CARCINOGENIC COMPARATIVE STUDY ON CB6F1 TG RASH2 MICE PRODUCED BY TWO BREEDING FACILITIES

K. Urano, K. Machida, M. Yoshimura, K. Kikuchi, T. Nomura and T. Usui *Central Institute for Experimental Animals, Kawasaki, Japan.*

CIEA SINCE 1952

Introduction:
High carcinogenic sensitivity and reproducibility for human carcinogens in CByB6F1-Tg(HRAS)2Jic mice (hereinafter rasH2 mice) have been validated by a large number of certification studies (Yamamoto *et al*., 1998; Usui *et al*., 2001). At present, this strain of mouse is produced by two breeding facilities, Taconic (Germantown, NY) and CLEA Japan, Inc. (Shizuoka, Japan), and supplied all over the world.
Although genetic and microbiological monitoring has been performed periodically to maintain a quality of breeding parents, and also all mice used for commercial purposes were checked to determine whether they have the transgene or not by PCR method, different results have sometimes been obtained due to differences in environmental factors such as temperature, microorganisms, diet, bedding and social order. To confirm phenotypic conformity of both mice produced at CLEA Japan,Inc. and Taconic, a 26-week carcinogenicity test was performed under the same protocol and environmental conditions in our facility.

Material & Method:
1.Animals
One hundred and twenty rasH2 mice from CLEA Japan Inc. and Taconic were divided into eight groups as shown in Table 1.
2.Treatment
Mice in vehicle group were injected with citrate buffer at pH 4.5 once intraperitoneally (10 ml/kg) and mice in MNU group were injected with MNU (N-methyl-N-nitrosourea, 75 mg/kg) once intraperitoneally.
3.Survival rate
Clinical observation was performed daily in all animals just after group formation to check for dead animals.
4. Pathological examination
All animals were necropsied at 26 weeks after administration, or at the time of death or emergency sacrifice. Liver, kidneys, spleen, heart, lungs, thymus and lymph nodes, stomach (forestomach and glandular stomach), bone marrow, skeletal muscle, skin, reproductive organs and sites of visual abnormalities were subjected to a histopathologic examination.
5. Statistical analysis
Differences in the incidences of each lesion between the two breeding facilities were tested by Fisher's exact test with $p < 0.05$ taken as significant.

Table 1 Group composition at present study

Vehicle				MNU			
CLEA Japan		Taconic		CLEA Japan		Taconic	
M	F	M	F	M	F	M	F
15	15	15	15	15	15	15	15

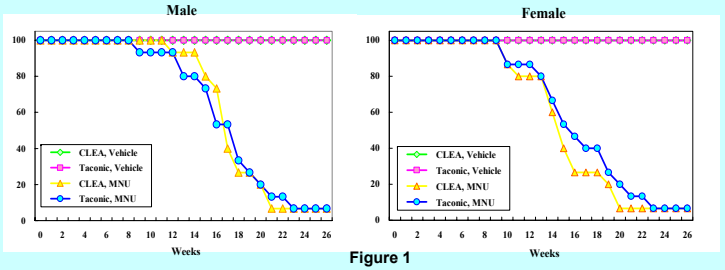

Figure 1

Table 2 Incidence of neoplastic changes in rasH2 mice produced by CLEA Japan and Taconic

Organs/Findings	Group	Vehicle				MNU			
	Breeder	CLEA Japan		Taconic		CLEA Japan		Taconic	
	Sex	M	F	M	F	M	F	M	F
	No. of mice	15	15	15	15	15	15	15	15
Lung									
Adenoma		1[a]	0	0	0	1	3	3	4
Liver									
Hemangioma		0	0	0	1	0	0	0	0
Squamous cell carcinoma metastasis		0	0	0	0	0	0	1	1
Kidneys									
Hemangioma		0	0	0	0	0	1	0	0
Hematopoietic system									
Malignant lymphoma		0	0	0	0	14	12	14	14
Spleen									
Hemangioma		1	0	0	0	1	2	0	0
Tymus									
Squamous cell carcinoma metastasis		0	0	0	0	0	0	1	1
Forestomach									
Papilloma		0	1	0	0	15	13	15	14
Squamous cell carcinoma		0	0	0	0	1	2	2	4
Glandular stomach									
Squamous cell carcinoma metastasis		0	0	0	0	0	0	1	2
Small Intestine									
Adenocarcinoma (jejunum)		0	0	0	0	1	0	0	1
Adenocarcinoma metaplasia (ileum)		0	0	0	0	0	0	0	1
Pancreas									
Squamous cell carcinoma metastasis		0	0	0	0	0	1	0	0
Adrenal glands									
Squamous cell carcinoma metastasis		0	0	0	0	0	1	0	0
Uterus									
Hemangioma		/	0	/	0	/	3	/	1
Vagina									
Papilloma		/	0	/	0	/	2	/	0
Squamous cell carcinoma metastasis		/	0	/	0	/	0	/	1
Skin (including nose, tail and auricle)									
Papilloma		0	0	3	0	11	8	11	9
Squamous cell carcinoma		0	0	0	0	0	1	0	1
Keratoacanthoma		0	0	0	0	2	5	5	6
Basal cell tumor		0	0	0	0	1	0	0	0
Melanoma		0	0	0	0	1	0	0	0
Oral cavity (including tongue and gum)									
Papilloma		/	/	/	/	7	2	1**	4
Thoracic cavity									
Papilloma (esophagus)		/	/	/	/	0	0	0	1
Squamous cell carcinoma metastasis (diaphragm)		/	/	/	/	0	1	1	0

/: not examined
a: No. of mice with finding
**: Significant difference at p<0.01 from CLEA Japan (Fisher's exact test)

Table 3 Incidence of non-neoplastic changes in rasH2 mice produced by CLEA Japan and Tacon

Organs/Findings	Group	Vehicle				MNU			
	Breeder	CLEA Japan		Taconic		CLEA Japan		Taconic	
	Sex	M	F	M	F	M	F	M	F
	No. of mice	15	15	15	15	15	15	15	15
Heart									
Thickening of serosa		0[a]	0	0	0	1	0	0	1
Lung									
Alveolar epithelial hyperplasia		3	2	3	1	6	4	3	8
Thickening of serosa		0	0	0	0	0	0	1	0
Liver									
Foci of cellular alteration		2	1	1	0	1	0	1	1
Focal necrosis		0	0	0	0	0	1	2	3
Extramedullary hematopoiesis		1	0	0	0	0	2	0	0
Hemosiderin deposition		0	0	0	0	1	1	0	0
Kidneys									
Hyaline cast		0	3	0	2	0	0	0	0
Fibrosis		0	0	0	0	0	1	0	0
Basophilic tubule		0	0	1	1	0	0	0	0
Cystic tubule		0	0	0	0	0	1	1	0
Spleen									
Melanin deposition		2	2	0	4	1	3	4	3
Extramedullary hematopoiesis		0	0	0	0	0	2	1	2
Thymus									
Hypoplasia		0	1	1	2	1	3	0	2
Bone marrow									
Increase of myeloid cells		0	0	0	0	0	2	1	2
Hypoplasia		0	0	0	0	0	3	0	1
Forestomach									
Squamous cell hyperplasia		0	0	0	0	6	7	10	7
Ulcer		0	0	0	0	1	0	7**	0
Glandular stomach									
Atypical hyperplasia		0	0	0	0	0	0	0	2
Small intestine									
Hyperplasia of lymphatic follicle (jejunum)		0	0	0	1	0	0	0	0
Skeletal muscle									
Myopathy		12	13	15	11	10	7	9	6
Uterus									
Endometrial hyperplasia		/	0	/	0	/	1	/	3
Vagina									
Squamous cell hyperplasia		/	0	/	0	/	1	/	1
Skin									
Squamous cell hyperplasia		0	0	0	0	4	3	5	4
Basal cell hyperplasia		0	0	0	0	1	0	0	2
Oral cavity (including tongue and gum)									
Squamous cell hyperplasia		/	/	/	/	3	1	4	7**

/: not examined
a: No. of mice with finding
**: Significant difference at p<0.01 from CLEA Japan (Fisher's exact test)

Result :
1. Survival rate (Figure 1)
The survival rate of the vehicle group was maintained at 100% for mice from both facilities at the completion of test. In the MNU group, MNU induced tumor death occurred from 9 to 12 weeks after administration, and the final survival rate for both facilities was 6.7%.

2.Pathological examination
Neoplastic changes (Table 2)
* In the vehicle group, only benign tumors in lungs, spleen, forestomach and skin were observed in a few mice in both facilities.
* In the MNU group, the incidence of forestomach papilloma in mice from CLEA or Taconic was 100 % (15/15) in males and 86.7% (13/15) in females, or 100 % (15/15) in males and 93.3 % (14/15) in females, respectively. The incidences of malignant lymphoma in both mice were 86.7 % (14/15 in males and 12/15 in females) and 93.3 % (14/15 in both sexes), and no significant difference in the incidence was observed. There was also no significant difference in the incidence of major MNU induced tumors in rasH2 mice such as lung adenoma and skin papilloma / keratoacanthoma. A significant difference in males for oral cavity papilloma was indicated due to all multi-site changes.

Non-neoplastic changes (Table 3)
* No significant difference was observed between the mice from both facilities in the vehicle group, although non-specific changes in lungs, liver, kidneys and thymus were observed in a few mice from both facilities.
* A high incidence of skeletal myopathy was observed in all groups, but there was no significant difference between mice from both facilities.
* Hyperplastic changes were found in forestomach, lungs and skin where major MNU induced tumors occurred, but there was no significant difference between mice from both facilities.
* Forestomach ulcer was considered a secondary change of papilloma /squamous cell carcinoma.
* A significant difference was observed in oral cavity papilloma in females due to all multi-site changes.

Comparison with historical data at CIEA

Table A Historical data of MNU induced tumor in rasH2 mice at CIEA

Organ		Tumor incidence (% n=45 M	F
forestomach	papilloma	93.2	97.7
	squamous cell carconoma	15.9	6.8
thymus	malignant lymphoma	88.4	81.8
skin	papilloma	68.9	53.3
spleen	malignant lymphoma	62.2	65.9
	hemangiosarcoma	8.9	2.3
born marrow	malignant lymphoma	58.1	53.3
lung	malignant lymphoma	75.6	68.9
	adenoma	15.6	6.7

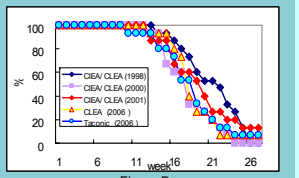

Figure B

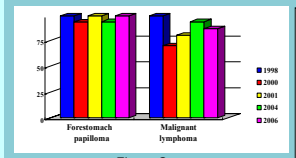

Figure C

Historical comparison :
In order to evaluate if the carcinogenic sensitivity of rasH2 mice has been maintained during last ten years, data of present study (described as 2006 in Figure B & C) were compared to those of historical data at CIEA.
Table A shows historical data of MNU induced tumor in rasH2 mice at CIEA.
Figure B shows survival rate of male rasH2 mice after MNU administration produced in 1998,2000 and 2001(including the data of present study).
Figure C shows tumor incidence, forestomach papilloma and malignant lymphoma were selected for representative, in rasH2 mice produced at CIEA/CLEA Japan after MNU administration.

These result indicate carcinogenic sensitivity of rasH2 mice has been well maintained since 1998.

Conclusion :
* Phenotypic conformity of rasH2 mice derived from Taconic and CLEA Japan, Inc. was confirmed by the present study.
* Carcinogenic sensitivity of rasH2 mice has been well maintained for last decade.

47th Annual Meeting of SOT(2008), Seattle, WA, USA