Extrascleral Tumor Extension Associated with Localized Scleral Melt following Plaque Brachytherapy for Uveal Melanoma: Clinical and Histologic Findings

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Keywords

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Abstract

Background/Aims: Our study aims to better characterize the clinical and histopathologic features of eyes with extrascleral uveal melanoma associated with scleral melt following brachytherapy. **Methods:** A retrospective review was performed on patients who had undergone ¹²⁵I (iodine-125) brachytherapy for uveal melanoma at our institution between 1992 and 2015. Patients with postradiation scleral necrosis who required enucleation were identified, and an analysis including a review of histopathology results was performed on those cases. **Results:** A total of 301 patients underwent plaque brachytherapy for uveal melanoma, of whom 31 required eventual enucleation. The histologic analysis showed extraocular extension through full-thickness scleral discontinuities in 6 cases. All but 1 (5 of 6) of these eyes exhibited either mitotic figures or Ki-67 positivity. Mitotic figures were noted in 4 specimens, including 3 eyes exhibiting mitoses within or adjacent to the extrascleral portion of the tumor. Two eyes exhibiting mitoses, as well as the case with Ki-67 positivity, also had clinical evidence of tumor

regrowth. **Conclusion:** We found evidence of mitotic activity at the area of scleral discontinuity in some eyes with and in some without clinical evidence of tumor regrowth. Protruding pigmented material in areas of scleral necrosis after plaque brachytherapy may represent actively proliferating tumors even without internal evidence of tumor regrowth.

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Introduction

Scleral necrosis is a known complication of plaque radiotherapy for uveal melanoma, with a reported incidence of 0–33% [1–5]. Proposed mechanisms include a direct necrotizing effect of radiation on the sclera, a secondary effect due to local ischemic inflammation from muscle disinsertion, inflammation related to tumor necrosis, regression of the tumor with undiagnosed scleral invasion, and an occult systemic autoimmune phenomenon [2, 4, 6]. Factors found to be related to postradiation scleral necrosis include the initial tumor thickness, radia-

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Table 1. Tumor characteristics

Patient No.	Initial tumor thickness; largest initial basal diameter, mm	Location (by clinical examination)	AJCC tumor stage; grade	Size of plaque margin, mm	Scleral dose, Gy	Plaque-to-enucleation time, months	Cell type	GEP	Months of follow-up after plaque	Metastatic disease at last follow-up	Clinical evidence of tumor regrowth	Size of extrascleral extension: largest basal diameter; thickness, mm	Mitotic activity	Ki-67 expression
1	7.8; 11.5	MP	ypT2c; G2	3.25	–	94	M	1A	95	No	Yes ESE noted on B-scan and MRI	4; 3	No	Few positive
2	10.5; 15.0	CB + C	ypT4e; G2	2.5	505	19	M	2	32	No	Yes Increased size of pigmented extrascleral portion	13; 8.5	Yes	Not used
3	8.5; 17.8	C + CB	ypT4e; G2	2.1	382	46	M	2	51	No	Yes Increased internal thickness on B-scan	9; 1	Yes	Not used
4	9.0; 18.0	CB + C	ypT4d; G2	2	475	14	M	2	74	No	No	3; 1	No	Not used
5	5.0; 7.2	CB	ypT4e; G2	3.4	–	92	M	–	95	No	No	5; 1.5	Yes	Negative
6	3.1; 15.0	P	ypT4e; G3	3.5	–	42	E	1B	42	Yes	No	12; 9	Yes	Not used

MP, mid-peripheral choroid; CB, ciliary body; C, choroid; P, posterior choroid; E, predominantly epithelioid; M, mixed; GEP, gene expression profile; ESE, extrascleral extension; –, no data available; AJCC, American Joint Committee on Cancer Staging Manual, ed 7 [24].

tion dose to the tumor base, ciliary body/anterior location of the tumor, and intraocular pressure [2, 4, 5].

The diagnosis of scleral thinning and necrosis is made clinically, and the diagnostic dilemma lies in differentiating herniation of an inactive tumor through the necrotic sclera from active tumor regrowth. Some authors have observed that tumor recurrence is associated with a concurrent increase in basal diameter or thickness of the tumor and a tumor shadow on transillumination, and they do concede that there can be scleral thinning combined with tumor recurrence [5]. Currently, treatment options for scleral thinning and necrosis include observation and scleral or corneal patch grafts, with the aim of preventing perforation of the globe (reported in 4–9% of cases of post-plaque-brachytherapy scleral thinning) [4, 5].

Our study aims to better characterize the clinical and histopathologic features of patients with presence of an extrascleral tumor associated with scleral melt following brachytherapy.

Methods

After approval by our institution's review board, a retrospective review was performed on the clinical records of patients who had undergone ¹²⁵I plaque brachytherapy for uveal melanoma between 1992 and 2015, in order to identify a subset of patients with scleral necrosis ultimately necessitating enucleation. The radiation dose prescription was 85 Gy to the tumor apex for tumors ≥5 mm, and 85 Gy to the center for tumors ≤5 mm, and the plaques were sized to allow a 2- to 3-mm margin on all sides of the tumor. The histopathology including immunohistochemical staining that had been performed on the enucleated globes was reviewed. For each globe, there were 25–60 H&E (hematoxylin-eosin)-stained sections (each 3–5 μm thick) derived from 2–3 paraffin-embedded tissue blocks (the pupil-optic nerve section and one or both calottes). The presence of morphologically intact melanoma cells was confirmed with Mart-1/tyrosinase-red immunostaining. Ki-67 immunostaining had been carried out on 2 of the 6 samples. Extrascleral extension was defined as the presence of morphologically intact tumor cells outside of the sclera. Gene expression profiling (GEP) data were also reviewed.

Results

The clinical, histopathologic, immunohistochemical, and molecular diagnostic (GEP) data are reported in Table 1. There were 301 cases of uveal melanoma treated with ¹²⁵I plaque brachytherapy identified in our institutional record review from 1992 to 2015. The rate of post-brachytherapy scleral necrosis ultimately requiring enucleation was 2% (6/301).

Fig. 1. Fundus (a, b) and external (c, d) photographs of case 2. Before (a) and 11 months after plaque brachytherapy (b). Scleral necrosis with an episcleral nodule 7 months (c) and 19 months (d) after plaque brachytherapy.

For these 6 cases, the mean patient age at plaque placement was 66 years (range 54–83), and the average follow-up from plaque placement was 65 months (range 32–95), with metastatic disease in 1 patient. No patients had an ocular history of scleritis or diseases predisposing to scleral melt. The average initial tumor thickness was 7.1 mm (range 3.1–10.5). By clinical evaluation, 4 tumors were anterior (exclusively, predominantly, or partially involving the ciliary body), 1 tumor was mid-peripheral, and 1 was posterior (including the peripapillary component). Three eyes had clinical evidence of tumor regrowth. Of these, 1 exhibited extraocular extension by B-scan as well as MRI without change in the intraocular portion, 1 had an increased intraocular tumor thickness as measured by B-scan without evidence of external change (although this patient had had a scleral patch graft placed), and 1 exhibited an increased size of the external pigmented lesion (Fig. 1). GEP showed class 1A in 1 case, class 1B in 1 case, and class 2 in 3 cases; it had not been performed in 1 case. Histologic analysis showed extraocular extension

of morphologically intact tumor cells through full-thickness scleral discontinuities in all (6/6) cases. Scleral discontinuities occurred in the region covered by the plaque in all (6/6) cases, with 1 case (case No. 3) exhibiting an additional discontinuity at the anterior edge of the plaque site near the corneoscleral limbus. In 4 of the 6 cases, the extrascleral portion of the tumor measured at least 5 mm in maximal dimension.

The specimens had varying proportions of morphologically intact tumor that stained positive with Mart-1/tyrosinase-red. The cell type was predominantly epithelioid in 1 specimen, and mixed spindle/epithelioid in 5 specimens. Mitotic figures (though sparse) were noted in 4 specimens, including 3 eyes exhibiting mitoses within or adjacent to the extrascleral portion of the tumor (Fig. 2). Of the specimens exhibiting mitoses, 2 were in patients with, and 2 in patients without, clinical evidence of tumor regrowth. Ki-67 staining was available for 2 of the 6 samples. Of these, 1 was positive for proliferative activity (no mitotic figures had been detected in this case),

Fig. 2. H&E sections of the enucleated globe from case 2. **a** Low-power view showing extrascleral extension through 3 scleral discontinuities (arrows). **b** High-power view of the boxed area from **a** showing a mitotic figure (arrow) within the neck of the tumor in the region of the scleral melt.

and in this case there was also clinical evidence of tumor regrowth. In the other case, Ki-67 staining was negative despite the identification of occasional mitotic figures.

Discussion

Scleral necrosis as a complication of irradiation of ocular tissues has been described as far back as the 1950s [7, 8]. Necrosis after plaque brachytherapy of any kind for malignant intraocular tumors has been reported in 0–33% of patients [1–5]. For uveal melanomas treated with ^{125}I brachytherapy, necrosis was seen in 11% of patients with predominantly ciliary body involvement [2], while Shields et al. [3] reported necrosis in 7% at 5 years and 9% at 10 years in patients with large posterior uveal melanomas. In our series, the rate of post-plaque-brachytherapy scleral necrosis requiring enucleation was found to be 2% (6/301), with the majority of these tumors (4/6) involving the ciliary body. Because our clinical record search was not designed to hit all cases of post-plaque-brachytherapy scleral necrosis, but only those cases ultimately necessitating enucleation, the rate of all cases of post-brachytherapy scleral necrosis in our patient population is likely higher than 2%, similar to other published data. A case-control study by Kaliki et al. [4] found that cases with scleral necrosis were more likely to have larger initial basal tumor diameters; however, on multivariate analysis, factors predictive of scleral necrosis included an initial tumor thickness ≥ 6 –7 mm, a peripheral tumor location (ciliary body), and a radiation dose ≥ 400 Gy to the sclera

[2, 5]. These studies did not comment on whether there was a relationship between scleral necrosis and tumor regrowth or treatment failure. In our cohort, 4 of the 6 cases had an initial tumor thickness ≥ 6 mm, 4 of the 6 involved the ciliary body, and 2 of 3 cases in which scleral dosing information was available had a radiation dose ≥ 400 Gy. The highest scleral dose (505 Gy) was associated with the largest initial tumor thickness (10.5 mm) and the largest basal dimension of extraocular extension (13 mm).

Whether or not protrusion of pigmented material represents extrascleral extension of a growing tumor or benign herniation of an inactive tumor likely carries prognostic weight. Both extrascleral tumor extension and post-irradiation tumor recurrence are associated with an increased risk of metastasis and death [9–11]. Furthermore, the presence of extrascleral extension is associated with an increased risk of local orbital recurrence following enucleation (HR = 3.2; 95% CI: 1.5–6.7; $p = 0.003$) [11]. All specimens in our study exhibited extraocular extension of morphologically intact tumor cells through the necrotic sclera, and 5 of the 6 cases exhibited some evidence of tumor cell proliferation, suggesting that the extrascleral extension in these 5 cases signified some degree of tumor regrowth, therefore possibly conferring an increased risk of orbital recurrence, metastasis, and death. However, it is presently unknown whether extrascleral extension via post-brachytherapy regrowth of a tumor through the necrotic sclera carries the same prognostic significance as extrascleral extension of an untreated tumor via the traditional route of emissary canal invasion.

Furthermore, it is possible that proliferative activity in the absence of clinical evidence of tumor regrowth (of which there were 2 cases in our series) may not have the same prognostic significance as proliferative activity in the context of a clinically expanding tumor (of which there were 3 cases), as it may be presumed in the former scenario that the tumor is proliferating at an extremely slow rate. Additionally, the prognostic value of the size of post-plaque-brachytherapy extraocular extension for metastasis cannot be determined from such a small cohort of cases. Of the 2 cases with the largest volume of extrascleral extension, one was the only case with metastatic disease at the time of data acquisition (extraocular extension measured: 12 mm basal $\times$ 9 mm thickness), while in the other case no clinically detectable metastatic disease had yet developed (extraocular extension measured: 13 mm basal $\times$ 8.5 mm thickness).

Currently, it is posited that the overall natural history of post-plaque-brachytherapy scleral necrosis is a benign one, and that in most cases protruding pigmented material represents herniation of an inactive tumor rather than tumor regrowth. One case series found necrosis and the volume of extrascleral pigmented lesions to be stable in 75%, whereas a case-control study of 73 cases found necrosis to be stable in 48%, while it was increasing in size or severity in 48% [4, 5]. These studies focused primarily on the natural history of scleral necrosis rather than the histopathology. Histopathology results were not reported in the study by Kaliki et al. [5] (no biopsies were reported, and no histology was reported for the 4 eyes requiring enucleation – 1 for endophthalmitis and 3 for neovascular glaucoma and/or blind painful eye). An analysis was carried out for 5 of 23 eyes in the case series by Radin et al. [4], wherein the authors reported that they did not find evidence of tumor cells in any of the 3 biopsies taken of the suspicious episcleral pigmented lesions (only macrophages were seen), and they did not comment on the presence/absence of tumor cell proliferative activity on histopathology of the 2 enucleated eyes.

In cases of scleral melt after plaque brachytherapy, it is hypothesized that the radiation effect would render residual tumor cells unviable. In studies in which histopathologic analyses were performed on post-enucleation specimens without scleral melt, less mitotic activity was found in post-plaque-brachytherapy eyes compared to those primarily enucleated, even though the authors note that whether this represents a direct cytotoxicity/radiation effect or slower-growing tumors at baseline is difficult to determine [12–14]. Histologic examinations and in vitro studies of melanoma cell growth after pre-enu-

cleation external beam radiation have found decreased mitotic and Ki-67 activity, and decreased cell adhesion, growth, and viability in cell culture, attributable to the radiation effect [15–17]. Meanwhile, another study found no mitotic figures and furthermore found evidence of “reproductive cell death” (defined as increased doubling time) in 3 fresh post-irradiation samples submitted for cell culture [18] (though it is worth noting that none of the samples submitted for cell culture were from eyes enucleated for suspicion of tumor regrowth). However, some authors have found evidence of mitotic activity after plaque radiotherapy, particularly in eyes with clinical evidence of tumor regrowth [19]. In the current study, proliferative activity was noted in 5 of the 6 specimens (mitotic figures in 4, and Ki-67 positivity in 1), including 3 eyes exhibiting mitoses within or adjacent to the extrascleral portion of the tumor. Furthermore, 2 of the specimens exhibiting mitosis were in eyes without clinical evidence of tumor regrowth at the time of enucleation. These findings demonstrate that even in the absence of other clinical measures for regrowth, protrusion of pigmented material may in fact represent tumor growth, even though it is likely to be quite slow in such cases.

In addition to the presence of mitotic figures, Ki-67 immunostaining is used as a marker of cell proliferation [20]. In our series, Ki-67 staining had been performed on 2 of the 6 samples. Of these, 1 demonstrated positive evidence of cell proliferation that had not been detected via examination for mitotic figures, correlating with clinical evidence of tumor regrowth in this case. The GEP in this case was class 1A. In the other case, Ki-67 staining was negative despite the identification of sparse mitotic activity on H&E staining, thereby indicating that the rate of tumor cell proliferation was very low (presumably <1 cell, on average, per histologic section), correlating with the absence of clinical evidence of tumor regrowth in that case. GEP data were not available for that specimen. It was not entirely necessary to retrospectively perform Ki-67 staining in the remaining cases, as the presence of mitotic activity had already been established via mitotic figures on routine histology in 3 of the remaining 4 cases. Also of note, heavy scattering of melanin pigment, some of which may be engulfed by melanophages, was often found in such post-brachytherapy cases, and it was therefore anticipated that the brown-colored chromogen of the Ki-67 stain used in our pathology laboratory, despite being combined with Mart-1-red to identify melanocytes, would be quite challenging to interpret. The future acquisition of a combined Ki-67-blue/Mart-1-red immuno-

stain in our laboratory would help facilitate assessment of the cell proliferation index in such cases.

Not only has class 1B and 2 GEP been associated with a higher tumor-related mortality rate, as has the presence of epithelioid morphology on histology, but class 2 GEP is also associated with higher Ki-67 expression and tumor cell proliferation [21, 22]. Among the 5 cases in our series in which GEP information was available, 4 specimens exhibited either class 1B or class 2, correlating for the most part with the high prevalence of epithelioid cells in our cases (5 with mixed cell morphology, 1 predominantly epithelioid). Only 1 case developed metastatic disease by the time of data acquisition, and the specimens in this case exhibited class 1B GEP and predominantly epithelioid cell morphology. Given the relationship between class 2 GEP and proliferative index, it may be posited that pigmented tissue in the region of scleral necrosis may be more likely to represent an active tumor if the tumor has an epithelioid cell morphology and expresses class 2 (or class 1B) GEP. However, the pre-radiation proliferation rate may not correlate with the likelihood of post-radiation proliferation: Chappell et al. [23] found that post-radiation recurrence was not predicted by GEP, and the small size of the current series precludes elucidation of the true relationship between GEP and the likelihood of post-brachytherapy cell proliferation.

In summary, in our series of enucleated eyes with scleral necrosis after brachytherapy for uveal melanoma, we found evidence of tumor cell proliferative activity in some eyes with, and in some eyes without, clinical evidence of tumor regrowth, and in these cases, the proliferative activity was generally identified in the vicinity of scleral discontinuity. Protruding pigmented lesions in ar-

eas of scleral necrosis after plaque radiotherapy may represent herniation of an inactive tumor in the majority of cases, and a limitation of our study is that it included only cases culminating in enucleation, with corresponding histologic confirmation, and therefore our results cannot necessarily be extrapolated to all cases of clinically observed post-plaque-brachytherapy scleral melt. Our study does, however, establish that at least some cases of extra-scleral pigmented lesioning in the vicinity of post-plaque-brachytherapy scleral melt may represent actively proliferating tumors even without internal evidence of tumor regrowth. Considerations regarding the management of scleral necrosis following plaque brachytherapy for uveal melanoma should include not only clinical observations of tumor growth, but perhaps also the GEP status, along with tumor cell morphology.

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Statement of Ethics

This retrospective chart review study was undertaken with IRB approval (#201312030) from Washington University in Saint Louis, MO, USA.

Disclosure Statement

The authors have no conflicts of interest related to this topic.

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