Original

Spontaneous Tumors in the Uterus, Testis and Other Organs of F₁ Hybrids between Donryu and F344 Rats

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Abstract: In order to assess the effects of genetic factors on spontaneous tumor development in various organs/tissues, especially the reproductive organs of Donryu and F344 rats, F₁ hybrids between these two strains were investigated. Litters were obtained by mating virgin Donryu or F344 females with F344 or Donryu males, respectively. They were weaned at 4 weeks of age and divided into two hybrid groups: Group A, (Donryu female × F344 male) F₁ (male n=49, female n=48) and group B, (F344 female × Donryu male) F₁ (male n=53, female n=56). These two groups of F₁ hybrids were observed with their parents (Donryu male n=19, female n=30; F344 male n=21, female n=29) until 120 weeks of age. The incidences of persistent estrus in both F₁ groups from 8 to 12 months of age were in between those for their Donryu and F344 mothers, being slightly higher in group A than in group B. They increased thereafter and reached about 90% and 80% at 18 months of age in groups A and B, respectively. In females of group A, the incidences of endometrial hyperplasias and adenocarcinomas were comparable with those in the Donryu strain, and higher than those in group B, whose values were in turn elevated as compared to their F344 mothers. In contrast, endometrial stromal polyps and Leydig cell tumors of the testes were observed with similar incidences in both hybrids as well as F344 rats, but at markedly lower levels in the Donryu strain. The frequency of mononuclear cell leukemias was very low in both F₁ groups of each sex. Various tumors were also observed in many other organs/tissues such as the pituitary, thyroid, pancreas, adrenals, mammary and prostate glands and their incidences were relatively high in both groups A and B, but their variation between the F₁ groups was small. These results indicate that development of uterine endometrial hyperplasias/carcinomas in F₁ hybrids might be related to hormonal conditions depending on the genetic background of the mothers, but other tumors are considered to be influenced by other factors. The effects of genetic background on the occurrence of spontaneous tumors in hybrids are very complex and differ not only with the site but also the tumor types. (*J Toxicol Pathol* 2001; **14**: 99–104)

Key words: Donryu, F344, F₁ hybrids, spontaneous tumors, uterus, testis

Introduction

It is generally well known that the types and incidences of spontaneous tumors in rats differ among strains because of different genetic background, and are also influenced by various factors such as foods, housing conditions, micro-organisms, and hormones¹. Previously, we reported high spontaneous yields of uterine endometrial adenocarcinomas in female Donryu rats², the tumors being associated with an age-related hormonal imbalance^{3,4}. Furthermore, in male Donryu rats, the spontaneous occurrence of testicular tumors is low². In Fischer 344 (F344) rats, in contrast, the most common spontaneous uterine tumor is of endometrial

stromal polyp and the incidence of endometrial adenocarcinoma is very low, while Leydig cell tumors of the testis are very frequent⁵.

In the present study, we investigated the types and incidences of spontaneous tumors in various organs/tissues of F₁ hybrids between Donryu and F344 rats with special reference to the reproductive system, to evaluate the effects of various factors including hormonal and genetic factors on spontaneous tumor development.

Materials and Methods

Animals

Crj:Donryu rats (male n=19, female n=30) and F344/DuCrj rats (male n=21, female n=29) at 4 weeks of age were purchased from Charles River Japan, Inc. (Atsugi, Kanagawa). The rats were housed 2 or 3 males and 3 or 4 females in plastic cages in an air-conditioned barrier-system animal room at 24 ± 1°C and 55 ± 5% humidity with a 12-hr

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light/dark cycle. They were maintained on a basal diet, CRF-1 (Oriental Yeast Ind. Co., Tokyo), and tap water *ad libitum* throughout the study.

Experimental design

At 12 weeks of age, single virgin female Donryu or F344 rats were caged with single male F344 or Donryu rats overnight, respectively. Litters were weaned at 4 weeks of age and divided into two hybrid groups: Group A, Donryu female $\times$ F344 male (Donryu $\times$ F344)F₁ and group B, F344 female $\times$ Donryu male (F344 $\times$ Donryu)F₁. They were housed separately by sex. The numbers of F₁ hybrids obtained were 49 males and 48 females in group A, and 53 males and 56 females in group B. These F₁ hybrids and their parents were observed until 120 weeks of age, when all survivors were sacrificed.

Body weights and clinical signs

For both hybrids and their parents, body weights were recorded once a month. All animals were observed daily for clinical signs and mortality.

Estrous cycles

For both female hybrids and their mothers, estrous cycles were checked by vaginal smears every 2 months from 4 to 18 months of age.

Organ weights and histological examination

All dead, moribund- or terminal-sacrificed animals, except dead animals with severe autolysis (group B: male 10, female 1), were necropsied, and the uterus, testes, and other main organs/tissues (pituitary, thyroid, lungs, liver, pancreas, kidneys, heart, spleen, ovaries, vagina, prostate, urinary bladder, skin, mammary glands, and bone marrow) and tumor masses were taken for histological examination. They were fixed in buffered neutral 10% formalin, and sections were routinely prepared and stained with hematoxylin and eosin (H & E) for microscopy. In the present study, uterine endometrial proliferative lesions were histologically classified into two categories: hyperplasia and adenocarcinoma, according to the criteria described in our previous reports^{3,4}. Statistical analysis was performed using the χ^2 test.

Results

Survival rates and growth curves

In both F₁ hybrid groups and their parents, many animals (group A: male 25, female 10; group B: male 24, female 22; Donryu: male 2, female 18; F344: male 2, female 12) were dead or moribund-sacrificed during the study.

Figures 1 and 2 show the growth curves for males and females of both F₁ hybrid groups and their parents. In both sexes, the mean body weights of Donryu rats were higher than those of F344 rats. Mean values for groups A and B were in between those for Donryu and F344 rats, with no significant differences (Figs. 1 and 2).

Fig. 1. Growth curves for male rats.

Fig. 2. Growth curves for female rats.

Fig. 3. Incidences of persistent estrus.

Estrous cycles

In female Donryu rats, disorder of estrous cycles was detected first at 6 months of age and prevalence of abnormalities (persistent estrus) increased thereafter with age. Almost all animals showed persistent estrus at 18 months of age (approximately 96%). Many female F344 rats, in contrast, showed a normal estrous cycle until the end of the observation period, except only a few demonstrating

persistent estrus. The incidences of persistent estrus in both female F₁ groups from 8 to 12 months of age were in between those of their Donryu and F344 mothers, which were slightly higher in group A than that in group B (Fig. 3). The incidence increased thereafter and reached about 96% and 80% at 18 months of age in groups A and B, respectively.

Histological examination

Uterine proliferative lesions: Table 1 summarizes the incidences of uterine endometrial lesions in females of both hybrids and their mothers at terminal sacrifice. Endometrial hyperplasia and adenocarcinoma lesions were more frequently observed in Donryu than in F344 rats. Adenocarcinomas occurred in Donryu rats with an incidence of 25% together with severe hyperplasia, but such lesions were not detected in F344 rats. In contrast, endometrial stromal polyps were more frequently observed in F344 rats. In F₁ hybrids, the incidences of total endometrial hyperplasias and adenocarcinomas in group A were comparable with those in the Donryu mothers. The incidences of both lesions in animals of group B were lower than those in group A, but slightly higher than the F344 maternal values, the differences being not significant. On

the other hand, in both hybrids, endometrial stromal polyps were observed with similar incidence, the incidences being comparable with that of F344 rats.

Leydig cell tumors: Leydig cell tumors of the testis were observed at very high and low incidences in F344 and Donryu rats, respectively, as shown in Table 1. In both F₁ hybrid groups, however, the incidences were very high, with no difference between the two groups.

Tumors in other organs/tissues: Tables 2 and 3 show the incidences of various tumors in other organs/tissues of both male and female hybrids. Various tumors were observed with relatively high incidences in sites such as the pituitary, thyroid, pancreas, adrenal, mammary, and prostate. In contrast, mononuclear cell leukemias were very rare in both F₁ groups of males and females.

Lesions in F₁ hybrids dead or moribund-sacrificed before the terminal: Lesions in F₁ hybrids died or moribund-sacrificed before the terminal are shown in Table 4. The trends for tumor incidences in all reproductive and other organs of both F₁ hybrids were in line with those observed at the terminal.

Table 1. Uterine Proliferative Lesions and Testicular Tumors in F₁ Animals and Their Parents (Terminal Stage)

		Group A		Group B		F ₀			
		[Donryu × F344]F ₁		[F344 × Donryu]F ₁		Donryu		F344	
		female 38	male 24	female 34	male 29	female 12	male 17 ^{a)}	female 17	male 19 ^{a)}
Uterus	Hyperplasia	+	11 (28.9)	–	6 (17.6)	3 (25.0)	–	1 (5.9)	–
		++	3 (7.9)	–	6 (17.6)	0 (0.0)	–	0 (0.0)	–
		+++	5 (13.2)	–	1 (2.9)	2 (16.5)	–	0 (0.0)	–
	Total	19 (50.0) ^{##}		13 (38.2) [#]		5 (41.7)		1 (5.9)	
	Adenocarcinoma	9 (23.7)	–	2 (5.9)	–	3 (25.0)	–	0 (0.0)	–
Endometrial stromal polyp		11 (28.9) [*]	–	10 (29.4) [*]	–	0 (0.0)	–	4 (23.5)	–
Testis	Leydig cell tumor	–	22 (91.7) ^{***}	–	25 (86.2) ^{***}	–	5 (29.4)	–	18 (94.7)

a): including animals died or moribund-sacrificed before the terminal.

* p<0.05, ***: p<0.001 Significant different from F₀ Donryu rats.

#: p<0.05, ##: p<0.01 Significant different from F₀ F344 rats.

(): %

Table 2. Other Tumors in F₁ Males

		Group A	Group B	
		[Donryu × F344]F ₁	[F344 × Donryu]F ₁	
		male 24	male 29	
Pituitary	Adenoma	19 (79.2)	19 (65.5)	
Thyroid	C-cell adenoma/carcinoma	1 (4.2)	4 (13.8)	
Pancreas	Islet adenoma/adenocarcinoma	6 (25.0)	6 (20.7)	
Adrenal gland	Cortical adenoma	1 (4.2)	1 (3.4)	
	Pheochromocytoma	2 (8.3)	4 (13.8)	
Prostate	Adenoma	2 (8.3)	4 (13.8)	
Mammary gland	Fibroma	3 (16.7)	4 (13.8)	
	Fibroadenoma	6 (25.0)	7 (24.1)	
Hematopoietic system	Mononuclear cell leukemia	0 (0.0)	1 (3.4)	(): %

Table 3. Other Tumors in F₁ Females

		Group A [Donryu × F344]F ₁ female 38	Group B [F344 × Donryu]F ₁ female 34
Pituitary	Adenoma	19 (50.0)	9 (26.5)*
Thyroid	C-cell adenoma/carcinoma	7 (18.4)	4 (11.8)
Adrenal gland	Cortical adenoma	1 (2.6)	3 (8.8)
Mammary gland	Fibroma	1 (2.6)	1 (2.9)
	Fibroadenoma	13 (34.2)	15 (44.1)
Hematopoietic system	Mononuclear cell leukemia	1 (2.6)	0 (0.0)

*: p<0.05 Significant different from Group A.

(): %

Table 4. Uterine Proliferative Lesions, Testicular and Other Tumors in F₁ Hybrids Dead or Moribund-Sacrificed before the Terminal

			Group A		Group B	
			[Donryu × F344]F ₁		[F344 × Donryu]F ₁	
			female 10	male 25	female 21	male 14
Uterus	Hyperplasia	+	3 (30.0)	—	3 (14.3)	—
		++	0 (0.0)	—	0 (0.0)	—
		+++	0 (0.0)	—	0 (0.0)	—
		Total	3 (30.0)	—	3 (14.3)	—
	Adenocarcinoma		2 (20.0)	—	1 (4.8)	—
	Endometrial stromal polyp		2 (20.0)	—	4 (19.0)	—
Testis	Leydig cell tumor		—	16 (64.0)	—	10 (71.4)
Pituitary	Adenoma		0 (0.0)	2 (8.0)	5 (23.8)	5 (35.7)
	Adenocarcinoma		1 (10.0)	15 (60.0)	6 (28.6)	7 (50.0)
Thyroid	C-cell adenoma/carcinoma		0 (0.0)	2 (8.0)	4 (19.0)	1 (7.1)
Pancreas	Islet adenoma/adenocarcinoma		0 (0.0)	2 (8.0)	0 (0.0)	3 (21.4)
Adrenal gland	Cortical adenoma		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
	Pheochromocytoma		1 (10.0)	1 (4.0)	0 (0.0)	3 (21.4)
Prostate	Adenoma		—	0 (0.0)	—	0 (0.0)
Mammary gland	Fibroma		0 (0.0)	4 (16.0)	3 (14.3)	2 (14.3)
	Fibroadenoma		4 (40.0)	4 (16.0)	6 (28.6)	0 (0.0)
Hematopoietic system	Mononuclear cell leukemia		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

(): %

Discussion

Strain-differences in the incidence and type of spontaneous tumors in rats are well known¹. In the present study, a high yield of uterine endometrial adenocarcinomas was characteristic of Donryu rats, although the most common spontaneous tumor of the uterus in F344 rats was of endometrial stromal polyp. On the other hand, in males, almost all F344 rats had interstitial cell (Leydig cell) tumors of the testis, but the incidence was very low in Donryu rats. These results are consistent with our previous reports^{1,2,5}.

In both male and female hybrids of these two strains, body weights were intermediate between the values for their parents. Although the histological types of main tumors in both hybrid groups of each sex to a certain extent reflected their strain background^{2,5}, there were no clear differences in the incidences of these tumors between groups A and B, except in terms of endometrial adenocarcinoma. The results

indicate that the effects of hybridization on spontaneous development of tumors in F₁ hybrids differ with the organ/tissue.

In females, the incidence of endometrial adenocarcinomas in group A was higher than that in group B, but not significant, and was comparable with that in the Donryu mother. In addition, the fact of only a slight difference between group B and F344 values indicates that the occurrence of endometrial adenocarcinomas in F₁ hybrids was mainly dependent on the genetic background of the mothers. Previously, we reported that the spontaneous occurrence of endometrial adenocarcinomas in Donryu rats was related to age-related hormonal disturbance, especially an increased E2:P ratio^{3,4}. The resultant constant high level of proliferative activity of epithelial cells⁶ plays an important role in the high yield of uterine endometrial adenocarcinomas in this rat strain, as reviewed recently⁷. In the present study, sequential changes of estrous cycle in both

parents were comparable with those observed previously^{3,4}, and the appearance and incidence of persistent estrus in female hybrids were intermediate, although higher in group A. These results may indicate that the difference in the incidence of endometrial carcinoma between the two groups of hybrids depends fundamentally on their hormonal condition, and persistent estrus (perhaps increased E2:P ratio) promotes development of the tumor. Although steroid hormone analysis was not performed in the present study, the histological finding of age-dependent ovarian atrophy with follicular cysts and loss of corpus lutea in both hybrids, especially in group A (the incidences in Group A: 52.1%, Group B: 25.5%), provides support for hormonal abnormality, in line with our previous reports^{3,4}. The early appearance of persistent estrus in group B may have played a role in slight increase of the carcinoma incidence, compared with the F344 mothers. In group A, however, the carcinoma incidence at the termination was comparable with that in the Donryu mothers, although the appearance and incidence of persistent estrus were delayed and lower, respectively. Other factors, besides persistent estrus, may thus also be important for development of this tumor. The data for endometrial stromal polyps did not point to any influence of hormonal disturbance but rather some other genetic trait of the F344 strain.

The incidences of other tumors such as pituitary, adrenocortical and mammary adenomas in both female hybrids were paradoxical. These tumors are known to be induced by estrogen⁸⁻¹⁰, and their spontaneous occurrence is generally slightly higher in Donryu than F344 rats^{1,2,5}. The reason for the increased pituitary adenomas in group A might be an increase in the E2:P ratio, but the lower incidences of adrenocortical adenomas and mammary fibroadenomas can not be explained. In women, a relationship between uterine adenocarcinomas and endocrine tumors such as pituitary tumors has been documented¹¹. Previously, we investigated the coincident occurrence of uterine adenocarcinoma with some endocrine tumors including pituitary and adrenocortical tumors as well as mammary tumors, but the coincidences in any of the cases were under the statistically expected values³. These results may indicate that the differences in these tumor incidences between groups A and B can not be simply explained by the hormonal condition.

In males, the most clear difference in incidences of spontaneous tumors between Donryu and F344 rats was found for interstitial cell tumors of the testis, as reported previously^{1,2,5}. While that in Donryu males was higher than in the past, it was still much lower than in both groups A and B, with incidences comparable with the F344 value. On the contrary, mononuclear cell leukemias were very low in both hybrids as in Donryu rats, this tumor being known to occur in F344 rats at relatively high incidence⁵. The reason that there were no differences in the incidence of these tumors between groups A and B is uncertain, although genetic factors would appear to be responsible for these findings.

It is well known that mononuclear cell leukemias are

one of the most common spontaneous tumors in F344 rats^{1,5}. In the present study, however, their occurrence was very few in both F₁ hybrids. This tumor is a fatal disease in animals but the tumor-incidences were also null in both hybrids before the terminal. The reason for very low incidence LGL leukemias in F₁ hybrids is uncertain.

In the present study, unfortunately, the number of hybrids and parents, especially the latter (male), examined at the termination was very small. In addition, parent rats were used once for a mating. Recently, we examined the effects of reproduction on spontaneous development of endometrial adenocarcinomas in Donryu rats, and showed that tumor development was suppressed in rats experiencing three pregnancies, associated with changes in the hormonal milieu, although no suppression was evident after a single reproductive experience¹². In the present study, the incidences of uterine proliferative lesions in Donryu rats were comparable with those in nulliparous animals reported previously¹². However, effects of copulation on the occurrence of Leydig cell tumors in male rats are uncertain.

In conclusion, effects of genetic backgrounds on the occurrence of spontaneous tumors in hybrids are very complex and differ not only with the site but also the tumor types, and the differences in tumor incidences between parents and F₁ hybrids or groups A and B depend on multifactorial inheritance, presumably involving genes determining both relative susceptibility and resistance. Further investigations including backcross experiments and gene mutation studies are needed to clarify the genes involved and the relative dominance. Interestingly, it was recently reported that alterations in the *K-ras* gene may be one of the important initiating events in spontaneous endometrial carcinoma development in the Donryu rat, like in man¹³. Elucidation how this could be linked to genetic variation is worthy of attention.

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