Carcinogenic susceptibility comparative study on rasH2 mice produced by two breeding facilities

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Background and purpose

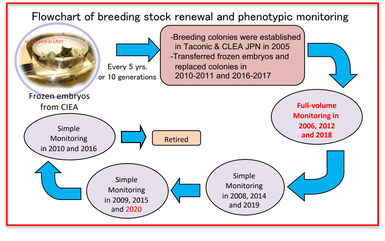
The CByB6F1-Tg(HRAS)2Jic mouse (rasH2 mouse) is a genetically engineered mouse containing the HRAS (c-Ha-ras) gene, developed by the Central Institute for Experimental Animals (CIEA).

The rasH2 mouse is produced at two breeding facilities, CLEA Japan, Inc. (Fuji, Shizuoka, Japan) and Taconic Bioscience (Germantown, NY, USA), and are supplied worldwide. The inserted gene is a prototype prepared by removing the point mutation sites from two HRAS (c-Ha-ras) genes obtained from human malignant melanoma and human bladder carcinoma tumors, followed by their recombination.

Animals are obtained by injection of the prototype gene into the pronucleus oocyte, and by backcrossing these animals more than 20 times with the C57BL/6J strain to match original strain.

F1 hybrid (F1 hybrid with the BALB/cByJ strain) , which display broader susceptibility than inbred lines, were used for the carcinogenicity studies, because it is ideal to detect carcinogenicity that many unspecified compound has to all organs.

The rasH2 mouse was produced by cross-breeding the BALB/cByJJic female and the C57BL/6JJic-Tg(HRAS)2Jic hemizygous male in order to prevent genetic drift. Both CLEA and Taconic colonies were renewed within 10 generations or 5 years from the introduction of a previous colony by rederivation from frozen embryos preserved in the CIEA.

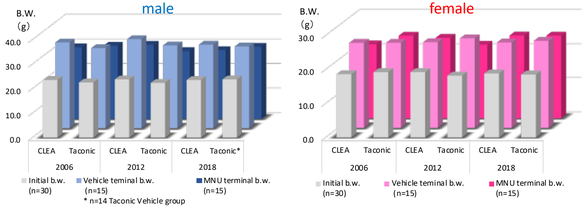


After the breeding colonies have been renewed, we regularly perform a carcinogenic susceptibility study to confirm the homogeneity of rasH2 mice produced by the two facilities. So far, we have renewed the breeding colonies twice since the colony established at the facilities.

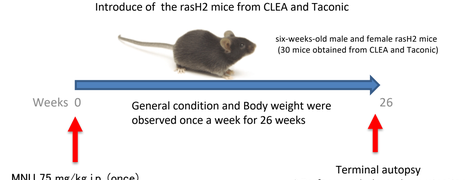
In this study, we investigated the carcinogenic susceptibility of the rasH2 mice derived from the breeding colonies replaced in 2016-2017. Therefore, we summarized the results this study and previous two studies (performed in 2006 and 2012).

Results

Table 1. Body weight of the Vehicle- and MNU-treated rasH2 mice in three studies



Study design



・When mice were identified as moribund during the study period, they were euthanized and almost all organs were investigated histopathologically.

Group composition

Supplier	Test compound	Sex	Number of animals	Observation period
CLEA Japan	Vehicle (citrate-buffered saline at pH 4.5)	Male	15	26 weeks
		Female	15	
	MNU (N-methyl-N-nitrosourea)	Male	15	
		Female	15	
Taconic Bioscience	Vehicle	Male	15	
		Female	15	
	MNU	Male	15	
		Female	15	

Table 2 Incidence(%) of proliferative lesions in the Vehicle- and MNU-treated male rasH2 mice in three studies.

Sex	male											
Year	2006		2012		2018		2006		2012		2018	
Breder	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic
Coompound	Vehicle						MNU					
No. of Animals examined	15	15	15	15	15	14	15	15	15	15	15	15
Forestomach												
Hyperplasia, squamous cell	0.0	0.0	0.0	0.0	0.0	0.0	40.0 (6)	66.7 (10)	6.7 (1)	20.0 (3)	20.0 (3)	20.0 (3)
Papilloma, squamous cell	0.0	0.0	0.0	0.0	6.7 (1)	0.0	100.0 (15)	100.0 (15)	80.0 (12)	73.3 (11)	100.0 (15)	100.0 (15)
Carcinoma, squamous cell	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)	13.3 (2)	13.3 (2)	6.7 (1)	13.3 (2)	6.7 (1)
Adenocarcinoma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)	0.0	0.0	0.0
Lung												
Hyperplasia, bronchiolo-alveolar	20.0 (3)	20.0 (3)	0.0	6.7 (1)	0.0	0.0	40.0 (6)	20.0 (3)	6.7 (1)	13.3 (2)	13.3 (2)	6.7 (1)
Adenoma, bronchiolo-alveolar	6.7 (1)	0.0	13.3 (2)	6.7 (1)	6.7 (1)	0.0	6.7 (1)	20.0 (3)	40.0 (6)	0.0	33.3 (5)	13.3 (2)
Methothelial hyperplasia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)
Carcinoma, bronchiolo-alveolar	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)	0.0	0.0	0.0
hemagioma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)	0.0	0.0	0.0
Hemolymphoreticular												
Granulocytic leukemia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)
Malignant lymphoma	0.0	0.0	0.0	0.0	0.0	0.0	93.3 (14)	93.3 (14)	80.0 (12)	93.3 (14)	93.3 (14)	73.3 (11)
Spleen												
Hemangioma	6.7 (1)	0.0	0.0	0.0	0.0	0.0	6.7 (1)	0.0	0.0	0.0	6.7 (1)	6.7 (1)
Hemangiosarcoma	0.0	0.0	0.0	6.7 (1)	6.7 (1)	7.1 (1)	0.0	0.0	13.3 (2)	6.7 (1)	0.0	0.0
Lymphocyte hyperplasia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Kidney												
Atypical tubule hyperplasia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)	0.0
Adenoma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)	6.7 (1)	0.0
Hemangioma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Skin/subcutis												
Hemangiosarcoma	0.0	0.0	0.0	0.0	6.7 (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hyperplasia, squamous cell	0.0	0.0	0.0	0.0	0.0	0.0	26.7 (4)	33.3 (5)	0.0	0.0	13.3 (2)	0.0
Papilloma, squamous cell	0.0	20.0 (3)	0.0	0.0	0.0	7.1 (1)	73.7 (11)	73.7 (11)	60.0 (9)	46.7 (7)	100.0 (15)	100.0 (15)
Carcinoma, squamous cell	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Keratoacanthoma	0.0	0.0	0.0	0.0	0.0	0.0	13.3 (2)	33.3 (5)	53.5 (8)	46.7 (7)	6.7 (1)	0.0
Melanoma	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)	0.0	0.0	0.0	0.0	0.0

N.E. : not examined Number in parentheses shows No. of animals showed the findings

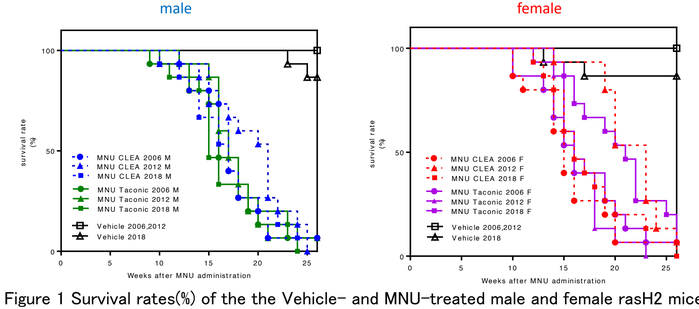


Figure 1 Survival rates(%) of the the Vehicle- and MNU-treated male and female rasH2 mice in three studies

Table 3 Incidence(%) of proliferative lesions in the Vehicle- and MNU-treated female rasH2 mice in three studies.

Sex	female											
Year	2006		2012		2018		2006		2012		2018	
Breder	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic	CLEA	Taconic
Coompound	Vehicle						MNU					
No. of Animals examined	15	15	15	15	15	15	15	15	15	15	15	15
Forestomach												
Hyperplasia, squamous cell	0.0	0.0	0.0	0.0	0.0	0.0	46.7 (7)	46.7 (7)	13.3 (2)	20.0 (3)	13.3 (2)	20.0 (3)
Papilloma, squamous cell	6.7 (1)	0.0	0.0	0.0	6.7	0.0	86.7 (13)	93.9 (14)	86.7 (13)	73.3 (11)	93.3 (14)	100.0 (15)
Carcinoma, squamous cell	0.0	0.0	0.0	0.0	0.0	0.0	13.3 (2)	26.7 (4)	0.0	0.0	13.3 (2)	0.0
Lung												
Hyperplasia, bronchiolo-alveolar	13.3 (2)	6.7 (1)	6.7 (1)	0.0	0.0	0.0	26.7 (4)	53.5 (8)	46.7 (7)	13.3 (2)	13.3 (2)	6.7 (1)
Adenoma, bronchiolo-alveolar	0.0	0.0	0.0	0.0	0.0	0.0	20.0 (3)	26.7 (4)	13.3 (2)	26.7 (4)	33.3 (5)	26.7 (4)
Methothelial hyperplasia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)	0.0	0.0	6.7 (1)
Carcinoma, bronchiolo-alveolar	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)	0.0	0.0	0.0
Hemolymphoreticular												
Histiocytic sarcoma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)	0.0
Malignant lymphoma	0.0	0.0	0.0	0.0	0.0	6.7 (1)*	80.0 (12)	93.3 (14)	86.7 (13)	86.7 (13)	73.3 (11)	66.7 (10)
Spleen												
Hemangioma	0.0	0.0	0.0	0.0	6.7 (1)	6.7 (1)	13.3 (2)	0.0	0.0	0.0	20.0 (3)	13.3 (2)
Hemangiosarcoma	0.0	0.0	0.0	6.7 (1)	0.0	6.7 (1)	0.0	0.0	13.3 (2)	20.0 (3)	0.0	0.0
Lymphocyte hyperplasia	0.0	0.0	0.0	0.0	6.7 (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Kidney												
Adenoma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)
Hemangioma	0.0	0.0	6.7 (1)	0.0	0.0	0.0	6.7 (1)	0.0	0.0	0.0	0.0	0.0
Skin/subcutis												
Hemangioma	0.0	0.0	0.0	0.0	0.0	6.7 (1)	0.0	0.0	0.0	0.0	0.0	0.0
Hemangiosarcoma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)
Hyperplasia, squamous cell	0.0	0.0	0.0	0.0	0.0	0.0	20.0 (3)	26.7 (4)	13.3 (2)	26.7 (4)	0.0	6.7 (1)
Papilloma, squamous cell	0.0	0.0	0.0	0.0	6.7 (1)	0.0	53.3 (8)	60.0 (9)	20 .0 (3)	20.0 (3)	80.0 (12)	66.7 (10)
Carcinoma, squamous cell	0.0	0.0	0.0	0.0	0.0	0.0	6.7 (1)	6.7 (1)	6.7 (1)	6.7 (1)	0.0	6.7 (1)
Keratoacanthoma	0.0	0.0	6.7 (1)	0.0	0.0	0.0	33.3 (5)	40.0 (6)	26.7 (4)	6.7 (1)	13.3 (2)	13.3 (2)

N.E. : not examined * : In jejunal Peyer's patch Number in parentheses shows No. of animals showed the findings

Summary

The average body weight of male and female rasH2 mice produced by CLEA and Taconic on arrival were similar to the previous two studies (2006, 2012). In the three studies (2006 ,2012 and 2018), the survival rates of the MNU and Vehicle group at week 26 were 0.0-13.3% and 86.6-100.0%, respectively.

In the MNU group, the incidence of forestomach squamous cell papilloma/carcinoma (major tumors in MNU-treated rasH2 mice) was 73.3.-100.0% in mice produced at both facilities. No significant difference in the incidence of forestomach tumors were found between the two facilities. And, the incidence of malignant lymphoma in mice produced at CLEA and Taconic was 73.3-93.3% and 66.7-93.3%, respectively. We also confirmed that incidences of major MNU-induced tumors in these three studies were similar to previous reports. (Takaoka *et ad.*, 2003)

Thus, carcinogenic conformity of rasH2 mice derived from the replaced breeding colonies in both facilities was confirmed in the present study. Furthermore, by comparing tumor incidences with those of previous similar studies, we found that the carcinogenic susceptibility of the rasH2 strain has been well-maintained for more than two decades.

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