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Genetic and Molecular Characterization of Uveal Melanoma Cell Lines

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Abstract

Summary—The recent identification of frequent activating mutations in *GNAQ* or *GNA11* in uveal melanoma provides an opportunity to better understand the pathogenesis of this melanoma subtype, and to develop rational therapeutics to target the cellular effects mediated by these mutations. Cell lines from uveal melanoma tumors are an essential tool for these types of analyses. We report the mutation status of relevant melanoma genes, expression levels of proteins of interest and DNA fingerprinting of a panel of uveal melanoma cell lines used in the research community.

Significance—This study represents the most comprehensive molecular analysis of uveal melanoma cell lines performed to date. The data confirms the mutually exclusive nature of *GNAQ* and *GNA11* mutations *in vitro*. The lack of *BRAF, NRAS, KIT, PI3K,* and *AKT* mutations reveal *GNAQ* and *GNA11* uveal melanoma cells to be distinct among melanoma types. The data provided is intended as a reference for investigators to select appropriate model systems and assist with authentication of uveal melanoma cell lines.

Keywords

uveal melanoma; GNAQ; GNA11

Introduction

Uveal melanoma is the most common primary intraocular cancer of the eye in adults. Approximately 40-50% of patients with primary uveal melanoma will develop metastases (Bedikian, 2006). Ninety-five percent of metastatic uveal melanomas involve the liver. Once liver metastases develop, patients have a median survival of about 6-9 months. Metastatic uveal melanoma has proven to be essentially refractory to biological and chemotherapy treatment (Singh et al., 2011). Thus, there is an urgent need to better understand the molecular underpinnings of uveal melanoma and develop effective therapeutics.

Recent studies have identified activating mutations in the heterotrimeric g-protein alpha subunits *GNAQ* or *GNA11* in the majority of uveal melanoma tumors (Van Raamsdonk et

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al., 2009, Van Raamsdonk et al., 2011), which occur in a mutually exclusive pattern. The most common site of mutation occurs in the Q209 position in either *GNAQ* (45%) or *GNA11* (32%). Less frequently mutations affect the R183 position in either *GNAQ* (3%) or *GNA11* (2%).

Generating uveal melanoma cell lines is difficult. As such, most functional analyses have been performed with a limited set of established cell lines. Folberg, et al., have reported karyotype and short tandem repeat analysis on seven commonly used uveal melanoma cell lines (OCM1, OCM3, OCM8, MUM2B, MUM2C, C918, M619), most obtained from original stock sources (Folberg et al., 2008). The analysis indicated that many of these cell lines previously believed to be distinct were actually derived from the same patients (OCM1 = MUM2C, OCM3 = OCM8, and M619 = C918 = MUM2B). Misidentification of cell lines is not unique to the uveal melanoma research field, as it has been proposed that many cell lines have been misclassified (Chatterjee, 2007, Lacroix, 2008). These unsettling findings highlight the need to authenticate the cell lines that are used in the uveal melanoma field and share this data with other researchers.

Apart from the proper authentication of cell lines, cost-effective technologies are now available to perform comprehensive genetic and molecular analysis of cell lines. This provides a tremendous opportunity to more fully characterize the mechanistic underpinnings that drive uveal melanoma proliferation, survival and metastasis. Equipped with this understanding researchers may have more confidence in conclusions derived from cell line experiments, both in the understanding of the basic biology of the cellular processes and data derived from therapeutic or molecular manipulation of cell lines.

In this study we analyzed a panel of 19 cell lines reported to be derived from uveal melanoma for mutation of relevant genes. Short-tandem repeat analysis and/or haplotype mapping was performed to determine the specific genetic identity of each cell line. We also report the expression of melanocyte-lineage/antigen protein markers of the cell line, as determined by us or others.

Results

Nineteen cell lines identified in the literature as derived from uveal melanoma tumors were investigated in this study (Table 1). Fifteen of the cell lines were noted to be generated from primary uveal melanoma tumors, whereas OMM1, OMM2.3 (a.k.a, OMM1.3), OMM2.5 (a.k.a, OMM1.5) and MUM2C are reported to originate from metastatic lesions (De Waard-Siebinga et al., 1995, Kan-Mitchell et al., 1989, Luyten et al., 1996, Maniotis et al., 1999, Nareyeck et al., 2009, Rummelt et al., 1998, Soulieres et al., 1991). The laboratory origin for each cell line is listed in Table S1. Cell lines were analyzed for the presence of hotspot mutations in exons 4 (R183) or 5 (Q209) of *GNAQ* or *GNA11* by Sanger sequencing of genomic DNA. We observed *GNAQ* or *GNA11* mutations in 11 of 19 cell lines examined. Mutations in GNAQ were either Q209L (92.1, MEL202, UPMM2) or Q209P (MEL270, OMM2.3, OMM2.5, UPMM3), whereas mutations in *GNA11* were only Q209L (UPMD1, UPMD2, OMM1). One line (MEL202) harbored a Q209L and a R210K mutation in *GNAQ*. Mutations at codon 183 (R183Q) were found in *GNAQ* in one cell line (UPMM1), whereas no *GNA11* R183 mutations were observed in any of the cell lines tested. All mutations in *GNAQ* and *GNA11* occurred in a mutually exclusive pattern. The BRAF (V600E) mutation was present in the OCM1, OCM3, OCM8, SP6.5, and MUM2C cell lines, as reported in prior studies, (Calipel et al., 2003, Folberg et al., 2008, Lefevre et al., 2004). No *GNAQ* or *GNA11* mutations were found in the BRAF-mutated cell lines.

We used Sequenom-based genotyping to interrogate mutations in a larger set of genes known to be mutated in other melanoma subtypes and cancer in general. None of the cell lines analyzed harbored other recurrent mutations found in melanoma (*KIT, NRAS, PI3K* or *AKT*) (Table S2a), or found in other cancer types (CDK4, CTNNB1, EGFR, FGFR3, GNAS, IDH1, IDH2, KRAS, MET) (Table S2b). *GNAQ, GNA11,* and *BRAF* mutations observed by Sanger sequencing in the uveal melanoma cell lines noted were also detected using the Sequenom-based platform.

The mRNA transcript and/or protein expression of melanocytic lineage markers (Melan-A/MART-1, gp100, tyrosinase, tyrosinase-related protein-1, dopachrome tautomerase, S100, HMB45, MCSP1) have been assessed by many methods in many of the cell lines analyzed in this study (van Dinten et al., 2005, Nareyeck et al., 2009). Studies that examined the mRNA transcripts levels utilized RT-PCR, while those that analyzed protein expression did so with immunohistochemistry or western blotting. We further determined the MCSP-1 expression status in 92.1, MEL202, MEL270, MEL285, MEL290, OMM1, OMM2.3, and OMM2.5 using flow cytometry (Table 1). All cell lines, with the exception of MEL285 and MEL290 have been shown to express at least two melanoma marker proteins. To authenticate the individual identity of each cell line, short-tandem repeat (STR) and/or haplotype mapping was performed on each cell line and results compared statistically to identify related cell lines (Tables S3, S4 and S5). Consistent with Folberg et al., our SNP data found identical fingerprints for OCM1 and MUM2C (39/39 match p-value 2.9269E-15), as well as OCM3 and OCM8 (33/33 p-value 1.0525E-12) Furthermore, the identical fingerprint of OCM-3 and OCM-8 was highly similar to SK-Mel28 (37/38 and 39/39 with p-values of 4.18E-15 and 2.44E-17, respectively). The three cell lines MEL270, OMM2.3, and OMM2.5, which were derived from primary and metastatic tumors from the same patient also showed similar fingerprints (Mel270 to OMM2.3, 35/38 p-value 3.2057E-10; Mel270 to OMM2.5, 34/36 p-value 1.0051E-10). The STR profile of the 92.1 cell line we tested differed from the STR profile reported in the European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI; Cambridge, UK) web site (http://www.ebi.ac.uk/ipd/estdab/directory.html). Statistical comparison of the STRs used in both studies reveals 7 of 10 STRs shared, indicating a high likelihood that the lines are related (p value = 0.038). In contrast, our STR profile of MEL202 identically matched that reported in the EMBL-EMI. Expectedly, the OMM2.3 and OMM2.5 cell lines reportedly derived from metastases of the primary uveal melanoma from which MEL270 was derived showed highly concordant STR profiles (Mel 270 to both OMM2.3 and OMM2.5 10/11 p-value = 0.036). The remaining cell lines showed distinct STR profiles.

Discussion

Our data show that at least some *GNAQ* and *GNA11* mutant uveal melanoma cells are capable of growth in culture using standard cell culture conditions. The cell lines highlighted in this study have mutually exclusive *GNAQ* or *GNA11* mutations and lack mutations in *BRAF, NRAS*, and *KIT*, consistent with the genotype observed in tissues. Prior studies have shown that both *GNAQ* and *GNA11* mutant cells activate the MEK/MAPK pathway *in vitro*. The lack of common *BRAF, NRAS,* or *KIT* mutations in *GNAQ* and *GNA11* mutant cells is thus consistent with the notion that redundant mutant proteins within the RAS/MEK/MAPK are not necessary for adequate activation of this signaling pathway. This is similar to the mutation patterns with other melanoma oncogenes, such as *BRAF, NRAS* and *KIT*.

The lack of *PI3K* or *AKT* mutations in uveal melanoma cell lines is informative. Activation of both the MEK/MAPK and PI3K/AKT pathways has been reported in uveal melanoma (Populo et al., 2010, Saraiva et al., 2005, Zuidervaart et al., 2005). Tumors with *BRAF* mutations, that activate the MEK/MAPK, usually have concurrent loss of PTEN activation,

or other aberrations that result in PI3K/AKT pathway signaling. As mutant *NRAS* is able to activate both the MEK/MAPK and PI3K/AKT pathways, cutaneous melanomas with *NRAS* mutations tend not to demonstrate PTEN loss or mutations in *PI3K* or *AKT (Ko et al.)*. The observation that *GNAQ* and *GNA11* mutant uveal melanoma cells have intact PTEN expression (unpublished data) and lack *PI3K* or *AKT* mutations is intriguing. Activation of GNAQ/11 signaling does not lead to an apparent increase in PI3K/AKT pathway activation (unpublished data) and published reports show active GNAQ to negatively regulate the PI3K/AKT pathway (Ballou et al., 2003, Howes et al., 2003, Ballou et al., 2006). These findings may suggest that activation of the PI3K/AKT pathway in uveal melanoma occurs by other means or is not a major requirement in tumorigenesis.

The frequency of *BRAF* (V600E) mutations in cell lines noted to be derived from uveal melanoma tumors is noteworthy. Multiple studies using standard PCR-based techniques have failed to identify *BRAF* mutations in uveal melanoma tumors (Cohen et al., 2003, Edmunds et al., 2003, Weber et al., 2003). However, using highly sensitive techniques, *BRAF* mutations have been identified in a subset of uveal melanoma, but appear to be present only in small populations of cells within a tumor (Janssen et al., 2008, Maat et al., 2008, Henriquez et al., 2007). In light of the later discovered oncogenes *GNAQ* and *GNA11*, additional studies revisiting these findings would be warranted. To date, mutations in *GNAQ, GNA11* or *BRAF* have been found in a consistently mutually exclusive pattern. If a small proportion of uveal melanomas indeed harbor *BRAF* mutations, it is conceivable that this proportion could be skewed under culture conditions. We have observed that cell lines derived from cutaneous melanoma with *BRAF* mutations generally tend to grow well in culture, relative to cell lines derived from cutaneous melanoma without *BRAF* mutations (Woodman, unpublished). Other cell lines (e.g., TP31, MKT-BR) noted to be derived from uveal melanoma tumors, but not investigated in this study, have been determined to have *BRAF* V600E mutations, as well (Calipel et al., 2003, Folberg et al., 2008, Lefevre et al., 2004). Uveal melanomas, of which more than 80% carry *GNAQ* or *GNA11* mutations, are likewise difficult to establish in culture. In some cases no distinction between ocular and uveal is made in referencing the site of origin from which the cell line was established. Ocular melanomas that do not arise from the uvea, primarily originating from the conjunctiva are biologically more similar to cutaneous melanomas, non-CSD and mucosal in particular, and should be considered distinct. In summary, considering the predominance of *GNAQ* and *GNA11* mutations in human uveal melanoma tumor samples as well as the fact, that a number of studies have failed to identify *GNAQ* mutations in large panels of other malignancies we believe the origin and validity of cell lines harboring *GNAQ* or *GNA11* mutations as derived from uveal melanoma is near certain. On the other hand, taking into account the questionable presence of *BRAF* mutations in uveal melanomas, and the high similarity of some of the here described *BRAF* mutant cell lines, both to each other, and for OCM3/OCM8 to SK-Mel28, a well known cutaneous melanoma cell line, the origin and representatively of these cell lines is less clear. Considering these findings, we believe future studies on uveal melanoma should always include cell lines shown here to harbor *GNAQ* or *GNA11* mutations

Multiple studies analyzing expression of melanoma markers have shown that the uveal melanoma cell lines highlighted in this study express similar markers to those observed in cutaneous melanoma cell lines. Noteworthy exceptions are the MEL285 and MEL290 cell lines. It has been reported that these cell lines and a subset of cutaneous melanoma cell lines do not express the typical melanoma-associated antigens (van Dinten et al., 2005). It is unclear whether the absence of these melanoma-associated antigens is a product of the expansion of these cells in culture or represents the antigenic nature of the tumors from which they were derived; however, it is of interest that this phenotype was observed in uveal melanoma cell lines lacking mutations in *GNAQ* or *GNA11.*

Our finding that the 92.1 cell line used here showed a partially identical STR profile to that on file at EMBL-EBI is noteworthy. Since the cell line we analyzed was obtained directly from the laboratory which in which it was generated, had a *GNAQ Q209L* mutation and expressed MCSP-1 by FACS, we believe this supports the authenticity of our cell line as derived from uveal melanoma. If the EMBL-EBI cell line authenticity is verified and the difference in the STR profile confirmed, the diverging changes must have emerged by genetic drift during culturing. This could potentially be worrisome as one might expect that this degree of genetic variation could result in biological behavior and interpretation of experimental results.

Functional analysis of *GNAQ* or *GNA11* mutations within uveal melanoma will be greatly enhanced by the use of cell lines that harbor these mutations. This study aimed to identify and more fully characterize uveal melanoma cell lines with *GNAQ* or *GNA11* mutations. We determined that *GNAQ* or *GNA11* mutant cell lines retain the mutually exclusive pattern of *GNAQ* or *GNA11* mutations observed in uveal melanoma tumors, and do not harbor other recurrent mutations (e.g., *BRAF, NRAS, KIT, PI3K*, or *AKT*) found in other types of melanoma. We observed that mutation spectrum in cell lines (Q209L, Q209P, and R183Q) does not significantly diverge from what we have found in human tumor tissues. In addition, DNA fingerprinting and melanoma cell markers were employed to authenticate the cell lines and re-confirmed the identical profiles of some cell lines. We also report for the first time the DNA fingerprints of many cell lines and determined each to be unique among the cell lines we tested. It is our desire that this information will be of utility to the research community and serve as a reference for future studies.

Methods

Cell Lines and Growth Conditions

The following cell lines were generously provided by the respective contributors: 92.1, MEL202, MEL270, MEL285, MEL290, OMM1, OMM2.3, OMM2.5, OCM1, OCM3, and OCM8 (Drs. Martine Jager and Bruce Ksander); UPMM1, UPMM2, UPMM3, UPMM4, UPMD1 (Dr. Gordon Nareyeck). Cells were grown in either RPMI + 5% FCS or 10% FCS. UPMM1-4 and UPMD1-2 cell lines were cultured in Ham F-12 Media with 10% FCS.

Flow Cytometry

Cultured cells were collected after brief trypsin treatment. Cells were washed twice with FACS Buffer (PBS and 1% BSA 0.1% NaAzide) prior to staining with anti human MCSP antibody conjugated with APC (Miltenyi Biotec, Auburn, CA). Cells were stained at 4°C for 15 minutes, and washed twice with FACS Buffer prior to fixation in 2% paraformaldehyde. Cells were analyzed on a FACs Canto (BD biosciences San Jose, CA) and data analyzed using FlowJo (Treestar Ashland, OR).

Sequenom-based SNP and Mutation Analysis

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) on the Sequenom platform was used to identify single nucleotide changes that result in known substitution mutations. DNA around the designated nucleotide change was first amplified by PCR then a primer extension reaction was run to determine the potential nucleotide base. Both the PCR primers and the extension primers are designed using the Sequenom Assay Design software. This program allows for muliplex reactions of up to 29 different SNPs per well. The initial PCR reactions are done in a 384 well format according to manufactures instructions. Then the PCR reactions are “cleaned up” using EXO-SAP also supplied by Sequenom. The primer extension reactions are done using Sequenom’s IPLEX chemistry according to their protocol. The IPLEX reactions are then desalted using

Sequenom's Clean Resin and spotted onto Spectrochip matrix chips using a Samsung Nanodispenser. The chips are then run on the Sequenom MassArray. The Sequenom Typer Software interprets the mass spectra that are generated and reports the SNPs based on expected masses. The quality and certainty of the call was rated by the program as A.Conservative, B.Moderate or C.Aggressive. Beneath which, the program does not make a nucleotide call. Grey shaded information in Supplementary Table S4 pertains to calls with the highest stringency, call A. Non-shaded calls had the lower quality call B. C quality calls were not added to the table. All spectra generated are run in duplicate and are visually inspected.

Short Tandem Repeat (STR) Analysis

Cell lines were validated by STR DNA fingerprinting using the AmpF*l*STR Identifiler kit according to manufacturer instructions (Applied Biosystems cat 4322288). The STR profiles were compared to known ATCC fingerprints (ATCC.org), to the Cell Line Integrated Molecular Authentication database (CLIMA) version 0.1.200808 (http://bioinformatics.istge.it/clima/) (Nucleic Acids Research 37:D925-D932 PMCID: PMC2686526) and to the MD Anderson fingerprint database. The STR profiles matched known DNA fingerprints or were unique.

HapMap Analysis

A fully detailed description of this procedure has been previously published (Janakiraman et al., 2010). In brief, the SNP calls were determined using the Sequenom platform as described above. SNP results from the cell lines were compared to each other and those deposited in the Memorial Sloan Kettering database, using a microsoft excel macro that reads in the assay results and does a pair wise comparison between all samples A detailed description of the microsoft excel macro and the algorithm applied has been previously published Janakiraman et al (Janakiraman et al., 2010). When applicable, the multiple testing artifact was corrected with the Bonferroni correction. Individual probabilities were multiplied by the number of sample pairs. Any sample pairs with a corrected probability of < 0.05 were consider unlikely to be unrelated. There is a 5% likelihood that the similarity between a pair of samples is not due to chance. Additionally, a simple clustering macro was applied to group samples likely to be related. In this procedure, samples are placed together in a cluster if they are related (at a corrected p-value of 0.05) to at least one other sample in the cohort.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Melanoma marker analysis of uveal melanoma cell lines

	92.1	MEL202	MEL270	OMM2.3	OMM2.5	UPMM1	UPMM2	UPMM3	OMM1	UPMD1	UPMD2	MEL285	MEL290	UPMM4	OCM-1	OCM-3	OCM-8	SP6.5	MUM2C
GNAQ	Q209L (626 A>T)	Q209L (626 A>T) R210K (629 G<A)	Q209P (626 A>C)	Q209P (626 A>C)	Q209P (626 A>C)	R183Q (548 G>A)	Q209L (626 A>T)	Q209P (626 A>C)	–	–	–	–	–	–	–	–	–	–	–
GNA11	–	–	–	–	–	–	–	–	Q209L (626 A>T)	Q209L (626 A>T)	Q209L (626 A>T)	–	–	–	–	–	–	–	–
BRAF	–	–	–	–	–	–	–	–	–	–	–	–	–	–	V600E	V600E	V600E	V600E	V600E
Primary	+	+	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	–
Metastatic	–	–	–	liver	liver	–	–	–	subcutaneous	–	–	–	–	–	–	–	–	–	liver
Melan-A/MART-1*<	+	nd	+	+	+	nd	nd	nd	+	nd	nd	–	–	nd	+	+	nd	+	nd
gp100*^	+	+	+	+	+	nd	nd	nd	+	nd	nd	–	–	nd	+	+	nd	nd	nd
Tyrosinase*^	+	+	+	+	+	nd	nd	nd	+	nd	nd	–	–	nd	+	+	nd	+	nd
Tyrosinase-related protein-1*	+	nd	+	+	+	nd	nd	nd	+	nd	nd	–	–	nd	+	+	nd	+	nd
Dopachrome tantomerase*	+	nd	+	+	+	nd	nd	nd	+	nd	nd	–	–	nd	+	+	nd	nd	nd
S100>	nd	nd	nd	nd	nd	+	+	+	nd	+	+	nd	nd	+	nd	nd	nd	nd	+
HMB-45<	nd	nd	nd	nd	nd	+	+	+	nd	+	+	nd	nd	+	nd	nd	nd	nd	nd
MAGE 1,2,3^	–	–	nd			nd	nd	nd	+	nd	nd	nd	nd	nd	+	nd	nd	nd	nd
MCSP-1>	+	+	+	+	+	+	+	+	+	+	+	–	+/–	+	nd	nd	nd	nd	nd

+ = positive, − = negative, nd = not determined, m = mRNA level determined, p = protein level determined, * determined by ref. Van Dinten (m,p), ^ determined by ref.Luyten (m), > determined by ref. Nareyeck (p), < determined by ref Wang (m,p)