

Concise Review

rasH2 mouse: reproducibility and stability of carcinogenicity due to a standardized production and monitoring system

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Abstract: The rasH2 mouse was developed as a model for carcinogenicity studies in regulatory science. Its phenotype is stable during high-volume production and over successive generations. To produce rasH2 mice, three strains of mice (C57BL/6J-TgrasH2, C57BL/6J, and BALB/cByJ) were maintained individually. Since the homozygous *c-HRAS* genotype is lethal, hemizygous transgenic mice were maintained by crossing with inbred C57BL/6J mice. After breeding, male B6-transgenic mice were mated with female BALB/cByJ mice to obtain transgenic mice. Pups that were rasH2-Tg (tg/wt) or rasH2-Wt (wt/wt) were confirmed by genotyping. Frozen embryos were preserved by the Central Institute for Experimental Animals (CIEA) and sent to two facilities, CLEA Japan and Taconic Biosciences, where the mice were produced. Production colonies are created in both facilities and supplied to customers worldwide. To prevent genetic drift, the colonies were renewed for up to 10 generations, and renewals were carried out four times every five years from 2005 to 2021. To ensure the uniformity and maintenance of the phenotype of rasH2 mice, the carcinogen susceptibilities were monitored in every renewal of colonies by CIEA based on a standard protocol of the short-term carcinogenicity study using the positive control compound N-methyl-N-nitrosourea (MNU). Furthermore, simple carcinogenicity monitoring targeting the forestomach, the organ most sensitive to MNU, was performed approximately once a year. Based on the optimally designed production and monitoring systems, the quality of rasH2 mice with reproducibility and stability of carcinogenicity is maintained and supplied globally. (DOI: 10.1293/tox.2021-0068; J Toxicol Pathol 2022; 35: 19–24)

Key words: rasH2 mouse, carcinogenicity, short-term study, production system, monitoring system

In 1990, the rasH2 mouse was developed by the Central Institute for Experimental Animals (CIEA) in collaboration with Tokai University and the National Cancer Center Research Institute¹. It is a hemizygous transgenic mouse carrying the human protooncogene *c-HRAS*¹ that has been used in a 26-week short-term carcinogenicity study².

A short-term carcinogenicity study using genetically modified mouse strains, including rasH2, was considered as an alternative to the conventional two-year mouse carcinogenicity study according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) S1B guidance “Testing for carcinogenicity of pharmaceuticals” issued in 1997. To agree on this guidance, a program for alternatives to carcinogenicity testing by the International Life Sciences Institute (ILSI)/Health and Environmental Sciences Institute (HESI) was

conducted as a global research collaboration^{3, 4}. Five genetically modified mouse strains, namely rasH2, p53+/-, Tg.AC, xpa-/-, and xpa-/-/p53+/-, were evaluated for 21 known carcinogenic or noncarcinogenic compounds in this program. These data were generalized in the international workshop in 2003, and the American, European, and Japanese regulatory agencies approved the use of genetically modified mouse strains for regulatory applications^{5, 6}. The value of rasH2 and p53+/- mice was recognized through subsequent applications and verifications^{6, 7}, and the number of short-term carcinogenicity studies using rasH2 mice has been increasing since 2010⁸.

In the development of laboratory animals for regulatory science, the creation of a strategy that will allow for high-volume production of mice with the same phenotype in many individuals over many generations, is important. With this in mind, we developed rasH2 mice and constructed optimally designed production systems to prevent genetic drift. In this review, we describe the development process for rasH2 mice and the production and monitoring systems for maintaining the quality of rasH2 mice for reproducibility and stability of carcinogenicity.

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Development of rasH2 mouse

The rasH2 mouse is genetically engineered with *c-HRAS* gene insertion. The transgene was constructed by ligating the normal restriction fragments of human activated *c-HRAS* oncogenes associated with urinary bladder carcinoma and melanoma with a single-point mutation at codons 12 and 61, respectively, to create a normal (protooncogene) *c-HRAS*^{4,9,10}. It contains a mutation in the last intron that induces high enhancer activity¹¹. The transgene is a prototype DNA genome that includes its own promoter and enhancer regions^{1,12}.

The rasH2 founder mouse was produced by integrating the transgene in F1 embryos of the DBA/2 and C57BL/6 mouse strains. The founder mouse was backcrossed with the C57BL/6J strain, and mass production was started when the backcross was 99.5% homozygous^{9,10}.

The target specificity of tumor development is mouse strain dependent. Therefore, to detect multi-tissue susceptibility in human carcinogenicity risk assessments, the rasH2 mouse was mated with another inbred strain to produce F1 hybrids that achieved specific carcinogen susceptibilities for each strain in the final stage of the production system. The BALB/cByJ strain, in which epithelial tumors are relatively highly induced and females have large litters and superior nursing abilities, was selected to produce the F1 hybrid of the rasH2 mouse. Through crossbreeding, rasH2 mice achieved wide-ranging carcinogen susceptibility^{13–16}.

Production and global supply systems of rasH2 mice

A flowchart of the rasH2 mouse production system is shown in Fig. 1. Three strains of mice, C57BL/6J-TgrasH2, C57BL/6J, and BALB/cByJ, were maintained, and the embryos were preserved under frozen conditions. As a foundation, living mice were recovered from cryopreserved embryos. Next, male C57BL/6J-TgrasH2 mice were mated with female C57BL/6J mice to obtain B6-transgenic mice for the expansion colony as the homozygous *c-HRAS* genotype is lethal¹. After breeding in the expansion colony, rasH2 [CBYB6F1-Tg(HRAS)2Jic] mice, which were used for short-term carcinogenicity studies, were produced. Male C57BL/6J-TgrasH2 (tg/wt) mice were mated with female BALB/cByJ mice to obtain transgenic mice with a CB6F1 background in the production colony. Pups that were heterozygous rasH2-Tg (tg/wt) or rasH2-Wt (wt/wt) were confirmed by individual genotyping of the tail tips. To prevent genetic drift, expansion colonies are renewed for up to 10 generations.

The rasH2 mice are produced in two facilities and are supplied globally (Fig. 2). CIEA preserves frozen embryos to supply the original rasH2 mice, and cryopreserved embryos are sent to CLEA Japan (Fuji, Shizuoka, Japan) and Taconic Biosciences (Germantown, NY, USA) to establish breeding colonies. Colonies are formed in both companies, and the rasH2 mice are supplied to customers in Asia by

CLEA Japan and in the USA and Europe by Taconic Biosciences.

Quality control of the rasH2 mice for reproducibility and stability of carcinogenicity

The renewal of breeding colonies and the phenotypic monitoring system of rasH2 mice are shown in Fig. 3. To prevent the appearance of substrains in international production, the breeding colonies in both facilities were renewed every 10 generations with living hemizygous male rasH2 mice restored from the original embryos cryopreserved at the CIEA. Until 2021, the expansion and production of colonies was renewed every five years and this has been carried out four times (Fig. 3B).

The main phenotypic characteristic of rasH2 mice was carcinogen susceptibility. During colony renewal, the carcinogen susceptibilities of the colonies in CLEA Japan and Taconic Biosciences were subjected to full-volume carcinogenicity monitoring at the CIEA using standard criteria with N-methyl-N-nitrosourea (MNU) as the positive control (Fig. 3A). Furthermore, simple carcinogenicity monitoring targeting the forestomach, the organ most sensitive to MNU, was performed approximately once a year (Fig. 3A). The treatment in simple carcinogenicity monitoring is the same as that for full-volume carcinogenicity monitoring.

Full-volume carcinogenicity monitoring was carried out based on a standard protocol of the short-term carcinogenicity study¹⁷. RasH2 mice were divided into two groups. In one group, the mice were given a single intraperitoneal injection of MNU at a dose of 75 mg/kg, while in the other group, the mice were treated with an equal volume of vehicle (citrate buffer, pH 4.5) and served as controls. All mice were sacrificed 26 weeks post-injection. The organs/tissues specified in the standard protocol were removed from each mouse and evaluated histopathologically using hematoxylin and eosin staining.

Full-volume carcinogenicity monitoring of rasH2 mice produced by CLEA Japan and Taconic Biosciences was performed three times: in 2006, 2012, and 2018. The incidence of major neoplastic lesions in rasH2 mice from both facilities is shown in Tables 1 and 2. The most common neoplastic lesions induced by MNU were forestomach neoplasms originating from stratified squamous epithelium. In the forestomach, nodular lesions were macroscopically and histologically observed as squamous cell papilloma or carcinoma (Fig. 4, Tables 1 and 2). Other common MNU-induced neoplastic lesions were malignant lymphoma mainly composed of thymic lymphoma in the hematopoietic system, bronchiolo-alveolar adenoma, or carcinoma in the lungs, and squamous cell papilloma, carcinoma, and keratoacanthoma on the skin (Fig 5, Tables 1 and 2). These neoplastic lesions were frequently observed in rasH2 mice produced by CLEA Japan and Taconic Biosciences throughout the validation period (Tables 1 and 2). In addition, we are currently conducting a fourth full-volume monitoring of renewal colonies that started breeding in 2020.

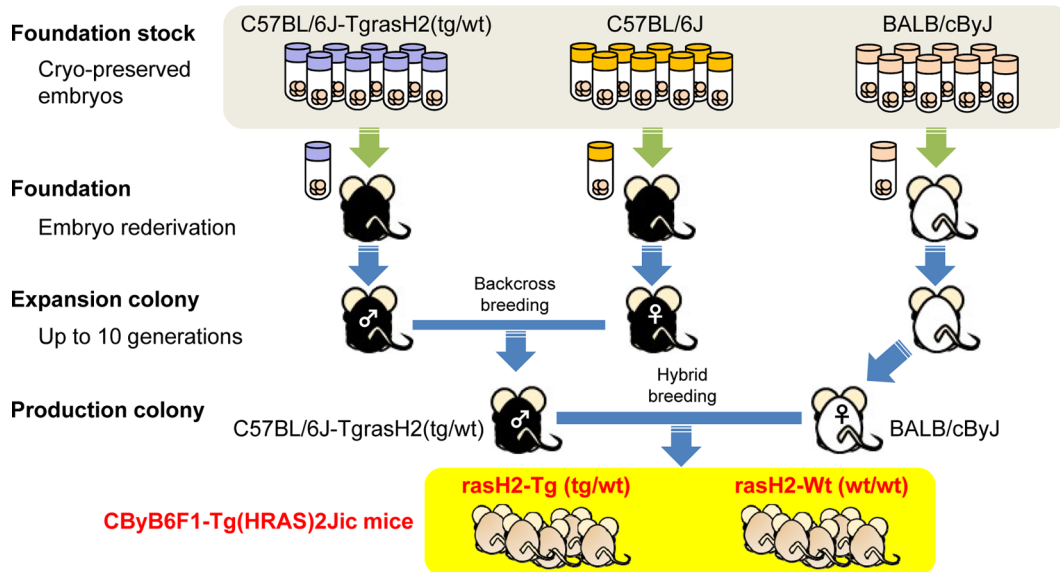


Fig. 1. Production system of rasH2 mice.

rasH2 mice are produced in three steps. Mouse embryos of three strains (C57BL/6J-TgrasH2, C57BL/6J, and BALB/cByJ) are preserved under frozen conditions. Hemizygous transgenic mice are crossed with inbred C57BL/6J mice. After breeding in the expansion colony, male B6-transgenic mice are mated with female BALB/cByJ mice to obtain transgenic mice with a CB6F1 background in the production colony. Pups that are heterozygous rasH2-Tg (tg/wt) or rasH2-Wt (wt/wt) are confirmed by individual genotyping of the tail tips. To prevent genetic drift, expansion colonies are renewed for up to 10 generations.

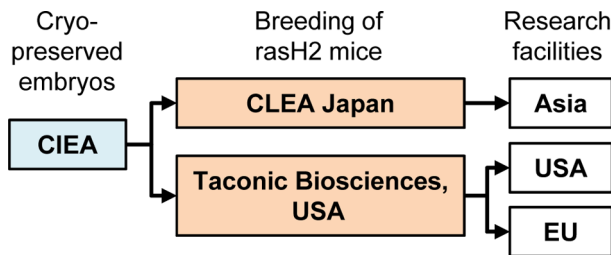


Fig. 2. Global supply system of rasH2 mice in 2021.

Cryopreserved embryos of rasH2 mice are preserved by CIEA and sent to CLEA Japan and Taconic Biosciences. The breeding colonies of rasH2 mice are created in both facilities, and rasH2 mice are supplied globally. To prevent the appearance of substrains in international production, breeding colonies are renewed every 10 generations.

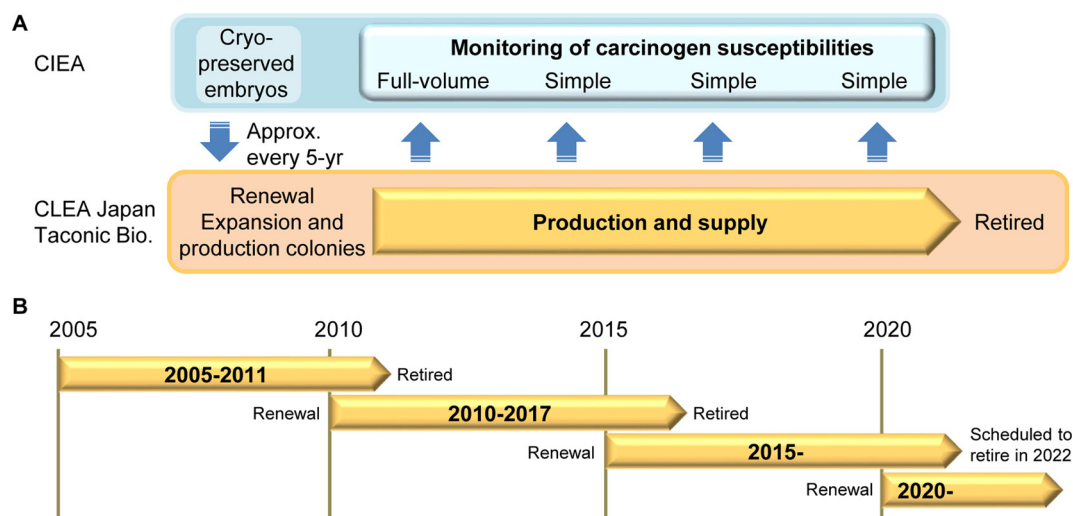


Fig. 3. Renewal of breeding colonies and phenotypic monitoring system for rasH2 mice.

A: To ensure the uniformity and maintenance of the phenotype of rasH2 mice, the carcinogen susceptibilities of both colonies in CLEA Japan and Taconic Biosciences are monitored at the CIEA using standard criteria. In addition, simple carcinogenicity monitoring targeting the forestomach, the organ most sensitive to N-methyl-N-nitrosourea (MNU), is performed approximately once a year.

B: The expansion and production colonies have been renewed four times every five years from 2005 to 2021.

Table 1. Incidence of Major Neoplastic Lesions in the Male rasH2 Mice Produced by CLEA Japan and Taconic Biosciences

Group	Vehicle		MNU										
	Year		2012		2018		2006		2012		2018		
	Breeder	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic
		No. of animals examined	15	15	15	15	15	14	15	15	15	15	15
Forestomach													
Papilloma, squamous cell	0	0	0	0	1 (7)	0	15 (100)	15 (100)	12 (80)	11 (73)	15 (100)	15 (100)	
Carcinoma, squamous cell	0	0	0	0	0	0	1 (7)	2 (13)	2 (13)	1 (7)	2 (13)	1 (7)	
Hematopoietic system													
Malignant lymphoma	0	0	0	0	0	0	14 (93)	14 (93)	12 (80)	14 (93)	14 (93)	11 (73)	
Lung													
Adenoma, bronchiolo-alveolar	1 (7)	0	2 (13)	1 (7)	1 (7)	0	1 (7)	3 (20)	6 (40)	0	5 (33)	2 (13)	
Carcinoma, bronchiolo-alveolar	0	0	0	0	0	0	0	0	1 (7)	0	0	0	
Skin/subcutis													
Papilloma, squamous cell	0	3 (20)	0	0	0	1 (7)	11 (73)	11 (73)	9 (60)	7 (47)	15 (100)	15 (100)	
Carcinoma, squamous cell	0	0	0	0	0	0	0	0	0	0	0	0	
Keratoacanthoma	0	0	0	0	0	0	2 (13)	5 (33)	8 (54)	7 (47)	1 (7)	0	

Numbers indicate the number of cases (the numbers in the parentheses indicate the percentage to the total number of cases for each tumor type).

Table 2. Incidence of Major Neoplastic Lesions in the Female rasH2 Mice Produced by CLEA Japan and Taconic Biosciences

Group	Vehicle		MNU										
	Year		2012		2018		2006		2012		2018		
	Breeder	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic
		No. of animals examined	15	15	15	15	15	15	15	15	15	15	15
Forestomach													
Papilloma, squamous cell	1 (7)	0	0	0	1 (7)	0	13 (87)	14 (93)	13 (87)	11 (73)	14 (93)	15 (100)	
Carcinoma, squamous cell	0	0	0	0	0	0	2 (13)	4 (27)	0	0	2 (13)	0	
Hematopoietic system													
Malignant lymphoma	0	0	0	0	0	1 (7)	12 (80)	14 (93)	13 (87)	13 (87)	11 (73)	10 (67)	
Lung													
Adenoma, bronchiolo-alveolar	0	0	0	0	3 (20)	0	3 (20)	4 (27)	2 (13)	4 (27)	5 (33)	4 (27)	
Carcinoma, bronchiolo-alveolar	0	0	0	0	0	0	0	0	1 (7)	0	0	0	
Skin/subcutis													
Papilloma, squamous cell	0	0	0	0	1 (7)	0	8 (53)	9 (60)	3 (20)	3 (20)	12 (80)	10 (67)	
Carcinoma, squamous cell	0	0	0	0	0	0	1 (7)	1 (7)	1 (7)	1 (7)	0	1 (7)	
Keratoacanthoma	0	0	1 (7)	0	0	0	5 (33)	6 (40)	4 (27)	1 (7)	2 (13)	2 (13)	

Numerals indicate the number of cases (the numbers in parentheses indicate the percentage of the total number of cases for each tumor type).

Conclusion

The rasH2 mouse, which was developed for use as a model for carcinogenicity studies in regulatory science, exhibits a stable phenotype during high-volume production and over successive generations. RasH2 mice are produced by two facilities in Japan and the USA and are supplied globally. The quality of rasH2 mice in terms of reproducibility and stability of carcinogenicity is controlled through optimally designed production and monitoring systems.

Disclosure of Potential Conflicts of Interest: All authors are researchers at the CIEA.

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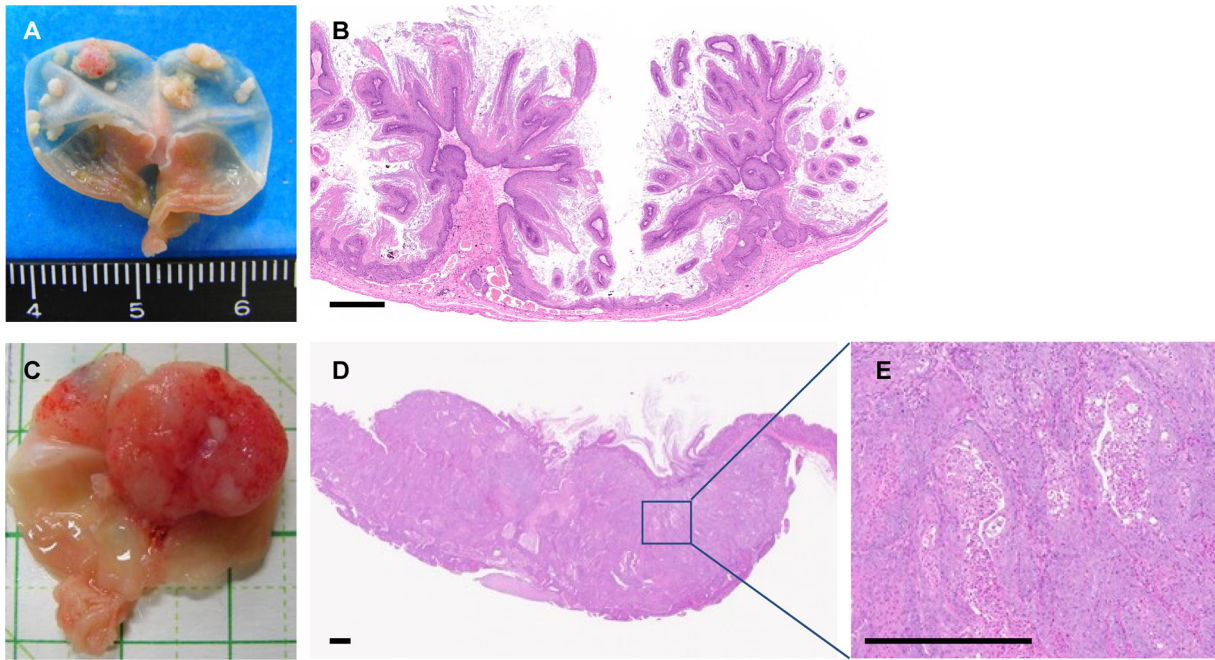


Fig. 4. Neoplastic lesions induced by N-methyl-N-nitrosourea (MNU) in the forestomach of rasH2 mice. White raised nodular lesions are macroscopically identified (A) and squamous cell papillomas are histologically observed (B). Large nodular lesion in the serosa is macroscopically (C) and histologically observed as squamous cell carcinoma (D, E). Scale bar=500 μ m.

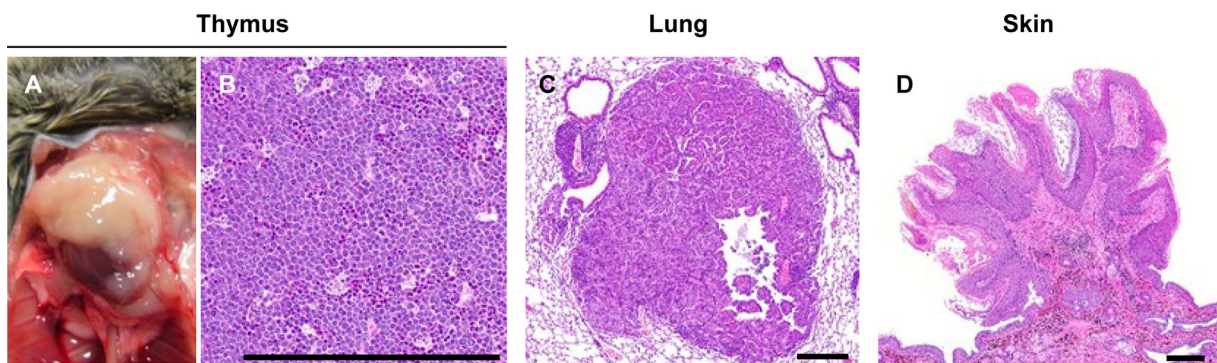


Fig. 5. MNU-induced neoplastic lesions in rasH2 mice. Malignant lymphoma with enlarged thymus (A, B), bronchiolo-alveolar adenoma in the lung (C), and papilloma in the skin (D), which are common neoplastic lesions induced by N-methyl-N-nitrosourea (MNU). Scale bar=250 μ m.

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