Effects of Reproduction on Spontaneous Development of Endometrial Adenocarcinomas and Mammary Tumors in Donryu Rats

Takaharu Nagaoka,[1] Kiyoshi Takegawa,[1] Masaki Takeuchi[1] and Akihiko Maekawa[2, 3]

[1]Safety Evaluation, Drug Development Laboratories, Pharmaceutical Research Division, Yoshitomi Pharmaceutical Industries Ltd., 14-1 Yamasaki, Fukusaki-cho, Kanzaki-gun, Hyogo 679-2296 and [2]Department of Pathology, Sasaki Institute, 2-2 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062

Effects of reproduction on spontaneous development of uterine endometrial adenocarcinomas and mammary tumors in Donryu rats were investigated. While the incidence of endometrial adenocarcinomas in Donryu rats was not influenced by a single reproductive experience (SRE), it showed a tendency to decrease in animals having three reproductive experiences (TRE), compared to the nulliparous case (NRE). In addition, both SRE and TRE animals showed delayed occurrence and decreased incidences and mean numbers of mammary tumors, along with reduced incidences of proliferative lesions in the pituitary gland and mucinous epithelium in the vagina. The appearance-time and incidences of persistent estrus in TRE rats were delayed and low, respectively, compared to the SRE and NRE values. The hormonal environment was altered in both groups, the prolactin level in TRE especially being decreased. These results suggest that suppression of the occurrence of endometrial adenocarcinomas and mammary tumors in rats experiencing reproduction is associated with change in the hormonal milieu.

Key words: Uterine adenocarcinoma — Mammary tumor — Reproduction — Donryu rat

Effects of reproduction on development of endometrial adenocarcinomas and breast cancers are well known in women, and it has long been suggested that the risk of uterine endometrial adenocarcinoma is lower in multiparous as compared to nulliparous or infertile individuals.[1–6] This is related to the number of pregnancies/deliveries.[3–6] With breast cancer, on the other hand, it is reported that the age of reproductive experience is one of the principal factors influencing this tumor and reproductive frequency probably has an independent effect on breast cancer risk.[7–11] However, there is no report concerning the effects of reproduction on development of these tumors in experimental animals. Previously, we described spontaneous tumors in Donryu rats.[12] This rat strain yields spontaneous endometrial adenocarcinomas at high incidence, and hormonal imbalance, particularly an age-related increase of the estrogen:progesterone (E_2:P) ratio may play an important causative role.[13, 14] Thus, the Donryu rat strain has been considered as a good animal model for human endometrial adenocarcinomas caused by endogenous estrogens, as reviewed quite recently.[15] High yields of spontaneous mammary tumors are also observed in females of this rat strain.[12]

In this paper, we describe the effects of reproduction on spontaneous development of endometrial adenocarcinomas and mammary tumors in the Donryu rat.

---

[3] To whom requests for reprints should be addressed.
  E-mail: amaekawa@sasaki.or.jp

MATERIALS AND METHODS

Animals Male and female Donryu rats were purchased from Nippon Rat Co., Ltd. (Urawa) and Charles River Japan Inc. (Hino) and housed 3 animals to an aluminum cage, in an air-conditioned barrier system animal room at $24\pm2°C$ with a relative humidity of $55\pm5\%$. They were maintained on basal diet, CRF-1 (Oriental Yeast Co., Ltd., Tokyo) throughout the experiments.

Experimental design

Experiment 1: Animals were divided into two groups, a no reproductive experience (NRE) group and a single reproductive experience (SRE) group. The NRE group consisted of 100 virgin females, and the SRE group had 37 rats which had experienced pregnancy, delivery and lactation once. For this, two 10-week-old virgin females showing proestrus were mated with a single normal male overnight and pregnancy was determined on the base of vaginal plugs the following morning. Pregnant rats were housed in plastic cages individually during the gestation period, and allowed to deliver. All dams reared their pups, which were adjusted to 8 animals including all females at 4 days of age, for 4 weeks. After weaning, all dams were maintained under the same conditions as for virgin rats. Survivors were killed at 27 months of age.

Experiment 2: Fifty-seven virgin females were allocated to the NRE group for comparison with 55 female rats with three reproductive experiences (TRE). Reproductive history was as follows: 3-month-old (12-week-old) virgin

females were mated, and allowed to deliver and lactate for 3 weeks. This was repeated for a total of three times before 7 months of age. Animals in both the NRE and TRE groups were then maintained until 28 months of age, when all the survivors were killed.

General condition, body weights and skin/subcutaneous nodules General condition was observed daily, and body weights were measured every month until 27–28 months of age. All animals were palpated once a week throughout the experimental period, and the location, size of palpable skin/subcutaneous nodules and time of the first observation were recorded.

Estrous cycle Estrous cycles were followed by means of vaginal smears from 3 months (7 months TRE group) to 15 months of age in all animals of each group in both experiments.

Examination of serum steroid levels

Experiment 1: Forty-three females in the NRE group and 23 females in the SRE group were utilized for examination of serum steroid hormone levels at 27 months of age.

Experiment 2: For sequential examination of the serum steroid hormone levels and uterine adenocarcinoma development, 18 NRE rats and 18 TRE rats were used. Groups of six rats were selected for examination at 9, 12, and 18 months of age, each animal being autopsied without selection of estrous stage. At 28 months of age, all surviving animals were killed and examined for hormone levels, in addition to histopathologically for lesions.

Before autopsy, blood was collected from the abdominal aorta under ether anesthesia, centrifuged at $1700g$ for 10 min and stored at $-80°C$ until assay. The serum values of 17β-estradiol (E_2) and progesterone (P) were estimated with estradiol cautoria (bioMerieux) (experiment 1), a double antibody estradiol kit (Diagnostic Product Corp., Los Angeles, CA) (experiment 2) and DPC progesterone kits (Diagnostic Product Corp.) respectively. In experiment 2, the prolactin values were additionally measured, with a rat prolactin (rPRL) [^{125}I] assay system (Amersham, Buckinghamshire, England).

Organ weights and histological examination All reproductive and other related organs and/or tissues, and skin/subcutaneous nodules, were removed and fixed in buffered 10% formalin. Before fixation, each nodule was measured for weight and length. Organ weights of the uterus, ovaries, adrenals and pituitary were also determined after fixation. Tissue sections were routinely prepared and stained with hematoxylin and eosin for microscopic examination. Uterine proliferative lesions were classified into adenocarcinoma and hyperplasia categories, according to our criteria reported previously.[14] Mammary, pituitary and adrenal proliferative lesions were diagnosed according to the criteria described in "Pathology of the Fischer Rat" edited by Boorman *et al.*[16]

Statistical analysis Data concerning the incidences of the lesions, survival rates and persistent estrous rates were statistically analyzed using the one-sided Fisher's exact probability test or χ^2 test. Other data were analyzed using Student's *t* test.

RESULTS

Survival rates In the NRE groups of experiments 1 and 2, survival rates began to reduce at 18 and 15 months of age, with a pronounced decline from 21 and 18 months, respectively. In contrast, in the SRE and TRE groups, first occurrence of animal death was observed at 21 months and 18 months of age, respectively. From 21 and 18 months to 27 and 28 months of age, 10–20% higher survival values were sustained in the SRE and TRE groups than in the respective NRE group, sometimes achieving significance ($P<0.05$) (Table I).

Body weights Body weights in the NRE and SRE groups were almost the same. However, the weight in the TRE group was lower than that in the corresponding NRE group, significantly so at 7–12 months of age.

Organ weights and hormone levels There were no clear differences in ovary, uterus, pituitary and adrenal weight values between the NRE and SRE or TRE groups in

Table I. Survival Rates

Group	Initial no. of animals	Survival rate (%)							
		7	9	12	15	18	21	24	27–28 (month)
Experiment 1									
NRE	100	100	100	100	100	95	86	69	47
SRE	37	100	100	100	100	100	97.3	89.2*	64.9
Experiment 2									
NRE	57	100	100	100	95.6	84.4	79.5	60.0	20.5
TRE	55	100	100	100	100	97.7	97.3*	78.4	37.8

NRE, no reproductive experience; SRE, single reproductive experience; TRE, three reproductive experiences.
∗ Significantly different from the NRE case ($P<0.05$).

Table II. Sequential Change in Persistent Estrous Incidence

Group	Initial no. of animals	Incidence (%)						
		5	6	7	8	10	12	15 (month)
Experiment 1								
NRE	100	6.0	10.0	21.0	72.0	73.0	83.0	86.9
SRE	37	16.2	16.2	35.1	62.2	86.5	91.0	97.3
Experiment 2								
NRE	57	24.6	21.1	45.6	73.6	90.2	94.1	90.7
TRE	55	—	—	32.7	43.6**	79.6	91.8	90.7

NRE, no reproductive experience; SRE, single reproductive experience; TRE, three reproductive experiences. ** Significantly different from the NRE case ($P<0.01$).
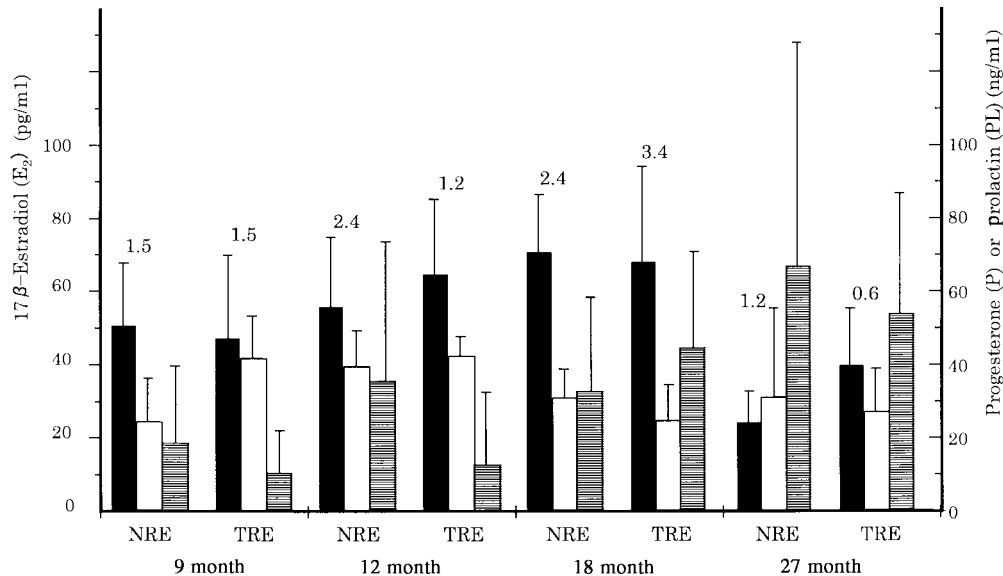

Fig. 1. Sequential changes in serum estrogen (E_2), progesterone (P) and prolactin (PL) levels of female Donryu rats in experiment 2. The points plotted are mean±SD (bars) values. The numbers above each column present the $E_2/P(10^{-3})$ values. E_2 ■, P ☐, PL ▤.

experiments 1 and 2. While the incidences of persistent estrus at each time point did not differ between the SRE and corresponding NRE groups, the incidence was lower in the TRE group than in the NRE group from 7 months to 10 months of age (Table II). In experiment 1, E_2 and P values in the SRE group were a little higher and lower, respectively, at 27 months of age than in the NRE group, but the differences did not achieve significance. In experiment 2, the prolactin value in the TRE group was lower than that in NRE group until 12 months of age (Fig. 1).

Histological findings for the uterus Table III summarizes quantitative data for uterine endometrial lesions. In experiment 1, the incidences of endometrial adenocarcinoma (Figs. 2, 3) and hyperplasia did not vary with the group. However, in experiment 2, the incidence of adeno-

carcinoma in the TRE group was 13.9%, compared to 29.7% in the NRE group, though this was not significant, and one adenocarcinoma with widespread invasion/metastasis was observed in the TRE group, whereas four were found in the NRE group (Fig. 4). Furthermore, sequential observation demonstrated the appearance and incidences of uterine proliferative lesions in the TRE group to be, respectively, later and lower than those in NRE group.

Cumulative palpable skin/subcutaneous nodule incidences and histological findings In the two NRE groups, palpable skin/subcutaneous nodules were first observed at 13 and 7 months of age, respectively, in experiments 1 and 2. Thereafter, the incidences in both groups increased with age, and 57% and 82% of the animals were found to have

Table III.　Sequential Histological Changes in the Uterus

Month		No. of animals	Incidence (%)			
			Adenocarcinoma (with metastasis)	Hyperplasia		
				+	++	+++
Experiment 1						
27	NRE	98	27.6 (0)	29.6	9.2	5.1
	SRE	37	27.0 (2.7)	24.3	13.5	8.1
Experiment 2						
9	NRE	6	0	16.7	16.7	0
	TRE	6	0	0	0	0
12	NRE	6	0	33.3	33.3	0
	TRE	6	0	16.7	0	0
18	NRE	6	16.7	33.3	50.0	0
	TRE	6	0	0	16.7	16.7
28	NRE	37	29.7 (10.8)	24.3	29.7	8.1
	TRE	36	13.9 (2.8)	16.7	41.7	11.1

NRE, no reproductive experience; SRE, single reproductive experience; TRE, three reproductive experiences.
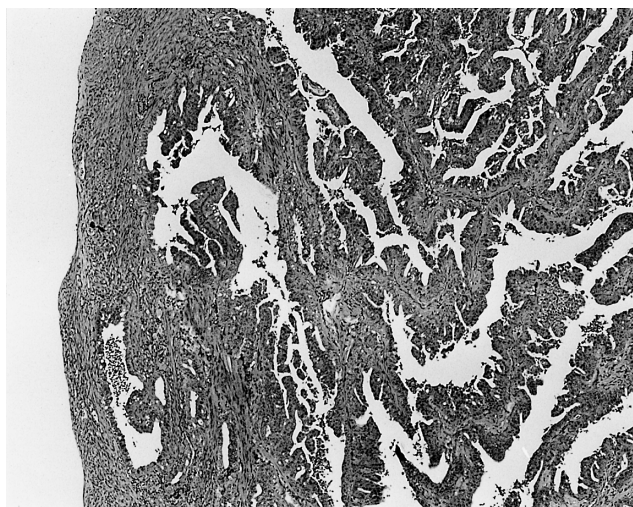

Fig. 2.　Well to moderately differentiated endometrial adenocarcinoma of a 28-month-old Donryu rat of the NRE group (experiment 2). The tumor exhibits irregular proliferation of atypical glands, with tumor cells infiltrating the muscularis. H&E, ×40.
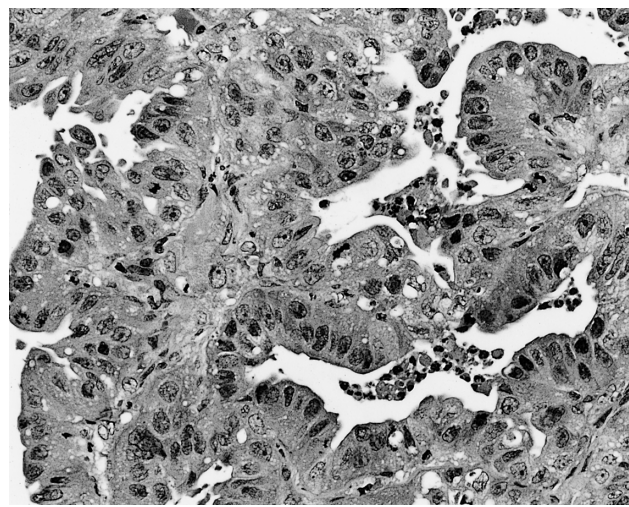

Fig. 3.　Higher magnification of Fig. 2. Tumor cells having large nuclei with prominent nucleoli show cellular and structural atypia. Mitotic figures are abundant. H&E, ×240.

palpable masses at 26 months of age. In contrast, first occurrence of palpable nodules was observed at 16 months or 9 months of age in the SRE and TRE groups, and the incidences increased only gradually thereafter, the values at 22 or 24–26 months being significantly ($P<0.05$) lower than in the relevant NRE group (Table IV). Histologically, the nodules were mostly derived from mammary glands. The incidences of mammary adenocarcinomas (Fig. 5) and atypical hyperplasias in the SRE and TRE groups were lower than in the NRE group. The total incidence of mammary tumors in TRE group was also significantly

decreased and the mean numbers of mammary tumors per animal in the SRE and TRE groups were low ($P<0.01$) (Table V).

Histological findings for other organs In both the SRE and TRE groups, the incidences of vaginal mucification were lower than in the NRE groups. In addition, the incidences of pituitary hyperplasia in the SRE group and adenomas in the TRE group were lower than in the respective NRE group, the former with statistical significance. The incidence of adrenal medullary hyperplasia in the TRE group was also significantly lower than that of the NRE group (Table VI).

DISCUSSION

It has been reported that experience of pregnancy, delivery and lactation decreases the risk of endometrial cancer and breast cancer development in women, dependent on the frequency and/or age of reproduction.[3–11] The risk of endometrial cancer is remarkably decreased in women experiencing 4 or more pregnancies/deliveries.[5] While the precise reasons for the association of multiple reproductions with decreased risk of endometrial cancer are still uncertain, they are presumed to be due to alteration in the hormonal milieu.

The present findings that spontaneous endometrial adenocarcinoma development was suppressed in rats with multiple reproductive experiences supports experimentally the decreased risk in multiparous women. In humans, it has been reported that relatively high E_2:P values increase the endometrial cancer risk.[17–19] The Donryu rat is known as a high incidence strain for spontaneous occurrence of endometrial adenocarcinomas, and the rat shows early appearance of persistent estrus and an increase of
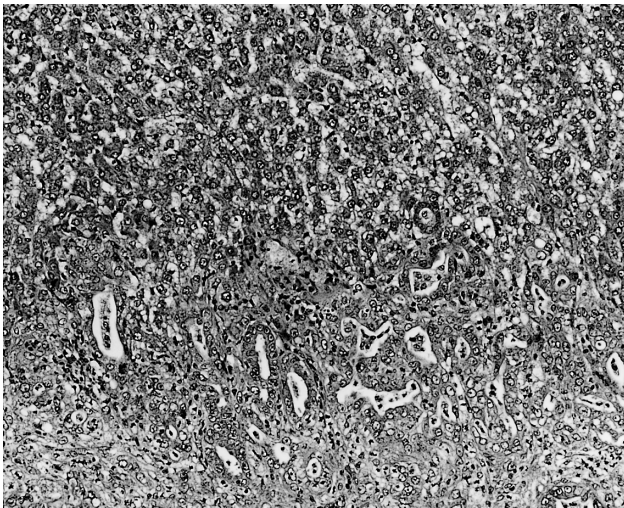

Fig. 4. Liver metastasis of an endometrial adenocarcinoma observed in a 28-month-old Donryu rat of the NRE group (experiment 2). H&E, ×120.
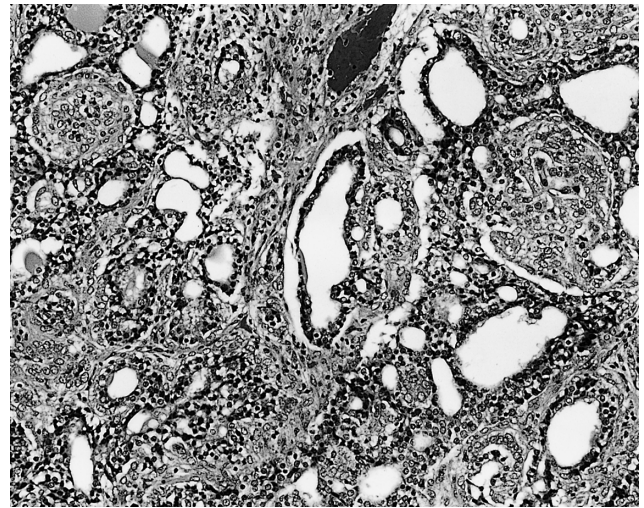

Fig. 5. Mammary adenocarcinoma in a 28-month-old Donryu rat of the NRE group (experiment 2). H&E, ×120.

Table IV. Cumulative Incidences of Palpable Skin/subcutaneous Nodules

Group	Initial no. of animals	Incidence (%)									
		7	9	11	13	16	18	20	22	24	26 (month)
Experiment 1											
NRE	100				6.0	9.0	15.0	31.0	38.0	53.0	57.0
SRE	37					2.7	8.1	16.2	21.9*	37.8	40.5
Experiment 2											
NRE	39	5.1	10.3	17.9	20.5	28.2	33.3	43.6	56.4	74.4	82.1
TRE	37		5.4	8.1	10.8	13.5	16.2	27.0	32.4	48.6*	54.1*

NRE, no reproductive experience; SRE, single reproductive experience; **TRE, three reproductive experiences.**
∗ Significantly different from the NRE case ($P<0.05$).

Table V.　Incidences of Proliferative Lesions in the Mammary Gland

Histological type of mammary proliferative lesion	Incidence (%)			
	Experiment 1		Experiment 2	
	NRE	SRE	NRE	TRE
No. of animals examined	98	37	39	36
Total no. of animals with tumors	74	24	33	22
Adenocarcinoma	2.0	0	7.7	2.7
Adenoma	22.8	16.2	5.1	0
Atypical hyperplasia	4.1	0	2.6	0
Fibroadenoma	38.6	32.4	51.3	58.3
Fibroma	8.9	16.2	20.5	2.7[*]
Total	75.5	64.9	84.6	61.1[*]
Mean no. of mammary tumors/animal	1.48	0.92[**]	1.79	0.95[**]
(mean±SD)	±1.31	±0.98	±1.45	±0.84

NRE, no reproductive experience; SRE, single reproductive experience; TRE, three reproductive experiences.
*, ** Significantly different from the NRE case (* $P<0.05$, ** $P<0.01$).

Table VI.　Histological Findings for the Ovary, Vagina and Endocrine Organs

Histological type of lesions	Incidence (%)			
	Experiment 1		Experiment 2	
	NRE	SRE	NRE	TRE
No. of animals	98	37	37	36
Ovary				
Absence, corpus luteum	61.2	78.4	73.0	83.3
Cyst	71.4	83.8	73.0	80.5
Atrophy	60.2	75.7	86.5	94.4
Vagina				
Cornification	29.6	40.5	37.8	47.2
Mucification	37.2	16.2	16.2	8.3
Pituitary				
Adenoma	22.4	27.0	32.4	19.4
Hyperplasia	34.7	16.2[*]	16.2	16.7
Adrenal				
Cortical adenoma	18.4	16.2	18.9	11.1
Cortical hyperplasia	45.9	48.6	2.7	5.6
Pheochromocytoma	4.1	2.7	16.2	13.9
Medullary hyperplasia	26.5	24.3	27.0	5.6[*]

NRE, no reproductive experience; SRE, single reproductive experience; TRE, three reproductive experiences.
* Significantly different from the NRE case ($P<0.05$).

E_2:P ratio with age, in contrast to F344 rats, a low incidence strain, in which normal estrous cycles last for a long period.[13, 14] The high incidence of spontaneous endometrial adenocarcinoma in Donryu rats is also probably due to the hormone imbalance. In the present study, while outcome of persistent estrus was not influenced by a single reproductive experience, the incidence was lower in the TRE as compared to the NRE group until 10 months of age. The fact that the incidence of vaginal mucification was decreased in the SRE and TRE groups points to relatively low progesterone levels.[13] However, the E_2 and P levels in both experiments showed no clear difference among the groups. Thus, the precise mechanisms underlying the decrease in incidence and invasion/metastasis of

endometrial adenocarcinomas in the TRE case remain to be clarified.

In the present study, the incidences of mammary tumor were also decreased in both the SRE and TRE groups. In humans, it is reported that the age of reproductive experience is one of the principal factors influencing this tumor, and reproductive frequency probably has an independent effect on breast cancer risk.[8-11] It is proposed that decrease of breast cancers in multiparous women is probably related either to long-lasting hormonal alterations or to differentiative changes that render the mammary tissue less susceptible to carcinogenic agents.[20] With regard to the hormonal aspect, certain hormonal changes may last for at least several years after pregnancy/delivery and lactation.[21-24] In experimental animals, however, clear hormonal changes after pregnancy and lactation have not been described and a normal estrous cycle begins again 1 week after lactation.[25] In the present study, the ages at the first reproduction were almost the same in the SRE and TRE groups, and this might be a reason for the lack of a clear difference in the incidences of mammary tumors between the two cases.

The prolactin level in the TRE group was found to be decreased from 9 months to 12 months of age, compared to the NRE values. In humans, prolactin levels also change after delivery.[23, 24] The fact that the incidences of pituitary hyperplasias/adenomas were low in SRE and TRE groups, in addition to mammary tumors, is interesting in this context. The incidence of adrenal medullary hyperplasia was also lower in the TRE group. We confirmed immunohistochemically that many of the pituitary tumors in Donryu rats were prolactinomas.[26] In experimental animals, it is well known that prolactin may play a very important role in mammary carcinogenesis[27, 28] and that pheochromocytomas are associated with hyperprolactinemia.[29, 30] In addition, progesterone acts as a promoter of mammary tumor development.[31, 32] The results thus suggest that relatively low levels of serum prolactin and progesterone in the SRE and TRE groups might have suppressed mammary tumor development, similar to the human case.

Recently, local estrogen production has been investigated by molecular methods. The conversion of C_{19} steroids to estrogens catalyzed by aromatase cytochrome P450, has been considered to play an important role in the progression of human estrogen-dependent neoplasms.[33] Aromatase immunoreactivity and mRNA expression are detectable in stromal or interstitial cells rather than in the carcinoma cells of uretine and mammary carcinoma in human, and are associated with malignant phenotype in both cancers.[34, 35] Consequently, aromatase overexpression in human uterine and breast carcinoma tissue is considered to occur as a result of carcinoma-stroma cell interactions, i.e. paracrine communication between stromal and carcinoma cells. Recently, we reported cell-type specific patterns of ER (estrogen receptor) mRNA expression in the uterus of Donryu rat.[36] Further studies concerning epithelial or carcinoma-stroma cell interactions in the uterus of rats are needed.

On the basis of experiments in rats, Russo et al.[20] have proposed that cellular differentiation of the mammary gland during full-term pregnancy and lactation protects against the subsequent development of breast cancer. In the uterine tissue too, uterine cell differentiation may also be an important determinant of tumor development influenced by multiple reproductive experiences.

In conclusion, rats that had experienced multiple pregnancy and lactation exhibited decreased development of endometrial adenocarcinomas and mammary tumors, probably owing to hormonal changes.

REFERENCES

1) Kelsey, J. L., Livolpsi, V. A., Holford, T. R., Fischer, D. B., Mostow, E. D., Schwartz, P. E., O'Connor, T. and White, C. A case-control study of cancer of the endometrium. *Am. J. Epidemiol.*, **116**, 333–342 (1982).

2) Parazzini, F., La Vecchia, C., Negri, E., Fedele, L. and Balotta, F. Reproductive factors and risk of endometrial cancer. *Am. J. Obstet. Gynecol.*, **164**, 522–527 (1991).

3) Brinton, L. A., Berman, M. L., Mortel, R., Twiggs, L. B., Barrett, R. J., Wilbanks, G. D., Lannom, L. and Hoover, R. N. Reproductive, menstrual, and medical risk factors for endometrial cancer: results from a case-control study. *Am. J. Obstet. Gynecol.*, **167**, 1317–1325 (1992).

4) Henderson, B. E., Casagrande, J. T., Pike, M. C., Mack, T., Rosario, I. and Duke, A. The epidemiology of endometrial cancer in young women. *Br. J. Cancer*, **47**, 749–756 (1983).

5) Inoue, M., Okayama, A., Fujita, M., Enomoto, T., Tanizawa, O. and Ueshima, H. A case-control study on risk factors for uterine endometrial cancer in Japan. *Jpn. J. Cancer Res.*, **85**, 346–350 (1994).

6) Kvåle, G., Heuch, I. and Ursin, G. Reproductive factors and risk of cancer of the uterine corpus: a prospective study. *Cancer Res.*, **48**, 6217–6221 (1988).

7) Ewertz, M., Duffy, S. W., Adami, H.-O., Kvåle, G., Lund, E., Meirik, O., Mellemgaard, A., Soini, I. and Tulinius, H. Age at first birth, parity and risk of breast cancer: a meta-analysis of 8 studies from the Nordic countries. *Int. J. Cancer*, **46**, 597–603 (1990).

8) Kelsey, J. L., Gammon, M. D. and John, E. M. Reproductive and hormonal risk factors—reproductive factors and

breast cancer. *Epidemiol. Rev.*, **15**, 36–47 (1993).

9) Layde, P. M., Webster, L. A., Baughman, A. L., Wingo, P. A., Rubin, G. L., Ory, H. W. and the Cancer and Steroid Hormone Study Group. The independent associations of parity, age at first full term pregnancy, and duration of breastfeeding with the risk of breast cancer. *J. Clin. Epidemiol.*, **42**, 963–973 (1989).

10) Leon, D. A. A prospective study of the independent effects of parity and age at first birth on breast cancer incidence in England and Wales. *Int. J. Cancer*, **43**, 986–991 (1989).

11) Negri, E., La Vecchia, C., Duffy, S. W., Bruzzi, P., Parazzini, F. and Day, N. E. Age at first and second births and breast cancer risk in biparous women. *Int. J. Cancer*, **45**, 428–430 (1990).

12) Maekawa, A., Onodera, H., Tanigawa, H., Furuta, K., Matsuoka, C., Kanno, J., Ogiu, T. and Hayashi, Y. Spontaneous neoplastic and non-neoplastic lesions in aging Donryu rats. *Jpn. J. Cancer Res.*, **77**, 882–890 (1986).

13) Nagaoka, T., Onodera, H., Matsushima, Y., Todate, A., Shibutani, M., Ogasawara, H. and Maekawa, A. Spontaneous uterine adenocarcinomas in aged rats and their relation to endocrine imbalance. *J. Cancer Res. Clin. Oncol.*, **116**, 623–628 (1990).

14) Nagaoka, T., Takeuchi, M., Onodera, H., Matsushima, Y., Ando-Lu, J. and Maekawa, A. Sequential observation of spontaneous endometrial adenocarcinoma development in Donryu rats. *Toxicol. Pathol.*, **22**, 261–269 (1994).

15) Maekawa, A., Takahashi, M., Ando, J. and Yoshida, M. Uterine carcinogenesis by chemicals/hormones in rodents. *J. Toxicol. Pathol.*, **12**, 1–11 (1999).

16) Boorman, G. A., Wilson, J. Th., van Zwieten, M. J. and Eustis, S. L. (19. Mammary gland), Mackenzie, W. F. and Boorman, G. A. (30. Pituitary gland), Hamlin, M. H., II and Banas, D. A. (31. Adrenal gland). *In* "Pathology of the Fischer Rat," ed. G. A. Boorman, S. L. Eustis, M. R. Elwell, C. A. Montgomery, Jr. and W. F. Mackenzie, pp. 295–313, 485–500, 501–518 (1990). Academic Press Inc., San Diego.

17) Fox, H. Endometrial carcinogenesis and its relation to oestrogens. *Pathol. Res. Pract.*, **179**, 13–19 (1984).

18) Lingeman, C. H. Hormones and hormonomimetic compounds in the etiology of cancer. *Recent Results Cancer Res.*, **66**, 1–48 (1979).

19) Zeil, H. K. Estrogen's role in endometrial cancer. *Obstet. Gynecol.*, **60**, 509–515 (1982).

20) Russo, J., Tay, L. K. and Russo, I. H. Differentiation of the mammary gland and susceptibility to carcinogenesis. *Breast Cancer Res. Treat.*, **2**, 5–73 (1982).

21) Bernstein, L., Pike, M. C., Ross, R. K., Judd, H. L., Brown, J. B. and Henderson, B. E. Estrogen and sex hormone-binding globulin levels in nulliparous and parous women. *J. Natl. Cancer Inst.*, **74**, 741–745 (1985).

22) Bernstein, L. and Ross, R. K. Endogenous hormones and breast cancer risk. *Epidemiol. Rev.*, **15**, 48–65 (1993).

23) Musey, V. C., Collins, D. C., Musey, P. I., Martino-Saltzman, D. and Preedy, J. R. K. Long-term effect of a first pregnancy on the secretion of prolactin. *N. Engl. J. Med.*, **316**, 229–234 (1987).

24) Yu, M. C., Gerkins, V. R., Henderson, B. E., Brown, J. B. and Pike, M. C. Elevated levels of prolactin in nulliparous women. *Br. J. Cancer*, **43**, 826–831 (1981).

25) Yohkaichiya, T., O'Connor, A. and de Kretser, D. M. Circulating immunoreactive inhibin, gonadotropin, and prolactin levels during pregnancy, lactation, and postweaning estrous cycle in the rat. *Biol. Reprod.*, **44**, 6–12 (1991).

26) Kitamura, T., Nishimura, S., Sasahara, K., Yoshida, M., Ando, J., Takahashi, M., Shirai, T. and Maekawa, A. Transplacental administration of diethylstilbestrol (DES) causes lesions in female reproductive organs of Donryu rats, including endometrial neoplasia. *Cancer Lett.*, **141**, 219–228 (1999).

27) Furth, J. The role of prolactin in mammary carcinogenesis. *In* "Human Prolactin," International Congress Series No. 308, ed. J. L. Pasteels and C. Robyn, pp. 233–248 (1973). Excerpta Medica, Amsterdam.

28) Welsch, C. W. and Nagasawa, H. Prolactin and murine mammary tumorigenesis: a review. *Cancer Res.*, **37**, 951–963 (1977).

29) Fernandez-Ruiz, J., Cebeira, M., Agrasal, C., Tresguerres, J. A. F., Esquifino, A. I. and Ramos, J. A. Effect of elevated prolactin levels on the synthesis and release of catecholamines from the adrenal medulla in female rats. *Neuroendocrinology*, **45**, 208–211 (1987).

30) Fernandez-Ruiz, J. J., Martinez-Arrieta, R., Hernandez, M. L. and Ramos, J. A. Possible direct effect of prolactin on catecholamine synthesis and release in rat adrenal medulla: *in vitro* studies. *J. Endocrinol. Invest.*, **11**, 603–608 (1988).

31) Sluyser, M. and van Nie, R. Estrogen receptor content and hormone-responsive growth of mouse mammary tumors. *Cancer Res.*, **34**, 3253–3257 (1974).

32) Young, S. Induction of mammary carcinoma in hypophysectomized rats treated with 3-methylcholanthrene, oestradiol-17β, progesterone and growth hormone. *Nature*, **190**, 356–357 (1961).

33) Sasano, H., Kaga, K., Sato, S., Yajima, A., Nagura, H. and Harada, N. Aromatase cytochrome P450 gene expression in endometrial carcinoma. *Br. J. Cancer*, **74**, 1541–1544 (1996).

34) Watanabe, K., Sasano, H., Harada, N., Ozaki, M., Niikura, H., Sato, S. and Yajima, A. Aromatase in human endometrial carcinoma and hyperplasia—immunohistochemical, *in situ* hybridization, and biochemical studies. *Am. J. Pathol.*, **146**, 491–500 (1995).

35) Sasano, H. and Ozaki, M. Aromatase expression and its localization in human breast cancer. *J. Steroid Biochem. Mol. Biol.*, **61**, 293–298 (1997).

36) Katsuda, S., Yoshida, M., Watanabe, T., Kuroda, H., Ando-Lu, J., Takahashi, M., Hayashi, H. and Maekawa, A. Estrogen receptor mRNA in uteri of normal estrous cycling and ovariectomized rats by *in situ* hybridization. *Proc. Soc. Exp. Biol. Med.*, **221**, 207–214 (1999).