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Review

Spontaneous and Induced *gpt* and Spi$^-$ Mutant Frequencies in *gpt* delta Transgenic Rodents

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Transgenic rodent mutation assays are useful models for investigating the genotoxicity of chemicals *in vivo*. Transgenic *gpt* delta mice contain multiple copies of chromosomally integrated lambda EG10 phage shuttle vector, which contains reporter genes that allow detection of mutations. This system can identify both point mutations by the *gpt* assay (6-thioguanine selection) and certain types of deletions using the Spi$^-$ assay. Transgenic *gpt* delta rats, which have the same lambda EG10 DNA copies as *gpt* delta mice, have also been developed. The average spontaneous *gpt* mutant frequency (MF) in both *gpt* delta mice and rats is approximately 4.5×10^{-6}. In the Spi$^-$ assay, the average spontaneous Spi$^-$ MF is approximately 2.7×10^{-6} in *gpt* delta mice, similar to that of *gpt* delta rats. More than 20 chemicals and irradiations have been analyzed with these systems, and this review summarizes the MFs and treatment conditions. The data demonstrate that these transgenic rodent models are useful for detection and analysis of point mutations and deletions *in vivo*.

Key words: *gpt* delta transgenic rodent, mutant frequency, *gpt* assay, Spi$^-$ assay

Development of *gpt* delta Transgenic Rodents

A number of transgenic rodent mutation assays using transgenic animals with reporter genes directly integrated into the chromosome (1,2) have been developed to investigate *in vivo* genotoxicity. In these systems, gene mutations occurred in the rodents can be detected by recovering the transgene, delivering the transgene into a bacterial host and analyzing the subsequent phenotype. These models permit not only quantification of mutations, but also allow identification of mutations at the sequence level in any tissue in the body. The *lacZ*, *lacI* and *cII* genes have been employed as reporter genes in transgenic rodent systems, such as the Muta^{TM}mouse, and the Big Blue^R mouse and rat (3–7). A transgenic ''*gpt* delta'' assay system was developed for the efficient detection of both point mutations and deletions (1,8). The lambda EG10 phage vector constructed for this system contains two positive selection methods: the *gpt* assay [6-thioguanine (6-TG) selection] using the *gpt* gene of *E. coli*, which mainly detects point mutations such as base substitutions and frameshifts, and the Spi$^-$ assay (Spi$^-$ selection) that utilizes the *red/gam* genes of lambda phage to detect deletions, including frameshifts (Fig. 1).

The principles and method of the *gpt* assay have been previously described (1,8,9). The *gpt* gene of *E. coli* encodes guanine phosphoribosyltransferase, which functions in the purine salvage pathway. This enzyme phosphoribosylates 6-TG, which is subsequently incorporated into DNA and exert toxicicty to the cells. *E. coli* cells expressing the wild-type *gpt* gene cannot survive on plates containing 6-TG, and only cells harboring mutations in *gpt* can form colonies on 6-TG plates. The coding region of *gpt* is only 456 bp, a suitable size for easy sequencing analysis. When an *E. coli* strain expressing Cre recombinase is infected with lambda EG10 rescued from DNA of *gpt* delta rodents, the lambda EG10 DNA plasmid region is efficiently excised by Cre-lox recombination, circularized and propagated as multi-copy plasmids carrying the *gpt* and chloramphenicol acetyltransferase (CAT) genes. Bacteria harboring mutated *gpt* genes can then be selected as colonies on plates containing 6-TG and chloramphenicol (Cm). The number of rescued phages can be determined by plating cells on plates containing Cm alone. The *gpt* mutant frequency (MF) is calculated by dividing the number of *gpt* mutant (6-TG^r) colonies by the number of rescued Cm-resistant colonies.

The Spi$^-$ assay is a unique selection technique that detects deletions rather than base substitutions. The methodology and characteristics of the chemical- and radiation-induced Spi$^-$ mutations have been described in detail (1,10). Spi$^-$ selection takes advantage of the restricted growth of wild type lambda phage in P2 lysogens (11), a phenotype known as Spi (sensitive to P2 interference). Only mutant lambda phages that are

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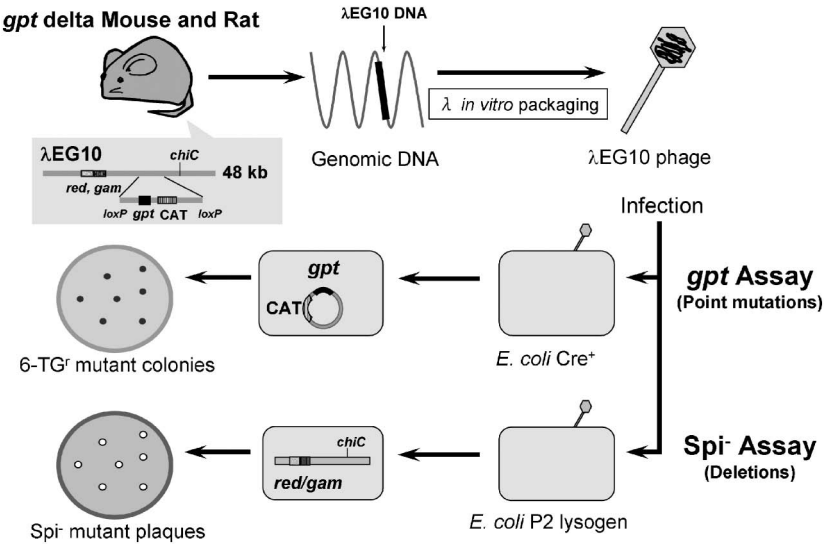

Fig. 1. Schematic outline of the *gpt* delta transgenic rodent mutation assays (1,8,10,34). Genomic DNA is extracted from transgenic rodent tissue, and lambda EG10 DNA is rescued as phage particles by *in vitro* packaging reactions. Two *E. coli* host strains are infected with the rescued lambda EG10 phages: *E. coli* expressing Cre recombinase for the *gpt* assay and the *E. coli* P2 lysogen for the Spi$^-$ assay.

deficient in the functions of both *gam* and *red* genes can grow in P2 lysogens and exhibit the Spi$^-$ phenotype. Simultaneous inactivation of both the *gam* and *red* genes is normally induced by deletions. Because of the size limitation for *in vitro* packaging reactions (there must be two cos sites separated by 38–51 kb of lambda DNA), Spi$^-$ selection can detect deletions up to 10 kb. Thus, the mutants are mostly intrachromosomal deletions.

The *gpt* delta transgenic mouse model employs both the *gpt* and Spi$^-$ assays (1,8). Lambda EG10 DNA was microinjected into the fertilized eggs of C57BL/6J mice. The transgenic mouse harbors approximately 80 copies of the transgene in a head to tail fashion at a single site in chromosome 17 and is maintained as a homozygote (9,12). The same lambda EG10 transgene was introduced into the genome of a Sprague-Dawley (SD) rat to establish the *gpt* delta rat (13). The *gpt* delta rat contains approximately 5 to 10 copies of the transgene in chromosome 4 and is maintained as a heterozygote. The outbred *gpt* delta SD rat was backcrossed with an F344 rat to establish an inbred *gpt* delta rat (F344).

Transgenic rodent mutation assays require the replication of the isolated DNA in a bacterial host. Thus, there is the possibility of *ex vivo* mutations arising in the bacterial host, not in a rodent, by lesions present in the isolated DNA. However, several observations suggest that these types of mutations are extremely rare (14). *E. coli* host strains used to detect mutant transgenes are *recA*$^-$, which greatly reduces the mutagenic potential of DNA lesions derived from a variety of mutagenic agents. In addition, the positive selection systems using *gpt*, Spi$^-$, *lacZ* and *cII* genes are unlikely to detect *ex vivo* mutations, since cells containing the wild type phages will be selected against before the mutations arise. There is a general consensus that *ex vivo* mutations unlikely to contribute in any significant way to spontaneous or induced MFs in a properly conducted assay (14). Indeed, the predominant type of spontaneous mutations in the *gpt* gene on a plasmid in *E. coli* cell is an insertion of IS*1*, which is a transposable element of *E. coli*. This insertion was rarely observed in the *gpt* mutations recovered from untreated *gpt* delta mice (9).

Spontaneous MFs in *gpt* and Spi$^-$ Assays

The spontaneous *gpt* MFs in various organs of *gpt* delta rodents were summarized in Table 1. Tissue type, age and sex are indicated. The experiments listed in Table 1 were performed in the same laboratory (Division of Genetics and Mutagenesis, NIHS). Therefore, variation of the values does not reflect inter-laboratory differences. The average spontaneous *gpt* MF in the *gpt* delta mice is 6.0×10^{-6}. No differences in MF among tissues were detected, except for the skin (epidermis and dermis), which shows a relatively higher MF. If epidermis and dermis are excluded from the calculation, the average MF is 4.6×10^{-6}. Although the number of studies that analyzed multiple tissues is limited, the spontaneous MFs were similar in various tissues (liver, spleen, colon, testis, brain and bone marrow) collected from the same animals (12). It is reported that the spontaneous MF values for male germline cells appear to be lower than that of somatic cells (14). The spontaneous MF in the testis of *gpt* delta rodents should be evaluated in more thoroughly. No significant differences were ob-

Table 1. Spontaneous *gpt* MFs of *gpt* delta rodents

	gpt MF ($\times 10^{-6}$)	No. of animal	Sex	Age (week)	Ref.
gpt delta Mouse (C57BL/6J)					
Liver	5.8	45	M&F	8–22	(12) (19) (35) (36) (37) U
Lung	3.4	15	M&F	11–13	(30)* (36)
Colon	7.4	11	M&F	19–22	(12) (19) (37)
Epidermis	11.5	6	M&F	13–18	(38) U
Bone marrow	2.9	5	M	13–22	(12) U
Kidney	5.7	5	M	17	U
Stomach mucosa	5.0	5	M	21	U
Spleen	3.3	3	M	22	(12)
Testis	3.0	3	M	22	(12)
Dermis	12.1	2	M&F	18	(38)
Brain	5.0	2	M	22	(12)
Average	6.0				
gpt delta Rat (SD)					
Liver	4.6	21	M&F	10–30	U
Mammary gland	4.4	5	F	10–12	U
Kidney	4.0	5	M	17	U
Average	4.5				
gpt delta Rat (F344)					
Liver	4.4	10	M	20–24	U
Average	4.4				

*Instead of mutant frequency, mutation frequency is cited. U: Unpublished data.

served in the spontaneous MFs in liver and colon between males and females (12). In most studies, the spontaneous *gpt* MFs in mice range around 5×10^{-6} whether the studies assessed mutant frequencies (not sequenced) or mutation frequencies (sequenced, and clonally corrected). This spontaneous MF is lower than that of other transgenic rodent mutation assays, such as Muta^{TM}mice and Big Blue^{R} mice, which are usually higher than 1×10^{-5}. The lower MF of the *gpt* assay may be caused by the different selection method and the sequence of the *gpt* gene. Phenotypic selection requires complete loss of enzymatic activity of the mutated *gpt* gene product; thus even if a mutation was introduced into the *gpt* gene, the residual enzymatic activity, if remains, may still be sufficient to catalyze enough 6-TG to kill the cells. It is reported that the spontaneous MF increases with age in most somatic cells in Muta^{TM}mice and Big Blue^{R} mice (15–18). In *gpt* delta mice, the spontaneous MF in the liver in 85 weeks old was 2-fold higher than in 19 week old (19). This tendency could differ in brain and germ tissue, where MFs do not increase with age in adult mice (20–22). Interestingly, the spontaneous MFs varied among the knockout mice. The spontaneous *gpt* MF in the liver of *ogg1* gene knockout mice, which lack 8-oxo-guanine DNA glycosylase activity, is significantly higher than that in wild type mice (23). IL-10-deficient mice, which spontaneously develop intestinal inflammation, have a *gpt* MF five times higher in the colon than that in wild type (24). In the transcription factor Nrf2-null (*nrf2*$^{-/-}$) mice, the spontaneous *gpt* MF in the lung was approximately three times higher than that in heterozygous (*nrf2*$^{+/-}$) or wild-type (*nrf2*$^{+/+}$) mice. The MF in the liver was higher in *nrf2*$^{-/-}$ and *nrf2*$^{+/-}$ mice than in *nrf2*$^{+/+}$ mice. In contrast, no difference in MF was observed in the testis among the three genotypes (25). These results suggest that the intracellular environment, including oxidative stress and/or detoxification systems, may contribute to spontaneous mutation. In the *gpt* delta rat, the average of spontaneous *gpt* MF is 4.4×10^{-6}. No difference was observed in MF between the SD (4.5×10^{-6}) and F344 (4.4×10^{-6}) backgrounds. Although the data are very limited, these values are comparable to those in mice. In contrast, Hayashi *et al.* reported that the spontaneous *gpt* MF in the liver of rats was lower than that of mice (13). The similar observation was reported in Big Blue mice and rats (7). Additional studies are needed to investigate the effect of genetic strain background, tissue type, and age on spontaneous MF.

Table 2 summarizes the spontaneous Spi^{-} MFs of *gpt* delta rodents. The average MF is 2.7×10^{-6} in mice and 2.8×10^{-6} in rat. No marked difference in MF has been observed between SD (2.9×10^{-6}) and F344 (2.8×10^{-6}) rats, although additional studies are needed to confirm this finding. In most experiments, the spontaneous Spi^{-} MFs of *gpt* delta mice were approximately 1 to 5×10^{-6},

Table 2. Spontaneous Spi$^-$ MFs of *gpt* delta rodents

	Spi$^-$ MF ($\times 10^{-6}$)	No. of animal	Sex	Age (week)	Reference
gpt delta Mouse (C57BL/6J)					
Liver	2.0	35	M&F	8–85	(12) (35) (37) U
Lung	2.8	16	M&F	11–12	(30)* U
Colon	2.4	6	M&F	22	(12)
Epidermis	1.5	6	M&F	13–18	(38) U
Bone marrow	5.4	5	M	13–22	(12) U
Kidney	5.1	9	M	10–17	(35) U
Spleen	3.2	17	M&F	10–22	(12) (35) U
Testis	2.2	4	M	10–22	(12) (35)
Brain	1.8	12	M&F	12–22	(12) U*
Average	2.7				
gpt delta Rat (SD)					
Liver	4.4	5	M	30	U
Kidney	1.3	5	M	17	U
Average	2.9				
gpt delta Rat (F344)					
Liver	2.8	3	M	24	U
Average	2.8				

*Instead of mutant frequency, mutation frequency is cited. U: Unpublished data.

which is lower than MFs of other transgenes, such as *lacZ* and *lacI*. Because the predominant types of point mutations induced *in vivo* are base substitutions, the spontaneous MF of Spi$^-$, which specifically detects deletion mutations, may be lower than that of *lacZ*, *lacI* or *gpt*. No difference in spontaneous Spi$^-$ MFs among tissues or between males and females was detected (12), although the number of studies evaluating these groups is limited. The effect of age on spontaneous Spi$^-$ MF has not been well characterized.

Interestingly, *Parp-1* knockout mice showed tendencies of higher Spi$^-$ MFs at 18 months of age, compared to *Parp-1*$^{+/+}$ mice, in the liver and brain (26). Several studies investigated the spontaneous Spi$^-$ MFs of *p53* and *Atm* knockout *gpt* delta mice; no significant differences were reported among these groups (27,28).

Induced MFs of the *gpt* delta Rodents

The *gpt* and Spi$^-$ assays have been validated in both mice and rats with various chemical mutagens/carcinogens, UV and ionizing radiations. The previously published experimental data from chemicals and physical (irradiation) agents are shown in Table 3. Among 23 agents, 4 chemicals showed a negative response. Acetaminophen is noncarcinogen and did not induce *gpt* mutations in the liver of SD rats. Di(2-ethyl-hexyl)phthalate (DEHP) and flumequine are known as nongenotoxic hepatocarcinogens. DEHP did not increase *gpt* or Spi$^-$ MFs in the liver of SD rats. Flumequine did not significantly increase *gpt* and Spi$^-$ MFs in the liver of B6C3F1 mice, a target organ of carcinogenicity in CD-1 mice. 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX) is strongly mutagenic in *Salmonella typhimurium* without metabolic activation. However, MX was not mutagenic in both *gpt* and Spi$^-$ assays in the liver or lung of C57BL/6J mice, although MX induced tumors in rats (29). These results suggest that the *in vivo* mutagenicity should be evaluated in a target organ of carcinogenicity to investigate the mechanism of carcinogenesis. Nineteen agents showed positive response in these assays. 2-Amino-3,8-dimethylimidazo[4,5-*f*]qoinoxaline (MeIQx) was used in a dose-response study. MeIQx induced *gpt* mutations in the liver of mice that were fed more than 30 ppm for 12 weeks. However, no significant induction was observed in mice fed 3 ppm. As an example of organ specificity, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP) induced *gpt* and Spi$^-$ mutations in the colon mucosa, spleen and liver, but not in the testis, brain and bone marrow. No significant differences were observed in the MFs in liver and colon of PhIP-treated mice between males and females. Dicyclanil may exhibit gender differences in mutagenicity, as the *gpt* MF in the liver was elevated only in females. Also interesting is the induction of *gpt* mutations but not Spi$^-$ mutations in the same organ by *N*-ethyl-*N*-nitrosourea (ENU) and 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) treatment. This may be because these are alkylating agents that preferentially induce base substitutions. On the other hand, a carbon ion beam induced Spi$^-$ muta-

Table 3. Summary of experimental data of *gpt* delta transgenic rodents treated with chemical or physical agents

Treatment	Animal	Dose	Route	Sex	Administration /sampling time (day)	Tissue	*gpt* or Spi$^-$	MF ($\times 10^{-6}$) Control /treatment	Fold increase	Response	Reference
Acetaminophen											
	gpt delta rat (SD)										
		10000 ppm	Diet	F	91/91	Liver	*gpt*	5.5/5.5	1.0	−	(39)
2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx)											
	gpt delta mouse (C57BL/6J)										
		300 ppm	Diet	M	84/84	Liver	*gpt*	6.2/52.9	8.5	+	(19)
						Colon	*gpt*	9.0/25.4	2.8	+	(19)
					546/546	Liver	*gpt*	12.2/238	19.5	+	(19)
		30 ppm	Diet	M	84/84	Liver	*gpt*	6.2/14	2.3	+	(19)
						Colon	*gpt*	9.0/11.7	1.3	−	(19)
		3 ppm	Diet	M	84/84	Liver	*gpt*	6.2/7.6	1.2	−	(19)
						Colon	gpt	9.0/9.3	1.0	−	(19)
						Testis	Spi$^-$	2.4/2.5	1.0	−	(19)
2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ)											
	gpt delta rat (SD)										
		300 ppm	Diet	F	91/91	Liver	*gpt*	5.5/188	34.2	+	(39)
2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)											
	gpt delta mouse (C57BL/6J)										
		400 ppm	Diet	M	91/105	Colon mucosa	*gpt*	5.7/111	19.5	+	(12) (40)
							Spi$^-$	3.7/45.6	12.3	+	(12) (40)
						Spleen	*gpt*	3.3/35	10.6	+	(12) (40)
							Spi$^-$	5.2/23.5	4.5	+	(12) (40)
						Liver	*gpt*	6.4/20.9	3.3	+	(12) (40)
							Spi$^-$	2.7/8.4	3.1	−	(12) (40)
						Testis	gpt	3.0/3.0	1.0	−	(12) (40)
							Spi$^-$	2.1/1.8	0.9	−	(12) (40)
						Brain	*gpt*	5.0/10.3	2.1	−	(12) (40)
							Spi$^-$	2.1/3.3	1.6	−	(12) (40)
						Bone marrow	*gpt*	2.0/11.9	6.0	−	(12) (40)
							Spi$^-$	1.9/5.5	2.9	−	(12) (40)
				F	91/105	Colon mucosa	*gpt*	8.6/122	14.2	+	(12) (40)
							Spi$^-$	1.1/29.8	27.1	+	(12) (40)
						Liver	*gpt*	4.4/38.6	8.8	+	(12) (40)
							Spi$^-$	2.1/9.9	4.7	+	(12) (40)
Aminophenylnorharman											
	gpt delta mouse (C57BL/6J)										
		20 ppm	Diet	M	84/98	Liver	*gpt*	6.6/68	10.3	+	(37)
							Spi$^-$	1.2/16	13.3	+	(37)
						Colon mucosa	*gpt*	6.2/29	4.7	+	(37)
		10 ppm	Diet	M	84/98	Liver	*gpt*	6.6/38	5.8	+	(37)
							Spi$^-$	1.2/12	10	+	(37)
						Colon mucosa	*gpt*	6.2/17	2.7	+	(37)

Table 3. (continued)

Treatment	Animal	Dose	Route	Sex	Administration /sampling time (day)	Tissue	*gpt* or Spi$^-$	MF ($\times 10^{-6}$) Control /treatment	Fold increase	Response	Reference
Benzo[*a*]pyrene (B[*a*]P)											
	gpt delta mouse (C57BL/6J)										
		2 mg/mouse	Intratracheal	M	1/14	Lung	*gpt*	5.5/31.3	5.7	+	(41)
		1 mg/mouse	Intratracheal	M	1/14	Lung	*gpt*	5.5/20.5	3.7	+	(41)
		0.5 mg/mouse	Intratracheal	M	1/14	Lung	*gpt*	5.5/13.9	2.5	−	(41)
	nrf2$^{+/-}$ *gpt* delta mouse (C57BL/6J)										
		1 mg/mouse	Intratracheal	M	1/14	Lung	*gpt*	4.8/14.7	3.1	+	(25)
	nrf2$^{-/-}$ *gpt* delta mouse (C57BL/6J)										
		1 mg/mouse	Intratracheal	M	1/14	Lung	*gpt*	14/29.3	2.1	+	(25)
	gpt delta rat (SD)										
		125 mg/kg	Intraperitoneal	M	1/7	liver	*gpt*	1.0/4.2	4.3	+	(13)
							Spi$^-$	0.5/4.6	9.3	+	(13)
		62.5 mg/kg	Intraperitoneal	M	1/7	liver	*gpt*	1.0/2.2	2.3	+	(13)
							Spi$^-$	0.5/1.2	2.4	−	(13)
Carbon ion beam											
	gpt delta mouse (C57BL/6J)										
		10 Gy	Irradiation	M	1/3	Liver	*gpt*	2.4/3.7	1.5	−	(35)
							Spi$^-$	2.2/7.4	3.4	+	(35)
						Spleen	Spi$^-$	1.8/5.2	2.9	+	(35)
						Kidney	Spi$^-$	2.6/3.9	1.5	+	(35)
	p53$^{+/+}$ *gpt* delta mouse (C57BL/6J)										
		10 Gy	Irradiation	M	1/3	Liver	Spi$^-$	3.6/9.8	2.7	+	(27)
		10 Gy	Irradiation	M	1/3		Spi$^-$	2.6/7.3	2.8	+	(27)
	p53$^{-/-}$ *gpt* delta mouse (C57BL/6J)										
		10 Gy	Irradiation	M	1/3	Liver	Spi$^-$	3.3/9.8	3.0	+	(27)
						Kidney	Spi$^-$	2.8/12.3	4.4	+	(27)
3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX)											
	gpt delta mouse (C57BL/6J)										
		100 ppm	Drinking water	M	84/84	Liver	*gpt*	2.2/1.5	0.68	−	(42)
							Spi$^-$	1.6/1.7	1.1	−	(42)
						Lung	*gpt*	1.9/2.1	1.1	−	(42)
							Spi$^-$	1.1/1.3	1.2	−	(42)
					546/546	Liver	*gpt*	3.4/3.6	1.1	−	(42)
				F	84/84	Liver	*gpt*	2.0/2.4	1.2	−	(42)
					546/546	Liver	*gpt*	2.5/2.7	1.1	−	(42)
		30 ppm	Drinking water	M	84/84	Liver	*gpt*	2.2/1.8	0.82	−	(42)
							Spi$^-$	1.6/2.0	1.3	−	(42)
						Lung	*gpt*	1.9/1.8	0.95	−	(42)
							Spi$^-$	1.1/0.8	0.72	−	(42)
				F	84/84	Liver	*gpt*	2.0/4.2	2.1	−	(42)
		10 ppm	Drinking water	M	84/84	Liver	*gpt*	2.2/2.3	1.0	−	(42)
							Spi$^-$	1.6/1.7	1.1	−	(42)
						Lung	*gpt*	1.9/2.5	1.3	−	(42)
							Spi$^-$	1.1/0.8	0.73	−	(42)

Table 3. (continued)

Treatment	Animal	Dose	Route	Sex	Administration /sampling time (day)	Tissue	*gpt* or Spi$^-$	MF ($\times 10^{-6}$) Control /treatment	Fold increase	Response	Reference
				F	84/84	Liver	*gpt*	2.0/2.2	1.1	−	(42)
Dicyclanil											
	gpt delta mouse (B6C3F1)										
		0.15%	Diet	M	91/91	Liver	*gpt*	4.2/4.8	1.1	−	(43)
							Spi$^-$	2.7/4.2	1.6	−	(43)
				F	91/91	Liver	*gpt*	4.8/22.3	4.6	+	(43)
							Spi$^-$	6.8/8.3	1.2	−	(43)
Di(2-ethyl-hexyl)phthalate (DEHP)											
	gpt delta rat (SD)										
		12000 ppm	Diet	F	91/91	Liver	*gpt*	5.5/3.3	0.6	−	(39)
							Spi$^-$	4.3/4.5	1.0	−	(39)
Diesel exhaust											
	gpt delta mouse (C57BL/6J)										
		3 mg/m^3 SPM	Inhalation	M	28/31	Lung	*gpt*	6.1/10.6	1.7	+	(44)
					84/87	Lung	*gpt*	5.9/19.0	3.2	+	(44)
						Testis	*gpt*	5.7/6.1	1.1	−	(45)
					168/171	Liver	*gpt*	5.6/6.7	1.2	−	(45)
						Lung	*gpt*	8.2/21.1	1.5	+	(45)
						Testis	*gpt*	5.8/11.7	2.0	+	(45)
		1 mg/m^3 SPM	Inhalation	M	84/87	Lung	*gpt*	5.9/18.4	3.1	+	(44)
		0.5 mg DEP	Intratracheal	M	1/14	Lung	*gpt*	6.6/19.7	3.0	+	(44)
		0.25 mg DEP	Intratracheal	M	1/14	Lung	*gpt*	6.6/14.0	2.1	+	(44)
		0.125 mg DEP	Intratracheal	M	1/14	Lung	*gpt*	6.6/11.6	1.8	+	(44)
		0.2 mg DEP extract	Intratracheal	M	1/14	Lung	*gpt*	6.6/17.8	2.7	+	(44)
		0.1 mg DEP extract	Intratracheal	M	1/14	Lung	*gpt*	6.6/12.8	1.9	+	(44)
		0.05 mg DEP extract	Intratracheal	M	1/14	Lung	*gpt*	6.6/9.7	1.5	−	(44)
1,6-Dinitropyrene											
	gpt delta mouse (C57BL/6J)										
		0.1 mg/mouse	Intratracheal	M	1/14	Lung	*gpt*	5.0/8.9	1.8	−	(46)
		0.05 mg/mouse	Intratracheal	M	1/14	Lung	*gpt*	5.0/19.2	3.8	+	(46)
		0.025 mg/mouse	Intratracheal	M	1/14	Lung	*gpt*	5.0/9.4	1.9	−	(46)
N-ethyl-*N*-nitrosourea (ENU)											
	gpt delta mouse (C57BL/6J)										
		100 mg/kg, bw	Intraperitoneal	F	1/14	Lung	*gpt*	6.4/55	8.6	+	(47)
	gpt delta mouse (B6C3F1)										
		200 ppm	Drinking water	M	28/56	Thymus	*gpt*	10.0/20.1	2.0	+	(48)
	gpt delta mouse (C57BL/6J × SWR, F1)										
		250 mg/kg, bw	Intraperitoneal	M&F	1/14	Small intestine	*gpt*	25/129	5.2	+	(49)
					1/28	Small intestine	*gpt*	25/127	5.1	+	(49)
		250 mg/kg, bw	Intraperitoneal	M&F	1/10	Small intestine	*gpt*	30/1285	42.8	+	(49)
		150 mg/kg, bw	Intraperitoneal	M&F	1/10	Small intestine	*gpt*	30/750	25	+	(49)
		50 mg/kg, bw	Intraperitoneal	M&F	1/10	Small intestine	*gpt*	30/395	13.1	+	(49)
		94 μg/mL	Drinking water	M&F	10/20–24	Small intestine	*gpt*	20/123	6.2	+	(49)
					30/40–44	Small intestine	*gpt*	20/402	20.1	+	(49)

Table 3. (continued)

Treatment	Animal	Dose	Route	Sex	Administration /sampling time (day)	Tissue	*gpt* or Spi$^-$	MF ($\times 10^{-6}$) Control /treatment	Fold increase	Response	Reference
	gpt delta mouse (BDF1)										
		150 mg/kg, bw	Intraperitoneal	M	1/7	Bone marrow	*gpt*	58/222	3.8	+	(8, 9)
	gpt delta rat (SD)										
		50 mg/kg bw	Intraperitoneal	M	5/7	Liver	*gpt*	1.7/16	9.4	+	(13)
					5/21	Liver	*gpt*	nd/20.3			(13)
					5/35	Liver	*gpt*	nd/24.9			(13)
					5/70	Liver	*gpt*	0.4/19.6	49	+	(13)
		100 mg/kg, bw	Intraperitoneal	M	1/7	Liver	*gpt*	2.3/16.8	7.3	+	(13)
							Spi$^-$	0.8/0.9	1.2	−	(13)
Ferric nitrilotriacetate (Fe-NTA)											
	gpt delta mouse (C57BL/6J)										
		3～5 mg iron/kg, bw	Intraperitoneal	M	5/7	Kidney	*gpt*	6/14	2.4	+	(50)
							Spi$^-$	8/13	1.7	+	(50)
Flumequine											
	gpt delta mouse (B6C3F1)										
		0.4%	Diet	M	91/91	Liver	*gpt*	8.0/10.1	1.3	−	(51)
				F	91/91	Liver	*gpt*	4.6/6.5	1.4	−	(51)
Gamma-ray											
	gpt delta mouse (C57BL/6J)										
		10 Gy	Irradiation	M	1/3	Liver	*gpt*	2.4/7.7	3.2	+	(35)
							Spi$^-$	2.2/3.0	1.4	−	(35)
		1.02 Gy (1.5 mGy/h)	Irradiation	F	31/31	Lung	*gpt*	4.2/2.7	0.6	−	(30)
							Spi$^-$	4.1/4.7	1.1	−	(30)
		0.68 Gy (1.0 mGy/h)	Irradiation	F	31/31	Lung	*gpt*	4.2/2.1	0.5	−	(30)
							Spi$^-$	4.1/4.1	1.0	−	(30)
		0.34 Gy (0.5 mGy/h)	Irradiation	F	31/31	Lung	*gpt*	4.2/4.7	1.1	−	(30)
							Spi$^-$	4.1/5.1	1.2	−	(30)
	gpt delta mouse (BDF1)										
		50 Gy	Irradiation	M	1/3	Spleen	Spi$^-$	$<$1.1/20	18$<$	+	(8) (34)
		10 Gy	Irradiation	M	1/3	Spleen	Spi$^-$	$<$1.1/12	11$<$	+	(8) (34)
		5 Gy	Irradiation	M	1/3	Spleen	Spi$^-$	$<$1.1/7	6$<$	+	(8) (34)
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)											
	gpt delta mouse (C57BL/6J)										
		2 mg/mouse	Intraperitoneal	M	4/32	Lung	*gpt*	3.1/26.5	8.5	+	(36)
				F	4/32	Lung	*gpt*	3.0/58.1	19.4	+	(36)
				F	4/18	Lung	*gpt*	4.2/14.3	3.4	+	(30)
							Spi$^-$	4.1/5.2	1.3	−	(30)
		100 mg/kg, bw	Intraperitoneal	F	1/14	Lung	*gpt*	6.4/62.2	9.7	+	(47)
Mitomycin C											
	gpt delta mouse (C57BL/6J)										
		4.0 mg/kg, bw	Intraperitoneal	M	1/14	Bone marrow	Spi$^-$	1.3/2.5	1.9	+	(52)
		2.0 mg/kg, bw	Intraperitoneal	M	1/14	Bone marrow	Spi$^-$	1.3/1.5	1.2	−	(52)
		1.0 mg/kg, bw	Intraperitoneal	M	1/14	Bone marrow	Spi$^-$	1.3/2.0	1.6	−	(52)
		0.5 mg/kg, bw	Intraperitoneal	M	1/14	Bone marrow	Spi$^-$	1.3/1.8	1.4	−	(52)

Table 3. (continued)

Treatment	Animal	Dose	Route	Sex	Administration /sampling time (day)	Tissue	*gpt* or Spi$^-$	MF ($\times 10^{-6}$) Control /treatment	Fold increase	Response	Reference
		1 mg/kg, bw	Intraperitoneal	M&F	5/12	Bone marrow	*gpt*	8.2/14.1	1.7	+	(53)
							Spi$^-$	1.8/5.2	2.9	+	(53)
					5/33	Bone marrow	*gpt*	8.2/9	1.1	−	(53)
							Spi$^-$	1.8/2.5	1.4	−	(53)
N-nitrosobis(2-hydroxypropyl)amine (BHP or DHPN)											
	Parp-1$^{+/+}$ *gpt* delta mouse (ICR/129Sv, C57BL/6J mixed)										
		2 mg/kg, bw	Intraperitoneal	M	1/7	Liver	*gpt*	5.7/89	15.6	+	(32)
							Spi$^-$	7.5/16	2.1	+	(32)
	Parp-1$^{-/-}$ *gpt* delta mouse (ICR/129Sv, C57BL/6J mixed)										
		2 mg/kg, bw	Intraperitoneal	M	1/7	Liver	*gpt*	10.0/66	6.6	+	(32)
							Spi$^-$	3.6/26	7.2	+	(32)
N-nitrosopyrrolidine											
	gpt delta rat (SD)										
		200 ppm	Drinking water	F	91/91	Liver	*gpt*	5.5/56.5	10.3	+	(39)
Potassium bromate ($KBrO_3$)											
	Ogg1$^{+/+}$ *gpt* delta mouse (129Sv×C57BL/6J, F1)										
		2 g/L	Drinking water	M&F	84/84	Kidney	*gpt*	4.6/9.9	2.2	+	(31)
	Ogg1$^{-/-}$ *gpt* delta mouse (129Sv x C57BL/6J, F1)										
		2 g/L	Drinking water	M&F	84/84	Kidney	*gpt*	2.9/28.8	9.9	+	(31)
	gpt delta rat (SD)										
		500 ppm	Drinking water	M	91/91	Kidney	*gpt*	9.4/15.7	1.7	−	(54)
							Spi$^-$	2.7/6.2	2.3	+	(54)
					7/7	Kidney	*gpt*	8.3/5.4	0.65	−	(54)
							Spi$^-$	2.9/3.6	1.2	−	(54)
					35/35	Kidney	*gpt*	8.3/7.2	0.87	−	(54)
							Spi$^-$	2.9/3.8	1.3	−	(54)
					63/63	Kidney	*gpt*	8.3/7.6	0.91	−	(54)
							Spi$^-$	2.9/9.9	3.4	+	(54)
					91/91	Kidney	*gpt*	8.3/7.8	0.93	−	(54)
							Spi$^-$	2.9/6.3	2.2	+	(54)
		250 ppm	Drinking water	M	91/91	Kidney	*gpt*	9.4/12.9	1.4	−	(54)
							Spi$^-$	2.7/3.1	1.1	−	(54)
		125 ppm	Drinking water	M	91/91	Kidney	*gpt*	9.4/7.7	0.82	−	(54)
							Spi$^-$	2.7/3.1	1.1	−	(54)
		60 ppm	Drinking water	M	91/91	Kidney	*gpt*	9.4/8.0	0.85	−	(54)
							Spi$^-$	2.7/1.6	0.59	−	(54)
	gpt delta rat (F344)										
		500 ppm	Drinking water	M	63/63	Kidney	*gpt*	1.2/4.3	3.6	−	(55)
							Spi$^-$	8.2/10.4	1.3	−	(55)
				F	63/63	Kidney	*gpt*	2.4/5.3	2.2	+	(55)
							Spi$^-$	4.5/8.9	2.0	−	(55)
Ultraviolet light B (UVB)											
	gpt delta mouse (C57BL/6J)										
		2.0 kJ/m^2	Irradiation	M&F	1/28	Epidermis	*gpt*	13.4/107	8.0	+	(38)

Table 3. (continued)

Treatment	Animal	Dose	Route	Sex	Administration /sampling time (day)	Tissue	*gpt* or Spi$^-$	MF ($\times 10^{-6}$) Control /treatment	Fold increase	Response	Reference
							Spi$^-$	1.1/1.4	1.3	−	(56)
						Dermis	*gpt*	12.1/104	8.6	+	(38)
		1.5 kJ/m^2	Irradiation	M&F	1/28	Epidermis	gpt	13.4/71.9	5.4	+	(38)
							Spi$^-$	1.1/2.2	2.0	+	(56)
						Dermis	*gpt*	12.1/92.7	7.7	+	(38)
		1.0 kJ/m^2	Irradiation	M&F	1/28	Epidermis	*gpt*	13.4/92.0	6.9	+	(38)
							Spi$^-$	1.1/3.7	3.4	+	(56)
						Dermis	*gpt*	12.1/113	9.3	+	(38)
		0.5 kJ/m^2	Irradiation	M&F	1/28	Epidermis	*gpt*	13.4/127	9.5	+	(38)
							Spi$^-$	1.1/3.5	3.2	+	(56)
						Dermis	*gpt*	12.1/37.8	3.1	+	(38)
		0.3 kJ/m^2	Irradiation	M&F	1/28	Epidermis	*gpt*	13.4/121	9.0	+	(38)
							Spi$^-$	1.1/2.5	2.3	+	(56)
						Dermis	*gpt*	12.1/29.2	2.4	+	(38)
X-ray											
	gpt delta mouse (C57BL/6J)										
		10 Gy	Irradiation	M	1/3	Liver	*gpt*	2.4/4.9	2.0	+	(35)
							Spi$^-$	2.2/4.8	2.2	−	(35)
	gpt delta mouse (B6C3F1)										
		1 Gy × 4/week	Irradiation	M	28/84	Thymus	*gpt*	10.0/4.9	0.49	−*	(48)
		0.2 Gy × 4/week	Irradiation	M	28/84	Thymus	*gpt*	10.0/5.9	0.59	−*	(48)
	Atm$^{+/+}$ *gpt* delta mouse (129, C57BL/6J mixed)										
		50 Gy	Irradiation	M&F	1/3	Liver	Spi$^-$	2.8/17.5	6.3	+	(28)
		10 Gy	Irradiation	M&F	1/3	Liver	Spi$^-$	2.8/7.6	2.7	+	(28)
		5 Gy	Irradiation	M&F	1/3	Liver	Spi$^-$	2.8/4.3	1.5	+	(28)
	Atm$^{-/-}$ *gpt* delta mouse (129, C57BL/6J mixed)										
		50 Gy	Irradiation	M&F	1/3	Liver	Spi$^-$	2.4/17.5	7.3	+	(28)
		10 Gy	Irradiation	M&F	1/3	Liver	Spi$^-$	2.4/5.4	2.3	+	(28)
		5 Gy	Irradiation	M&F	1/3	Liver	Spi$^-$	2.4/5.1	2.1	+	(28)

MF: Mutant frequency. Instead of mutant frequency, mutation frequency is cited for the references No. 30, 31, 34, 41, 46 and 48. Administration: administration period (days); Sampling time: Days counted from the first day of administration to sampling day; SPM: Suspended particle matter; DEP: Diesel exhaust particles; *The MF was reduced.

tions in the liver of mice, but not *gpt* mutations, possibly due to the efficient induction of DNA double strand breaks by carbon particle irradiation that leads to deletions. It should be noted that chronic gamma-ray irradiation at low dose rates (0.5, 1.0 and 1.5 mGy/h) did not cause a significant increase in *gpt* and Spi^- MFs in the lungs of mice; however, DNA sequencing analysis of the recovered Spi^- mutants showed a dose-dependent induction of large deletions over 1 kb (30). This suggests that sequence analysis is a useful method to identify the specific type of mutations. Determining the mutational spectrum could be important in providing mechanistic information of the mutagenic agents. The induced MFs were modified in various knockout mice. $KBrO_3$ treatment for 12 weeks gave rise to increased *gpt* MF in the kidney of *ogg1* knockout mice higher than that of wild type mice (31). In the Nrf2-null ($nrf2^{-/-}$) mice, a single intratracheal instillation of benzo[*a*]pyrene (B[*a*]P) increased *gpt* MF in lung higher than that of B[*a*]P-treated $nrf2^{+/-}$ mice (25). The p53 defect markedly enhanced the Spi^- MF in the kidney of mice exposed to carbon-ion irradiation. The enhancement of Spi^- MF in kidney of $p53^{-/-}$ mice was primarily due to an increase in complex or rearrangement-type deletions (27). After X-ray irradiation, *Atm* status did not significantly affect either the induced Spi^- MF or the type of Spi^- mutations in the mouse liver (28). After treatment with an alkylating agent, *N*-nitrosobis(2-hydroxypropyl)amine (BHP), the Spi^- MF in the liver of $Parp\text{-}1^{-/-}$ mice increased 1.6-fold higher than that of $Parp\text{-}1^{+/+}$ mice. In contrast, the *gpt* MFs in the liver of $Parp\text{-}1^{-/-}$ and $Parp\text{-}1^{+/+}$ mice after BHP treatment were both elevated and there was no significant difference between these genotypes (32).

Remarks

Transgenic mutation assays can evaluate mutagenesis *in vivo* in a broad range of tissues using neutral reporter genes that are integrated into the genome of the animal model. Many agents have been assayed using this approach over the past several decades (14). The data were weighted by strong mutagens. In these cases, shorter treatment time such as a single administration was sufficient to detect mutagenic effects. However, the majority of mutagenic chemicals in the environment are likely to be weak mutagens. The International Workshop on Genotoxicity Testing (IWGT) provided recommendations for the assays, treatment protocols and post-treatment sampling procedures for regulatory assessment of safety (2,33). Based on observations that mutations accumulate with each treatment, a repeated-dose regimen for a period of 28 days is strongly encouraged, with sampling at 3 days following the final treatment. Because this treatment protocol is commonly used in toxicological testing, transgenic rodents may be used for 28-day repeat dose toxicity assays for analysis of both genotoxicity and pathological alterations. However, it has not conclusively been determined if the 28-day treatment period is sufficient to detect a significant increase of MF of weak mutagens. Also uncertain is the effect of daily compared to weekly administrations on the MF, and whether 3-day sampling time after repeated administrations is sufficient in both slowly and rapidly dividing tissues. Published reports in which a 28 consecutive daily treatment protocol was applied are limited. It will be particularly important to evaluate mutagenesis using this protocol in different tissues, particularly for weak mutagens. Flexibility of route of administration and tissues for analysis is an advantage of *in vivo* transgenic rodent assays. Researchers can select the most appropriate mode of administration based on absorption, distribution and metabolism. Humans are chronically exposed to most environmental chemicals at low doses. Long-term exposure of a low dose is an important approach to investigate the observed threshold effects or adaptive responses. In addition, humans are exposed to a variety of chemical and physical agents; these factors may interact and the action of one agent may be influenced by exposure to another agent. The risk from combined exposure to more than one agent could be more complicated, and worth further investigation.

To analyze the various types of *in vivo* mutations, *gpt* delta transgenic rodents were established to detect deletions as well as point mutations. Because most carcinogenesis studies are undertaken in rats rather than mice, the *gpt* delta rat could be useful to investigate the mechanisms of carcinogenesis in target organs. The mouse model is useful to investigate how specific genes function in these processes, as specific gene knockout mice can be crossed and studied. Understanding how the specific molecular alterations induced by chemicals in the various tissues will be helpful for elucidating the molecular mechanisms underlying environmental mutagenesis and carcinogenesis.

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