Cellular Radiosensitivity of Primary and Metastatic Human Uveal Melanoma Cell Lines

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Purpose. To investigate the radiosensitivity of uveal melanoma cell lines by a clonogenic survival assay, to improve the efficiency of the radiation regimen.

Methods. Four primary and four metastatic human uveal melanoma cell lines were cultured in the presence of conditioned medium. After single-dose irradiation (0–12 Gy), colonies were allowed to form for 6 to 14 days. Two cutaneous melanomas cell lines were also tested for comparison. The survival curves were analyzed by the linear quadratic (LQ) model, and the surviving fraction at a dose of 2 Gy (SF_2), the SF at 10 Gy (SF_{10}), the ratio of initial irreparably damaged DNA (α-coefficient) to the capacity to repair sublethally damaged DNA (β-coefficient), and the plating efficiency were calculated.

Results. The melanomas displayed a wide range of initial irreparable DNA damage (α-component), as well as a capacity for repair of sublethal DNA damage (β-component), which ultimately resulted in a wide range of α/β ratios. These findings were similar in both primary and metastatic melanomas and were comparable with data obtained from two cutaneous melanomas.

Conclusions. Cell lines obtained from primary and metastatic human uveal melanomas displayed a wide range of radiosensitivity, similar to that published for cutaneous melanomas. Translating these data to the clinical setting indicates that a fractionated dose of 8 to 10 Gy administered in three to four fractions, as currently delivered in many centers, should be sufficient to eradicate tumors of approximately 1 cm^3. (*Invest Ophthalmol Vis Sci.* 2002;43:2561–2565)

Uveal melanomas are the most common primary intraocular malignancies in adults in the Western world, with an annual incidence of six cases per million.[1] Radiotherapy is commonly used as the first choice for treatment of small- and medium-sized melanomas and offers patients an eye- and vision-sparing alternative to enucleation. The introduction of plaque radiotherapy has stimulated the search for an optimal radiation dose to improve local control rates and reduce side effects.[2–5] High local control rates of more than 90% have been achieved with this modality,[6,7] but failures of up to 25% have also been reported.[8,9] Factors such as radiation dose, dose rate, tumor size, tumor location, and dose distribution within the tumor all influence the clinical outcome.[10,11]

External beam radiotherapy has also been tested, including proton beam irradiation,[12] helium ion therapy,[13] and stereotactic irradiation with a gamma knife[14,15] or a linear accelerator.[16] With these modalities, again, high local control rates of more than 90% have been achieved.[13,16] Despite this range of radiotherapy modalities and high local control rates, an average of 16% to 20% of the patients undergo subsequent enucleation, due to local tumor recurrence or uncontrollable neovascular glaucoma.[6] However, the majority of these enucleations (80%) are due to radiotherapy-related complications.[13] Acute and late complications of ocular radiation have been reviewed extensively by Finger.[6] Treatment of the posteriorly located tumors with plaque radiotherapy has been primarily associated with such complications as retinopathy and optic neuropathy. In contrast, after external beam irradiation, complications of the adnexal and anterior ocular segments are more common.

To reduce side effects without diminishing tumor control rates, studies are needed to optimize the effectiveness of radiation treatment. However, little knowledge is available on the intrinsic cellular radiosensitivity of uveal melanomas. A better understanding of the radiosensitivity of these tumors would improve the effectiveness of radiation schedules implemented by ocular and radiation oncologists.

Much more is known about intrinsic cellular radiosensitivity in cutaneous melanomas. Rofstad[17] reviewed a large number of in vitro and in vivo studies of malignant cutaneous melanomas and reported a wide variation in radiosensitivity. This variation could be explained partially by tumor size, but differences in intrinsic radiosensitivity were also a prominent feature.

So far, only limited data have been published[18–20] on the cellular radiosensitivity of uveal melanoma cell lines treated with external beam irradiation. In this article, we present data on the cellular radiosensitivity of four primary and four metastatic human uveal melanoma cell lines, as well as data for two malignant cutaneous melanoma cell lines.

Materials and Methods

Cell Lines and Culture Conditions

In total, four primary uveal melanomas—OCM-1,[21] Mel202, Mel270,[22] and 92-1[23]—and four metastatic uveal melanomas—OMM1,[24] OMM2-2, OMM2-3, and OMM2-6—were tested together with the human cutaneous melanomas MelSK28 and Bowes (CRL-9607; American Type Culture Collection [ATCC], Manassas, VA). Exponentially growing cultures were harvested and diluted in six-well plastic plates at the required concentrations and incubated overnight before irradiation. After irradiation, uveal melanoma cells were grown in RPMI 1640 medium with 10% fetal calf serum (FCS) diluted with conditioned medium at 1:1 (vol/vol). Conditioned medium was collected from passage of stock cultures and sterilized by filtration through a 0.22-μm pore size membrane (Millipore, Bedford, MA). Cutaneous melanoma

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TABLE 1. Parameters for Uveal and Cutaneous Melanomas after X-irradiation for Cells Cultured in Conditioned Medium

Cell Type*	Plating Efficiency (%)†	α-Coefficient (Gy^{-1})	β-Coefficient (Gy^{-2})	α/β Ratio (Gy)‡	SF_2	$SF_{10} \times 10^4$
Uveal melanoma						
OCM-1						
(100–3,200)	38.9 ± 2.7	−0.07761	−0.05405	1.5 ± 0.2	0.69	20.67
Mel 270						
(800–25,600)	12.5 ± 3.0	−0.55204	−0.02137	26.2 ± 2.9	0.30	4.72
OMM2–2						
(200–6,400)	50.1 ± 4.0	−0.35727	−0.04056	9.3 ± 1.0	0.42	4.86
OMM2–3						
(400–12,800)	18.8 ± 1.9	−0.32630	−0.02799	12.7 ± 1.5	0.48	23.29
OMM 2–6						
(400–12,800)	30.6 ± 5.7	−0.27427	−0.03296	9.2 ± 1.2	0.51	23.84
Mel202						
(200–25,600)	25.7 ± 6.9	−0.30645	−0.03078	10.2 ± 0.7	0.48	21.49
92-1						
(400–12,800)	4.0 ± 1.6	−1.82498	—	—	0.026	0.00012
OMM-1						
(800–102,400)	13.2 ± 1.0	−0.67356	—	—	0.26	11.88
Cutaneous melanoma						
MelSK28						
(200–6,400)	29.5 ± 3.2	−0.15871	−0.02933	6.0 ± 1.0	0.65	109
Bowes						
(400–3,200)	60.3 ± 9.7	−0.18841	−0.11696	1.6 ± 0.4	0.43	0.0126

* Range of cells plated per well shown in parentheses.
† Data represent mean ± SEM from cell survival curves, obtained from at least three separate experiments with various cell concentrations.
‡ Mean ± SEM.

cells were cultured in DMEM containing 10% FCS. Cells were grown for 6 to 14 days to allow colonies to form. At 7 days after irradiation, the medium was refreshed.

A wide range in plating efficiency was observed in both the primary and the metastatic uveal melanoma cells. In the presence of conditioned medium, higher plating efficiencies were obtained compared with cells cultured in standard medium (data not shown). Increasing increments of cell concentration did not improve the plating efficiency in all cell lines used, which suggests that the given cell concentrations in conjunction with conditioned medium produced an optimal result.

Irradiation and Clonogenic Assay

Two six-well plastic plates containing different cell lines were placed next to each other and irradiated simultaneously in an 18 × 24-cm field at a dose rate of 0.66 Gy/min using a 200-kV x-ray machine operating at 20 mA. The x-ray machine contained a 1-mm Cu filter resulting in a half value layer (HVL) of 1.6 mm Cu. Delivered doses were checked with thermoluminescent dosimetry (TLD), which indicated a less than 3% dose variation over the irradiated field. Single doses of 0 to 12 Gy were used, with one six-well plate per dose level containing two consecutive levels of cell concentration. After irradiation, cells were incubated for logarithmic growth of the colonies. After culturing, cells were fixed with methanol-acetic acid 3:1 (vol/vol) for 10 minutes followed by staining with hematoxylin. Colonies were counted under the microscope at a magnification of ×40. Colonies containing more than 50 cells were regarded as positive.

Data Analysis

For each dose point, the number of positive colonies obtained from three wells was averaged. These mean values were corrected for the plating efficiency and used to calculate the cell survival for each dose level (Excel; Microsoft, Redmond, WA). On the basis of these cell survival data, cell survival curves were computer fitted to the linear quadratic (LQ) model, by using the Slide Write Plus program (Advanced Graphics Software, Inc., Encinitas, CA). In this LQ model,[25–27] the surviving fraction (SF) is related to total dose (D) according to $SF = \exp(-\alpha D - \beta D^2)$. The α-coefficient is regarded as an estimate of the initial irreparable DNA damage, and the β-coefficient represents the capacity for DNA repair of sublethal damage. Both components are cell and tissue specific.

Because fewer colonies were counted at higher doses, the relative contribution of each dose level to the cell survival curve was estimated, by using a weighting factor: (total number of plated cells × number of colonies)/(total number of plated cells − number of colonies).

Data for each cell line from at least three experiments were averaged for each cell concentration (Table 1). At the various cell concentrations, best-fitting values for α- and β-coefficients and for the SF at 2 Gy (SF_2) and at 10 Gy (SF_{10}) were calculated as estimates of the intrinsic radiosensitivity of each cell line. The α- and β-coefficients were used to derive the α/β ratio, the dose at which the contribution of the linear and quadratic components are equal. For each cell survival parameter, possible differences related to cell concentration were examined with Student's *t*-test for significance. If no significant difference with change in cell concentration was reached (two sided, $P > 0.05$), data were averaged.

RESULTS

The cell survival curves for the various uveal and cutaneous melanomas are presented in Figure 1. The parameters associated with these survival curves are presented in Table 1. The plating efficiency of these cell lines demonstrated a wide variation and ranged from as low as 4% (92-1) to 50% to 60% (Mel2-2 and Bowes). For all cell lines tested, the cell survival parameters listed in Table 1 did not show significant differences at different levels of cell concentration. Therefore, for each parameter we combined the data obtained from experiments at different cell concentrations. In all cases the plating efficiency had no influence on the α- or the β-coefficients or on the α/β ratios (correlation coefficient < 0.3).

A wide range of α-coefficients was found, with very high and low values for OCM-1 ($-0.07761\ Gy^{-1}$) and for 92-1 ($-1.82498\ Gy^{-1}$), respectively. Estimates for the α-coefficients are reflected in the SF_2, with high levels of survival indicative of radioresistant cell lines and lower survival of the more radiosensitive cell lines. The calculated β-coefficients ranged

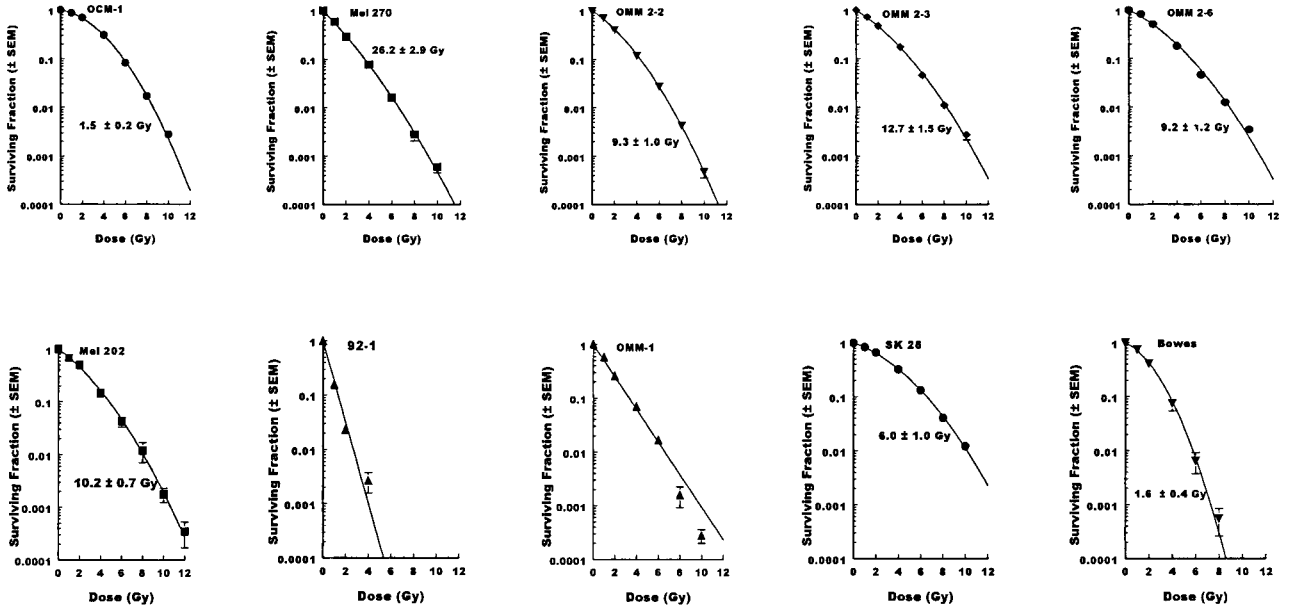

FIGURE 1. Cell survival curves for various primary (OCM-1, Mel 270, Mel 202, 92-1) and metastatic (OMM2-2, OMM2-3, OMM2-6, OMM-1) uveal melanomas and cutaneous melanomas (MelSK28, Bowes). Cells were cultured in standard medium in the presence of conditioned medium (1:1 vol/vol). If standard errors are not visible, they fall within the symbol. Data are survival ratios ± SEM.

from -0.02 Gy^2 to -0.05 Gy^2, except for the cutaneous melanoma Bowes cell line (Table 1). This resulted in α/β ratios of approximately 10 Gy for both primary and metastatic uveal melanomas, except for OCM-1, for which a low α/β ratio of 1.5 Gy was calculated, which indicated the cells were spared by dose fractionation. In contrast, for Mel270 a very high α/β ratio of 26.2 Gy was obtained in conjunction with a low SF_2, suggesting a relatively radiosensitive cell line and a low SF after dose fractionation. For the cell lines OMM-1 and 92-1, a very low α-component was obtained, but no value could be determined for the β-component; hence, no α/β ratio was calculated (Table 1). This resulted in very low SF_2 and SF_{10}, indicating that these cell lines were very radiosensitive.

In both cutaneous melanomas, estimates of the α-coefficient were very similar but much higher than that of most of the uveal melanoma cell lines. However, the β-components of the cutaneous and uveal cell lines differed by a factor of approximately 4, with a much lower value for Bowes (-0.11696 Gy^{-2}). This resulted in low α/β ratios of 6.0 Gy and 1.6 Gy for MelSK28 and Bowes, respectively.

DISCUSSION

Radiotherapy is commonly used as the primary choice of treatment for small- and medium-sized human uveal melanomas, because of high local control rates and the potential sparing of the eye and vision. Currently, a range of radiotherapy modalities is applied, but knowledge is still lacking concerning the cellular radiosensitivity and hence the dose per fraction and number of fractions required for a curative regimen. Limited data have been published on the intrinsic radiosensitivity of uveal melanoma cell lines.[18–20]

In this report, we present data regarding the radiosensitivity of primary and metastatic human uveal melanoma cell lines, treated with external beam irradiation and compare them with data obtained in cutaneous melanoma cell lines.

Although caution must be exercised in using data obtained from these in vitro studies in clinical practice, studies have shown that in vitro survival curves of many tumor cell lines seem to resemble the radiation sensitivity of these tumors in vivo.[28] Especially parameters related to the initial part of the cell survival curve, such as the α-component and SF_2, correlated with the clinical radioresponsiveness of human tumors.[28]

The cell lines displayed a wide range of α/β ratios in both the primary and metastatic melanomas. In general cells with a low α/β ratio are spared by dose fractionation and by low-dose-rate brachytherapy, yielding lower control rates, and thus these cells would be better treated with hypofractionation. However, a hypofractionation radiation regimen has to be balanced against adverse ocular normal tissue toxicity. A low α/β ratio was found in the uveal cell line OCM-1 and in the cutaneous lines MelSK28 and Bowes, which indicated they were spared by dose fractionation. For these cell lines, a relatively high α-component was calculated, as reflected in the high SF_2 levels, which were indicative of radioresistance. A similar high radioresistance was reported in other uveal melanomas, with SF_2 higher than 0.95, and even a positive α-component was calculated; hence, an estimate for the α/β ratio could not be determined.[17,18]

For cutaneous melanomas, a wide range of α/β ratios, from 0.5 to 60 Gy, have been reported.[17,29] Approximately 66% of the presented cases had α/β ratios less than 10 Gy,[17] suggesting that higher-than-conventional doses per fraction would be required for effective treatment. Our results in cutaneous melanomas are in agreement with these published data. Analysis of clinical data for human cutaneous melanomas and for lymph node lesions with histologically verified recurrent or metastatic malignant melanoma, irradiated with doses per fraction ranging from 1.8 to 10 Gy, showed a low α/β ratio of 0.57 Gy.[30] This analysis supported the necessity of higher-than-conventional doses per fraction of approximately 6 Gy recommended for this type of tumor.

Cell lines displaying high α/β ratios have a reduced DNA repair capacity, and thus cell survival is less influenced by dose fractionation. In this situation hyperfractionation or low-dose brachytherapy would be a viable option, because there is preferential sparing of normal tissue cells over tumor cells. The metastatic cell lines OMM2-2, OMM2-3, and OMM2-6 had similar, relatively high, α/β ratios (>9 Gy). All three metastatic cell lines were derived from the primary cell line Mel270, which

itself had an even higher α/β ratio of approximately 26 Gy. The Mel270 cell line was obtained from a recurrence after radiotherapy and enucleation became inevitable. This indicated that radiotherapy was inadequate, resulting in regrowth of original tumor cells, or that preferentially radioresistant tumor cells survived. A high α/β ratio in conjunction with a low SF_2 suggests that the former of the two options is the most likely.

For two cell lines (92-1 and OMM-1), no α/β ratio could be calculated, because the β-component was infinite small, indicating that repair of sublethal DNA damage was virtually absent. These two cell lines appeared very radiosensitive with very low α-components and SF_2. Although repair of DNA damage hardly occurred in these tumor cells, rendering dose fractionation ineffective, dose fractionation has a substantial advantage in reducing normal tissue toxicity.

The β-coefficient in all cell lines ranged from -0.02 to -0.05 Gy^{-2}, except for the cutaneous melanoma line Bowes, which showed a very low coefficient (-0.117 Gy^{-2}). This low β-component indicated a lower level of cell survival compared with that for OCM-1, which had a very similar low α/β ratio. Thus, for the Bowes cell line, dose fractionation would be appropriate (low α/β ratio), and smaller doses per fraction would be adequate (low β-component and SF_2).

From these data, it can be calculated that for the more radioresistant uveal cell lines, single doses of approximately 17 to 20 Gy are necessary to eradicate 10^9 cells, which is the equivalent of a tumor of approximately 1 cm^3. Based on the LQ model it is calculated that dose fractionation amounting to three to four fractions of 8 to 10 Gy is required for elimination of 10^9 cells. Thus, the clinically applied schedule of 10 Gy in five fractions should be adequate for tumors of this size.

Several factors, such as apoptosis, cell cycle disruptions, or a combination of these factors, could be underlying mechanisms for the wide range of radiosensitivity observed in the uveal melanomas. Cell cycle disruption, and in particular loss of the G_2-cell cycle checkpoint due to mutations and DNA methylation in the *p16* gene, could influence radiosensitivity. A *p16* mutation was reported in uveal melanomas[31] that correlated with increased radiosensitivity after UV irradiation. In recent publications on cutaneous melanomas, absence of the tumor-suppresser gene *p16*, a cell cycle inhibitor gene,[32] correlated with increased intrinsic radioresistance,[33]—a phenomenon also demonstrated in other tumor cell lines.[34] Similar *p16* mutations in uveal melanomas could correlate with changes in radiosensitivity after ionizing radiation. Nauss NC, et al. (personal communication, 2002) reported mutations in and DNA methylation of the *p16* gene in several of the uveal melanoma cell lines. Mel270 and its metastatic cell lines OMM2-2, OMM2-3, and OMM2-6, as well as 92-1 and OMM-1, are all methylated in the *p16* gene. The cell line OCM-1 appears to have a heterozygous mutation, whereas Mel202 contains a homozygous mutation in the *p16* locus. In the cutaneous melanoma MelSK28, the *p16* gene is not methylated[35] and does not contain mutations. Therefore, it can be concluded that DNA methylation of the *p16* gene does not correlate with radiosensitivity in these uveal melanoma cell lines after ionizing radiation.

A higher capacity for sublethal DNA damage repair after radiation would improve cell survival and consequently would be reflected in increased radioresistance. The capacity for DNA damage repair can be studied in more detail by using dose fractionation. In two cell lines, the process of DNA damage repair appeared to be absent; hence, a detailed dose fractionation study for all the uveal human melanoma cell lines examined in this study is currently in progress.

References

1. Egan KM, Seddon JM, Glynn RJ, Gragoudas ES, Albert DM. Epidemiologic aspects of uveal melanoma. *Surv Ophthalmol.* 1988;32:239–251.
2. Lommatzsch PK. Results after beta-irradiation (106Ru/106Rh) of choroidal melanomas: twenty years' experience. *Am J Clin Oncol.* 1987;10:146–151.
3. Potter R, Janssen K, Prott FJ, et al. Ruthenium-106 eye plaque brachytherapy in the conservative treatment of uveal melanoma: evaluation of 175 patients treated with 150 Gy from 1981–1989. *Front Radiat Ther Oncol.* 1997;30:143–149.
4. Tjho-Heslinga RE, Davelaar J, Kemme HM, et al. Results of ruthenium irradiation of uveal melanomas: the Dutch experience. *Radiother Oncol.* 1999;53:133–137.
5. Seregard S. Long-term survival after ruthenium plaque radiotherapy for uveal melanoma: a meta-analysis of studies including 1,066 patients. *Acta Ophthalmol Scand.* 1999;77:414–417.
6. Finger PT. Radiation therapy for choroidal melanoma. *Surv Ophthalmol.* 1997;42:215–232.
7. De Potter P, Shields CL, Shields JA. et al. Impact of enucleation versus plaque radiotherapy in the management of juxtapapillary choroidal melanoma on patient survival. *Br J Ophthalmol.* 1994;78:109–114.
8. Lommatzsch PK, Lommatzsch R. Treatment of juxtapapillary melanomas. *Br J Ophthalmol.* 1991;75:715–717.
9. Packer S, Stoller S, Lessor ML, et al. Long-term results of 125I irradiation of uveal melanoma. *Ophthalmology*. 1992;99:767–774.
10. Char D, Quivey J, Castro J, Kroll S, Philips T. Helium ions versus iodine 125 brachytherapy in the management of uveal melanoma: a prospective, randomized, dynamically balanced trial. *Ophthalmology.* 1993;100:1547–1554.
11. Quivey JM, Augsburger J, Snelling L, Brady LW. 125I plaque therapy for uveal melanoma: analysis of the impact of time and dose factors on local control. *Cancer* 1996;77:2356–2362.
12. Egger E, Zografos L, Munkel G, Bohringer T, Bercher L, Chamot L. Results of proton radiotherapy for uveal melanomas. *Front Radiat Ther Oncol.* 1997;30:111–122.
13. Char DH, Kroll SM, Castro J. Ten-year follow-up of helium ion therapy for uveal melanoma. *Am J Ophthalmol.* 1998;125:81–89.
14. Marchini G, Gerosa M, Piovan E, et al. Gamma knife stereotactic radiosurgery for uveal melanoma: clinical results after 2 years. *Stereotact Funct Neurosurg.* 1996;66(suppl 1):208–213.
15. Logani S, Helenowski TK, Thakrar H, Pothiawala B. Gamma knife radiosurgery in the treatment of ocular melanoma. *Stereotact Funct Neurosurg.* 1993;61(Suppl 1):38–44.
16. Zehetmayer M, Kitz K, Menapace P, et al. Local tumour control and morbidity after one to three fractions of stereotactic external beam irradiation for uveal melanoma. *Radiother Oncol.* 2000;55:135–144.
17. Rofstad EK. Radiation biology of malignant melanoma. *Acta Radiol Oncol.* 1986;25:1–10.
18. Logani S, Cho AS, Ali BH, et al. Single-dose compared with fractionated-dose radiation of the OM431 choroidal melanoma cell line. *Am J Ophthalmol.* 1995;120:506–510.
19. Logani S, Cho AS, Su LD, et al. Effects of gamma radiation on the OM431 human ocular melanoma cell line. *Exp Eye Res.* 1995;60:603–605.
20. Soulières D, Rousseau A, Tardif M, et al. The radiosensitivity of uveal melanoma cells and the cell survival curve. *Graefes Arch Clin Exp Ophthalmol.* 1995;233:85–89.
21. Kan-Mitchell J, Mitchell MS, Rao N, Liggett PE. Characterization of uveal melanoma cell lines that grow as xenografts in rabbit eyes. *Invest Ophthalmol Vis Sci.* 1989;30:829–834.
22. Chen PW, Murray TG, Salgaller ML, Ksander BR. Expression of MAGE genes in ocular melanoma cell lines. *J Immunother.* 1997;20:265–275.
23. De Waard-Siebinga I, Blom DJ, Griffioen M, et al. Establishment and characterization of an uveal-melanoma cell line. *Int J Cancer.* 1995;62:155–161.
24. Luyten GP, Naus NC, Mooy CM, et al. Establishment and characterization of primary and metastatic uveal melanoma cell lines. *Int J Cancer.* 1996;66:380–387.

25. Douglas BG, Fowler JF. The effect of multiple small doses of x rays on skin reactions in the mouse and a basic interpretation. *Radiat Res.* 1976;66:401–426.
26. Dale RG. The application of the linear-quadratic dose-effect equation to fractionated and protracted radiotherapy. *Br J Radiol.* 1985;58:515–528.
27. Thames HD, Withers HR, Peters LJ, Fletcher GH. Changes in early and late radiation responses with altered dose fractionation: implications for dose-survival relationships. *Int J Radiat Oncol Biol Phys.* 1982;8:219–226.
28. Malaise EP, Fertil B, Chavaudra N, Guichard M. Distribution of radiation sensitivities for human tumor cells of specific histological types: comparison of in vitro to in vivo data. *Int J Radiat Oncol Biol Phys.* 1986;12:617–624.
29. Marshall ES, Matthews JH, Shaw JH, et al. Radiosensitivity of new and established human melanoma cell lines: comparison of [3H]thymidine incorporation and soft agar clonogenic assays. *Eur J Cancer.* 1994;30A:1370–1376.
30. Bentzen SM, Overgaard J, Thames HD, Overgaard M, et al. Clinical radiobiology of malignant melanoma. *Radiother Oncol.* 1989;16:169–182.
31. Milligan A, Gabrielli BG, Clark JM, Hayward NK, Ellern KA. Involvement of p16CDKN2A in cell cycle delays after low dose UV irradiation. *Mutat Res.* 1998;422:43–53.
32. Piepkorn M. Melanoma genetics: an update with focus on the CDKN2A(p16)/ARF tumor suppressors. *J Am Acad Dermatol.* 2000;42:705–722.
33. Matsumura Y, Yamagishi N, Miyakoshi J, Imamura S, Takebe H. Increase in radiation sensitivity of human malignant melanoma cells by expression of wild-type p16 gene. *Cancer Lett.* 1997;115:91–96.
34. Fu XY, Zhang SW, Ran RQ, Shen ZH, Gu JX, Cao SL. Restoration of the p16 gene is related to increased radiosensitivity of p16-deficient lung adenocarcinoma cell lines. *J Cancer Res Clin Oncol.* 1998;124:621–626.
35. Gonzalgo ML, Bender CM, You EH, et al. Low frequency of p16/CDKN2A methylation in sporadic melanoma: comparative approaches for methylation analysis of primary tumors. *Cancer Res.* 1997;57:5336–5347.