Oestrogen inactivation in the colon: analysis of the expression and regulation of 17β-hydroxysteroid dehydrogenase isozymes in normal colon and colonic cancer

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Summary Epidemiological data suggest that oestrogen contributes to the aetiology of colonic cancer. Furthermore, recent studies have suggested that local hormone metabolism may play a key role in determining colonic responsiveness to oestrogen. To further clarify this mechanism we have characterized the expression and regulation of isozymes of 17β-hydroxysteroid dehydrogenase (17β-HSD) in vitro and in situ. Immunohistochemistry was used to confirm expression of the type 2 and 4 isozymes of 17β-HSD (17β-HSD2 and 4) in normal colonic epithelial cells. Parallel studies suggested that both isozymes were abnormally expressed in colonic tumours and this was confirmed by Western blot analyses. Abnormal expression of 17β-HSD2 and 4 proteins was also observed in Caco-2, HT-29 and SW620 colonic cancer cell lines, although the overall pattern of oestrogen metabolism in these cells was similar to that seen in primary colonic mucosal tissue. The predominant activity (conversion of oestradiol to oestrone) was highest in Caco-2>SW620>HT-29, which correlated inversely with the rate of proliferation of the cell lines. Regulatory studies using SW620 cells indicated that the most potent stimulator of oestradiol to oestrone inactivation was the antiproliferative agent 1,25-dihydroxyvitamin D3 (1,25D3), whilst oestradiol itself inhibited 17β-HSD activity. Both oestradiol and 1,25D3 decreased mRNA for 17β-HSD2 and 4. Data indicate that the high capacity for inactivation of oestrogens in the colon is associated with the presence of 17β-HSD2 and 4 in epithelial cells. Abnormal expression of both isozymes in colonic cancer cells and the stimulation of oestrogen inactivation by the antiproliferative agent 1,25D3 highlights a possible role for 17β-HSD isozymes as modulators of colonic cell proliferation. © 2000 Cancer Research Campaign

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Age and sex differences in the incidence of gastrointestinal cancers suggest the involvement of sex steroids. Specifically, postmenopausal loss of oestrogens in women appears to be associated with a lower risk of colonic cancer (Langman, 1967; Michael and Potter, 1982). These observations have been supported by studies in vitro which have highlighted the ability of active oestrogen (oestradiol, E2) to stimulate the growth of colonic cancer cell lines (Xu and Thomas, 1994; Di Domenico et al, 1996). In spite of this, the precise mechanism by which oestrogens influence colonic cancer in vivo remains unclear, principally because of conflicting reports concerning the role and expression of receptors for E2 (oestrogen receptors, ER) as determinants of oestrogen responsiveness in the colon (Francavilla et al, 1987; Jacobs et al, 1996). Di Domenico and colleagues suggested that the oestrogen-dependent growth of colonic cancer cells in vitro is dependent on ER expression (Di Domenico et al, 1996). However, although we have previously demonstrated differential responses to E2 in pre-malignant and malignant cell lines (Singh et al, 1994), we were unable to correlate this with differences in ER expression (Singh et al, 1994, 1998). A further paradox is provided by epidemiological data which show that hormone replacement therapy (HRT) is associated with a lower risk of colonic cancer (Calle et al, 1995; Newcomb and Storer, 1995; Persson et al, 1996), although this may reflect differences in the composition and route of administration of HRT regimes.

To clarify these observations we have carried out a series of investigations that have focused on the concept of 'pre-receptor regulation' as the principal determinant of oestrogen responsiveness in the colon. Analogous to well documented studies in breast cancer (O’Neill et al, 1988; Sasano et al, 1996), we have postulated that local steroid metabolism in the colon may play a key role in modulating the effects of oestrogens by determining the tissue availability of active E2. In recent studies using tissue biopsies we have shown that the normal colonic mucosa has a high capacity for metabolism of E2 (English et al, 1999). Furthermore, the predominant metabolic activity, inactivation of E2 to oestrone (E1), was significantly decreased in paired tumour biopsies. Conversion of E2 to E1 is catalysed by the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD) for which several isozymes have been identified (Peltoketo et al, 1999). The presence of 17β-HSD activity in the colon appears to be due to expression of the type 2 and 4 isozymes of 17β-HSD (17β-HSD2 and 4), and expression of mRNA for the latter was shown to be significantly decreased in tumours compared to normal mucosae. In this report we have used in vitro model systems and tissue analysis in situ to examine further the relationship between 17β-HSD expression, colonic cell
proliferation and tumour development. Data provide further
evidence for the importance of 17β-HSD2 and 4 as attenuators of
E1 bioavailability in the colon, and emphasize a possible role for
17β-HSD2 and 4 in the pathogenesis of colon cancer.

MATERIALS AND METHODS

Immunohistochemical studies
Colonic tumour and paired normal mucosal tissue were obtained
with agreement from the local ethical approval committee. Five-
micron thick, formalin-fixed tissue sections were cut and placed on
coated glass slides. Sections were de-waxed and endogenous
peroxidase activity quenched with 3% hydrogen peroxide. Sections
were then incubated in donkey serum (Binding Site, Birmingham,
UK) diluted 1/10 in PBS (15 min), followed by primary antibody
diluted in PBS (1 hour). Antisera used were as follows: 17β-HSD2
monoclonal antiserum (1/500 dilution), a kind gift of Dr S
Andersson (South Western Medical Center, Dallas, USA), was
produced with a synthetic carboxyterminal peptide [C]RALRMP-
NYKKKAT, corresponding to amino acids 375-387 in the human
17β-HSD2 protein; 17β-HSD4 monoclonal antibody (1:200 dilu-
tion), a kind gift of Dr J Adamski (GSF, Neuherberg, Germany)
was prepared against the porcine 17β-HSD4 which cross-reacts
with human, rat and mouse tissues. After washing, slides were
incubated for 30 min with a biotinylated universal secondary anti-
body (Binding Site), diluted 1/100 in PBS, and binding visualised
using ABC reagent (Binding Site) and 3,3'-diaminobenzidine
(Sigma Chemical Co, Poole, UK). After staining, slides were
washed and counterstained in Mayer’s haematoxylin.

Cell culture
Colonic carcinoma cell lines (SW620, Caco-2 and HT-29) were
routinely maintained in Dulbecco’s Modified Eagles Medium
(DMEM), supplemented with 5% fetal calf serum (FCS) (both
Life Technologies Ltd, Paisley, UK). Experimental cultures were
carried out using phenol red-free DMEM supplemented with 5%
charcoal-stripped FCS in the presence of absence of treatments
(1–100 mM) which included: E1, E2, progesterone (Prg), dexam-
ethasone (DEX), dihydrotestosterone (DHT), testosterone (T)
(all Sigma) and 1,25-dihydroxyvitamin D3 (a kind gift from Dr M
Uskokovic, Hoffman LaRoche, Nuttley, New Jersey).

Measurement of 17β-HSD activity
Interconversion of E2 to E1 via 17β-HSD was assessed using previ-
ously reported methods (Hughes et al, 1997). Briefly, the substrates
used were 3H-oestradiol (3H-E2) (specific activity: 110 Ci/mmol;
Amersham, Little Chalfont, Buckinghamshire, UK) for measure-
ment of oxidative 17β-HSD activity (E2 to E1), and 3H-oestrone
(3H-E1) (specific activity; 80 Ci/mmol; Amersham) for reductive
interconversion of E2 to E1 via 17β-HSD (E1 to E2). Assays were
incubated in triplicate using substrate concentrations of 25 nM and
2 μM. Medium was changed to serum-free DMEM 2 hours prior to enzyme assay
and cells were then incubated in a further aliquot of serum-free medium
containing 3H-E2 or 3H-E1. Reaction mixtures were extracted
in chloroform and then separated on silica thin layer chromatography
(TLC) plates in chloroform:ethyl-acetate (80:20 v/v). Conversion
of tritiated steroid was measured using a Bioscan System 200
imaging TLC plate scanner (Bioscan Inc, Washington DC, USA),
and the fractional conversion of E1 to E2, or E2 to E1, calculated.
Residual cell monolayers were lysed and proteins analysed using
standard Biorad protein assay (Biorad, Hemel Hempstead, UK).
Activity was expressed as pmol product h−1 mg protein−1.

Analysis of cell proliferation
Colonic cells were incubated with 0.5 μCi 3H-thymidine (specific
activity 80 Ci mmol−1; Amersham) for the last 6 hours of culture
incubation. Unlabelled thymidine was added for the last 5 minutes
to displace any non-specific uptake of 3H-thymidine. Cells were
then washed in PBS and cellular proteins precipitated with cold
5% trichloroacetic acid. After removing the liquid layer, an aliquot
of 0.1 M sodium hydroxide was added to the cells, and radio-
activity in the resulting solubilized nuclear material was deter-
mined by scintillation counting. Data were reported as mean ±
standard deviation of radioactive counts per minute (cpm) (n = 4).

Analysis of 17β-HSD isozyme mRNA expression
RNA extraction and RT-PCR analysis
Total RNA was extracted from cultured cells using RNazol (AMS
Biotechnology, Witney, UK), according to an adapted guani-
dinium-isothiocyanate method (Hughes et al 1997). Reverse
transcription of RNA was performed using a Promega Reverse
Transcription System (Promega Corp., Madison, WI) using previ-
ously reported methods (Hughes et al 1997). PCR analysis of 17β-
HSD mRNA expression was carried out using the following
primers for 17β-HSD types 1 to 4: 17β-HSD1: (5’ primer) 5’TGG
CTT ATG AGA GAA GAT GTT GG3; (3’ primer) 5’CAT
GCC GGA CTT GTT GG3 (bp 1460–1809); 17β-HSD2: 5’TGG
CTT ATG AGA GAA GAT GTT GG3; (3’ primer) 5’AGG
CTT ATG CGA GAG TCT GG3; 3’ primer) 5’CAT
GCC GGT GAC GTA GTT GG3 (bp 445–1038); 17β-HSD4: 5’TGG
CTT ATG AGA GAA GAT GTT GG3; (3’ primer) 5’AGG
CTT ATG CGA GAG TCT GG3; 3’ primer) 5’CAT
GCC GGT GAC GTA GTT GG3 (bp 445–1038); 17β-HSD3:
5’ACA ATG TGC GAA GAA CC3; 5’GAA GTC CTT GCT
GGC TAA CG3 (bp 445–1038); 17β-HSD3: 5’ACA ATG
TGC GAA GAA CC3; 5’GAA GTC CTT GCT GGC
TAA CG3 (bp 445–1038); 17β-HSD3: 5’ACA ATG TGC
GAA GAA CC3; 5’GAA GTC CTT GCT GGC
TAA CG3 (bp 445–1038). PCR reactions were set up in PCR buffer containing
50 mM KCl, 10 mM Tris-HCl (pH 9.0) and 0.1% Triton X-100, 1.5 mM MgCl2,
0.2 μM of each dNTP, 0.5 μM (17β-HSD1, 2 and 3) or 0.4 μM
(17β-HSD4) of primers and 1 μ of Taq DNA polymerase.
Amplification of cDNA was performed using an initial denatura-
tion step of 95°C followed by either 30 cycles of 95°C (1 min);
60°C (1 min); 72°C (1 min); 72°C (1 min); 72°C (1 min); 72°C (1 min); 72°C (1
minute) 17β-HSD4). A final elonga-
tion step of 72°C for 7 minutes was also included.

Northern blot analysis of 17β-HSD expression
Northern blot analysis of 17β-HSD mRNA expression was carried
out using aliquote (10 μg) of total RNA from each cell line. RNA
was separated by denaturing gel electrophoresis and blotted onto
Hybond N nylon filters (Amersham). After fixation by UV irradia-
tion, filters were probed using previously reported methods
(Hughes et al, 1997), and then exposed to Dupont Cronex film
(Dupont/NEN, Boston) for various time periods, before develop-
ment of autoradiographs.

Western blot analysis
Colonic mucosae, tumour tissue and cell lines were homogenized
in the presence of the protease inhibitor PMSF (Sigma) (0.5 mM)
and then centrifuged at 4°C and 6500 rpm for 5 min. Aliquots of
the resulting supernatants, corresponding to cytoplasmic prepara-
tions, were then denatured at 95°C in 2% SDS, 10% glycerol,
62.5 mM Tris (pH 6.8) and size-separated on 10% SDS-PAGE
gels. Proteins were transferred to Immobilon P membrane (0.4
μm; Millipore Corp, Bedford, MA). The resulting membranes
were blocked by incubating overnight in PBS containing 10%
bovine serum albumin (Sigma). Immunoactivity was detected
by incubation with primary antibody (17β-HSD2 diluted 1:100;
17β-HSD4 diluted 1:200) followed by peroxidase-conjugated anti-
mouse secondary antibody (Amersham). The reaction detected by
enhanced chemiluminescence (ECL, Amersham Pharmacia
Biotech, Buckinghamshire, UK). The sizes of the reactive
immunoproteins were estimated by comparison to the mobility of
protein standards (Amersham).

Data analysis

Immunohistochemistry and Western blot analyses were carried out
using paired normal and tumour samples (n = 3). Immuno-
histochemistry data were shown as a single pair of representative
sections. Assays for 17β-HSD activity were carried out in tripli-
cate and cell proliferation assays in quadruplicate. Data from both
were reported as mean ± standard deviation (SD). Figures show
typical experiments that were successfully repeated at least three
times. Statistical calculations were performed using one-way
analysis of variance (ANOVA) with the statistical software
package Sigma-Stat3 (Jandel Scientific, Germany).

RESULTS

Expression of 17β-HSD2 and 4 in the colon

Immunocytochemical analysis of proteins for 17β-HSD2 and 4 in human
tissue was carried out using colon sections as well as posi-
tive and negative control tissues (Figure 1). Analysis of positive
control tissue (placenta) showed expression of 17β-HSD2 in
stromal cells but not syncytiotrophoblasts, while 17β-HSD4 was
detectable in both stromal and trophoblastic cells (Figure 1A and
1D). No staining was detected for either isozyme in testis (data not
shown). In the colon, 17β-HSD2 and 4 were found mainly in
colon epithelial cells with increased immunoreactivity towards
the luminal surface (Figure 1D and 1E). In contrast to normal
colonic epithelium, expression of 17β-HSD2 and 4 was relatively
weak in colonic mucosa adjacent to a tumour, and weaker still in
tumour tissue itself (Figure 1C and 1F).

Western blot analysis of 17β-HSD2 and 4 indicated that the
pattern of protein expression for these isozymes was different in
tumour biopsies when compared to paired normal mucosal tissue
(Figure 2). In normal mucosa and positive control tissue (placenta), 17β-HSD2 was expressed as a single protein species of
45 kDa. However, in each of the paired tumour samples an addi-
tional band of approximately 50 kDa was also detected. Similar
data were obtained following Western analysis of 17β-HSD4
(Figure 3). In control tissue (placenta) and normal colonic
mucosa a single protein species of 32 kDa was detected, with an
additional band of approximately 46 kDa in tumour specimens.
Statistical analysis showed no significant difference in expression
in normal and tumour tissue for both 17β-HSD2 and 17β-
HSD4 (P = 0.386 and 0.318 respectively).

Analysis of 17β-HSD activity in vitro

Further analysis of the expression and regulation of 17β-HSD2
and 4 in colonic epithelial cells was carried out using three colonic
cancer cell lines. Initial enzyme activity studies revealed a similar
pattern of oestrogen metabolism to that previously described in
primary colonic mucosa (English et al, 1999). In Caco-2, SW620
and HT-29 cells the predominant 17β-HSD activity was oxidative
conversion of E2 to E1. Using 50 nM E2 as substrate the highest
activity was observed in Caco-2 cells (52 ± 5.0 pmol h⁻¹ mg
protein⁻¹), followed by SW620 cells (23 ± 2.0 pmol h⁻¹ mg
protein⁻¹), and HT-29 cells (8 ± 1.0 pmol h⁻¹ mg protein⁻¹). Kinetic
analysis of 17β-HSD activity in SW620 cells indicated that the
affinity constant (Kₘ) was 400 nM E2, with a maximal enzyme
activity (Vₘₐₓ) of 190 pmol h⁻¹ mg protein⁻¹. Further enzyme
assays using lysates from SW620 cells demonstrated that the
conversion of E2 to E1 in these cells was dependent upon NAD+ as
a co-factor (data not shown). Reductive activity (conversion of E1
to E2) was highest in Caco-2 cells (8.4 ± 3.8 pmol h⁻¹ mg
protein⁻¹), followed by HT-29 (4.1 ± 2.3 pmol h⁻¹ mg protein⁻¹)
and SW620 (2.5 ± 1.1 pmol h⁻¹ mg protein⁻¹).

All three cell lines were used in further studies to investigate the
regulation of E2 inactivation in colonic cells. Cells were incubated
for 24 hours in charcoal-stripped, phenol red-free medium in the
presence of various steroid hormones (all at 100 nM). Data shown
in Figure 3 indicated that, following 24 h treatments, only 1,25D₃
and E₂, itself were able to modulate 17β-HSD activity. In Caco-2
and SW620, E₂ significantly inhibited oxidative 17β-HSD activity.
In contrast, in SW620 and HT-29 cells, 1,25D₃ potently stimulated
17β-HSD activity. Caco-2 cells showed a similar trend which was
not statistically significant at this time point. Further studies were
carried out to investigate these responses in more detail and
compare changes in 17β-HSD activity with effects on colonic cell
proliferation. Dose–response experiments using SW620 cells
confirmed the sensitive up-regulation of oxidative 17β-HSD
activity by 1,25D₃, as well as the inhibitory effects of E₂ (Figure
4). However, it was noted that low doses (1 nM) of E₂ and testos-
sterone (T) also produced a significant decrease in 17β-HSD
activity. Although these effects were observed after 24 hours, we
were unable to detect any significant changes in cell proliferation
until 72 hours of treatment; at this time point antiproliferative
effects were observed following treatment with 1,25D₃, or E₂
(Figure 4). Similar observations were also made using Caco-2
cells, and in both cell lines none of the treatments had a significant
effect on reductive 17β-HSD activity (E₁ to E₂ conversion) (data
not shown).

17β-HSD isozyme expression in colonic cancer cells

RT-PCR analyses indicated that transcripts for 17β-HSD1–4 were
detectable in all three cell lines (Figure 5A). However, Northern
blots probed with purified cDNAs generated by RT-PCR
confirmed the presence of mRNA for 17β-HSD2 and 4, but not
17β-HSD1 and 3. Data in Figure 5B show Northern blot analyses
of 17β-HSD2 and 4 mRNA expression in SW620 cells. Single
transcripts corresponding to the reported mRNA species for 17β-
HSD2 (1.3 kb) and 17β-HSD4 (3.0 kb) were detected. The expres-
sion of mRNA for both isozymes was inhibited by 24 hour treat-
ment with 1,25D₃, E₂, and E₂, but not progesterone (all at 100 nM).
Further analysis of the effects of 1,25D₃ indicated that lower doses
of the hormone (1 nM and 10 nM) also down-regulated mRNA
levels for 17β-HSD2 and 4 (data not shown). The effects of 1,25D₃ on expression of 17β-HSD2 and 4 were studied further using Western blots and isozyme-specific antibodies. Data in Figure 6 show that proteins corresponding to both isozymes were readily detectable in SW620 cells. In untreated cells two principal species were detected for 17β-HSD2 and 4. In each case the smallest protein band (45 kDa for 17β-HSD2, 32 kDa for 17β-HSD4) corresponded to the reported size of each isozyme. However, a larger species (approximately 50 kDa for 17β-HSD2, 46 kDa for 17β-HSD4) was also observed which was not present in positive control tissue (placenta). Treatment with 1,25D₃ (1–100 nM) for 24 h did not appear to have any effect on the expression of the protein species for either 17β-HSD2 or 4.

DISCUSSION

Sex hormones play a key role in the pathophysiology of some cancers. In particular, oestrogen has been shown to play a major
role in the development, progression and treatment of breast cancer (Colditz, 1998; Osborne, 1998). This has led to the use of synthetic receptor agonists such as tamoxifen as a treatment regime for ER-positive breast tumours (McDonnell, 1999). However, another approach to this type of therapy has been to regulate ER responses by modulating the availability of endogenous ER ligand within tumour tissue. Local synthesis of oestrogens as a result of endogenous aromatase, 17β-HSD and oestrone sulphatase activity has been demonstrated in endometrial (Maentausta et al, 1992), prostate (Elo et al, 1996) and breast tumours (Pasqualini et al, 1996). As a consequence, novel anti-cancer therapies have been aimed at controlling the local build-up of oestrogens in tumours by inhibiting the activity of specific steroidogenic enzymes, particularly aromatase and oestrone sulphatase (Brodie et al, 1999). Epidemiological evidence suggests that the incidence of colorectal cancer is also influenced by sex hormones (Langman, 1967; Michael and Potter, 1982; Calle et al, 1995; Newcomb and Storer, 1995; Persson et al, 1996). In common with breast cancer, normal and neoplastic colonic mucosae show differential responses to E2, although this does not appear to be due to dysregulation of ER expression (Singh et al, 1994, 1998).

In more recent studies we have postulated that enhanced oestrogen responsiveness in colonic tumours may be due to decreased inactivation of E2 by isozymes of 17β-HSD (English et al, 1999). Immunohistochemical data presented here indicate that 17β-HSD2 and 4 are localized predominantly within the luminal surface of the normal colonic epithelium, supporting previous reports of 17β-HSD2 expression in the gastrointestinal epithelium of the mouse (Mustonen et al, 1998), and human fetus (Takeyama et al, 2000). It would therefore appear that oestrogen inactivation by locally expressed 17β-HSD isozymes is a feature of normal gastrointestinal biology. The most likely function of these isozymes within colonic epithelial cells is that they form part of a mechanism that protects the colon against environmental or bacterially-synthesized steroids. In recent studies, 17β-HSD2 has been shown to metabolize several orally administered steroidal compounds, including those used in oral contraceptives and HRT (Puranen et al, 1999). It was also interesting to note the presence of 17β-HSD2 and 4 in colonic crypts. Pluripotent stem cells occur in the crypt base and daughter cells migrate upwards undergoing a series of divisions before full maturation. In view of the mitogenic nature of E2, this suggests that 17β-HSD2 and 4 may also play a role in modulating epithelial cell development.

Western blot analysis of normal colon of biopsy specimens confirmed the specificity of the 17β-HSD antisera used for immunohistochmistry studies. However, in tumour material and colonic cancer cell lines, additional larger protein species for 17β-HSD2 and 17β-HSD4 were also detected. Previous studies of 17β-HSD expression in breast cancer tissue, indicated the presence of enzymes with molecular weights in the range of 50–80 kDa in addition to a 35 kDa enzyme with different properties from those of the 35 kDa enzyme with the same molecular weight in adipose tissue (Mann et al, 1999). To date no similar studies have been carried out on colorectal cancer tissue. However, it is possible to speculate that the presence of tumour-specific hydroxysteroid dehydrogenase proteins may alter co-factor availability, or result in the quenching of specific substrates. This may help to explain the decreased capacity for oestrogen inactivation that we have described previously in colonic tumours (English et al, 1999). It is also important to note that 17β-HSD4 is a 80 kDa multi-domain protein, which has previously been reported to undergo processing to the 32 kDa protein detected in target tissues. Evidence for further processing into additional fragments has been demonstrated in rat tissue by peroxisome proliferator chemicals (Fan et al, 1998). Tumour specific processing of this particular isozyme may therefore occur by an as yet unidentified mechanism, resulting in species that competitively alter oestrogen inactivation.

Experiments in vitro confirmed that the overall pattern of oestrogen metabolism in these cells was similar to that observed with primary human colonic tissue, namely 17β-HSD-mediated inactivation of E2 to E1. Levels of E2 metabolism varied between the cell lines but only the antiproliferative agent 1,25D3 and T, and E1 had any significant effect on enzyme activity. E1-mediated regulation of oxidative 17β-HSD activity has not previously been reported in other tumour tissues although, in breast cancer cells, E1 has been shown to significantly inhibit 17β-HSD activity (Mehta and Gupta, 1993; Peltoketo et al, 1996). The decreased 17β-HSD activity observed following treatment of Caco-2 and SW620 cells.
Figure 4  Effects of steroid hormones on 17β-HSD activity and cell proliferation in SW-620 cells. Cell were treated with 1–100 nM concentrations of oestradiol (E$_2$), oestrone (E$_1$), testosterone (T), progesterone (Prg), or 1,25-dihydroxyvitamin D$_3$ (1,25D$_3$) for either 24 hours (17β-HSD activity) or 72 hours (cell proliferation). Data shown are means ± SD (n = 3 for 17β-HSD activity, n = 4 for ³H-thymidine). * = significantly different from control cells, $P < 0.05$; ** = significantly different from control cells, $P < 0.01$.

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with E₂ and E₁ correlated with decreased expression of mRNAs for both 17β-HSD2 and 4. In contrast, although 1,25D₃ also downregulated the expression of mRNAs for 17β-HSD2 and 4, its overall effect on enzyme activity in HT-29 and SW620 cells was to stimulate E₂ inactivation. It therefore seems likely that there are different mechanisms involved in regulating oestrogen metabolism in colonic cells. The effect of E₁ and E₂ appears to be due to direct inhibition of 17β-HSD transcription. In contrast, responses to 1,25D₃ were similar to those we have previously described for HL60 leukaemic cells and normal human keratinocytes; treatment with 1,25D₃ produced a similar rapid induction of E₂ inactivation that was also associated with decreased 17β-HSD₄ mRNA expression (Hughes et al, 1997; Mountford et al, 1999). These observations, together with the Western analyses presented here suggest that the stimulation of oestrogen metabolism by 1,25D₃ in colonic cancer cells may not be mediated via direct regulation of 17β-HSD4 mRNA expression. The contribution of other 17β-HSD isozymes to cell proliferation and differentiation remains unclear. The type 1, 3, 5 and 7 isozymes show predominantly reductive (E₁ and E₂) activity and are therefore unlikely candidates, particularly as we were unable to demonstrate significant amounts of mRNA for 17β-HSD1 and 3 in colonic mucosae and cancer cell lines. However, conversion of E₁ to E₂ was detectable in these samples. Although this was relatively low compared to oxidative activity (E₂ to E₁ conversion) the possibility remains that a variety of 17β-HSD enzyme activities are found in the colon, including possible novel isozymes of 17β-HSD.

In previous reports we have postulated that decreased E₂ inactivation may make a significant contribution to the enhanced cell proliferation associated with colonic tumours (English et al, 1999). Here we have shown that the antiproliferative effects of 1,25D₃ are preceded by sensitive up-regulation of E₂ inactivation. In contrast, known mitogenic agents such as E₁ demonstrated...
apparent autocrine inhibition of 17β-HSD activity. Similar inhibition of E2 inactivation was also observed with low doses of E1 or T, although the precise mechanism for this remains unclear. Other groups have demonstrated pro-proliferative responses to E2 in colonic cells (Xu and Thomas, 1994; Di Domenico et al., 1996). Although we were unable to confirm this effect of E2 in our models systems, it was interesting to note that treatment with E2 at concentrations of 100 nM or greater significantly inhibited cell proliferation. These data support previous studies in which we have shown that generation of E2 through the action of 17β-HSD isozymes may act as a novel component of cell differentiation processes (Hughes et al., 1997; Mountford et al., 1999). Thus, stimulation of 17β-HSD activity by established differentiating agents may not only decrease the availability of mitogenic E2 but could also enhance local concentrations of antiproliferative E1.

A putative role for E1, as a novel antiproliferative agent may help to resolve the paradox associated with the epidemiology of oestrogens and colon cancer. On the one hand, an increased ratio of circulating E2/E1 in postmenopausal women is associated with decreased risk of colon cancer. Conversely, HRT has also been shown to protect against colon cancer. A clear relationship between the composition of HRT and effects on colon cancer has yet to be fully described. However, it is important to recognize that the principal prescribed HRTs (Premarin/Prempak C) contain delta-8-E1 sulphate rather than E2. Consequently, within the colon, the most likely metabolite that will be derived from this treatment is E1 and not E2. Importantly, the immunohistochemistry data presented here indicate that orally administered HRT regimes that contain E1 may lead to the generation of increased local levels of E1 in the colon. The discrete expression of 17β-HSD2 and 4 in the epithelial cells of the colonic mucosa provides an efficient barrier for inactivation of ingested steroids including the E2 present in some oral HRT regimes. Other components of HRT preparations such as progesterone appeared to be without effect in terms of 17β-HSD expression/activity, or cell proliferation. It is possible that progesterone itself may be subject to local metabolism but as yet the potential effects of this on colonic cells remain unclear.

In summary we have presented further evidence for the important role of 17β-HSD isozymes as modulators of oestrogen effects in the colon. Localization of 17β-HSD2 and 4 to the epithelial layer of the colon and the presence of these isozymes in epithelial cell lines highlights a potential role as protective barrier against ingested oestrogens. In addition, regulatory studies have confirmed the association between colonic cell proliferation and 17β-HSD activity, further emphasizing the possible importance of E2 inactivation in the aetiology of colon cancer. Further studies to define the cellular impact of HRT, and to identify other 17β-HSD isozymes in the colon will help to clarify the role of hormone metabolism in colonic cell development, but may also provide a novel target for improved therapies for colonic cancer.

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REFERENCES

Brodie A, Lu Q and Long B (1999) Aromatase and its inhibitors. J Steroid Biochem Mol Biol 69: 205–210
Calle EE, Miracle-McMahill HL, Thun MJ and Heath Jr CW (1995) Estrogen replacement therapy and risk of fatal colon cancer in a prospective cohort of postmenopausal women. J Natl Cancer Inst 87: 517–523
Colditz GA (1998) Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. J Natl Cancer Inst 90: 814–823
Di Domenico M, Castoria G, Bilancio A, Migliaccio A and Auricchio F (1996) Estradiol activation of human colon carcinoma-derived Caco-2 cell growth. Cancer Res 56: 4516–4521
Eli JP, Akinola LA, Poutanen M, Vihko P, Kylloinen AP, Lakkunen O and Vihko R (1997) Characterization of 17β-hydroxysteroid dehydrogenase isozyme expression in benign and malignant human prostate. Int J Cancer 66: 37–41
English MA, Kane CF, Cruickshank N, Langman MJS, Stewart PM and Hewison M (1999) Loss of estrogen inactivation in colon cancer. J Clin Endocrinol Metab 84: 2080–2085
Fun L-Q, Cattley RC and Corton JC (1998) Tissue-specific induction of 17β-hydroxysteroid dehydrogenase type IV by peroxisome proliferator chemicals is dependent on the peroxisome proliferator-activator receptor α. J Endocrinol 158: 237–246
Forniticheva J, Baker ME, Anderson E, Lee GY and Aziz N (1998) Characterization of keto, a new 17 beta-hydroxysteroid dehydrogenase, and its expression in gonadal tissues. J Biol Chem 273: 22664–22671
Francavilla A, Di Leo A, Polimeni L, Conte D, Barone M, Fanizza G, Chiumarulo C, Ricco G and Rubino M (1987) Nuclear and cytosolic estrogen receptors in human colon carcinoma and in surrounding non-cancerous colonic tissue. Gastroenterology 93: 1301–1306
Hughes SV, Robinson E, Bland R, Lewis HM, Stewart PM and Hewison M (1997) 1,25-Dihydroxyvitamin D3 regulates estrogen metabolism in cultured keratinocytes. Endocrinol 138: 3711–3718
Jacobs E, Watson SA, Hardcastle JD and Robertson IFR (1996) Oestrogen and progesterone receptors in gastrointestinal cancer cell lines. Eur J Cancer 32: 2348–2353
Langman MJS (1967) Current trends in the epidemiology of cancer of the colon and rectum. Proc R Soc Med 60: 210–212
Maantausta O, Boman K, Isomaa V, Stendahl U, Backstorn T and Vihko R (1992) Immunohistochemical study of the human 17 beta-hydroxysteroid dehydrogenase and steroid receptors in endometrial adenocarcinoma. Cancer 70: 1551–1555
Mann VZ, Newton CJ and Tait GH (1991) 17β-hydroxysteroid dehydrogenases in human breast tissues: purification and characterization of soluble enzymes and the distribution of particulate and soluble forms in adipose, non-adipose and tumour tissues. J Mol Endocrinol 7: 45–55
McDonnell DP (1999) The molecular pharmacology of SREMs. Trends Endocrinol Metab 10: 301–311
McMichael AJ and Potter JD (1982) Colon cancer and sex hormones. Lancet I: 1190–1192
Mehta RR and Das Gupta TK (1993) Regulation of 17 beta-hydroxysteroid dehydrogenase in a newly-established human breast carcinoma cell line. Steroids 58: 301–311
Mountford JC, Bunce CM, Hughes SV, Drayson MT, Webb D, Brown G and Hewison M (1999) Estrogen potentiates myeloid cell differentiation: A role for 17β-hydroxysteroid dehydrogenase in modulating haemopoiesis. Exp Hematol 27: 451–460
Moutson MJ, Poutanen MH, Kellomkumpu S, de Launoit Y, Isomaa VV, Vihko RK and Vihko R (1998) Mouse 17 beta-hydroxysteroid dehydrogenase type 2 mRNA is predominately expressed in hepatocytes and in surface epithelial cells of the gastrointestinal and urinary tracts. J Mol Endocrinol Metab 78: 197–204
Newcomb PA and Storer BE (1995) Postmenopausal hormone use and risk of large bowel cancer. J Natl Cancer Inst 87: 1067–1071
O’Neill JS, Elton RA and Miller WR (1988) Aromatase enzyme in adipose-tissue from breast quadrants – a link with tumour site. BMJ 296: 741–743
Osborne CK (1998) Steroid hormone receptors in breast cancer management. British J Cancer (2000) 83(4), 550–558
© 2000 Cancer Research Campaign
Peltoketo H, Vihko P and Vihko R (1999) Regulation of estrogen action: Role of 17 beta-hydroxysteroid dehydrogenase. *Vitamins and Hormones* **55**: 353–398

Persson I, Yuen J, Bergvist L and Schairer C (1996) Cancer incidence and mortality in women receiving estrogen and estrogen-progestin replacement therapy – long term follow-up of Swedish cohort. *Int J Cancer* **67**: 327–332

Puranen TJ, Kurkela RM, Lakkakorpi JT, Poutanen MH, Itaranta PV, Melis JPJ, Ghosh D, Vihko RK and Vihko PT (1999) Characterization of molecular and catalytic properties of intact and truncated human 17\(\beta\)-hydroxysteroid dehydrogenase type 2 enzymes: intracellular localization of the wild-type enzyme in the endoplasmic reticulum. *Endocrinol* **140**: 3334–3341

Sasano H, Frost AR, Saitoh R, Harada N, Poutanen M, Vihko R, Bulun SE, Silverberg SG and Nagura H (1996) Aromatase and 17 beta-hydroxysteroid dehydrogenase type 1 in human breast carcinoma. *J Clin Endocrinol Metab* **81**: 4042–4046

Singh S, Paraskeva C, Gallimore PH, Sheppard MC and Langman MJS (1994) Differential growth response to oestrogen of premalignant and malignant colonic cell lines. *Anticancer Res* **14**: 1037–1042

Singh S, Poulsom R, Hanby AM, Rogers LA, Sheppard MC and Langman MJS (1998) Expression of oestrogen receptor and oestrogen-inducible genes pS2 and ERD5 in large bowel mucosa and cancer. *J Pathol* **184**: 153–160

Takeyama J, Suzuki T, Hirasawa G, Muramatsu Y, Nagura H, Inuma K, Nakamura J, Kimura K, Yoshihama M, Harada N, Andersson S and Sasano H (2000) 17\(\beta\)-hydroxysteroid dehydrogenase type 1 and 2 expression in the human fetus. *J Clin Endo Metab* **85**: 410–416

Xu X and Thomas ML (1994) Estrogen receptor-mediated direct stimulation of colon cancer cell growth in vitro. *Mol Cell Endo* **105**: 197–201