Ultrastructure of the Hormone-dependent N-Nitrosomethylurea-induced Mammary Carcinoma of the Rat

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ABSTRACT

Hormone dependency of the N-nitrosomethylurea-induced mammary tumor in rats has been demonstrated by ovariectomy. Tumors showing a clear reduction in size in response to ovariectomy have been used in an ultrastructural study. Histologically, the tumor is an adenocarcinoma. The multilayered tumor parenchyma contains myoepithelial cells and highly and less-well-differentiated cells. The adluminal cells tended to be the most differentiated and were secretory in nature. After ovariectomy, junctional complexes of the luminal cells showed little change, but intercellular adhesion among the less-well-differentiated cells appeared weakened by the altered endocrine milieu; consequently, the parenchyma appeared less compact. Cellular degeneration occurred randomly and affected all cell types. However, the least affected cells were the poorly differentiated cells with a high nuclear-cytoplasmic ratio and few cytoplasmic organelles. The findings suggest that the latter may be the hormone-independent cell subpopulation in the N-nitrosomethylurea-induced mammary tumor.

INTRODUCTION

NMU has recently been introduced as a carcinogen to induce mammary gland neoplasia in rats. In contrast to DMBA-induced mammary tumors, the NMU-induced carcinoma frequently metastasizes to bone and spleen and occasionally to other sites. Due largely to the propensity to metastasize, tumors induced by NMU have been suggested as the more complete model of the 2 for human mammary carcinoma (6).

The cytoarchitecture of this tumor has been described as that of an adenocarcinoma or papillary carcinoma, and the tumor parenchyma appears to consist of both epithelial and myoepithelial cells. To the author's knowledge, the ultrastructural features of this tumor have not been described. Accordingly, in this paper, the fine structure of the NMU-induced mammary carcinoma is described before and after ovariectomy. Particular attention has been paid to the cellular components of the epithelia and their differential response to hormone deprivation.

MATERIALS AND METHODS

At 50 days of age, 30 female Fischer CEF rats were given the first of 3 monthly injections of NMU (ICN Pharmaceuticals, Inc.) in accordance with the method of Guittino et al. (6). Mammary tumors were palpable in about 70% of the treated rats 2 to 7 months after the first injection. In most cases, a single tumor developed although multiple tumors are not uncommon. After the tumor reached a maximal linear dimension of 2 to 3 cm, rats were ovariectomized. Tumor size was measured 3 times a week. One week after ovariectomy, those tumors showing clear reduction in size, i.e., greater than 20%, were designated 'hormone dependent.' A total of 4 such tumors and 4 tumors from intact rats was used in the study. Immediately after excision, tissues were thinly sliced and immersed in a glutaraldehyde-paraformaldehyde fixative for 90 min at 4°C. After an overnight wash in 1% cacodylate buffer (pH 7.3), the specimens were postfixed in 1% osmium tetroxide, dehydrated in ascending concentrations of alcohol, and embeded in Araldite 502. Thin sections were cut with a diamond knife and stained with uranyl acetate and lead citrate before being examined on a Philips 300 electron microscope.

RESULTS

All tumors examined were diagnosed histologically as either adenocarcinomas or papillary carcinomas. The epithelioid cell nests varied in size and shape; one representative field is illustrated in Fig. 1. Central to the pseudolobular formations were lumina of different dimensions. Ultrastructural examination revealed that many lumina contained large amounts of osmiophile proteinaceous secretory material. The tumor proper is of multilayered cellular architecture (Fig. 3). Most of the parenchymal cells have a large pale-staining nucleus and scant cytoplasmic organelles. Between the lining epithelium and the peripherally located, darkly stained myoepithelial cells are layers of undifferentiated cells. The width of these layers varies considerably; on occasion they may be absent.

These cell types can be distinguished ultrastructurally by their location as well as by the organelles they contain. The myoepithelial cells can be distinguished by their fusiform outline and their numerous cytoplasmic filaments which give the cytoplasm a greater opacity (Fig. 3). Cells lining the luminal surfaces are specialized for protein synthesis and secretion. Their Golgi complexes often appear active, containing many small clear vesicles and electron-dense granules. These vesicles and granules usually accumulate between the Golgi complex and the luminal surface. The large nucleus is basally situated. Lipid droplets, when present, are located supranuclearly. Junctional complexes are present between these cells, and their luminal surfaces are covered by a few microvilli. Other organelles include a few scattered mitochondria and occasional stacks of rough endoplasmic reticulum. The remaining cells appear less differentiated and lack polarity. Their large nuclei display finely dispersed chromatin and are centrally located. The scant cytoplasm contains inconspicuous Golgi complexes, a few wisps of rough endoplasmic reticulum, some mitochondria, and a few aggregates of polysomes. The undif-

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2 The abbreviations used are: NMU, N-nitrosomethylurea; DMBA, 7,12-dimethylbenz(a)anthracene.

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Differentiated cells are well interdigitated and are anchored to one another by desmosomes.

After ovariectomy, reduction of tumor volume is reflected in the decreased dimensions of the tumor. Foci of frank necrosis are readily discerned by light microscopy, and there is a tendency for peripheral sparing (Fig. 2). At the fine structural level, the adluminal cells still maintain surface modifications such as microvilli, and the cells adhere to one another through junctional complexes. Unlike the tumors in intact animals, the synthetic activity of the tumor cells appears greatly diminished (Fig. 4). Beneath this cell layer, the parenchymal cells display varying degrees of detachment from one another. Although desmosomes and gap junctions are still preserved on many cell surfaces, large clear clefts are seen between adjacent cells. In addition, many of these cells have begun to accumulate lipid droplets and myelin-like bodies. Degenerative changes are seen in both myoepithelial cells and the parenchymal cells (Fig. 5). It is not unusual, however, to find an apparently healthy, hormone-independent cell in the midst of areas of affected cells (Fig. 6). No definite pattern is apparent in the distribution of endocrine-sensitive and endocrine-insensitive cells within the tumor. In the periphery of tumor aggregates, reduplicated basal laminae are found. The surrounding stroma is heavily infiltrated with lymphocytes, plasma cells, and mast cells. The occurrence of these inflammatory cells showed a tendency to infiltrate the tumor itself and to concentrate close to basal laminae.

DISCUSSION

Despite the reported tendency of NMU-induced tumors to metastasize (6), their ultrastructure appears similar to that of the well-known DMBA-induced mammary tumor (12). Incomplete basal laminae of tumor cells have been suggested to be related to the ease of metastasis of certain human mammary carcinomas (13). In this study, continuous laminae appeared to be the rule rather than the exception. Thus, defects other than in formation of basal laminae may be more important in permitting metastasis in the NMU-induced mammary carcinoma.

Histological changes in DMBA-induced mammary tumors after ovariectomy were studied initially by Young et al. (17), and ultrastructural responses to this procedure were subsequently reported (15) in the same tumor model. Hydropic change, including the presence of watery cytoplasm and vacuole formation, characterizes endocrine-sensitive cells 2 days after endocrine deprivation. Scott et al. (15) also reported focal detachment between cells. In the present study, degenerative changes in luminal, undifferentiated, and myoepithelial cells were observed. Most of the changes suggested either depressed synthetic capacity or increased autophagic activity rather than hydropic change. A longer interval between ovariectomy and tumor excision in the present study may account in part for the difference in subcellular effects. The loosening of the tumor parenchyma suggests that cellular adhesion in an...
endocrine-dependent tumor may be hormonally regulated. The gap junction is known to provide ionic coupling and to facilitate metabolic interaction between adjacent cells (1, 5, 8). Hormonal modulation of the size and number of gap junctions between interstitial cells of the rat ovary has recently been demonstrated (2). While the gap junction itself may not be directly responsible for intercellular adhesion, changes in hormone-dependent cells may be communicated to adjacent cells via the gap junction. It is possible, therefore, that the detachment of previously tightly adherent cells may reflect metabolic uncoupling between cells with different endocrine requirements.

The existence of distinctive subpopulations of breast carcinoma cells has been demonstrated by fluorescent histochemical methods (9), through cell kinetic studies (7, 10), and in vitro systems (3, 4, 14). The differential attrition of the parenchymal cells of the NMU-induced mammary tumor after ovariectomy supports the concept that cellular heterogeneity occurs in solid tumors. Maynard et al. (11) studied the relation in human mammary tumors between estrogen receptor levels and histological grade and concluded that better-differentiated tumors rarely lack receptors. In the estrogen receptor-positive patient, administration of nafinoxidine, an antiestrogenic compound, was effective in reducing the tumor mass. Ultrastructural analysis of cutaneous metastases revealed that those cells resisting changes in the endocrine milieu appear less well differentiated (16). The present findings corroborate these studies and indicate that less-well-differentiated cells, i.e., cells containing scant amounts of cytoplasm and few cell organelles, are more resistant to the withdrawal of endogenous hormones. These tumor cells may well represent the "hormone-independent" cells of the NMU-induced mammary tumor.

REFERENCES

1. Anderson, E., and Albertini, D. F. Gap junctions between the oocyte and other cells present show a high nuclear-cytoplasmic ratio. Portions of myoepithelial cells (M) are peripherally situated. Note the presence of normal basal laminae (arrowheads). × 4,000. Inset, adluminal junctional complexes. × 7,000.

Fig. 3. NMU-induced mammary tumor from intact rat. Apical portion of the adluminal cells in the tumor acinus contains many electron-opaque, secretory granules (arrows). Other cells present show a high nuclear-cytoplasmic ratio. Portions of myoepithelial cells (M) are peripherally situated. Note the presence of normal basal laminae (arrowheads). × 4,000. Inset, adluminal junctional complexes. × 7,000.

Fig. 4. NMU-induced mammary tumor from ovariectomized rat. Secretory activity of the adluminal cells is markedly inhibited. Lysosomal complexes (LY) are common in these cells. The tendency to accumulate lipid droplets (L) is illustrated by 3 cells in this field. × 4,100. Inset, multilayered basal lamina seen in the peripheral tumor acini. × 19,000.

Fig. 5. Parts of NMU-induced mammary tumor from ovariectomized rat. A peripherally located aggregation of myoepithelial cells showing evidence of detachment from one another. Similar to the other parenchymal cells, a varying degree of lipid accumulation is shown. × 4,100.

Fig. 6. NMU-induced mammary tumor from ovariectomized rat. Poorly differentiated cells show little response to the changing endocrine milieu. The small foci of detachment (arrows) between cells suggest that intercellular adhesion is weakened. × 4,400.
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