Uveal Melanocytomas

Genetic Comparison With Uveal and Dermal Melanomas

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Objective: Melanocytomas of the eye are typically benign tumors that may be associated with nevi and melanomas. In this study, we assessed the genetic data of melanocytomas and compared them with nevi and melanomas of both the eyes and the skin.

Design: We microdissected 8 melanocytomas, 13 uveal melanomas, and 10 cutaneous melanomas and analyzed loss of heterozygosity markers on chromosome bands 1p36, 6q22-23.3, 9p21, and 10q23, which represent genetic loci associated with advanced dermal melanocytic lesions.

Results: There was no loss of heterozygosity in any of the melanocytomas. However, many loss of heterozygosity events were found in uveal and cutaneous melanomas, most frequently involving chromosome 1 damage followed by chromosome 9 and 10 alterations.

Conclusion: Based on the absence of loss of heterozygosity in melanocytomas, specifically the locus that is lost most often in dysplastic nevi of the skin, we conclude that melanocytomas represent an entity that is different from melanomas or may be similar to that of dermal nevi.

Clinical Relevance: Our results confirm that melanocytomas represent nonagressive lesions that do not demand radical surgery.


METHODS

Three groups of cases were selected from the archives of the pathology departments at Wills Eye Hospital (Philadelphia, Pa), the University of Pennsylvania (Philadelphia), and Duke University Medical Center (Durham, NC). The first group comprised 8 cases of uveal melanocytoma, the second comprised 13 cases of posterior uveal melanoma, and the third comprised 10 cases of cutaneous melanoma, all of which were at least Clark level IV. All cases had been processed routinely for diagnostic histopathologic features, and the sections had been stained with hematoxylin-cosin. After retrieval, representative sections from each case were reviewed to confirm the initial histopathologic diagnosis. Before microdissection, the slides were also screened to determine whether adequate quantities of representative tumor and control tissue were present.
MOLECULAR EVALUATION FOR LOH

After hematoxylin-eosin staining, sections of each sample were microdissected using an LM200 LCM system (Arcturus Engineering, Santa Clara, Calif). DNA samples were extracted from the microdissected cell specimens by proteinase K digestion, followed by phenol/chloroform/isoamyl alcohol extraction. Corresponding normal tissue and stroma samples were extracted and used as controls. Quantification of DNA was undertaken using a primate-specific alpha satellite probe assay (Quantigene, Foster City, Calif). Polymerase chain reaction amplification was performed using the following primers for loci on chromosome bands 1p36, 6q22-23.3, 9p21, and 10q23: D1S1612—5’ GAA-AGA-AAG-AAG-AAG-GAG-GG-3’ and 5’/-5HEX/TGC-GCT-CTT-CTG-CTG-3’; D9S171—5’-A-GCA-CTG-AGA-TGG-GCC-CA-3’ and 5’/-5HEX/AGC-TTT-GGC-AGG-AGA-3’; D6S1038—5’-GCA-GCC-ATG-CTG-GGA-3’ and 5’/-5HEX/GAT-CTT-TCA-TTT-GCA-3’.

GENE AMPLIFICATION

Each polymerase chain reaction contained 1 to 2 ng of DNA, 1× Taq buffer, 1.5mM magnesium chloride, 200µM each nucleotide, 1.5 U of Taq polymerase (Promega Corp, Madison, Wis), and 0.25µM each primer in a total volume of 50 µL. A total of 30 cycles were carried out in a GeneAmp PCR System 9700 (PerkinElmer Inc, Wellesley, Mass), with denaturation for 60 seconds at 94°C, annealing for 60 seconds at 55°C, and extension for 60 seconds at 72°C. The polymerase chain reaction products were analyzed using capillary electrophoresis (ABI Prism 310 and 3100 Genetic Analyzers; Applied Biosystems). Loss of heterozygosity was defined as either an absence of or a significant reduction (>49%) in alleles in the study sample compared with normal tissue from the same patient. Homozygotes and cases in which DNA did not amplify for technical reasons were considered noninformative.

STATISTICAL EVALUATION

Data were compared using the paired t test comparison with Bonferroni correction. P < .05 was considered statistically significant.

RESULTS

Three groups of cases were analyzed for the presence of LOH. A representative case with LOH is depicted in Figure 1. Eight cases of melanocytoma were available for analysis. All were well-circumscribed, intensely pigmented tumors composed of large, uniform, polygonal cells with copious quantities of maximally pigmented cytoplasm that tended to obscure nuclear details (Figure 2). Bleached sections disclosed bland nuclei and a low nuclear-cytoplasmic ratio. Three typical melanocytomas harbored foci composed of nests of less-pigmented cells with pleomorphic nuclei and prominent nucleoli (Figure 3). Overall, genetic analysis disclosed 22 LOH-informative loci in the melanocytoma group, but no LOH was detected in any of the 8 melanomas in this group. In regions that demonstrated normal cytologic features, LOH at chromosomes 1 (D1S1612), 6 (D6S1038), 9 (D9S171 and IFNA), and 10 (D10S185) was detected in none of 7, 3, 5, and 2 cases, respectively. In cytologically atypical areas, LOH in chromosomes 1 and 9 was seen in none of 3 and 2 cases, respectively; no informative data were obtained for chromosomes 6 and 10. The uveal melanoma group comprised 13 cases, each of which harbored at least 1 analyzable genetic result. Overall, 41 LOH-informative loci were obtained. Loss of heterozygosity was identified in 8 of the 41 informative samples in the uveal melanoma group. Compared with melanocytoma, this difference in LOH events was statistically significant (P = .04). Of the 13 cases, 5 showed the presence of at least 1 LOH event; in 8 cases, no LOH was ob-
observed. Four LOH events involving chromosome 1 were identified in 12 cases (33%) examined with probes for that chromosome \( (P = .04 \text{ vs melanocytoma}) \); LOH events involving the locus on chromosome 6, either marker on the short arm of chromosome 9, and the locus on chromosome 10 were observed in 2 (50%) of 4, 2 (17%) of 12, and 0 of 7 cases, respectively. The dermal melanoma group comprised 10 cases of cutaneous melanoma. Four instances of LOH identified in this group yielded 35 LOH-informative loci. Of the 10 cases, 2 demonstrated at least 1 LOH event and 8 did not show LOH. There were 2 of 10 LOH events observed on chromosome 1 \( (P = .09 \text{ vs ocular melanoma}) \); LOH events involving chromosome 6, either marker on chromosome 9p, and chromosome 10 were observed in 1 (17%) of 6 cases, 1 (11%) of 9 cases, and 0 of 5 cases, respectively.

Regarding individual polymorphic markers, LOH involving the locus on chromosome 1 was observed most frequently in uveal and dermal melanomas (6 times). Three respective examples of LOH involving 9p21 and D6S1038 were identified.

**COMMENT**

In the skin, the relative absence of genetic alterations in benign nevi and increased genetic damage in dysplastic nevi and malignant melanomas have been observed. The genetic alteration identified most frequently in early dermal dysplastic melanocytic lesions is LOH at 9p21, which is strongly associated with p16, an important factor in the cyclin-dependent kinase system. Loss of heterozygosity at 9p21 is thought to represent an early genetic alteration involved in the development of dermal melanomas because it has frequently been noted in dysplastic nevi and melanomas but not in congenital nevi. Park and associates found LOH at 9p21 in 78% of dysplastic dermal nevi, but they identified no LOH at that locus in regular nevi. Similarly, Boni et al demonstrated LOH at 9p21 or lp in 47% of atypical and dysplastic nevi but in no typical compound nevi. In contrast to the early genetic alterations at 9p21, LOH events at 1p36 and 11q23 have been reported to represent a characteristic of progressive and metastatic lesions. Boni et al observed no LOH at 1p in compound nevi but identified LOH at that locus in atypical and dysplastic nevi. Similarly, LOH at 10q23 has been described as a frequent event in primary and metastatic malignant melanomas.

The frequency of LOH in ocular melanomas is less well established, and even fewer studies have addressed the genetic abnormalities in benign melanocytic lesions of the eye. Merbs and Sidransky and Kumar et al reported that LOH at 9p21 was the most frequent genetic alteration seen in ocular melanomas, with frequencies ranging from 24% to 71% of cases. Sisley et al demonstrated loss or partial deletions of the short arm of chromosome 1 in ciliary body melanomas and concluded that alterations at chromosome 1 are important in the progression of some uveal melanomas. Another study showed LOH at chromosome arm 6p in 65% of uveal melanomas. This is different from our observations of 16% LOH at 6p in this group. Reasons for this discrepancy may be based on our relatively low number of cases and the fact that we used only 1 chromosomal marker, chromosome arm 6p, whereas other groups included up to 3 markers of this genetic area.

Melanocytomas are deeply pigmented lesions that classically are associated with the optic disc but that may arise in any part of the uveal tract. Lesions within or adjacent to the optic disc are considered to be congenital hamartomas, derived from an excess accumulation of uveal melanocytes in the lamina choroidalis. Multiple studies have proposed that melanocytomas are of nevic origin. The clinical behavior of melanocytomas is apparently benign, with only rare cases spawning malignant melanocytic lesions. Joffe et al followed up 27 patients with melanocytomas of the optic disc for periods ranging from 1 to 19 years. In 15% of the patients, there was a slight increase in tumor size, but no malignant transformation was noted. Growth of an iris melanocytoma has also been reported in the iris of a 9-year-old boy. Magnocellular nevus is an older term for melanocytoma that reflects the generally benign nature of these lesions and the histologic similarity with nevocellular nevi of the dermis. However, rare reports documenting the development of melanoma developing from preexisting melanocytoma and melanocytomas with associated melanoma have been published. On the contrary, benign melanocytomas that undergo spontaneous necrosis can produce visual loss without the presence of melanoma. We performed laser capture microdissection in 8 uveal melanocytomas, 13 posterior uveal melanomas, and 10 cutaneous melanomas to characterize the presence of LOH in melanocytomas compared with malignant melanomas. Use of the laser capture technique allowed us to sample appropriate lesional and control tissue for analysis and to minimize the possibility of contamination. Moreover, the technique allowed us to dissect and sample small, histologically atypical loci within melanocytomas. The results of this study indicate that LOH events are statistically significantly more frequent in cutaneous and uveal melanomas than in melanocytomas. Not a single example of LOH was observed in any of the 8 melanocy-
tomomas studied, either in areas of typical tumor or within atypical foci of melanomalike cells that were found in several lesions. Failure to observe LOH in these histologically atypical areas may indicate that the latter may represent relatively amelanotic foci in typical melanocytomas rather than foci of genetically atypical cells. We found an equal frequency of genetic alterations at 1p36, a possible marker for late tumor development, in ocular and dermal melanomas but not in melanocytomas.

We conclude, based on the absence of LOH in melanocytomas specifically at the locus that is lost in early dysplastic dermal melanocytic lesions, that ocular melanocytomas represent an entity different from ocular melanomas. The present results do not allow speculation as to the nature of similarities or differences between melanocytomas and melanomas. However, one can speculate that based on these results and clinical behavior, melanocytomas of the uveal tract may be similar to benign nondysplastic nevus of the skin. Further studies must be performed to evaluate genetic alterations in melanocytomas with associated dedifferentiation toward malignant melanomas.

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REFERENCES