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Pituitary Role in the Growth of Metastasizing MRMT-1 Mammary Carcinoma in Rats[1]

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SUMMARY

MRMT-1 is a mammary carcinoma induced in immunologically impaired female Sprague-Dawley rats fed 3-methylcholanthrene. Its biological characteristics include transplantability to syngeneic normal rats and spontaneous metastases to various organs. Hypophysectomy performed 48 hr after tumor inoculation resulted in tumor regression accompanied by the disappearance of the metastasis in lungs of all the animals. The hypophysectomized animals were given replacement treatments, such as transplantation of pituitary homogenates, pituitary homograft underneath the renal capsule, or prolactin administration, and the inhibited tumor growth was markedly reactivated, with the reappearance of lung metastasis. These results indicate that MRMT-1 mammary carcinoma is prolactin dependent.

INTRODUCTION

A previous paper (5) reported that a mammary carcinoma called MRMT-1 was transplantable into normal syngeneic female rats and was easily metastasized to regional lymph nodes and lungs of all animals after s.c. transplantation. As the tumor grew at the site of transplantation, additional characteristics appeared; the constant estrous cycle of all tumor-bearing rats became irregular from about 3 weeks after transplantation, and the state of diestrus continued to the end of the host's life.

The studies reported here were designed to investigate quantitatively and qualitatively the alterations among organs accompanying tumor growth and to determine whether the growth and metastasis of MRMT-1 can be exaggerated by replacement treatment after remission was induced by hypophysectomy.

MATERIALS AND METHODS

Rat Mammary Carcinoma, MRMT-1. Four-week-old Sprague-Dawley female rats were thymectomized and/or splenectomized and fed 200 mg of 3-methylcholanthrene from 7 weeks of age by a modification of the method of Kim (7). In addition to these treatments, the early-appearing tumors were excised in order to select by isoimmunity the late-appearing ones that were less antigenic. The latter were easily transplanted into normal syngeneic female rats with metastases to remote organs. This metastasizing capacity of the tumor became an inherent characteristic in syngeneic normal rats of succeeding generations of transplantation. In 1 of the 4 transplantable mammary carcinomas obtained (MRMT-1), many cancer cells were histologically detected in circulating blood from 24 hr following s.c. transplantation of the tumor at the inguinal region and were seen trapped in pulmonary capillaries 1 week later, setting up metastasized foci in the lungs. Spontaneous metastases to axillary and lumber lymph nodes and to lungs were found in all animals by gross observation within 5 weeks after tumor transplantation.

Experimental Procedures. Five-week-old outbred (closed colony maintenance) female rats (CLEA Japan Inc., Tokyo, Japan) received s.c. implantation of a 50-mg fragment of MRMT-1 in the right inguinal region. In the 1st half of the experiment, MRMT-1 bearing rats were killed sequentially after tumor implantation, and their blood was collected in sterile tubes. After clotting, serum was separated for radioimmunoassay of prolactin. Serum prolactin levels were determined using reagents provided by the National Institute of Arthritis and Metabolic Diseases. The radioimmunoassay for rat prolactin was performed according to the procedure described by Niswender *et al.* (13). After exsanguination, the pituitary glands, adrenals, ovaries, thymi, and spleens were removed for weighing and histological examination. Tumor weight was also determined at sacrifice. The organs were fixed in 10% neutral formaldehyde solution, sectioned by the conventional method, and stained with hematoxylin and eosin for histological examination. Pituitary glands were fixed in Heidenhain's solution, stained with periodic acid-Schiff reagent-Orange G after preparation of the paraffin section, and examined by light microscope.

In the latter half of the experiment, 74 rats were hypophysectomized in the transauricular method (16) 48 hr after tumor inoculation. Following this ablative procedure, the animals were divided into 4 groups. (*a*) Thirty-one hypophysectomized rats were used as controls for observing regression of tumor growth. (*b*) Nineteen of the hypophysectomized rats received an s.c. injection of pituitary homogenate on their backs once a day for 4 days from 24 hr after hypophysectomy. Pituitary glands were removed from 350 syngeneic female rats and homogenized in 7 ml of 0.85% NaCl solution with a glass homogenizer. Thus, 0.1-ml aliquots of the homogenate contained extracts of nearly 5 pituitaries. (*c*) Eight rats had 2 pituitaries each, derived from normal syngeneic animals of the same age, grafted underneath the kidney capsule at the time the tumor was implanted. They

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were hypophysectomized 48 hr after these treatments. (*d*) The remaining 16 hypophysectomized rats received replacement treatment with prolactin (Teikoku Zoki Co., Ltd., Tokyo, Japan) at 10 to 20 IU daily for 24 days from the day after hypophysectomy. In addition to the animals receiving ablative treatment, 33 intact rats had MRMT-1 implanted as a growth control for each experiment conducted. Every week after tumor implantation, 2 mutually orthogonal diameters of progressively growing tumors were measured with vernier calipers and the mean values were calculated to obtain tumor growth curves. Four weeks later, all the rats were killed and primary s.c. tumors were excised and weighed. The lungs that were selected for study of metastasis were examined by the method of Wexler (19). Metastases appeared as white nodules against the black substance of normal lung tissue following injection of dilute India ink through the trachea before fixation of the whole lung with Fekete's solution. For identification of tumor deposits, the lungs were scrutinized with a stereomicroscope.

The rats were housed in a temperature-controlled (24° ± 1°), artificially illuminated (12 hr/day) room and were given a basal chow diet CA-1 (CLEA Japan Inc., Tokyo, Japan) and water *ad libitum* throughout the experiment. Vaginal smears were taken daily of all rats during the experiment in order to follow estrous cycles.

RESULTS

Changes in Serum Prolactin Levels and Organ Weight Induced by Progressive Growth of MRMT-1. The relation of serum prolactin levels to the growth of tumors is shown in Table 1. An initial steep increase of prolactin levels after tumor implantation was obtained in tumor-bearing rats. However, these levels declined gradually with advancing tumor growth and reached values lower than those of the normal controls 3 weeks after tumor implantation. Table 2 discloses a correlation between tumor growth and weight increase in the adrenals and the ovaries until the 21st day after tumor implantation. Although the weight of pituitaries decreased in the 1st week after tumor implantation, this weight was not significantly different from that of the controls. Thereafter, the pituitaries gained weight until the end of the 3rd week at a rate higher than that of the controls. On the other hand, the thymus showed a constant decrease in weight and finally disappeared within 5 weeks after tumor implantation. However, the spleen showed weight gain until the death of the tumor-bearing animal and was largest in the middle of the experimental period.

Table 1

Relationship between serum prolactin levels and progressive growth of MRMT-1

Days after implantation	Group[a]	No. of rats	Av. tumor wt (g)	Serum prolactin levels (ng/ml)
7	C-I	3	0	84.1 ± 43.5[a, b]
	E-I	3	3.9 ± 0.1[c]	329.6 ± 37.3[b]
14	C-II	3	0	108.9 ± 3.0
	E-II	3	10.4 ± 0.2	108.0 ± 3.9
21	C-III	3	0	121.4 ± 8.3
	E-III	5	57.7 ± 3.8	66.2 ± 21.5

[a] C, normal control group; E, MRMT-1-implanted group.
[b] a/b = $p < 0.01$.
[c] Mean ± S.E.

Histological Changes in Endocrine Organs. When the estrous cycle followed by vaginal smears turned to constant diestrus, the histology of the ovaries from tumor-bearing rats was characterized by atrophy of the follicular apparatus and interstitium, and by hyperplasia of corpora lutea. In the anterior pituitary glands, the number of acidophilic cells increased markedly. On the other hand, periodic acid-Schiff-positive basophilic cells and chromophobes decreased gradually as the tumor continued to grow. These results indicated that the growth and metastasis of MRMT-1 might depend upon the activity of adenohypophysis.

Effect of Hypophysectomy on MRMT-1. A group of 10 rats in which MRMT-1 was implanted underwent hypophysectomy 48 hr after inoculation. As shown in Chart 1, tumor growth ceased in these rats immediately after the operation, in contrast to the tumor growth of intact controls, and it remained in this arrested condition from the 2nd week. Moreover, the tumor growth in one-half of the hypophysectomized animals completely regressed in the 4th week after tumor inoculation. Necropsy showed that lung metastasis, which appeared in all of the control rats, was completely inhibited in the experimental group.

Tumor Response to the Pituitary Homogenate after Hypophysectomy. Twenty-nine rats bearing MRMT-1 were hypophysectomized 48 hr after tumor inoculation. After the next 24 hr, the pituitary homogenate was injected daily for 4 days in doses of 0.1 ml/rat for a group of 9 rats and 0.05 ml/rat for a group of 10 rats. The remaining 10 rats received no replacement treatment, but only injections of the 0.85% NaCl vehicle.

Chart 2 shows that, within a week after tumor inoculation, tumor growth in the animals that received pituitary homogenate was activated in response to the amount given, as opposed to that of the hypophysectomized controls, and it became nearly comparable to the growth in the intact control animals. Thereafter, tumor growth subsided along with the withdrawal of this homogenate, but it recovered at the end of the experiment to +60% of that of the hypophysectomized controls in the group given 0.1 ml/rat and to +11% in the group given 0.05 ml/rat. Furthermore, necropsy showed that lung metastasis, which had been perfectly inhibited by hypophysectomy, had reappeared in 4 of 9 rats of the group given the higher dose (Table 3).

Tumor Response to Pituitary Homografts underneath the Kidney Capsule after Hypophysectomy. The tumor grew conspicuously in hypophysectomized rats that had received 2 pituitary glands during the experiment. In the 4th week after tumor implantation, the percentage of change of tumor growth increased to +73% of that of the hypophysectomized control animals (Chart 3). Spontaneous metastases in lungs appeared in nearly 60% of the hypophysectomized rats with pituitary graft, but they were not found at all in the hypophysectomized control animals, as shown in Table 4.

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Table 2

Comparison of changes in organ weight between normal control and MRMT-1-implanted female rats

Days after inoculation	Group[a]	No. of rats	Av. tumor wt (g)	Av. anterior pituitary wt (mg)	Av. adrenal wt (mg)	Av. ovarian wt (mg)	Av. thymus wt (g)	Av. spleen wt (g)
7	C-I	3	0	7.5 ± 0.5[b]	50.5 ± 2.5	78.3 ± 5.8	0.5 ± 0.03	0.6 ± 0.06
	E-I	3	3.9 ± 0.1	5.9 ± 0.4	44.2 ± 3.8	92.0 ± 8.3	0.5 ± 0.04	0.6 ± 0.02
14	C-II	3	0	8.0 ± 0.5	42.4 ± 4.0	91.0 ± 6.5	0.6 ± 0.04	0.6 ± 0.02[a, c]
	E-II	3	10.4 ± 0.2	7.5 ± 0.4	51.5 ± 2.6	93.0 ± 5.1	0.7 ± 0.03	1.4 ± 0.1[h]
21	C-III	3	0	9.8 ± 0.5	62.1 ± 4.8[a]	91.1 ± 9.0	0.5 ± 0.04	0.7 ± 0.6[i]
	E-III	19	57.7 ± 3.8	9.5 ± 0.6	77.3 ± 3.6[b]	94.2 ± 3.5	0.4 ± 0.01	2.8 ± 0.2[j]
35–57	C-IV	3	0	9.9 ± 0.6	65.0 ± 5.1[c]	100.0 ± 7.1[e]	0.5 ± 0.03	0.6 ± 0.5[k]
	E-IV	14	79.9 ± 6.0	9.0 ± 0.4	116.1 ± 6.3[d]	78.5 ± 6.1[f]	0	1.6 ± 0.1[l]

[a] C, normal control group; E, MRMT-1-implanted group.
[b] Mean ± S.E.
[c] a/b, e/f = $p < 0.05$; c/d, g/h, i/j, k/l = $p < 0.01$.

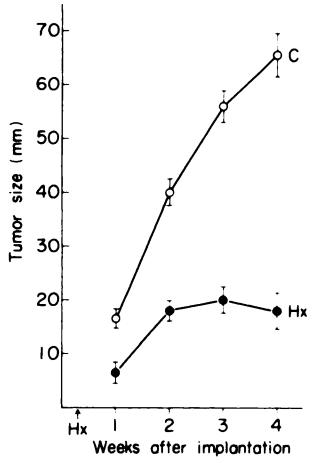

Chart 1. Effect of hypophysectomy on growth of MRMT-1. Note the regression of tumors following surgery. *Tumor size*, mean value calculated from 2 orthogonal diameters of each tumor. *Bars*, S.E. *C*, intact control; *Hx*, hypophysectomy.

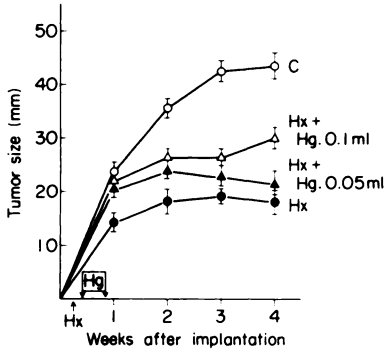

Chart 2. Tumor response to the pituitary homogenate in hypophysectomized female rats. Each dose of the homogenate was injected daily for 4 days beginning the day following hypophysectomy. *Tumor size*, mean value of the 2 orthogonal diameters of each tumor. *Bars*, S.E. *C*, intact control; *Hx*, hypophysectomy; *Hg*, pituitary homogenate.

Tumor Response to Prolactin Administration after Hypophysectomy. Twenty-three rats bearing MRMT-1 were hypophysectomized 48 hr after tumor inoculation and were divided into the following 3 subgroups. Starting from 24 hr after the ablative operation, 8 rats received daily s.c. injections of 10 IU/day on their backs for 24 days, another group of 8 was given the same hormone at 20 IU/day, and the remaining 7 rats were given the 0.85% NaCl vehicle only. Compared with the tumor growth in intact rats inoculated with MRMT-1, the difference in tumor size in those given prolactin was obviously due to the influence of hypophysectomy in the initial stage. However, their tumors grew similarly to those in the intact rats and showed an increase

Table 3

Effect of daily s.c. administration of pituitary homogenate for 4 days on lung metastasis induced by MRMT-1 in hypophysectomized rats

Group of rats given implants of MRMT-1	Metastasis in lung		
	Present	Absent	Total
Intact control (unoperated)	8	0	8
Hypophysectomized			
Pituitary homogenate injection (0.05 ml/rat)	0	10	10
Pituitary homogenate injection (0.1 ml/rat)	4	5	9
0.85% NaCl injection	0	10	10

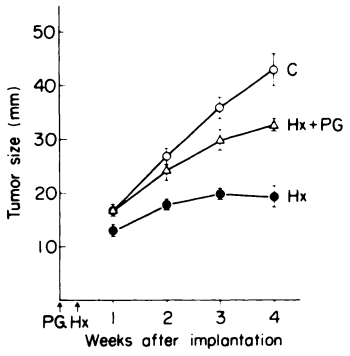

Chart 3. Tumor response to pituitaries grafted under the kidney capsule in hypophysectomized female rats. Two pituitaries, derived from normal syngeneic rats of the same age, were transplanted into each animal at the time MRMT-1 was implanted. *Tumor size*, mean value calculated from 2 orthogonal diameters of each tumor. *Bars*, S.E. *C*, intact control; *PG*, pituitary graft; *Hx*, hypophysectomy.

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of more than +70% over that in the hypophysectomized control rats. Chart 4 gives the results of these experiments. Metastatic nodules in lungs reappeared in 50% of the rats that received the higher dose of prolactin and in 25% of those given the lower dose (Table 5).

DISCUSSION

According to previous investigators, many mammary carcinomas induced by 7,12-dimethylbenz(a)anthracene or 3-methylcholanthrene are hormone dependent in rats (6, 9, 10). Nearly 100% regression of both primary and transplanted mammary carcinomas has been reported with hypophysectomy and bilateral oophorectomy-adrenalectomy (10, 14) or with prolactin antiserum treatment (2). These regressed tumors were reactivated promptly by grafts of mammotropic pituitary tumor, median eminence lesions, or pituitary grafts (11, 12, 17). Since these treatments elevated prolactin levels in blood (4, 18), such chemically induced rat mammary tumors as mentioned above were prolactin dependent.

In this study, an initial increase with consequent depletion in serum prolactin levels observed in all the tumor-bearing rats had a direct relationship with advanced growth of MRMT-1. This initial rise in serum prolactin could be a stress effect resulting from tumor implantation, since many types of stressful stimuli have been observed to induce prolactin release (11). Then the decrease in serum prolactin that occurred after the initial increase could be interpreted as prolactin binding by progressively growing mammary carcinomas. The same interpretation was introduced by Bogden *et al.* (1) in a study on 13762 MT. Removal of the hypophysis resulted in regression of MRMT-1; many replacement treatments, such as transplantation of pituitary homogenates, pituitary homograft under the kidney capsule, or prolactin administration reactivated the inhibited tumor growth with lung metastasis, the extent depending on the dose. These results indicate that this carcinoma is definitely prolactin dependent. However, complete recovery of tumor growth did not result from prolactin administration alone in hypophysectomized rats. Considering that the pituitary homografts implanted underneath the kidney capsule are free from hypothalamic inhibition and that they secrete large amounts of prolactin but very little adrenocorticotropic hormone, thyroid-stimulating hormone, growth hormone, follicle-stimulating hormone, or luteinizing hormone (11), growth hormone might have a small supplemental role in the growth of the prolactin-dependent MRMT-1, since the increased number of acidophilic cells of the anterior pituitary could release growth hormone in some amount besides the prolactin released in tumor-bearing rats. This assumption will be clarified in the future. Continuous adrenal hypertrophy and thymolysis in rats with advancing MRMT-1 growth suggests that there is augmented release of adrenocorticotropic hormone by the stresses of tumor transplantation and growth. However, this hormone is not regarded as necessary for MRMT-1 growth, since tumor growth is neither enhanced nor inhibited by adrenalectomy (unpublished data of the author).

As far as this study is concerned, the conclusions reached are that growth of the primary tumor easily responds to a hormone or hormones in the host and that metastasis formation is strongly affected by the growing tumor size in the early phase after tumor implantation. However, the mechanism of tumor dissemination and agents of specific effects participating in the formation of tumor metastasis must be elucidated. With respect to tumor dissemination, the following have been reported using several spontaneously metastasizing rat tumors: the role of concomitant immunity followed by immunoselection of metastasizing cells in the tumor host, the lack of an immunogenic coat on metastasizing cells for escape from immunological interference of tumor hosts, and the difference in enzyme level between metastasizing and nonmetastasizing tumor cells (3, 8, 15). Thus, MRMT-1 can also be used as an experimental tool like other spontaneously metastasizing tumors for studying agents that affect the formation of metastases without influencing the primary tumor growth.

Table 4

Effect of pituitary grafts implanted underneath kidney capsule on lung metastasis induced by MRMT-1 in hypophysectomized rats

Group of rats given implants of MRMT-1	Metastasis in lung		
	Present	Absent	Total
Intact control (unoperated)	8	0	8
Hypophysectomized			
Pituitary graft	5	3	8
Nongraft	0	4	4

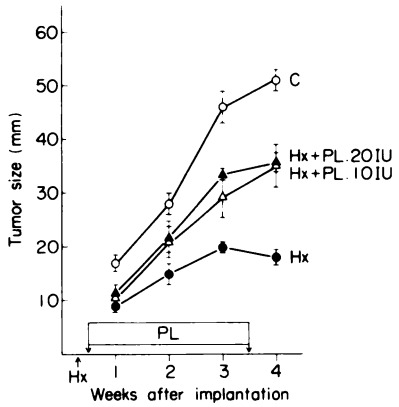

Chart 4. Tumor response to prolactins in hypophysectomized female rats. Each dose of prolactin was injected daily for 24 days from the day after hypophysectomy. *Tumor size*, mean value calculated from 2 orthogonal diameters of each tumor. *Bars*, S.E. *C*, intact control; *Hx*, hypophysectomy; *PL*, prolactin.

Table 5

Effect of daily s.c. administration of prolactin for 24 days on lung metastasis induced by MRMT-1 in hypophysectomized rats

Group of rats given implants of MRMT-1	Metastasis in lung		
	Present	Absent	Total
Intact control (unoperated)	7	0	7
Hypophysectomized			
Prolactin injection (10 IU/rat)	2	6	8
Prolactin injection (20 IU/rat)	4	4	8
0.85% NaCl injection	0	7	7

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