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Chemotherapeutic Profiles of Human Tumors Implanted in SCID Mice Showing Appreciable Inconsistencies with Those in Nude Mice

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Abstract: Sensitivities to antitumor drugs of human tumor xenografts (HTXs) implanted in C.B-17-scid and in BALB/cA-nu were compared to examine whether genetic backgrounds of immune deficiency of the host mice influenced the chemotherapeutic profiles of implanted tumors. In a total of 25 pairs of corresponding experiments with each host mouse strain (5 HTXs $\times$ 5 drug treatment groups), we obtained consistent results in 23 (92.0%) experiments consisting of 10 which were both significantly effective and 13 which were both ineffective, although the remaining two (8.0%) experiments showed inconsistent results. A human T-cell lymphoma cell line, LM-2-JCK, implanted in nude mice, was resistant to treatment with 65 mg/kg of cyclophosphamide, but this tumor showed sensitivity to the same treatment when implanted in either SCID mice or mice with a recombination activating gene 2 defect [BALB/cA-TgH(Rag2)], suggesting that the genetic immune background of the host mouse should not be overlooked as a factor affecting tumors.

Key words: drug sensitivity, human neoplasm, immune deficient mouse

Human tumor xenografts (HTXs) established in nude mice are widely used in the field of evaluation of new drugs for cancer treatment [2, 3, 7, 8, 12]. However, nude mice do not always accept tumor tissues from patients [9]. The SCID mouse (*scid/scid*), a mutant strain with severe combined immune deficiency, is a potential new recipient of HTX, and it has been reported that the SCID mouse has advantages over the nude mouse in the establishment of certain types of human tumor [4, 6]. SCID mice bearing HTXs may

therefore become a new model for the evaluation of antitumor drugs. In addition, recent gene and reproductive engineering technology has produced mice with similar phenotypes, but different mechanisms, to SCID mouse, such as the recombination activating gene 2 (*Rag2*) knockout mouse [10].

In this study, we compared sensitivities to antitumor drugs of HTXs in SCID mice with those in nude mice which have been widely used in new drug evaluation research in order to validate the use of the SCID mouse

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in this field.

Mice: BALB/cA-*nu* was obtained from CLEA Japan Inc. (Tokyo). C.B-17-*scid* was a gift from Dr. Bosma, Fox Chase Cancer Center, Philadelphia and bred in our animal quarters. Rag2 knockout mice with BALB/cA background (BALB/cA-Rag2^{-/-}, N8) were established by mating the original TgH(Rag2) mouse donated by Dr. Shinkai, The Howard Hughes Medical Institute, Boston [10] and BALB/cA, and were used when inconsistent results were observed in experiments with BALB/cA-*nu* and C.B-17-*scid*. The mice used were females at 6–8 weeks of age. We reared the mice in accordance with the animal care and use guidelines of the Central Institute for Experimental Animals.

Tumors: Five HTX lines [7], a gastric cancer line: 4-1ST, a non-small cell lung cancer line: LC-11-JCK, a breast cancer line: MC-8-JCK, a myeloblastoma line: MB-1, and a T-cell lymphoma line: LM-2-JCK, were used.

Drugs: Mitomicin C (MMC) and 5-fluorouracil (5-FU) were supplied by Kyowa Hakko Kogyo Co. (Tokyo). Cyclophosphamide (CPM) was provided by Shionogi & Co. (Osaka). They were dissolved in sterile 0.85% NaCl solution just before use. The dose and schedule were single administration of 260 mg/kg or 65 mg/kg for CPM, 6.7 mg/kg or 1.7 mg/kg for MMC, and administration of 19 mg/kg of 5-FU for 5 consecutive days.

Treatment protocol: We examined sensitivity to drugs by the method previously described [11]. In brief, a fragment of tumor was inoculated into the right flank of the mice. Tumor size was measured with calipers twice a week, and tumor volume was calculated by the formula: Tumor volume = Length $\times$ (Width)²/2 (mm³). We shaved the hair coat of mice other than nude mice to measure tumor size more precisely. The mice were divided into groups (n=6) when tumor size had reached 100 to 300 mm³, and were administered drugs or control NaCl solution intravenously. We calculated the T/C (%), where T and C were the relative tumor growth rate of the drug treated group and the control group, respectively. Mann-Whitney *U*-test (*p*<0.01, one sided) was used for statistical determination, and the consistency of the significantly effective (SE) sensitivity or not (NSE) was analyzed between two strains.

Since the growth rate of implanted tumors affects the T/C values [7], we first analyzed growth curves of HTXs

implanted in nude mice and SCID mice, but no significant difference was observed (data not shown). Thus we examined sensitivities of HTXs to antitumor drugs (Table 1, experiment 1). In 25 pairs (5 HTXs $\times$ 5 drug treatment groups) of corresponding experiments with each host mouse strain, we obtained consistent results in 23 (92.0%) experiments which consisted of 10 experiments with both SE and 13 with both NSE. The remaining two (8.0%) experiments, however, showed inconsistent results: The T/C values for MC-8-JCK treated with 5-FU were 28% (SE) in C.B-17-*scid* and 52% (NSE) in BALB/cA-*nu*, and those for LM-2-JCK treated with 65 mg/kg of CPM were 28% (SE) and 99% (NSE), respectively (Table 1). A further examination of HTXs in C.B-17-*scid* mice showed 52% (NSE) for 5-FU with MC-8-JCK and 40% (SE) for CPM with LM-2-JCK (Table 1, experiment 2). In addition, when we examined sensitivities to CPM of LM-2-JCK implanted in Rag2 knockout mice with BALB/cA background, the T/C value was 34% with SE (Table 1, experiment 3). Furthermore, when we performed similar experiments with BALB/cA-*nu* [3, 7], the T/C value for MC-8-JCK to 5-FU was 47% (SE), and that for LM-2-JCK to CPM was 87% (NSE).

The reason why these inconsistent results were obtained was not able to be clarified. However, Maruo *et al.* [5] reported that LM-2-JCK took more efficiently in CBA/N-*nu* and NIH(S) type III nude mice, both carrying partial defects in B-cell function. CPM is known to have suppressive effects on the immune system [1]. It is suspected that improved susceptibility of the tumor in mice with B-cell dysfunction was related to a change in the sensitivity to CPM. Therefore, in the case of LM-2-JCK, the results may appear to indicate that the tumor line was rather highly sensitive to CPM when implanted in SCID mice and Rag2 knockout mice, but was resistant when implanted in nude mice, and the inconsistency was induced by the genetic backgrounds of the immune deficiency of the host mice.

Reproducibility of the chemotherapeutic profile of the tumor in the original patient in the corresponding HTX appears to be an essential factor in determining whether the HTX model is a good model for cancer treatment. Fiebig [2] compared tumor response to chemotherapy in nude mice and in patients, and reported that HTXs in nude mice had made possible correct predictions of resistance to therapy in 57/59 (96.6%)

Table 1. Sensitivities of human tumor xenografts to antitumor drugs

	Tumor	Drug	Dose ^a mg/kg	Mouse Strain	R.T.G. ^b		T/C ^c %	Consistency
					Control	Treated		
Exp.1	4-1ST	CPM	260	C.B-17- <i>scid</i>	10.44 ± 4.82 ^d	3.87 ± 1.36	37*	SE/SE ^e
				BALB/cA- <i>nu</i>	8.51 ± 2.36	4.04 ± 0.76	47*	
			65	C.B-17- <i>scid</i>	8.08 ± 2.56	8.56 ± 2.68	106	NSE/NSE
				BALB/cA- <i>nu</i>	9.14 ± 1.11	7.71 ± 1.81	84	
		MMC	6.7	C.B-17- <i>scid</i>	8.41 ± 1.79	2.60 ± 0.76	31*	SE/SE
				BALB/cA- <i>nu</i>	9.94 ± 0.94	3.07 ± 1.04	31*	
			1.7	C.B-17- <i>scid</i>	11.94 ± 5.40	8.76 ± 3.58	73	NSE/NSE
				BALB/cA- <i>nu</i>	9.94 ± 0.94	7.63 ± 1.98	77	
		5FU	19	C.B-17- <i>scid</i>	8.85 ± 3.03	7.84 ± 3.32	89	NSE/NSE
				BALB/cA- <i>nu</i>	6.93 ± 1.77	5.15 ± 2.14	74	
	LC-11	CPM	260	C.B-17- <i>scid</i>	3.23 ± 0.93	2.32 ± 1.25	72	NSE/NSE
				BALB/cA- <i>nu</i>	3.05 ± 0.73	2.62 ± 0.44	86	
			65	C.B-17- <i>scid</i>	3.23 ± 0.93	2.50 ± 0.34	78	NSE/NSE
				BALB/cA- <i>nu</i>	3.05 ± 0.73	2.99 ± 0.68	98	
		MMC	6.7	C.B-17- <i>scid</i>	3.23 ± 0.93	1.30 ± 0.24	40*	SE/SE
				BALB/cA- <i>nu</i>	4.30 ± 1.20	2.13 ± 0.77	49*	
			1.7	C.B-17- <i>scid</i>	3.23 ± 0.93	2.19 ± 0.64	68	NSE/NSE
				BALB/cA- <i>nu</i>	4.30 ± 1.20	3.46 ± 0.77	80	
		5FU	19	C.B-17- <i>scid</i>	3.47 ± 0.95	2.70 ± 0.56	78	NSE/NSE
				BALB/cA- <i>nu</i>	3.56 ± 0.75	2.48 ± 0.91	70	
	MB-1	CPM	260	C.B-17- <i>scid</i>	7.31 ± 2.72	1.25 ± 0.36	17*	SE/SE
				BALB/cA- <i>nu</i>	7.14 ± 2.98	1.76 ± 1.12	25*	
			65	C.B-17- <i>scid</i>	7.31 ± 2.72	4.01 ± 2.57	55	NSE/NSE
				BALB/cA- <i>nu</i>	7.14 ± 2.98	5.29 ± 2.76	74	
		MMC	6.7	C.B-17- <i>scid</i>	7.34 ± 3.27	0.0 ± 0.0	0*	SE/SE
				BALB/cA- <i>nu</i>	5.81 ± 1.73	0.0 ± 0.0	0*	
			1.7	C.B-17- <i>scid</i>	7.64 ± 3.04	0.04 ± 0.11	1*	SE/SE
				BALB/cA- <i>nu</i>	7.28 ± 2.95	0.15 ± 0.25	2*	
		5FU	19	C.B-17- <i>scid</i>	6.77 ± 2.69	4.44 ± 2.34	66	NSE/NSE
				BALB/cA- <i>nu</i>	5.84 ± 1.12	7.37 ± 1.94	126	
Exp. 2	MC-8	CPM	260	C.B-17- <i>scid</i>	5.33 ± 1.04	1.67 ± 0.87	31*	SE/SE
				BALB/cA- <i>nu</i>	5.16 ± 1.06	1.95 ± 0.48	38*	
			65	C.B-17- <i>scid</i>	5.49 ± 2.62	3.66 ± 1.44	67	NSE/NSE
				BALB/cA- <i>nu</i>	5.74 ± 1.90	4.68 ± 1.07	81	
		MMC	6.7	C.B-17- <i>scid</i>	5.33 ± 1.04	0.78 ± 0.25	15*	SE/SE
				BALB/cA- <i>nu</i>	5.16 ± 1.06	0.55 ± 0.15	11*	
			1.7	C.B-17- <i>scid</i>	5.33 ± 1.04	3.07 ± 0.63	57	NSE/NSE
				BALB/cA- <i>nu</i>	5.16 ± 1.06	2.81 ± 1.01	54	
		5-FU	19	C.B-17- <i>scid</i>	5.37 ± 0.61	1.50 ± 0.57	28*	SE/NSE
				BALB/cA- <i>nu</i>	5.74 ± 1.90	3.00 ± 0.74	52	
	LM-2	CPM	260	C.B-17- <i>scid</i>	28.66 ± 17.20	0.96 ± 0.60	3*	SE/SE
				BALB/cA- <i>nu</i>	13.28 ± 5.69	0.85 ± 0.68	6*	
			65	C.B-17- <i>scid</i>	28.66 ± 17.20	8.04 ± 3.12	28*	SE/NSE
				BALB/cA- <i>nu</i>	13.28 ± 5.69	13.16 ± 6.53	99	
		MMC	6.7	C.B-17- <i>scid</i>	28.72 ± 0.26	0.0 ± 0.0	0*	SE/SE
				BALB/cA- <i>nu</i>	23.25 ± 9.16	1.12 ± 0.77	5*	
			1.7	C.B-17- <i>scid</i>	28.72 ± 10.26	20.84 ± 6.70	73	NSE/NSE
				BALB/cA- <i>nu</i>	23.25 ± 9.16	20.55 ± 7.87	88	
		5-FU	19	C.B-17- <i>scid</i>	37.48 ± 11.53	28.05 ± 11.20	75	NSE/NSE
				BALB/cA- <i>nu</i>	23.25 ± 9.16	19.13 ± 7.48	82	
Exp. 3	MC-8	5-FU	19	C.B-17- <i>scid</i>	6.27 ± 1.69	3.25 ± 0.44	52	
				LM-2	18.88 ± 4.22	7.48 ± 2.51	40*	
	LM-2	CPM	65	C.B-17- <i>scid</i>				
Exp. 3	LM-2	CPM	65	BALB/cA- <i>Rag2</i> ^{-/-}	22.37 ± 15.32	7.61 ± 3.29	34*	

*: Significantly effective as determined by the Mann-Whitney *U*-test ($p < 0.01$, one sided). ^a: Doses were expressed as single doses. 5-FU was given for 5 consecutive days, the others were given in a single administration. ^b: Relative tumor growth, expressed as V_{14}/V_0 where V_{14} is the tumor volume at day 14 and V_0 is the initial tumor volume when treatment was started (day 0). ^c: Ratio of the relative tumor growth in the treated group to that in the control (untreated) group. ^d: Mean ± Standard deviations. ^e: Tumor response in C.B-17-*scid*/Tumor response in BALB/cA-*nu*. SE: Significantly effective, NSE: Not effective.

patients and response in 19/21 patients (90.5%). We and many others also have reported that HTXs in nude mice have reproducibility of the clinical response to therapy [3, 7, 8, 11, 12]. In this study, it is noted that sensitivities to drugs of HTXs implanted in SCID mice were in good agreement with those implanted in nude mice in almost all cases, although the genetic background may influence them in some cases.

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