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Original Paper

Oestrogen and Progesterone Receptors in Gastrointestinal Cancer Cell Lines

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Expression of sex steroid receptors by gastrointestinal (GI) tumours may indicate a role for hormonal manipulation in their management. A panel of seven established cell lines derived from primary human GI tumours were assayed for oestrogen and progesterone receptor (ER and PgR) expression by ligand binding, immunocytochemistry and enzyme-immunoassay methods. It was possible to demonstrate very low levels of both ER and PgR in the GI cell lines using enzyme immunoassay (EIA), with levels of PgR generally higher than those for ER. Neither ER nor PgR were detected in cytosols made from the GI cell lines in a classical dextran coated charcoal ligand binding assay. Similarly, immunohistological analysis (Abbott ERICA, PgRICA) of cultured cell preparations or of frozen and paraffin sections of xenograft tumour failed to demonstrate receptors. This confirms that low PgR levels are measurable by EIA in GI tumour cell lines. Upregulation of PgR expression in tumours by exposure to oestradiol *in vitro* was not observed. Copyright © 1996 Elsevier Science Ltd

Key words: progesterone, oestrogen, receptor, gastric, pancreatic, colorectal, neoplasm, human tumour cell-line

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INTRODUCTION

THERE ARE age and sex differences in the incidence of colorectal and gastric cancer [1–2]. The risk is increased for women before the menopause, by celibacy and if they bear few or no children. This may reflect hormonal influence in either carcinogenesis or tumour progression. Oestrogen and progesterone receptor (ER and PgR) expression has been detected in gastrointestinal (GI) tumours and cell lines, although the levels reported vary considerably from study to study as discussed below. One possible explanation could be the different methods used to assess receptor expression.

Ligand binding methods have demonstrated ER and PgR, but only in a minority of primary tumours tested. Alford and associates [3] found both ER and PgR in 30% of primary colon tumours. Sica and associates [4] evaluated both colorectal (23% ER, 43% PgR) and stomach (14% ER, 25% PgR) cancers. Benz and associates [5] also used ligand binding methods and showed expression of ER in four pancreatic tumour cell lines studied.

Monoclonal antibodies recognising ER and PgR have been used to assess receptor expression by enzyme immunoassay and by immunocytochemical staining. Taylor and associates [6] found no measurable ER on tissue sections in a series of primary pancreatic biopsies by immunocytochemistry using the ERICA kit (Abbott Laboratories), and corroborated these findings by an alternative method using isoelectric focusing. Hendrickse and associates [7] reported both ER and PgR expression (0–11.3, 0.3–10.2 fmol/mg protein, respectively) in a series of 17 primary colon tumours and in five colonic cell lines (ER 1.2–10.4, PgR 9.1–63.2 fmol/mg protein) measured by an enzyme immunoassay (EIA) kit (Abbott Laboratories). These receptor concentrations are low relative to those reported in breast cancers. In ER-EIA positive breast tumours, median values were between 423 and 538 fmol/mg when grouped by histological grade, lymph node staging or tumour size [8]. Mean levels of ER and PgR in breast adenocarcinomas measured by ligand binding were 32–174 and 222–256

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fmol/mg protein depending on menopausal status [9]. Hendrickse found that PgR concentrations were consistently higher than those for ER in both colon tumours and cell lines indicating the possibility of endocrine regulation. However, they failed to upregulate PgR on exposure to levels of oestradiol which had previously been shown to upregulate PgR in MCF7 cells.

We assayed a panel of 7 GI cell lines for ER and PgR using ligand binding, enzyme immunoassay and immunocytochemistry with a view to correlating receptor status with response to hormone therapy.

MATERIALS AND METHODS

Cell lines and culture

The established cell lines evaluated included the gastric lines RD19 and MKN45G [10], the pancreatic lines PAN-1 and MIA-Pa-Ca-2, the colorectal lines C146 and C170 [11] and a metastatic variant C170HM2 [12]. MIA-Pa-Ca-2 was derived from the European Collection (ECACC) and all the others were derived from primary tumour specimens in the Cancer Research Laboratories and the Cancer Studies Unit, Nottingham University, U.K. The MCF7 breast cell line was obtained from the Tenovus Institute, Cardiff, U.K. and from Dr John Nelson, Queens University, Belfast, Northern Ireland. These were designated MCF7T and MCF7B, respectively. The T47D breast cell line was obtained from Professor Carmichael (Clinical Oncology Unit, City Hospital, Nottingham, U.K.). Growth medium (GM) for routine cell culture was RPMI 1640 with 2 mM glutamine (Gibco, Irvine, U.K.), supplemented with 10% heat inactivated fetal calf serum (FCS, Sigma, Poole, Dorset, U.K.). Cells were harvested for assay or passaged at subconfluence using 0.025% EDTA in phosphate buffered saline (PBS). Assays were set up in a steroid depleted basic medium (BM) composed of RPMI 1640 (phenol red free) supplemented with 2 mM glutamine plus 10% FCS treated with dextran coated charcoal to remove endogenous steroids.

In vivo

The established human cell lines were grown as xenografts in nude mice (Harlan-Olac, Bicester, U.K., 4–6 weeks of age). The grafts were initiated by subcutaneous (s.c.) injection of $5–10 \times 10^6$ cells (C146, RD19) into the left flank. After 2–3 weeks, palpable tumours were excised for assay and portions immediately either flash frozen or prepared as blocks for frozen sections in liquid nitrogen and stored at −80°C, or fixed in formal calcium and processed into paraffin blocks. The work involving animals conformed to UKCCCR guidelines.

Cytosol preparation

Cytosols were prepared from cells harvested from cultures approaching confluence. Preservation of receptors, which are heat labile, was ensured by performing all further manipulations at 4°C using prechilled solutions and equipment. Pellets of $2–5 \times 10^7$ cells were washed in PBS and sonicated for 10 s on ice in 1 ml sonication buffer (10 mM Tris-HCl, pH 7.4, 1.5 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol) for use in the ligand binding/DCC assay. Sodium molybdate (5 mM) was added to this buffer in some repeated assays to increase the proportion of soluble receptor available (by inhibiting receptor activation/binding to chromatin). For EIA analysis, the homogenisation buffer specified in the Abbott protocol was used to prepare the cytosols (10 mM Tris–HCl, pH 7.4, 1.5 mM EDTA, 5 mM Na_2MoO_4, 1 mM monothioglycerol).

Flash frozen pieces of xenograft tumour were allowed to thaw on a bed of dry ice only sufficiently to allow them to be sliced thinly using a scalpel blade without defrosting completely. The slices were transferred into glass universals with approx. 5–10 times volume homogensisation buffer and homogenised using an Ultra-Turrax homogeniser for 2–3 separate 10 s bursts on ice to prevent warming.

For both cells and tumours, the supernatant was retained and pooled after pelleting and washing of the gross cell debris in the same buffer. This was then ultracentrifuged at 100 000*g* for 1 h. Aliquots were assayed immediately, or after a single freeze–thaw cycle on storage at −80°C or below for a maximum period of 4 weeks.

Routinely, cytosols with protein concentrations of 1–4 mg/ml (at the higher end of the range recommended in the kit protocols) were used for receptor analysis. This was based on the premise that only low levels of receptors were expected from previously reported work and thus may be detected more readily in more concentrated cytosols.

Ligand binding by dextran coated charcoal (DCC) assay

Cytosols were prepared from cells grown in GM and protein concentrations determined as above. They were used in a conventional DCC binding assay using the methods of Green and Leake [13]. Briefly, cytosol was incubated overnight at 4°C with 0.8–12.0 nM tritiated oestradiol (Amersham) to measure ER; or with 2.0–20.0 nM tritiated promegestone (NET-555 [17 alpha-methyl-^{3}H]-R5020*, NEN Du-Pont de Nemours, Germany, spec. act. 85.3 Ci/mmol) to assess PgR. Non-specific binding was calculated from parallel incubations also containing a 100-fold molar excess of cold competitor ligand DES (diethylstilbestrol) or promegestone, respectively). Free and bound radioactive ligand were separated by incubation with dextran coated charcoal followed by centrifugation, and the bound radioactivity in the supernatant counted. Total inputs counts and background levels were determined from a series of tubes containing buffer instead of cytosol. Scatchard plots were generated from the data. Cell lines were assayed at least three times. Cytosols made from MCF7B, MCF7T and T47D cells were used as controls.

Immunocytochemistry (ERICA/PgRICA)

Immunocytochemical analysis for ER and PgR was performed using Abbott Monoclonal Antibody Kits (Abbott Diagnostics, Maidenhead, Berks, U.K.) and the techniques used were those reported for fixing and staining breast cancer material [14]. Three different methods were used to prepare the slides.

(1) Frozen sections of xenograft or primary tumour.
(2) Cytospins of freshly harvested cultured cells. Cells were suspended (10^4 in 200 µl phosphate buffered saline) and deposited on glass slides using a Shandon Cytospin 2 centrifuge (1500 rpm/5 min) then air dried prior to fixation.

(3) Cells in monolayer culture. Cultured cells ($1–4 \times 10^5$ in 100 µl GM) were incubated for 4 h at 37°C on sterile glass slides placed in petri dishes to allow adherence, the slides flooded with GM and incubated 24–48 h then washed in serum-free medium before fixation.

In a fourth method, paraffin sections of xenograft tumour were exposed to pronase (Sigma) digestion (0.06% in PBS) to expose ER [15], then blocked with swine serum and incubated overnight with anti-ER monoclonal antibody. For PgR, blocked sections were incubated with anti-PgR monoclonal antibody, but the pronase digestion step omitted. Slides were developed using biotin ABC reagents (DAKO Ltd., High Wycombe, U.K.) followed by diaminobenzidine (DAB) and hydrogen peroxide. This was followed by a 10 min incubation with copper sulphate (0.5% in 0.8% sodium chloride), followed by ethyl green counterstain.

Enzyme immunoassay (EIA) analysis for ER and PgR

Cytosols were analysed for receptors using an EIA kit (Abbott Diagnostics) as previously reported for breast cancer [8]. Cytosols were prepared as above from xenograft tumours or from cultured cells grown to subconfluence in GM or BM. As cultures reached subconfluence, cells were harvested and pellets of intact cells stored at −80°C. Cytosols were assayed by EIA on the day of preparation and again after a single freeze–thaw cycle on storage at −80°C for up to 18 days.

Regulation of PgR levels

Exposure of MCF7 to oestradiol has been reported to up-regulate progesterone receptor expression [16, 17]. Cells were cultured in BM for one week. The medium was replenished such that cells were exposed to oestradiol at a final concentration of 10^{-9} M for 4–7 days. Oestradiol at 10^{-9} M is physiological, has been shown to stimulate growth of MCF7B control cells, and is consistent with Kd levels measured for MCF7B in the ligand binding assays.

Table 1. Oestrogen receptor (ER) determinations by ligand binding

Cell line	*n*	ER B_{max} (fmol/mg)	K_d (nM)
Breast			
MCF7B	5	166.3 ± 65.7	4.7 ± 3.9
MCF7T	4	-ve	
T47D	2	7.7 ± 1.0	1.2 ± 0.8
Gastric			
RD19	5	-ve	
MKN45G	3	-ve	
Pancreatic			
MIA-Pa-Ca-2	3	-ve	
PAN-1	3	-ve	
Colorectal			
C146	5	-ve	
C170	3	-ve	
C170HM2	3	-ve	

n, number of determinations. Mean values ± SE are given for ER (B_{max} = fmol/mg cytosolic protein) with K_d values calculated from Scatchard plots. Cytosols were prepared on the day of assay for at least one determination for each cell line, and only six were stored frozen for longer than 2 weeks.

Table 2. Progesterone receptor (PgR) determinations by ligand binding

Cell line	*n*	PgR B_{max} (fmol/mg)	K_d (nM)
Breast			
MCF7B	3	82.3 ± 28.5	1.5 ± 1.0
MCF7T	1	39	6.2
	2	-ve	
T47D	8	542 ± 109	1.6 ± 0.5
Gastric			
RD19	5	-ve	
MKN45G	3	-ve	
Pancreatic			
MIA-Pa-Ca-2	4	-ve	
PAN-1	5	-ve	
Colorectal			
C146	5	-ve	
C170	3	-ve	
C170HM2	3	-ve	

n, number of determinations. Mean values ± SE are given for PgR (B_{max} = fmol/mg cytosolic protein) with K_d values calculated from Scatchard plots. Cytosols were prepared on the day of assay for at least one determination for each cell line (T47D $n = 4$), and only four were stored frozen for longer than 2 weeks.

Cytosol preparations from these cells were assayed for PgR by EIA.

RESULTS

Ligand binding—DCC method

MCF7B and T47D control breast cell lines were positive for oestrogen receptors (ER, mean values 166.3 and 7.7 fmol/mg protein for MCF7B and T47D, respectively, Table 1) and for progesterone receptors (PgR, mean values for MCF7B and T47D of 82.3 and 542 fmol/mg protein, respectively, Table 2). The MCF7T control cell line had no detectable ER, and PgR at 39 fmol/mg protein in only one of three determinations. In all assays, at least one of the breast cell lines expressed receptors but all GI cell line determinations were negative for both ER (Table 1) and PgR (Table 2) whether cytosols were prepared from cultured cell pellets or from xenograft tumour. There was no significant difference ($P = 0.72$) in receptor levels measured in freshly prepared cytosols or after frozen storage (mean PgR levels ± SE for T47D fresh or frozen of 582 ± 187 and 492 ± 147 fmol/mg, respectively, both $n = 4$).

ERICA/PgRICA

Cell lines. MCF7B cells were stained for both ER and PgR. T47D cells were stained weakly for ER and strongly for PgR. MCF7T cells and all the gastric cell lines did not stain for either ER and PgR whether assayed as cell monolayers grown on slides (MCF7T, RD19, PAN-1, MIA-Pa-Ca-2, C146, C170), as cytospin preparations (MKN45G, PAN-1, C170) or as frozen sections of xenograft tumour (C146, RD19). The use of copper sulphate enhancement techniques on the paraffin sections also failed to reveal either ER or PgR in the C146 or RD19 xenografts.

EIA for ER and PgR

The range of receptor levels in the GI cell lines for both ER and PgR were similar whether cytosols were prepared from cells grown in GM, grown in BM, or from xenograft

Table 3. Enzyme-immunoassay (EIA) data for ER and PgR

Cell line	Receptor concentration (fmol/mg protein) ER mean ± SE	*n*	PgR Mean ± SE	*n*
Controls				
Breast				
MCF7T	6.4 ± 3.2	(10)	25.3 ± 5.1	(9)
MCF7B	127.7 ± 25.3	(11)	460.0 ± 20.5	(11)
T47D	52.2 ± 13.5	(12)	549.3 ± 56.8	(11)
Test				
Gastric				
RD19	4.9 ± 2.1	(9)	9.2 ± 2.3	(9)
MKN45G	8.2 ± 6.2	(5)	5.5 ± 1.9	(5)
Pancreatic				
MIA-Pa-Ca-2	6.3 ± 3.2	(5)	9.2 ± 4.4	(5)
Pan-1	4.7 ± 2.2	(8)	9.3 ± 2.7	(9)
Colorectal				
C146	4.4 ± 2.9	(7)	4.0 ± 1.9	(9)
C170	7.5 ± 3.9	(7)	57.4 ± 19.0	(7)
C170HM2	<1.5	(5)	7.7 ± 5.7	(5)

Mean receptor concentrations (±SE) are given in fmol/mg cytosolic protein and the number of determinations (*n*) shown for each cell line. The lower sensitivity limit of the assay is ER 1.5; PgR 1.7 fmol/mg.

tissue. The data sets ($n = 5$ to $n = 12$ depending on cell line) were therefore combined. Mean ER and PgR concentrations (fmol/mg cytosolic protein) ±SE are indicated in Table 3. The controls supplied with the EIA kits gave values within the acceptable range (± 30%, data not shown). The lower sensitivity limit of the assay is 1.5 fmol/mg for ER and 1.7 fmol/mg for PgR, and breast cancers are usually considered receptor-positive if above 5 fmol/mg.

The controls (MCF7B and TD47D cell lines) had high levels of PgR receptors in most assays (mean 460 ± 20.5 and 549.3 ± 56.8 fmol/mg PgR, respectively). MCF7B had higher concentrations of ER than T47D (means (± SE) 127.7 ± 25.3 and 52.2 ± 13.5 fmol/mg, respectively). MCF7T expressed low levels of ER (mean 6.4 ± 3.2 fmol/mg) and PgR (mean 25.3 ± 5.1 fmol/mg). There was no evidence for loss of either receptor in the control cell lines (MCF7B, T47D) in cytosols stored for up to 18 days prior to assay compared with fresh cytosol preparations.

PgR concentrations were measured in all seven of the GI cell lines at low levels (mean values ranging between 4.0 and 57.4 fmol/mg depending on the cell line). Using the criteria for breast cancer (positive over 5.0 fmol/mg), 52% of PgR determinations ($n = 47$) for the GI cell lines were positive. This figure rises to 61% if a threshold of twice the lower sensitivity limit (3.4 fmol/mg) for the assay is used.

ER were detected in six of the seven GI cell lines (all except C170HM2) with mean ER levels of between 4.4 and 8.2 fmol/mg. Twenty-one per cent of ER determinations ($n = 46$) for the GI cell lines were positive by the criteria for breast cancer (>5 fmol/mg), with 28% having values greater than twice the lower sensitivity limit for the assay (3.0 fmol/mg).

Regulation of PgR (measured by EIA)

At least two PgR evaluations ($n = 2$–6) were available for each sample (Table 4). PgR were assayed in cytosols prepared from cells grown in steroid depleted medium with or

Table 4. The effect of oestradiol on PgR levels

Cell line	PgR receptor concentration (fmol/mg protein) Without E2 Mean ± SE	*n*	With E2 Mean ± SE	*n*
Control				
Breast				
MCF7B	367.3 ± 13.0	(3)	487.5 ± 15.6	(8)
Test				
Gastric				
RD19	10.6 ± 4.4	(3)	8.5 ± 3.0	(6)
MKN45G	6.4 ± 3.7	(2)	5.0 ± 2.7	(3)
Pancreatic				
MIA-Pa-Ca-2	18.0 ± 8.0	(2)	3.3 ± 1.1	(3)
Pan-1	11.2 ± 6.1	(3)	8.3 ± 3.1	(6)
Colorectal				
C146	8.3 ± 6.8	(2)	2.3 ± 0.4	(5)
C170	21.7 ± 3.3	(3)	84.2 ± 26.5	(4)
C170HM2	1.9 ± 0.1	(2)	11.6 ± 9.5	(3)

Cytosols were assayed by EIA from cells grown 'without E2' (steroid depleted medium) or 'with E2' (plus 1 nM oestradiol). Mean receptor concentrations (±SE) are given in fmol/mg cytosolic protein and the number of determinations (*n*) shown for each cell line.

without the addition of oestradiol (10^{-9} M). It was possible to upregulate PgR expression in the MCF7B cell line (from a mean (± SE) of 367.3 ± 13 to 487.5 ± 15.6 fmol PgR/mg protein when cultured without or with oestradiol, respectively). PgR levels in the GI cancer cell lines were not upregulated on exposure to oestradiol (Table 4). One of two C170 cytosols prepared from cells cultured with E2 showed an increase in PgR (mean PgR 110.7 ± 2.3 fmol/mg, $n = 3$) but a second C170 cytosol did not (PgR 4.9 fmol/mg) when compared with a mean value of 21.7 fmol/mg for cytosol prepared from C170 cells cultured without E2. Three other C170 cytosols assayed in a preliminary study also failed to show upregulation of PgR in response to E2 (3.05 ± 1.06 fmol/mg PgR, $n = 4$, data not included in Tables 3 and 4). These particular GI cell lines were not growth stimulated by oestradiol *in vitro*, whereas MCF7B cells were (data not shown).

DISCUSSION

The use of classical ligand binding assays is now often supplemented by techniques such as immunocytochemical and enzyme immunoassays. These use specific antireceptor monoclonal antibodies and are proving to be invaluable tools in the field of breast cancer. There are a number of reports in the literature comparing the alternative techniques, mainly on breast tissues. Berger and associates [18] found immunochemical staining for PgR comparable with ligand binding methods. Marchal and colleagues [19] found good correlation between values obtained by DCC and EIA methods when measuring ER. Freyschuss and associates [20] also found good correlation between values obtained by ligand binding and EIA methods, but also found EIA to be more accurate than ligand binding in detecting low-level ER expression in rat hepatocytes. In a recent breast cancer study, Robertson and colleagues [21] reported that some breast tumours had low-level ER expression detectable by EIA, but were negative by ERICA. However, ERICA was a more accurate predictor of therapeutic response in that the tumours with low ER expression by EIA and negative by ERICA did not subsequently respond to tamoxifen. Thus, in the latter two studies, EIA appeared to be the more sensitive method for detecting low levels of ER expression.

This study supports the findings that the EIA for ER and PgR is a more sensitive technique for demonstrating low levels of receptor expression than either ERICA/PgRICA or the ligand binding assay. ER and PgR were not detected in the GI tumour cell lines by ligand binding. The monoclonal antibodies used in immunocytochemistry and EIA specifically recognise the receptors whether or not they are occupied by ligand, so it is not possible to distinguish between functional and non-functional receptors whereas ligand binding methods measure only functional receptors. This may explain why PgR levels in MCF7B were usually higher when measured by EIA (Table 3) than by ligand binding (Table 2). In comparing the two methods which utilise monoclonals to detect receptors, the EIA may be more sensitive than the ERICA/PgRICA method because presentation of the receptors for antibody binding is in a concentrated cytosolic form. Low receptor density on individual tumour cells may bind insufficient antibody to give a signal by immunocytochemistry, even when additional enhancement is incorporated into the staining protocol. This could explain the failure to detect receptors by immunocytochemistry both in the GI tumour cell lines and in a previous study of a series of primary gastric and colorectal tumours.

The levels of ER and PgR measured by EIA in the seven GI cell lines reported in this study are near the lower threshold of detection by the assay and some values fall below the lowest cut-off points generally accepted for breast tumours (i.e. 5–10 fmol/mg cytosolic protein for ER and PgR). Nevertheless, low-level receptor expression was consistently seen in our series of experiments. Overall, there was a trend for PgR levels to be higher than those for ER in the GI cell lines. This corroborates the findings of Hendrickse and associates [7] who found this pattern in both colorectal tumours and normal mucosa as well as in five colorectal cell lines studied. His tumour samples would have been subject to steroid hormone levels normally encountered in the body. Most of our samples were prepared from cells maintained in routine growth medium (not steroid stripped) so would also have been exposed to residual oestrogen levels including the oestrogenic effects of phenol red. In both situations, this weak oestrogenic stimulus may be sufficient both to downregulate ER expression and to promote PgR production, and could be an explanation for the observed pattern of PgR concentrations exceeding those for ER. The relatively high PgR values obtained for MCF7B by EIA probably reflect such oestrogenic upregulation.

PgR expression was upregulated in the ER positive, oestrogen sensitive breast line MCF7B. This latter result is in keeping with previous studies. Satyaswaroop and associates [22] have reported *in vivo* data showing that a primary endometrial adenocarcinoma with high ER concentrations did upregulate PgR expression when grown in nude mice given oestradiol. However, like Hendrickse and associates [7], we were unable to measure upregulation of PgR expression by oestradiol in any of the GI cell lines *in vitro* except in one instance. The one C170 cytosol preparation where PgR was upregulated was, therefore, unusual for this cell line. However, the fact that similar results were obtained on three separate occasions suggests that this was a genuine finding and not a technical error. The GI tumour cell lines will grow in nude mice with or without oestrogen supplementation.

There are a number of possible explanations for the failure of the GI tumour cell lines to upregulate their PgR in response to oestradiol *in vitro*. The cells may express too few receptors to be able to respond adequately to hormone stimulation. The oestrogen priming may need adjustment (e.g. timing and/or concentration used) to achieve stimulation. Alternatively, there may be no response because the receptors are not functional. The fact that Singh and associates [1, 2] have demonstrated the presence of mRNA for both ER and PgR in gastric and colorectal tumours is not an indication that these are functional receptors. The levels of mRNA they found were similar in benign tumours and normal tissues of gastric and colorectal origin. They also corresponded to the mRNA levels for these receptors found in normal breast and endometrium. However, there was poor correlation between the amount of ER protein and ER-mRNA in the gastric tissues. Additional areas of the ER promoter region have been sequenced, and it is suggested

that there is a complex multiple promoter directing different ER transcripts [23]. If such differential transcription does occur, depending on the cell type involved, it could well determine both quantity and functional activity of receptor expression. It may be that steroid receptors in GI cancer are not classically functional.

In summary, we have shown that we can measure low levels of PgR in GI tumour cell lines by enzyme immunoassay, and that these levels are consistently higher than the detected levels of ER, but we have not been able to measure upregulation of PgR in response to oestradiol stimulation. This latter finding raises questions about the functionality of the ER in GI cell lines, concerning their inability either to bind ligand or to induce ER mediated genes such as PgR as seen in breast tissue. Future studies should focus on determining whether sex steroid receptors are functionally normal, and whether ER mediated pathways are intact in GI tumours.

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