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Original Article

Background data of 2-year-old male and female F344 *gpt* delta rats

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Abstract: Although *gpt* delta rats, as reporter gene-transgenic rats, were originally developed for *in vivo* mutation assays, they have also been used to evaluate chemical carcinogenesis and comprehensive toxicity. Therefore, it is necessary to accumulate background data on carcinogenicity and general toxicity in *gpt* delta rats. Here, we investigated the background data of 110-week-old male and female F344 *gpt* delta rats and wild-type rats. There was no effect of reporter gene transfection on animal survival rates and body weights during the experiment. The relative weight of male *gpt* delta rat adrenals was significantly higher than that of wild-type rats, possibly due to the higher incidence of pheochromocytoma. There were no intergenotype differences in the incidence of nonneoplastic lesions in both sexes, including chronic progressive nephropathy and focus of cellular alteration in the liver, which had a higher incidence in both genotypes. Additionally, the significantly higher incidence of adrenal pheochromocytoma in male *gpt* delta rats than that in wild-type rats was likely incidental because of the lack of differences in the incidences of preneoplastic (male and female) and neoplastic (female) adrenal lesions in both genotypes. Other neoplastic lesions in both sexes showed no intergenotype differences in incidence rates, although large granular lymphocytic leukemia in the spleen and Leydig cell tumors in the testes of males showed higher incidence rates. Overall, there were no effects of reporter gene transfection on the spectrum of spontaneous lesions in F344 *gpt* delta rats, thus supporting their applicability in evaluating chemical toxicity and carcinogenicity. (DOI: 10.1293/tox.2020-0060; J Toxicol Pathol 2021; 34: 23–31)

Key words: *gpt* delta rat, F344 rat, background data

Introduction

Although various genotoxicity tests have been developed, *in vivo* mutation assays using reporter gene transgenic rodents are promising tools to evaluate chemical mutagenicity owing to the involvement of *in vivo* metabolism and target organs. The *gpt* delta rodents are an established reporter gene-transgenic animal model used for *in vivo* mutation assays and were originally developed by Nohmi *et al.*[1]. These *gpt* delta rats are transfected with 5–10 copies of lambda EG10, including a reporter gene, which insert themselves into chromosome 4 of the somatic and germ cells of systemic organs[2–4]. The F344, Sprague-Dawley (SD), and Wistar rat strains have been established as background strains for *gpt* delta rats. In particular, reporter gene mutation assays using *gpt* delta rodents have many advantages. For example, these rats can be used for the detection of point and deletion mutations via *gpt* and Spi$^-$ assays, respectively[5].

Detailed information on the types of mutations that are induced by chemicals has enabled us to utilize *gpt* delta rats to investigate the underlying mechanisms of carcinogenesis. In fact, combined assays for evaluation of DNA modification and/or the microenvironmental status of cells with reporter gene mutation assays have been used to provide evidence of the mechanisms of chemical carcinogenesis[6–11]. In addition, we have developed a medium-term animal model that is capable of simultaneously detecting *in vivo* mutagenicity and tumor-promoting activities in the liver and kidney[12, 13]. However, these applications of *gpt* delta rats require knowledge of the sensitivity to chemical carcinogens of the background strain. Our previous studies have demonstrated that medium-term exposure of *gpt* delta rats to various hepatocarcinogens induced the development of glutathione *S*-transferase placental form (GST-P) foci to the same degree as those observed in wild-type (WT) rats[12–15]. As it is impossible to examine the carcinogenic sensitivity of the *gpt* delta rats to all chemical carcinogens, evaluation of spon-

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taneous tumor spectra could provide valuable information.

Notably, *gpt* delta rodents are able to provide data on the general toxicity of test chemicals and have enabled us to evaluate comprehensive toxicity, including general toxicity and *in vivo* genotoxicity, in a single study, conforming to the 3R's principle of animal use in laboratory experiments[16, 17]. In a previous study of medium-term administration of some chemicals, toxicological responses in *gpt* delta rats were found to be identical to those in WT rats with the same background[18]. However, no reports have described spontaneous age-related lesions and their effects on chronic toxic responses.

In the current study, we aimed to obtain background data required to expand the applications of *gpt* delta rats by evaluating parameters related to general toxicity and carcinogenicity in *gpt* delta rats and WT rats of the same background strain (F344 rats).

Materials and Methods

Experimental animals and housing conditions

The protocol was approved by the Animal Care and Utilization Committee of the National Institute of Health Sciences (Approval number: 208). Male and female 5-week-old specific pathogen-free F344/NSlc rats and F344/NSlc-Tg (*gpt* delta) rats carrying approximately five tandem copies of the transgene lambda EG10 per haploid genome were obtained from Japan SLC, Inc. (Shizuoka, Japan) and acclimated for 1 week prior to testing. The rats were housed in polycarbonate cages with hardwood chips for bedding in a conventional animal facility. Three or four rats were housed in each cage. Animals were maintained under controlled temperature (23 ± 2°C), relative humidity (55 ± 5%), air changes (12 times/h), and lighting (12-h light-dark cycle) conditions with free access to a basal diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. Male and female 6-week-old F344 and F344 *gpt* delta rats were maintained for 104 weeks (n = 75). At the end of each experiment, the rats were anesthetized using isoflurane, then euthanized by exsanguination via transection of the abdominal aorta.

Organ weights and histopathological assessment

Complete necropsies were performed for all animals, and their internal organs, i.e., brain, thymus, lungs, heart, spleen, liver, adrenal glands, kidneys, and gonads, were weighed. These organs and additional tissues (skin, mammary gland, sternum with marrow, femur with marrow, mandibular and mesenteric lymph nodes, salivary glands, aorta, trachea, tongue, esophagus, stomach, small and large intestines, pancreas, urinary bladder, epididymis, seminal vesicles, prostate gland, bulbourethral glands, uterus, vagina, pituitary gland, thyroid glands, parathyroid glands, spinal cord with vertebrae, trigeminal nerve, sciatic nerve, harderian glands, femoral skeletal muscle, and nasal cavity) were fixed in 10% neutral buffer formalin, and paraffin-embedded sections were routinely prepared and stained with hematoxylin and eosin for histopathological examination. Bony tissues, including the nasal cavity, vertebrae, sternum, and femur, were decalcified with a mixture of 10% formic acid and 10% buffered formalin for up to 2 weeks. As previously reported, histopathological grading for non-proliferative lesions was performed as follows: (1) minimal, (2) mild, (3) moderate, (4) marked, and (5) severe[19]. Number of lesions of focal medullary hyperplasia and benign pheochromocytoma in adrenals of male WT and *gpt* delta rats were counted and their calculated multiplicity per affected rat.

Statistical analysis

Variations in body weights, organ weights (both absolute and relative weights), and multiplicity of focal medullary hyperplasia and benign pheochromocytoma lesions in male WT and *gpt* delta rats were analyzed by assessing the variance for homogeneity using F-tests. Student's t-tests and Welch's t-tests were used to assess the homogeneous and heterogeneous data, respectively. For comparison of histopathological changes between *gpt* delta and WT rats, incidences were analyzed with Fisher's exact probability test.

Results

Survival rates and body weights

There were no intergenotype differences in the survival rates of both male and female rats during the experiment (Fig. 1A). Thirty-seven male WT, 42 male *gpt* delta, 20 female WT, and 14 female *gpt* delta rats were found dead or were euthanized when moribund. Figure 1B shows the body weight curves. Body weights of male *gpt* delta rats were significantly higher than those of corresponding WT rats from the start of the experiment until week 4. However, body weights of male *gpt* delta rats were significantly lower than those of WT rats from 10 weeks onwards until the end of the experiment. There were no intergenotype differences in body weights in female rats during the experiment.

Organ weights

The results of organ weight assessments are shown in Table 1. The data from moribund-sacrificed or found-dead animals were excluded. The absolute weights of the brains of male and female F344 *gpt* delta rats were significantly lower than those of WT rats. Relative weights of the brains, lungs, hearts, adrenals, and kidneys of male *gpt* delta rats were significantly higher than those of male WT rats. Absolute and relative weights of the lungs and livers of female *gpt* delta rats were significantly higher than those of female WT rats.

Histopathological examination

Some organs could not be evaluated owing to the loss of materials during processing or specimen preparation. The missing organs and their numbers are listed in Supplementary Table 1.

Histopathological findings of nonneoplastic lesions are shown in Table 2. The incidence rates of all nonneoplastic lesions did not show intergenotype differences. Histopatho-

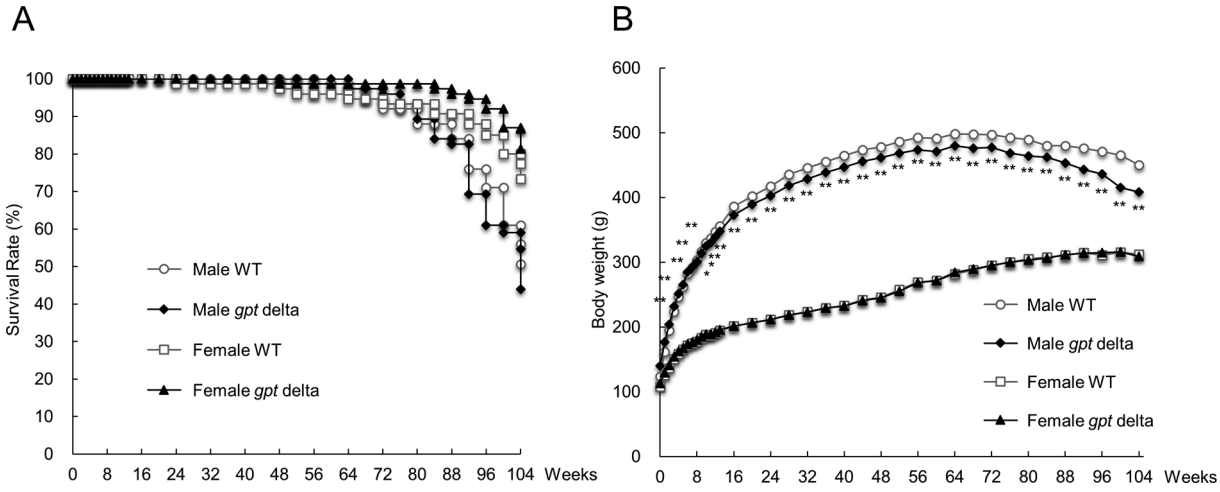

Fig. 1. Survival (A) and body weight curves (B) for wild-type (WT) F344 rats and F344 *gpt* delta rats housed for 2 years. *, **: $P<0.05$, 0.01 versus male WT rats.

Table 1. Organ Weights of Wild-type (WT) F344 Rats and F344 *gpt* Delta Rats Bred for 2 Years

	Male		Female	
	WT	*gpt* delta	WT	*gpt* delta
Effective no.of animals	38	33	55	61
Final body weight (g)	450.3 ± 32.7[a]	408.5 ± 52.5**	312.4 ± 32.7	309.1 ± 39.4
Absolute (g)				
Brain	2.12 ± 0.04	2.07 ± 0.06**	1.89 ± 0.12	1.87 ± 0.06[#]
Lungs	2.27 ± 0.62	2.41 ± 0.67	1.10 ± 0.15	1.22 ± 0.30[##]
Heart	1.22 ± 0.16	1.45 ± 0.92	0.76 ± 0.08	0.77 ± 0.07
Spleen	3.33 ± 3.69	3.23 ± 3.27	0.76 ± 0.56	1.07 ± 1.19
Liver	15.35 ± 2.67	15.22 ± 3.44	8.77 ± 1.11	9.28 ± 1.18[#]
Adrenals	0.09 ± 0.07	0.12 ± 0.14	0.06 ± 0.05	0.06 ± 0.01
Kidneys	3.11 ± 0.43	3.04 ± 0.58	1.87 ± 0.19	1.91 ± 0.14
Testes	4.71 ± 1.82	4.11 ± 1.89	-	-
Ovaries	-	-	0.053 ± 0.011	0.052 ± 0.011
Relative (g%)				
Brain	0.47 ± 0.04	0.52 ± 0.08**	0.61 ± 0.08	0.62 ± 0.10
Lungs	0.51 ± 0.17	0.60 ± 0.20*	0.36 ± 0.06	0.41 ± 0.18[#]
Heart	0.27 ± 0.04	0.37 ± 0.33**	0.25 ± 0.03	0.25 ± 0.05
Spleen	0.76 ± 0.88	0.81 ± 0.82	0.25 ± 0.21	0.38 ± 0.50
Liver	3.42 ± 0.59	3.74 ± 0.85	2.81 ± 0.25	3.05 ± 0.52[##]
Adrenals	0.02 ± 0.02	0.03 ± 0.04*	0.02 ± 0.01	0.02 ± 0.01
Kidneys	0.69 ± 0.09	0.75 ± 0.13*	0.60 ± 0.07	0.63 ± 0.12
Testes	1.04 ± 0.38	1.03 ± 0.41	-	-
Ovaries	-	-	0.017 ± 0.003	0.017 ± 0.003

Where [a]: Mean ± S.D.; *, **: $P<0.05$, 0.01 vs. male WT; [#, ##]: $P<0.05$, 0.01 vs. female WT.

logical findings of neoplastic lesions are listed in Table 3. The incidence of benign pheochromocytoma in male *gpt* delta rats was significantly higher than that in male WT rats. The multiplicity of focal medullary hyperplasia and benign pheochromocytoma in affected male rats showed no intergenotype differences (Table 4). The incidence rates of other tumors showed no intergenotype differences. Representative photographs of nonneoplastic and neoplastic lesions are shown in Fig. 2 and 3, respectively.

Discussion

As background data could be informative for investigating the toxicity and carcinogenicity of environmental chemicals, a comparison of the survival rates, body and organ weights, and the incidence of spontaneous lesions in reporter gene-transgenic rats and WT rats was performed. There are known to be strain differences in the spectrum of spontaneous lesions in rats[20, 21]. Moreover, F344 rats have

Table 2. Sites and Nonneoplastic Lesions in Wild-type (WT) F344 Rats and F344 *gpt* Delta Rats Bred for 2 Years

Sites and types of non-neoplastic lesions			Male		Female	
			WT	*gpt* delta	WT	*gpt* delta
	Effective no. of animals		75	75	75	75
Pituitary				[73][a]		
	Hyperplasia, pars distalis	-	14	16	14	12
Thyroid			[74]			
	Hyperplasia, C-cell, focal	-	16	20	16	23
Adrenal	Hyperplasia, medullary, focal	-	27	33	11	15
	Hyperplasia, cortical, focal	-	15	21	17	21
Pancreas			[70]		[74]	[74]
	Atrophy, acinar cell, diffuse	(1)[b]	6	12	3	4
	Atrophy, acinar cell, focal	(1, 2)	7, 1	9, 3	8, 0	5, 1
	Hyperplasia, acinar cell	-	0	2	3	3
	Hyperplasia, islet cell	-	4	3	1	0
Liver	Infiltration, mononuclear	(1, 2, 3)	8, 2, 0	9, 1, 0	20, 8, 0	17, 5, 1
	Angiectasis	(1)	0	1	3	2
	Degeneration, cystic	(1)	3	4	0	0
	Hyperplasia, bile duct	(1, 2, 3, 4)	7, 21, 35, 12	6, 23, 36, 8	25, 37, 5, 0	17, 43, 7, 0
	Focus of cellular alteration	-	63	59	66	68
	Basophilic cell	-	62	53	66	68
	Eosinophilic cell	-	7	5	6	4
	Clear cell	-	11	6	5	8
	Hyperplasia, hepatocellular, non-regenerative	-	2	1	3	4
Lung/bronchus	Hyperplasia, bronchiolo-alveolar	-	2	3	0	3
Kidney	Chronic progressive nephropathy	(1, 2, 3, 4)	19, 22, 10, 4	14, 19, 13, 6	6, 1, 0, 0	9, 2, 1, 0
Bone marrow	Atrophy, focal	(1, 2)	3, 0	1, 3	7, 2	4, 5
	Inflammation, granulomatous	(1, 2)	3, 0	3, 0	4, 3	6, 3
Thymus			[53]	[57]	[67]	[68]
	Involution, age-related	(1, 2, 3, 4)	3, 6, 21, 23	1, 9, 23, 20	5, 4, 13, 45	1, 5, 14, 48
Heart	Necrosis/inflammatory cell infiltrate, cardiomyocyte	(1)	3	4	0	0
Testis				[72]		
	Atrophy, tubular	(1, 2, 3)	8, 16, 31	7, 14, 30	-	-
	Hyperplasia, Leydig cell, focal	-	53	57	-	-
Prostate	Hyperplasia, atypical	-	17	21	-	-
Ovary					[73]	[74]
	Atrophy	(1, 2)	-	-	17, 1	18, 5
Uterus						[74]
	Hyperplasia, glandular, focal	-	-	-	3	4
Eye			[73]	[73]		
	Degeneration, lens fiber	(1, 2, 3)	21, 6, 0	25, 5, 0	19, 7, 7	19, 6, 1
Nasal cavity	Eosinophilic globules	(1, 2)	37, 2	35, 2	38, 7	43, 6

Where [a]: Numbers in square bracket are for animals examined microscopically; [b]: Numbers in parenthesis indicate the grades of lesion: (1) Minimal, (2) Mild, (3) Moderate, (4) Marked.

Table 3. Sites and Types of Tumors in Wild-type (WT) F344 Rats and F344 *gpt* Delta Rats Bred for 2 Years

Sites and types of tumors		Male		Female	
		WT	*gpt* delta	WT	*gpt* delta
Effective No. of rats		75	75	75	75
Brain	Meningioma, malignant	0	1	0	0
	Astrocytoma, malignant, high grade	0	0	1	0
Pituitary			[73][a]		
	Adenoma, pars distalis	15	17	30	25
	Carcinoma, pars distalis	0	0	1	0
	Adenoma, pars intermediate	0	0	1	1
Thyroid		[74]			
	Adenoma, C-cell	9	9	5	6
	Carcinoma, C-cell	0	1	0	2
	Carcinoma, follicular cell	1	0	1	0

Table 3. Continued

Sites and types of tumors		Male		Female	
		WT	*gpt* delta	WT	*gpt* delta
Adrenal	Pheochromocytoma, benign	16	30*	1	1
	Pheochromocytoma, malignant	0	2	0	0
	Pheochromocytoma, complex, malignant	0	1	0	0
	Adenoma, cortical	0	0	1	1
		[70]		[74]	[74]
Pancreas	Adenoma, islet cell	6	5	0	1
	Carcinoma, islet cell	1	0	1	0
	Adenoma, acinar-islet cell	0	1	1	0
Liver	Adenoma, hepatocellular	1	2	0	0
	Carcinoma, hepatocellular	0	1	0	0
Lung	Adenoma, bronchiolo-alveolar	5	5	3	2
Spleen	Hemangiosarcoma	0	1	0	0
Kidney	Adenoma	0	0	1	0
	Carcinoma, transitional cell	1	0	0	0
Urinary bladder		[74]	[74]		[74]
	Papilloma, transitional cell	7	0	0	2
Hematopoietic organ	Large granular lymphocyte leukemia	31	36	9	17
	Lymphoma	3	3	1	2
	Thymoma, benign	0	0	0	1
Heart	Schwannoma, intramural	1	0	0	0
Oral cavity	Ameloblastoma	0	1	0	0
Tongue	Papilloma, squamous cell	0	0	0	1
Forestomach	Carcinoma, squamous cell	0	1	0	0
Testis			[72]		
	Adenoma, Leydig cell	66	65	-	-
Prostate gland	Adenoma	2	1	-	-
Ovary				[73]	[74]
	Tumor, granulosa cell, malignant	-	-	1	0
					[74]
Uterus	Polyp, endometrial stromal	-	-	10	17
	Sarcoma, endometrial stromal	-	-	1	1
Vagina					[73]
	Polyp, vaginal	-	-	1	1
Mammary gland		[58]	[58]		[74]
	Fibroadenoma	4	3	22	16
	Adenoma	0	0	0	1
	Adenocarcinoma	0	0	0	1
Skin		[74]			[74]
	Keratoacanthoma	3	3	0	0
	Tumor, basal cell, benign	2	0	0	0
	Carcinoma, basal cell	1	0	0	0
	Carcinoma, squamous cell	1	1	0	1
	Adenoma, sebaceous cell	2	0	0	0
	Tumor, hair follicle, benign	1	1	0	0
Subcutis	Fibroma	5	3	1	3
	Fibrosarcoma	0	0	1	2
	Fibrosarcoma, pleomorphic	0	1	0	0
	Lipoma	0	1	1	0
	Hemangioma	0	0	0	1
	Adenoma, preputial/clitoral gland	3	6	5	1
	Adenocarcinoma, preputial gland	0	2	-	-
Bone	Osteosarcoma	1	0	0	1
Thoracic/abdominal cavity	Mesothelioma, malignant	2	1	0	0
	Sarcoma, histiocytic	1	1	0	0
	Fibrosarcoma	1	0	0	0
	Sarcoma, NOS	0	1	0	0

Where [a]: Numbers in square bracket are for animals examined microscopically; *: $P<0.05$ vs. male WT.

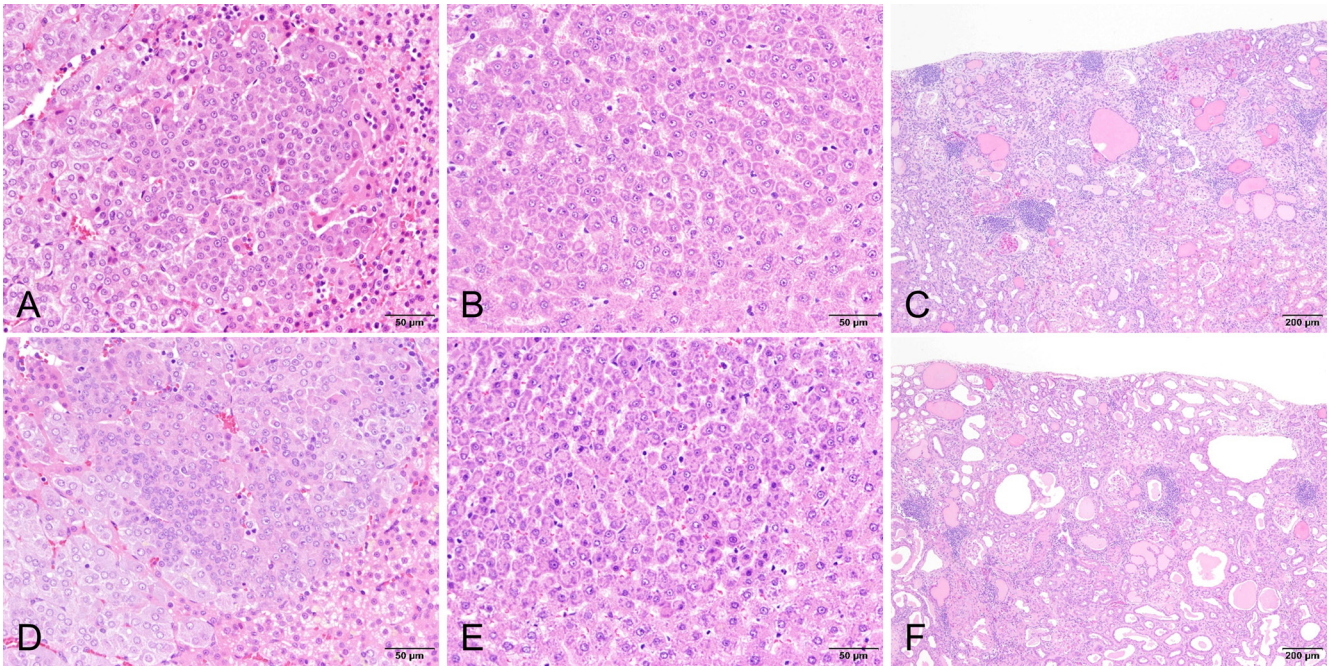

Fig. 2. Representative photographs of nonneoplastic lesions observed in wild-type F344 rats (A–C) and F344 *gpt* delta rats (D–F). (A and D) Focal medullary hyperplasia in the adrenal glands. (B and E) Focus of cellular alteration in the liver. (C and F) Chronic progressive nephropathy in the kidneys.

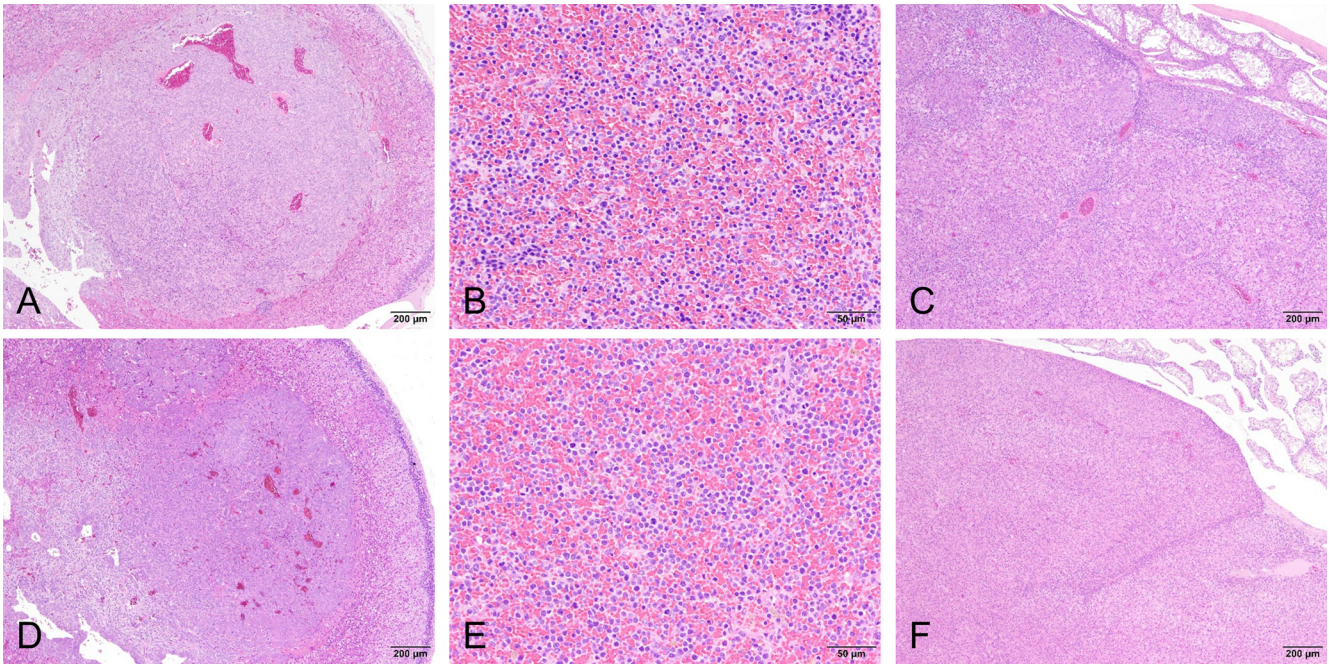

Fig. 3. Representative photographs of neoplastic lesions observed in wild-type F344 rats (A–C) and F344 *gpt* delta rats (D–F). (A and D) Benign pheochromocytoma in the adrenal glands. (B and E) Large granular lymphocyte leukemia in the spleen. (C and F) Leydig cell adenoma in the testes.

been used in long-term toxicological and carcinogenicity studies for many years, and abundant background data have been accumulated. Therefore, in the current study, F344 *gpt* delta rats were selected among the three background strains of *gpt* delta rats.

For both sexes, there were no intergenotype differences

Table 4. Multiplicity of Medullary Hyperplasia and Benign Pheochromocytoma in Adrenals of Male Wild-type (WT) and *gpt* Delta Rats

	Multiplicity in affeteced rats	
	Hyperplasia, medullary, focal	Pheochromocytoma, benign
WT	1.44 ± 0.80	1.19 ± 0.40
gpt delta	1.27 ± 0.45	1.37 ± 1.49

Values are mean ± SD.

in survival rates throughout the experimental period. Body weights of male *gpt* delta rats were significantly higher than those of WT rats from the start of the experiment until week 4 and were significantly lower from week 10 onwards, until the end of the experiment. These outcomes were inconsistent with those in a previous validation study[18] wherein the body weights of F344 and SD *gpt* delta rats were found to be lower than those of corresponding WT rats at the beginning of the experiment (at 6 weeks of age). As the differences observed in the current study were smaller, despite the observed statistical significance, the differences in growth curves observed in male *gpt* delta rats seemed to be incidental.

There were significant differences in the absolute and/or relative weights of various organs, including the brain, lungs, heart, adrenal glands, and kidneys of male *gpt* delta rats and the brain, lungs, and liver in female *gpt* delta rats. However, the incidence and severity of spontaneous lesions in all organs, except the adrenal glands, were almost identical between the two genotypes, as described below. Thus, it is unlikely that these fluctuations had a biological significance. However, increases in the relative weights of the adrenal glands in male *gpt* delta rats were thought to be associated with increases in the incidence of benign pheochromocytoma, as described below.

Several spontaneous nonneoplastic lesions, such as chronic progressive nephropathy in the kidney, bile duct hyperplasia, focus of cellular alteration in the liver, and eosinophilic globules in the nasal cavity, frequently occur in aged F344 rats[22–26]. In the current study, a higher incidence of spontaneous nonneoplastic lesions reported in F344 rats were also observed in *gpt* delta rats, and there were no intergenotype differences in their incidence. Additionally, the incidence rates of rare nonneoplastic lesions in F344 rats also showed no intergenotype differences.

The histopathological analysis of neoplastic lesions revealed that the incidence of benign pheochromocytoma in male *gpt* delta rats was significantly higher than that in male WT rats. Historical control data from the National Toxicology Program (NTP) showed that control incidence of benign pheochromocytoma in male F344 rats was 98/696 (14.1%) with an inter-study range of 8–22%[27]. In this study, the incidence of pheochromocytoma in male *gpt* delta rats was 30/75 (40%), which was higher than that of control data obtained from NTP. However, the incidence of benign pheochromocytoma in female *gpt* delta rats did not differ from that in female WT rats. In addition, adrenal focal medullary hyperplasia, a preneoplastic lesion of pheochromocytoma[28], showed no intergenotype differences in male and female *gpt* delta rats. In addition, multiplicity of focal medullary hyperplasia and benign pheochromocytoma in affected animals showed no intergenotype differences. These results indicated that the increased incidence of benign pheochromocytoma in male *gpt* delta rats may be incidental, rather than an effect of transfection of the reporter gene. Various spontaneous tumors have higher incidence rates in aged F344 rats, e.g., large granular lymphocyte leukemia, Leydig cell adenoma in the testis, anterior lobe adenoma in the pituitary gland, and fibroadenoma in the female mammary gland[29–32]. In the current study, these tumors were observed in *gpt* delta rats of both sexes to some extent. Notably, no intergenotype differences were observed. Additionally, the incidence of infrequent neoplastic lesions in F344 rats showed no intergenotype differences.

In conclusion, spontaneous lesions discovered in *gpt* delta rats with the F344 background strain seemed to be identical to those in WT rats. These findings supported the applicability of *gpt* delta rats as appropriate tools to evaluate the toxicity and carcinogenicity of environmental chemicals.

Disclosure of Potential Conflicts of Interest: The authors declare no potential, real, or perceived conflicts of interest with respect to the research, authorship, and/or publication of this article.

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