Histopathology, Morphometry, and Nuclear DNA Content of Iris Melanocytic Lesions

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Purpose. To compare the histologic, morphometric and nuclear DNA content of a group of benign and malignant melanocytic lesions of the iris.

Methods. Forty-four surgically excised melanocytic lesions of the iris were histologically classified as nevus or melanoma. Morphometric analysis using a digital filar micrometer (LaSICO 1602N-10 and 5-4A) measured the mean size of the 10 largest nucleoli, and Feulgen staining and image cytometry (CAS 200 Cell Analysis Systems) analyzed the nuclear DNA ploidy in the lesions. Patient follow-up information was obtained whenever possible.

Results. Sixteen cases were histologically classified as nevi and twenty-eight cases as melanoma. The mean of the 10 largest nucleoli of the nevi was smaller than the mean among the melanomas (1.772 μm [SD = 0.366] and 2.773 μm [SD = 0.565], respectively). Feulgen staining revealed that all lesions were diploid, with the exception of two hyperdiploid and two hypodiploid melanomas. Of the patients with follow-up information available, none with nevi developed a metastasis and two with melanoma died of metastatic disease.

Conclusions. The histologic classification of iris melanocytic lesions (i.e., nevus versus melanoma) correlates to nucleolar size (P < 0.001) but not to nuclear DNA ploidy. Invest Ophthalmol Vis Sci. 1995;36:745–750.

A curious aspect regarding the clinical behavior of uveal melanoma is that primary melanoma located in the iris exhibits relatively benign behavior compared to melanoma located in the posterior compartment. The overall 5-year mortality rate for patients with primary ciliary body or choroidal melanoma is approximately 30%, whereas that for patients with primary iris melanoma is approximately 2% to 3%. It is unknown if the difference in the clinical behavior is because iris melanoma is intrinsically less aggressive, the physical location and/or size at the time of detection of iris melanoma provides less potential for metastasis, the host–immune response causes more suppression of metastatic potential of iris versus ciliary body–choroidal melanoma, or whether any other factor is causally related to the uncommon occurrence of metastasis from iris melanoma.

Jakobiec and Silbert reviewed the clinicopathologic features of 189 iris melanocytic lesions that had originally been classified as melanoma and reclassified the lesions as 176 nevi and 13 melanomas using a new classification scheme. Their study raises the possibility that iris melanoma has been reported to have a low metastatic potential because many surgically excised iris melanocytic lesions previously classified as melanomas were really nevi. To determine if Jakobiec’s and Silbert’s histopathologic classification of iris melanocytic lesions corresponds to measurable nuclear features, we examined a series of melanocytic lesions of the iris with regard to histology, morphometry, and nuclear DNA ploidy.

MATERIALS AND METHODS

Specimens
Forty-four melanocytic iris lesions accessioned in the L. F. Montgomery Ophthalmic Pathology Laboratory,
Emory University School of Medicine (Atlanta, GA) between 1955 and 1991 were evaluated. Patient age, sex, involved eye, eye color, and presence of glaucoma in the involved eye at the time that tissue was excised for histopathologic examination were recorded when the information was available. Five-micron thick sections stained with hematoxylin and eosin were examined. Specimens heavily pigmented were bleached with potassium permanganate. Confidential follow-up questionnaires were sent to referring physicians for all patients in the study; questions concerned survival, recurrence, presence of metastatic disease, and length of follow-up. The Atlanta Surveillance and Epidemiology Endpoint Registry and the Georgia Tumor Registry also provided follow-up information about patients. Histopathologic examination, morphometry, and DNA content studies of the specimens were performed in a masked fashion by independent observers.

### Histopathology

All specimens were examined and classified by an experienced ophthalmic pathologist (HEG). The type of surgical specimen (enucleation or resection) and overall configuration of the tumor (focal or diffuse) were recorded. The classification scheme used is that proposed by Jakobiec and Silbert and, in brief, corresponds to the following: melanocytosis (N1), melanocytoma (N2), epithelioid cell nevus (N3), intrastromal spindle cell nevus (N4), spindle cell nevus with surface plaque (N5), borderline spindle cell nevus (N6), spindle cell melanoma (M7), spindle and epithelioid cell melanoma (M8), and epithelioid cell melanoma (M9).

### Morphometry

A reproducible method for estimating the malignant potential of uveal melanoma from routine hematoxylin and eosin-stained slides, as proposed by McCurdy and coworkers, was used. Briefly, a single hematoxylin and eosin-stained section from each patient was used with a Zeiss (Carl Zeiss, Oberkochen, Germany) microscope, a 2X Zeiss optivar, a 100X Zeiss Plan 1.25 N.A. oil immersion objective, and 10X oculars. The operator (JHO) used the mechanical stage for manual control and the X/Y coordinates to determine a 5-mm path through the center of each tumor parallel to its long axis. Nucleoli along this path were measured with a digital filar micrometer (LaSICO model 1602 A-10) linked to an HP model computer via a LaSico N-KB interface, which was calibrated using a Reichart-Jung micrometer. Measurements were obtained along the horizontal axis only, and nucleoli were included only if they could be included in one plane. An average of 20 nucleoli were measured per tumor; only the 10 largest were used for comparison to determine the mean of the 10 largest nucleoli (MLN). All 44 patients were measured twice for reproducibility.

### Image Cytometric Quantitation of Nuclear DNA Content

Five-micron paraffin sections of the iris lesions were deparaffinized, hydrated to distilled water, and bleached with potassium permanganate before staining by the Feulgen method outlined in the CAS Quantitative DNA staining kit (Cell Analysis Systems, Elmhurst, IL). Digitized images from these slides were created and evaluated using the CAS 200 (Cell Analysis Systems). This interactive video image cytometer incorporates a modified optical microscope and an IBM enhanced computer system. After standardization and calibration using a CAS calibration slide of rat hepatocytes with known DNA content, nuclei for analysis and spleen control slides were selected by the operator (PD). A pathologist (CC) identified 150 to 200 tumor nuclei and up to 15 stromal or inflammatory cell nuclei as an internal diploid control to be counted on each test slide. A DNA index was calculated for each lesion, and histograms of nuclear optical density of tumor and internal control cells were generated. The nuclear optical density is proportional to the nuclear DNA content. An aneuploid peak was defined as a peak separate from the peak obtained with the internal or between-batch diploid control. Ploidy definitions used were: hypodiploid, DI <0.9; diploid, DI 0.9 to 1.1; near diploid, DI 1.1 to 1.2; hyperdiploid, DI 1.2 to 1.9; tetraploid, DI 1.9 to 2.1; and hypertetraploid, DI >2.1.

### Statistical Analysis

Student’s t-test and Wilcoxon rank sum statistics were used to evaluate differences between nevi and melanomas in the average size of the 10 largest nucleoli. Kendall and Spearman rank correlations were used to identify the relationship between grade of a lesion using Jakobiec’s and Silbert’s classification scheme and the mean size of the 10 largest nucleoli. Fisher’s exact test was used to evaluate independence of nevi and melanomas in demographic characteristics. All analyses were computed using the BMDP statistical software.

### RESULTS

The results are summarized in Table 1. The patients ranged from 6 to 83 years of age (median, 52 years of age). There were 21 males and 23 females. The iris melanocytic lesion was in 21 left eyes and 20 right eyes, with the laterality not specified in 3 eyes. The colors of the irides were 20 blue, 3 brown, 2 grey, and 19 not recorded. Glaucoma was recorded in 8 eyes.
There were 29 lesions focally resected, and 15 were removed by enucleation. Five diffuse melanomas were noted, all in enucleated eyes. In total, 16 nevi and 28 melanomas were included in the study. The histologic classification of all 44 lesions was as follows: N4 spindle cell nevus with stromal component, 4 patients; N5 spindle cell nevus with surface plaque, 5 patients; N6 border nevus, 7 patients; M7 malignant melanoma, spindle cell type, 3 patients; M8 malignant melanoma, mixed cell type, 24 patients; M9 malignant melanoma, epithelioid cell type, 1 patient.

The mean of the MLN for nevi was significantly reduced from the mean of the MLN for melanomas. The mean MLN for nevi was 3.399 microns, whereas the mean MLN for melanomas was 3.931 microns. The difference was statistically significant (p < 0.01).

Nevus Melanoma

**FIGURE 1.** The mean of the 10 largest nucleoli (MLN) at 95% confidence intervals comparing 16 iris nevi with 28 iris melanomas. MLN = mean of the 10 largest nucleoli.

smaller (1.772 μm; SD 0.378 μm) than the mean among melanomas (2.773 μm; SD 0.575μm) \((P < 0.001)\) (Fig. 1). Further, the MLN increased with increased atypia according the Jakobiec and Silbert classification \(^4\) \((r_{\text{ Kendall }} = 0.65)\) (Fig. 2).

DNA content studies showed that 34 lesions were diploid (21 melanomas, 13 nevi), one melanoma was hypodiploid, one melanoma was diploid−hypodiploid, two melanomas were diploid−hyperdiploid, and one melanoma was near diploid. Follow-up information was available from 18 patients (3 of 16 with nevi, 15 of 28 with melanomas) ranging in age from 2 to 21 years, with a median of 5 years. All three patients with nevi and 13 of 15 with melanoma had no metastatic disease; two patients with melanoma died of metastatic melanoma 5 and 6 years after diagnosis, respectively. The two patients who died of melanoma had the two largest MLNs in the study.

**DISCUSSION**

It is well recognized that the mortality rate is higher for patients with primary ciliary body and/or choroidal melanoma than for patients with primary iris melanoma.\(^2\) Geise and Robertson reviewed the literature in 1985, found 1036 patients reported with primary iris melanoma, and added 7 from the Mayo Clinic, for a total of 1043 patients.\(^2\) Only 31 of the 1043 patients succumbed to metastatic disease, yielding a relatively low mortality rate of 3%. The cause-specific mortality rate is probably lower than 3% because a number of the 31 patients clinically had presumed metastatic disease without histopathologic documentation.\(^1\) The relative benign course of iris melanoma has been confirmed by others.\(^1,7\) The fact that iris melanomas are malignant, yet behave in a relatively benign fashion, has led to controversy regarding clinical management.\(^5,8\)

Several studies have attempted clinically to differentiate benign from malignant melanocytic lesions of the iris.\(^6,12\) The clinical findings in our patients are in concurrence with previous studies showing that benign and malignant melanocytic iris lesions are found approximately equally in either sex and eye.\(^6,7\) One study has shown that patients with lighter irides may have an increased risk for iris melanoma than patients with darker irides.\(^14\) Of the 25 patients in our series whose iris color was recorded, 20, 3, and 2 had blue, brown, and grey irides, respectively. The proportion of patients with blue irides was similar in patients with nevi (90%) and in patients with melanomas (73%) \((P = 0.63)\). This suggests that although patients with light irides may be at increased risk for melanoma, they also may be at increased risk for nevus. Therefore, the presence of a pigmented lesion in a light iris is not a clinical sign indicative of melanoma. Glaucoma was recorded to be present in the involved eye in 8 of 44 patients. The melanocytic lesion was locally resected in 29 patients, and the involved eye was enucleated in 15 patients. The decision to resect versus enucleate was based on numerous clinical considerations, such as visual potential, age and general health of the patient, extent of the tumor, presence of glaucoma. It is interesting to note that 14 of the 15 enucleated eyes had melanomas and that one enucleated eye had a...
diffuse nevus and glaucoma (patient 4). This may suggest a correlation between histologic grade of the lesion and degree of severity of clinical involvement. Rapidity of growth, satellite lesions, and infiltration of contiguous structures may be clinical clues of malignant potential; however, these features were not evaluated in patients in this study.

Several authors have recognized the importance of histologic classification with regard to management of patients with iris melanocytic lesions. Jakobiec and Silbert reclassified 189 iris melanocytic lesions originally classified as melanoma and, using a new scheme, classified only 13 of the 189 as melanoma. We classified 28 of the 44 melanocytic lesions in our series as melanoma. It is unknown why there was a higher percentage of melanomas in our series than Jakobiec’s and Silbert’s series, although one possibility is that regional differences in indications for surgical removal of iris melanocytic lesions may have played a role. We also retrospectively reviewed the masked histologic classifications in light of the nucleolar measurements. The lesions were all classified as they had been when they were masked, although the most difficult to classify were those that fit into either of the borderline nevus or the malignant melanoma–spindle cell categories (e.g., patients 16 and 17). To investigate further the validity of the Jakobiec and Silbert classification scheme, we compared the nucleolar and DNA content features of the lesions.

Computerized histologic assessment has thus far shown that variation in nucleolar size or, more recently, the 10 MLN is the most useful morphometric criteria with regard to long-term survival in patients with primary ciliary body and/or choroidal melanoma. The value of the mean of the MLNs of iris melanomas in our study (approximately 2.8 μm) is virtually identical to the mean of the MLNs of ciliary body and choroidal melanomas (2.8 μm and 2.7 μm, respectively). Additionally, measurements of DNA content of ciliary body and/or choroidal melanoma has been performed using flow cytometric techniques in two studies. An abnormal amount of nuclear DNA in the tumors has been correlated with patient prognosis in one of those studies. A method other than flow cytometry to measure DNA content is the image cytometric quantitation of the optical density of Feulgen-stained nuclei. This method is particularly applicable if only small amounts of archival material are available, as in our study. Despite the lack of statistical significance, DNA ploidy values may be useful when they are aneuploid because aneuploidy was found only in melanomas in our study. Unfortunately, many melanomas were diploid. Measurements of nucleolar organizing regions in iris melanocytic lesions may also be of diagnostic significance. We were unable measure the nucleolar organizing regions in this study because of limitations in the amount of archival material available.

Our study evaluated intrinsic properties of the melanocytic iris lesions, including histopathologic classification, morphometry, and nuclear DNA content. Extrinsic factors that may have affected tumor behavior, including tumor location and host response, were not evaluated. Features related to location and host response include tumor size (the anterior chamber may be more confining than the posterior compartment, and anterior chamber tumors may be detected earlier than posterior tumors), anatomic configuration of surrounding structures, and inflammatory response. Any or all of these extrinsic factors not evaluated in this study may be causally related to the relative benign behavior and slow growth of iris melanomas.

Follow-up information was available for 18 patients in our study, and the only metastatic tumor deaths occurred in two patients with a mixed-cell type melanoma (patients 42 and 45). These fatalities had the second from the highest and the highest values of MLNs.

The findings in our study show that the Jakobiec and Silbert histopathologic classification of primary iris melanocytic lesions correlates with morphometric properties of the lesions and not necessarily with nuclear DNA ploidy. This is in agreement with previous studies showing morphometric analysis to be a better predictor of survival and correlating better with cell type than DNA content in ciliary body and/or choroidal melanoma. Further studies, including anatomic differences between iris and ciliary body–choroidal melanoma and host response, may help define differences in clinical behavior.

Key Words

iris, melanoma, nevus, morphometry, DNA

References

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