Neoplastic transformation of mammary epithelial cells in rats is associated with decreased apoptotic cell death

Anne Shilkaitis, Albert Green, Vernon Steele1, Ronald Lubet1, Garry Kellogg and Konstantin Christov2

Department of Surgical Oncology, University of Illinois at Chicago, 840 South Wood Street MC 820, Chicago, IL 60612 and National Cancer Institute, Division for Chemoprevention, Bethesda, MD, USA

Previous studies have shown that terminal end buds (TEBs) in the murine mammary gland have high proliferative activity and demonstrate apoptotic cell death (ACD). Since TEBs are considered the place of origin of most chemically induced mammary carcinomas, we hypothesized that the development of hyperplastic and premalignant lesions in TEBs is associated with either a further increase in cell proliferation and/or with a decrease in ACD. To test this hypothesis we used the N-methyl-N-nitrosourea (MNU) carcinogenesis model in rats, where the occurrence of mammary tumors is preceded by hyperplastic and premalignant lesions arising mostly in TEBs, as well as in ducts and alveoli. The percentage of proliferating cells, as evaluated by 5-bromodeoxyuridine labeling (BrDU-LI), was similar in TEBs to those in terminal endbud hyperplasia (TEBH), CIS, and carcinomas (CA), whereas the percentage of apoptotic cells (apoptotic index, AI) was relatively high in TEBs and decreased in TEBH, CIS, and CA. This indicates that neoplastic transformation of mammary epithelial cells in TEBs is not associated with an increase in cell proliferation, but with a decrease in ACD. In addition to TEBH, hyperplastic lesions developed in ductal branching areas (ductal hyperplasia, DH) and alveolar structures (alveolar hyperplasia, AH). However, BrDU-LI in both DH and AH was lower than in TEBH, whereas the AI values were similar, suggesting that TEBH has a higher potential for progression and malignant transformation than DH and AH. In mammary tumors apoptotic cells were rare in the peripheral, proliferative areas, but frequent close to the necrotic areas, suggesting that intratumoral factors may significantly affect ACD. Thus, it appears that dissociation between cell proliferation and apoptosis occurs in the hyperplastic stages of mammary carcinogenesis and that neoplastic transformation of mammary epithelial cells is associated with decreased ACD but not with increased cell proliferation.

Materials and methods

Animals

Virgin female Sprague–Dawley [Hsp:(SD/BR)] rats were obtained from Harlan Sprague–Dawley (Indianapolis, IN) at 42 days of age and, after 1 week of quarantine, were randomized by weight and injected with MNU. Beginning 3 weeks after MNU administration, the animals were palpated weekly to monitor mammary tumor appearance. The animals were fed 4% Purina Chow diet and had free access to water. To assess the incidence and frequency of mammary carcinogenesis model in rats has been widely used hyperplastic and premalignant lesions the animals were killed at different time points after removal of hormone stimulation (10). We also found that various hyperplastic lesions differ in their response to 4-(hydroxyphenyl)retinamide, a well-known inhibitor of mammary carcinogenesis, suggesting different sensitivity of mammary epithelial cells in these lesions to chemopreventive and antitumor agents (11,12). Data have also been published indicating the negative role of apoptosis in the initiating phase of dimethylbenzanthracene (DMBA)-induced mammary carcinogenesis in stromolysine gene knockout mice (13). To the best of our knowledge, no data have been published on cell proliferation and apoptosis in various hyperplastic and premalignant lesions in the MNU carcinogenesis model.

In the present study, we hypothesize that deregulation of cell proliferation and apoptosis occurs in the early stages of mammary carcinogenesis and that various hyperplastic lesions may differ in their potential for progression and malignant transformation. Data supporting this hypothesis are presented.

Abbreviations: ACD, apoptotic cell death; AH, alveolar hyperplasia; AI, percentage of apoptotic cells; BrDU, 5-bromodeoxyuridine; BrDU-LI, percentage of cells labeled by BrDU; CA, carcinoma; CIS, carcinoma in situ; DH, ductal hyperplasia; DMBA, dimethylbenzanthracene; MECs, mammary epithelial cells; MNU, N-methyl-N-nitrosourea; TEBs, terminal end buds; TEBH, terminal end bud hyperplasia.
Table I. Experimental groups and frequency of mammary gland lesions*  

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>No. of lesionsb</th>
<th>Week killed after MNU administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 (n = 10)</td>
<td>7 (n = 10)</td>
</tr>
<tr>
<td>1. TEBs</td>
<td>55</td>
<td>3.8</td>
</tr>
<tr>
<td>2. TEBH</td>
<td>44</td>
<td>2.0</td>
</tr>
<tr>
<td>3. DH</td>
<td>33</td>
<td>1.0</td>
</tr>
<tr>
<td>4. AH</td>
<td>6</td>
<td>0.2</td>
</tr>
<tr>
<td>5. CIS</td>
<td>49</td>
<td>0.3</td>
</tr>
<tr>
<td>6. CA</td>
<td>106</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*Fifty-five animals were used in this experiment. The number of animals (n) per time point is given as well.

bNumber of lesions, including tumors, was assessed by histological examination. In some animals CA were discovered at autopsy only. The animals were injected i.v. with 50 mg/kg body wt MNU at the age of 50 days and killed 5, 7, 9, and 12 weeks later. Various hyperplastic lesions and CIS were identified on whole mount preparations. Significance of differences in frequency between various groups: TEBH versus DH, P < 0.001; TEBH versus AH, P < 0.0001; TEBH versus CIS, not significant; CIS versus CA, P < 0.0001.

Whole mounts and histomorphology of mammary glands

The animals were killed by CO₂ narcosis and the skin with all mammary glands (six pairs) attached was removed and fixed in 10% phosphate-buffered formalin for 24 h. Tumors or any abnormal masses in the mammary glands were removed and cut into two halves. One part was fixed in 10% neutral formalin for histomorphological study and estimation of 5-bromodeoxyuridine (BrdU)-labeled and apoptotic cells and the other part was frozen in liquid nitrogen for other analyses. After fixation, the glands were dissected from the skin, stained in alum-carmine, dehydrated in ethanol, defatted in histoclear and observed under a dissection microscope as described earlier (14). Mammary carcinomas were classified using the criteria proposed by Russo et al. (15). Hyperplastic and premalignant lesions according to their origin and

Fig. 1. Frequency of various mammary gland lesions in animals killed at different time points (5, 7, 9 and 12 weeks) after MNU administration. The frequency of lesions and their topology were assessed by a whole mount procedure followed by histological examination. The frequency of lesions represent the mean number per carcinogen-treated animal.

Whole mounts and histomorphology of mammary glands

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Role of apoptosis in mammary carcinogenesis

morphology were divided into terminal end bud hyperplasia (TEBH), DH, alveolar hyperplasia (AH), and CIS (Table I).

**Cell proliferation**

Proliferating cells in various mammary gland lesions and carcinomas were labeled with 50 mg/kg body wt BrdU (Sigma Co., St. Louis, MO), injected i.p. 2 h before killing of the animals. BrdU-labeled cells were identified with an anti-BrdU monoclonal antibody (Beckton Dickinson, Palo Alto, CA) and an ABC kit (10). More than 1000 cells were scored to assess the percentage of cells labeled by BrdU (BrdU-LI). In CIS and carcinoma (CA), BrdU-LI were estimated in the peripheral, proliferating tumor areas (close to the stroma, <50 μm from the stroma and about 5–6 cell layers from the tumor capsule or stroma) only. Because of the small size of terminal end buds (TEBs), the percent of BrdU-labeled or apoptotic cells was evaluated after scouring all TEBs in mammary gland.

**Programmed cell death (apoptosis)**

The percentage of apoptotic cells (apoptotic index, AI) was evaluated using the TUNEL method (16) as recommended in the ApopTag *in situ* hybridization kit (Oncor Co., Gaithersburg, MD). The top sections on each slide, which were incubated without digoxigenin-dUTP, were used as negative controls. Sections from mammary glands of rats 6 days after ovariectomy (provided by Oncor Co.) were used as positive controls. The slides were counterstained with methyl green. BrdU-labeled cells were identified with in situ hybridization in the proliferating areas and close to the necrotic areas (5–6 cell layers from the tumor capsule or stroma).

**Statistical analysis**

Statistical analysis was performed for significant differences in the occurrence of various lesions and carcinomas across time. Incidence was evaluated by a Mantel–Haenszel 2 test that tested whether the differences in incidence were constant over time (17). Differences in tumor frequency were assessed by Poisson regression, treating days post-carcinogen administration as an ordered categorical variable (18). The Spearman rank test was used to assess the correlation between BrdU-LI and AI values in various lesions and tumors.

**Results**

**Most hyperplastic lesions developed in TEBs**

Animals were killed 5, 7, 9, and 12 weeks after MNU administration and the frequency of TEBs and various hyperplastic lesions, CIS, and CA evaluated (Table I and Figure 1). TEBH and DH arose from luminal cells, which developed heterogeneous structures of alveolar, papillary, or solid type (Figure 2A, arrow). Normal TEBs in control animals were symmetrical in shape and composed of a homogeneous population of luminal cells with high proliferative activity (Figure 2B, arrow). In AH, alveoli formed nodular structures with a small amount of stroma between alveoli (Figure 2C). CIS was mostly of comedo or alveolar type and was composed of dysplastic cells adjacent to those of ductal CIS in human breast (Figure 2D).

From the data presented in Table 1 it is evident that normal TEBs could be identified in mammary glands of animals killed 5 and 7 weeks after MNU administration but they were rare at later time points (9–12 weeks). Hyperplastic lesions (TEBH, DH and AH) were more frequent in animals killed 5 and 7 weeks after MNU administration and then decreased. CIS appeared to increase between weeks 5 and 7 and then remained at the same level in the week 9 and 12 groups, whereas the frequency of CA constantly increased between weeks 5 and 12 (Figure 1). The number of hyperplastic lesions (TEBH, DH and AH) per animal, when combined for all time points, was more than 3 times higher than CIS and about 2.5 higher than

![Fig. 2](https://via.placeholder.com/150) (A) Various hyperplastic lesions (TEBH, arrow) and papillomas (P) in mammary gland of an animal killed 5 weeks after MNU administration. Hematoyxlin and eosin staining. ×100. (B) BrdU-labeled cells in TEBs (arrow). Note the high number of dark stained nuclei, which represent BrdU-labeled cells. The slide was counterstained with methyl green. ×200. (C) A mammary carcinoma (CA) and alveolar hyperplasia (AH). Note a much higher number of BrdU-labeled cells in the carcinoma as compared with alveolar hyperplasia. The slide was counterstained with methyl green. ×200. (D) Carcinoma *in situ* (CIS) in an animal killed 7 weeks after MNU administration. CIS is of comedo type. Note the high number of proliferating cells close to the basal lamina. The slide was counterstained with methyl green. ×100. (E) Two apoptotic cells in a ductal hyperplastic lesion (DH) (arrow). There is a lack of apoptotic cells in a lesion with the characteristics of CIS. Apoptotic cells were identified by the TUNEL method. The slide was counterstained with methyl green. ×200. (F) Apoptotic cells in a mammary carcinoma. Note the small number of apoptotic cells (with dark stained nuclei) among tumor parenchyma. The slide was counterstained with methyl green. ×200. (G) A high number of apoptotic cells (with dark stained nuclei) in the central tumor area surrounding necrotic tissue (N). A single apoptotic cell is visible (arrow) in the tumor periphery. The slide was counterstained with methyl green. ×200.
Statistical differences. BrdU-LI values: TEBs versus TEBH, not significant; TEBs versus CIS, not significant; TEBs versus CA, not significant; TEBH versus CIS, not significant; TEBH versus CA, not significant; CIS versus CA, not significant. Apoptosis (AI): TEBs versus TEBH, P < 0.0001; TEBs versus DH, P < 0.05; TEBH versus CIS, not significant; TEBH versus CA, not significant; CIS versus CA, not significant. R: TEBs versus TEBH, P < 0.001; TEBH versus DH, P < 0.001; DH versus AH, not significant; TEBs versus CIS, P < 0.0001; TEBH versus CIS, P < 0.001; CIS versus CA, P < 0.05.

In two recently studied CA (not involved in this study), a high number of apoptotic cells (AI > 5.0%) were found, suggesting spontaneous cell suicide in this tumor model.

CA, suggesting that not all hyperplastic lesions progress and develop malignant tumors.

**Hyperplastic lesions differ in their proliferating activity but not in apoptotic cell death (ACD)**

In order to understand which of the hyperplastic lesions have a higher potential for progression and malignant transformation, we assessed their proliferating activity and ACD. We hypothesized that the higher the ratio (R) between proliferating (BrdU-LI) and apoptotic (AI) cells, the higher potential of hyperplastic lesions for progression and malignant transformation. The data presented in Table II show that among various hyperplastic lesions, BrdU-LI in TEBH (21.8 ± 9.4%) was significantly higher than in DH (7.0 ± 3.1%, P < 0.001) and AH (5.3 ± 1.4, P < 0.001), whereas the values of apoptotic cells were similar (0.3 ± 0.2, 0.4 ± 0.2, and 0.3 ± 0.2, respectively). Differences between proliferating and apoptotic cells also affected the BrdU-LI/AI ratio (R), which was higher in TEBH (62.4 ± 5.3) than in DH (23.3 ± 6.2, P < 0.001) and AH (17.6 ± 5.8, P < 0.001) (Figure 3). These data suggest that the place of origin of hyperplastic lesions may determine their proliferative activity and that mammary epithelial cells in TEBH have a higher potential for malignant transformation than those in DH and AH.

**Neoplastic transformation of MECs in TEBs is not associated with increased proliferation but with decreased ACD**

To test the hypothesis that malignant transformation of MECs is associated with deregulation in the balance between proliferating and apoptotic cells, we compared BrdU-LI and AI between TEBs, TEBH, CIS, and CA. From the data shown in Table II it is evident that BrdU-LI values in TEBs, TEBH, CIS, and CA were similar or close, whereas AI values were relatively high in TEBs and decreased in TEBH (P < 0.001), CIS (P < 0.001), and CA (P < 0.001). This indicates that dissociation between cell proliferation and apoptosis occurs in the early, proliferative stages of mammary carcinogenesis and that neoplastic transformation of mammary epithelial cells in TEBs is not associated with increased cell proliferation but with decreased ACD. The differences in the values of BrdU-labeled and apoptotic cells reflected their ratio, R. The BrdU-LI/AI ratio increased in TEBH (62.4 ± 5.3, P < 0.001), CIS (82.8 ± 12.7, P < 0.001), and CA (73.8 ± 18.2, P < 0.001) as compared with normal TEBs (40.6 ± 7.8).

BrdU-LI and AI values in CIS were similar to those in CA (24.8 ± 8.2 and 26.5 ± 11.5% for BrdU-LI, and 0.3 ± 0.2 and 0.4 ± 0.2 for AI, respectively). Spearman rank analysis did not find a direct correlation between BrdU-LI and AI values in hyperplastic lesions, CIS, and CA.

In evaluating the topology of BrdU-labeled and apoptotic cells in various mammary gland lesions and tumors, we observed that they were randomly distributed in most hyperplastic lesions. However, in some CIS of comedo type and in most CA, BrdU-labeled cells predominated in the tumor periphery (Figure 2D). Apoptotic cells were rare in proliferating areas and in the periphery of tumors (Figure 2E and F), but their number increased in the areas close to necrotic regions or within the areas of tissue disintegration (Figure 2G). Because of the variability in distribution of proliferating and apoptotic cells in CIS and CA, the BrdU-LI and AI values given in Table II were evaluated within the peripheral proliferating areas only (5–6 cell layers from the tumor capsule or stroma).

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**Table II. Cell proliferation and apoptosis in TEBs, TEBH, DH, AH, CIS and CA**

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Animals with lesions</th>
<th>Lesion (%)</th>
<th>BrdU-LI (%)</th>
<th>AI (%)</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEBs</td>
<td>20</td>
<td>55</td>
<td>23.6 ± 5.0, 9.1–30.2</td>
<td>0.7 ± 0.3, 0.0–1.3</td>
<td>40.6 ± 7.8, 25.2–48.8</td>
</tr>
<tr>
<td>TEBH</td>
<td>29</td>
<td>44</td>
<td>21.8 ± 9.4, 11.0–33.2</td>
<td>0.3 ± 0.2, 0.0–1.3</td>
<td>62.4 ± 5.3, 9.6–40.6</td>
</tr>
<tr>
<td>DH</td>
<td>30</td>
<td>34</td>
<td>7.0 ± 3.1, 3.3–12.2</td>
<td>0.4 ± 0.2, 0.0–0.8</td>
<td>23.3 ± 6.2, 15.5–31.4</td>
</tr>
<tr>
<td>AH</td>
<td>6</td>
<td>6</td>
<td>5.3 ± 1.4, 0.8–7.7</td>
<td>0.3 ± 0.1, 0.1–0.4</td>
<td>17.6 ± 5.8, 9.8–25.8</td>
</tr>
<tr>
<td>CIS</td>
<td>22</td>
<td>25</td>
<td>24.8 ± 8.2, 10.6–38.3</td>
<td>0.3 ± 0.2, 0.1–0.9</td>
<td>82.8 ± 12.7, 15.0–77.8</td>
</tr>
<tr>
<td>CA</td>
<td>20</td>
<td>32</td>
<td>26.5 ± 11.5, 14.0–38.8</td>
<td>0.4 ± 0.2, 0.1–0.9a</td>
<td>73.8 ± 18.2, 23.4–83.5</td>
</tr>
</tbody>
</table>

BrdU-LI, percent of cells labeled by BrdU in various hyperplastic lesions (TEBH, DH and AH) as well as in proliferating areas of CIS and ICA (see text); AI, percent of apoptotic cells evaluated in the above lesions as well as in proliferating areas of CIS and ICA; TEBs, normal terminal end buds in control animals; TEBH, terminal end bud hyperplasia; DH, ductal hyperplasia involving ductal lateral branching areas; AH, alveolar hyperplasia; CIS, carcinoma in situ; ICA, invasive carcinoma.

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**Fig. 3.** The ratio R between BrdU-labeled (BrdU-LI) and apoptotic (AI) cells in various mammary lesions. The means ± SD are given in Table II. Note the much higher R value in CIS and CA as compared with those in TEBs, DH and AH.
Apoptotic cells are increased in areas close to necrotic regions
Since apoptotic cells have recently been used as end-point biomarkers in breast cancer chemoprevention and therapy studies, it was important to assess whether intratumoral factors may affect their frequency. In order to obtain quantitative information on the differences in distribution of apoptotic cells among tumor parenchyma, tumors with defined necrotic areas were selected and AI values were evaluated separately in the areas close to (within 5–6 cell layers from the necrotic area) and far from the necrotic area, in the tumor periphery. It was found that the values of AI were significantly higher in the areas close to the necrotic area than in the proliferating areas ($P < 0.001$) (Figure 4). Apoptotic cells close to the necrotic area (N) were mostly in groups, whereas those in the tumor periphery were identified as single cells (Figure 2G, arrow). In the necrotic area nuclear fragments positively stained for apoptotic cells were observed, indicating that ACD is also involved in the development of necrosis.

Discussion
In this study we induced hyperplastic and premalignant (CIS) lesions in mammary glands of rats and assessed their proliferative activity and ACD. To the best of our knowledge this is the first study focused on cell proliferation and apoptosis in hyperplastic and premalignant stages of MNU-induced mammary carcinogenesis in the rat. The MNU carcinogenesis model was used for several reasons: (i) tumors arise from ductal epithelial cells, as in most human breast carcinomas; (ii) the occurrence of tumors is preceded by hyperplastic and premalignant lesions, which mostly develop in TEBs; (iii) proliferative activity and ACD in TEBs are relatively high, suggesting dissociation of these two cellular events in the course of carcinogenesis; (iv) histogenesis, morphology, and progression of hyperplastic, premalignant, and malignant lesions are similar in many aspects to those of human breast cancer (13).

In earlier studies using the DMBA carcinogenesis model, Russo et al. (19) developed the concept that mammary carcinomas arise from TEBs, whereas benign lesions, like hyperplastic alveolar nodules, fibroadenomas, and adenomas, arise from alveolar cells. Our data, although generated in a different (MNU) carcinogenesis model, support this hypothesis and show that hyperplastic lesions in TEBs (TEBH) were most frequent, followed by those in ductal branching areas (DH) and alveoli (AH). Hyperplastic lesions and CIS in mammary gland of rats have also been described by others, however, in their studies different carcinogens, treatment protocols, and ages of animals at the time of carcinogen administration were used (5–7,20–23).

We found that the decrease in the frequency of hyperplastic lesions (TEBH, DH and AH) in the animals killed 9 or 12 weeks after MNU administration, as compared with those killed at the earlier time points (5 or 7 weeks), was concomitant with the increase in CIS and CA (Figure 1). This suggests that only some hyperplastic lesions will progress and develop into malignant tumors. In order to assess the potential of various hyperplastic lesions for progression and malignant transformation we compared the numbers of proliferating and apoptotic cells. BrdU-LI was much higher in TEBH than in DH and AH, whereas the AI values were similar, suggesting a higher turnover rate and potential for progression and malignant transformation of the former than of the latter two. This was also supported by the higher BrdU-LI/AI ratio in TEBH than in DH and AH.

When the number of BrdU-labeled cells in TEBH was compared with those in normal TEBs, CIS, and CA, surprisingly no significant difference or relatively small differences were found. At the same time, AI values were relatively high in TEBs and decreased in TEBH, CIS, and CA (Table II). These data suggest that neoplastic transformation of mammary epithelial cells in TEBs is not associated with increased cell proliferation but with decreased ACD. Thus, it appears that dissociation of cell proliferation and apoptosis occurs in the early, hyperplastic stages of mammary carcinogenesis and persists, probably at different levels, in CIS and CA.

Our data for the high proliferative activity of mammary epithelial cells in TEBs supports some previous studies where, instead of BrdU, $[^3]$H]thymidine was used as a marker of proliferating cells. $[^3]$H]thymidine-labeled cells in TEBs were in the range of those observed in our study (24–27). In the late 1970s Russo and co-workers, using electron microscopy, described dark and light cells in TEBs and suggested that in the early stages of mammary carcinogenesis these cells were replaced by intermediate cells (28,29). However, the authors did not discuss the role of apoptosis in this process, nor did they follow cell proliferation and apoptosis in the various hyperplastic and premalignant stages of mammary carcinogenesis. They also used electron microscopy to address cell kinetics phenomena, which is not the best means to examine the proliferation of cell populations. Finally, they used DMBA as carcinogen, which has, at the DNA level, quite a different mechanism of action to that of MNU.

We found that in TEBs not only the numbers of proliferating cells, but also of apoptotic cells were high. Recent studies on Balb/c mice indicated high values of ACD (>11%) in TEBs, as well as variability in the distribution of apoptotic cells in various zones of TEBs (30). It has been suggested that the high ACD in TEBs is a physiological phenomenon and that it is involved in differentiation of TEBs into acinar and ductal structures, which have low proliferative activity and ACD.

The role of ACD in mammary carcinogenesis is still unclear and contradictory. A decrease in ACD as compared with normal MECs has recently been described in DMBA-induced mammary carcinoma (31). However, in this study no information was given on heterogeneity in the distribution of apoptotic

Fig. 4. Differences in the percentage of apoptotic cells in mammary carcinomas when estimated in the peripheral and central (close to the necrotic region) tumor areas ($P < 0.001$).
cells among tumor parenchyma. Using pituitary isografted mice as a model for induction of mammary carcinogenesis, we observed higher ACD in carcinomas than in hyperplastic ductal and alveolar lesions, but we did not consider the effect of necrosis on ACD (10). It has been reported that a high ACD in the initiating phase of mammary carcinogenesis may eliminate the aberrant cell clones and thus suppress the neoplastic process (32).

In this study we also addressed questions of variability in the distribution of apoptotic cells in mammary tumors. The high numbers of apoptotic cells in some tumors might not be associated with the phenotype of the tumor cells per se, but more with intratumoral factors (hypoxia, nutritional deficiency and lack of growth factors) that may stimulate ACD. Recent in vitro in vivo studies have shown that hypoxia may affect ACD and that tumors resistant to hypoxia are more aggressive and have a poorer prognosis than those sensitive to hypoxia (33,34). It appears that in mammary tumors, and possibly in other malignant tumors, at least three types of cell death could be distinguished: (i) ACD that occurs in the proliferating areas and reflects the phenotype of tumor cells; (ii) ACD that occurs close to the necrotic areas and probably depends on intratumoral factors; (iii) necrotic cell death. In assessing the growth potential and the probability of progression of various mammary gland lesions, the contribution of necrosis as a negative factor in tumor growth should be taken into account as well (35).

In conclusion, in this study we obtained data indicating that neoplastic transformation of mammary epithelial cells in TEBs, which are considered the place of origin of most mammary carcinomas, is not associated with increased cell proliferation but with decreased ACD. We also found that dissociation of cell proliferation and apoptosis occurs in the early stages of mammary carcinogenesis and that various hyperplastic lesions differ in their potential for progression and malignant transformation. The low numbers of apoptotic cells in the peripheral, proliferating tumor areas and the increase in apoptotic cells close to necrotic areas requires a selective approach in employing apoptosis in tumors as end-points in chemoprevention and therapy studies.

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