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Influence of Host Sex on the Growth of Human Melanoma (40940)

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Abstract. The growth of estrogen receptor-positive and -negative human melanoma cells was compared in male and female athymic mice. The growth of all receptor-negative and receptor-positive tumors carried in male mice was significantly greater than receptor-positive tumors in female mice. The receptor-positive tumor growth rate in male mice was inhibited by exogenous estradiol while receptor-negative tumors were not. The results suggest that the favorable influence of sex on the natural history of human melanoma may be restricted to hormonally responsive lesions.

A hormonal influence on the natural history of human melanoma is suggested by the improved survival rate in women (1). While there does not appear to be a significant sex difference in the frequency of melanoma (2) or in the incidence of receptor for estradiol in melanoma cytosols (3), suggestive evidence for a similar or shorter length of history of primary lesions in men (4, 5) argues for a more rapid development of the disease in males. We have defined a physiologic basis for this apparent sex difference in survival rates by demonstrating that the growth of estradiol receptor-positive (RPos) and -negative (RNeg) human melanoma cell lines in male and female athymic mice is significantly different.

Materials and methods. The growth and metastasis of cloned RPos (K_d, $3 \times 10^{-10} M$) and RNeg human melanoma cell lines (6) of male origin were studied in two groups of adult male and female athymic mice. Mice (Laboratory Supply Co., Indianapolis, Ind.) were housed in filter cages in a laminar flow hood under a 14-hr light:10-hr dark lighting regimen at constant temperature. All animals received sterile mouse chow and water *ad libitum*. Tumor incidence and initial growth phase of latency (defined as the length of time in days to onset of palpable tumor; approximately 1 to 2 mm in diameter) were monitored daily. Growth rate (tumor volume) and metastatic spread were determined following subcutaneous (sc) inoculation (5×10^5 cells) on the flanks of adult mice. Tumor size was determined by vernier caliper measurement of each subcutaneous mass. Initial growth rates were established by measuring tumor diameter until Day 17, when all animals had sufficient tumor mass to determine volume accurately. One-half the longest and shortest tumor diameter was taken as the value of r in the formula $4/3\pi r^3$ used to estimate tumor volume.

Two additional groups of male mice received each cell line and were injected sc with 17β-estradiol (E_2, 0.01 μg) every other day beginning the day after tumor cell inoculation. Control groups received only the sterile corn oil vehicle. All mice were sacrificed 35 days post tumor cell inoculation, 24 hr after the last injection of steroid or vehicle, when the tumors of all mice bearing RNeg and male mice carrying RPos lesions produced initial signs of morbidity in the host. Tumors were excised and weighed, and portions were taken for histologic examination. Additional specimens of each tumor were immediately frozen for receptor characterization (7). All major organs were examined for gross and microscopic evidence of metastasis and the incidence was recorded. All growth data were analyzed by analysis of variance (ANOVA) followed by Student–Neuman–Keuls sequential range test.

Results. Histologic examination revealed that all tumors possessed an extremely thin capsule, were moderately vascular, and were virtually devoid of lymphocytes. Tumor cells also had a high mitotic index,

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TABLE I. BIOLOGIC BEHAVIOR OF ESTROGEN-RESPONSIVE (RPos) AND -UNRESPONSIVE (RNeg) HUMAN MELANOMA IN MALE AND FEMALE ATHYMIC MICE

Tumor	No. of mice	Sex	Treatment	Tumor incidence (%)	Tumor latency (days)	Tumor weight (g)	Incidence of metastasis (%)
RNeg	7	F	—	100	6.3 ± 0.6[a,b]	8.9 ± 2.1[c]	43
	7	M	—	100	6.5 ± 0.6[d]	7.3 ± 1.6	26
	5	M	0.01 μg E_2	100	6.8 ± 0.2	11.7 ± 2.6	40
RPos	8	F	—	100	3.8 ± 0.5	3.7 ± 1.0[e]	13
	8	M	—	100	4.5 ± 0.3	9.6 ± 1.8[f]	17
	5	M	0.01 μg E_2	80	4.2 ± 0.6	4.5 ± 0.9	0

Note. Growth data were analyzed by ANOVA. Differences between groups were tested for significance by Student–Neuman–Keuls sequential range test. Fisher's exact probability test was used to determine the significance of sex and treatment on tumor and metastatic incidence.

[a] Mean ± SE.

[b] $P < 0.01$ tumor RNeg vs tumor RPos (female mice).

[c] $P < 0.05$ tumor RNeg vs tumor RPos (female mice).

[d] $P < 0.02$ tumor RNeg vs tumor RPos (male mice).

[e] $P < 0.05$ tumor RPos (female mice) vs tumor RPos (male mice).

[f] $P < 0.05$ tumor RPos (male mice) vs tumor RPos (male mice treated with E_2).

high nuclear to cytoplasmic volume, and a large number of melanosomes. Although neither sex nor receptor content significantly influenced tumor incidence, receptor-negative tumors had a slower initial growth phase in both sexes when compared to RPos tumors which were not influenced by estradiol treatment (Table I). The growth rate of cell lines RNeg and RPos in male and female mice is shown in Fig. 1.

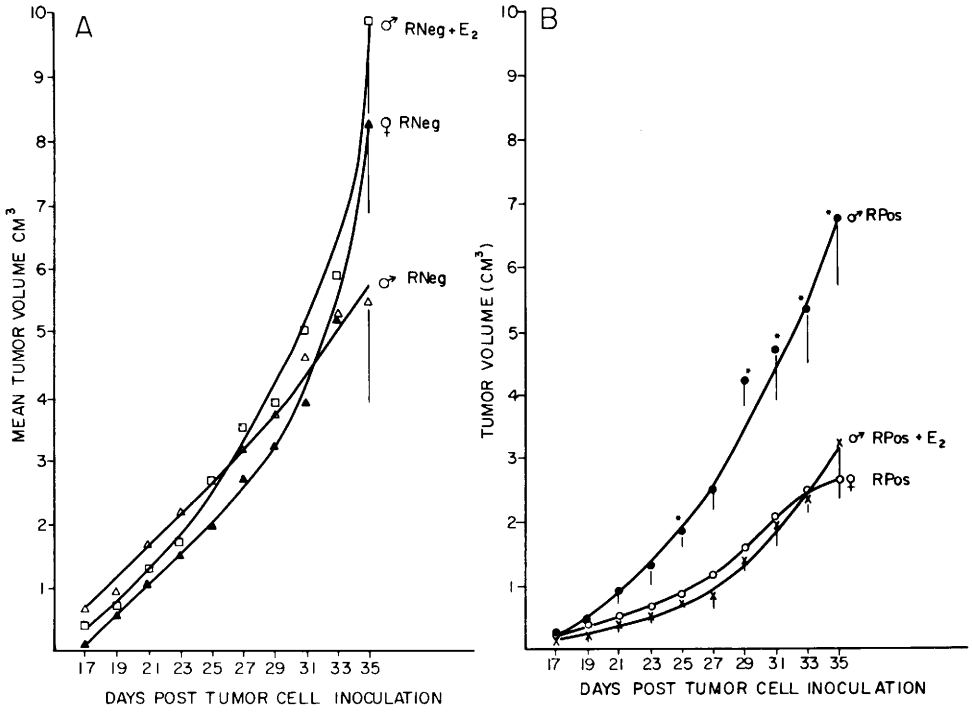

FIG. 1. Growth rates of (A) estrogen receptor-negative (RNeg) and (B) receptor-positive (RPos) melanoma in male and female athymic mice treated with 0.01 μg of estradiol sc every other day. All values are reported as means ± SE *$P < 0.05$ vs male RPos + E_2 and female RPos.

Once established, all RNeg tumors and those RPos tumors carried in male mice exhibited a more rapid growth rate than RPos tumors carried in female mice. This increase in growth was also reflected in final tumor weight (Table I). While estradiol had no effect on the growth of RNeg tumors in male mice (Fig. 1A), it reduced the growth rate of RPos tumors in males to a rate comparable to its growth in female mice (Fig. 1B). Although no significant sex difference in the incidence of liver metastases was apparent, line RNeg had a higher overall incidence than line RPos (35 vs 15%) (Table I). There was no evidence of liver metastasis in estradiol-treated male mice bearing RPos tumors. Sucrose density gradient analysis of RPos cytosols demonstrated a peak at 4 to 5 s that was suppressible by diethylstilbestrol (DES) and not altered by host sex. Scatchard analysis (Fig. 2) (8) of RPos cytosols revealed that the binding affinity of tumor cytosols from untreated male mice (K_d, 3.5×10^{-10} M) was twice that of untreated female mice (K_d, 7.0×10^{-10} M). A significantly greater amount (38.6 fmole/mg of protein) of estradiol bound to cytosols from untreated female mice than untreated male mice (4.0 fmole/mg of protein) (9). Estradiol administration increased slightly the amount of estradiol (6.5 fmole/mg of protein) bound to RPos cytosols in male mice without substantially altering binding affinity. Scatchard analysis of receptor-negative tumor cells was not possible as no affinity of estrogen for its receptor could be demonstrated in male or female mice.

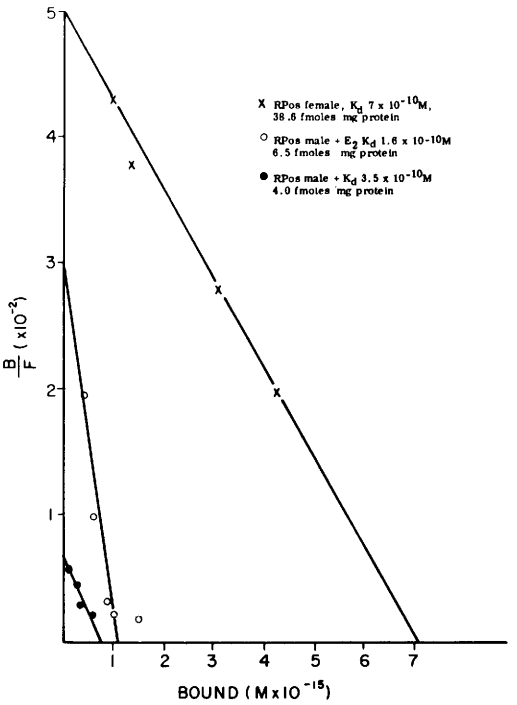

FIG. 2. Scatchard plot of binding [^{3}H]estradiol to human melanoma cell cytosol. Estradiol (0.01 μg) was administered in corn oil sc every other day for 35 days and mice were sacrificed 24 hr following the last injection. Analysis revealed a single class of high-affinity binding sites in tumor cytosols from control and estradiol-treated mice.

Discussion. The demonstration of cytoplasmic receptors for all classes of steroid hormones in specimens of melanoma tissue (10) provided initial evidence that a direct effect of steroids on the behavior of human melanoma was possible. Our recent observations of a direct dose-related effect of estrogen on several cloned human melanoma cell lines positive for estrogen receptor *in vitro* (6) supported this observation. The possibility that a steroid-responsive subset of melanoma patients, much like that in breast cancer patients (11, 12), exists is further strengthened by the early clinical observation that an objective, albeit minimal incidence (11%) of remission can follow the administration of a steroid with anti-estrogen and glucocorticoid activity (13). Since steroid receptor analysis was not available during this clinical study (13), the overall response rate may have been higher in a selected population.

Although host sex has previously been reported to influence melanoma growth in rodents (14, 15), the present studies provide the first experimental evidence substantiating clinical data that the growth of human melanoma may be sex dependent. Binding studies suggest that, in untreated animals, tumor growth may be influenced by the increased amount of estrogen receptor in tumor cells carried in female mice. It is also clear from our observations that the initial growth phase (latency) of receptor-negative lesions is rapid and not influenced by exogenous estradiol. This observation is analogous to that of estrogen

receptor-negative human mammary lesions, which exhibit a greater incorporation of [^{3}H]thymidine than estrogen receptor-positive lesions (16). The growth of estrogen receptor-positive human melanoma, unlike the RNeg tumor, is altered *in vivo* by the administration of estrogen.

Taken together our observations suggest that epidemiologic and clinical evidence of a more favorable prognosis in female patients with melanoma may be contingent upon the hormonal responsiveness of an individual lesion (17).

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