Effects of a Pure Antiestrogen on Apoptosis and Proliferation within Human Breast Ductal Carcinoma in Situ

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ABSTRACT

Adjuvant antiestrogen (AE) therapy has been proposed for all women with ductal carcinoma in situ (DCIS). However, many cases of DCIS are of the high-grade, estrogen receptor (ER)-negative subtype that are unlikely to respond to AE treatment. Hormonal agents work by increasing apoptosis and/or decreasing cell proliferation; therefore, we studied the effect of a pure AE on levels of apoptosis and proliferation in human DCIS xenografts using an in vivo model.

Women (n = 23) with mammographic microcalcification suggestive of DCIS were identified at the time of surgery (day 0), a sample of representative tissue was obtained, divided into multiple 2 × 2 × 1-mm xenografts, and implanted s.c. into female BALB/c nu/nu mice (eight xenografts/mouse). Day 0 grafts underwent immunohistochemical assessment of ER status. Fourteen days after implantation, four xenografts were retrieved and mice were randomly divided into one of three treatment groups: (a) insertion of a slow release 2-mg 17β-estradiol pellet; (b) weekly 5-mg injections of the pure AE Faslodex (Zeneca Pharmaceuticals); and (c) injections of a control vehicle oil alone. After 2 weeks of treatment, the remaining four xenografts were retrieved from each mouse. Retrieved xenografts containing DCIS were assessed for morphological evidence of apoptotic cell death [apoptotic index (AI)] and cell proliferation (by immunohistochemical detection of the Ki67 proliferation antigen LI).

Both AI and LI were higher in the day 0 specimens of 16 ER+ DCIS lesions compared with 7 ER+ DCIS lesions (mean values, 1.47% versus 0.32% and 20.6% versus 3.1%; both P < 0.0001). AI and LI values within ER− DCIS did not differ between xenografts exposed to 17β-estradiol or AE treatment compared with the controls or pretreatment values (mean AI and LI in estradiol-treated, antiestrogen-treated, and control groups 1.04% versus 0.98% versus 1.29% and 17.2% versus 20.5% versus 17.7% respectively). In contrast, treatment of mice bearing ER+ DCIS xenografts with 17β-estradiol raised both the AI (1.03% versus 0.40%, P = 0.03) and LI (11.0% versus 5.1%, P = 0.007) compared with controls. AE therapy of ER+ DCIS xenografts did not affect proliferation but resulted in higher apoptosis than in controls (0.9% versus 0.4% respectively, P = 0.04).

AE therapy should be reserved for patients with estrogen receptor-positive DCIS.

INTRODUCTION

A rise in the diagnosis of DCIS has occurred since the introduction of a mammographic breast screening program. The detection of smaller, more localized mammographically detected lesions in clinically asymptomatic women has allowed increasing use of wide local excision for the treatment of women diagnosed with screen-detected DCIS (1). Following local excision, postoperative adjuvant radiotherapy has been shown to reduce local recurrence rates (2).

The NSABP group has recently published data examining the role of the AE tamoxifen in the treatment of DCIS following breast-conserving surgery and localized radiotherapy (3). Tamoxifen reduced the incidence of ipsilateral invasive breast cancer recurrence by 44%, and they recommended that adjuvant tamoxifen be used for all women undergoing breast-conserving surgery for DCIS. However, no information regarding ER status of tumors was given in this study. In women with ER− invasive breast carcinoma, tamoxifen has been shown to provide little benefit (4, 5), and our recently published in vivo model has demonstrated that ER− DCIS is, likewise, hormone independent (6). In the NSABP B-24 trial, one in three women were reported unable to complete the course of tamoxifen treatment due to a combination of unacceptable effects of the drug or “personal reasons.” There was also an excess of endometrial cancer and thromboembolic events in the treated group compared with controls (3). In women with unresponsive DCIS such adverse effects of tamoxifen assume greater clinical concern.

Although in vitro studies have shown a definite apoptotic effect of AE therapy on both ER+ and ER− cell lines (7, 8), the clinical relevance of these studies are unknown. Additional studies of the effects of AEs on DCIS at the cellular level may aid distinction between DCIS lesions likely to respond, or not, to hormonal therapy and would potentially be clinically useful. The absence of cell lines for DCIS led us to develop an in vivo animal model in which human DCIS xenografts could be subjected to hormonal manipulation (6). We showed that oestriol exposure generated increased cell proliferation in ER+, but not ER− DCIS (6) and hypothesized that proliferative drive in ER− DCIS was unlikely to be controlled by estrogen or AE therapy. To test this hypothesis, we have used the same model to expose human DCIS xenografts to pure AE therapy and examined the effects on rates of cell proliferation and apoptotic cell death. We show that neither cell proliferation nor apoptotic cell death is affected by AE exposure in ER− DCIS, in direct contrast to ER+ DCIS.

MATERIALS AND METHODS

Patients. Participants in this study were women who had attended the Nightingale Breast Screening Assessment Clinic (Manchester, United Kingdom) or the symptomatic clinic at the University Hospital of South Manchester from August 1995 to August 1998. Women whose mammograms showed widespread microcalcification indicative of DCIS (minimum 3-cm mammographic diameter) with or without associated lesions were identified prior to surgery, and, on the day of surgery, tissue was obtained for use in the study. Tissue was not taken from women who had localized DCIS (<3-cm mammographic diameter) because it was felt that histological assessment of the biopsy specimen would be compromised. Approval to remove a small sample of tissue from pathological specimens from women undergoing excision of widespread DCIS was granted by the South Manchester Medical Research Ethics Committee.

Treatment of Tissue Samples. A small (1–2 cm3) sample of representative tissue was immediately removed from the surgically excised specimen, stripped of excess fat, placed in DMEM with 4.5 g/liters of glucose and without sodium pyruvate (Life Technologies, Inc., Paisley, Scotland) at room temperature, and transported to the laboratory without delay. The tissue was then placed in fresh DMEM in a Petri dish and dissected into 2 × 2 × 1-mm xenografts using a scalpel blade. Depending on the volume of tissue available,
between 10 and 100 xenografts were randomly picked from the Petri dish and fixed with 4% formaldehyde solution for 24 h, followed by storage in 70% alcohol until paraffin embedding. These samples, representing the DCIS excised from each patient, were labeled as “day 0 specimens” and were reserved for histological review, immunostaining, and apoptotic cell counts.

The remaining xenografts were implanted into nude mice for hormonal manipulation.

Animals. Intact, female athymic nude mice (BALB/c nu/nu), 9–10 weeks of age, were obtained from the breeding colony at the Paterson Institute for Cancer Research. The mice were housed under conventional conditions with a 12-h cycle of light and dark (lights off 1900–0700 h) in filter top cages and supplied ad libitum with normal feed, water, and bedding during the experiments. Irradiated bedding, irradiated food, and filtered water were used during production of the nude mice. Animal care and all surgical procedures were performed in strict accordance with Home Office Regulations and United Kingdom Scientific Procedures (1986) Act.

Implantation of DCIS into Nude Mice. Each patient’s sample was divided and implanted into ~5–24 (median, 14) mice depending on the volume of tissue available. Transplantation of xenografts into the nude mice was completed within 90 min of removal of tissue from the patient. Two small midline skin incisions were made across the dorsal skin through which eight xenografts were symmetrically placed (two by each scapula and two by each hindquarter). Halothane inhalation anesthesia was used for each procedure (2–4% halothane in oxygen; Halovet Vaporiser, International Market Supplies, Congleton, United Kingdom).

Removal of xenografts involved reanesthetizing the mice and use of sharp dissection for excision of grafts at the appropriate time point. On removal from the mice, each graft was immediately fixed in 4% formaldehyde solution for 24 h, followed by storage in 70% alcohol until paraffin embedding.

Hormonal Manipulation of Xenografts.

Fourteen days after implantation of DCIS tissue four xenografts were removed from each mouse, which was then randomly placed into one of three independent treatment groups:

1. Estrogen Administration. A s.c. tunnel was made to the base of the tail to allow the placement of a slow release silastic encapsulated estrogen pellet containing 2 mg of 17β-estradiol (E-8875; Sigma Chemical Co., St. Louis, MO). The preparation, use, and effects on serum estradiol of these pellets has been previously described in detail (5, 9).

2. AE Administration. The AE used was Faslodex [7α-9-(4, 4,5,5,5-pentafluoropentylsulfinyl)nonyl]estra-1,3,5, (10)-triene-3,17β-diol (ICI 182 780), kindly donated by Dr. A. Wakeling (Zeneca Pharmaceuticals, Cheshire, United Kingdom). This compound has pure antiestrogenic activity and no partial estrogen agonist properties both in vitro and in vivo (10) and has also been shown to have potent antiestrogenic effects in a nude mouse model using breast cancer cell lines as xenografts (11). Five milligrams of Faslodex suspended in 1 ml of propylene glycol-based vehicle oil was injected s.c. into the nape of the neck (without the use of anesthetic) in the appropriate mice 14 and 21 days after implantation of the xenografts.

3. Control Group. Control mice received 1 ml of vehicle oil injections injected in a similar fashion to AE on days 14 and 21.

After the 14-day treatment period, four remaining xenografts were retrieved from each mouse.

Histological Evaluation of Xenografts.

All day 0 specimens and each xenograft were embedded in paraffin blocks. H&E-stained, 3-μm sections from each block were examined by a single experienced breast pathologist (W. F. K.) for the presence of DCIS; those containing DCIS were assessed for apoptosis and Ki67 antigen immunogenicity (as a marker for cell proliferation). In addition, day 0 specimens were evaluated immunohistochemically for ER status.

For each experiment, the number of day 0 specimens containing DCIS was used as an indication of the proportion of the xenografts expected to contain DCIS tissue. For example, in an experiment in which 33% of the day 0 specimens contained DCIS, then one in three retrieved xenografts would be expected to return DCIS. However, if only one in six retrieved xenografts contained DCIS, then a 50% return would be recorded.

Assessment of Apoptotic Cell Death. H&E-stained sections of DCIS samples were examined using light microscopy for morphological evidence of apoptosis. The criteria used to identify apoptotic cells are well recognized (12, 13) and include condensation of chromatin initially at the margins of the nucleus; condensation of the cytoplasm (chromophilia); detachment from surrounding cells, indicated by the appearance of a characteristic halo around the dying cell; and cytoplasmic budding to form membrane-bound fragments (apoptotic bodies).

To obtain the AI, a minimum of 500 cells (1000 cells for day 0 specimens) were counted, using ×40 Planapo oil lens and a Zeiss microscope, and the number of cells displaying apoptotic morphology were expressed as a percentage of the total number counted, as described previously (14).

Immunohistochemical Determination of Ki67 Nuclear Antigen. A standard three-layered streptavidin-avidin-biotin horseradish peroxidase method was used with diaminobenzidine (DAKO Ltd., High Wycombe, United Kingdom) as the chromogen. The primary antibody was a polyclonal rabbit antihuman Ki67 antigen (DAKO A047, 1:30 dilution). A swine antirabbit biotin-labeled polypeptide (DAKO E431, 1:400 dilution) was used as the secondary antibody. Ki67 immunostaining was predominantly nuclear with little cytoplasmic uptake. The intensity of staining was variable, but this was not assessed separately, and the cells were judged as positive or negative. Ki67 and apoptosis assessment of retrieved xenografts were performed by investigators (A. G., W. F. K.) blinded to the treatment group.

Assessment of ER Status. A standard three-layered streptavidin-avidin-biotin horseradish peroxidase method was used with a mouse antihuman ER primary antibody (DAKO M7047, 1:100 dilution) and a biotinylated rabbit antimouse secondary antibody (DAKO E413, 1:350 dilution). Staining was predominantly nuclear with little or no cytoplasmic staining. A minimum of 1000 malignant cells were counted, and lesions were considered ER+ if >5% of cells were positively stained for ER.

Assessment of PR Status. Day 28 xenograft sections from ER+ experiments were labeled for PR using serum from the PR-ICA kit (Abbot Diagnostics). A standard three-layered streptavidin-avidin-biotin horseradish peroxidase method was used with an overnight application of rat monoclonal primary antibody (1:4 dilution) and use of a biotinylated rabbit antirat secondary antibody at 1:100 dilution. Similar to ER, staining was predominantly nuclear with little or no cytoplasmic staining. A minimum of 500 cells were assessed, and positively stained nuclei were expressed as a percentage of the total number counted.

Statistical Methods. For each DCIS case, a comparison was made between DCIS samples retrieved from the three treatment groups and also between the three different time points [day 0, day 14 (pretreatment), day 28 (treated)]. The tissue samples obtained at each assessment day for the three study groups were all considered statistically independent. Differences between the groups and between the assessment days were evaluated by use of ANOVA. Both AI and LI were found to follow a skewed non-Normal distribution and were converted to natural logarithms for analysis. Transformation to logₑ gave an adequate approximation to Normality for all analysis in the model. The results obtained were then detransformed into the original units for presentation and are presented as geometric means and their 95% CIs. Pairwise comparisons indicated by the F-ratios from the ANOVA were investigated by Tukeys multiple comparison test. A comparison between AI and LI in day 0 samples was explored using the Pearson correlation following transformation to logₑ and confirmation by scatterplot of a robust linear relationship. Comparisons of the day 0 results between ER− and ER+ samples and between samples displaying some or no comedo-necrosis were carried out using simple ANOVA, as were comparisons of mouse weights, which are presented as the true (arithmetic) means and their 95% CIs for each treatment group. All significance tests were two-sided and used the conventional 5% significance level.

RESULTS

Breast tissue from 23 women has been used in this study (median age, 58 years; range, 18–84 years). Ten women (44%) were identified via the UK national breast screening program with asymptomatic widespread mammographic microcalcification; the remainder (56%) presented symptomatically with palpable breast lumps. Fourteen women (61%) were diagnosed as having purely DCIS, and nine (39%) were diagnosed with invasive carcinoma and DCIS.

Histopathology of Nonimplanted Day 0 Specimens. Sixteen of the 23 experiments (70%) contained ER− DCIS, and 7 cases (30%) contained ER+ DCIS (median number of cells staining positive for...
ER, 53%; range, 22–82%). Eleven (69%) of the 16 ER− women and 2 (29%) of the 7 ER+ women presented symptomatically. In only one case did the ER status of the xenografts not correlate with that of the histological slides (i.e., there was 95% concordance between ER status of day 0 xenografts and clinical tissue samples from which day 0 tissue was obtained). The DCIS in 17 women (74%) was designated as high grade, and three each (13%) as intermediate and low grade. According to the criteria of Page et al. (15), one of seven of the oestrogen receptor-positive DCIS samples and 15 of 16 of the oestrogen receptor-negative DCIS samples were of the comedo histological subtype, all of which demonstrated evidence of comedo-necrosis, thus displaying a significant association between ER status and presence of comedo-necrosis (P < 0.001; degrees of freedom = 1; two-sided Fishers exact test). The remaining seven samples were all of the noncomedo variety, none of which showed evidence of necrosis. The mean AI was higher in those tissue samples displaying evidence of necrosis than in those without necrosis (mean AI, 1.34% versus 0.43%; f[1.35] = 19.9, P < 0.001). A clear association between comedo-necrosis and raised AI has been noted previously (14, 16).

The 23 breast biopsies gave rise to a total of 546 day 0 specimens (i.e., nonimplanted xenografts), of which 151 (28%) contained foci of DCIS (median number containing DCIS, 24%; range, 7–67%).

Overall DCIS Retrieval from Xenografts. The 23 experiments produced a total of 2444 xenografts, of which 2321 (95%) were retrieved (median value for each experiment, 97%; range, 50–100%). Of these, 763 (33%) contained foci of DCIS.

The median retrieval of DCIS per experiment was 92% of that expected (range, 33–203%). DCIS was retrieved from day 14 xenografts in 91% (21 experiments) and from day 28 xenografts in 100% of the 23 experiments.

Apoptosis (Fig. 1)

ER− DCIS. The mean AI in day 0 ER− specimens (i.e., tissue representative of that excised from patients at the time of surgery) was 1.47% (95% CI, 1.21–1.77%), over 4-fold higher than in ER+ DCIS day 0 specimens (see below; P < 0.001). No change in AI was noted in pretreated xenografts obtained on day 14 [mean AI, 1.16% (95% CI, 0.93–1.43%)]. AI did not alter following exposure to either 17β-estradiol [mean AI, 1.04% (95% CI, 0.64–1.67%)] or AE [mean AI, 0.98% (95% CI, 0.64–1.50%)] when compared with control xenografts [mean AI, 1.29% (95% CI, 1.08–1.54%)] or to day 14 specimens (f[4, 109] = 1.3; P = 0.28).

ER+ DCIS. The mean AI in day 0 ER+ DCIS specimens was 0.32% (95% CI, 0.22–0.45%) and 0.38% in day 14 specimens (95% CI, 0.27–0.56%). AE treatment for 14 days resulted in a rise in AI to a mean of 0.90% (95% CI, 0.48–1.69%), significantly higher than both the control group [mean AI, 0.40% (95% CI, 0.34–0.48%)] and the pretreatment day 14 specimens (f[4,64] = 7.0; P < 0.001). Those xenografts subjected to 17β-estradiol exposure also showed an elevation of AI to 1.03% (95% CI, 0.58–1.84%), higher than control and pretreatment values (f[4,64] = 7.0; P < 0.001).

Cell Proliferation (Fig. 2)

ER DCIS. Mean epithelial cell proliferation, as measured by the LI, in day 0 ER− DCIS lesions was 20.6% (95% CI, 18.5–22.9%), 6-fold higher than in ER+ DCIS day 0 specimens [3.1% (95% CI, 2.2–4.2%), P < 0.001], and had not changed by day 14 [mean LI, 17.1% (95% CI, 15.0–19.6%)]. LI remained high in control ER− DCIS xenografts retrieved on day 28 [mean LI, 17.7% (95% CI, 14.6–21.4%)] and was unaffected by AE or 17β-estradiol exposure [mean LI, 20.5% (95% CI, 14.4–29.3) and 17.2% (95% CI, 14.2–20.7%), respectively]; f[4,250] = 1.21; P = 0.31.

ER+ DCIS. Treatment of mice bearing ER+ DCIS xenografts with 17β-estradiol resulted in a sharp elevation of LI to a mean of 11.0% (95% CI, 8.9–13.7%), significantly higher than pretreatment day 14 values [4.7% (95% CI, 3.5–6.4%)] and control specimens [mean LI, 5.1% (95% CI, 4.1–6.4%)]. LI was not affected by exposure to AE therapy for 14 days [mean LI, 6.8% (95% CI, 6.3–7.3%)]. A positive correlation was seen between apoptotic and proliferative indices in all day 0 specimens (Pearson correlation coefficient, r = 0.5, P = 0.02).

The antiestrogenic effects of Faslodex on ER+ DCIS xenografts was confirmed by immunohistochemical assessment of PR within day 28 xenografts. Expression of the PR protein, an estrogen-regulated gene product (17), was reduced in Faslodex-treated xenografts [geometric mean PR score, 18.17% (95% CI, 17.99–18.24%)] compared with control xenografts (mean PR score, 43.38% (95% CI, 34.12–54.6%)), estrogen-treated xenografts [mean PR score, 46.53% (95% CI, 41.68–51.94%)], or day 0 specimens [mean PR score, 54.6% (95% CI, 41.68–51.94%)] and was unaffected by AE or 17β-estradiol exposure [mean LI, 20.5% (95% CI, 14.4–29.3) and 17.2% (95% CI, 14.2–20.7%), respectively]; f[4,94] = 11.0; P < 0.001.

Mouse Weights. Mean mouse weights at day 0 were 24.0 g (95% CI, 23.6–24.5 g), increasing to 25.0 g (95% CI, 24.4–25.6 g; f[1.38] = 6.8, P = 0.01) on day 14 when treatment was commenced.

Fig. 1. Apoptotic cell death in ER− (solid symbols and solid line) and ER+ DCIS (open symbols and dotted line). Apoptosis was assessed by morphological assessment of H&E sections of xenografts retrieved from mice exposed to 17β-estradiol, AE, or a control vehicle oil. An AI was calculated by noting the number of cells showing morphological evidence of apoptotic cell death [see Kerr et al. (12)] as a percentage of total number of cells counted in each retrieved xenograft containing DCIS. Results are presented as geometric mean values with their 95% CIs. The number of xenografts used for each time point is presented beneath the chart for ER− and ER+ DCIS. At day 0, ER− DCIS displays a much higher AI than ER+ DCIS. In both subtypes the AI does not change significantly in the 14-day pretreatment period. Treatment was commenced on day 14. Hormonal manipulation of ER− DCIS had no effect on apoptotic cell death compared with control or pretreatment xenografts. ER+ DCIS displayed a significant rise in cell death when exposed to 17β-estradiol or AE therapy.
AE EFFECTS ON HUMAN BREAST DCIS

There was no difference in the weight of mice treated by AE injections [mean weight, 25.7 g (95% CI, 24.0–26.8 g)] compared with the control group [mean weight, 25.8 g (95% CI, 24.9–26.0 g)], but was raised by treatment with an estradiol implant [mean weight, 28.0 g (95% CI, 26.3–29.7 g); f(2,38) = 6.6, P = 0.004].

DISCUSSION

To aid further investigation into effects of AE on DCIS, we have developed an in vivo model in which specimens of human breast DCIS have been shown to survive as xenografts and have undergone hormonal manipulation. Using this model, we found that ER– DCIS had a much lower resting cell proliferation rate than ER+ DCIS and showed no change following 14 days of either estradiol or AE therapy. In contrast, ER+ DCIS xenografts displayed a marked increase in the Ki67 proliferation index when subjected to estradiol exposure, although AE treatment had no effect on cell proliferation. In vitro studies of the growth inhibitory properties of Faslodex have shown a cytostatic effect with accumulation of cells in the G0/G1 phase (10). If this same effect were seen in vivo, then a fall in the LI would be expected in ER+ DCIS xenografts exposed to AEs. The lack of such a finding in our model may be due to the short period of AE treatment or to the dose of AE used (based on work using a similar mouse model; Ref. 11). However, Brüntner et al. (20) found that the effects of AE treatment of ER+ MCF-7 cells in vitro resulted in cell accumulation in the G1 phase of the cell cycle (i.e., cytostatic effect), but treatment in an in vivo model (MCF-7 cell xenografts) had no effect on cell cycle kinetics. Nevertheless, AE treatment of in vivo MCF-7 tumors resulted in tumor regression, and Brüntner et al. (20) suggested that, in the absence of changes in proliferation kinetics, tumor shrinkage must be due to an increase in cell loss.

AE EFFECTS on Apoptosis. Several studies, in vitro and in vivo, have demonstrated that apoptotic cell death can be induced in breast cancer cells by estrogen withdrawal or by AE administration (21, 22). Cells exhibiting apoptosis display a characteristic morphology (12, 13) that may be recognized by light microscopy. We have used these properties to determine an AI for each xenograft containing DCIS, thus allowing comparisons between treatment groups.

ER– DCIS had a high resting AI compared with ER+ DCIS and remained unaltered after 14 days of treatment with either estradiol or AE therapy. In contrast, ER+ DCIS xenografts showed a marked increase in apoptotic cell death when subject to estradiol or AE exposure compared with pretreatment and control groups. Whereas estradiol treatment resulted in an accompanying rise in cell proliferation, no such effect was seen following AE treatment. Previous in vivo studies showed no effect of AE on cell cycle distribution (i.e., no antiproliferative effect) in MCF-7 cell xenografts, yet tumor regression was induced (20). Our data, using human DCIS xenografts, suggests that this regression is due to induction of the apoptotic process.

Other methods for identifying apoptosis include ISEL and terminal deoxynucleotidyl transferase-mediated nick end labeling. These processes are thought to simplify detection of apoptotic cells by incorporating biotinylated deoxyuridine at DNA strand breaks within apoptotic nuclei. However, doubts over the specificity of these methods in distinguishing between apoptotic and necrotic cell death are well documented. ISEL detection of DNA strand breaks
is not specific for apoptotic cell death and can be positive in cells that show no evidence of apoptosis (23), as well failing to stain cells showing quite classical features of programmed cell death (24). Both the ISEL and terminal deoxynucleotidyl transferase-mediated nick end labeling assays can fail to discriminate between apoptotic and necrotic cell death (23, 25), and there may be a discrepancy between the two staining techniques in the detection of apoptosis in breast cancer tissue (26). In our own laboratory, we have noted that ISEL staining gives false positive rates of 0.3–1.6% and false negative rates of 17.3–35% (27). While accepting that morphological assessment of apoptotic counts may detect only cells in the latter stages of the apoptotic process, we consider that light microscopic identification of cells displaying the recognized morphology of apoptosis is the most reliable and reproducible method of evaluating apoptotic cell counts (28).

Decreased detection of estrogen-regulated gene products following treatment of breast cancer cells with pure AEIs has been documented previously and is thought to be due to the attenuated transcriptional activity of the pure AE-ER complex (11). Our model clearly demonstrated the negative effects of Faslodex on immunohistochemical expression of the estrogen-regulated PR protein (18) within oestrogen receptor-positive DCIS xenografts. This finding has also been noted in human subjects (29) using the same AE and verifies our model as a valid system of delivering Faslodex to DCIS xenografts for the purposes of endocrine manipulation and investigation.

A positive association between apoptosis and cell proliferation has been documented previously (14, 30) and is also seen in our experiments within DCIS in day 0 specimens (i.e., breast biopsy tissue). Dysregulation of the critical balance between cell proliferation and cell death by processes that favor proliferation or impairs apoptosis can result in progression of benign lesions to frankly invasive tumors (31). However, if the balance is tipped in favor of cell death over proliferation, then tumor regression may be expected (32).

The NSABP B-24 study randomized women with DCIS who underwent breast-conserving surgery with adjuvant radiotherapy to receive tamoxifen or placebo (3). The study demonstrated a reduction in ipsilateral invasive cancer (but not DCIS) recurrences after the addition of tamoxifen. Unfortunately, information regarding ER status of DCIS lesions was not provided. Our data suggest that the benefit of endocrine manipulation will be limited to women with ER+ DCIS lesions. Women with ER− DCIS are unlikely to respond to AE exposure, and in these patients the side effect profile of tamoxifen therapy would make such treatment difficult to justify.

To achieve a greater response to treatment in women with ER− DCIS, a target other than the ER may need to be used. The c-erbB-2 oncogene has been shown to be present on 90–100% of comedo DCIS lesions (33). Antibodies to this receptor molecule have been shown in vitro to induce cellular differentiation (34) and in vivo to inhibit tumor growth when radiolabeled (35). The c-erbB-2 oncogene may, therefore, represent a potentially effective target for antigen-specific immunotherapy of breast DCIS. Studies are currently underway in our laboratory, using the described model, to investigate this possibility.

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