescence system. The level of the ERα and ERβ proteins were normalised with the ones of the β-actin protein for each tissue sample. Western blot analysis could not be carried out for ERR proteins due to a lack of commercially available specific antibodies.

3.4. Statistical analysis

Mean relative gene expressions between matched normal and tumour samples were tested using the two-tailed Wilcoxon signed-rank test, while the Spearman's rank correlation test was used to study the relationships between two different gene expression profiles. The associations between mRNA levels and clinical parameters were tested by non-parametric Kruskal–Wallis test. A multiple-comparison Dunnett test was used among three groups of patients with different tumour grade or with different histological differentiation. In this statistical analysis we have used the differences of the values (called Delta value) obtained by subtracting from each tissue sample the score of the gene expression in the pathological tissue with the one of the surrounding normal mucosa.

The relative mRNA levels were expressed as means ± SEM. Two-sided $P \leqslant 0.05$ was considered as statistically significant.

4. Results

4.1. Expression analysis of the ER subtypes

The ERβ and ERα mRNA levels were significantly higher in normal mucosa than in tumour tissues (Fig. 2). ERα/ERβ mRNA ratio was higher in colorectal tumour tissue compared to adjacent normal compartment (Fig. 2). The Western blotting results were consistent with the m-PCR data, even if the protein levels of both ERs had been evaluated in only 20 patients (Fig. 3).

A positive correlation between ERα and ERβ mRNA expressions ($r = 0.31$, $P = 0.02$) was observed in normal mucosa, but this correlation was negative in tumour compartment ($r = 0.17$, $P = 0.22$). The ERα and ERβ mRNA levels were significantly lower in tumours with stage II to stage IV (Fig. 4(a)). The same trend was observed for tumours with histological differentiation (Fig. 4(b)).

No relationship was observed between the two ERs and other clinicopathological characteristics (data not shown).

4.2. Expression analysis of the ERR subtypes

ERRα and ERRγ mRNAs were detected in tumour tissue and adjacent normal mucosa by qualitative PCR analysis, whereas the ERRβ mRNA was not found.

The quantitative PCR analysis showed a greater level of ERRα in tumour tissue than in surrounding normal mucosa (Fig. 2). Positive correlation between ERRα

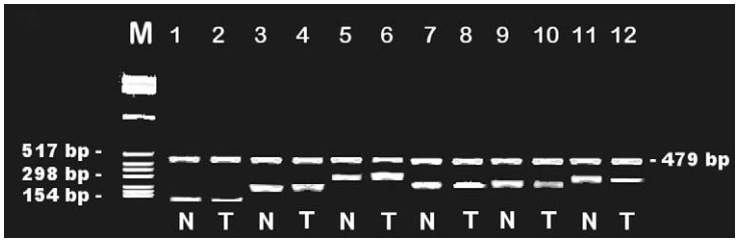

Fig. 1. Representative multiplex PCR in colorectal tumour (T) and matched normal (N) tissue samples. The conditions of multiplex PCR (m-PCR) are described in Section 2. The sizes of the multiplex PCR products are: 149, 230, 332 and 298 bp for oestrogen receptor alpha (ERα), beta (ERβ), oestrogen receptor-related receptor alpha (ERRα) and gamma (ERRγ), respectively; 309 bp for peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) and 329 bp for peroxisome proliferator-activated receptor gamma (PPARγ). The PCR product of the ERRβ mRNA (302 bp) is not shown as it was not found in any of the tissue samples examined. Lanes 1–12: ERα, ERβ, ERRα, ERRγ, PGC-1 and PPARγ PCR products in tumour tissue (T) and adjacent normal mucosa (N), respectively. The band of 479 bp is the PCR product of the β-actin mRNA used as control. M: Molecular weight marker.

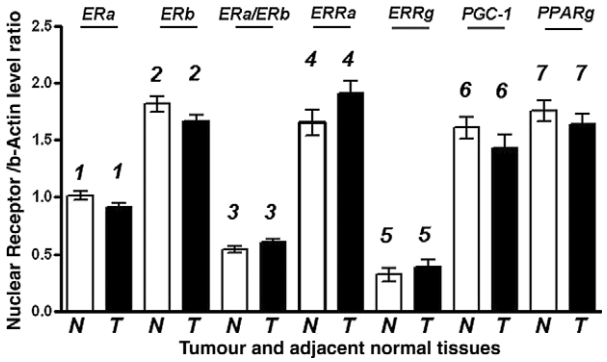

Fig. 2. Comparison of nuclear receptor mRNA levels in matched normal (N) and tumour (T) compartments in human colorectal cancer. ERa, oestrogen receptor alpha; ERb, oestrogen receptor beta; ERa/ERb, oestrogen receptor alpha/oestrogen receptor beta ratio, ERRa, oestrogen receptor-related receptor alpha; ERRg, oestrogen receptor-related receptor gamma; PGC-1, peroxisome proliferator-activated receptor gamma coactivator-1; PPARg, peroxisome proliferator-activated receptor gamma. Signals were quantified and normalised as described in Section 3.4. The relative mRNA levels are expressed as means ± SEM. Differences between matched normal and tumour tissues were tested using the two-tailed Wilcoxon signed rank test: $^{1,1}P = 0.011$; $^{2,2}P = 0.028$; $^{3,3}P = 0.018$; $^{4,4}P = 0.0018$; $^{5,5}P = 0.1$ ($n = 24$); $^{6,6}P = 0.014$; $^{7,7}P = 0.028$.

and ERα levels was found, but only in normal tissue compartment ($r = 0.63$, $P = 0.02$), and no correlation was observed among the expression of the other nuclear receptors (data not shown). The distribution of the ERRα mRNA levels according to the TNM stage and histological differentiation exhibited an interesting trend. The ERRα increased with the worsening of the colorectal tumour disease, as well as with the differentiation of malignant colon cells (Fig. 4(a) and (b)). No

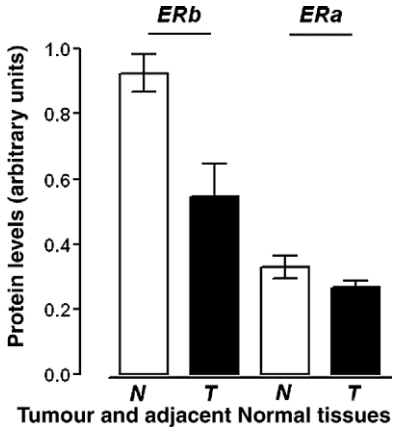

Fig. 3. The Western blotting analysis of the oestrogen receptor alpha and beta (ERα and ERβ) proteins. The graph shows mean ± SEM of ERα and ERβ protein distribution in tumour (T) and adjacent normal (N) mucosa samples of 20 patients. The patients were selected as follows: 6 patients with tumour grade II, 7 with tumour grade III and 7 with tumour grade IV. The *x*-axis shows the ERα and ERβ protein values after normalisation with β-actin. Differences between tumour tissue and matched normal tissue samples were tested using the two-tailed Wilcoxon signed-rank test: $P = 0.03$ and 0.09 for ERβ and ERα, respectively.

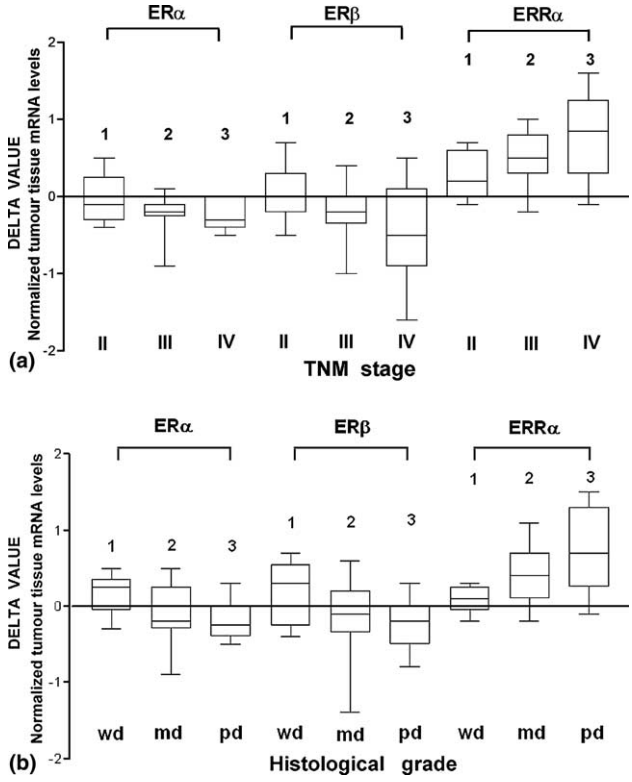

Fig. 4. (a) Distribution of oestrogen receptor alpha (ERα), oestrogen receptor beta (ERβ) and oestrogen receptor-related receptor alpha (ERRα) mRNA levels according to TNM stage within the colorectal tumour samples. Box and whisker plots are shown for each group of tumour. Delta value is the difference between the value of the tumour tissue and the value of the adjacent normal mucosa of the same patient (see Section 3.4). Groups II–IV represent the patients with tumour grade II ($n = 37$), grade III ($n = 28$) and grade IV ($n = 15$), respectively. The multiple-comparison Dunnett test was used to analyse differences among groups. ERα: $^{1,2}P = 0.056$, $^{1,3}P = 0.037$; ERβ: $^{1,2}P = 0.012$, $^{1,3}P = 0.002$; ERRα: $^{1,2}P = 0.048$, $^{1,3}P = 0.012$. (b) Distribution of ERα, ERβ and ERRα mRNA levels in well (wd; $n = 15$), moderately (md; $n = 34$) and poorly (pd; $n = 32$) differentiated tumours. ERα: $^{1,2}P = 0.048$, $^{1,3}P = 0.004$; ERβ: $^{1,2}P = 0.07$, $^{1,3}P = 0.01$; ERRα: $^{1,2}P = 0.047$, $^{1,3}P = 0.006$.

relationship was observed between ERRα and other clinicopathological parameters (data not shown). ERRγ mRNA was expressed at very low levels, approximately 30% of the patients ($n = 24$) and no changes were observed between pathological and normal tissue compartments. Unfortunately, the number of patients who showed the ERRγ mRNA was too low for permitting further statistical analysis about this nuclear receptor.

4.3. Expression analysis of PGC-1 and PPARγ

PGC-1 and PPARγ expression in the tumours was significantly decreased in comparison to normal mucosa (Fig. 2).

Moreover, the PGC-1 and PPARγ mRNA levels positively correlated each other in the normal mucosa ($r = 0.66$, $P = 0.001$) and in the tumour tissue with a

trend toward significance ($r = 0.43$, $P = 0.059$). No correlation was observed between PGC-1 and PPARγ or other nuclear receptors examined (data not shown).

5. Discussion

5.1. ERα and ERβ expression

Although it is undoubtedly true that the human colorectal tumour is a complex process, epidemiological and experimental data indicate that oestrogens and their cognate receptors (ERα and ERβ) play an important role in both growth and development of the human colorectal mucosa.

In this study, we found in normal colorectal mucosa a positive correlation in the gene expression between ERα and ERβ subtypes. This suggests that the ER-mediated gene transcription in normal colorectal mucosa is regulated by cross-talk between these two receptors, as recently reported by Lindberg and colleagues [25] in animal models. The decrease of ERβ and ERα from normal to pathological colorectal tissues at mRNA level, is also confirmed at protein level by Western blotting analysis. As to ERα proteins, this trend had been also described in one of our previous studies [26]. Of note, we previously reported an up-regulation of ERβ mRNA expression in colorectal tumour tissue as compared to surrounding normal mucosa [8]. The discrepancy between the present study and the previous one [8] may depend on the different cohort of the patients analysed. In fact, herein we have considered a larger number of patients with tumour at stages III and IV. The decrease of the two ER subtypes and lack of correlation in tumour tissue compartment underline the importance of the ERs in human colorectal tract. In addition, the increase of the ERα/ERβ value in tumour tissue, as compared to adjacent normal mucosa, confirms more and more the protective role exerted by the ERβ against colorectal carcinoma development.

These findings agree with data published by Konstantinopoulos and colleagues [9] who showed a correlation between progressive decline of ERβ proteins and degree of colorectal cancer cell differentiation by immunohistological methods. The positive correlation between ERα and ERRα mRNA levels in normal mucosa suggests two hypotheses: (a) the transcriptional activation of two different nuclear receptors has a common regulatory pathway in normal tissue, and (b) the transcriptional activation of ERRα may be ERα-mediated or vice versa, as demonstrated by an *in vitro* study [27]. Although a functional relationship between these nuclear receptors can be tested in subsequent, more-extensive studies, these data seem to contribute to the better understanding of the complex mechanism of the oestrogen signalling pathway modulation in human colorectal tissue.

5.2. ERR expression

Another family of nuclear receptors, linked to the ERs, is the ERR family. Unfortunately, the studies on the ERRs have been undertaken mostly *in vitro* and clinical data which might allow an assessment of their biological relevance is often lacking. The present study demonstrates, for the first time, the presence of ERRα and ERRγ mRNAs in both normal and tumour colorectal tissues, whereas the ERRβ mRNA, the third member of this family, was not detected by PCR analysis.

The absence of the ERRβ mRNA in human colorectal tissue may reflect the inactivation of the ERRβ gene during adult life or the instability of its messenger that is readily degraded in colonic cells. To date, we cannot answer this question. While the ERRγ messenger is present at very low levels and with similar amount in both tumour and adjacent tissue compartments, the increase of the ERRα expression in tumour tissues reveals a possible involvement of this nuclear receptor in human colorectal carcinoma. In fact, the ERRα seems to emerge as a novel potential modifier of the malignant colorectal tract because the change in the expression of its messenger is associated either with tumour progression or with the different histopathological stages of tumour disease. In addition, the decline in the expression of both ER subtypes and the increase of ERRα levels in tumour compartment could have a profound effect on oestrogen-stimulated gene activity. Although the factors that determine ERRα's transcriptional activity *in vivo* have not yet been identified, the very low levels of ERRγ or its absence in the same samples of colorectal tissue suggest that this receptor does not affect ERRα activity. In fact, an *in vitro* study has demonstrated that the heterodimerization between ERRα and ERRγ, in contrast to ERRα homodimerization, inhibits the transcriptional activity of both receptors [28]. Up to now, no specific biomarkers have been discovered for the human colorectal cancer. Our data suggest that the ERRα expression may be used as a potential biomarker in the progression of the colorectal tumour disease. In this direction, Ariazi and colleagues [23] have shown an inverse association between ERRα expression and oestrogen responsiveness of the human breast tumours, proposing that ERRα is an unfavourable biomarker for this tumour.

5.3. PGC-1 and PPARγ expression

We have investigated the relative distribution of PGC-1 expression in human colorectal tissue as some *in vitro* studies have demonstrated an interaction between PGC-1 and ERRα [17] and between PGC-1 and ERs [18]. Our study shows a decline in PGC-1 mRNA levels in human colorectal carcinoma when compared to adjacent normal tissue, and no correlation was found between PGC-1 and other nuclear receptors. Since only

the PGC-1 mRNA levels have been investigated in this study, the interaction between PGC-1 and ERRα or between PGC-1 and ERα could also occur in human colorectal cells at protein level. In fact, Schreiber [17] have described that PGC-1 converts ERRα from a factor with little or no transcriptional activity to a potent regulator of gene expression by a physical interaction between the two proteins. Therefore, further investigations are needed to understand whether activation of PGC-1 by specific ligands can also alter the transcriptional regulation of ERα or ERRα.

This study was also designed to define the relative PPARγ expression in human colorectal tissue. We observed a decrease of the PPARγ mRNA levels in colorectal tumour compartment as compared to adjacent normal mucosa. Although performed on a reduced number of samples, a recent study reports a decrease of the PGC-1 expression from normal to tumour tissues, whereas no changes were observed between these two tissue compartments for PPARγ mRNA levels [29]. However, these data seem to be in contrast with the definition of "tumour-suppressor" given to PPARγ by some *in vivo* and *in vitro* pre-clinical models [20]. The positive correlation between PGC-1 and PPARγ expression, that we have found in both tumor tissue and surrounding normal mucosa, suggests that these gene are co-regulated in both tissue types. Therefore, the decreased PGC1 expression in tumor tissue, as compared to normal mucosa, may be responsible for the decreased expression of PPARγ. Finally, the lack of the correlation between PPARγ and oestrogen receptors seem to provide the first evidence *in vivo* of a negative PPARγ-ERR or PPARγ-ERs cross-talk in gene regulation, even if the ability of PPARγ to interfere with ER or ERR action needs to be further explored.

Conflict of interest statement

None declared.

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References

1. McDonnell DP, Norris JD. Connections and regulation of the human estrogen receptor. *Science* 2002, **296**, 1642–1644.
2. Hodgson SF, Watts NB, Bilezikian JP, et al. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the prevention and treatment of postmenopausal osteoporosis: 2001 edition, with selected updates for 2003. *Endocr Pract* 2003, **9**, 544–564.
3. Di Leo A, Messa C, Cavallini A, et al. Estrogen and colorectal cancer. *Curr Drug Targets-Immune, Endocrine Metab Disor* 2001, **1**, 1–12.
4. Chlebowski RT, Wactawski-Wende J, Ritenbaugh C, et al. Estrogen plus progestin and colorectal cancer in postmenopausal women. *N Engl J Med* 2004, **350**, 991–1004.
5. Campbell-Thompson M, Lynch IJ, Bhardwaj B. Expression of estrogen receptor (ER) subtypes and ERbeta isoforms in colon cancer. *Cancer Res* 2001, **61**, 632–640.
6. Foley EF, Jazaeri AA, Shupnik MA, et al. Selective loss of estrogen receptor beta in malignant human colon. *Cancer Res* 2000, **60**, 245–248.
7. Witte D, Chirala M, Younes A, et al. Estrogen receptor beta is expressed in human colorectal adenocarcinoma. *Hum Pathol* 2001, **32**, 940–944.
8. Cavallini A, Messa C, Pricci M, et al. Distribution of estrogen receptor subtypes, expression of their variant forms, and clinicopathological characteristics of human colorectal cancer. *Dig Dis Sci* 2002, **47**, 2720–2728.
9. Konstantinopoulos PA, Kominea A, Vandoros G, et al. Oestrogen receptor beta (ERβ) is abundantly expressed in normal colonic mucosa, but declines in colon adenocarcinoma paralleling the tumour's dedifferentiation. *Eur J Cancer* 2003, **39**, 1251–1258.
10. Pettersson K, Gustafsson J-A. Role of estrogen receptors beta in estrogen action. *Annu Rev Physiol* 2001, **63**, 165–192.
11. Giguere V, Yang N, Segui P, et al. Identification of a new class of steroid hormone receptors. *Nature* 1988, **331**, 91–94.
12. Hong H, Yang L, Stallcup MR. Hormone-independent transcriptional activation and coactivator binding by novel orphan nuclear receptor ERR3. *J Biol Chem* 1999, **274**, 22618–22626.
13. Zhang Z, Teng CT. Estrogen receptor alpha and oestrogen receptor-related receptor alpha1 compete for binding and coactivator. *Mol Cell Endocrinol* 2001, **172**, 223–233.
14. Horard B, Vanacker J-M. Estrogen receptor-related receptors: orphan receptors desperately seeking a ligand. *J Mol Endocrinol* 2003, **31**, 349–357.
15. Vanacker JM, Pettersson K, Gustafsson J-A, et al. Transcriptional targets shared by estrogen receptor-related receptors (ERRs) and estrogen receptor (ER)α, but not by ERβ. *EMBO J* 1999, **18**, 4270–4279.
16. Sanyal S, Matthews J, Bouton D, et al. Deoxyribonucleic acid response element-dependent regulation of transcription by orphan nuclear receptor estrogen receptor-related receptor gamma. *Mol Endocrinol* 2004, **18**, 312–325.
17. Schreiber SN, Knutti D, Brogli K, et al. The transcriptional coactivator PGC-1 regulates the expression and activity of the orphan nuclear receptor ERRα. *J Biol Chem* 2003, **278**, 9013–9018.
18. Tcherepanova I, Puigserver P, Norris JD, et al. Modulation of estrogen receptor-α transcriptional activity by the coactivator PGC-1. *J Biol Chem* 2000, **275**, 16302–16308.
19. Wang X, Kilgore MW. Signal cross-talk between estrogen receptor alpha and beta and the peroxisome proliferator-activated receptor gamma1 in MDA-MB-231 and MCF-7 breast cancer cells. *Mol Cell Endocrinol* 2002, **194**, 123–133.
20. Bull AW. The role of peroxisome proliferator-activated receptor gamma in colon cancer and inflammatory bowel disease. *Arch Pathol Lab Med* 2003, **127**, 1121–1123.
21. Kraus RJ, Ariazi EA, Farrell ML, et al. Estrogen-related receptor alpha 1 actively antagonizes oestrogen receptor-regulated transcription in MCF-7 mammary cells. *J Biol Chem* 2002, **277**, 24826–24834.
22. Luo J, Sladek R, Carrier J, et al. Reduced fat mass in mice lacking orphan nuclear receptor estrogen-related receptor alpha. *Mol Cell Biol* 2003, **23**, 7947–7956.
23. Ariazi EA, Clark GM, Mertz JE. Estrogen-related receptor α and estrogen-related receptor γ associate with unfavourable and

favourable biomarkers, respectively, in human breast cancer. *Cancer Res* 2002, **62**, 6510–6518.
24. Henegariu O, Heerema NA, Dlouhy SR, et al. Multiplex PCR: critical parameters and step-by-step protocol. *BioTechniques* 1997, **23**, 504–511.
25. Lindberg MK, Moverare S, Skrtic S, et al. Estrogen receptor (ER)-beta reduces ERalpha-regulated gene transcription, supporting a "ying yang" relationship between ERalpha and ERbeta in mice. *Mol Endocrinol* 2003, **17**, 203–208.
26. Di Leo A, Messa C, Russo F, et al. Prognostic value of cytosolic estrogen receptors in human colorectal carcinoma and surrounding mucosa. Preliminary results. *Dig Dis Sci* 1994, **39**, 2038–2042.
27. Liu D, Zhang Z, Gladwell W, et al. Estrogen stimulates estrogen-related receptor α gene expression through conserved hormone response elements. *Endocrinology* 2003, **144**, 4894–4904.
28. Huppunen J, Aarnisalo P. Dimerization modulates the activity of the orphan nuclear receptor ERRγ. *Biochem Biophys Res Commun* 2004, **314**, 964–970.
29. Feilchenfeldt J, Brundler MA, Soravia C, et al. Peroxisome proliferator-activated receptors (PPARs) and associated transcription factors in colon cancer: reduced expression of PPARgamma-coactivator 1 (PGC-1). *Cancer Lett* 2004, **203**, 25–33.