Inheritance of Sinclair Swine Cutaneous Malignant Melanoma

Robert G. Tissot, Craig W. Beattie, and Max S. Amoss, Jr.

Center for Genetics [R. G. T.], Division of Surgical Oncology [C. W. B.], University of Illinois College of Medicine at Chicago, Chicago, Illinois 60612, and the Department of Veterinary Physiology and Pharmacology, Texas A & M University, College Station, Texas 77843 [M. S. A.]

ABSTRACT

Genetic studies of familial human cutaneous malignant melanoma have failed to support a single mode of inheritance. To eliminate the complexities of genetic heterogeneity, we have turned to appropriate animal models to gain insights into possible genetic mechanisms that may be applicable to the human. Cutaneous malignant melanoma of Sinclair miniature swine is an inherited malignancy with many of the histopathological characteristics of human melanoma. The actual mode of inheritance of the melanoma has not been determined. Recently, we initiated experiments to characterize the swine major histocompatibility complex in melanoma- and nonmelanoma-bearing animals. These experiments led to the discovery of two loci that are involved in the expression of exophytic melanomas. The first locus lies within the swine major histocompatibility complex where one particular haplotype produces a phenotype in which the effects of a second locus are fully penetrant. The second locus segregates independently of the major histocompatibility complex. The melanoma-producing allele at this second locus is inherited in the heterozygous state and requires a somatic mutation of the normal allele to initiate tumor development.

INTRODUCTION

The mode of inheritance of familial human cutaneous malignant melanoma has been difficult to establish. Some investigators have reported evidence for multifactorial inheritance (1-5), while others have interpreted their studies as supporting an autosomal dominant mode of inheritance with reduced penetrance (6-9). Wallace et al. (2) point out that although the two hypotheses are not really different in kind, they differ significantly when counseling within a family with multiple affected individuals. Bale et al. (10) used a life table approach to reexamine the family data of Greene et al. (7). They reported evidence for a highly penetrant autosomal dominant gene with pleiotropy, the same gene being responsible for the expression of dysplastic nevus and melanoma; however, serious challenges to their analysis have been presented (11). Caporuso et al. (12) have recently presented evidence that hereditary malignant melanoma and dysplastic nevus syndrome may be a chromosome instability disorder, thus introducing yet another possible mode of inheritance. Taken as a whole, these studies suggest that human malignant melanoma may involve some degree of genetic heterogeneity, i.e., more than one mode of inheritance may be involved in melanoma expression in different families. Since human melanoma may consist of several genetic subtypes, one method of gaining insight into the genetics of human melanoma is to determine the mode of inheritance in an animal model that closely resembles the human disease situation, where genetic heterogeneity can be experimentally controlled. The SSCM has been shown to histopathologically resemble cutaneous malignant human melanoma (13-16). Melanomas in Sinclair swine are present at birth in about 75% of the animals, but additional tumors can develop during the first 6 weeks of life. Multiple primary tumors are also common in these swine and spontaneous regression, accompanied by changes in host cellular immunity, occurs in a high frequency of animals that survive to puberty (14, 16).

There is little doubt that SSCM is an inherited disease (17, 18). We report here a plausible mode of inheritance and present evidence that the SLA plays a significant role in the ability to express the tumor with at least one other as yet unidentified locus also playing a major role in melanoma expression.

MATERIALS AND METHODS

Swine. The Sinclair miniature swine (SMS-TAMU) used in these experiments were from the Texas A & M University herd that was derived from stock maintained at the Sinclair Comparative Research Farm of the University of Missouri and maintained for approximately 15 years as a closed colony. No formal genetic attempts to increase the frequency of exophytic melanoma or the degree of expression in affected animals have been made prior to our present experiments. Since the herd has been maintained as a relatively small breeding unit for several generations, with few founding animals, some inbreeding may have occurred, but the herd cannot be considered an inbred strain. All pigs are identified at birth and the number of tumors at birth and at weaning are recorded. It has been our experience in the SMS-TAMU that few, if any, exophytic tumors develop after weaning.

MLC Typing. Typing of the SLA complex was accomplished using a one-way mixed lymphocyte typing test (MLC) modified from the method of Vaiman et al. (19). Blood samples were obtained at Texas A & M University with the use of sterile materials, although aseptic technique was not complete. Twenty ml of heparinized blood per animal were shipped, on ice, by overnight delivery to the University of Illinois at Chicago for typing. All subsequent procedures used sterile techniques.

The lymphocytes were harvested, divided into two aliquots, and the portion that was to serve as stimulator cells was irradiated with 2500 R from a cobalt source. Inactivated stimulator cells (5 x 10^8) and responder cells (5 x 10^6) were combined in 96-well Linbro round bottom culture plates (Flow Laboratories) and incubated for 4 days at 37°C in a 98% relative humidity, 5% CO_2 atmosphere. One μCi of [methyl-3H] thymidine (RPI, Mount Prospect, IL) was then added to each well and incubation was continued for an additional 18 to 20 h. All tests were run as triple replicates, usually involving 8 animals per group. The cells were harvested on an automated sample harvester and counted in a liquid scintillation counter. The stimulation index, "T" statistic (20), and percentage of maximum stimulation were calculated to determine stimulation or lack of stimulation.

RESULTS

Using MLC typing and pedigree relationships over the last 3 generations, we have been able to identify 4 SLA-D haplotypes in the homozygous state. SLA-D is the region within the SLA complex that controls the major MLC response in swine (19). We have arbitrarily identified these as haplotypes A, B, C, and D until a more definitive serological typing can be completed. Because of limitation on animal numbers we have not been able to maintain all 4 haplotypes as homozygotes, but all 4, as well as
as at least one other haplotype, are still segregating within the present herd.

Very early in our studies we recognized that there appeared to be a relationship between a particular homozygous SLA haplotype, haplotype B, and the ability to express melanoma. Testing the hypothesis that homozygous B/B animals are significantly more likely to have melanoma than non-B haplotype animals, even though both groups of animals came from melanoma × melanoma matings, resulted in the data shown in Table 1. These data show that the B haplotype, in the homozygous state, plays a significant role in melanoma expression.

We then characterized the mating animals of the herd for the haplotypes detected by the MLC response. This provided us with the ability to group offspring on the basis of the probability of having the B haplotype. The results are shown in Table 2. It is clear that the various matings involving the B haplotype have significantly differing probabilities of expressing melanoma, χ² for homogeneity = 23.77, P < 0.001, and the more likely that an offspring expresses at least one B haplotype, the more likely it is of having melanoma. Thus, the B haplotype of the SLA complex plays a significant role in tumor expression. It is also clear from the data in Table 2 that factors other than the B haplotype must also be involved in melanoma expression since no mating class produced all affected or all nonaffected offspring.

Since the SLA complex alone could not account for the presence or absence of melanoma, we turned to a two-locus hypothesis by using human familial retinoblastoma as a model for the second locus (21, 22). The familial retinoblastoma model predicts that if one defective gene or gene deletion is inherited in the germ line, a mutation of the normal allele must occur in a somatic cell to produce a loss of normal function, resulting in tumor expression. All tumor-bearing swine would have one affected allele or gene deletion and one normal allele in their germ line, but would not have a functional normal allele in their melanoma cells. When the data of Table 2 are examined with the use of this two-locus model, the expected values are extremely close to those observed. The results, shown in Table 3, also demonstrate that haplotype B is not absolutely required for tumor expression. Some non-B haplotype animals have exophytic melanomas, but in animals where the B haplotype is present, the second locus is fully penetrant. This is not true of the A, C, D, and other haplotypes segregating within the herd.

The assumption of inherited heterozygosity at the second, tumor-initiator locus can be examined directly by progeny testing animals from melanoma × melanoma matings. If all animals are heterozygous, each melanoma-bearing animal from a melanoma × melanoma mating should produce some nonmelanoma offspring when mated to an unrelated SSCM animal. To date, we have progeny tested 18 SSCM animals in matings where the B haplotype is present. All 18 have produced at least one nonmelanoma, B haplotype offspring, strongly supporting the hypothesis that SSCM animals are heterozygous for the second locus. Furthermore, the segregation ratio among the B haplotype offspring of the 18 progeny tested animals was 23 nonmelanoma and 41 melanoma, not significantly different from the 21.33 nonmelanoma and 42.67 melanoma expected, χ² = 0.193.

The two-locus hypothesis can be further tested if one assumes that somatic mutation is a random event. The number of primary tumors in pigs with at least one tumor should be randomly distributed when corrected for ascertainment bias and classified by SLA haplotype. We corrected for ascertainment bias by subtracting one tumor per affected pig, i.e., all pigs that had one tumor were classified as having none, those with two tumors were classified as having one, etc. Table 4 shows the observed and expected distributions of the number of animals expressing multiple tumors, grouped by haplotype class. Within each group, B/B, B/non-B, and non-B/non-B, the distribution of extra primary tumors does not differ significantly from a random Poisson distribution.

**Discussion**

Our analysis of the results on the inheritance of Sinclair swine melanoma reported by Hook et al. (18) indicated to us that their data were most consistent with a multifactorial mode of inheritance. Our observations are consistent with the control of melanoma expression occurring at a single locus in animals with at least one SLA haplotype, B. Two major differences between the original Sinclair Comparative Research Farm herd and the SMS-TAMU herd may explain this apparent inconsistency. First, we are focusing only on the exophytic form of the melanoma. Second, the SMS-TAMU herd was derived from a melanoma × melanoma mating classified for the B haplotype of the SLA complex.
Heritable melanocytic lesions have also been reported in other breeds of swine (23, 24). Nordby (23) reported a brief pedigree that supported multifactorial inheritance in an unidentified commercial breed. Hordinski et al. (24) reported a melanoma in Duroc swine, a phenotypically red breed, that superficially shares some of the pathophysiological characteristics of SSCM. Their pedigree data support a Mendelian dominant mode of inheritance, although segregation analysis failed to confirm this hypothesis. We cannot say if either of these diseases is a variant of the SSCM found in the SMS-TAMU herd since the authors did not include histopathological studies in their reports.

At the present time, we cannot say with certainty that the SLA-D locus is the responsible element that produces a phenotype where the second locus can be fully penetrant. The SLA-D region has been shown to be quite complex (25) and the B haplotype may be serving only as a marker locus. However, it is clear that the cellular immune system plays a role in the spontaneous regression observed in affected pigs (26–28) and immunosuppression has been shown to enhance tumor growth (29). It is plausible, therefore, that the immune system may also play a role in allowing tumor cells to develop or escape normal surveillance mechanisms. If so, a case could be built for a locus within the SLA-D region functioning as a nonresponder immune response gene in B haplotype animals. Our current outcross and backcross mating studies should be helpful in evaluating this hypothesis.

Disease comparisons between species should always be interpreted with caution. Although SSCM resembles human cutaneous malignant melanoma histopathologically, there are some obvious differences which need to be emphasized. The most significant of these may be the time of melanoma expression. SSCM is often present at birth and rarely develops in animals beyond the age of puberty. On the other hand, human melanoma is seldom present before puberty and is usually expressed during the middle decades of adult life. SSCM is clearly an inherited disease while human cutaneous malignant melanoma may be familial or it may be sporadic, induced by exogenous factors. Thus, our genetic findings in SSCM may have some relevance to human melanoma, but the expression of melanoma in humans is clearly more complex than it is in Sinclair swine.

Several human studies have shown an association of melanoma with specific HLA haplotypes; however, a consistent pattern of association has not emerged (30–33). The study most closely resembling our findings was reported by Pollack and Livingstone (34), who found a relationship between an HLA-D allele and melanoma expression; however, this association was not statistically significant when corrected for the number of tests. Our results indicate that this relationship may need to be reexamined if a proper human population can be defined.

To date, we have not identified a marker for the second locus. Our hypothesis of a mutant allele or gene deletion, lethal in the homozygous state in the zygote, but producing melanoma when somatic cell mutation results in a loss of functional gene product in a melanocyte precursor cell was designed to provide an explanation of how multiple primary tumors might arise in an inherited disease. Since every cell in a tissue or organ is not affected, some somatic event must be required for individual tumors to develop at different sites and at different times. We have hypothesized that one allele for melanoma expression can be inherited at this second locus, but the other allele must arise by somatic mutation. Multiple independent somatic events at this locus would lead to multiple primary tumors. Very early somatic mutation, or inherited homozygosity, would result in such an affected embryo that early lethality would result. Hence, all affected pigs surviving to term carry a single germ line allele for melanoma expression. There have been several human tumors reported where chromosomal deletions lead to tumors that may be inherited in a similar fashion. Among them are retinoblastoma (21, 22, 35), bladder cancer (36), hepatoblastoma, rhabdomyosarcoma, Wilms’ tumor (37), and uveal melanoma (38). To our knowledge, the only karyotypic studies of SSCM reported only the ploidy of the melanoma cells and failed to address the question of chromosomal deletions (39, 40). Our hypothesis fits the mating data of Table 2, the progeny testing experiments, and the random distribution expectations of additional primary tumors shown in Table 4. We believe this is convincing evidence for its acceptance.

The importance of our finding of only two major genetic components in the inheritance of the Sinclair melanoma is quite evident. Multifactorial inheritance implies many small gene effects working in concert to produce a particular phenotype, in this case, melanoma. Multifactorial inheritance does not lend itself to further analysis by the techniques of molecular biology. On the other hand, major gene effects, such as those found in our studies, imply controlling elements in the induction and growth of this congenital neoplasm that can be isolated and studied at the molecular level.

REFERENCES


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