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Short Communication

Direct Action of Estrogen on Sequence of Progression of Human Preneoplastic Breast Disease

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We have used the MCF10AT xenograft model of human proliferative breast disease to examine the early effects of estradiol exposure on morphological progression of preneoplastic lesions and to define the step(s) in the morphological sequence at which estrogen may act. The effects of estradiol on neoplastic progression of estrogen-receptor-positive MCF10AT cells in the orthotopic site were examined in ovariectomized female nude mice that received subcutaneous administration of implants of 17β-estradiol or placebo pellets. At 10 weeks, histological analysis of the lesions derived from the estrogen-supplemented group revealed that 92% of lesions displayed histological features of atypical hyperplasia, carcinoma *in situ*, or invasive carcinoma, and the remaining 8% exhibited histological features of moderate hyperplasia. These highly proliferative lesions are in marked contrast to the control group in which 60% of samples displayed no evidence of hyperplasia. In contrast with control xenografts, estrogen-exposed xenografts demonstrated extensive areas of papillary growth, adenosis-like areas, prominent host inflammatory infiltration, and angiogenesis. Our results suggest that estrogen exerts a growth-promoting effect on benign or premalignant ductal epithelium by enhancing 1) the frequency of lesion formation, 2) the size of lesions, 3) the speed of transformation from normal/mild hyperplasia to those with atypia, 4) the degree of dysplasia, and 5) angiogenesis. *(Am J Pathol 1998, 152:1129–1132)*

The development of strategies for breast cancer prevention depends on improved understanding of the molecular and cellular events that lead to transformation and neoplastic progression of human breast epithelial cells. Epidemiological and experimental evidence suggests that breast cancer risk is related to the duration of estrogen exposure during puberty, the early postmenarchial period, and the menopausal period.[1,2] The effects of estrogen on the proliferation of target breast cells are believed to be mediated through transactivation of specific genes that are recognized by the estradiol-estrogen receptor (E_2-ER) complex.[3] This process stimulates DNA synthesis, cell division, and production of biologically active proteins, such as pS2, transforming growth factor-α, and epidermal growth factor,[4] which influence cell growth and differentiation. Exposure to estrogen may contribute to mammary carcinogenesis by stimulating proliferation of a clone of precancerous cells or by increasing the chance of spontaneous mutation. Alternatively, estrogen could decrease cell cycle transit time so that a spontaneous mutation becomes fixed before repair. An additional possibility is that estrogen may have a direct genotoxic effect.[5,6] Thus, despite wide agreement that estrogen is involved in the etiology of breast cancer, there is uncertainty as to the precise role of estrogen in the biology of breast cancer induction. Much of this difficulty can be ascribed to the lack of relevant model systems to understand the mechanism of early estrogen action in human mammary tumorigenesis.

In the present study we have used the MCF10AT xenograft model of human proliferative breast disease[7] to 1) examine the influence of estrogen exposure on the morphological progression of preneoplastic lesions and 2) define the step(s) in the morphological sequence at which estrogen may act. Previous studies have shown that MCF10AT cells grow progressively in immunodeficient mice, in which over a period of several months they

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undergo a sequence of histological changes. The intermediate changes mimic those seen in breasts of women at high risk of developing breast cancer (eg, atypical hyperplasia) and culminate in a significant proportion of grafts with carcinoma *in situ* and frankly invasive cancer.[7] In the absence of estrogen supplementation, proliferative lesions develop in the xenograft but progress only sporadically to atypical or frankly malignant lesions within intervals ranging from 7 to 56 weeks.[8] These data suggest that additional promotional events may be required for the eventual development of neoplasia in hormonally unsupplemented animals.

Materials and Methods

The effects of 17β-estradiol (E_2) on neoplastic progression of ER-positive MCF10AT cells[9,10] were examined in E_2-supplemented ovariectomized female nude mice. A total of 10^7 MCF10AT1 cells were suspended in Matrigel (Collaborative Research, Bedford, MA) and inoculated subcutaneously into the mammary fatpad region of ovariectomized nude mice 5 days after subcutaneous administration of implants of 1.7 mg/60-day release E_2 (treated; 13 mice) or placebo (control; 5 mice) pellets (Innovative Research, FL). Animals were observed twice a week and palpated for lesion formation at the injection site once a week, beginning 5 weeks after injection of MCF10AT1 cells. Pellets were replaced at 7 weeks, and all mice sacrificed at 10 weeks after injection by cervical dislocation. National Institutes of Health guidelines for proper and humane use of animals were observed. Tissues from the injection site were removed, and lesions were weighed with portions of each fixed in neutral buffered formalin and embedded in paraffin for histological examination. Histological grading of lesions was done as described previously.[8,11–13] The categories were as follows: 0, simple epithelium; 1, mild hyperplasia; 2, moderate hyperplasia; 3, atypical hyperplasia; 4, carcinoma *in situ* (CIS); 5, invasive carcinoma. Each lesion was graded according to the most advanced (deviant from normal) morphological pattern observed within it.

Results and Discussion

At 10 weeks, histological analysis of all tissues derived from control MCF10AT1-injected mice showed a considerable range of proliferative activity (Figure 1). The results were similar to previous reports.[7,8] Three of five of the control MCF10AT1 tissues displayed histological characteristics of grades 0/1, one lesion exhibited features of grade 3, and the remaining sample had no epithelial inclusions (Figure 1). In contrast, samples derived from 10-week E_2-supplemented MCF10AT1-injected mice were uniformly larger (~100 to 250 mg *versus* 450 to 550 mg), and 100% contained ductal epithelial structures. Histological analysis of the lesions derived from the estrogen-supplemented group revealed that 92% of lesions (Figure 1) displayed histological features of atypical hyperplasia (5/13), CIS (5/13), or invasive carcinoma (2/13),

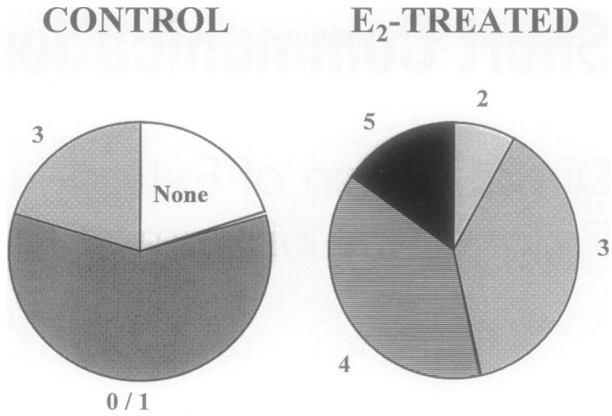

Figure 1. Distribution frequency of different grades of progression of MCF10AT1 lesions from the orthotopic sites of control and estrogen-supplemented mice at 10 weeks of implantation. Frequency distribution pattern represents data obtained from 5 control and 13 estrogen-exposed mice. Numbers indicate grade of lesion: grade 0/1, simple/mild hyperplasia; grade 2, moderate hyperplasia; grade 3, atypical hyperplasia; grade 4, carcinoma *in situ*; grade 5, invasive carcinoma; none, no persistent epithelial lesion detected.

and the remaining sample exhibited histological features of moderate hyperplasia (grade 2). These highly proliferative lesions are in marked contrast to the control group in which the majority (60%) of samples displayed no evidence of hyperplasia (Figure 2). The histological grading of the estrogen-stimulated lesions is shown in Table 1. In contrast to previous studies with control xenografts,[8] estrogen-exposed xenografts demonstrated extensive areas of papillary (as opposed to the cribriform growth (Figure 3A) as well as adenosis-like areas, often with noticeable eosinophilic intraluminal secretion (Figure 3B). Estrogen-treated xenografts also differed from previous studies[8] by virtue of demonstrating prominent host inflammatory infiltration and angiogenesis (Figure 3C). These results suggest that estrogen exerts a growth-promoting effect on benign or premalignant ductal epithelium by enhancing 1) the frequency of lesion formation, 2) the size of lesions, 3) the speed of transformation from grades 0/1 to grades 3 and higher, and 4) the degree of dysplasia. At least part of this growth-promoting effect of estrogen appears to arise from its effects on angiogenesis, as control xenografts from this and previous studies[8] did not show angiogenesis. The dramatic increase in growth and advanced histological grades of progression, concomitant with its remarkable effect on angiogenesis, suggests that one of the mechanisms by which estrogen acts as a mammary tumor promoter could be through its effect on expression of angiogenesis-regulating factors, *viz*, vascular endothelial growth factor and its receptor. In fact, a rapid induction of vascular endothelial growth/permeability factor expression by E_2 has been demonstrated in dimethylbenz(a)anthracene-induced estrogen-dependent tumors.[14] A recent study has demonstrated that augmentation of basic fibroblast growth factor-induced angiogenesis by exogenous E_2 in female mice requires functional estrogen receptors.[15] In this context, it is interesting to note that, although parental MCF10A cells are ER-negative, MCF10A cells expressing mutant Ha-*ras*, *viz*, MCF10AT cells, ex-

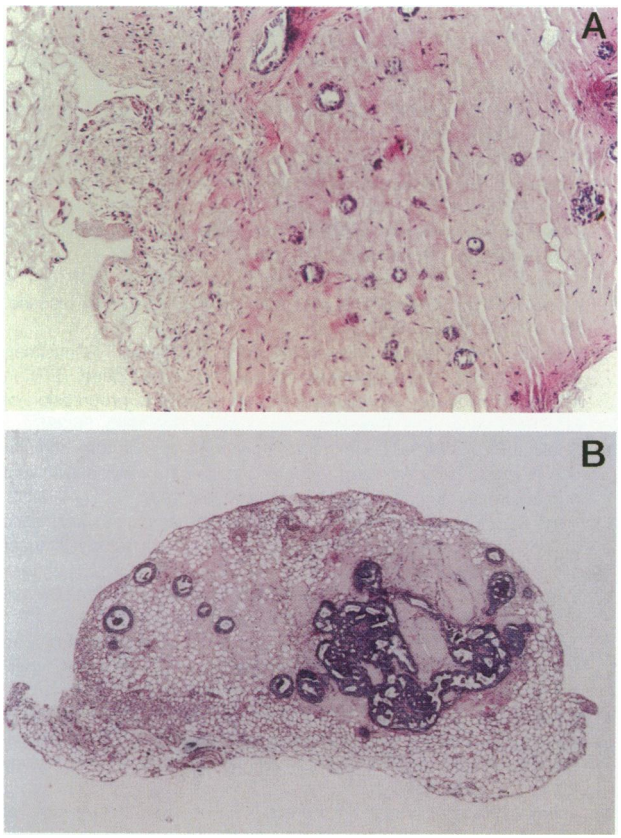

Figure 2. Histological features of MCF10AT1 lesions derived from control and estrogen-supplemented mice. **A:** Control lesion at 70 days of injection. Note that majority of the lesion consists of epithelial ducts that are lined by one layer of epithelial cells. H&E; magnification, ×10. **B:** Lesion from estrogen-treated nude mouse at 35 days af injection of MCF10AT1 cells. Note that the bulk of the lesion is displaying features of grade 3, although few ducts with simple epithelia and some with papillary bridging (grade 2) are still present. H&E; magnification, ×10.

hibit spontaneous gain in expression of functionally active estrogen receptor[9,10]; however, the extent of contribution of the E_2-ER complex to angiogenesis remains to be established.

Table 1. Distribution (%) of Histological Grades by Extent in Estrogen-Exposed MCF10AT1 Xenografts

	Distribution (%)				
Case	Grade 0/1	Grade 2	Grade 3	Grade 4	Grade 5
1	0	50	0	50	0
2	0	60	20	20	0
3	0	100	0	0	0
4	10	30	30	30	0
5	20	40	40	0	0
6	10	20	20	30	20
7	20	50	30	0	0
8	20	40	20	20	0
9	10	60	30	0	0
10	0	30	40	30	0
11	0	0	0	0	100
12	10	60	30	0	0
13	20	30	50	0	0

Grade 0/1, simple/mild hyperplasia; grade 2, moderate hyperplasia; grade 3, atypical hyperplasia; grade 4, carcinoma in situ; grade 5, invasive carcinoma.

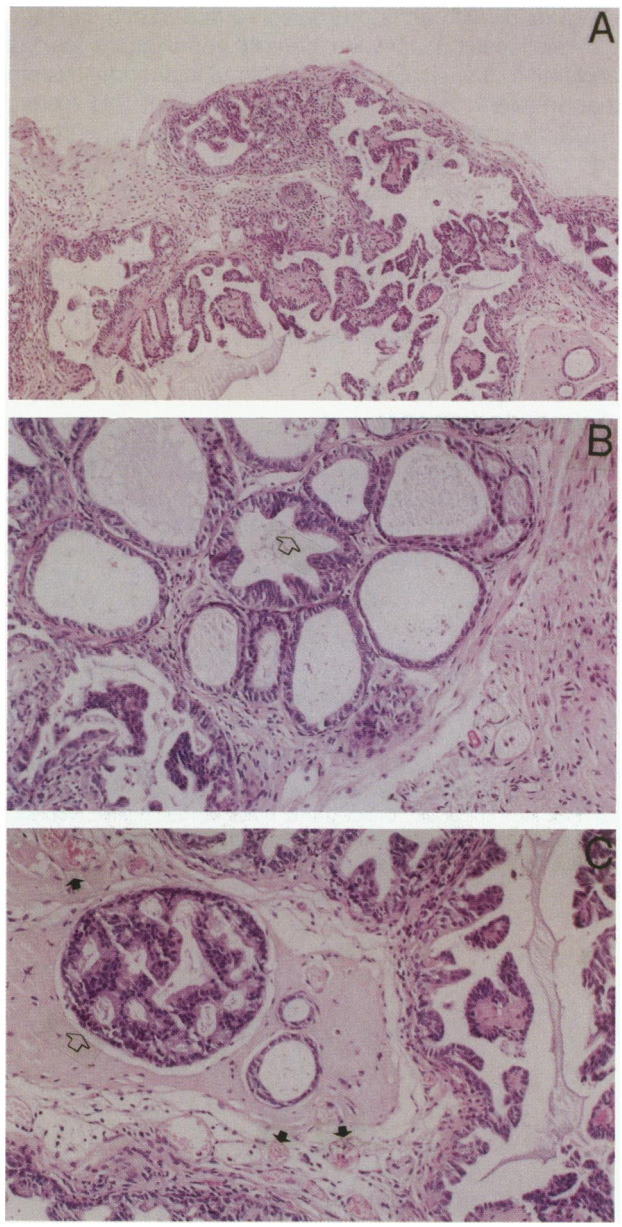

Figure 3. Morphological alterations induced by exposure of MCF10AT1 lesions to 17β-estradiol. **A:** Florid papillary hyperplasia within a cystically dilated space. Cystic dilation was characteristic of estrogen-treated lesions. H&E; magnification, ×50. **B:** Glandular adenosis. Note that the central gland demonstrates mild hyperplasia that is indicated by an **arrow**. H&E; magnification, ×100. **C:** Angiogenesis. Note the presence of numerous blood vessels (indicated by **solid arrow**) in a lesion that shows an extended duct (**open arrow**) at grade 4 and surrounding areas of papillary hyperplasia. H&E; magnification, ×100.

Previous studies have explored the MCF10AT model for human proliferative breast disease and development. However, in these studies there was 1) nonuniformity of progression to cancer and 2) a prolonged delay in most hosts, and 3) the overall rate of cancer induction was ~30%.[8] In contrast, in the presence of estrogen, this model exhibits premalignant and malignant lesions at high frequency in a relatively short time frame. The demonstration of a direct action of estrogen on the sequence of neoplastic progression of ER-positive MCF10AT cells

provides us with an unprecedented opportunity to study the relationships between benign/premalignant lesions and frankly invasive breast carcinoma in a model system that permits *in vivo* and *in vitro* manipulation. This system will allow evaluation of specific gene products (estrogen and cell cycle regulated) that are selectively modulated during progression. Moreover, as there is heterogeneity within individual lesions, with some areas displaying normal ducts or mild hyperplasia and others with atypia (Figure 3), the significance of the association of a specific gene product with histological grades of progression may be assessed more reliably.

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