rasH2 Transgenic Mice

Expression of the human c-Ha-ras oncogene provides a mouse model for sensitive and rapid carcinogenicity testing and investigating the role of ras oncogenes in tumorigenesis

rasH2 Transgenic Mouse Model Applications (Taconic model 001178)

The rasH2 Mouse Model carries the human c-Ha-*ras* oncogene in addition to the endogenous murine Ha-*ras* oncogene. The gene, recognized in both species as capable of transforming normal cells into a neoplastic phenotype following its mutation,[1] confers an unusually high susceptibility to tumor formation in rasH2 Transgenic Mice. Applications of the model include:

- Rapid, cost-effective, and sensitive transgenic mouse alternative to the traditional 2-year mouse bioassay for regulatory carcinogenicity testing and medical device testing
- Evaluating the roles of promoters in oncogene-initiated carcinogenesis
- Evaluating concordance in outcome of chemical mutagenicity testing between the *Salmonella* assay and *in vivo* carcinogenicity tests
- Determining the impact of point mutations in the Ha-ras oncogene on tumorigenesis
- Determining relative roles of the *ras* oncogene family in tumor formation
- Investigating molecular mechanisms of hemangiosarcoma generation
- Characterizing lung tumor formation
- Discovery of the role of the Ha-*ras* gene product, p21, in neoplasia
- Evaluating and designing tumor prevention or regression therapies

Features of the rasH2 Transgenic Mouse

- Transgenic mice are hemizygous for the human c-Ha-*ras* oncogene; wildtype (nontransgenic)** littermates serve as controls
- All mice retain the endogenous murine Ha-*ras* oncogene[2]
- Transgenic mice have normal organ/body weight ratios and blood chemistry/ hematology[3]
- Slightly smaller body weights (80% and 90% of non-transgenic male and female mice, respectively)[3]
- No indication of pre-neoplastic cell stages or tumors at 6 months of age[2]
- Total *ras*-encoded p21 proteins expressed at 2-3 times normal levels in all tissues[2]
- Rapid (within 6 months) and statistically significantly elevated incidence of tumor formation with exposure to many mutagenic (and non-mutagenic) chemicals (by *Salmonella* assay) compared to vehicle-treated transgenic mice[4,5]
- Significantly higher tumor formation rates on chemical exposures than non-transgenic controls[4]
- Generally non-responsive to currently tested non-carcinogens (by *Salmonella* assay)[4]
- Minimal spontaneous hepatocellular adenomas, forestomach squamous cell papilloma, malignant lymphomas in the hematopoietic system, splenic hemangio-sarcoma, squamous papillomas in the skin, lung adenomas and harderian gland adenomas, develop during life stage applicable to carcinogenicity testing (first 35 weeks)[5]
- Incidences of spontaneous tumor formation in older mice (up to 18 months) are low except for lung adenomas (5-7%), hemangiomas of the spleen (4-8%), and forestomach papillomas (1-3%)[7]
- Point mutations in the transgenic Ha-*ras* gene are present in some, but not all spontaneous[2] and chemically-induced[8, 9] tumors
- Elevated levels of transgene expression were detected in all genotoxin-induced tumors in the

* CIEA designates littermates of rasH2 hemizygous mice (001178-T) as nontransgenic or non-Tg. Taconic refers to rasH2 littermates without the transgene as wild type (001178-W). CIEA designates wild type to mean the F1 hybrid of the BALB/cByJ female x the C57BL/6J male. Taconic refers to these mice as F1 hybrid mice.

rasH2, suggesting that the overexpression of transgenic c-Ha-ras is responsible for accelerated tumor development[10, 11]

- No point mutations in the transgenic Ha-*ras* gene are found in normal (non-neoplastic) tissues of transgenic mice.[2]

Results of 26-week ILSI HESI ACT studies in the rasH2 mice[1]

Chemicals Tested		Tumor Response
Human Carcinogens		
	Phenacetin	+
	Cyclophosphamide	+
	Melphalan	+/-
Immunosuppressive Human Carcinogens		
	Cyclosporin A	+/-
Human Hormone Carcinogens		
	Diethlstibestrol	+
	17-estradiol	-
Non-genotoxic rodent-only Carcinogens		
Based on epidemiology	Clofibrate	+$
	Phenobarbital	-
	Reserpine	-
	Dieldrin	-
	Methapyrilenee	-
Baased on mechanism	Haloperidol	-
	Chlorpromazine	-
	Metaproterenol	-
	Wy-14643	+$
	DEHP	+$
	Sulfamethoxazole	-
Non-genotoxic Non-carcinogens		
	Sulfisoxazole	-
	Mannitol	-
	Ampicillin	-

Susceptibilities of rasH2 mice for mutagenic or non-mutagenic carcinogens[2]

Chemicals Tested #		Tumor Response
Mutagenic Carcinogens		
	p-Cresidine	+
	Cyclophosphamide	+
	DEN	+
	1,2 Dimethylhydrazine	+
	4HAQO	+
	MNNG	+
	MNU	+
	4NQO	+
	NNK	+
	Phenacetin	+
	4,4'-Thiodianiline	+
	Thiotepa	+
	Vinyl carbamate	+
	4-Vinyl-l-cylohexene diepoxide	+
Non-mutagenic Carcinogens		
	Benzene	+
	Ethyl acrylate	+
	Ethylene thioureau	+
	1,1,2-Trichloroethane	-
Mutagenic Non-carcinogens		
	p-Anisidine	-
	8-Hydroxyquinoline	-
	4-Nitro-o-phenylenediamine	+/-
Non-mutagenic Non-carcinogens		
	Resorcinol	-
	Rotenone	-

+: Positive, +/-: Equivocal, -: Negative

$: The three peroxisome proliferators showed positive results in inducing hepatocellular adenoma in male rasH2 mice with no detectable mutations in the ras transgene.

#: Mutagenic status determined by Salmonella assay; carcinogenicity status by trans-species rodent bioassay.

1) Usui, T. et al., (2001) CB6F1-rasH2 mouse: overview of available data. *Toxicologic Pathology*, Vol. 29, Suppl: 90-108.
2) Yamamoto, S., et al., (1998) Validation of Transgenic Mice Carrying the Human Prototype c-Ha-ras gene as a Bioassay Model for Rapid Carcinogenicity Testing. *Environmental Health Perspectives*, Vol. 106, Suppl 1:57-69.

Scientific Profile of the rasH2 Transgenic Mouse Model

Sensitivity and reproducibility of tumorigenesis. The rasH2 model has been tested in the International Life Science Institute Health and Environmental Science Institute (ILSI/HESI) Alternative Carcinogenicity Testing (ACT) project. Results demonstrate rasH2 mice are sensitive to both genotoxic and non-genotoxic human carcinogens and show no response to non-carcinogens.

Regulatory acceptance in US, EU and Japan The rasH2 mouse has been exemplified as a transgenic mouse model for alternative short-term carcinogenicity testing in guidelines set by The International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) (Topics S1B). Based on the results of the ILSI/HESI international collaborative research project, regulatory agencies in Japan, USA and Europe have recognized the utility of the rasH2 transgenic mouse model for human carcinogenicity risk assessment. Worldwide regulatory agencies have approved carcinogenicity protocols using alternative transgenic models, including the $p53^{+/-}$, TG.AC and rasH2 models.

National Toxicology Program (NTP) strategy NTP has verified that the accuracy in the NTP program for evaluation of carcinogens could be improved by combining "alternative short-term carcinogenicity using genetically modified animals" with the "2-year rat carcinogenicity bioassay" in comparison with the conventional long-term bioassay in rodents.

Dozens of mutagenic and non-mutagenic carcinogens have been tested in 6-month protocols using rasH2 Transgenic Mice.[3-6, 9, 12] The transgenic mice develop malignant tumors at rates that are statistically significantly greater than controls and non-transgenic littermates after exposure to many known carcinogens. These carcinogens include those that are both genotoxic and non-genotoxic human carcinogens. In these studies, rasH2 Transgenic Mice were non-responsive to the half-dozen non-carcinogens tested to date.[4, 5]

The rasH2 model is ideal for carcinogenicity testing of medical devices and implants. Standard 2 year rodent bioassays may be confounded by the tendency of mice and rats to develop solid-state tumors such as sarcomas by 11-24 months.[15] Whereas $p53^{+/-}$ mice have been shown to develop sarcomas an implantation sites,[16] rasH2 mice developed no inflammatory or neoplastic changes at the site of IC tag implantation in a 26 week short term carcinogenicity study.[17] The rasH2 model may thus be used for 26 week carcinogenicity testing of medical devices and implants without the solid-state tumor response.

There is little spontaneous tumor formation up to 35 weeks of age in these mice.[2, 5, 6] In older mice (over 18 months), spontaneous tumor formation remains low, with the exception of some tissue-specific tumors.[7] Most common are lung adenomas (5-7%), hemangiomas (especially of the spleen: 4-8%), and forestomach papillomas (1-3%). Protocols for carcinogenicity testing therefore can be structured to utilize young mice, whereas investigative or drug-discovery studies focusing on innate neoplastic transformation can utilize mice at all ages.

In humans, point mutations of the endogenous Ha-*ras* oncogene, especially at codons 12 and/or 61, are found in several tumors, and such mutations can transform cells *in vitro*.[1] Thus far, few studies have evaluated the incidence of mutations in Ha-*ras* genes in tumors of rasH2 Transgenic Mice, but this is an important line of investigation in understanding the complex mechanisms of tissue-specific tumorigenesis.

Current data indicate that point mutations in the transgene are only partly responsible for high rates of tumorigenesis in rasH2 Transgenic Mice. For example, spontaneous liver tumors (occurring in about 6% of mice) had point mutations at codon 61 in either the endogenous or the transgenic gene, depending on the tumor type, although some tumors had no

mutations at this locus.[8] In another study, lung tumors generated by vinyl carbamate rarely had mutations in the transgene (endogenous Ha-*ras* was not evaluated).[9, 13] And in the F2 progeny of the original founder, Ha-*ras* transgenic mouse,[2] of which about 50% developed spontaneous tumors, some tumors contained a transgene point mutation at codon 61 (e.g., all angiosarcomas and some lung adenocarcinomas) or codon 12 (some skin papillomas), while other tumors had no mutations at these typically mutated loci. Together these data suggest that high susceptibility to tumorigenesis in transgenic mice results in part from yet unidentified mechanisms, such as loss of heterozygosity.[4]

In many other aspects of their physiology, rasH2 Transgenic Mice appear normal. They have normal organ/body weight ratios and blood chemistry/hematology[3], although they are slightly smaller (body weights of 80% and 90% compared to non-transgenic males and females, respectively).[3] Mutational analyses of several tissues in rasH2 Transgenic Mice showed no point mutations of the transgene in normal (non-neoplastic) tissue[2], although total *ras*-encoded p21 proteins were expressed at 2-3 times normal levels in all tissues.[2]

Origin of the Model

The rasH2 Transgenic Mouse Model was developed by Dr. Tatsuji Nomura and colleagues of the Central Institute for Experimental Animals (CIEA) in Kawasaki, Japan.[2] Transgenic founder mice were created by injection into zygotes (from C57BL/6 x DBA/2J crosses) of a c-Ha-*ras* gene, which later was found to have become integrated into the murine transgenic genome as 3 copies in tandem array. These mice previously have been referred to in the literature as $CB6F_1$ TgHras2 mice.[14]

The injected transgene consisted of a prototype human c-Ha-*ras* sequence containing a human promoter region. This transgene was a hybrid construct, created by ligation of portions of two activated human c-Ha-*ras* genes, each of which were isolated from tumors (malignant melanoma and bladder carcinoma). But the hybrid transgene was constructed such that the portion contributed by each tumor gene lacked the mutated region. Thus, the ligated, human hybrid c-Ha-*ras* sequence contained no mutations at position 12 or 61. However, it retained a point mutation within the last intron, from one of the tumor genes, which conferred a high level of expression. The transgene was not able to transform mouse NIH3T3 cells, which is a standard bioassay for transformational activity of the Ha-*ras* ongogene.[14]

Male transgenic founder mice were backcrossed to C57BL/6J females, and offspring inherited the transgene in expected Mendelian fashion.[2] Studies of spontaneous tumor formation were conducted in the original F1 and F2 mice[2], but animals used for published carcinogenicity studies have been generated by crossing male transgenic mice with BALB/cByJ females to generate F1 transgenic offspring (confirmed by polymerase chain reaction or Southern blot analyses).[3-6, 14]

Taconic rasH2 Transgenic Mice available for end user studies are hemizygous progeny of hemizygous transgenic males (from a C57BL/J-based rasH2 expansion colony) crossed with non-transgenic (inbred) BALB/cByJ females. The wild type (non-transgenic) mice produced as littermates to the rasH2 hemizygous mice are the recommended experimental control animals.

Ready for Your Experiments

Taconic's Transgenic Models are produced in Isolator Barrier Unit (IBU™) facilities. Mice are shipped in Taconic Transport Cages (TTC ™) and come with an up-to-date health report documenting their Murine Pathogen Free (MPF™) health status. Barrier housing conditions are recommended for maintenance of rasH2 Transgenic Mice.

Related Mouse Models from Taconic

Taconic provides a number of mouse models relevant to carcinogenicity testing, toxicology, and cancer research. Call or fax for information about these additional models:

- **K6-ODC Microinjected Mouse (models 000993 and 003000)** – over-expressing

ornithine decarboxylase (ODC) in epidermal root sheath keratinocytes, with resulting skin abnormalities and epidermal papilloma formation after exposure to initiators only (promoters not required); allows for dramatic reduction in time, expenditure, and animals required for effective carcinogenicity testing

- **v-Ha-ras (TG.AC) OncoMouse™ Micro-injected Mouse (model TGAC)** – carrying an activated v-Ha-*ras* oncogene fused to a murine zeta-globulin promoter, conferring high sensitivity to tumor promotion through a v-Ha-*ras* mechanism; allows for dramatic reduction in time, expenditure, and animals required for effective carcinogenicity testing
- **TSG-p53® Targeted Mutation Mouse (models P53N4, P53N5 and P53N12) –** carrying one or two nonfunctional copies of the well-established tumor suppresser and pro-apoptotic *p53* gene, leaving mice highly susceptible to tumorigenesis; allows for dramatic reduction in time, expenditure, and animals required for effective carcinogenicity testing
- **TSG-p53®/Big Blue® Mouse (model P53BB)** – heterozygous for the *p53* gene and carrying the recoverable Big Blue (*LacI*) transgene, useful for simultaneous short-term carcinogenesis and mutagenesis testing in target and distal tissues

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* Did not use RasH2 mice in protocol

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