Assessment of Hormone Dependence of Comedo Ductal Carcinoma In Situ of the Breast

Philip A. Holland, W. Fiona Knox, Christopher S. Potten, Anthony Howell, Elizabeth Anderson, Andrew D. Baildam, Nigel J. Bundred

Background: Ductal carcinoma in situ (DCIS) represents 20%-30% of breast cancers detected by clinical screening (i.e., mammography). More than 50% of DCIS lesions may be estrogen receptor negative and, therefore, hormone independent. However, the role of estrogen in the natural history of DCIS is unknown. Purpose: A novel in vivo (i.e., xenograft) model was developed to determine to what degree DCIS lesions depend on estrogen for growth. Methods: Specimens of breast tissue were collected from 52 women during diagnostic or therapeutic surgical procedures. Portions of each specimen were randomly selected and analyzed by histology and thymidine labeling (to measure cell proliferation). The remainder of each specimen was implanted into five to 18 athymic BALB/c nu/nu mice (depending on the amount of tissue available), with eight pieces of approximately 2 mm x 2 mm x 1 mm implanted at different locations on the back of each mouse. Half of the mice received implants containing estrogen (2 mg 17β-estradiol), and the other half received placebo implants. Levels of cell proliferation in xenografts, recovered after 14, 28, 42, or 56 days in the mice, were measured by thymidine labeling or by immunohistochemistry through use of an antibody specific for the Ki-67 nuclear antigen. Immunohistochemistry was also used to measure the levels of estrogen receptor in the tissue specimens. Serum 17β-estradiol levels in the mice were measured by radioimmunoassay. Results: Initial levels of cell proliferation were approximately 10-fold higher in 10 specimens with estrogen receptor-negative, comedo (i.e., more malignant in appearance) DCIS than in four specimens with estrogen receptor-positive DCIS (mean proliferation indices: 22% versus 1.9%, respectively; two-sided P<.001). Xenografts from the majority of specimens survived up to 56 days in the mice and maintained good architectural and cellular preservation. Estrogen treatment of the xenograft-bearing mice had no effect on the high level of cell proliferation observed in estrogen receptor-negative, comedo DCIS specimens (two-sided P = .89). In contrast, increased levels of cell proliferation in response to estrogen supplementation were measured in three estrogen receptor-positive, noncomedo DCIS specimens (two-sided P<.001). However, even with estrogen treatment, cell proliferation levels in estrogen receptor-positive DCIS specimens did not reach those seen in estrogen receptor-negative DCIS specimens. Conclusion and Implication: Estrogen receptor-negative, comedo DCIS lesions appear to be estrogen independent; therefore, antiestrogen (e.g., tamoxifen) therapy may not benefit patients with comedo DCIS. [J Natl Cancer Inst 1997;89: 1059-65]
versity Hospital of South Manchester, U.K. during the period of September 1993 through August 1995. Women (n = 52) were included in this study if suspicious mammographic microcalcifications, with or without an associated lesion, were detected. A preoperative diagnosis of cancer, using fine-needle cytology or corecut biopsy, was usually obtained.

Approval to remove tissue from pathologic samples in this study was granted by the South Manchester Medical Research Ethics Committee.

Samples of suspected DCIS were collected during both diagnostic and therapeutic surgical procedures.

**Diagnostic procedures.** When a preoperative diagnosis was unavailable, but extensive suspicious mammographic microcalcification was present, representative tissue was removed and implanted into nude mice. Tissue was not removed from patients undergoing a diagnostic needle localization procedure for localized microcalcification (<2 cm), since histologic processing of the excision margin status. A preoperative diagnosis of cancer, using fine-needle cytology or corecut biopsy, was usually obtained.

**Therapeutic procedures.** Such procedures were used for extensive DCIS lesions confirmed preoperatively by histopathology. Therapeutic excision was by wide local excision for smaller lesions (<4 cm) and simple mastectomy for larger lesions involving more than one quadrant.

**Re-excision biopsies.** If histologically confirmed DCIS had been incompletely excised from the case patient at initial diagnostic biopsy, tissue was also taken during re-excision or mastectomy.

After removal of tissue, surgical specimens were submitted for formal histopathologic evaluation.

**Surgical Procedures**

Breast tissue from 52 women was used in the study. The median age of the women was 57.5 years (range, 18-77 years). Eleven tissue samples were obtained after a diagnostic incision biopsy and 25 samples were obtained after therapeutic excisions for DCIS. Tissue was also collected after re-excision (n = 3) or mastectomy (n = 13) for previously incompletely excised DCIS lesions (Table 1).

**Animals**

Intact, adult, female, 9-10-week-old, athymic nude mice (BALB/c nu/nu) were obtained from the breeding colony at the Paterson Institute for Cancer Research. They were housed under conventional conditions with a 12-hour cycle of light and dark (lights off from 7 PM to 7 AM) in filter-top cages. They were supplied ad libitum with irradiated feed and water, and bedded with wood shavings and sawdust. The animals and surgical procedures were performed in accordance with Home Office Regulations and the UK Scientific Procedures (1986) Act. Halothane inhalation anesthesia (2%-4% halothane in oxygen; Halovet Vapouriser, International Market Supplies, Congleton, U.K.) was used for all procedures. Approximately eight (median) mice (range five to 18) were used for each woman’s tissue samples. In total, 470 mice were used.

**Treatment of Tissue Samples**

For preparation of tissue specimens for grafting in mice, for histology, and for thymidine labeling, a 1- to 2-cm³ area of breast tissue, containing microcalcifications, was taken at the time of either diagnostic or therapeutic excision biopsy. The fresh tissue was stripped of excess fat and immediately divided under sterile conditions into three or four smaller portions and placed in Dulbecco’s modified Eagle medium (DMEM), with 4.5 g/L glucose and without sodium pyruvate (Gibco Life Technology, Paisley, Scotland, U.K.), at a concentration of 10 μCi/mL—for 1 hour at 37 °C. The vials were gently shaken at regular intervals to aid tissue penetration by the [3H]Tdr. After this incubation, the tissue was removed from the medium and fixed in buffered formalin at room temperature for 24 hours.

**Implantation of Tissue (DCIS) Into Nude Mice**

Each patient’s sample was divided between five and 18 mice (depending on the volume of tissue available; median number, eight). Transplantation of xenografts onto the mice was completed within 90 minutes of removal of tissue from the patient. Two small midline skin incisions were made across the dorsal skin through which eight tissue pieces were symmetrically placed. To retrieve these xenografts at the appropriate time point, we reanesthetized the mice and excised each graft using sharp dissection. The grafts were then processed for thymidine labeling and histology as described. Blood was obtained from each of the mice at the conclusion of the experiment from which serum (an average volume of 1 mL) was prepared and stored at −70 °C until assay.

**Estrogen Administration**

On the day of DCIS implantation, another subcutaneous tunnel was made at the base of the tail.

<p>| Table 1. Histopathologic diagnosis of surgical specimens from patients and histopathology of implanted material* |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| <strong>A) Final histopathologic diagnosis of surgical specimens from patients studied compared with histopathology on nonimplanted day-0 xenografts (%)</strong> |</p>
<table>
<thead>
<tr>
<th>No. (%)</th>
<th>Normal/benign</th>
<th>DCIS only</th>
<th>DCIS + invasive</th>
<th>Invasive only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histology of surgical specimens</td>
<td>52 (100)</td>
<td>3 (5.8)</td>
<td>29 (55.8)</td>
<td>14 (26.9)</td>
</tr>
<tr>
<td>Histology of day-0 grafts</td>
<td>52 (100)</td>
<td>19 (36.5)</td>
<td>17 (32.7)</td>
<td>8 (15.4)</td>
</tr>
<tr>
<td><strong>B) Histopathology of implanted material related to the type of operation from which the material was obtained</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. (%)</td>
<td>Normal/benign</td>
<td>DCIS only</td>
<td>DCIS + invasive</td>
<td>Invasive only</td>
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<tr>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Diagnostic biopsy</td>
<td>11 (21.2)</td>
<td>9 (17.3)</td>
<td>1 (1.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Definitive procedure†</td>
<td>4 (7.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (5.8)</td>
</tr>
<tr>
<td>Wide local excision</td>
<td>21 (40.4)</td>
<td>2 (3.8)</td>
<td>11 (21.2)</td>
<td>3 (5.8)</td>
</tr>
<tr>
<td>Mastectomy</td>
<td>3 (5.8)</td>
<td>1 (1.9)</td>
<td>1 (1.9)</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td>Re-excision procedure‡</td>
<td>13 (25)</td>
<td>7 (13.5)</td>
<td>4 (7.7)</td>
<td>1 (1.9)</td>
</tr>
</tbody>
</table>

*Histopathologic diagnosis of the specimens removed from the 52 patients from whom tissue was subsequently implanted. Day-0 grafts were randomly selected and were not implanted into the mice but were part of the specimen that had been selected for implantation. The day-0 grafts give a guide to how frequently ductal carcinoma in situ (DCIS) was implanted into the mice. Part B shows the likelihood of DCIS being implanted in relation to the type of procedure by which the histologic specimen was taken.

†Total DCIS cases implanted from definitive procedures: 17 of 25 = 68%.

‡Total DCIS cases implanted from re-excision procedures: 7 of 16 = 43.8%.
allowing placement of a slow-release, silastic-encapsulated estrogen pellet containing 2 mg 17\beta-estradiol (E-8875; Sigma Chemical Co., St. Louis, MO) in half of the mice (within each DCIS case). The preparation and use of these pellets have been described previously (6). Silastic implants containing no estrogen were implanted into control mice.

**Histologic Evaluation of Grafts**

For each day-0 sample (nonimplanted grafts), two nonserial, 3-\(\mu\)m-thick sections were cut from each block and subsequently stained with hematoxylin–eosin (H & E). All tissue specimens were reviewed by a single experienced breast pathologist, and each graft was evaluated for the presence of DCIS. The proportion of randomly selected day-0 grafts containing malignant tissue was used to calculate the number of implanted grafts likely to contain foci of DCIS or invasive carcinoma. For example, if only one (20\%) in five day-0 grafts contained DCIS and/or invasive cancer, then the take rate would be 75\% if five mice were each implanted with eight grafts and six xenografts were retrieved containing DCIS.

Blocks containing grafts retrieved from the animals at each time point were evaluated in an identical manner. Overall, 43 (82.7\%) of the 52 surgical specimens from which experimental material was taken contained DCIS, either alone (\(n = 29\)) or in combination with invasive carcinoma (\(n = 14\)) (Table 1, A).

After pathologic evaluation, unstained nonserial sections of grafts containing DCIS were processed for cell proliferation studies by use of [\(\text{\text{H}}\)]thymidine autoradiography and Ki-67 immunostaining. Day-0 grafts were also evaluated immunohistochemically for estrogen receptors (ERs).

DCIS cell survival and proliferation were assessed by the use of in vitro thymidine labeling and Ki-67 immunostaining. The nuclear grade of the DCIS lesions was assessed as described previously (5).

**Preparation of Autoradiographs for Thymidine Labeling**

Autoradiographs were prepared by a well-validated technique in regular use in our laboratory (6). A cell was considered labeled when three or more silver grains were seen overlying a nucleus. A section was scored only if there were clearly labeled cells within a focus of DCIS. In addition, grafts and DCIS foci were assessed for adequacy of penetration by tritiated thymidine. Background levels were deemed as satisfactory if there was one grain per nucleus or fewer.

Fields for scoring were selected out of focus at low power, then cells were counted at \(x400\) magnification with a 10 \(\times\) 10-square graticule and cell counter.

At least 1000 malignant epithelial cells were counted for each focus of DCIS. If fewer than 1000 cells were present, additional nonserial sections were cut from the same block and autoradiographs were prepared until the desired numbers of cells were counted. Each focus of DCIS within a particular graft was counted separately. An overall percent labeling index (positive cells/total number of cells counted) was calculated in each case.

**Immunohistochemical Determination of Ki-67 Nuclear Antigen**

A standard three-layered streptavidin–avidin–biotin–horseradish peroxidase method was used with diaminobenzidine (DAKO Ltd., High Wycombe, U.K.) as the chromogen. The primary antibody was a polyclonal rabbit anti-human Ki-67 antigen (DAKO A047, 1:30 dilution), which is suitable for use on formalin-fixed, paraffin-embedded tissue. The secondary antibody was a swine anti-rabbit, biotin-labeled antibody (DAKO E431, 1:400 dilution). A section was scored if the initial H & E-stained section showed foci of DCIS with clearly identifiable malignant epithelial cells. Fields for scoring were selected out of focus at low power, then cells were counted independently by two observers, at \(x400\) magnification with a graticule and cell counter.

The staining was localized predominantly to the nuclei with little cytoplasmic staining. The intensity of staining was variable, but this was not assessed separately, and the cells were judged as either positive or negative. Nuclei with any detectable staining above background levels (i.e., the levels observed for negative controls that were not incubated with primary antibody) were scored as positive. At least 1000 malignant epithelial cells were counted for each focus of DCIS. If fewer than 1000 cells were present, additional nonserial sections were cut from the same block until the desired numbers of cells were counted. Each focus of DCIS within a particular graft was counted separately. An overall percent labeling index (positive cells/total number of cells counted) was calculated in each case.

**Assessment of ER Status**

A three-layered streptavidin–avidin–biotin–horseradish peroxidase method was used. The primary antibody was a mouse anti-human ER (DAKO M7047, 1:100 dilution); the secondary antibody was a biotinylated rabbit anti-mouse immunoglobulin (DAKO E413, 1:350 dilution). Staining was localized predominantly to the nuclei with little or none in the cytoplasm. Sections were assessed quantitatively by two observers, and the interobserver agreement was good. At least 1000 epithelial cells were counted for each sample, and the overall percentage of positive cells was calculated for each specimen. Lesions were considered positive for ER if more than 5\% of the malignant cells showed staining of any intensity above that of the negative controls.

**Radioimmunoassay of Serum Estrogen**

Serum 17\(\beta\)-estradiol concentrations were measured by use of an in-house specific radioimmunoassay as previously described (6). The intra-assay coefficient of variation was 5\% or less for this assay whereas the interassay coefficient of variation was 13\%.

**Statistical Methods**

For each DCIS case, a comparison was made between DCIS samples retrieved from estrogen-treated and control mice at each assessment time point. The tissue samples obtained at each assessment day for the two study groups were all considered to be statistically independent. Thus, differences between the groups and between the assessment days were evaluated by use of a one-way analysis of variance (ANOVA). Since the model residuals indicated that the proliferation-rate data tended to follow a positively skewed, log-normal distribution, these data were converted to natural logarithms for analysis. Adequate model (residual) fits were obtained for all analyses under this transformation. The results obtained were detransformed into the original units for presentation, which is in the form of geometric means and their 95\% confidence intervals (CIs). Pairwise comparisons indicated by the F-ratios from the ANOVAs were investigated by the Tukey multiple comparison test. All significance tests were two-sided, using the conventional 5\% significance level.

**Results**

**Histopathology of Nonimplanted Day-0 Specimens in Relation to the Type of Surgery**

Randomly selected, nonimplanted day-0 specimens were evaluated for the presence or absence of DCIS (Table 1, B) to give an indication of the success with which DCIS was obtained from the different surgical procedures; these specimens were subsequently implanted into nude mice. Of 43 breast specimens containing DCIS at surgery, only 25 (58\%) of the xenografts processed for implantation contained DCIS either alone (\(n = 17\)) or in conjunction with invasive carcinoma (\(n = 8\)). In the remaining 18 cases, microcalcification or epithelial proliferation was seen that did not have conclusive diagnostic features of DCIS, and subsequently DCIS was not obtained from any mouse explant. Three hundred two (22.3\%) of 1355 day-0 grafts from confirmed DCIS cases contained foci of DCIS.

The architectural subtypes and ER status of implanted DCIS cases are shown in Table 2. Overall, 1812 of 1956 xenografts were successfully retrieved, with a median of 94.5\% (range, 48\%-100\%) of the grafts being retrieved at the end of each experiment (Table 2). No sign of immunologic rejection was seen in any of the xenografts.

**DCIS Survival at Each Time Point**

In each experiment and at each time point, survival of DCIS tumor cells was confirmed by two independent markers of cell proliferation (thymidine labeling

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Of the 25 cases of DCIS implanted, tissue was removed 14, 28, 42, and 56 days after implantation in 18 experiments and 14 and 28 days after implantation in seven experiments. DCIS survival was 100% at 14 days, 92% (23 of 25 cases) at 28 days, and 83% (15 of 18 cases) at 56 days. At each time point, both comedo and noncomedo subtypes of DCIS were retrieved, with good architectural preservation and cellular morphology. The latter seven experiments had a shorter time scale to allow removal of four grafts at days 14 and 28 to increase the number of data points for these two time points.

Overall DCIS Survival

Overall DCIS survival was estimated on the basis of the percentage of retrieved grafts containing foci of DCIS (19%) and compared with the percentage of randomly selected nonimplanted day-0 grafts, containing foci of DCIS (21.5%), in each case.

For the 25 cases of DCIS that were implanted, the median overall DCIS survival within the grafts was 75% (range, 17%-100%) of that expected from nonimplanted day-0 grafts, whereas the overall median survival of xenografts of invasive carcinoma was only 16% of those implanted (range, 0%-100%). When DCIS and invasive carcinoma coexisted, the survival of the DCIS component was good, whereas that of the invasive component was invariably poor. Similar retrieval rates of DCIS from estrogen-treated and control mice were achieved. In total, 361 xenografts containing foci of DCIS were retrieved from the mice.

Despite the overall good survival of DCIS xenografts, in 12 of 25 experiments, the amount of grafts containing DCIS implanted (as predicted from nonimplanted day-0 grafts) and therefore retrieved was too low to provide sufficient numbers of cells at each time point to allow statistical comparison of cell proliferation between the different time points for estrogen-treated and control mice. Subsequently, all tissue was removed at two time points (days 14 and 28) to study the effect of estrogen stimulation in detail; this was possible for 10 cases of pure comedo DCIS and three cases of noncomedo DCIS. All cases of comedo DCIS in this group were ER negative (eight were high nuclear grade and two were intermediate grade), whereas all of the cases of noncomedo DCIS were ER positive (one was high nuclear grade and two were low nuclear grade).

ER-Negative, Comedo DCIS

Epithelial cell proliferation was approximately 10-fold higher in ER-negative than in ER-positive DCIS (22% versus 1.9% [see below]; \( P \leq .001 \)). The measured Ki-67 cell proliferation index for 10 cases of comedo DCIS at day 0 was a mean of 22.0% (95% CI = 19.7%-24.6%). It showed a nonsignificant decline at day 14 to 17.5% (95% CI = 14.6%-21.0%) but at day 28 remained at 17.2% (95% CI = 13.9%-21.2%) in the estrogen-treated group compared with 17.3% (95% CI = 13.9%-21.5%) in the control group (\( P = .89 \)) (Fig. 2). Cell proliferation remained high at 56 days in both groups at 19.6% (95% CI = 12.9%-30.0%). All 10 comedo DCIS cases were negative for ER as determined by immunohistochemical staining (Table 2).

ER-positive DCIS (Cribriform and Papillary)

ER-positive DCIS had a mean proliferation rate at day 0 of 1.9% (95% CI =
4.0% (95% CI 4.05 and P negative DCIS (both control and estrogen-treated ER-elevation in cell proliferation with time for that there was a statistically significant elevation in the control mice (P 0.05).

In the ER-positive DCIS implanted into control mice, a significant proliferation rates at days 14 and 28. In the ER-positive DCIS implanted into control mice, a significant elevation of proliferation rate occurred, but the proliferation rate in control-treated mice was significantly lower at both days 14 and 28 than in the estrogen-treated mice.

1.1%-2.6%), which increased to 3.7% (95% CI = 2.3%-6.2%) by day 14 and 4.0% (95% CI = 2.9%-5.5%) by day 28 in the control mice (P 0.05).

Ki-67 immunostaining demonstrated that there was a statistically significant elevation in cell proliferation with time for both control and estrogen-treated ER-negative DCIS (P=0.05 and P=0.001, respectively). Cell proliferation in estrogen-treated DCIS was significantly higher than that in control DCIS reaching a peak on day 14 (Fig. 2; f[4.65] = 29.7; P=0.001), corresponding to the peak serum 17β-estradiol seen in the mice. Stimulation in the control group was significantly less than that seen in the estrogen-treated group (f[4.65] = 29.7; P<0.001). Stimulation in the control mice was presumably due to endogenous mouse 17β-estradiol, which has a concentration similar to that of the follicular phase of normal premenopausal women (7).

**Serum 17β-Estradiol Levels**

Before estradiol supplementation, the geometric mean serum 17β-estradiol concentration in intact mice was 125 pmol/L (95% CI = 90.0-174.2 pmol/L), which is equivalent to the early follicular phase of normal premenopausal women (7). After estradiol treatment, the median serum estradiol level increased sharply to 1422.3 pmol/L (95% CI = 1144.2-1768.0 pmol/L) at day 14 and 1266.5 pmol/L (95% CI = 960.3-1670.2 pmol/L) at 28 days after implantation (data not shown). These levels are equivalent to midcycle levels of normal premenopausal women. Serum estradiol levels fell to 707 pmol/L (95% CI = 574.4-870.3 pmol/L) at 42 days and 794 pmol/L (95% CI = 623.4-1011.0 pmol/L) at 56 days but remained statistically higher than day-0 levels (P<0.001). These latter levels are equivalent to luteal peak levels of premenopausal women (and were statistically lower than days 14 and 28; P<0.001) (7).

**Mouse Weights**

Mean mouse weights at day 0 were 24.9 g (95% CI = 23.6-25.6 g). They increased to 28.3 g (95% CI = 27.5-29.1 g) in the estrogen-treated mice at day 14 compared with 26.1 g (95% CI = 25.5-26.8 g) (f[4.4] = 6.03; P<0.001); thereafter, up to day 56, weight gain between the groups did not differ.

**Discussion**

This is the first model system for the in vivo study of breast cancer in which specimens of human DCIS have been shown to survive as xenografts in mice and to be capable of hormonal manipulation.

The DCIS model was adopted from a model described previously by us (6), in which normal human breast tissue xenografted and maintained by the athymic nude mouse responded to the hormone-induced growth stimulus following implantation of estradiol pellets on the dorsum of the mice.

Because the presenting feature of the DCIS lesions was mammographic microcalcification, it is not surprising that comedo DCIS was the predominant histologic subtype found. Micropapillary and cribriform subtypes less often microcalcify and are usually occult mammographically (3). Hence, only four cases were of the noncomedo subtype. The majority of pure comedo lesions were ER negative, and the few pure cribriform and micro papillary lesions examined were all ER positive.

Irrespective of the pathology of the xenograft, overall graft retrieval was excellent. The median overall DCIS survival within the grafts at the end of each experiment was 75% of that predicted from day-0 grafts. These survival figures might be expected in a model of normal breast tissue but are in contrast to those obtained with human breast carcinomas, which are difficult to maintain and grow in nude mouse models (8-10). The reported take rate of human tumors implanted into
mice varies with tumor type, being approximately 48% for lung carcinomas, 14% for gastric carcinomas, and 7%-14% for breast carcinomas (8-10). We found that the overall survival of invasive carcinoma was only 16% of expected. The reason for the failure of invasive breast cancer to survive in vivo is unknown; this is surprising, since malignant cells within foci of DCIS appear to survive intact. Breast tumors typically contain relatively few malignant cells encased in a dense fibrous supporting stroma. In organ culture systems, this stroma prevents diffusion of nutrients and metabolites to and from the cells, except to those on the periphery of the tissue (17). Because xenografted tissue relies on diffusion until vascular continuity with the host is established, malignant cells may die, thus reducing the clonogenic cell population below that necessary to establish a xenograft. It is possible that DCIS lesions produce a weaker stromal reaction in the surrounding breast tissue than carcinomas, allowing better diffusion of oxygen and nutrients into the graft and facilitating the development of a host vascular supply. Alternatively, since nude mice have non-killer cell activity, a cell-mediated, immunity-based rejection may occur in invasive tumors but not in DCIS lesions.

Thymidine labeling was found to be an unreliable method of assessing cell proliferation. The poor penetration of thymidine into DCIS lesions has also been shown in other studies (12,13). Immunohistochemical assessment of cell proliferation using antibody to Ki-67 avoided the problems of poor tissue penetration and was found to be a reliable method of determining the level of cell proliferation in DCIS lesions.

Epithelial proliferation in the ER-negative DCIS was higher at day 0 than the ER-positive DCIS. Ki-67 proliferation rates in unstimulated normal breast tissue are low (usually <1%-4%) (8) and ER-negative DCIS has a higher baseline proliferation rate (6). The major difference in cell proliferation between ER-positive DCIS and normal breast epithelium is the increase in the level of cell proliferation with time in unstimulated ER-positive DCIS. By contrast, unstimulated ER-negative DCIS or normal breast epithelium shows no change in the level of cell proliferation; this finding demonstrates the inherent sensitivity of ER-positive DCIS to estrogen. The increase in proliferation in ER-positive DCIS in control mice was significantly lower than that seen in estrogen-supplemented mice when serum levels of estrogen are approximately to those seen in premenopausal women (7). The contrast in the response to estrogen between ER-positive and ER-negative DCIS is stark and, although ER-positive DCIS should respond to antiestrogen therapy, the same cannot be expected of ER-negative DCIS.

The role of estrogen in the natural history of DCIS is unknown, as is the role of endocrine therapy in preventing relapse and progression to invasive carcinoma. The incidence of ER positivity in DCIS has been found to be similar to that in invasive carcinoma, leading to speculation that ER-positive invasive carcinoma originates from an ER-positive precursor lesion (14,15). However, in the U.K., approximately 50% of all screen-detected DCIS lesions are of a purely comedo or a predominantly comedo subtype (1), and pure noncomedo lesions represent only 23% of cases. In several studies (14,16), 60% of the DCIS cases expressed detectable ER, but only 20%-30% of comedo DCIS—compared with 60% of cribriform, solid, and micropapillary categories—showed ER expression (14,15). Within invasive breast carcinomas, not all detectable receptors are functional; approximately 70% of all ER-positive invasive breast cancers show growth alteration with hormone manipulation (17). If this success rate is applicable to DCIS, then only 42% of DCIS patients may benefit from antiestrogen therapy. The majority of comedo lesions are c-erb B2 and epidermal growth factor receptor positive; in contrast, noncomedo DCIS is usually negative. The failure of estrogen to increase proliferation in comedo DCIS implies that it is an unlikely promoter of ER-negative DCIS cell proliferation, especially since ER-positive DCIS showed a significant increase in cell proliferation after estrogen supplementation.

Although the number of patients included in this study is relatively small, a suitable model for human DCIS has not been described previously. With continuing experience, this model would appear to be ideal to investigate potential promoters and inhibitors of comedo DCIS cell proliferation. If these promoters and inhibitors can be identified, then new treatment strategies can be developed to inhibit the proliferation of estrogen-independent comedo DCIS.

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Benzene and the Dose-Related Incidence of Hematologic Neoplasms in China

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Background: Benzene is a widely distributed environmental contaminant known to cause leukemia, particularly acute nonlymphocytic leukemia, and perhaps other hematologic neoplasms and disorders. Few epidemiologic studies, however, have been able to address relationships between the extent of benzene exposure and the level of risk. Purpose: A large cohort study was carried out in China to evaluate the risks of developing specific hematologic neoplasms and selected related disorders in relationship to quantitative estimates of occupational benzene exposure. Methods: A cohort of 74828 benzene-exposed and 35805 unexposed workers employed from 1972 through 1987 in 12 cities in China was identified and followed to determine the incidence of hematologic neoplasms and related disorders. Estimates of benzene exposure were derived from work histories and available historic benzene measurements. Existing pathologic material and supporting medical records were reviewed to establish diagnoses of disease. Relative risks (RRs) (i.e., ratios of incidence rates for specific hematologic neoplasms and related disorders in the benzene-exposed group to incidence rates in the unexposed group) were determined by use of Poisson regression analysis, with stratification by age and sex. Results: For workers historically exposed to benzene at average levels of less than 10 parts per million (ppm), the RR for all hematologic neoplasms combined was 2.2 (95% confidence interval [CI] = 1.1-4.2), and, for the combination of acute nonlymphocytic leukemia and related myelodysplastic syndromes, the RR was 3.2 (95% CI = 1.0-10.1). For individuals who were occupationally exposed to benzene at constant levels of 25 ppm or more, the RR for the combination of acute nonlymphocytic leukemia and related myelodysplastic syndromes was 7.1 (95% CI = 2.1-23.7). Workers with 10 or more years of benzene exposure had an RR of developing non-Hodgkin’s lymphoma of 4.2 (95% CI = 1.1-15.9), and the development of this neoplasm was linked most strongly to exposure that had occurred at least 10 years before diagnosis (i.e., distant exposure) (P for trend = .005, two-sided). In contrast, the risk for the combination of acute nonlymphocytic leukemia and related myelodysplastic syndromes was significantly increased among those with more recent benzene exposure (P for trend = .003, two-sided), but it was not linked to distant exposure (P for trend = .51, two-sided). Conclusions: The results of this study suggest that benzene exposure is associated with a spectrum of hematologic neoplasms and related disorders in humans. Risks for these conditions are elevated at average benzene-exposure levels of less than 10 ppm and show a tendency, although not a strong one, to rise with increasing levels of exposure. The temporal pattern of benzene exposure appears to be important in determining the risk of developing specific diseases. [J Natl Cancer Inst 1997;89:1065-71]

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See ‘Notes’ following ‘References.’