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Morphology of MXT Mouse Mammary Tumors. Correlation with Growth Characteristics and Hormone Sensitivity*

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Abstract—*The transplantable MXT mouse mammary tumor has been a useful tool for studying endocrine mechanisms underlying mammary tumor growth. It is our experience, however, that this model is unstable during serial transplantation. This paper analyses this variability from the viewpoints of histology and estrogen receptor content and indicates that these parameters should always be checked before planning experimental work. It is advised that a more homogeneous material is needed and that this goal should be achieved by clonal selection before transplantation.*

INTRODUCTION

OUR UNDERSTANDING of the hormone dependence of breast cancer has been improved by studying murine mammary tumor models. Thus, investigation of the 7,12-dimethylbenz(a)anthracene (DMBA)-induced rat mammary carcinoma [1,2] revealed that besides the ovarian hormones [3–6], prolactin [4–12] participates in the regulation of tumor development and growth.

The transplantable MXT mouse mammary tumor is another useful tool [13–15]. It contains estrogen receptors (ER) and its growth is modulated by ovarian and pituitary hormones. The original tumor arose in a urethane-treated female BD2F1 (C57BL × DBA2F1) mouse carrying a pituitary isograft under the renal capsule [16]. Its morphological appearance was that of a ductal papillary carcinoma resembling human breast cancers. During the last 6 yr three tumors deriving from this original carcinoma were introduced into our laboratory. Marked morphological modifications were observed during serial transplantations. The aim of this paper is to describe these modifications and correlate them with the growth characteristics and hormone sensitivity of the tumor.

MATERIALS AND METHODS

Animal and tumor transplantation procedure

In 1979, 1981 and 1984 (C57BL × DBA2f)F1 mice bearing MXT tumors were received in our laboratory for subsequent transplantations. In 1979 animals were kindly provided by Dr D. Medina (Baylor College, Houston, TX); tumors obtained from these animals will be designated MED throughout. In 1981 and 1984, animals were from Dr A. E. Bogden (Mason Research Institute, Worcester, MA). Tumors from this source were designated BOG.I and BOG.II respectively.

MXT tumors were maintained in 8—10-week-old female BD2F1 mice (20–25 g) (IFFA-CREDO, France) by monthly serial transfers. In each transfer tumors of about 1 cm^3 were pooled and minced into 15-mm^3 pieces under sterile conditions; some pieces were taken for histological examination and estrogen receptor (ER) measurement. Tumor fragments were inoculated subcutaneously on each flank in the axillary fat pad through a trochar (gauge 13). Fifteen (T1–T15), 36 (T1–T36) and 16 (T1–T16) transfers were respectively studied for MED, BOG. I and BOG.II.

Estrogen receptor assay

Samples of ~500 mg tumor tissue stored in liquid nitrogen were used for estrogen receptor assay. The {^{3}H}estradiol ({^{3}H}E_2) binding capacity of their cytosol fraction was measured using the dextran-coated charcoal method previously described [17]. Receptor levels were expressed as fmol/mg cytosol protein. Protein concentration was estimated by the Bio-Rad assay using bovine

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serum albumin as standard.

Histological procedures

Tumor pieces were fixed in Bouin-Hollande fluid. After washing and embedding in paraffin, 4-μm sections were stained using hematoxylin and eosin (H – E) or hematoxylin, eosin and saffran (H–E–S).

Tumour size

For growth experiments tumors were inoculated in intact mice or in mice ovariectomized 15 days earlier. Tumor size was measured weekly by means of a caliper and expressed as 'surface' by multiplying two perpendicular diameters.

RESULTS

Morphology

MED tumor transplants. From T1 to T9 the tumor was a differentiated adenocarcinoma showing acini varying in size and lined by a single layer of cuboidal or columnar epithelium (Fig. 1); stromata were scarce. Tumor cells were uniform, moderately large with round or oval nuclei, and containing 1–3 nucleoli. The chromatin was diffuse and mitoses were moderately frequent.

In transplants T10, T11 and T12, between the glandular elements, more undifferentiated, slightly eosinophilic tumor cells were found. Nuclear pleiomorphism was evident and mitotic figures were more common. The connective tissue was more abundant. Significant inflammatory pattern was observed.

The fibrovascular stroma was markedly enlarged in T13–T15 but some acini were still recognized (Fig. 2).

BOG.I tumor transplants. The first transplants of the original tumor (T1–T20) closely resembled MED histological appearance: they were well-differentiated adenocarcinomas. Progressively, however, the feature of a well-differentiated adenocarcinoma was lost. Transplants T21–T31 presented a characteristic pattern of intermingled small glandular elements and poorly differentiated polygonal cells with large nuclei and a moderate amount of pale cytoplasm: a characteristic of poorly differentiated adenocarcinoma (Fig. 3). From T32 the poorly differentiated adenocarcinoma was invaded by spindle-shaped cells. Mitotic figures were common. Late transplants (T32–T36) were completely devoid of glandular acini.

BOG.II tumor transplants. The initial histological aspect of BOG.II was that of an anaplastic carcinoma. Moreover, BOG.II transplants were composed of two recognizable epithelial tumor cells. Type A subpopulation consisted of polygonal epithelial cells (Fig. 4). Type B subpopulation consisted of sheets and interspersed bands of spindle-shaped cells with large elongated nuclei containing prominent nucleoli (Fig. 5). This picture was similar to that observed in BOG.I T32–T36. As the tumors became bulky, wide areas of necrotic tissue and numerous polymorphonuclear leukocytes were detectable. This phenomenon, encountered in other models, could be explained by the rapid growth rate of the epithelial cancer cells not followed by a similar connective and vascular growth pattern. Capillaries were lined by flattened endothelial cells with their elongated nuclei that could be distinguished from the tumor cells. Invasion of striated muscles was observed in some regions. Mitotic figures were numerous (Fig. 6).

Tumor growth and survival of the animals

Figure 7 provides representative growth patterns and response to ovariectomy of each tumor type. Corresponding survival of tumor-bearing animals is shown in Fig. 8. Comparison of these figures shows that death appears more rapidly in mice bearing the faster growing transplant.

MED tumor transplants. The growth of well-differentiated carcinomas of early transplants was slow and characterized by an extreme sensitivity to ovariectomy (Fig. 7, MED). Thus the operation largely reduced the tumor growth and, as a consequence, totally suppressed the death of the animals (Table 1). On the contrary, tumors with large fibrovascular stroma displayed a weak sensitivity to the ovariectomy as shown by the total absence of prolongation of the survival (Table 1).

BOG.I tumor transplants. Growth of the BOG.I tumors (T1–T31) was faster than that of the well-differentiated carcinomas (MED). They were also less sensitive to ovariectomy as shown by their higher growth rate in castrated animals (Fig. 7) and weak influence on the survival rate (Fig. 8, 'BOG.I'). On the T32–T36 transplants ovariectomy failed to influence growth at all.

BOG.II tumor transplants. These anaplastic tumors were characterized by an extremely high growth rate, as well as by a total absence of response to ovariectomy (Fig. 7). Survival of the tumor-bearing animals was shorter than with the well-differentiated carcinomas (MED) (Fig. 8).

Estrogen receptor levels

MED tumor transplants. Cytoplasmic ER contents of differentiated carcinomas of the early tumors transplants (T1–T9) were quite constant (40 fmol/mg protein). The progressive appearance of connective tissue (T10–T12) in the subsequent transplants was associated with a marked reduc-

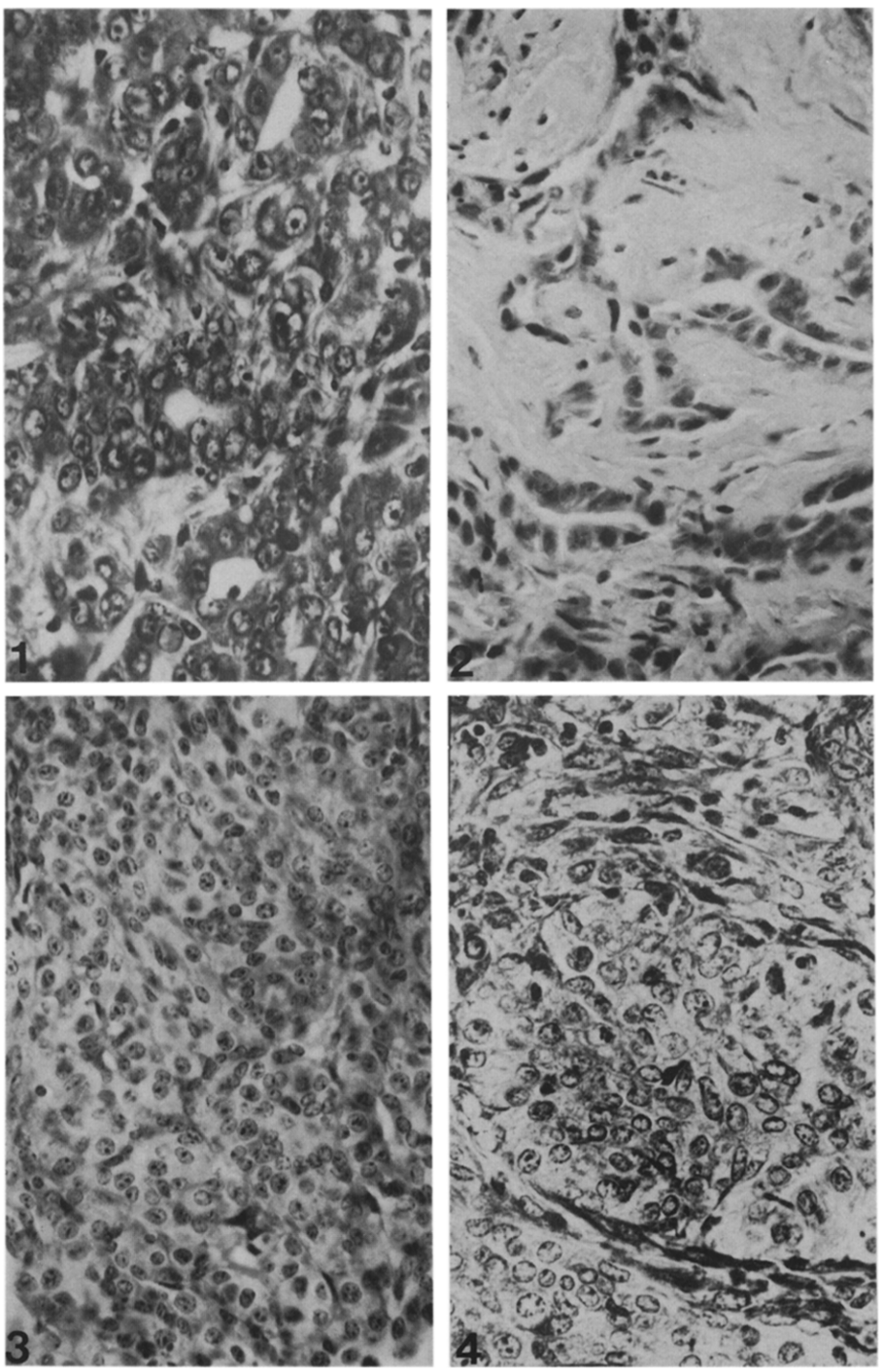

Fig. 1. Early histologic aspect of MED transplants. Well-differentiated carcinoma. Acini vary in size and shape. Stroma is scarce (H–E, × 1000).

Fig. 2. Late histologic aspect of MED transplants. Stroma is profuse and contain some acini (H–E–S, × 1000).

Fig. 3. BOG.I transplant. Histological aspect of poorly differentiated carcinoma (H–E, × 1000).

Fig. 4. BOG.II transplant. Histological aspect of polygonal epithelial tumor cells (H–E, × 1000).

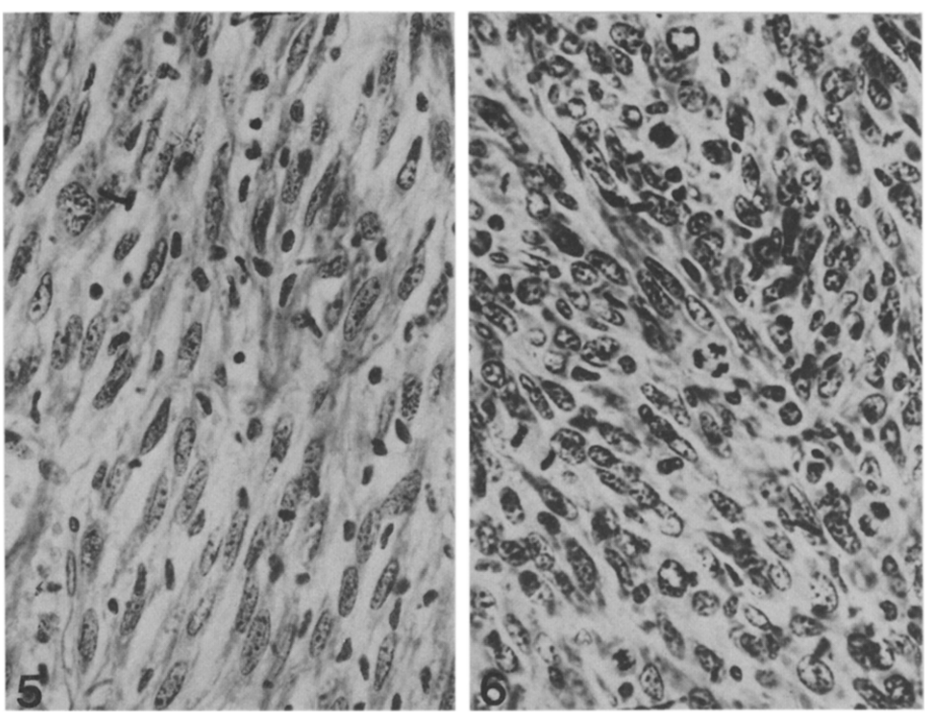

Fig. 5. BOG.II transplant. Same slide as in Fig. 4. Histological aspect of spindle-shaped epithelial tumor cells (H–E, × 1000).
Fig. 6. BOG.II transplant. Histological aspect showing numerous mitotic figures. An atypical mitose is seen in the centre of the figure (H–E, × 1000).

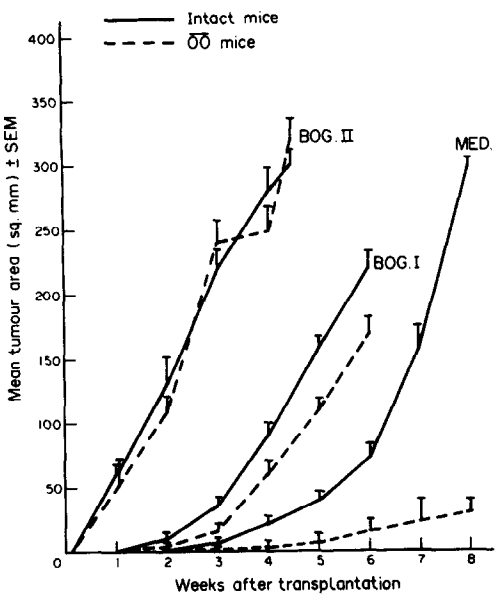

Fig. 7. Comparison of tumor growths between representative transplants of well-differentiated (MED, T4), poorly differentiated (BOG. I, T25) and anaplastic adenocarcinomas (BOG.II, T4). The last contains polygonal and spindle-shaped tumor cells. MED and BOG.I transplant growths are slowed down by ovariectomy. Castration has no effect on BOG.II transplant growth.

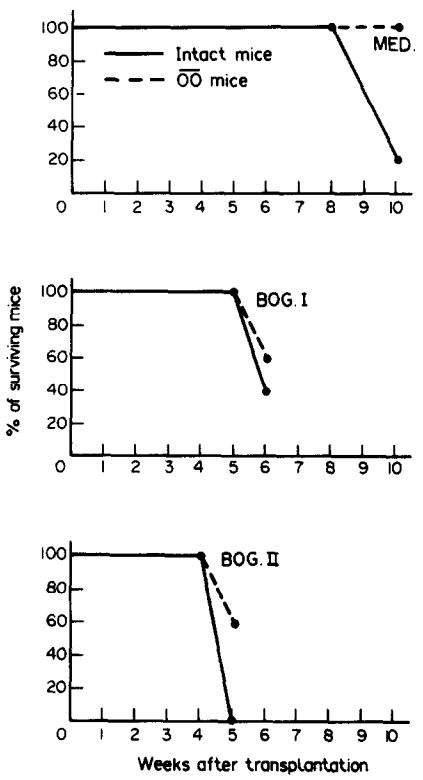

Fig. 8. Comparison between survival of the well-differentiated- (MED, T4), poorly differentiated- (BOG.I, T25) and anaplastic carcinoma (BOG.II, T4) transplant-bearing intact and ovariectomized mice. Corresponding tumor growth rates are given in Fig. 7.

tion of receptor concentration. Tumors with large fibrovascular stroma of the late transplants (T13–T15) were totally devoid of detectable receptors (Fig. 9).

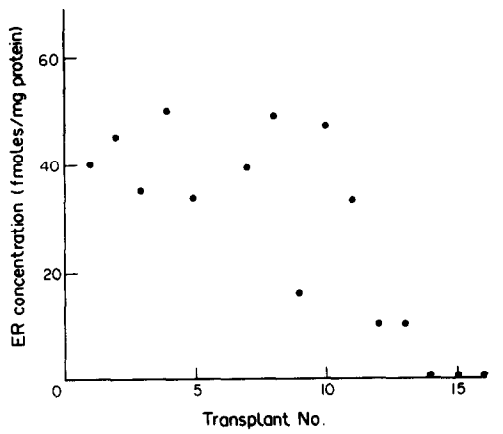

Fig. 9. ER content evolution of the MED transplant during serial transplantations.

Table 1. Number of surviving animals (groups of 15), 8 and 10 weeks after tumor inoculation.

	8 weeks (%)	10 weeks (%)
T4–T8 (MED, ER+) (acinar-riched aspect)		
Control	90	58
Ovariectomized mice	100	100
T15 (MED, ER−) (fibrovascular stroma abundant aspect)		
Control	87	60
Ovariectomized mice	53	33

BOG.I tumor transplants. ER content of differentiated or poorly differentiated carcinomas of the first 31 transplants were characterized by a receptor concentration fluctuating within a rather large range (25–100 fmol/mg protein) without any tendency to increase or decrease with time (T1 = 64; T1–T31: mean = 64 fmol/mg protein). Interestingly, tumors with low or high ER levels displayed the same histological pattern. Anaplastic tumors of the late transplants (T32–T36), in wich two cell populations were observed, were characterized by a low concentration of ER (T32 = 40; T33 = 20; T34 = 14; T35 = 6; T36 = 6 fmol/mg protein).

BOG.II tumor transplants. ER levels of all tumors were of the same order of magnitude as BOG.I of the late transplants (T1–T16: mean = 20 fmol/mg protein).

DISCUSSION

The importance of murine tumors for the study of breast cancer has been recognized for a number of years [7, 18]. The MXT mouse mammary tumor model, which was originally described as ductal papillary carcinoma, resembling human

mammary cancer, is relevant as an experimental tool for research on the hormone dependence mechanisms in the human [19]. The present study indicates, however, that this model has limitations and should be used with caution.

The histological appearance of the three MXT tumors investigated changed during serial transfers. Morphological transformation seems a usual property of the transplantable mammary tumors since it was also reported in other models such as DMBA-induced adenocarcinoma of rats [20] or pregnancy-dependent tumors in GR mice [21]. Early MED (T1–T9) and BOG.I (T1–T20) MXT transplants corresponded to well-differentiated ductal carcinomas without important stroma [16]. This feature progressively disappeared: connective tissue become abundant in MED passages. BOG. I (T32–T36) shifted to a poorly differentiated carcinoma similar to that observed in BOG.II transplants. Moreover, the later anaplastic tumors contained at least two histologically recognizable epithelial tumor cell subpopulations, a polygonal cell population and a spindle-shaped one. Recent data have shown that only the former display sensitivity to estrogen [22]. The cellular heterogeneity of this model is not unexpected if we refer to recent publications [23–26]. Furthermore, even when the cells appear histologically homogeneous in human or animal neoplasms, they are known to be heterogeneous with respect to a variety of biological criteria, such as growth rate [27], hormone receptors [28–30], vulnerability to cytotoxic agents [31, 32], antigenic properties and immunogenicity [33] and culture characteristics [34–36].

Estrogen receptor assessment provides further proof of heterogeneity and lack of stability of MXT carcinomas during serial passages. ER progressively disappeared when MED tumors shifted from a well-differentiated adenocarcinoma to an anaplastic carcinoma. ER of BOG.I remained at a constant level until an histological aspect similar to BOG.II appeared. At that time receptor concentration fell to a value similar to that found in BOG.II. Assessment of the hormone sensitivity of these tumors indicates that the reduction in ER content was associated with a loss of response to ovariectomy. From the foregoing, it is thus clear that the acinar aspect of the tumor is associated with a significant ER content. MED tumors with extensive stroma (T13–T15) were devoid of receptors. This observation is reminiscent of the situation found in tumors growing in castrated animals which show the same histological pattern [14].

Our data agree with those of Meyer *et al.* [37], who showed that rapid growth rates of cellular replication are associated with a low incidence of ER. Well-differentiated carcinoma (early MED and BOG.I) contain larger amounts of ER and have a lower growth rate than anaplastic ones (late BOG.I and BOG.II).

The present work indicates that the MXT mammary tumor model is a valuable tool for the study of estrogen dependence of breast cancer if morphological controls and receptor measurements are performed at each transplantation. Isolation of a biologically homogeneous tumor material (i.e. clonal selection) should overcome this difficulty.

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