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The Role of Myoepithelial Cells in the Morphogenesis of Induced Mammary Tumours

J. Tóth, J. Sugár, and O. Csuka
Research Institute of Oncopathology, Budapest, Hungary

Summary. The localization and cytomorphology of myoepithelial (ME) cells and their role in the morphogenesis of the mammary gland tumours of Wistar rats induced by 7,12-Dimethylbenz-a-anthracene-DMBA- were studied. Cells which do not participate in secretion and contain cytoplasmic myofibrillar bundles in a typical arrangement are considered to be of ME origin. In the histogenesis of induced mammary gland tumours no definite role can be attributed to mature ME cells or their precursors. Decreased differentiation is associated with reduced numbers of ME cells. No ME cells can be detected in the anaplastic, stromafree portions of the solid tumour. The sarcomatous component of the induced carcinosarcomas originates from connective tissue. ME cells may give rise to leiomyoma-like tumours comparable with the human benign mammary myoepithelioma. The atrophic areas of mammary gland tumours consisted mostly of preserved ME cells. The ME cells of induced mammary gland tumours were, in every respect, identical with the normal ME cells of control mammary glands.

Key words: Induced mammary tumours – Myoepithelial cells – Morphogenesis.

Introduction

The origin and character of mammary gland tumours of rats induced by carcinogenic hydrocarbons have given rise to much controversy. Since human mammary tumours of ME origin have been reported (Hamperl, 1970; Cameron et al., 1974; Tóth, 1977) we wished to study the role of this cell type in the histogenesis of experimentally induced tumours.

In this work cells of mammary gland tumours representing different histological structures and degrees of differentiation were analysed for origin and charac-

Send offprint requests to: J. Tóth, M.D., Research Institute of Oncopathology, H-1122 Budapest, XII., Ráth György u. 7–9, Hungary

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ter as it is of primary importance, in regard to hormone dependence and biological behaviour, whether the mammary gland tumour is of epithelial or myoepithelial origin (Fink et al., 1968; Hamperl, 1970; Murad, 1971; Murad and Haam, 1972).

Materials and Methods

Single doses (20–50 mg/kg) of DMBA suspended in olive oil were administered by gastric intubation to 264 outbred Wistar (W/H-Riop) female rats aged 50–52 days. The tumours thereby induced were examined after 6 to 18 months and ranged from pin-head size to about 2 cm diameter.

Altogether 235 tumours were examined together with the mammary glands of 5 virgin, 5 pregnant and 2 lactating animals and those of rats treated with 4×5 mg/kg Syntestrin, (4,4′-dihydroxy-α, β-diethyl-stilben-dipropionate) as controls. The specimens of mammary gland and tumour were fixed in 4% formaldehyde and Carnoy solutions.

Sections embedded in paraffin were stained with haematoxylin eosin, iron haematoxylin (Heidenhain), van Gieson stain, and azophloxin according to Puchtler (1974). Some of the sections were deparaffinized and treated with amylacetate and covered with a mixture of aniline oil and Canada balsam (1:1), (Aniline reaction of Orbán and Romhányi, 1962). Sections were studied 24–28 h later under an Opton polarising microscope.

For electron microscopy 1 mm^3 portions were cut from 15 control mammary glands and from 50 tumours. Samples were fixed in 2.5% buffered glutaraldehyde for 30 min at 4° C. The rest of the specimens was postfixed in 1% OsO_4, dehydrated and embedded in Durcupan (ACM, Fluka). Semi-thin sections were contrasted with uranylacetrate and lead citrate and examined with JEM-6C type electron microscope.

Results

Control Examinations

In the mammary glands of pregnant and lactating rats, and those treated with Syntestrin, spindle-shaped ME cells, staining bright red with azophloxin, were visible between the epithelial cells and the basal lamina. In the cytoplasm of ME cells, myofilaments showed positive birefringence with the aniline reaction. Accumulation of ME cells or their transformation into secretory cells was not observed during gestation and lactation.

During gestational proliferation, however, some clear cells with scanty organelles were detected amongst the epithelial and ME cells.

These cells contained some myofilaments and a poorly developed Golgi apparatus in the cytoplasm and micropinocytotic vesicles in the plasma membrane. They lacked secretion granules and were considered as "wandereing" or ME precursor cells (Fig. 1).

Induced Tumours of the Mammary Gland

Seventy-nine cases of highly differentiated secretory and non-secretory alveolar adenomas were studied, five of them by electron microscopy.

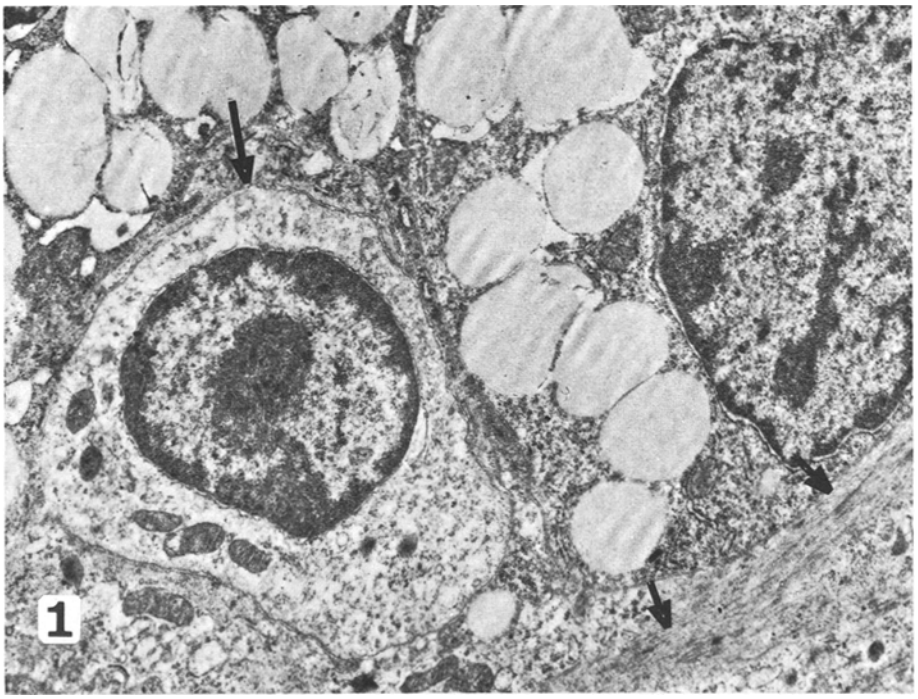

Fig. 1. Lactating mammary gland of Wistar/H-Riop rat. Epithelial cell filled with secretory granules, and an immature, precursor myoepithelial (*ME*) cell (*arrow*). In its vicinity there is a mature peripheral ME cell with typical bundles of cytoplasmic myofilaments (*arrows*). ×11,250

In the stromal connective tissue there were glandular acini lined with epithelial cells. Spindle-shaped, elongated ME cells were seen under the epithelial layer (Fig. 2). Most of the adenomas were secretory in character. The electron micrographs of ME cells revealed the presence of well developed myofilamentous bundles of medium electron density in the cytoplasm and showed ME cells were anchored to the basement membrane by hemidesmosomes. Mitotic figures or focal accumulation of ME cells were not encountered. In the secretory tumours, ME cells were more easily distinguished from epithelial cells by their lack of secretory granules.

Dedifferentiated Secretory and Non-Secretory "Malignant" Tumours of the Mammary Gland

A total of 77 cases were examined, 31 by electron microscopy.

Tubular, alveolar, glandular, papillary, solid and sarcoma-like tumours had very little stromal connective tissue. In these tumours ME cells were present only at the junction between parenchyma and stroma. It was not possible to demonstrate myofilaments ultrastructurally with azophloxin in the sarcomatous tumours and carcinosarcomas.

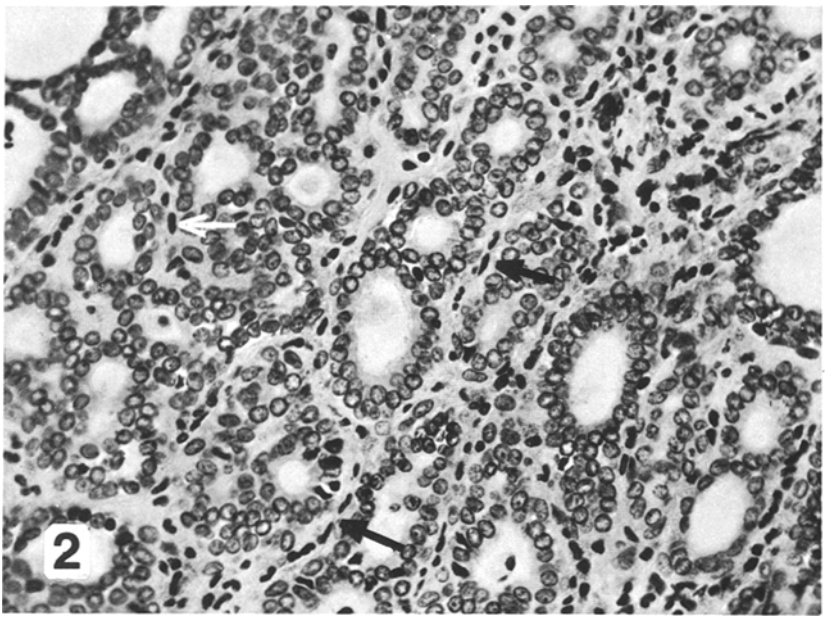

Fig. 2. Differentiated adenoma. ME cells appear around the glandular acini (*arrows*). Azophloxin stain (Puchtler). ×350

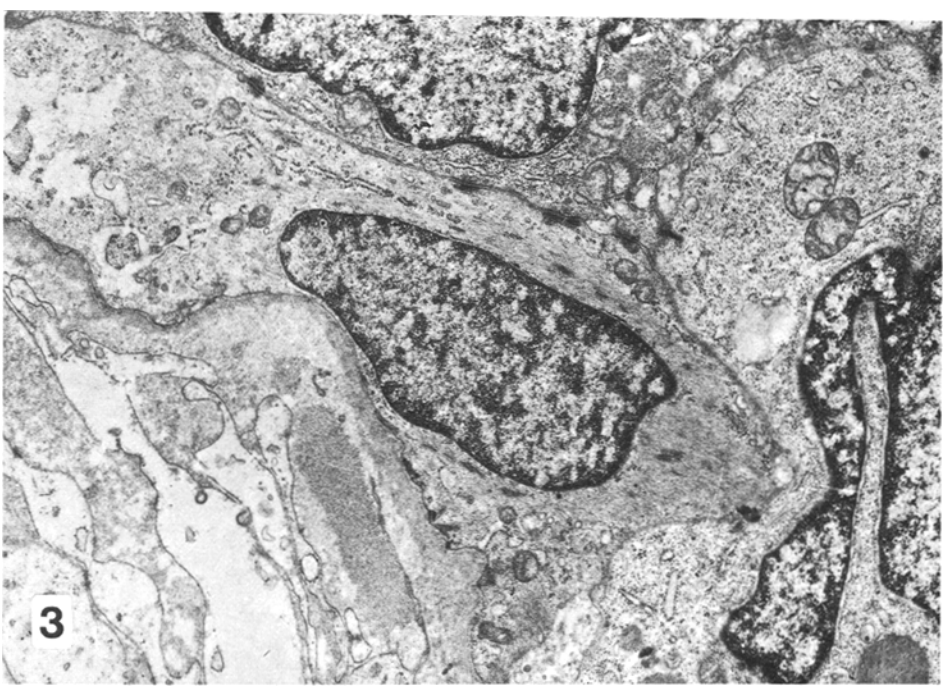

Fig. 3. Dedifferentiated, non-metastasizing mammary "carcinoma". At the periphery of the neoplasm there are mature ME cells. Basement membrane is irregular or absent. Polymorphic tumour and ME cells connected by desmosomes. ×12,250

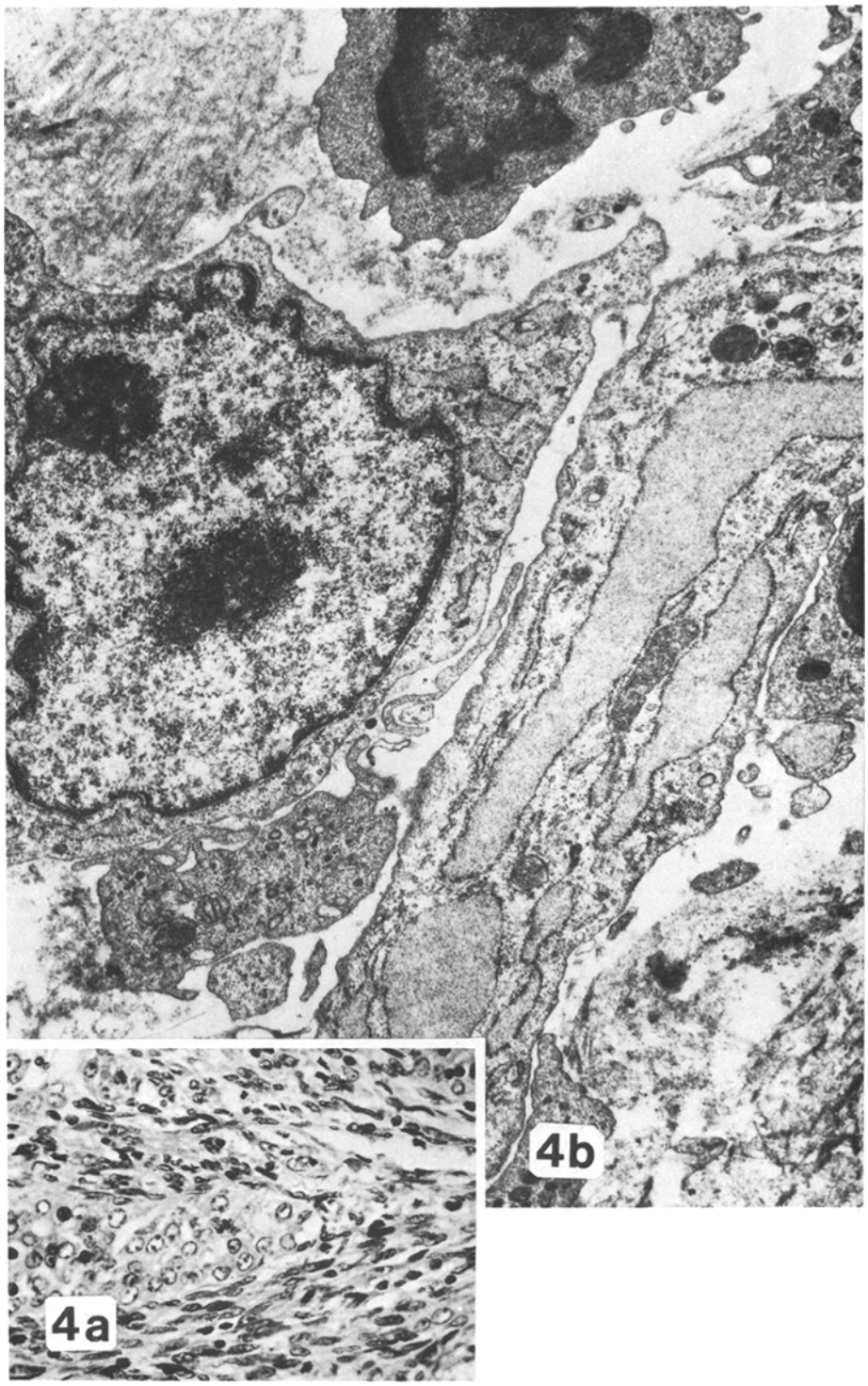

Fig. 4a and b. Carcino-sarcoma. **a** Inset: no ME cells among the sarcomatous and carcinomatous components. H-E. ×150. **b** Fibroblast-like tumour cells are loosened, and show abundant rough surfaced endoplasmic reticulum, and cytoplasmic protrusions on the cell surfaces. ×12,250

With the electron microscope, decreased detachment of tumour and ME cells and the presence of few desmosomes were observed.

The basement membrane was usually widened and irregular and in some cases the lamina densa was multilayered in the dedifferentiated secretory tumours, ME cells often assumed the form of a dense myofilamentous bundle located at the periphery of a tumour cell nest or at the margin of an acinus (Fig. 3). Normal or mitotic ME cells did not occur in these tumours.

In two old, atrophic tumours, some areas of the dilated acini were lined with flattened pykotic columnar epithelium. There were some parts of the glandular wall, however, where only peripheral ME cells were preserved.

Metastasizing Mammary Tumours

Twenty-one cases were examined, four by electron microscopy.

Their histological structure was identical with that of the dedifferentiated tumours. In carcinosarcomas, flattened ME cells occurred only occasionally at the periphery of tumour cell nests or glands. Quite often no ME cells were seen in large areas of the tumour.

By electron microscopy, the structure of ME cells appeared normal and mitotic figures or cells undergoing malignant transformation were not observed.

Tumour cells in the mesenchymal component of metastasizing carcinosarcomas and induced sarcomatous tumours were spindle-shaped or polygonal. The cell nuclei were large and lobular and the nuclear chromatin abundant and clumped. The cytoplasm contained large amount of rough-surfaced endoplasmic reticulum. Myofilamentous bundles were not apparent. In some cells, however, microfilaments were visible arranged in parallel to the longitudinal axis of the cells (Fig. 4a, b).

Discussion

In breast tumours of varying histological types and degrees of differentiation, ME cells were observed only at the periphery of alveolar acini and of solid tumour cell nests or at the junction between parenchyma and stroma. Neither precursor nor mature ME cells showed secretory transformation, confirming the observation of Murad and Pretlow (1975) and, on this basis, ME cells and epithelial elements can be distinguished. During neoplastic transformation of ME cells, the cytoplasmic myofilaments increase in number and become thicker, and, the Golgi apparatus enlarges (Pulley, 1973; Bomhard and Sandersleben, 1975; 1976). We observed none of these ultrastructural features in the ME studied, and focal accumulation or neoplastic proliferation of ME cells were not encountered. ME cells were frequently found in the highly differentiated mammary adenomas induced but seldom or never seen in the differentiated non-metastasizing and metastasizing tumours.

The cells of the sarcomatous component of carcinosarcomas and of mesenchymal mammary tumours were fibroblast-like, with ultrastructural features

suggesting a connective tissue origin and character. Very rarely, cytoplasmic microfilaments were observed in these tumour cells indicating a myofibroblastic character (Feiner and Kaye, 1976). In the cytoplasm of epithelial type tumour cells there were thin perinuclear microfilaments and bundles of thicker and dense tonofilaments. These filaments are thought to play a role in the motility and invasiveness of tumour cells (Schenk, 1975).

We do not believe that a true adenoma develops from ME cells. Wandering (Kurosumi et al., 1968 or precursor Tandler, 1965; Radnor, 1972), ME cells, however, may give rise to tumour development. In such instances this may result in tumour development.

In such instances this may result in leiomyoma or leiomyosarcoma-like tumours (Dévényi, 1959; Cameron et al., 1974; Schlotke, 1975; Bomhard and Sandersleben, 1976; Tóth, 1977).

The occurrence of ME cells in induced tumours of the rat mammary gland has been observed by many investigators (Archer, 1969; Murad and Haam, 1971, 1972; Murad, 1971). Archer (1969) attributed no importance to ME cells in the morphogenesis of such tumours. The tumour induced by DMBA was considered by Murad and Haam (1971) to be neoplasm of ductal epithelial origin. Later, in 1972, however, the same authors reported neoplastic proliferation of ME cells induced by this means.

Tumour cells, in which myofilaments cannot be demonstrated, may be supposed to be of ME origin only on the basis of certain enzymological and morphological similarities (Gould et al., 1975). Induced mammary gland tumours of mice are considered by Russo et al. (1976) to be of epithelial origin on the basis of Na^+ and K^+ dependent ATP-ase reaction.

Contrary to the opinion of Murad and Haan, the Mg^{++} dependent ATP-ase reaction cannot be regarded as a suitable method for the identification of ME cells (Tóth et al., 1972; Russo and Wells, 1977). Similarly, a positive alkaline phosphatase reaction does not prove a ME origin of tumour cells (Pulley, 1973; Hamperl, 1970).

Our results suggest that most of the mammary gland tumours induced in rats are of epithelial origin. The sarcomatous component of carcinosarcomas and the sarcomatous mammary tumours are not of ME origin.

An origin from myoepithelial cells can be proven only when typical myofilamentous bundles are present in the cytoplasm of tumour cells.

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