Human Breast Carcinoma (MCF-7) Serially Transplanted into Nude Mice

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ABSTRACT: The tumor cells (0.5 ml, 1×10^7) of MCF-7 line were inoculated into the subcutaneous tissue or intraperitoneum of female BALB/c nude mice. Primarily tansplanted mice were treated with 17β-estradiol dipropionate (E_2) in a dose of 5 mg/kg and 17α-hydroxy progesterone caproate (Pg) in a dose of 250 mg/kg once a week. After the transferable strain was established, tumors were transplanted into female and male mice treated with E_2, Pg, and E_2+Pg. The tumors treated with E_2 or E_2+Pg grew exponentially while tumors in the other group regressed. Pg was assumed to play some role in the growth of MCF-7, in the presence of estrogen. Although cytosol estrogen receptors (ERc), nuclear estrogen receptors (ERn), and progesterone receptors (PgR) were detected by dextran coated-charcoal method and exchange assay in the growing tumors, ERn and PgR of regressing tumors was usually negative. This MCF-7 strain in nude mice may be a promising animal model for studying chemo-hormone therapy for human breast carcinomas.

KEY WORDS: human breast carcinoma, nude mice, hormone dependency

INTRODUCTION

In case of human tumor xenografts, nude mice systems have been developed to evaluate chemotherapeutic agents, *in vivo*.[1-3] The utility of this system in breast carcinomas was, however, limited because of a relatively low transplantability of human breast carcinomas into nude mice.[4] In particular, transplantable breast carcinomas with hormone receptors were rarely reported,[5,6] and it was therefore difficult to apply this system for an evaluation of hormonal agents. We now report the establishment of human breast carcinoma (MCF-7) with hormone receptors, serially transplanted into nude mice.

The MCF-7 cell line[7] was kindly provided by Dr. Y. Nomura of the National Kyushu Cancer Center Hospital. The tumor cells (0.5 ml, 1×10^7) suspended in RPMI-1640 medium containing 10 per cent fetal bovine serum and 100 units of penicillin/streptomycin were inoculated into subcutaneous tissue (s.c.) or the intraperitoneum (i.p.) of female BALB/c nude mice from Central Institute for Experimental Animals (Kawasaki, Japan). The mice were maintained under specific pathogen-free conditions using laminar air flow racks, in the Experimental Animal Center of our university, and pro-

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Table 1. Effect of Hormones on the Growth and Hormone Receptors of MCF-7 in Nude Mice

Treatment	Tumor Doubling Time (Days)		Hormone Receptors (f-mole/mg protein)		
	Female	Male	ERc	ERn	Pg
EP	7.84±1.95	7.84±1.27	24.3	20.4	103.2
E_2	6.90±1.01	11.11±0.71*	25.0	19.5	132.2
Pg	−51.03±5.34**	−13.29±0.39	20.5	0	0
None	−17.80±4.25	ND	UD	UD	UD

Abbreviations: E_2, 17β-estradiol dipropionate 5 mg/kg once weekly i.m.; Pg, 17α-hydroxy progesterone caproate 250 mg/kg once weekly i.m.; EP, E_2+Pg; ERc, cytosol estrogen receptor; ERn, nuclear estrogen receptor; PgR, progesterone receptor; ND, not done; UD, undetected.

*$P<0.05$ *vs* male EP, female EP, and female E. **$P<0.001$ *vs* male Pg and control female.

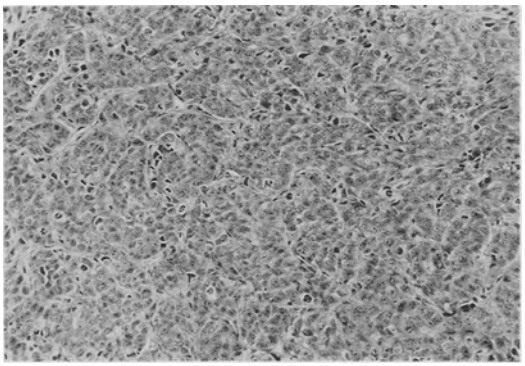

Fig. 1. Histology of MCF-7, showing a common ductal carcinoma with trabeculae and small nests of polygonal cells. (H.E. ×220).

vided sterilized water and food *ad libitum*. Primarily transplanted mice were treated with 17β-estradiol dipropionate (E_2) in a dose of 5 mg/kg and 17α-hydroxy progesterone caproate (Pg) in a dose of 250 mg/kg intramuscularly (i.m.) once weekly for 4 or 5 weeks starting one week after the tumor inoculations. Drugs were dissolved in 0.1 ml of sesame oil and given i.m. into the thigh.

Serial tumor transfers were accomplished by inoculation into the subcutaneous tissue, using a trocar needle. Tumors were transplanted into both sexes of nude mice treated with E_2, Pg, or E_2+Pg (EP), once weekly starting from 1 week after the tumor inoculations. Tumors were also inoculated into untreated female nude mice. Tumor doubling times were calculated during the exponental growth phase or regression phase. At the end of the experiments, routine hematoxylin-eosin sections of the tumor were examined. Hormone receptors including cytosol estrogen receptors (ERc), nuclear estrogen receptors (ERn), and progesterone receptors (PgR) were detected in the grown tumors of female mice, by dextran coated-charcol method and exchange assay.[8,9]

Primarily transplanted tumors grew progressively, in both i.p. and s.c. injected female mice treated with EP. In the i.p. injected mice, some small nodules of tumor cells were evident and the ascites contained few cellular components. Both tumors originating from i.p. and s.c. injected mice were transferred in a subcutaneous solid form and the strain from s.c. injected mice was used for the experiments.

Tumor doubling time during the exponential growth phase and regression phase is shown in Table 1. Tumors transplanted into both sexes of mice treated with EP grew exponentially, after a latent period of almost 2 weeks. Although MCF-7 transplanted into female mice treated with E_2

alone showed much the same growth as the MCF-7 treated with EP, the exponential growth of MCF-7 in male mice treated with E_2 alone was retarded significantly, in comparison with EP and female E_2 only treated groups. The histological features of these tumors were similar, showing common ductal carcinoma with trabeculae and small nests of polygonal cells (Fig. 1). Hormone receptor assay revealed positive ERc, positive ERn, and positive PgR in these tumors of four groups, as shown in Table 1. Tumors transplanted into untreated female nude mice, and male mice treated with Pg alone regressed within 2 weeks after the tumor inoculation, and tumor cells were not histologically evident. Although tumors transplanted into female nude mice treated with Pg regressed, this regression was significantly retarded compared with tumors transplanted into untreated female mice. ERn and PgR of tumors in female mice treated with Pg alone were usually negative.

In 1980, Shafie and Liotta[10] reported the successful transplantation of MCF-7 cells into nude mice, with a genetic background of BALB/c NIH. The MCF-7 formed tumors when injected into untreated female nude mice and the 17β-estradiol treatment increased the growth. In our experiments, although the MCF-7 grew exponentially, depending on the exogenic estrogens, tumors in the untreated female nude mice disappeared within 2 weeks. This discrepancy is probably genetically-related or perhaps in related to the different supply of MCF-7 cells. Additionally, from the results that the growth of MCF-7 in male mice treated with E_2 alone was retarded significantly compared with EP treated group, and that the regression of MCF-7 in female mice treated with Pg alone was retarded from untreated female mice, it was considered that Pg plays some role in the growth of MCF-7, in transition from *in vitro*[7] to *in vivo*, and these results corresponded well to the hormone dependency of MCF-7 *in vivo*. In a previous report,[11] human breast carcinoma, Br-10 [5] with ERc and without PgR and successfully transplanted into untreated female nude mice, was suppressed by anti-estrogenic tamoxifen. As PgR is considered to be a processed product of the estrogen receptor system,[8] the MCF-7 (ER+, PgR+) may have a more complete estrogen receptor system than the Br-10 (ER+, PgR−). The different growth of both tumors in untreated female nude mice revealed the higher estrogen dependency of MCF-7 than the Br-10, with a good correlation of their hormone receptors.

As MCF-7 requires exogenic estrogen for growth, there are difficulties to be overcome when attempting to use this tumor as a screening target of hormonal agents. As exogenic estrogens were administered during the experiments, it may be difficult for competitive inhibitors of estrogen, like tamoxifen to exhibit anti-cancer effects in this system, because tamoxifen will be difficult to conjugate to ER in the higher level of estrogens. The minimum requirement of estrogen for the exponential growth of MCF-7 has to be determined before this strain can be validly used as a screening target tumor, for both chemotherapeutic and hormonal agents.

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