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Rat Mammary Tumors from Carcinogen-induced Nodules and their Responsiveness to Ovariectomy*

EVELYN M. RIVERA† and SRINIVASAN VIJAYARAGHAVAN
Department of Zoology, Michigan State University, East Lansing, Michigan 48824, *U.S.A.*

Abstract—*The research objective was to determine whether DMBA-induced rat mammary tumors derived from transplanted mammary nodules differ in ovarian-responsiveness from tumors originating randomly from* in situ *mammary parenchyma. Nodule-derived tumors were from hyperplastic alveolar outgrowths in mammary fat pads, which were either first generation outgrowths (primary nodule outgrowths) or generations 3–6 of a tumorigenic nodule line (nodule outgrowth line T18). Tumors were induced in primary outgrowths by secondary exposure to DMBA* in vivo *or* in vitro. *The nodule line was not re-exposed to DMBA because of its high oncogenicity* (80–85%). *Despite the histological similarity of the tumor derivatives, primary nodule outgrowths and the nodule outgrowth line developed a higher proportion of ovarian-independent to ovarian-dependent tumors* (1:3 *and* 3:4 *respectively) than did* in situ *mammary tissues* (1:9). *These results are consistent with the observation that nodule outgrowths were themselves ovarian-independent.*

INTRODUCTION

HYPERPLASTIC alveolar nodules are the most common type of dysplasia induced by dimethylbenz(a)anthracene (DMBA) in rat mammary glands [1, 2]. These lesions are transplantable in gland-free mammary fat pads of syngeneic hosts, where they proliferate and produce hyperplastic nodule outgrowths [1]. Although their capacity to undergo tumor transformation is limited [1, 3], it is markedly increased by alterations of their growth environment [4] and by re-exposure to DMBA [5]. Histological studies indicate that nodule-derived tumors are mammary adenocarcinomas [1, 4], but little else is known of their growth and behavioral properties. Since DMBA-induced rat mammary tumors are generally ovarian-dependent [2, 3, 6], it was of interest to determine the responsiveness to ovariectomy of nodule-derived mammary tumors. The present study shows that a higher proportion of ovarian-independent mammary tumors develop from nodule outgrowths than from *in situ* mammary glands and that nodules themselves are partially autonomous.

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†To whom reprint requests should be addressed.

MATERIALS AND METHODS

Rats

Two sublines of Lewis strain rats, separately maintained and inbred by brother–sister matings in our laboratory since 1976, were used in this study. Most of the experiments were performed on strain LEW/SsN provided by the National Institutes of Health, Bethesda, MD, the remainder on strain LEW/Sim from Simonsen's Laboratories, Gilroy, CA.

Induction of mammary nodules and tumors

Virgin females, 45–50 days of age, were fed 20 mg of DMBA (Sigma Chemicals, St. Louis, MO), as described before [4]. A high incidence of mammary nodules and tumors develop within 3–5 months after DMBA feeding. The resultant tumors are designated as *in situ*-derived mammary tumors to distinguish them from those derived from nodules, which were randomly selected from the mammary glands and transplanted as described below.

Primary nodule outgrowths

Primary nodule outgrowths were first generation hyperplastic tissues, which developed

from nodules transplanted individually into gland-free mammary fat pads of 9–12-week-old syngeneic virgin hosts by the method of DeOme *et al.* [7], as modified for the rat by Rivera *et al.* [4]. Of the 20 mammary tumors, 17 were induced in the outgrowths by feeding 5–20 mg of DMBA to the hosts 1–3 weeks after transplantation of nodules. The remaining 3 tumors were induced by culturing nodules for 5 days in medium 199 (Microbiological Associates, Walkersville, MD) supplemented with DMBA (1 μg/ml) and insulin, corticosterone and prolactin (5, 1 and 5 μg/ml respectively) before transplantation into the fat pads of untreated hosts. The tumors were randomly selected from those which developed and, since the ratios of ovarian-dependent to ovarian-independent tumors did not differ significantly according to method of induction, the results were combined in Table 1.

Nodule outgrowth line T18

This serially-transplantable nodule line originated from the outgrowth of a DMBA-induced nodule in our laboratory. It has been maintained by transplanting 10–20 pieces of hyperplastic outgrowth at each generation into a corresponding number of gland-free mammary fat pads. Since 80–85% of the outgrowths developed tumors, this line was not re-exposed to DMBA. Tumors from outgrowth generations 3–6 were studied. The histology of the outgrowths was typical of hyperplastic alveolar nodules [8] and tumors did not develop until about 15 weeks after outgrowth transplantation.

Table 1. Differential responsiveness to ovariectomy of DMBA-induced mammary tumors from various mammary sources

Source of tumors	No. of tumors studied	Tumor responsiveness Ovarian-dependent	Ovarian-independent
Primary nodule outgrowths	20*	13(65%)	7(35%)
Nodule outgrowth line T18	19*	5(26%)	14(74%)
In situ mammary tissue	26†	23(88%)	3(12%)

*The number of tumors studied corresponds to the number of outgrowths studied, each outgrowth having produced a single tumor mass.

†These 26 tumors were from 24 mammary glands of 14 rats.

In situ *mammary tissues*

Tumors from these tissues were those induced in *in situ* mammary parenchyma 3–5 months after DMBA feeding as described above. No further exposure to the carcinogen was provided. Tumors in this category may be considered 'randomly derived', since it is not known whether they arose from nodule or ductal portions of the mammary gland. Nevertheless, it was desirable to include in this study tumors which develop in non-transplanted tissues.

As summarized in Table 1, the 3 sources of mammary tumors were: first generation outgrowths of mammary nodules (primary nodule outgrowths), generations 3–6 of outgrowths derived from a single mammary nodule (nodule outgrowth line T18) and *in situ* mammary parenchyma (non-transplanted).

Evaluation of the effects of ovariectomy on tumors

When tumors were about 1.5–2.0 cm in diameter the ovaries were removed bilaterally through dorsal slits in the body wall. After ovariectomy tumor size was estimated 2–3 times weekly by measuring the largest diameter and the smallest diameter perpendicular to it. Tumors were considered ovarian-dependent if their size was reduced by about 50% within 2–3 weeks after ovariectomy [3, 6]. Those which continued to grow were provisionally classified as ovarian-independent. This classification was confirmed or rejected after autopsy, when all tumors were cut into slices to determine the proportion of the tumor mass represented by viable epithelium. In a few cases tumors which were apparently growing contained only a peripheral rim of viable tissue surrounding a necrotic mass. These were reclassified as ovarian-dependent tumors. Representative portions of tumors were fixed in Carnoy's solution and processed for histological examination. Three tumors, found after histology to be fibroadenomas, were eliminated from final evaluation; only mammary adenocarcinomas were considered in this study.

Evaluation of the effects of ovariectomy on the maintenance and growth of mammary nodules

To determine whether intact ovaries were required for the formation and maintenance, respectively, of nodule outgrowths in gland-free mammary fat pads, host rats were ovariectomized 4 weeks before and 8 weeks after nodules were transplanted. Animals were terminated 8 weeks and 4 weeks, respectively, after ovariectomy and the mammary fat pads stained and examined as described before [4].

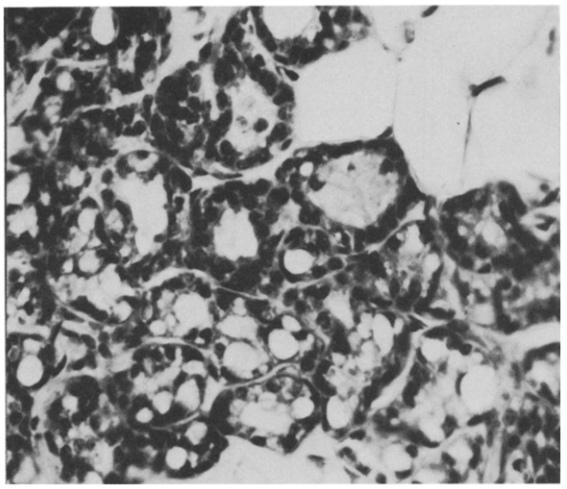

Fig. 1. *Section of transplanted mammary nodule in gland-free mammary fat pad of ovariectomized host. Note maintenance of alveolar structure and secretory appearance* (×340).

RESULTS

Table 1 shows that only 12% of mammary tumors which developed in *in situ* mammary tissues were ovarian-independent. In contrast, although most of the tumors derived from primary nodule outgrowths were ovarian-dependent, there were 3 times more (35%) ovarian-independent tumors than from *in situ* mammary glands. Even more striking were the results obtained with tumors from nodule outgrowth line T18, where nearly 75% were ovarian-independent with no significant differences among different generations.

Table 2 shows that the majority (86%) of nodule transplants survived in ovariectomized hosts (Fig. 1), although they failed to proliferate outgrowths. When ovariectomy was delayed for 8 weeks to allow proliferation, the outgrowths were largely maintained compared to those of intact controls, with only a slight reduction in the extent of lobuloalveolar development.

The tumors were mammary adenocarcinomas in which considerable histological variation was found [9], regardless of tumor source.

DISCUSSION

The finding that primary nodule outgrowths generated a higher ratio (1:3) of ovarian-independent mammary tumors than did *in situ* mammary tissues (1:9) opens a new perspective of the differing potentials of mammary tissue. Whether nodules are the source of ovarian-independent tumors in DMBA-treated mammary glands is difficult to test directly in view of their limited potential to form tumors without modification of their growth environment [4, 5]. Since only some 10–15% of untreated nodule outgrowths develop tumors, the possibility that secondary exposure to DMBA itself contributes to the development of ovarian-independence is difficult to assess. However, in a limited study (data not shown) 4 out of 6 tumors derived from untreated nodule outgrowths were ovarian-independent. In addition, there is indirect evidence to suggest that it is the responding tissue rather than secondary exposure to the carcinogen *per se* which determines tumor phenotype. Of 6 randomly selected tumors induced in ductal mammary outgrowths by secondary exposure to DMBA, all regressed after ovariectomy (Rivera, unpublished observations).

That nodules gave rise to both hormone-responsive and -independent tumors may be attributed to nodule variability, a known property of these lesions [10]. On the other hand, the fidelity with which nodule line T18 generated a 3:4 ratio of ovarian-independent to -dependent tumors is consistent with previous studies on the stability of nodule outgrowth lines [10–12].

It had been difficult to reconcile observations of the ovarian-independence of nodules and their outgrowths *in vivo* [13] and *in vitro* [4] with the finding that the majority of DMBA-induced mammary tumors are ovarian-dependent [11]. By exploring the ovarian-responsiveness of differently derived mammary tumors, our study revealed a closer correspondence between nodules and their tumor derivatives than between nodules and tumors derived from unspecified mammary tissue sites.

Table 2. Effects of ovariectomy on viability and growth of mammary nodules in gland-free mammary fat pads

Time of ovariectomy	No. of samples transplanted*	No. of takes	No. of samples with outgrowth
Before transplantation	42	36	0
8 weeks after transplantation	12	12	11
Control (intact ovaries)	10	10	10

*Nodules were transplanted singly into the right and left gland-free inguinal mammary fat pads of syngeneic females.

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