AN ALTERNATIVE HYPOTHESIS FOR OBSERVED MORTALITY RATES DUE TO METASTASIS AFTER TREATMENT OF CHOROIDAL MELANOMAS OF DIFFERENT SIZES

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ABSTRACT

Purpose: To propose an alternative hypothesis for the observed differential survival of patients with small, medium, and large choroidal melanomas based on recently uncovered cytogenetic evidence about melanocytic choroidal tumors.

Methods: Review and analysis of published data.

Results: Recent evidence has shown that recurring nonrandom cytogenetic abnormalities are present within virtually all cytomorphologically malignant cells that compose choroidal melanomas and that certain individual cytogenetic abnormalities and combinations of these abnormalities are important prognostic factors for metastasis and metastatic death. Although these cytogenetic abnormalities are strongly correlated with recognized clinical prognostic factors (tumor size, intraocular tumor location) and histomorphologic prognostic factors (melanoma cell type, vascular mimicry pattern) for metastasis, most laboratories have found these cytogenetic abnormalities to be much more robust indicators that metastasis will or will not develop than these clinical and histopathologic factors. In most series of uveal melanomas evaluated by current cytogenetic methods, approximately 30% to 60% of the tumors have cytogenetic abnormalities indicative of high likelihood of metastasis posttreatment. Evidence suggests that these abnormalities are more frequent in larger tumors than in smaller ones. Survival analyses of uveal melanoma patients whose tumors have been evaluated cytogenetically have shown rates of metastasis that approach 100% for patients with a tumor exhibiting monosomy 3 or a class 2 gene expression profile but are very low for those with a tumor that did not exhibit these cytogenetic abnormalities.

Conclusion: The better prognosis of patients with smaller choroidal melanomas is likely to be attributable to a lower probability of cytogenetic abnormalities indicative of metastatic capability among smaller tumors and not to effectiveness of treatment at preventing metastasis.

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INTRODUCTION

Many studies over the past 50-plus years have demonstrated that patients with smaller choroidal melanomas at the time of treatment have a better survival prognosis than those with larger tumors. This differential survival is generally attributed to greater effectiveness of treatment when it is provided earlier in the natural history of the choroidal tumor. The theory behind this attribution holds that tumor cell entry into the bloodstream sufficient to cause metastasis is a time-dependent function (ie, the longer a choroidal melanoma is present, the higher the probability that tumor cell entry into the bloodstream sufficient to result in metastasis will have occurred by the time the primary tumor is treated). According to proponents of this theory, removal of the primary tumor (eg, enucleation) or local tumor destruction (eg, plaque radiotherapy, proton beam irradiation) prior to the date by which intravascular dissemination of a sufficient number of tumor cells to result in metastasis has occurred results in a cure, whereas the same treatments provided after that date will fail to prevent metastasis. The purpose of this study is to propose an alternative hypothesis for the observed differential survival of patients with small, medium, and large choroidal melanomas that is based on recently uncovered cytogenetic evidence about melanocytic choroidal tumors.

METHODS

Published survival data, cytogenetic information, and data regarding circulating melanocytic tumor cells in patients with choroidal melanoma were reviewed and analyzed and are presented below. No new or original data are presented.

RESULTS

GENERALLY ACCEPTED CONCEPTS ABOUT CHOROIDAL MELANOMAS

As a starting point for a discussion of the impact of treatment of the primary tumor on survival in patients with choroidal melanoma, several generally accepted concepts about such tumors are presented.

Limited Natural History Data Regarding Choroidal Melanomas

Although various individuals have recognized and commented on the limited natural history data that exists for patients with primary choroidal melanoma over the years, Zimmerman should be credited with emphasizing this point most effectively for the current generation of ophthalmologists.[1-3] As Zimmerman has indicated, in the absence of high-quality outcomes data on a large group of completely untreated patients with choroidal melanomas representative of the entire spectrum of such tumors, the unbiased impact of any treatment (including enucleation) relative to the natural history of the disease cannot be determined.

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Relationship Between Primary Tumor Size and Survival Prognosis

Multiple investigators over the years have observed that patients with larger choroidal melanomas at the time of initial treatment have a substantially higher cumulative actuarial probability of developing metastasis and dying of that metastasis than do patients with smaller tumors.[4-10] In the absence of natural history data (see preceding paragraph), this observation is commonly considered surrogate evidence of the effectiveness of treatment, provided it is not delivered "too late" in the natural history of the patient's disease.

Relationship Between Histomorphologic Features of Primary Tumor and Survival Prognosis

During the latter half of the 19th century and first quarter of the 20th century, it gradually became apparent to a number of clinician-scientists that patients with histopathologically more atypical melanocytic choroidal tumors appeared to have a worse survival prognosis following enucleation than did those who had less histopathologic atypia. However, Callender[4,11] is generally credited with being the first to demonstrate statistically that there was a strong correlation between the classification of a melanocytic choroidal tumor according to an arbitrary ordinal categorical histomorphologic scale and death from metastasis. Multiple investigators have shown that the melanoma cell type assigned according to the Callender classification (or the modified Callender classification currently in use throughout the world) is strongly correlated with tumor size, tumor involvement of the ciliary body, and other histomorphologic prognostic factors, such as the vasculogenic mimicry pattern of the tumor.[4,12-14]

Relationship Between Intraocular Tumor Location and Survival Prognosis

Multiple investigators over the years have also observed that patients with a histomorphologically malignant (see Appendix for definition of *histomorphologic malignancy*) melanocytic choroidal neoplasm that involves the ciliary body anteriorly have a higher cumulative actuarial probability of developing metastatic melanoma and dying of that metastasis than do those whose intraocular tumor was confined to the choroid.[5,6,8,15,16] Ciliary body involvement by a histomorphologically malignant melanocytic choroidal tumor has in turn been strongly correlated with melanoma cell type, tumor size, and vasculogenic mimicry pattern of the tumor.

Unreliability of Conventional Prognostic Factors for Prediction of an Individual Patient's Survival

Although both size of the intraocular tumor and melanoma cell type (ordinal categorical scale of extent of cytomorphologic atypia of component melanocytic cells) of melanocytic choroidal neoplasms are clearly associated with the probabilities of metastasis and metastatic death, even both types of factors together do not allow ophthalmologists and oncologists to predict with any degree of reliability which patients will actually develop or not develop metastasis. Some patients with relatively large melanocytic choroidal neoplasms that contain markedly atypical melanocytes (epithelioid melanoma cells) do not develop metastasis following enucleation (or other treatments), whereas other patients with relatively small melanocytic choroidal neoplasms that contain only moderately atypical melanocytic cells (spindle melanoma cells) on histologic study develop metastasis and die of that metastasis.

RELEVANT NEW OBSERVATIONS ABOUT CHOROIDAL MELANOMAS

During the past 15 years, a great deal of new information regarding the cytogenetic characteristics of choroidal melanoma cells has been accumulated. Following are a list and comments on some of these new observations that are relevant to our discussion of the impact of treatment.

Cytogenetic Abnormalities Within Choroidal Melanoma Cells

Virtually all of the cells that comprise cytomorphologically or histomorphologically malignant choroidal melanocytic tumors exhibit multiple cytogenetic abnormalities.[17-24] The detected cytogenetic abnormalities are not randomly distributed but seem to affect several specific chromosomes (most notably chromosomes 1, 6, 3, and 8) preferentially.

Association Between Cytogenetic Abnormalities Within Choroidal Melanoma Cells and Metastasis

Some of the cytogenetic abnormalities within cytomorphologically or histomorphologically malignant melanocytic choroidal tumors are significantly associated with metastasis and metastatic death. The cytogenetic abnormality (see Appendix for definition of *cytogenetic abnormality*) that has been associated with metastasis and metastatic death most consistently from laboratory to laboratory is a complete loss of one of the two copies of chromosome 3 (ie, monosomy 3).[19,22,23,25-32] Partial deletions of either the short (p) or long (q) arm of chromosome 3 (or both) have also been associated with metastasis but on a less consistent basis.[23] When monosomy 3 or a partial deletion of chromosome 3 (or functional monosomy 3 [isodisomy 3]) is present and coupled with a partial or complete deletion of chromosome 1p or chromosome 8p, the association between the cytogenetic abnormalities and metastasis is even stronger.[19,27] Several laboratories have observed a mortality rate due to metastasis of over 50% within 5 years and approaching 100% by 10 years or less in patients whose tumor showed monosomy 3 vs less than 10% among patients whose tumor did not show this abnormality. In contrast, Aalto and coworkers[27] indicated that 21% of their patients with nonmetastasizing uveal melanomas (all of whom had been followed for at least 15 years following enucleation) had monosomy 3. To our knowledge, no other group has published similar favorable survival among patients with monosomy 3.

Frequency of Cytogenetic Abnormalities Associated With Metastasis Within Tumor Cells of Choroidal Melanomas

In most series of posterior uveal melanomas evaluated by current cytogenetic methods, approximately 30% to 60% of the tumors have been found to have cytogenetic abnormalities indicative of high likelihood of metastasis following initial treatment.[19,23,25,27,30,33] When patients with uveal melanoma known to have metastasized were evaluated cytogenetically, approximately 73% of this subgroup were shown to have monosomy 3.[27] In contrast, most series have found few if any patients whose choroidal melanoma did not exhibit monosomy 3 who developed metastasis or died of metastasis.[25,29,31]

Correlations Between Cytogenetic Abnormalities Associated With Metastasis and Conventional Clinical and Pathologic Prognostic Factors

The cytogenetic abnormalities associated with metastasis and metastatic death in patients with primary choroidal melanomas are significantly correlated with larger tumor size, ciliary body involvement, epithelioid cells, and prominent vasculogenic mimicry patterns (loops and networks).[33-37] However, according to Prescher and coworkers,[25] monosomy 3 is a substantially stronger predictor of metastasis than any of these conventional clinical and histopathologic prognostic factors.

Association Between Cytogenetic Abnormalities Within Tumor Cells and Features of Choroidal Melanoma Other Than Metastasis

While some cytogenetic abnormalities within uveal melanocytes (eg, complete loss of one chromosome 3) appear sufficient to convey metastatic capability on those cells, others appear to cause some but not all of the features that comprise metastatic capability.[20,30,38] These features include conversion of choroidal melanocytes into cytomorphologically malignant cells, impairment or loss of cell cycle regulation of tumor cells, invasion or migration of tumor cells into the lumina of tumoral blood vessels, avoidance of detection of circulating malignant cells and their elimination by immunologic mechanisms, establishment of metastatic colonies in various organs, and/or growth of the cells composing those colonies, resulting in eventual replacement and/or destruction of the affected organs or organs. One of the best examples of a limited cytogenetic abnormality is a gain of chromosome 6p, which has been associated with conversion of choroidal melanocytes into spindle B cell melanoma cells but not with worsened survival prognosis (unless monosomy 3 is also present).

Gene Expression Profiling of Choroidal Melanoma Cells

Gene expression profiling of histomorphologically malignant choroidal melanomas enables investigators to classify these tumors into 2 prognostic groups, one with an extremely low probability of future development of clinical metastasis (class 1 gene expression profile) and the other with an extremely high probability of future metastasis (class 2 gene expression profile).[33,35,39,40] Although cytogenetic abnormalities of choroidal melanoma cells identified by methods such as karotype analysis, fluorescence in situ hybridization, and comparative genomic hybridization are frequently distributed nonuniformly from region to region of the primary choroidal tumor, the gene expression profiles of the tumor cells have been found to be much more homogeneously distributed in different regions of the primary tumor in most reported cases to date.[33,35] Consequently, classification of an individual's risk of developing metastasis by gene expression profiling appears to substantially be more reliable than any prognostic classification based on generally accepted clinical and/or histomorphologic prognostic factors or on the presence or absence of monosomy 3 within the tumor cells.

Circulating Malignant Cells in Patients With Primary Choroidal Melanoma

Circulating malignant cells are detectable in many patients with a cytomorphologically or histomorphologically malignant choroidal melanoma prior to or at the time of initial treatment.[41-43] Such cells are not detectable in the blood of persons without a cytomorphologically or histomorphologically malignant uveal (or cutaneous) melanoma. Although the presence of circulating malignant cells appears to correlate with death from metastatic melanoma, many patients with a primary choroidal melanoma in whom circulating malignant cells have been detected have not gone on to develop metastatic disease. This observation supports our previous comment that intravascular dissemination of choroidal melanoma cells is not equivalent with metastasis.

DISCUSSION

Recent evidence indicates that certain recurring, nonrandom cytogenetic abnormalities (alone or in combination) and the gene expression profiles these abnormalities produce are much more reliable prognostic factors for metastasis and metastatic death in patients with histomorphologically malignant melanocytic choroidal tumors (ie, choroidal melanomas) than conventional clinical and histopathologic prognostic factors. In most series of posterior uveal melanomas evaluated by current cytogenetic methods to date (most of which have been medium-sized and large tumors according to the Collaborative Ocular Melanoma Study size classification), approximately 30% to 60% of the tumors have exhibited cytogenetic abnormalities and gene expression profiles indicative of high likelihood of metastasis following initial treatment. Studies of metastasis and metastatic death among uveal melanoma patients whose tumors have been evaluated cytogenetically have shown rates of metastasis that approach 100% within 10 years among those whose tumor exhibited monosomy 3 or a class 2 gene expression profile, but very low rates of metastasis among uveal melanoma patients whose tumor did not show these cytogenetic abnormalities. The extremely high rate of metastasis among patients having a choroidal melanoma associated with monosomy 3 or a class 2 gene expression profile suggests that metastasis is an almost completely penetrant outcome of these cytogenetic abnormalities. Several studies have also shown that the proportion of tumors with such abnormalities is greater among larger tumors than among smaller ones. Because choroidal melanomas that have developed cytogenetic abnormalities which render them more malignant are likely to grow faster on average than melanomas that have not (because of escape from normal cell cycle regulation), the proportion of all choroidal melanomas that exhibits monosomy 3 and/or class 2 gene expression profile will be progressively higher as one goes from small to medium to large to extra-large tumors.

From published information from multiple laboratories, it appears that 30% to 40% of medium-sized melanomas, 50% to 60% of large choroidal melanomas, and over 70% of extra-large choroidal melanomas will exhibit monosomy 3 (including those with functional monosomy 3) and/or a class 2 gene expression profile predictive of high probability of future metastasis. Although relatively few small choroidal melanomas have been evaluated to date by cytogenetic methods, one can extrapolate from the published

data above that about 10% to 20% of small choroidal melanomas will also exhibit such cytogenetic abnormalities. These figures correspond quite closely with the asymptotic cured fractions that have been reported among patients with primary choroidal melanoma treated by enucleation for small, medium, large, and extra-large choroidal melanomas, respectively.[44]

Taken together, these observations suggest that choroidal melanomas which have developed cytogenetic abnormalities making them capable of metastasizing (ie, have attained metastatic capability) have probably already metastasized by the time they are detectable clinically. In contrast, tumors that have not developed such cytogenetic abnormalities will not have metastasized and can therefore be cured; unfortunately, most of these patients would probably have survived without metastasis even if treatment for the primary tumor had not been provided.

The clinical implication of the preceding inference is that (1) treatment (including enucleation) is likely to be ineffective as a preventative of metastasis in patients having a melanoma already capable of metastasizing cytogenetically, and (2) treatment is likely to be effective in preventing metastasis only for patients whose choroidal melanoma has not developed such cytogenetic abnormalities but will do so in the future as the tumor continues to grow. At present, we have no information on the frequency of such cytogenetic transformation. However, the fact that we observe a relatively stable cured fraction after a certain tumor size suggests that such occurrence may be infrequent.

In conclusion, the better prognosis of patients with smaller choroidal melanomas is likely to be attributable to a lower probability of cytogenetic abnormalities indicative of metastatic capability among smaller tumors and not effectiveness of treatment at preventing metastasis.

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APPENDIX

Several terms that may be unfamiliar to readers are used throughout the text of this article. This appendix provides definitions for these terms.

cytomorphologic malignancy: cellular morphologic atypia of cells comprising a tumor that is sufficient in extent to warrant pathologic classification of those cells as malignant by most well-trained, experienced pathologists.

histomorphologic malignancy*:* morphologic atypia of the cells and supporting elements composing a tumor that is sufficient in extent to warrant pathologic classification of that tumor as malignant by most well-trained, experienced pathologists.

metastatic capability: the capacity of cells composing a primary neoplasm to (1) leave the primary tumor and enter the bloodstream, (2) survive within the circulating blood, (3) colonize and survive in a receptive peripheral organ, and (4) proliferate uncontrollably within the metastatic site(s) at some point in time following establishment of the colony.

cytogenetic abnormality*:* an *abnormality of chromosome number* (as revealed by metaphase karyotyping or tests such as fluorescence in-situ hybridization or chromosomal in-situ hybridization) and/or *chromosome morphology*, including gains (duplications), losses (deletions), and translocations of portions of chromosomes (as detected by methods such as metaphase karyotyping, comparative genomic hybridization, microsatellite analysis, and restriction fragment length polymorphism analysis), and/or an *underexpression or overexpression of selected tumor cell associated genes* (as revealed by gene expression profiling) relative to normal cells of the same type or lineage.

PEER DISCUSSION

DR FREDERICK L. FERRIS III: Dr Augsburger and coauthors have posed an attractive hypothesis to identify patients at highest risk for metastasis from ocular malignant melanoma. He notes that traditionally our discussions with patients about their mortality risk from metastatic melanoma have been based on clinical factors such as tumor size or intraocular tumor location as well as histologic factors such as melanoma cell type or vascular mimicry pattern. There is ample data in the literature to document that these factors are associated with an increased mortality risk. However, these risk factors also demonstrate the difference between associations and "cause-and-effect" relationships.

It is virtually impossible to prove cause-and-effect relationships with observational studies. However, if there is clear evidence of biologic plausibility as well as clear evidence, generally from multiple studies, of a direct association of the risk factor with the outcome, one is tempted to believe the relationship may be cause-and-effect. An obvious example of this is the combined evidence suggesting that cigarette smoking causes lung cancer.

In the case of melanoma, we have had to rely on various physical and histologic attributes of the melanoma in assessing a patient's mortality risk. Dr Augsburger has now demonstrated that important cytogenetic abnormalities may be indicative of a high likelihood of metastasis. He has shown that these cytogenic abnormalities are also associated with the attributes of the tumor that have been previously used as risk factors.

When various potential risk factors are correlated with each other and with the outcome, they are considered confounders. One approach to deal with potential confounders is to do the appropriate statistical adjustment. These statistical methods should help further test Dr Augsburger's hypothesis. The cytogenetic abnormalities in the melanomas from several different large series of patients must be identified, along with clinical and histologic risk factors. These cohorts must have adequate follow-up to determine the incidence of metastases and death. The hypothesis can be tested by assessing the associations of the risk factors with the outcome and adjusting for the confounders. Dr Augsburger's hypothesis also has the advantage of having more direct biologic plausibility as a cause-and-effect risk factor than current risk factors. I look forward to the careful evaluation of this exciting new genetic hypothesis. If these observations are replicated in multiple studies, it will change the way we evaluate and talk to our patients with malignant melanoma.

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DR. GEORGE O. WARING, III: I would like to comment on something the discussant said and on the importance of replication. We all learned in high school and college that the essence of science is replication. I am the editor of the *Journal of Refractive Surgery* and, if there are any other editors here, they can confirm what we commonly have reviewers telling us, "oh, we already know that, so and so published that last year or two years ago, therefore this paper should not be published, even though it is ok". I would like to make a plea that the publishing studies that replicate findings are an important function of our journalistic responsibilities, as Rick said.

DR. HANS E. GROSSNIKLAUS: No conflicts. I have a comment on your models of melanoma progression regarding the nevus to melanoma model, the stem cell to melanoma model, the combined model, and other considerations on the progression of

micrometastatic melanoma in the recipient organ, particularly the liver. This probably depends on intrinsic properties of the micrometastasis, and it is likely that changes in the host response play a role in progression of clinical metastatic disease.

DR. JAMES J. AUGSBURGER: I would like to thank Dr. Ferris for his pertinent remarks about confounding factors and surrogate variables. Dr. Grossniklaus may be correct about the role of host factors in development of metastatic uveal melanoma; however, the ability of monosomy 3 and Class 2 gene expression profile to predict which patients will develop metastasis suggests that host factors may be of limited importance in this process.