[CANCER RESEARCH 48, 1708–1711, April 1, 1988]

Growth of Sinclair Swine Melanoma as a Function of Age, Histopathological Staging, and Gonadal Status[1]

Max S. Amoss, Jr.,[2] Salve G. Ronan, and Craig W. Beattie

Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas 77843-4466 [M. S. A., C. W. B.]; Departments of Pathology and Dermatology, University of Illinois School of Medicine at Chicago, Chicago, Illinois 60612 [S. G. R.]; and Division of Surgical Oncology, University of Illinois College of Medicine at Chicago, West Side Veterans Administration and Cook County Hospitals, and the Hektoen Institute for Medical Research, Chicago, Illinois 60612 [C. W. B.]

ABSTRACT

We have analyzed the association between host hormonal status and growth rate of congenital exophytic melanomas of Sinclair swine. The growth of multiple exophytic lesions during the first year of life of intact males, orchiectomized males, intact females, and ovariectomized females was quantitated using a proliferative index which assigned a numerical value to fixed increments of gains or losses in tumor volume. The proliferative index from 6 wk (gonadectomy at 6 wk) to 52 wk of age of each treatment group was statistically increased from 0 ($P < 0.01$) except that of gonadectomized females. The proliferative indices from lesions in gonadectomized females were significantly lower than those from intact males, intact females, and gonadectomized males. A total of 93 exophytic tumors from 63 swine were biopsied and histopathologically staged according to the degree of progression or regression and analyzed as a function of animal age. There were no Stage I and only two Stage II lesions at the time biopsies were taken. Twenty-six of 32 (81.2%) Stage III tumors were found in swine of both sexes less than 26 wk of age of which 71.8% were found <10 wk of age, while 20 of 29 (68.9%) were Stage IV, and only 3 of 32 (9%) were Stage V lesions present in this age group ($P < 0.001$, χ^2). Only 20.7% of Stage IV tumors were present prior to 6 wk of age. Preliminary results suggest that castration of either sex also altered tumor histopathology. Our data suggest that a reduction in gonadal steroid secretion was associated with a decrease in exophytic tumor growth rate and regression in animals of both sexes during the first year of life in Sinclair swine. The effect in female swine was due to significant reduction in the proliferative index over the first 6 mo of age.

INTRODUCTION

SSCM[3] presents a unique model for investigating melanoma growth, metastasis, and the phenomenon of spontaneous regression. Initial reports describing the biology of these tumors suggested an overall tumor incidence of 20 to 85% (1–3) with no sex or site preference (4). A significant number of swine present with multiple, congenital, primary lesions which exhibit significantly different growth rates (4). At various stages of postpartum development, these lesions are histopathologically similar to human junctional and compound nevi and malignant cutaneous melanoma (5, 6). A significant number of swine exhibit metastasis to regional lymph nodes, liver, and lungs (Footnote 4; Ref. 6). Approximately 35% of all primary lesions have been reported to regress following puberty, which can be accompanied by the appearance of a halo nevus and a generalized vitiligo (5, 6).

Five histopathologically distinct stages (I to V) of tumor development and regression have been described (5). Stages I to III are associated with progressive lesions and have previously been reported to be present during the first 6 mo of life. Latter stage tumors (IV and V) are initially associated with an invasion of pigment-laden macrophages and a decline of tumor cells (Stage IV) followed by an increase in keratinocytes and fibroblasts which is often associated with depigmentation (Stage V). The regression in Sinclair swine melanoma which has been reported to be accompanied by significant decreases in tumor volume apparently occurs in close proximity to puberty (5), although the hormonal events which characterize puberty in these animals are just beginning to be described.[5] Observations made on the Sinclair swine herd maintained at Texas A&M University indicate that, if metastasis occurs, it does so just prior to or immediately after weaning at 6 wk of age. These significant changes in tumor dynamics, potentially coincident with major changes in the hormonal status of the developing animal, led us to consider the hypothesis that gonadal steroid hormones may influence the natural history of SSCM.

Results from the present series of experiments suggest an association between swine age, histopathological stage, and gonadal steroids in the natural history of exophytic malignant melanoma of Sinclair swine.

MATERIALS AND METHODS

Black male and female Sinclair swine with a history of exophytic melanomas were mated. All animals were maintained on 14% hog chow (Producers Coop, Bryan, TX) and water *ad libitum*. Standard vaccinations and routine veterinary health care were provided. At 6 wk of age (weaning), piglets of both sexes were either bilaterally gonadectomized or sham gonadectomized and placed in groups of ten animals each. In these miniature swine, there was no difference in growth rate between intact and gonadectomized animals.

Tumor Volume. Immediately after farrowing, all piglets were checked for the presence of exophytic lesions and width, length, and depth measured on individual tumors. All tumors of each animal placed in a treatment group were measured at 2-wk intervals for 52 wk, and tumor volumes were calculated (7). The wide variation in tumor volumes within and between animals, irrespective of sex, which has confronted tumor biologists working with Sinclair swine, was solved by assigning a numerical value to fixed increments of gains or losses in individual tumor volumes. This value was termed the PI (Table 1). Tumor volume obtained at 6 wk of age (weaning or gonadectomy when appropriate) was used as the initial or base-line value. The subsequent volume of individual tumors measured at biweekly intervals was expressed as a percentage of the 6-wk (initial) volume and an appropriate PI assigned. Biweekly PIs were then analyzed as a function of swine chronological age, and regression coefficients were calculated for each group within specific time frames over the first year of life.

Histopathological Staging. Histopathological staging as a function of age was achieved by analyzing biopsy specimens of additional tumors that were not incorporated into growth studies. These tumors were

Received 4/20/87; revised 8/17/87, 12/2/87; accepted 12/15/87.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by NIH-NCI CA 33764, CA 31046, and the Texas Agricultural Experiment Station.

[2] To whom requests for reprint should be addressed, at Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843-4466.

[3] The abbreviations used are: SSCM, Sinclair swine cutaneous malignant melanoma; PI, proliferative index; PLM, pigment-laden macrophages; IM, intact males; IF, intact females; GDXM, gonadectomized males; GDXF, gonadectomized females; R^2, regression coefficient; SMS-TAMU, Sinclair melanoma swine-Texas A&M University herd.

[4] Das Gupta *et al.* Comparative histopathology of primary cutaneous melanoma of Sinclair swine, submitted for publication.

[5] Amoss *et al.* Plasma levels of gonadal steroid hormones during development of Sinclair miniature swine, submitted for publication.

Table 1 *Determination of PI*

% of change[a] in tumor volume	PI
0–50	0
51–100	0.5
101–250	1
251–500	2
501 greater	3

[a] $\frac{\text{Tumor volume at time } t}{\text{Tumor volume at 6 wk of age}} \times 100.$

randomly obtained from animals different from those in the PI study but treated identically. This was done to ensure that there would be no effect on PI due to biopsy. The biopsy specimen, which included the tumor-skin interface and the entire cross-section of the tumor, was immediately fixed in 10% neutral formalin:0.9% NaCl solution. Specimens were embedded in paraffin, cut at 5 μm, stained with hematoxylin, and counterstained with eosin. An equal number of sections were treated with 10% hydrogen peroxide for 24 to 48 h prior to staining to reduce interference from the heavy melanin pigmentation within these cells. Since the tumors in the Texas A&M herd are essentially all exophytic, they were assigned to one of four standardized histopathological stages (5) which were modified to include a quantitative assessment of the number of tumor cells within each lesion. Stage I (which was not analyzed) is characterized by an essentially flat lesion composed of epithelioid, heavily melanotic spindle-shaped cells, an occasional PLM and lymphocyte located within the dermis, and no fibroblastic invasion of the exophytic lesion (1, 6). Stage II tumors, which appear as the initial exophytic lesion in postpartum swine in the TAMU herd, are exophytic, contain 90 to 100% mononuclear and multinucleated tumor cells, have not penetrated the papillary dermis and panniculus, and are not as yet ulcerative with a central core of exfoliating pigment. Melanocytes are grouped occasionally in sheets and surround neurovascular bundles. State III lesions contain 50 to 100% tumor cells and are high proliferative, deeply invasive, usually ulcerated, and exfoliating pigment in larger lesions with extensive infiltration of the dermis by melanoma cells and PLM which comprise essentially the remaining number of cells in the lesion. Stage IV describes the early and middle stages of regression where tumor cells comprise <50% of total tumor, pigment is lost with additional depigmentation of the surrounding epidermis, and there is a heavy infiltration of fibroblasts. PLM comprise >50% of total tumor. Stage V is the final stage of regression, with virtually complete loss of tumor cells, dermal fibrosis, and extensive numbers of PLMs deep in the dermis and panniculus. Exfoliation of pigment is absent, and the entire area may be depigmented. A halo nevus and extensive epidermal vitiligo may be present.

Statistical Analysis. Growth curves were analyzed using a general linear model (8), and regression coefficients were calculated. Tumor volume (PI) was analyzed over the entire 52-wk period of the study for each group and then analyzed from 6 to 26 wk and 28 to 52 wk (pre- and postpuberty). The incidence of a particular histopathological stage in different age brackets was analyzed by χ^2.

RESULTS

The proliferative indices obtained over the first year of life of the four groups yielded the following slopes: IM = 0.0175; IF = 0.0158; GDXM = 0.0142; and GDXF = 0.0009 (Table 2; overall $R^2 = 0.57$). The slope of the PIs of IM, IF, and GDXM were significantly different from zero ($P < 0.01$), while that of GDXF was not. The slope of the PI of GDXF was significantly different from the other three groups ($P < 0.001$). Inspection of individual tumor volumes and PI suggested that tumor growth became static or diminished in all groups after 26 wk, irrespective of sex. Growth data were, therefore, reanalyzed using half-year time frames, *i.e.*, from 6 to 26 wk and from 28 to 52 wk at biweekly intervals. Tumor volume at 6 wk was again used as the initial value for each analysis.

Analysis of the PI over the first 26 wk yielded the same patterns as the analysis over wk 6 to 52; however, the slopes of the regression lines were greater within each treatment group (Table 2) with an overall $R^2 = 0.66$. Although the relative rankings of the slopes of the PIs were similar to that calculated for growth over 6 to 52 wk of age, the PI of GDXF was again significantly different from IF (<0.01) and IM ($P < 0.05$).

The PI observed from wk 28 to 52, however, was quite different (Table 2). The slopes of PI from wk 28 to 52 of IM, GDXM, and GDXF were significantly different from zero (<0005),but none was significantly different from each other. The rate of decline of the PI slowed in IM, IF, and GDXM, whereas the PI of the GDXF remained essentially static.

Histopathological staging (II to V) of biopsies of 93 exophytic melanomas from 63 swine during the first year of life (Table 3) revealed that Stage III and IV tumors were observed primarily before 26 wk of age (81.2% and 68.9%, respectively), whereas Stage V tumors were found in animals greater than 26 wk of age (90.6%) (χ^2; $P < 0.001$ for both Stage III *versus* Stages V and IV *versus* Stage V). Two additional tumors present at birth were classified as Stage II. Additional analysis of histopathological stage as a function of age (Table 4) suggested a definite tendency toward regression very early post weaning. Whereas Stage III lesions were uniformly distributed during the first 10 wk of life (<6 wk, 37.5%; 7 to 10 wk, 34.3%), there was a 2-fold increase in the number of Stage IV lesions with age in swine of both sexes less than 6 wk of age (20.7%) compared to swine 10 wk of age (44.8%). As noted, the majority (90.6%) of Stage V lesions appeared after 6 mo of age. A histogenetic analysis (as related to volume) as a function of treatment has recently been completed in swine between 10 and 16 wk of age.[6] The histopathological stage of lesions from subjects >26 wk of age was determined in intact and castrated swine of both sexes and is illustrated in Table 5. Although the numbers of gonadectomized animals are small, in swine older than 26 wk of age, there was a 60% increase in the percentage of Stage IV lesions in gonadectomized pigs of both sexes (40%) compared to intact swine (24%) with only a slight decrease in the total incidence of Stage V lesions (48% GDX *versus* 54% intact) in swine of both sexes. It would appear that two components contribute to this increase. GDXF show a reduction in Stage V lesions with little change in Stage III tumors. However, in castrated male swine the incidence of Stage III lesions decreases (68%), while that of Stage V lesions remains the same. This suggests that castration at 6 wk may alter the histopathological course of the disease in male and female swine and that this appears to be inversely related.

DISCUSSION

The usefulness of SSCM as a model of human disease depends, in part, upon the identification of similar tumor-responsive perturbations in both species. Clinical and epidemiological evidence suggests that host endocrine status may influence the natural history of human melanoma (9–20).

Whereas there are studies which suggest that the overall frequency of human melanoma (10, 11) does not appear sex related, others provide evidence for frequency differences between pre- and postmenopausal women (12). Additional studies indicate a significantly improved survival rate of women compared to men (13–18). One large study found that women, irrespective of age, level of invasion, lesion site, etc., survived longer than men and suggested the difference reflected the effect

[6] C. W. Beattie, S. G. Ronan, and M. S. Amoss, manuscript in preparation.

Table 2 *Comparison of the slopes of the regression of the proliferative indices of SSCM of intact and gonadectomized swine*

Group	Av. no. of tumors	R^2	Slope	P (vs. 0)	P (vs. other groups)
6–52 wk of age (overall $R^2 = 0.5665$)					
IM	13.8	0.6555	0.0175	0.0001	NS[a], NS, <0.001, NS, <0.001, >0.003
GDXM	12.6	0.5619	0.0142	0.0001	
IF	19.5	0.4451	0.0158	0.0001	
GDXF	14.8	0.0045	0.0009	0.7706	
6–26 wk of age (overall $R^2 = 0.6619$)					
IM	14.8	0.7323	0.0392	0.0004	NS, NS, <0.05, NS, <0.001, NS
GDXM	12.6	0.6011	0.0342	0.0016	
IF	20.0	0.7716	0.0541	0.0001	
GDXF	16.0	0.1100	0.0107	0.2875	
28–52 wk of age (overall $R^2 = 0.6485$)					
IM	13.1	0.3148	0.0137	0.0300	NS, NS, NS, NS, NS, NS
GDXM	12.6	0.3417	0.0129	0.0408	
IF	19.2	0.0571	0.0058	0.0072	
GDXF	13.8	0.3201	0.0129	0.0397	

[a] NS, not significant.

Table 3 *Histopathological staging of exophytic Sinclair swine melanoma (representing 93 tumors from 63 animals)*

	Stage III	Stage IV	Stage V
Total no. of tumors	32	29	32
Age			
<26 wk	26 (81.2)[a]	20 (68.9)	3 (9)
>26 wk	6 (18.8)	9 (31.3)	29 (90.6)
χ^2	$P < 0.001$	$P < 0.001$	

[a] Numbers in parentheses, percentage of the total number of lesions of that stage.

Table 4 *Age-related distribution of the histopathological stages of SSCM*

	Age (wk) <6	7><10	11><20	21><26	>26	Total tumors
Stage III	12 (37.5)[a]	11 (34.3)	1 (3.1)	2 (6.3)	6 (18.8)	32
Stage IV	6 (20.7)	13 (44.8)	1 (3.4)	0	9 (31.0)	29
Stage V	0	2 (6.3)	1 (3.1)	0	29 (90.6)	32

[a] Numbers in parentheses, percentage of total at that stage.

Table 5 *Effect of gonadectomy on the histopathological stages of SSCM in swine of both sexes older than 26 wk of age*

Treatment	Sex	Total tumors	Histopathological stage III	IV	V
Intact	M	21	6 (28)[a]	2 (10)	13 (62)
	F	38	7 (18)	12 (32)	19 (50)
	Total		13 (22)	14 (24)	32 (54)
GDX	M	11	1 (9)	3 (27)	7 (64)
	F	14	2 (14)	7 (50)	5 (36)
	Total		3 (12)	10 (40)	12 (48)

[a] Numbers in parentheses, percentage of total number of tumors in each sex treatment group.

of a different steroid environment (17). Sex consistently appears as an independent, positive, prognostic variable for survival (12), particularly in women with extremity lesions (17, 18). This apparent estrogenic effect on melanoma may be receptor mediated as a receptor for estrogen in melanoma appears functional in a variety of species (19–23) including humans. Recent observations also suggest that receptor incidence may also be associated with an increase in survival in women (24).

We have shown that gonadectomy of females at 6 wk of age significantly decreased the PI when compared to that of the other three groups. The effect was most apparent during the first 26 wk of life of females during which time the decrease in tumor PI was highly significant. A similar phenomenon has been reported for hamster MM1 melanoma (25).

SSCMs are histopathologically similar to human cutaneous malignant melanoma (1, 5, 6, 26). In the SMS-TAMU, the vast majority of SSCMs are present as exophytic lesions at birth which either grow, metastasize and kill the host, or regress. Regression appears to be the major response of the lesion in this animal model. Interestingly, regression results in a quantitative increase in tumor PI which is associated with lymphocytic and macrophage infiltration and progression to Stage IV and V lesions during the first year of life. The initial events of regression appear to occur earlier than previously reported (5, 27), and initial signs may be present in a small number of tumors that are present at birth. An initial report of a major and minor variant, with respect to the morphological development of SSCM, depending upon the age of onset suggested the majority of progressive lesions comprising the first three histologic stages (I to III) arose over the first 6 mo of age, with the majority of regressing tumors (Stages IV and V) appearing by 8 mo of age (5). The "minor variant" pattern appears to be expressed in the majority of swine in the Texas A&M University herd, suggesting that the progression of the tumor through Stages I to III may be occurring *in utero*. Therefore, the signal(s) triggering growth, development, and regression may be elaborated very early in the postnatal period or even *in utero*.

Of importance in analyzing the role of the steroid milieu on SSCM regression are the observations that (*a*) there is a significant decrease in PI (tumor growth) following ovariectomy but not orchiectomy in prepubertal swine and (*b*) a retardation in the progression of tumors from Stage IV to Stage V occurs in gonadectomized female swine, but an enhancement of the progression from Stage III to Stage IV occurs in gonadectomized male swine. Although gonadectomy leads to an increase in Stage IV lesions in both sexes, the mechanism(s) by which this occurs appears to be different. This may reflect the significant effect of castration on PI in female swine and absence of effect in males. In summary, it would appear that the changes in PI following gonadectomy of prepubertal females and the apparent retardation of regression associated with changes in the steroid hormone environment suggest gonadal steroids may play a role in the natural history of SSCM.

ACKNOWLEDGMENTS

The excellent technical assistance of O. Baukus, A. Shilakaitis, C. Chastain, and M. Quintanilla is gratefully acknowledged as is the expert secretarial assistance of R. Macke and M. Garza.

REFERENCES

1. Flatt, R. E., Middleton, C. C., Tumbleson, M. E., and Perez-Mesa, C. Pathogenesis of benign cutaneous melanoma in miniature swine. JAMA, *253:* 936–941, 1968.
2. Hook, R. R., Jr., Aultman, M. D., Adelstein, E. H., Oxenhandler, R. W., Millikan, L. E., and Middleton, C. C. Influence of selective breeding on the incidence of melanomas in Sinclair miniature swine. Int. J. Cancer, *24:* 668–672, 1979.
3. Strafus, A. C., Dommert, A. R., Tumbleson, M. E., and Middleton, C. C. Cutaneous melanoma in miniature swine. Lab. Anim. Care, *18:* 165–169, 1968.
4. Middleton, C. C., and Hook, R. R., Jr. The miniature pig: a melanoma model. Lab. Anim., *10:* 48–53, 1981.
5. Millikan, L. E., Boylon, J. L., Hook, R. R., Jr., and Manning, P. J. Melanoma in Sinclair swine: a new animal model. J. Invest. Dermatol., *62:* 20–30, 1974.
6. Oxenhandler, R. W., Adelstein, E. H., Gaigh, J. P., Hook, R. R., Jr., and Clark, W. H. Malignant melanoma in the Sinclair miniature swine. Am. J. Pathol., *96:* 707–714, 1979.
7. Mori, S., Clarkson, B., Masle, E., and Lawrence, W. An experimental perfusion system using rabbit Vx-2 carcinoma. J. Surg. Res., *2:* 268–282, 1962.
8. General Linear Model SAS Users Guide, Statistics Version, Ed. 5, Chap. 20, pp. 433–506. Cary, NC: SAS Institute, Inc., 1985.
9. McGovern, V. J., Shaw, H. M., and Milton, G. W. Prognosis in patients with thin malignant melanoma: influence of regression. Histopathology, *7:* 673–680, 1983.
10. Davis, N., McLeod, R., Beardmore, R., Little, Z., Quinn, J., and Holt, R. Melanoma is a word, not a sentence. Aust. N. Z. J. Surg., *46:* 188–196, 1976.
11. Balch, C. M., Soong, S. J., Shaw, H. W., and Milton, G. W. An analysis of prognostic factors in 4,000 patients with cutaneous melanoma. *In:* C. M. Balch and G. W. Milton (eds.), Cutaneous Melanoma, pp. 321–352. Philadelphia: J. B. Lippincott, 1985.
12. Nathanson, L., Hall, T. C., and Farber, S. Biological aspects of human malignant melanoma. Cancer (Phila), *20:* 650–655, 1967.
13. Olsen, G. The malignant melanoma of skin. New theories based on a study of 500 cases. Acta Chir. Scand. Suppl., *365:* 1–222, 1966.
14. Shaw, H. M., Milton, G. W., Farago, G., and McCarthy, W. G. Endocrine influences on survival from malignant melanoma. Cancer (Phila.), *42:* 669–677, 1978.
15. Rampen, R. H. J., and Mulder, J. H. Malignant melanoma: an androgen-dependent tumour? Lancet, *1:* 562–565, 1980.
16. Rampen, R. Malignant melanoma: sex differences in survival after evidence of distant metastasis. Br. J. Cancer, *42:* 52–57, 1980.
17. Reintgen, D. S., Paull, D. E., Seigler, H. F., Cox, E. B., and McCarty, K. S., Jr. Sex related survival differences in instances of melanoma. Surg. Gynecol. Obstet., *159:* 367–372, 1984.
18. Beattie, C. W., Shaw, H. M., McCarthy, W. H., and Milton, G. W. Sex as a prognostic factor in melanoma (abstract). Proceedings of the First International Conference on Skin Melanoma, 67A, 1985.
19. Chaudhuri, P. K., Walker, M. J., Beattie, C. W., and Das Gupta, T. K. Endocrine correlates of human malignant melanoma. J. Surg. Res., *26:* 214–219, 1979.
20. Chaudhuri, P. K., Walker, M. J., Briele, H. A., Beattie, C. W., and Das Gupta, T. K. Incidence of estrogen receptor in benign nevi and human malignant melanoma. JAMA, *244:* 791–793, 1980.
21. Walker, M. J., Chaudhuri, P. K., Das Gupta, T. K., and Beattie, C. W. Influence of host sex on the growth of human melanoma. Proc. Soc. Exp. Biol. Med., *165:* 96–99, 1980.
22. Markland, F. S., and Horn, D. Animal model systems for malignant melanoma: steroid hormone receptor studies. J. Supramol. Struct., *13:* 35–46, 1980.
23. Schleicher, R. L., Hitselberger, H. M., and Beattie, C. W. Inhibition of hamster melanoma growth by estrogen. Cancer Res., *47:* 453–459, 1987.
24. Walker, M. J., Beattie, C. W., Briele, H. A., Patel, M. K., Ronan, S. G., and Das Gupta, T. K. Estrogen receptors in malignant melanoma. J. Clin. Oncol., *5:* 1256–1261, 1987.
25. Proctor, J. W., Auclair, B. G., and Stokoswski, L. Endocrine factors and the growth and spread of B16 melanoma. J. Natl. Cancer Inst., *57:* 1197–1198, 1976.
26. Millikan, L. E., Hook, R. R., Jr., and Manning, P. J. Gross and ultrastructural studies in a new melanoma model: the Sinclair swine. Yale J. Biol. Med., *46:* 631–645, 1973.
27. Hook, R. R., Jr., Aultman, M. D., Millikan, L. E., Oxenhandler, R. W., and Adelstein, E. H. Development and regression of cutaneous malignant melanoma in Sinclair (S-1) miniature swine. Yale J. Biol. Med., *50:* 5610–A, 1977.