

# Characterization of three cell lines derived from fine needle biopsy of choroidal melanoma with metastatic outcome

Barry L. Burgess,<sup>1</sup> Nagesh P. Rao,<sup>2</sup> Ascia Eskin,<sup>3</sup> Stanley F. Nelson,<sup>3</sup> Tara A. McCannel<sup>1</sup>

<sup>1</sup>Department of Ophthalmology, University of California, Los Angeles, The Jules Stein Eye Institute, Los Angeles, CA; <sup>2</sup>Department of Pathology and Laboratory Medicine, University of California, Los Angeles, Los Angeles, CA; <sup>3</sup>Department of Human Genetics, University of California, Los Angeles, Los Angeles, CA

**Purpose:** To report three low-passage cell lines from primary choroidal melanoma with metastatic outcome, which were stable for cytogenetic patterns and expression profiles of the primary melanoma.

**Methods:** In patients with choroidal melanoma, transscleral fine needle aspiration biopsy (FNAB) was performed immediately before plaque placement for <sup>125</sup>iodine brachytherapy or immediately after enucleation. Cells were examined for cytopathology, evaluated by fluorescence in-situ hybridization (FISH) for the centromere of chromosome 3, analyzed by 250K whole genome Mapping Array and U133 plus 2.0 Expression Array, and placed in cell culture. At passage 3, the cell lines were analyzed by Mapping Array and Expression Array.

**Results:** Three cell lines were propagated from primary choroidal melanomas in three patients who subsequently developed metastasis. Two cell lines were stable for the entire chromosomal aberration pattern of the respective primary tumor. In the third, necrotic material from the biopsy prevented further analysis, yet resulted in a stable cell line. Each cell line had chromosome 3 loss, 6q loss, 8p loss, multiple 8q gain, and 16q loss. Additionally, two cell lines had chromosome 6p gain. Two cell lines had RNA expression profiles similar to the respective primary tumors; the third cell line had a similar RNA expression profile relative to the other two cell lines.

**Conclusions:** FNAB of primary choroidal melanomas resulted in highly characterized, low-passage cell lines, which were stable for the cytogenetic patterns and expression profiles found in the primary tumor. These cell lines represent novel tools for the study of metastatic choroidal melanoma biology.

Choroidal melanoma (melanoma arising primarily from the ciliary body or choroid) is the most common primary intraocular malignancy in adults. Despite treatment with brachytherapy, external beam radiation or enucleation, long-term follow-up is associated with melanoma-related death in approximately 50% of patients [1].

Most strongly correlated with increased risk of metastasis are cytogenetic aberrations and gene expression abnormalities in melanoma cells [2-5]. Loss of one copy of chromosome 3 (monosomy 3), loss of one copy of chromosome 8p and class II gene expression profile are most strongly associated with a high-risk of metastasis [4,6-10].

Melanoma cytogenetic abnormalities provide information regarding the fundamental molecular biology of choroidal melanoma and may uncover potential genes for targeted therapy [11,12]. Recognizing the potential value of studying melanoma cytogenetic aberrations and associated gene expression, this report presents development and characterization of low-passage choroidal melanoma cell lines that were stable for the cytogenetic patterns and gene expression profiles found in primary choroidal melanomas that resulted in metastatic disease.

## METHODS

All studies were performed in accordance with the United States Health Insurance Portability and Accountability Act (HIPAA) of 1996. All participants gave informed consent and all studies were approved by the Office of the Human Research Protection Program (Institutional Review Board) of the University of California, Los Angeles, Los Angeles, CA.

**Tissue collection:** In patients with primary choroidal melanoma and no clinical evidence of metastasis, transscleral fine needle aspiration biopsy (FNAB) was performed immediately before plaque placement for <sup>125</sup>iodine brachytherapy or immediately after enucleation. As described elsewhere, cells obtained from the biopsy were examined for cytopathology, evaluated by fluorescence in-situ hybridization (FISH) for the centromere of chromosome 3, analyzed by 250K Mapping Array (Affymetrix, Santa Clara, CA) for chromosomal copy number variation, U133 plus 2.0 Arrays (Affymetrix) for gene expression, and placed in cell culture [11-13].

**Cell culture:** Triturated biopsy cells were cultured in a growth medium of DMEM, 10% human AB serum, 5 µg/ml bovine insulin and glutamine-pen-strep at 37 °C, 7% CO<sub>2</sub>. Primary cultures were seeded in 12.5 cm<sup>2</sup> vented flasks and grown to confluency (about 12 weeks) replacing half of the medium every 3 days.

---

Correspondence to: Tara A. McCannel, M.D., Ph.D., 100 Stein Plaza, Los Angeles, CA, 90095; Phone: (310) 206-7484; FAX: (310) 794-7904; email: TMcCannel@jsei.ucla.edu

TABLE 1. CHOROIDAL MELANOMA CELL CULTURES.

	Number
<b>59 Melanomas with Chromosome 3 Loss</b>	
Successful propagation and characterization of cell lines	3 (5%)
Successful early propagation but not yet characterized	6 (10%)
Failure to propagate beyond first passage	40 (68%)
Failure to propagate	10 (17%)
<b>42 Melanomas with Chromosome 6p Gain/Disomy 3</b>	Number
Failure to propagate	42 (100%)

These spontaneous cultures were expanded into 75 cm<sup>2</sup> flasks and evaluated at passage 3. Briefly, trypsinized cells were stabilized in RNAProtect Cell Reagent (Qiagen, Valencia, CA) and analyzed by 250K Mapping Array and U133 plus 2.0 Expression Array (Affymetrix) as described hereafter. Beginning at passage 3, portions of the cell lines were cryogenically preserved for use in future studies and passaged in cell culture to document the characteristics of continued proliferation.

**Nucleic acid analyses:** Genomic DNA and total RNA were sequentially isolated from trypsinized cell cultures using an AllPrep DNA/RNA Mini Kit (Qiagen). Isolated DNA was quantified using a NanoDrop ND-1000 Spectrophotometer (NanoDrop, Wilmington, DE). No DNA sample was subjected to whole genome amplification techniques. DNA copy number was assessed using GeneChip Human 250K NSPI Mapping Arrays (Affymetrix). Probe preparation, hybridization, and reading were performed by the UCLA DNA Microarray Core according to the standard 96-well protocol published by Affymetrix. Copy number variation was computed using Genome Console software from Affymetrix [11].

RNA was quantified on a NanoDrop Spectrophotometer and analyzed on a 2100 Bioanalyzer (Agilent, Santa Clara, CA) for integrity. RNA had an A260/280 ratio of >1.90 and RNA integrity number (RIN) of 8.5 or higher as determined by the 2100 Bioanalyzer. Prepared RNA was hybridized to GeneChip Human Genome U133 Plus 2.0 Arrays (Affymetrix) at the UCLA DNA Microarray Core Facility using the standard Affymetrix protocol.

**GNAQ mutation:** Additionally, the nucleotide sequence of exon 5 of the guanine nucleotide binding protein (G protein) q polypeptide (*GNAQ*) was determined for both cell line genomic DNA and available germ line DNA from blood samples via PCR and sequencing on an ABI 3130 Genetic Analyzer using BigDye 2.0 chemistry (Life Technologies, Carlsbad, CA), and intronic sequence primers 5'-TTC CCT AAG TTT GTA AGT AGT GC-3' and 5'-AGA AGT AAG TTC ACT CCA TTC C-3' encompassing exon 5 of *GNAQ*.

## RESULTS

Cell cultures from choroidal melanomas with chromosome 3 loss (monosomy 3) showed variable propagation (Table 1).

Among choroidal melanomas with chromosome 6p gain in the absence of chromosome 3 loss, all cell cultures failed to propagate under the growth conditions employed. Each of the three cell lines were derived from two pooled fine needle aspirates containing approximately 110, 500, or 350 viable cells for MEL20-06-039, MEL20-06-045, or MEL20-07-070, respectively, as determined by counts of adherent cells obtained 24 h after initial seeding in a 12.5 cm<sup>2</sup> flask. In each case epithelioid morphology was initially observed. Macrophages and erythrocytes were present initially, but rapidly degraded. No fibroblasts were observed. At confluency, each culture was expanded into a 75 cm<sup>2</sup> flask. All subsequent passages were split 1:3 in 75 cm<sup>2</sup> flasks with 1×10<sup>7</sup> cells removed and aliquoted for cryopreservation.

The three characterized cell lines with monosomy 3 were from patients with choroidal melanoma who developed clinical evidence of melanoma metastasis <1.5 years after primary melanoma diagnosis and treatment; all three patients subsequently died from liver failure due to metastatic disease. All three cell lines demonstrated stable propagation through at least passage 6 over a period of 2.5 years. Two of the three cell lines persisted in melanin production beyond passage 6 and none of the cultures exhibited contact inhibition in overgrowth conditions (Figure 1).

Cytogenetically, two of the three cell lines (MEL20-06-039 and MEL20-07-070) demonstrated the chromosomal aberration pattern present in the respective primary melanoma, including monosomy 3, 6q loss, 8p loss, multiple 8q gain and 16q loss (Figure 2). The third cell line (MEL20-06-045), derived from a biopsy that showed necrotic material, had a chromosomal aberration pattern that was nearly identical with the other two cell lines. Additionally, the MEL20-06-045 cell line and both the MEL 20-07-070 cell line and primary tumor exhibited chromosome 6p gain. Moreover, two of the cell lines (MEL20-06-039 and MEL20-07-070) had RNA expression array characteristics similar to the respective primary tumor for the 25 most overexpressed and the 20 most under-expressed genes from a comparative gene list composed of genes most overexpressed and under-expressed in an integrated analysis of choroidal melanoma with chromosome 3 loss versus 6p gain in the absence of chromosome 3 loss (Figure 3A,B) [11]. The third cell line (MEL20-06-045) had a similar profile of gene expression



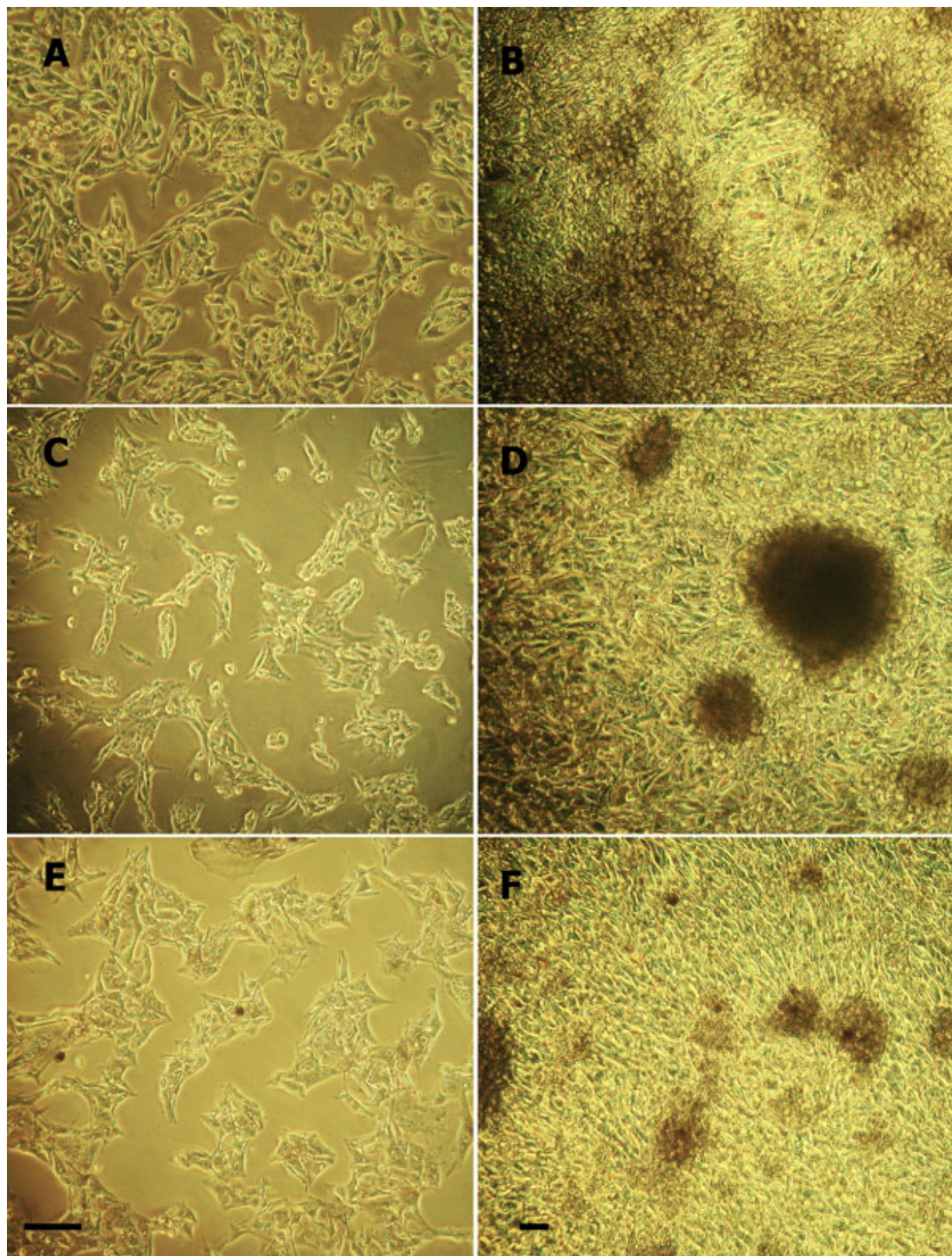


Figure 1. Phase contrast photomicrographs of cell lines in subconfluent and overgrowth conditions. At passage 3, cell lines MEL20-06-039 (A and B), MEL20-06-045 (C and D) and MEL20-07-070 (E and F) show early stage propagation (A, C, and E) and late stage confluent propagation with heaped up cells and no evidence of contact inhibition (B, D, and F). Melanin production is evident in two cell lines (MEL20-06-045 and MEL20-07-070). Original magnification (A, C, E) 200 $\times$ , (B, D, F) 100 $\times$ . Scale bar is 30  $\mu$ m.



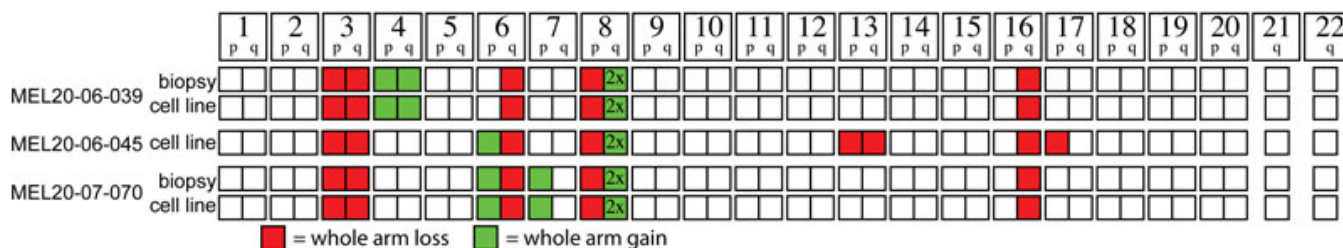


Figure 2. Comparison of Mapping Array results for chromosomal copy number for primary tumor biopsies and their cell lines, where available. Copy number variation analysis, as determined by Affymetrix Genotyping Console, showed identical patterns of aberration between primary tumor biopsies and corresponding cell lines. Gains (green boxes) and losses (red boxes) were largely whole arm in extent. 2± designations signify a total of 4 or more copies of chromosome 8q were detected. All samples had monosomy 3, 6q loss, 8p loss, multiple gains in 8q, and 16q loss. MEL20-06-045 and MEL20-07-070 also had 6p gain.

when compared to the two primary melanomas and the other two cell lines (Figure 3C).

Sequence analysis of exon 5 of *GNAQ* demonstrated heterozygous mutations in codon 209 in two of the three cultures (resulting in either Q209L or Q209P) and wild-type sequence in the third culture (Figure 4). Codon 209 sequence analysis of blood samples from the patients with Q209L and wild-type sequence revealed only wild-type sequence in both patients. No blood sample was available from the patient with Q209P gene mutation.

## DISCUSSION

This report presents for the first time, to our knowledge, cell lines characterized by microarray and grown from choroidal melanoma in patients who developed clinical metastases. Cell line characterization at passage 3 with 250K Mapping Arrays showed that each of the three cell lines and the two respective primary melanomas had common cytogenetic characteristics for monosomy 3, 6q loss, 8p loss, multiple 8q gain, and 16q loss. Two of the cell lines, MEL20-06-045 and MEL20-07-070 also demonstrated gains in chromosome 6p. Analysis of gene expression with U133 Plus 2.0 Arrays showed that two of the cell lines expressed gene characteristics similar to the respective primary melanoma for the 25 most overexpressed genes and the 20 most under-expressed genes in a comparative gene list composed of genes most overexpressed and under-expressed in an integrated analysis of choroidal melanoma with chromosome 3 loss versus 6p gain in the absence of chromosome 3 loss [11]; the third cell line had a profile of gene expression similar to the two primary melanomas and the other two cell lines. Potentially relevant functional classes of biologic activity include G-protein coupled signaling, calcium response pathways, cell adhesion marker expression, retinoic acid response pathways and regulation of palmitoylation [11].

Furthermore, sequence analysis from exon 5 of *GNAQ* demonstrated heterozygous mutation of codon 209 in two of the three cell lines and the wild-type sequence in the third. *GNAQ* codon 209 mutations in choroidal melanoma have been shown to be important in primary melanoma tumor development [14,15].

Choroidal melanoma cell lines derived from melanomas that progressed to clinical metastases and patient death with cytogenetic characteristics and gene expression profiles closely corresponding to the respective primary melanoma are important for at least two reasons. First, there is no animal model of spontaneous uveal melanoma that accurately replicates the course of the human disease [16]. Transgenic animal models of human disease do not reproduce the histogenesis of human melanoma and animal melanoma differs substantially from human uveal melanoma [17,18].

Second, cancer evolves through a process of step-wise accumulation of genetic alterations that result in uncontrolled cell proliferation and a lack of response to normal apoptotic stimuli. Genetic alterations include deletions, duplications/amplifications, translocations and point mutations that cause loss-of-function or gain-in-function and lead to altered protein expression. Availability of cell lines that closely correspond to the cells of metastasis-causing choroidal melanomas may serve as important in vitro models for the study of melanoma biology [19].

Characterization of the choroidal melanoma cell lines in this report extends the initial attempt at uveal melanoma cell culture by Kirby [20] in 1929, the report of six continuous cell lines from choroidal and ciliary body melanomas by Albert et al. [21]. in 1984 and subsequent reports of cell lines derived from uveal melanomas [22-28]. Cell lines from primary ciliary body and choroidal melanomas have been characterized by cellular morphology, relative growth rate, vasculogenic cord formation, immunohistochemistry, major histocompatibility complex (MHC), and cytogenetic analysis of cell lines with karyotype study and determination of monosomy 3 status by microsatellite analysis. Cell lines derived from ciliary body and choroidal melanoma metastases have been studied by Luyton et al. [25,27] using similar methods including karyotype analysis. Adding an additional dimension, this report presents analysis of primary choroidal melanomas and their cell lines with high resolution microarrays to compare cytogenetic characteristics and gene expression profiles.



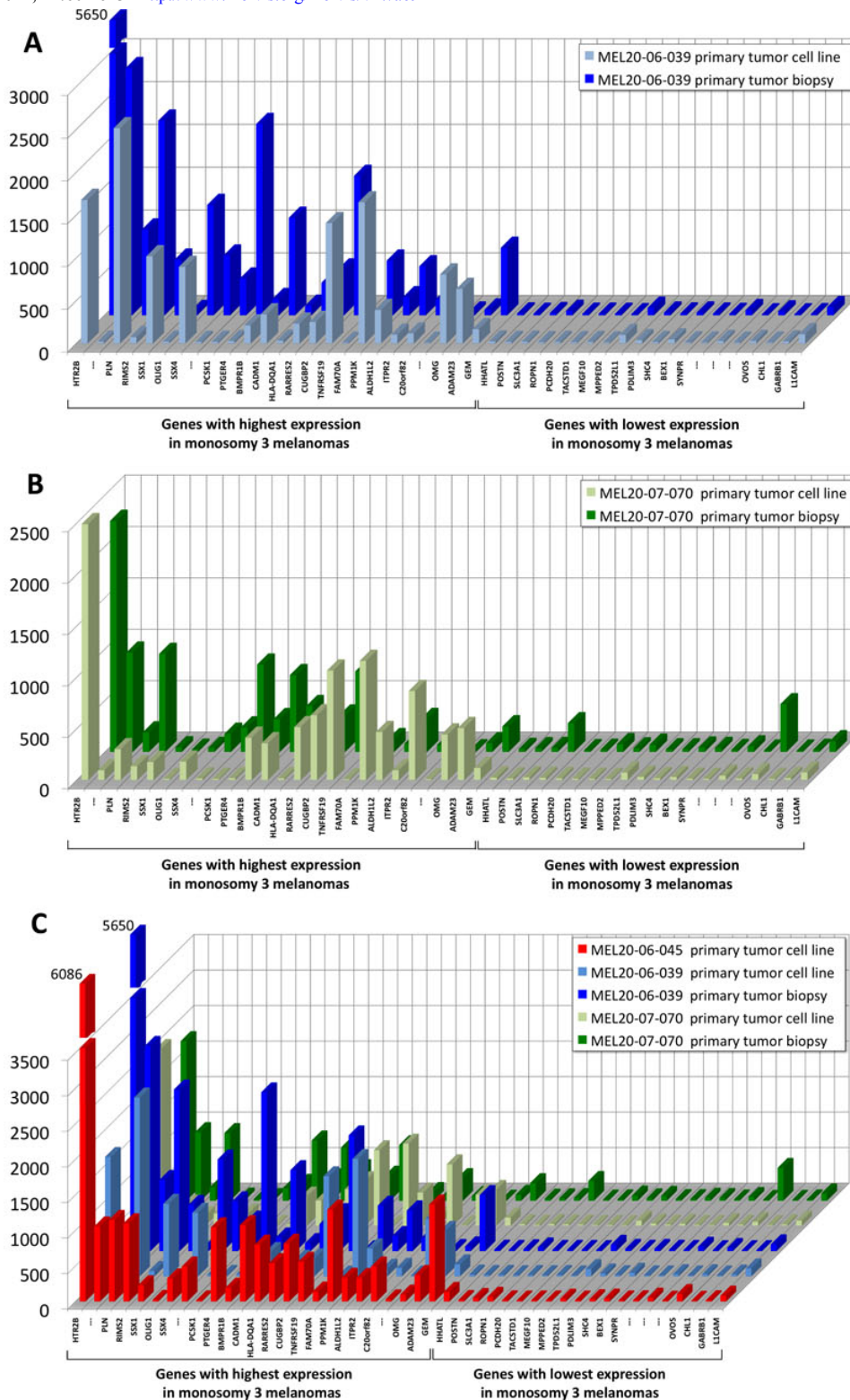


Figure 3. Comparison of expressional characteristics between primary melanomas and their corresponding cell lines. Normalized expressional values from Affymetrix GeneChip HG U133 plus 2.0 arrays for 25 genes significantly upregulated in monosomy 3 tumors and 20 genes significantly down-regulated or silenced in monosomy 3 melanomas compared to melanomas with 6p gain in the absence of chromosome 3 loss were selected to compare expressional variation between primary tumor biopsies and the corresponding cell lines. **A**: MEL20-06-039; **B**: MEL20-07-070; **C**: MEL20-06-045 cell line is shown in combination with MEL20-06-039 and MEL20-07-070 to provide a comparison of this cell line's expressional profile with those for which primary tumor expressional characteristics are known. Probeset identifiers and genomic locations of the listed genes are detailed in Table 2.

TABLE 2. GENES UPREGULATED, DOWN-REGULATED, OR SILENCED IN MONOSOMY 3 TUMORS.

Genes significantly upregulated in monosomy 3 tumors		
Probe set ID	Gene symbol	Location
206638_at	<i>HTR2B</i>	chr2q37.1
229823_at	—	chr8q22.3
204939_s_at	<i>PLN</i>	chr6q22.31
206137_at	<i>RIMS2</i>	chr8q22.3
206626_x_at	<i>SSX1</i>	chrXp11.22
228170_at	<i>OLIG1</i>	chr21q22.11
211425_x_at	<i>SSX4</i>	chrXp11.23
230650_at	—	chr8q13.3
205825_at	<i>PCSK1</i>	chr5q15
204897_at	<i>PTGER4</i>	chr5p13.1
229975_at	<i>BMPR1B</i>	chr4q22.3
209031_at	<i>CADM1</i>	chr11q23.2
212671_s_at	<i>HLA-DQA1</i>	chr6p21.32
209496_at	<i>RARRES2</i>	chr7q36.1
202158_s_at	<i>CUGBP2</i>	chr10p14
227812_at	<i>TNFRSF19</i>	chr13q12.12
219895_at	<i>FAM70A</i>	chrXq24
235061_at	<i>PPMIK</i>	chr4q22.1
231202_at	<i>ALDH1L2</i>	chr12q23.3
202660_at	<i>ITPR2</i>	chr12p11.23
235182_at	<i>C20orf82</i>	chr20p12.1
243339_at	—	chr5q33.1
238720_at	<i>OMG</i>	chr17q11.2
244463_at	<i>ADAM23</i>	chr2q33.3
204472_at	<i>GEM</i>	chr8q22.1
Genes significantly down-regulated or silenced in monosomy 3 tumors		
Probe set ID	Gene symbol	Location
223572_at	<i>HHATL</i>	chr3p22.1
210809_s_at	<i>POSTN</i>	chr13q13.3
205799_s_at	<i>SLC3A1</i>	chr2p21
231535_x_at	<i>ROPN1</i>	chr3q21.1
232054_at	<i>PCDH20</i>	chr13q21.31
201839_s_at	<i>TACSTD1</i>	chr2p21
232523_at	<i>MEGF10</i>	chr5q23.2
205413_at	<i>MPPED2</i>	chr11p14.1
203786_s_at	<i>TPD52L1</i>	chr6q22.31
238592_at	<i>PDLIM3</i>	chr4q35.1
235238_at	<i>SHC4</i>	chr15q21.1
218332_at	<i>BEX1</i>	chrXq22.1
230303_at	<i>SYNPR</i>	chr3p14.2
236936_at	—	chr8p11.23
236300_at	—	chr12p12.2
242206_at	—	chr7q11.21
228245_s_at	<i>LOC100132881</i>	chr12p13.31
234583_at	<i>CHL1</i>	chr3p26.3
207010_at	<i>GABRB1</i>	chr4p12
204584_at	<i>LICAM</i>	chrXq28

Recognition that cancer is fundamentally a genetic disease emphasizes the importance of precisely characterizing the cytogenetic and gene expression of primary choroidal

melanomas and the corresponding cell lines. Choroidal melanoma cell lines with genetic alterations that reflect their primary tumor provide malleable in vitro models. These



models enable mechanistic dissection of melanoma biology, identification of “driver” genes and insight regarding therapeutic targets [29].

Strengths of the three cell lines described in this report for in vitro study of choroidal melanoma biology and testing of therapeutic targets relate to (1) origin from primary choroidal melanomas that developed clinical evidence of metastasis <1.5 years after treatment of the primary tumor, (2) demonstration of cellular propagation through at least 6 passages, (3) documentation of equivalent cytogenetic aberrations and similar gene expression patterns in the primary melanomas and in the cell lines at passage 3, and (4) preservation of cell lines by freezing at passage 3 to provide highly characterized cell lines for future studies.

Limitations of the three cell lines as surrogates for study of melanoma biology, metastasis and therapy relate to (1) the limited but potentially significant differences in chromosomal aberration and gene expression between primary melanomas and corresponding cell lines, (2) the likelihood that, with long-term propagation and multiple passages, cell lines will undergo culture-related evolution in both chromosomal structure and gene expression, and (3) recognition that cancer biology is influenced by the in vivo micro-environment that is not replicated with in vitro models. However, the fact that the cell lines differ slightly between themselves may not be as important as having a diverse genetic background under which therapeutic drugs may be tested to determine biologic pathways in tumor development.

In summary, FNAB of primary choroidal melanomas that developed clinical metastases resulted in the development of three low-passage, highly characterized cell lines that exhibited the chromosomal aberrations and gene expression profiles present in the primary choroidal melanomas. These cell lines, with portions frozen beginning at passage 3 and portions continuing to propagate, provide in vitro models for study of choroidal melanoma biology, metastasis and therapy.

#### ACKNOWLEDGMENTS

Tara A. McCannel, M.D., Ph.D., the corresponding author, has had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The authors would like to thank Dr. Bradley R. Straatsma for his thoughtful comments on the manuscript. Funding to support this work was provided from a Career Development Award from the American Association of Cancer Research, the George and Ruth E. Moss Trust, and an unrestricted grant from Research to Prevent Blindness.

#### REFERENCES

1. Kujala E, Makitie T, Kivela T. Very long-term prognosis of patients with malignant uveal melanoma. *Invest Ophthalmol Vis Sci* 2003; 44:4651-9. [PMID: 14578381]
2. Prescher G, Bornfeld N, Hirche H, Horsthemke B, Jöckel KH, Becher R. Prognostic implications of monosomy 3 in uveal melanoma. *Lancet* 1996; 347:1222-5. [PMID: 8622452]

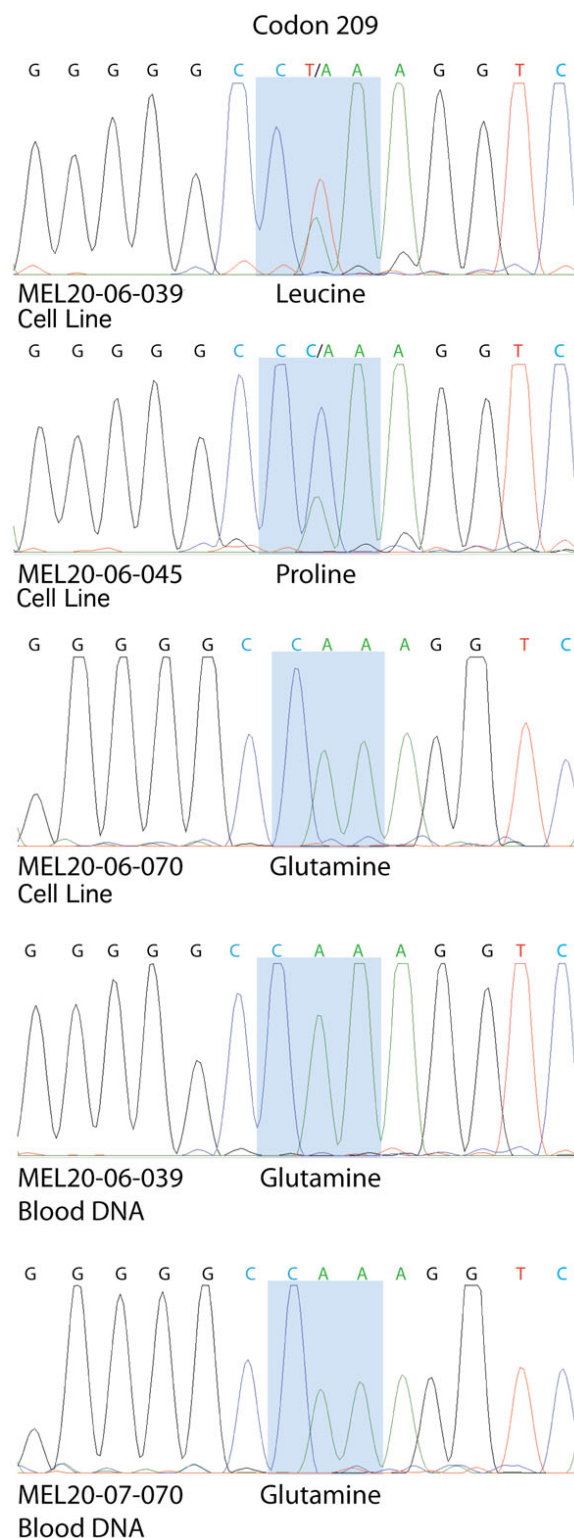


Figure 4. Sequence analysis of codon 209 of *GNAQ*. Heterozygous somatic mutations of Q209L and Q209P were identified in MEL20-06-039 and MEL20-06-045 cell lines, respectively. The MEL20-07-070 cell line was wild-type as were blood samples from the patients MEL20-06-039 and MEL20-07-070. No blood sample was available from patient MEL20-06-045.

3. White VA, Chambers JD, Courtright PD, Chang WY, Horsman DE. Correlation of cytogenetic abnormalities with the outcome of patients with uveal melanoma. *Cancer* 1998; 83:354-9. [PMID: 9669819]
4. Tschentscher F, Hüsing J, Hölter T, Kruse E, Dresen IG, Jöckel KH, Anastassiou G, Schilling H, Bornfeld N, Horsthemke B, Lohmann DR, Zeschnigk M. Tumor classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities. *Cancer Res* 2003; 63:2578-84. [PMID: 12750282]
5. Sisley K, Tattersall N, Dyson M, Smith K, Mudhar HS, Rennie IG. Multiplex fluorescence in situ hybridization identifies novel rearrangements of chromosome 6, 15, and 18 in primary uveal melanoma. *Exp Eye Res* 2006; 83:554-9. [PMID: 16684523]
6. Worley LA, Onken MD, Person E, Robirds D, Branson J, Char DH, Perry A, Harbour JW. Transcriptomic versus chromosomal prognostic markers and clinical outcome in uveal melanoma. *Clin Cancer Res* 2007; 13:1466-71. [PMID: 17332290]
7. Petrausch U, Martus P, Tönnies H, Bechrakis NE, Lenze D, Wansel S, Hummel M, Bornfeld N, Thiel E, Foerster MH, Keilholz U. Significance of gene expression analysis in uveal melanoma in comparison to standard risk factors for risk assessment of subsequent metastases. *Eye* 2008; 22:997-1007. [PMID: 17384575]
8. van Gils W, Lodder EM, Mensink HW, Kiliç E, Naus NC, Brüggewirth HT, van Ijcken W, Paridaens D, Luyten GP, de Klein A. Gene expression profiling in uveal melanoma: Two regions on 3p related to prognosis. *Invest Ophthalmol Vis Sci* 2008; 49:4254-62. [PMID: 18552379]
9. Mensink HW, Kiliç E, Vaarwater J, Douben H, Paridaens D, de Klein A. Molecular cytogenetic analysis of archival uveal melanoma with known clinical outcome. *Cancer Genet Cytogenet* 2008; 181:108-11. [PMID: 18295662]
10. Damato B, Dopierala J, Klaasen A, van Dijk M, Sibbring J, Coupland SE. Multiplex ligation-dependent probe amplification of uveal melanoma: correlation with metastatic death. *Invest Ophthalmol Vis Sci* 2009; 50:3048-55. [PMID: 19182252]
11. McCannel TA, Burgess BL, Rao NP, Nelson SF, Straatsma BR. Identification of candidate tumor oncogenes by integrative molecular analysis of choroidal melanoma fine-needle aspiration biopsy specimens. *Arch Ophthalmol* 2010; 128:1170-7. [PMID: 20837802]
12. McCannel TA, Burgess BL, Nelson SF, Eskin A, Straatsma BR. Genomic identification of significant targets in ciliochoroidal melanoma. *Invest Ophthalmol Vis Sci*. 2010 [PMID: 20837802]
13. Young TA, Burgess BL, Rao NP, Gorin MB, Straatsma BR. High density genome array is superior to fluorescence in-situ hybridization analysis of monosomy 3 in choroidal melanoma fine needle aspiration biopsy. *Mol Vis* 2007; 13:2328-33. [PMID: 18199974]
14. Van Raamsdonk CD, Bezroukove V, Green G, Bauer J, Gaugler L, O'Brien JM, Simpson EM, Barsh GS, Bastian BC. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature* 2009; 457:599-602. [PMID: 19078957]
15. Onken MD, Worley LA, Long MD, Duan S, Council ML, Bowcock AM, Harbour JW. Oncogenic mutations in GNAQ occur early in uveal melanoma. *Invest Ophthalmol Vis Sci* 2008; 49:5230-4. [PMID: 18719078]
16. Folberg R, Kadkol SS, Frenkel S, Valyi-Nagy K, Jager MJ, Pe'er J, Maniotis AJ. Authenticating cell lines in ophthalmic research laboratories. *Invest Ophthalmol Vis Sci* 2008; 49:4697-701. [PMID: 18689700]
17. Tolleson WH, Doss JC, Latendresse J, Warbritton AR, Melchior WB Jr, Chin L, Dubielzig RR, Albert DM. Spontaneous uveal amelanotic melanoma in transgenic Tyr-RAS+ Ink4a/Arf<sup>-/-</sup> mice. *Arch Ophthalmol* 2005; 123:1088-94. [PMID: 16087843]
18. Wilcock BP, Peiffer RL Jr. Morphology and behavior of primary ocular melanomas in 91 dogs. *Vet Pathol* 1986; 23:418-24. [PMID: 3750735]
19. Stuart D, Sellers WR. Linking somatic genetic alterations in cancer to therapeutics. *Curr Opin Cell Biol* 2009; 21:304-10. [PMID: 19328671]
20. Kirby DB. Tissue culture in ophthalmic research. *Trans Am Ophthalmol Soc* 1929; 27:33.
21. Albert DM, Ruzzo MA, McLaughlin MA, Robinson NL, Craft JL, Epstein J. Establishment of cell lines of uveal melanoma. *Invest Ophthalmol Vis Sci* 1984; 25:1284-99. [PMID: 6386741]
22. 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; 30:829-34. [PMID: 2722439]
23. Aubert C, Rouge F, Reillaudou M, Metge P. Establishment and characterization of human ocular melanoma cell lines. *Int J Cancer* 1993; 54:784-92. [PMID: 8325707]
24. De Waard-Siebinga I, Blom DJ, Griffioen M, Schrier PI, Hoogendoorn E, Beverstock G, Danen EH, Jager MJ. Establishment and characterization of a uveal-melanoma cell line. *Int J Cancer* 1995; 62:155-61. [PMID: 7622289]
25. Luyten GP, Naus NC, Mooy CM, Hagemeyer A, Kan-Mitchell J, Van Drunen E, Vuzevski V, De Jong PT, Luijckx TM. Establishment and characterization of primary and metastatic uveal melanoma cell lines. *Int J Cancer* 1996; 66:380-7. [PMID: 8621261]
26. Nareyeck G, Zeschnigk M, Prescher G, Lohmann DR, Anastassiou G. Establishment and characterization of two uveal melanoma cell lines derived from tumors with loss of one chromosome 3. *Exp Eye Res* 2006; 83:858-64. [PMID: 16750193]
27. Zuidervaart W, Hensbergen PJ, Wong MC, Deelder AM, Tensen CP, Jager MJ, Gruijs NA. Proteomic analysis of uveal melanoma reveals novel potential markers involved in tumor progression. *Invest Ophthalmol Vis Sci* 2006; 47:786-93. [PMID: 16505008]
28. Nareyeck G, Zeschnigk M, Bornfeld N, Anastassiou G. Novel cell lines derived by long-term culture of primary uveal melanomas. *Ophthalmologica* 2009; 223:196-201. [PMID: 19212147]
29. Lin WM, Baker AC, Beroukhi R, Winckler W, Feng W, Marmion JM, Laine E, Greulich H, Tseng H, Gates C, Hodi FS, Dranoff G, Sellers WR, Thomas RK, Meyerson M, Golub TR, Dummer R, Herlyn M, Getz G, Garraway LA. Modeling genomic diversity and tumor dependency in malignant melanoma. *Cancer Res* 2008; 68:664-73. [PMID: 18245465]



