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Establishment of a Tcrb and Trp53 Genes Deficient Mouse Strain as an Animal Model for Spontaneous Colorectal Cancer

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Abstract: *A congenic C57BL/6JJcl-Tcrb^{tm1Mom}Trp53^{tm1} (Tcrb $^{-/-}$: Trp53 $^{-/-}$) mouse lacking T-cell receptor beta chain (TCRβ) and transformation related protein 53 (p53) has been established at the N8th generation of backcrossing male Tcrb $^{-/-}$:Trp53 $^{-/-}$ mice, which had been obtained by mating a Tcrb $^{-/-}$ mouse with a Trp53 $^{-/-}$ mouse, with female C57BL/6JJcl mice. In the mice deficient for the both genes, occurrence of tumor masses was observed mostly in the cecum with high frequency as examined at 3 months of age. The majority of the masses had histologic features of hyperplasia or dysplasia while occasional lesions were noted to be adenocarcinomas invading the submucosa (invasive adenocarcinoma). As examined at 4 months of age and thereafter, all mice had 4–5 colorectal tumors per animal, the lesions being located mainly in the cecum and, histopathologically, all the obvious neoplastic growths in the regions examined were invasive adenocarcinomas. The Tcrb and Trp53 genes deficient mouse strain which develops spontaneous colorectal carcinoma with fairly high frequency at early age would be useful as an animal model for colorectal cancer.*

Key words: *animal model, colorectal cancer, T-cell receptor beta chain (TCRβ), Transformation related protein 53 (p53)*

Introduction

Immune system failure of the intestinal mucosa has pathogenetic implication in the etiology of inflammatory bowel disease (IBD) represented by ulcerative colitis and Crohn's disease, wherein involvement of T cells has been documented [1, 2, 19].

T-cell receptor beta chain (TCRβ) is one of the molecules that constitute the T-cell receptor, and lack of this molecule results in homeostatic defects of the intestinal mucosal immune system, leading to inflammations in the intestinal tract. The *Tcrb* gene deficient (*Tcrb* $^{-/-}$) mouse which has been produced for the purpose of clarifying the mechanisms of T cell genesis and differentiation is known to serve as an animal model for IBD that presents with lesions analogous to those of ulcerative colitis in humans [14].

The *Trp53* gene is causative of Li-Fraumeni syn-

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drome (LFS) characterized by hereditary, frequent occurrence of diverse malignant neoplasms [10], and is also an anti-oncogene (tumor suppressor gene) of which mutations are frequently detected in nonhereditary malignant tumors [7]. The protein encoded by the *Trp53* gene functions to arrest the cell cycle in response to DNA damage or to induce apoptosis [8, 23]. The *Trp53* deficient (*Trp53* $^{-/-}$) mice produced to analyze the function of the *Trp53* gene have been reported to exhibit spontaneous development of malignant lymphomas of various organs and angiosarcoma with high incidences [5, 12].

Ulcerative colitis is recognized as a risk factor for the development of colorectal carcinoma [4, 21], and *Trp53* gene mutations have been demonstrated with high frequency at sites of such tumors [22, 24].

Preliminary observations confirmed that TCRβ:p53 deficient (*Tcrb* $^{-/-}$: *Trp53* $^{-/-}$) mice obtained by mating *Tcrb* $^{-/-}$ mice [13] with *Trp53* $^{-/-}$ mice [5] were very likely to develop spontaneous colorectal tumors besides thymoma.

Since the target mouse originates in a chimeric embryo which has been derived from strain 129 mouse Es cell and strain C57BL/6 mouse embryo, it is of importance to render such animals genetically homogeneous in order to explore the effects of the gene(s) introduced, or to develop an animal model for human disease.

The TCRβ:p53 deficient mice obtained were genetically heterogeneous. In our study to derive a genetically homogeneous laboratory animal strain, therefore, we attempted to breed a congenic strain via introduction of *Tcrb* and *Trp53* genes into the C57BL/6JJcl mouse. In addition, the TCRβ:p53 deficient mice of midway generations in the line breeding were examined for occurrence and distribution of colorectal tumors.

This report presents the results of the study demonstrating that the genetic background of the mutant mouse became replaced with C57BL/6JJcl through the successive line-breeding generations up to N8 and that histopathologic examination of N4 generation mice revealed occurrence of colorectal carcinomas with a fairly high frequency.

Materials and Methods

Animals:

Male TCRβ:p53 deficient mice were derived, by breeding, from a colony consisting of 3 males and 3 females carrying *Tcrb* and *Trp53* genes disrupted by the targeting mutation method which had been produced at and introduced from the laboratory of Dr. Tonegawa, Massachusetts Institute of Technology, Cambridge, MA, USA. They were mated with female specific pathogen-free (SPF) C57BL/6JJcl (Clea Japan, Inc., Tokyo) mice, and the offspring *in utero* were aseptically transferred to a vinyl isolator upon cesarean section on day 19 of gestation and nursed thereafter by germ-free foster dams (germ-free BALB/cYit). After weaning, the pups were co-housed with female SPF C57BL/6JJcl mice and orally administered an aqueous solution of fresh feces from the latter to allow establishment of their intestinal bacterial flora. The animals were maintained in a vinyl isolator under SPF condition, and supplied *ad libitum* with 10kGy-sterilized pellet feed (FR-1; Funabashi Farms Co., Ltd., Chiba) and with tap water sterilized by autoclaving (126°C, 30 min).

The animal room was maintained at an ambient temperature and relative humidity of 24 ± 2°C and 55 ± 5%, respectively, with a 12-hr light: 12-hr dark cycle (on from 8:30 a.m. to 8:30 p.m.).

TCRβ:p53 deficient (Tcrb $^{-/-}$: Trp53 $^{-/-}$) Mice Production:

The congenic strain was produced by backcrossing at every consecutive generation TCRβ:p53 deficient mice heterozygous (*Tcrb* $^{-/+}$: *Trp53* $^{-/+}$) for *Tcrb* and *Trp53* genes with the partner strain C57BL/6JJcl (*Tcrb* $^{+/+}$: *Trp53* $^{+/+}$). Genotypes of *Tcrb* and *Trp53* genes were determined by amplification using the polymerase chain reaction (PCR) method. Genomic DNA was prepared in the usual manner by extraction from a homogenate of tail tip tissue of about 1 cm cut from the animal under Nembutal anesthesia. The PCR conditions employed for the *Tcrb* gene were as described by Stanford *et al.* [20], and those for the *Trp53* gene as reported by Donehower *et al.* [5]. The resulting PCR products were electrophoresed on agarose gel strips to determine their respective genotypes. As the *Tcrb* $^{-/-}$: *Trp53* $^{-/-}$ mice initially obtained varied in coat color, ranging from the characteristic of strain 129 mice to black, agouti, chocolate, etc., those with the same black coat color as C57BL/6J mice were selected for use in mating.

The mice were mated at 8 weeks, and bred using the concurrent pregnancy scheme. Pups were weaned between days 18 to 21 post-partum.

The congenic strain mice of backcross N1, N3, N5 and N8 generations were examined genetically for genetic homogeneity at the Genetics Division of the ICLAS Monitoring Center (Central Institute for Experimental Animals, Kawasaki, Kanagawa).

For the production of *Tcrb* $^{-/-}$: *Trp53* $^{-/-}$ mice, mating was carried out using as breeding stock those mice correspondent to N4+F1 to N4+F2 generations yielded in the course of congenic strain production. Mating was started at 8 weeks of age, and pups were weaned between days 18 to 21 post-partum.

Macroscopical examination:

Thirty-four male, 3- to 7-month-old *Tcrb* $^{-/-}$: *Trp53* $^{-/-}$ mice were sacrificed by cutting abdominal aorta under ether anesthesia, and all thoracic and abdominal organs were examined macroscopically. The large intestine extending from the terminal ileum (ileocecal region) to the anorectum was removed, and cut longitudinally along the mesenterium throughout. The opened large intestine was washed with physiological saline to rinse off its contents, and fixed in 10% neutral buffered formalin. Stereomicroscopic examination was made of the intestinal mucosa with 0.2% methylene blue stain. The small intestine from the duodenum to the ileocecal region was also removed, cut open along the mesenterium, and examined macroscopically.

Histopathological examination of masses in the large intestines:

Specimens of intestines from 14 mice among those with masses in the intestinal mucosa removed at necropsy were subjected to histopathologic examination. Twenty-three representative neoplastic lesions that were relatively large or showing uneven surfaces (10 lesions from 3 mice aged 3 months, one lesion from a 4-month old mouse, 6 from 4 mice aged 5 months, 4 from 4 mice aged 6 months, and 2 from 2 mice aged 7 months), were cut to appropriate sizes and embedded in paraffin using routine methods. Microscopical examination of these tissues was carried out on 3- to 5-μm paraffin-wax sections stained with hematoxylin and eosin (H-E).

Results

Production of a congenic strain:

A congenic mouse carrying destructed *Tcrb* and *Trp53* genes with the C57BL/6J genetic background was named C57BL/6JJcl-*Tcrb*^{tm1Mom}*Trp53*^{tm1} strain. Successive generations have reached the N8th level so far. The animals of the F0 generation showed polymorphisms at 7 biochemical marker loci (*Pep3, Mup1, Gpi1, Hbb, Es1, Es3*, and *Es10*) in their genetic profile. With advancing generations of the congenic strain, the variations were reduced to 3 loci (*Pep3, Hbb* and *Es3*) at the N3 generation, and to a single locus (*Es3*) at the N5 generation. At N8, all the gene markers became fixed in C57BL/6JJcl while the genotype originating in strain 129 mouse has entirely vanished.

Gross findings:

Macroscopic examination revealed lesions in the large intestine (cecum to rectum) among TCRβ:p53 deficient mice, which included proctocele, mucosal thickening of the large intestine (poorly demarcated lesions), and masses in the mucosa of the large intestine (discrete, nodular, elevated lesions) (Fig. 1). These changes were evident at 3 months of age and thereafter, with cumulative incidences of 20.6% (7/34) for proctocele, 50% (17/34) for thickening of the intestinal mucosa, and 100% (34/34) for masses in the mucosa of the large intestine (4–5 masses/animal) at 7 months of age (Table 1). The incidence of mucosal masses by location was 94.1% (32/34) for the cecum and 61.8% (21/34) for the colon. Besides the lesions in the large intestines, enlargement of the thymus was observed in 76.5% (26/34) of the TCRβ:p53 deficient mice at ≥4 months of age (Table 1). No grossly overt masses were detected in the small intestines of the animals examined.

Histopathological findings of masses in the large intestines:

The masses noted macroscopically in the large intestines presented histologic features of hyperplasia (the glands are lengthened and there is increased mitotic activity, but without cellular atypia and structural atypia: deepened crypts with an increased cellular density but no evidence of cellular atypia of constituent cells or structural atypias such as distortion and branching of crypts), dys-

Table 1. Macroscopical changes in TCRβ:p53 deficient mice

Age (Months)	Number of mice examined	Number of mice with abnormal findings			
		Large intestine			Thymus
		Proctocele	Thickening	Mass	Enlargement
3	3	1	3	3	0
4	5	0	0	5	5
5	14	5	11	14	11
6	9	1	1	9	7
7	3	0	2	3	3
Total	34	7	17	34	26

Table 2. Microscopical findings of masses in large intestine of TCRβ:p53 deficient mice

Age (Months)	Animal No	Segment of large intestine	Number of masses examined	Microscopical findings
3	1	Colon	2	Hyperplasia, Dysplasia
		Cecum	2	Hyperplasia, Adenocarcinoma[a)]
	2	Colon	3	Dysplasia
	3	Cecum	3	Dysplasia
4	4	Cecum	1	Adenocarcinoma
5	5	Cecum	3	Adenocarcinoma
	6	Cecum	1	Adenocarcinoma
	7	Cecum	1	Adenocarcinoma
	8	Cecum	1	Adenocarcinoma
6	9	Cecum	1	Adenocarcinoma
	10	Cecum	1	Adenocarcinoma
	11	Cecum	1	Adenocarcinoma
	12	Cecum	1	Adenocarcinoma
7	13	Cecum	1	Adenocarcinoma
	14	Cecum	1	Adenocarcinoma

a) Invasive carcinoma.

plasia (cellular atypia of varied degrees and structural atypias such as distortion and branching of crypts), or adenocarcinoma (histologic characteristics of dysplasia and submucosal invasion of neoplastic cell: invasive carcinoma) (Table 2, Fig. 2). The masses in the TCRβ:p53 deficient mice at 3 months of age were mostly either hyperplasia or dysplasia, and only occasionally adenocarcinomas. In animals at ≥4 months of age the lesions were all adenocarcinomas (Table 2).

Discussion

Reports of spontaneous tumors of the small intestines or large intestines are few. C57BL/6J-*Apc*^{Min} mice [9, 15] and *Apc* gene knockout mice [6, 16, 17] have been reported to show occurrence of spontaneous tumors in the alimentary tract. Tumors in these mice are largely found in the small intestines while tumors of the large intestines are low in multiplicity. Recently, it has been documented by Daniel *et al.* that IL-10 deficient mice develop colitis and colorectal carcinoma [3], and Rudolph *et al.* have described that mice defective

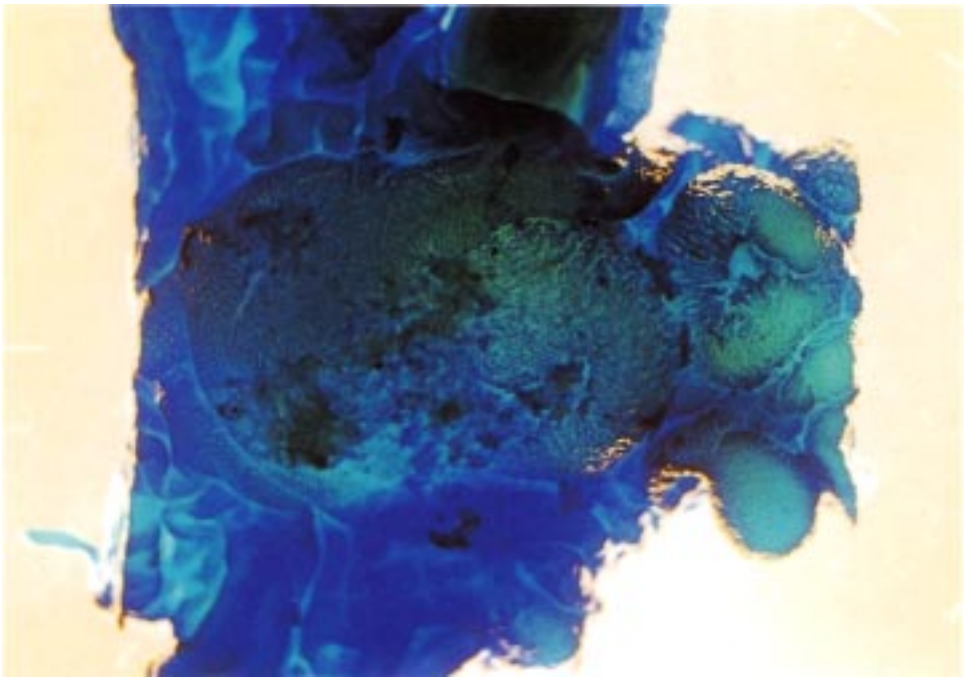

Fig. 1. Stereoscopic microphotograph of cecum from a 3-month-old TCRβ:p53 deficient mouse, showing nodular lesion (mass) in ileocecal region. Methylene blue, × 8.

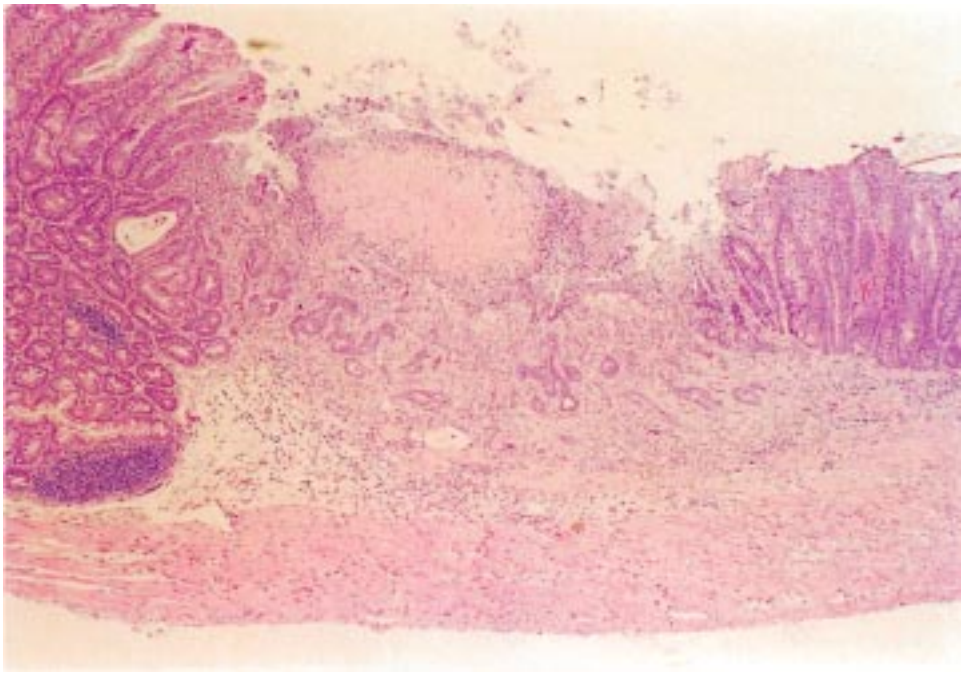

Fig. 2. Section of mass shown in Fig. 1, indicating invasive growth of dysplastic glands (invasive carcinoma) into submucosa and ulcer. H-E, × 52.

in G protein subunit α_{i2} also are prone to develop colitis and colorectal cancer [18].

Having confirmed that TCRβ:p53 deficient mice developed not only colitis but also colorectal carcinoma at a strikingly early stage of growth and with fairly high frequency, we attempted to line-breed this strain. The TCRβ:p53 deficient mice have become established as a genetically homogeneous, congenic strain (C57BL/6JJcl-*Tcrb*^{tm1Mom}*Trp53*^{tm1}) identical in genetic profile with the C57BL/6JJcl strain [11] at N8. The finding that colorectal carcinomas and enlargement of the thymus were noted with high frequency among TCRβ:p53 deficient mice of a midway generation (N4) in the line breeding indicates that *Tcrb* and *Trp53* gene actions were retained during the course of the line-breeding. No spontaneous occurrence of colorectal carcinoma has been reported for C57BL/6JJcl, and the strain is considered to bear no gene which by itself constitutes a background for carcinogenesis. Therefore, the occurrence of colorectal cancer in this mouse is considered to be due to the deficiency of both *Tcrb* and *Trp53* genes. It was observed that colorectal carcinoma occurred in animals derived from the N8 generation with practically equal frequency to that in animals of the N4 generation (unpublished data). Therefore, it appears that the present attempt to line-breed a rodent model prone to spontaneously develop colorectal carcinoma with a high incidence has been successful.

The primary objectives of this study were to establish a congenic mouse strain and to histopathologically verify whether mice of the strain develop colorectal carcinoma. The histopathological examination, confined to macroscopically evident masses, revealed that invasive adenocarcinomas were found in all TCRβ:p53 deficient mice examined at ≥4 months of age. Thus, the TCRβ:p53 deficient mice are considered useful as an animal model for colorectal cancer since there is no established mouse strain reported that spontaneously develops colorectal carcinoma in the early stage of postnatal growth with a high incidence.

We plan to pursue serial histopathologic and oncogenetic assessments of the neoplastic growth over time, with a view to clarifying the oncogenetic process in these mutant mice. Further investigations will also include biological characterization of those tumor cells, e.g. metastatic propensity, growth characteristics *in vitro*, and that in nude mice.

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