Alteration of oestradiol metabolism in myc oncogene-transfected mouse mammary epithelial cells

NT Telang1, F Arcuri2, OM Granata3, HL Bradlow1, MP Osborne1 and L Castagnetta2,3

1Strang Cancer Research Laboratory, The Rockefeller University, New York, USA; 2Hormone Biochemistry Laboratory, University of Palermo School of Medicine, Palermo, Italy; 3Experimental Oncology and Molecular Endocrinology Units, Palermo Branch of IST – Genova, c/o ‘M. Ascoli’ Cancer Hospital Centre, Palermo, Italy.

Summary Targeted overexpression of the c-myc oncogene induces neoplastic transformation in immortalized, non-tumorigenic mouse mammary epithelial cells (MMEC). Experiments in the present study were conducted to examine whether cellular transformation induced by c-myc oncogene is associated with altered metabolism of 17β-oestradiol (E₂). The parental, MMEC and the stable c-myc transfected (MMEC/myc₃) cell lines were compared for major oestrogen metabolic pathways, namely E₂ and E₁ interconversion, and C2- and C16α-hydroxylation by both high-pressure liquid chromatography (HPLC) analysis and the 3H release assay using specifically labelled [C2-³H]E₂ or [C16α-³H]E₂. The reductive conversion of E₂ to E₁ was about 14-fold and 12-fold higher than the oxidative conversion of E₂ to E₁ in MMEC and MMEC/myc₃ cells respectively. However, in MMEC/myc₃ cells, both reductive and oxidative reactions were decreased by about 32% and 12% relative to those seen in the parental MMEC cells (P = 0.0028). The extent of C16α-hydroxylation was increased by 164.3% (P < 0.001), with a concomitant 48.4% decrease (P < 0.001) in C2-hydroxylation in MMEC/myc₃ cells; this resulted in a fourfold increase in the C16α/C2 hydroxylation ratio in this cell line. Thus, a persistent c-myc expression, leading to aberrant hyperproliferation in vitro and tumorigenesis in vivo, is associated with an altered oestrogen metabolism. However, it remains unclear whether this represents a result of oncogene expression/activation or is rather a consequence of phenotypic transformation of the cells.

Keywords: c-myc expression; oestradiol metabolism; mammary carcinogenesis

It is well recognized that oestrogens exert a profound influence on mammary epithelial cell growth, differentiation and neoplastic transformation (Fishman et al., 1980; Prudhomme et al., 1984; Maurais-Jarvis et al., 1986; Siiteri et al., 1986). The molecular and biochemical mechanisms important for oestrogen responsiveness and the influence of altered oestrogen responsiveness on mammary cell carcinogenesis, however, are not fully understood. Our earlier studies on immortalized, non-tumorigenic mouse mammary epithelial cell lines have shown that transfection of the cell line with myc or Ras oncogenes results in neoplastic transformation. Before tumorigenesis in vivo, myc as well as Ras transfectants exhibit aberrant hyperproliferation in vitro (Telang et al., 1990, 1991; Suto et al., 1992). Thus, persistent oncogene expression and aberrant hyperproliferation may represent molecular and cellular biomarkers for neoplastic transformation.

The conventionally recognized markers for oestrogen responsiveness include (1) functional activity of oestrogen receptor as determined by receptor–ligand binding; (2) modulation of transcriptional activity, growth and induction of progesterone receptor (Prudhomme et al., 1984; Maurais-Jarvis et al., 1986; Siiteri et al., 1986; Dubik and Shiu, 1992); (3) reversible suppression of growth by hormone antagonists (Clark et al., 1977; Maurais-Jarvis et al., 1986; Siiteri et al., 1986; Dubik and Shiu, 1992). Our recent studies on Ras-initiated MMEC/pH06T cells as well as on Ras-transformed T1/Prl cells have shown that the oncogene-initiated and tumorigenically transformed cells are responsive to E₂, as also shown by their ability to metabolize the hormone and by reversible growth inhibition upon treatment with the non-steroidal anti-oestrogen tamoxifen (Telang et al., 1991; Suto et al., 1992).

Mammary epithelial cells initiated independently with chemical carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) and Ras oncogenes exhibit elevated oestrogen metabolism via the formation of C16α-hydroxylated metabolites (Telang et al., 1991, 1992; Suto et al., 1993). In addition, it has been proposed that the oestrogen-mediated stimulation of growth of breast tumour-derived MCF7 cells may involve transactivation in the c-myc promoter region (Dubik and Shiu, 1992). It is not clear whether these molecular and metabolic alterations characterize the initiated phenotype or represent a late-occurring, post-initiation event in a rapidly growing tumour cell phenotype.

The experiments in the present study were designed to (1) establish the validity of oestrogen metabolism as an endocrine biomarker for tumorigenic transformation in myc oncogene-transfected mammary epithelial cells; and (2) elucidate the relationship between myc expression, the extent of cellular metabolism of E₂, and tumorigenic transformation. To this end, we have compared the extent of E₂ metabolism in the spontaneously immortalized, non-tumorigenic mammary epithelial cell line MMEC and the stable transfected MMEC/myc₃, that expresses activated c-myc proto-oncogene and is highly tumorigenic.
MATERIALS AND METHODS

Cell lines

The non-tumorigenic mammary epithelial cell line MMEC was established from the mammary tissue of a 6- to 8-week-old virgin female BALB/c mouse. The stable myc transfectant MMEC/myc3 was obtained by transfection of a recombinant myc construct comprising the second and third coding exons expressed from a MLV-LTR promoter in a NEO-derived vector. DM-myc, and expansion of a stable G-418-resistant clone in the presence of 400 µg mL⁻¹ G-418, which is cytotoxic to the parental MMEC (Telang et al, 1990). Routinely, MMEC and MMEC/myc3 cells were maintained in DME/F12 medium supplemented with heat-inactivated 10% fetal bovine serum, 4 mM l-glutamine and 5 µg mL⁻¹ insulin. The stock MMEC/myc3 cells were maintained in the presence of 400 µg mL⁻¹ G-418 to eliminate the accumulation of spontaneous revertants. For the experiments measuring the cellular metabolism of E₂, the parental MMEC and the myc transfectant MMEC/myc3 cells were cultured in the absence of G-418 for 72 h to exclude the possibility of interference of the antibiotic with E₂ metabolism.

Growth characteristics

The growth pattern of MMEC and MMEC/myc3 cells was determined by a trypan blue exclusion test and haemocytometer counts for viability and growth. In addition, population doubling time (PDT), anchorage-independent growth (AIG) and mammary fat pad tumorigenicity assays were performed according to the published procedures (Ganguly et al, 1982; Telang et al, 1979, 1990, 1991). PDT was determined from the linear portions of the growth curves generated for at least 4 days after plating 5 × 10⁵ cells cm⁻². AIG was evaluated by determining the number of anchorage-independent, tridimensional colonies formed in 0.33% agar at an initial seeding of 1.0 × 10⁶ cells, and the data were expressed as colony-forming efficiency (CFE, %) at day 14. Tumorigenicity was determined by counting the number of palpable tumours in mammary fat pads after the injection of 1.0 × 10⁶ cells as a single 20-µl bolus into parenchyma-free mammary fat pads of syngeneic recipients.

c-myc expression and oestrogen receptor content

The relative expression of transfected (exogenous) c-myc oncogene was determined by the Northern blot analysis of RNA from the parental MMEC and the stable transfectant MMEC/myc3 cell lines essentially according to the method published previously (Telang et al, 1990, 1991). A [³²P]-labelled, nick-translated 1.8 kb SacI fragment of human c-myc spanning the second exon was used as the probe. The blots were scanned and the hybridization signal was quantified by arbitrary scanning units (ASU) normalized to 20 µg of RNA loaded. The oestrogen receptor content of MMEC and MMEC/myc3 cells was determined by the ligand binding assay (Castagnetta et al, 1992) and was expressed as fmol of oestrogen receptor protein (ERP) per 10 µg of DNA.

HPLC analysis of 17β-HSD activity

The relative extent of 17β-HSD activity was determined by measuring the interconversion of E₁ and E₂ in the two cell lines. T-25 flasks containing approximately 1.0 × 10⁶ MMEC and MMEC/myc3 cells were incubated for 24 h in serum-free, phenol red-free and G-418-free medium in the presence of 2 µCi mL⁻¹ [6,7-³H(N)]E₂ (specific activity 42.3 Ci mmol⁻¹), final concentration 4.6 × 10⁻⁸ M) or 2 µCi mL⁻¹ [6,7-³H(N)]E₁ (specific activity 41.9 Ci mmol⁻¹, final concentration 4.8 × 10⁻⁸ M). The incubation medium was collected, and 1-ml aliquots were extracted with 9:1 ethyl-ether:acetone. The extracts were analysed by reverse-phase HPLC (C18 column, 4.6 i.d. × 250 mm) under isocratic conditions (acetonitrile: 0.05 M citric acid, 40:60) at a flow rate of 1 ml min⁻¹ using a computer-aided optimized mobile phase (D’Agostino et al, 1985; Castagnetta et al, 1986). The detection of E₁ metabolites was carried out using a UV detector and a three-channel radiometric detector, both on-line to HPLC as described previously.

Table 1 Biomarker status of MMEC and MMEC/myc3 cells

| Type of biomarker          | MMEC | MMEC/myc3 |
|----------------------------|------|-----------|
| G418 resistance            | –    | +         |
| C-myc expression           | –    | 15.0 ± 2.6|
| Oestrogen receptor         | 12.5 ± 3.9 | 5.0 ± 1.7 |
| Population doubling        | 24.3 ± 0.5 | 18.3 ± 0.1 |
| Anchorage independence     | 0.01 ± 0.005 | 1.33 ± 0.075 |
| Tumorigenicity             | –    | +         |

*Growth in 400 µg mL⁻¹ G418. *Arbitrary scanning units for 2.8 kb (exogenous) transcript hybridizing to [³²P]-labelled c-myc probe. *Fmol ER protein per 10 µg DNA. *Percentage colony forming efficiency 0.33% agar. *Tumour formation after mammary fat pad transplantation.

Table 2 Effect of c-myc oncogene expression in 17β-hydroxy steroid dehydrogenase (17β-HSD) activity in mammary epithelial cells

| Cell line      | E₁ formed | E₂ formed |
|----------------|-----------|-----------|
| MMEC           | (n = 12)  | (n = 11)  |
| MMEC/myc3      | 22.14 ± 2.72 | 1.47 ± 0.13 |
|                | 15.14 ± 3.39 | 1.30 ± 0.13 |
|                | d.f. 11    | d.f.10    |
|                | f = 1.46   | f = 3.40   |
|                | P = 0.0028* | P = 0.05* |

* Determined after 24 h incubation with [6,7-³H(N)]E₂ or [6,7-³H(N)]E₁ and HPLC analysis of conversion products. *Two-tailed Student t-Test. Values represent mean ± s.d. pmol 10 µg⁻¹ DNA.
(Castagnetta et al., 1986, 1991). The cells were lysed in 0.1% sodium dodecyl sulphate (SDS), and DNA content was determined (Carruba et al., 1994). The resulting data were normalized for total radioactivity and expressed as pmol 10 mg-1 cellular DNA or fmol ml-1 after correction for equal cell numbers.

Separate experiments were carried out to inspect the time and dose dependence of oestrogen metabolism in both MMEC and MMEC/myc3 cells. To this end, 5 x 10^5 cells were incubated in the presence of 1 nM tritiated E2 for 3, 6, 12, 24, 48 and 72 h or exposed to increasing concentrations (0.1, 1, 10 and 100 nM) of

![Figure 2 Time course of E2 metabolism in MMEC (A) and MMEC/myc3 (B) cells. Cells (5 x 10^5) were incubated in the presence of 1 nM [6,7,3H(N)]E2 for 3, 6, 12, 24, 48 and 72 h. Each data point represents the mean ± s.d. of duplicate experiments, performed in triplicate, after correction for equal cell numbers. (●) E2; (○) E2 - OHE1; (△) 16αOHE2.](image)

![Figure 3 Dose-dependent E2 conversion to E2 in MMEC and MMEC/myc3 cells. Cells were incubated for 24 h in the presence of increasing concentrations of [6,7,3H(N)]E2. Percentage values represent the mean of triplicate determinations corrected for total radioactivity and cell numbers. (■) unconverted E2; (●) E2 formed.](image)

the same radioactive oestrogen for 24 h, using exactly the same experimental conditions and procedures described above.

**Radiometric assay for E2 metabolism**

The relative extent of E2 metabolism via the C2- and C16α-hydroxylation pathways was measured by determining 3H2O formation in cells incubated for 48 h in the presence of [C2-3H]E2, or [C16α-3H]E2 (final concentrations 5.6 x 10^4 d.p.m., 8.0 x 10^4 m) in a medium lacking serum, phenol red and G-418. Aliquots of 500 μl of incubation medium were diluted to 3.5 ml with water, and the lyophilized sublate was counted for 3H radioactivity in a liquid scintillation counter (Telang et al., 1991, 1992; Suto et al., 1992, 1993). The 3H release from [C2-3H]E2, or [C16α-3H]E2, to form 3H2O provides an indirect measurement of regiospecific hydroxylation of the steroid leading to the stoichiometric formation of 2-hydroxyestrone (2-OHE1) or 16α-hydroxyestrone (16α-OHE1) (Fishman and Martucci, 1980; Fishman et al., 1980, 1995; Telang et al., 1991, 1992; Suto et al., 1992, 1993; Telang, 1996).

**Statistical analysis**

The data were analysed for statistical significance of the differences between cell types and treatment groups by unpaired two-tailed Student t-test, using the Statview 4.01 statistical software. Probability values of less than 0.05 were considered significant.

**RESULTS**

**Growth characteristics of MMEC and MMEC/myc3 cells**

The proliferative status, including AIG and tumorigenic potential, of MMEC and MMEC/myc3 cells, is presented in Table 1. The MMEC cell line exhibited toxicity to the aminoglycoside antibiotic
Table 3  Oncogene-mediated alteration of 17β-oestradiol (E2) metabolism in mouse mammary epithelial cells

| Cell line | E2 metabolism* | 2-OHE, formed (n = 12) | 16α-OHE, formed (n = 12) | C16α/C2 ratio |
|-----------|----------------|------------------------|--------------------------|---------------|
| MMEC      |                | 44.29 ± 5.71           | 20.00 ± 2.86             | 0.45          |
| MMEC/myc3 |                | 22.86 ± 2.86           | 52.86 ± 7.14             | 2.31          |

* Determined by 3H2O formed after a 48 h incubation with [C2-3H]E2 or [C16α-3H]E2. Two-tailed Student t-test; values are mean ± s.d. fmol 10 μg−1 DNA.

G-418, lacked the expression of exogenous myc-specific 2.8 kb RNA transcript, exhibited a population doubling time of 24.3 ± 0.5 h, lacked anchorage-independent growth in vitro and lacked the ability to form tumours when transplanted into syngeneic recipients. These cells, however, exhibited a persistent ability for ductal morphogenesis at the transplant site (data not shown). In contrast, the MMEC/myc3 cell line did not exhibit any G-418 cytotoxicity, expressed the exogenous 2.8 kb transcript (15.0 ± 2.6 ASU 20 μg−1 RNA) and showed a shorter population doubling time of 18.3 ± 0.1 h (d.f. 5, t = 3.40, P = 0.01). Furthermore, MMEC/myc3 cells also showed a 132-fold increase in AIG relative to that observed in MMEC and were highly tumorigenic, exhibiting a 90% tumour incidence at 12 weeks after transplantation (data not shown). These results essentially confirm our earlier report (Telang et al, 1990), suggesting that the expression of activated c-myc confers neoplastic transformation to mammary epithelial cells. In addition to overexpression of exogenous c-myc, the MMEC/myc3 cells exhibited a substantial reduction in oestrogen receptor levels. Thus, whereas the oestrogen receptor content of parental MMEC was 12.5 ± 3.9 fmol 10 μg−1 DNA, it was decreased to 5.7 ± 1.7 fmol 10 μg−1 DNA (d.f. 5, t = 4.07, P = 0.001) in the transfected MMEC/myc3 cells, resulting in about a 60% reduction of ERα levels.

The experiment designed to establish the biological significance of altered E2 metabolism examined whether treatment of MMEC/myc3 cells with oestrogen metabolites 16α-OHE, or 2-OHE, affects aberrant proliferation as shown by anchorage-independent growth. Treatment of MMEC/myc3 cells with 16α-OHE, resulted in about a 152% increase (d.f. 11, t = 4.07, P = 0.002) in anchorage-independent colony formation. In contrast, treatment with 2-OHE resulted in a 12.6% decrease (d.f. 11, t = 3.41, P = 0.01) in anchorage-independent colony formation.

17β-HSD activity in MMEC and MMEC/myc3 cells

The effect of c-myc oncogene on intrinsic 17β-HSD activity was evaluated by comparing the relative extent of interconversion of E2 and E1 in MMEC and MMEC/myc3 cells. It is clear from the data presented in Table 2 that the reductive pathway of E2 to E1 dominates over the opposing oxidative pathway of E1 to E2 conversion, the reductive reaction being about 14-fold and 12-fold greater than the oxidative conversion in MMEC and MMEC/myc3 cells respectively. However, in MMEC/myc3 cells, both reductive and oxidative reactions are found to be significantly reduced, being about 32% (d.f. 11, t = 4.16, P = 0.0028) and 12% (d.f. 10, t = 3.40, P = 0.05) lower relative to those observed in MMEC cells.

Typical HPLC profiles of oestrogen metabolism in MMEC and MMEC/myc3 cells are illustrated in Figure 1.

Time course experiments (3–72 h) were specifically designed to compare E2 conversion to E1 in parental MMEC and c-myc-transfected cells. As shown in Figure 2, the extent of the reductive pathway of 17β-HSD is significantly reduced (from three- to 4.5-fold) in MMEC/myc3 cells. It is of interest that maximum E1 formation in the latter cell line was observed at 24 h (15.3%) or 48 h (20.4%), whereas it was steadily increasing with time in MMEC cells. Consistency in DNA values and cell counts was ensured for reproducibility of data.

Parallel experiments carried out on MMEC and MMEC/myc3 cells using increasing precursor concentrations (from 0.1 up to 100 nm) showed that the proportion of E1 formed remained relatively unchanged using either 1, 10 or 100 nm E2 in both MMEC (33–35%) and MMEC/myc3 cells (8–10%), whereas it was remarkably greater (46% in MMEC cells and 17% in MMEC/myc3 cells) at the lowest E2 concentration (0.1 nm) used (see Figure 3).

Nevertheless, the extent of E1 formation was again significantly (from 2.6- to 4.6-fold lower) in MMEC/myc3 cells with respect to the parental MMEC cells.

E2 hydroxylation in MMEC and MMEC/myc3 cells

The oestrogen metabolism was compared by radiometric determination of the relative extent of E2 conversion via the C2- and C16α-hydroxylation pathways (Table 3). The two cell lines exhibited persistent metabolic competence to convert E2. In parental MMEC cells, the extent of conversion of [C2-3H]E2 and of [C16α-3H]E2 was 0.32 ± 0.04% and 0.14 ± 0.02% (per 106 cells) respectively (mean ± s.d., n = 12). In MMEC/myc3 cells, the extent of conversion of [C2-3H]E2 was decreased to 0.16 ± 0.02%, while that of [C16α-3H]E2 was increased to 0.37 ± 0.05%. To maintain consistency with the data from the experiments on interconversion of E2 and E1, the data from E1 metabolism are expressed as amounts of 2-OHE, and 16α-OHE, formed. The data presented in Table 3 demonstrate clearly that MMEC/myc3 cells exhibit about a 164.5% increase (d.f. 11, t = 5.25, P = 0.001) in 16α-OHE formation, with a concomitant 48.3% decrease (d.f. 11, t = 4.23, P = 0.001) in 2-OHE formation. This results in a fourfold increase in the C16α/C2 hydroxylation ratio.

DISCUSSION

Altered endocrine status of the mammary tissue plays an important role in the expression of tumorigenic phenotype (Telang et al, 1979; Ganguly et al, 1982; McCormick et al, 1982; Mauvais-Jarvis et al, 1986; Sitieter et al, 1986; Welsch, 1987; Castagnetta et al, 1992; Fishman et al, 1995; Telang, 1996). The experiments designed in the present study have used the immortalized, non-tumorigenic MMEC and the tumorigenic myc-transfected MMEC/myc3 cells to understand the relationship between oestrogen metabolic pathways and myc-mediated tumorigenic transformation better.

The MMEC/myc3 cell line exhibits enhanced expression of the cellular markers for transformation, namely aberrant hyperproliferation in vitro before tumorigenicity in vivo. We have observed previously that (1) non-cancerous mammary tissue exhibits increased C16α-hydroxylation of E2 to diverse carcinogenic agents.
(Telang et al. 1991, 1997; Suto et al. 1992; Fishman et al. 1995; Telang 1996); (2) exposure to 16α-OHE₁, results in genotoxic DNA damage and aberrant proliferation in non-cancerous mammary epithelial cells (Telang et al. 1992); (3) specific E₁ metabolites modulate proliferation in cells pretreated with chemical carcinogens or those derived from mammary carcinoma (Schneider et al. 1984; Suto et al. 1993); and (4) mechanistically distinct classes of chemopreventive agents inhibit aberrant proliferation and induce C2-hydroxylation of E₁ (Suto et al. 1992, 1993; Telang et al. 1997). These observations taken together support the concept that E₁ metabolism may represent a biochemical/endoocrine marker for mammary carcinogenesis and its prevention.

Interconversion of E₁ and E₂ has been reported to be altered in the neoplastic breast tissue owing to a change in intrinsic 17β-HSD activity (Pollow et al. 1977; Prudhomme et al. 1984; Gompel et al. 1986; Vermeulen et al. 1986; Tait et al. 1989; Poutanen et al. 1992; Pasqualini et al. 1996), which also appears to be different according to the hormone-responsive status of cancer cells (Castagnetta et al. 1995, 1996). This evidence is also relevant for other target cells of steroids (Carruba et al. 1997). The relative extent of 17β-HSD-mediated interconversion of E₁ and E₂ revealed interesting differences between MMEC and MMEMVC cells. Overall, the reductive conversion of E₁ to E₂ was remarkably greater than the opposing oxidative pathway in both MMEC and MMEMVC cells. However, both reactions were significantly lower in MMEC/mvc3 cells with respect to the parental MMEC cells. This could be, only partially, a reflection of the sustained increase of 16α-hydroxylation of E₁ seen in MMEMVC cells in association with the persistent expression of the mvc oncogene.

Results from time course experiments and those obtained using increasing concentrations of precursor confirmed that the extent of E₁ reduction to E₂ is consistently and significantly lower in MMEMVC cells than that observed in MMEC cells, regardless of incubation time and dose of precursor used.

The alteration in 17β-HSD activity observed in the present study raises the possibility that deregulated mvc expression may have preferentially suppressed the reductive isoform of 17β-HSD enzyme(s), resulting in an altered oestrogen substrate utilization by MMEMVC cells, as has been reported in other systems (Pollow et al. 1977; Strobl and Lippman, 1979; Tait et al. 1989; Poutanen et al. 1993). The oestrogen receptor status is critical for the genesis and/or evolution of a transformed cell phenotype and, as such, modulation of the receptor status may coincide with progression of hormone-dependent tumours to a hormone-independent status (Abul-Hajj, 1979; McCormick et al. 1982; Prudhomme et al. 1984; Welsch, 1985, 1987; Mauvais-Jarvis et al. 1986; Siteri et al. 1986; Ball et al. 1988; Castagnetta et al. 1995; Nguyen et al. 1995). In this context, it is interesting to note that MMEMVC cells that express exogenous c-myc also exhibit about a 60% decrease in oestrogen receptor content relative to the parental MMEC cells.

The experiments in the present study (designed to inspect the metabolic pathways subsequent to the formation of E₁) demonstrated clearly that the two cell lines are able to metabolize E₁ via the mutually exclusive C2-hydroxylation and C16α-hydroxylation in a manner similar to that previously observed in mammary epithelial cells that are initiated with the Ras oncogene or with the chemical carcinogen DMBA (Suto et al. 1992; Telang et al. 1991, 1992). Furthermore, HPLC analysis confirmed that the incubation of MMEMVC cells with close to a physiological E₁ concentration also resulted in an appreciable 16α-OHE₁ formation. Consistent with the observed cellular effects of specific E₁ metabolites in carcinogen/oncogene-initiated or carcinoma-derived cells (Schneider et al. 1984; Suto et al. 1992, 1993; Telang et al. 1992), 16α-OHE₁ and 2-OHE₁ were also effective in modulating growth response of c-myc oncogene-transfected cells in the present model.

Overall, MMEMVC/mvc3 cells exhibit strikingly enhanced proliferative activity and persistence of the transformed phenotype that appear to be associated with (1) altered equilibrium of E₁ to E₂, conversion and consequent reduction in E₂ production; and (2) increased ratio of C16α/C2 hydroxylation with consequent possible overstimulation of cell proliferation induced by both increased 16αOHE₁, level and decreased 2OHE₁. However, concerning the relationship between aberrant hyperproliferation, altered oestrogen metabolism and c-myc deregulated expression, it remains unclear whether this represents a result of oncogene expression/activation or is rather a consequence of phenotypic transformation of the cells.

ACKNOWLEDGEMENTS

This study is supported in part by NIH grants CA 44741 and CA 29502. Department of Defence grant DAMD 17-94-J-4208, the Irving A Hansen Memorial Foundation and the Iris and B Gerald Cantor Fund, the Associazione Italiana per la Ricerca sul Cancro (AIRC) and Consiglio Nazionale delle Ricerche (CNR). F Arcuri is the recipient of a fellowship from AIRC. The authors wish to thank Milan Zvanovec for expert technical assistance and Linda Winter for the preparation of the manuscript.

REFERENCES

Abul-Hajj YJ (1979) Relationship between estrogen receptors, 17β-hydroxysteroid dehydrogenase and estrogen content in human breast cancer. Steroids 34: 217–225

Ball RK, Ziemiecki A, Schonenberger CA, Reichmann E, Redmond SMS and Groner B (1988) V-myc alters the response of a cloned mouse mammary epithelial cell line to lactogenic hormones. Mol Endocrinol 2: 133–142

Carruba G, Leake RE, Rinaldi F, Chaimers D, Comito L, Sorci C, Pavone-Macaluso M and Castagnetta L (1994) Steroid-growth factor interaction in human prostate cancer. 1. Short-term effects of transforming growth factors on growth of human prostate cancer cells. Steroids 59: 412–420

Carruba G, Adamsky J, Calabro M, Miceli MD, Cataliotti A, Bellavita V, Lo Bue A, Polito L and Castagnetta LA (1997) Molecular expression of 17β-hydroxysteroid dehydrogenase types in relation to their activity in intact human prostate cancer cells. Mol Cell Endocrinol 131: 51–57

Castagnetta L, Granata OM, Lo Casto M, D'Agostino G, Mitchell F and O'Hare MJ (1986) Steroid profiles and optimization of high performance liquid chromatographic analytical procedure. Ann NY Acad Sci 464: 316–330

Castagnetta L, Granata OM, Lo Casto M, Calabro M, Arcuri F and Carruba G (1991) Simple approach to measure metabolic pathways of steroids in living cells. J Chromatogr 572: 25–39

Castagnetta L, Trani A, Carruba G, Fecarotta E, Palazzotto G and Leake RE (1992) The prognosis of breast cancer patients in relation to the estrogen receptor status of both primary disease and involved nodes. Br J Cancer 65: 167–170

Castagnetta L, Granata OM, Farruggio R, Cannella S, Montesanti A, Oliveri G, Sorci C, Mestì M and Carruba G (1995) Oxidative and reductive pathways of estrogens in hormone responsive and non-responsive human breast cancer cells in vitro. J Steroid Biochem Mol Biol 53: 367–374

Castagnetta LA, Granata OM, Taibi G, Lo Casto M, Comito L, Oliveri G, Di Falco M and Carruba G (1996) 17β-hydroxysteroid oxidoreductase activity in intact cells significantly differs from classical enzymology analysis. J Endocrinol 150: S73–S78

Clark JH, Pasko Z and Peck EJ (1977) Nuclear binding and estrogen receptor complex: relation to the agonistic and antagonistic properties of estrol. Endocrinology 100: 91–96

D'Agostino G, Castagnetta L, Mitchell F and O'Hare MJ (1985) Computer-aided mobile phase optimization and chromatogram simulation in HPLC: a review. J Chromatogr 388: 1–23

© Cancer Research Campaign 1998

British Journal of Cancer (1998) 77(10), 1549–1554
Dubik D and Shiu RPC (1992) Mechanism of estrogen activation of c-myc oncogene expression. *Oncogene* 7: 1587–1594
Fishman J and Martucci CP (1980) Differential hydroxylations of estrone and estradiol in mam. *J Clin Endocrinol Metab* 51: 611–615
Fishman J, Bradlow HL, Schneider J, Anderson KE and Kappas A (1980) Radiometric analysis of oxidation in man: sex differences in estradiol metabolism. *Proc Natl Acad Sci USA* 77: 4957–4960
Fishman J, Osborne MP and Telang NT (1995) The role of estrogen in mammary carcinogenesis. *Ann N Y Acad Sci* 768: 91–100
Ganguly N, Ganguly R, Mehta NM and Banerjee MR (1982) Growth and differentiation of hyperplastic outgrowths derived from mouse mammary epithelial cells transformed in organ culture. *J Natl Cancer Inst* 68: 453–463
Gompel A, Malet C, Spritzer P, Lalardite JP, Kutten F and Maurais-Jarvis P (1986) Progesteron effect on cell proliferation and 17β-hydroxysteroid dehydrogenase activity in normal human breast cells in culture. *J Clin Endocrinol Metab* 63: 1174–1180
McCormick DL, Mehta RG, Thompson CA, Dinger N, Caldwell JA and Moon RC (1982) Enhanced inhibition of mammary carcinogenesis by combination N-(4-hydroxyphenyl) retinamide and ovariectomy. *Cancer Res* 42: 509–512
Mauvais-Jarvis P, Kutten F and Gompel A (1986) Estradiol/progesterone interaction in normal and pathologic breast cells. *Ann N Y Acad Sci* 464: 152–167
Nguyen BL, Chetrite G and Pasqualini JR (1995) Transformation of estrone and estradiol in hormone-dependent and hormone-independent human breast cancer cells. *Breast Cancer Res Treat* 34: 139–146
Pasqualini JR, Chetrite G, Blacker C, Feinstein M-C, Delalonde L, Talbi M and Maloche C (1996) Concentration of estrone, estradiol, estrone sulfate, and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer patients. *J Clin Endocrinol Metab* 81: 1460–1464
Pollow K, Bouquoi E, Baumann J, Schmidt-Gollwitzer M and Pollow B (1977) Comparison of the in vitro conversion of estradiol-17beta to estrone in normal and neoplastic human breast tissue. *Med Cell Endocrinol* 6: 333–348
Poutanen M, Montcharmont B and Vilko R (1992) 17β-hydroxysteroid dehydrogenase gene expression in human breast cancer cells: regulation of expression by a progesterin. *Cancer Res* 52: 290–294
Poutanen M, Meitinnen M and Vilko R (1993) Differential estrogen substrate specificities for transiently expressed human placental 17β-hydroxysteroid dehydrogenase and an endogenous enzyme expressed in cultured COS-1 cells. *Endocrinology* 133: 2639–2644
Prudhomme JF, Malet C, Gompel A, Lalardie JP, Ochou P, Maurais-Jarvis P and Kutten F (1984) 17β-hydroxysteroid dehydrogenase activity in human breast epithelial cell and fibroblast cultures. *Endocrinology* 114: 1483–1489
Schneider J, Huh MM, Bradlow HL and Fishman J (1984) Antiestrogen action of 2-hydroxysterone on MCF-7 human breast cancer cells. *J Biol Chem* 159: 4840–4845
Siiteri PK, Sinberg N and Murai J (1986) Estrogens and breast cancer. *Ann NY Acad Sci* 464: 100–105
Strobl JS and Lippman ME (1979) Prolonged retention of estradiol by human breast cancer cells in tissue culture. *Cancer Res* 39: 3319–3327
Suto A, Bradlow HL, Wong GYC, Osborne MP and Telang NT (1992) Persistent estrogen responsiveness of Ras oncogene-transformed mouse mammary epithelial cells. *Steroids* 57: 262–268
Suto A, Bradlow HL, Wong GYC, Osborne MP and Telang NT (1993) Experimental down-regulation of intermediate biomarkers of carcinogenesis in mouse mammary epithelial cells. *Breast Cancer Res Treat* 27: 193–202
Tait G, Newton CJ, Reed MJ and James VHT (1989) Multiple forms of 17β-hydroxysteroid oxidoreductase in human breast tissue. *Mol Cell Endocrinol* 2: 71–80
Telang NT (1996) Oncogenes, estradiol biotransformation and mammary carcinogenesis. *Ann N Y Acad Sci* 784: 277–287
Telang NT, Banerjee MR, Iyer AP and Kundu AB (1979) Neoplastic transformation of epithelial cells in whole mammary gland in vitro. *Proc Natl Acad Sci USA* 76: 5886–5890
Telang NT, Osborne MP, Sweeterlitsch L and Narayan R (1990) Neoplastic transformation of mouse mammary epithelial cells by deregulated myc expression. *Cell Regul* 1: 863–872
Telang NT, Narayan R, Bradlow HL and Osborne MP (1991) Coordinated expression of intermediate biomarkers for tumorigenic transformation in Ras-transfected mouse mammary epithelial cells. *Breast Cancer Res Treat* 18: 155–163
Telang NT, Suto A, Wong GYC, Osborne MP and Bradlow HL (1992) Induction by estrogen metabolite 6α-hydroxy estrone of genotoxic damage and aberrant proliferation in mouse mammary epithelial cells. *J Natl Cancer Inst* 84: 634–638
Telang NT, Kadare M, Bradlow HL and Osborne MP (1997) Estradiol metabolism: an endocrine biomarker for modulation of human mammary carcinogenesis. *Environ Health Perspect* 105: 559–564
Vermeulen A, Deslypere JP, Paridaens R, Leclercq G, Roy F and Heuson IC (1986) Aromatase, 17β-hydroxysteroid dehydrogenase and intratissular sex hormone concentrations in cancerous and normal glandular breast tissue in postmenopausal women. *Eur J Cancer Clin Oncol* 22: 515–525
Welsch CW (1985) Host factors affecting the growth of carcinogen-induced rat mammary carcinomas: a review and tribute to Charles Brenton Huggins. *Cancer Res* 45: 3415–3443
Welsch CW (1987) Dietary retinoids and chemoprevention of mammary gland tumorigenesis. In *Cellular and Molecular Biology of Breast Cancer*. Medina D, Kidwell W, Heppner G and Anderson E (eds), pp. 495–508. Plenum Press: New York