Identification of Unique MEK-Dependent Genes in GNAQ Mutant Uveal Melanoma Involved in Cell Growth, Tumor Cell Invasion, and MEK Resistance

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Abstract

Purpose: Metastatic uveal melanoma represents the most common intraocular malignancy with very poor prognosis and no effective treatments. Oncogenic mutations in the G-protein α-subunit q and 11 have been described in about 85% of uveal melanomas and confer constitutive activation. Multiple signaling pathways are induced as a consequence of GNAQ/11 activation, which include the MEK/ERK kinase cascade. We analyzed the transcriptional profile of cell lines treated with a mitogen-activated protein (MAP)/extracellular signal–regulated (ERK) kinase (MEK) inhibitor to identify gene targets of activated GNAQ and to evaluate the biologic importance of these genes in uveal melanoma.

Experimental Design: We conducted microarray analysis of uveal melanoma cell lines with GNAQ mutations treated with the MEK inhibitor selumetinib. For comparison, we used cells carrying BRAFV600E and cells without either mutation. Changes in the expression of selected genes were then confirmed by quantitative real-time PCR and immunoblotting.

Results: We found that GNAQ mutant cells have a MEK-dependent transcriptional output and identified a unique set of genes that are downregulated by MEK inhibition, including the RNA helicase DDX21 and the cyclin-dependent kinase regulator CDK5R1 whereas Jun was induced. We provide evidence that these genes are involved in cell proliferation, tumor cell invasion, and drug resistance, respectively. Furthermore, we show that selumetinib treatment regulates the expression of these genes in tumor tissues of patients with metastatic GNAQ/11 mutant uveal melanoma.

Conclusions: Our findings define a subset of transcriptionally regulated genes by selumetinib in GNAQ mutant cells and provide new insights into understanding the biologic effect of MEK inhibition in this disease.

Introduction

The RAS/RAF/MEK/ERK pathway is often activated by genetic alterations in upstream signaling molecules, such as RAS and BRAF. Hyperactivation of this signaling cascade results in dysregulated cell proliferation and malignant transformation of a number of human tumors (1). Recently, the presence of somatic activating mutations in the heterotrimeric G-protein α-subunit q (GNAQ) and 11 (GNA11) in 46% and 30% of primary uveal melanomas, respectively, has been shown (2–4). These mutations occur in codon 209 in the RAS-like domain, resulting in loss of GTPase activity and constitutive activation. Approximately 85% of ocular melanomas arise from melanocytes within the uveal tract, which consists of the iris, ciliary body, and choroid of the eye (5), and account for a significant rate of deaths. Uveal melanoma is biologically distinct from cutaneous melanoma, as recurrent chromosomal abnormalities have been detected in chromosomes 3 and 8 (6), as well as a very high tendency to metastasize to the liver (7). NRAS mutations have not been detected in uveal melanoma (8), and BRAF mutations are considered rare (9, 10); however, activation of the mitogen-activated protein kinase (MAPK) pathway by mutant GNAQ/11 appears to be critical for the development of this disease (2). G-protein α-subunits transduce external signals from 7-transmembrane domain G-protein–coupled receptor (GPCR) to intracellular signaling pathways. The canonical target of GNAQ/11 stimulation is phospholipase C-β, which, in turn, stimulates inositol lipid signaling [i.e., calcium and protein kinase C (PKC)], in response to mitogenic GPCR agonists (11, 12). The constitutively active mutant GNAQ and GNA11 have been reported to activate the extracellular signal–regulated (ERK)
Translational Relevance

Uveal melanoma is the most common primary intraocular malignancy in adults. Metastasis occurs frequently and there are no effective therapies. Recently, it has been shown that 85% of uveal melanomas have oncogenic mutations in the GNAQ/11, which activate the mitogen-activated protein kinase (MAPK) pathway. Here, we analyzed the transcriptional profile of GNAQ mutant cell lines treated with selumetinib, an MAP/extracellular signal–regulated (ERK) kinase (MEK) inhibitor, currently in clinical trial for uveal melanoma.

We found that these cells have a MEK functional activation signature and a unique set of MEK-dependent genes involved in proliferation, cell invasion, and drug resistance. These genes are also regulated in pre- and posttreatment tumor biopsies obtained from patients with uveal melanoma treated with selumetinib, and their expression may correlate to clinical activity. Our findings provide new insights into the biologic effect of selumetinib in this disease and may have profound implications for the clinical development of MEK inhibitors in uveal melanoma.

pathway (2, 4), and knockdown of mutant GNAQ in uveal melanoma cells resulted in MAPK inhibition, reduced growth, and induced apoptosis (2). However, the transcriptional output of ERK signaling downstream of mutant G-proteins is not well characterized. Targeting MAP/ERK kinase (MEK) with allosteric small-molecule inhibitors has been reported to be effective in suppressing cell growth and, in some cells, inducing apoptosis. In particular, melanoma, thyroid, and non–small cell lung cancer with mutant BRAFV600E have been shown to be sensitive to MEK inhibitors (13–15). In a large number of tumor types treated with the MEK inhibitor selumetinib (AZD6244), Dry and colleagues have reported transcriptional signatures that predict MEK addiction or drug resistance (16). In another study, using microarray analysis of cells treated with a MEK inhibitor, Pratilas and colleagues have found genes, including members of the dual specificity phosphatase and sprouty gene families, that were differentially regulated by MEK inhibition in BRAFV600E cells but not in receptor tyrosine kinase–driven tumor cells with similarly elevated levels of pERK (17). They also showed that BRAFV600E cells have elevated ERK-dependent transcriptional output and disabled feedback inhibition of RAF/MEK signaling. Here, we report that uveal melanoma cells with GNAQ mutations are highly sensitive to MEK inhibition with selumetinib. Expression microarray analysis identified a MEK-dependent transcriptional profile that is, in part, similar to that of BRAFV600E melanoma cells. In addition, we identified several genes unique to GNAQ mutant cells, which are involved in proliferation and tumor cell invasion. Furthermore, pre- and posttreatment tumor biopsies from an ongoing clinical trial of selumetinib in patients with uveal melanoma indicate that these genes are transcriptionally regulated and may correlate with clinical benefit.

Materials and Methods

Cell culture

Omm1.3, Mel202, and Mel270 have been kindly provided by Dr. Bruce Ksander (Harvard Medical School, Boston, MA). OCM1A and 92.1 were from Dr. William Harbour (Washington University, St. Louis, MO). OCM3, Mel290, and C918 were from Robert Folberg (University of Illinois, Chicago, IL). Uveal melanoma cell lines have been sequenced for the presence of activating mutations in codons 209 (exon 5) and 183 (exon 4) of GNAQ and GNA11. Two cell lines had Q209L mutation (92.1, Mel202), whereas Omm1.3 and Mel270 had Q209P mutation. None had GNA11 mutations. Cells were cultured in RPMI medium supplemented with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin and maintained at 37°C in 5% CO₂. Cells were treated with selumetinib (AZD6244, graciously supplied by AstraZeneca).

Cell viability assays

Cells were plated in 96-well plates and treated with the indicated concentrations of selumetinib, PD0325901, or dimethyl sulfoxide (DMSO) in triplicates. Viability was assessed after 4 days of treatment using the Cell Counting Kit 8 (CCK8) from Dojindo Molecular Technologies according to the manufacturer’s instructions. Survival is expressed as a percentage of untreated cells. The calculations of combination index values were conducted using the CompuSyn software (ComboSyn; ref. 18).

Microarray data analysis

Cells were treated in triplicate with 250 nmol/L selumetinib, or 0.01% DMSO as control, for 8 hours. Following RNA extraction with TRIzol reagent (Invitrogen), cDNA was synthesized in the presence of oligo(dT)24-T7 from Genset Corp. cRNA was prepared using biotinylated UTIP and CTP and was hybridized to Human HT-12 oligonucleotide Illumina arrays in triplicates. We used a single array slide for each cell line to minimize the effect of experimental artifact. Differential expression analysis was conducted to identify genes whose expression is affected by the treatment by comparing the posttreatment expression versus the pretreatment expression. The array data were log₂-transformed and quantile-normalized. For each cell line, gene expression was compared between time points using the empirical Bayesian method and the R LIMMA package. An empirical Bayes t test was applied to each gene (19). Linear models and empirical Bayes methods were used for assessing differential expression in microarray experiments and a P value cutoff of 0.0001 was used to select differentially expressed genes. There are about 47,000 markers on the Illumina array and 5 genes are expected by chance to have a Pvalue<0.0001. Data are deposited at GEO accession no. GSE33655.
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Immunoblotting
Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer supplemented with protease inhibitor cocktail tablets (Roche Diagnostics) and 1 mmol/L Na2VO4. Equal amounts of protein were loaded on 4% to 12% PAGE gels (Invitrogen). Polyvinylidene difluoride (PVDF) membranes were blocked with 5% nonfat dried milk and probed with pERK, ERK, cyclin D1, CDK5R1 (pS3), c-Jun, and α-tubulin (Cell Signaling); SPRY2, DUSP6, ETV5, and DDX21 (Abcam).

Quantitative real-time PCR
Reverse transcription of 1 μg of RNA was done using the SuperScript III First-Strand Synthesis System (Invitrogen). Quantitative real-time PCR (qRT-PCR) assays were done on the 7300 Real Time PCR System (Applied Biosystems). TaqMan gene expression assays, which include gene-specific probe primer sets (Applied Biosystems), were used to detect the indicated genes and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)/hypoxanthine phosphoribosyltransferase (HPRT) mRNA. The relative expression of each gene was calculated by the ΔΔCt method.

RNA interference–mediated gene knockdown
The list of siRNA is reported in the Supplementary Methods. siRNAs were transfected using Lipofectamine RNAiMAX reagent (Invitrogen). After transfections, cells were counted using a Nexcelom cell counter and plated in 96-well plates for cell viability assays. The statistical significance of the experimental results was determined by the 2-sided t test.

Tissue sample collection and analysis
Matched tumor biopsies were collected from patients with metastatic uveal melanoma (clinicaltrials.gov; no. NCT01143402) before and after 14 days of selumetinib treatment. Flash-frozen specimens were then lysed in RIPA lysis buffer and analyzed by immunoblotting. The protocol was approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center (New York, NY), and all patients signed informed consent forms.

Results

The MEK inhibitor selumetinib inhibits cell viability of GNAQ mutant uveal melanoma
Using a panel of uveal melanoma cell lines expressing GNAQ209L/P, mutant BRAFV600E or wild-type (WT) for both, we investigated the effects of MEK inhibition on cell viability with selumetinib. As shown in Fig. 1A, the both, we investigated the effects of MEK inhibition on cell viability at nanomolar concentrations (IC50 > 0.1 μmol/L). The BRAFV600E cells were the most sensitive, with IC50 < 0.05 μmol/L, whereas cells without either BRAF or GNAQ mutations exhibited little sensitivity, with IC50 > 1 μmol/L. This corresponded to inhibition of pERK with selumetinib in the sensitive mutant cells (Fig. 1B; Supplementary Fig. S1A), whereas induction of pMEK is consistent with relief of negative feedback (20). The effect of MEK inhibition on GNAQ mutant uveal melanoma showed an accumulation in the G1 phase of the cell cycle (Supplementary Fig. S1B).

GNAQ downregulation by siRNA induced a decrease in expression of pERK in the GNAQ209L/P cells (Supplementary Fig. S2A). This corresponded to a decrease in cell viability which was not appreciably increased by selumetinib (Supplementary Fig. S2B). Suppression of GNAQ in WT and BRAFV600E cells did not inhibit pERK (Supplementary Fig. S2A) and did not affect cell viability with or without selumetinib (Supplementary Fig. S2B). In addition, overexpression of a GNAQ209L plasmid in a WT cell line (Supplementary Fig. S2C) sensitized the cells to selumetinib, as compared with cells with an empty vector (Supplementary Fig. S2D). These results confirm that GNAQ209L/P signals to MEK and specifically renders mutant cells susceptible to MEK inhibition, as reported by Van Raamsdonk and colleagues (2).

Expression profile of GNAQ209L/P cells compared with BRAFV600E MEK signature
To identify the transcriptional profile of MEK inhibition in GNAQ209L/P cells and potential novel targets of GNAQ209L/P signaling, we used microarray gene expression analysis. Cells from each genetically defined subgroup of uveal melanoma (3 cell lines with GNAQ209L/P, 1 line with BRAFV600E, and 1 cell line WT for both) were treated with 250 nmol/L selumetinib or vehicle for 8 hours. Genes whose change upon MEK inhibition exceeded defined statistical thresholds for all 3 GNAQ209L/P cell lines were considered significant and defined the ERK-dependent transcriptional output of GNAQ209L/P uveal melanoma cells. A total number of 387 genes met the significance level of P ≤ 0.0001 in the GNAQ209L/P cells. Identical parameters were used to define the set of genes regulated differentially by MEK inhibitor in the BRAFV600E and WT cell lines, and a direct comparison was conducted to evaluate common genes among the genetic subgroups. Of the 387 genes determined to have changed significantly in response to selumetinib, 308 were differentially expressed only in GNAQ209L/P cells, whereas 29 overlapped with BRAFV600E MEK-dependent genes. 42 overlapped with genes meeting significance in WT cells, and 8 genes were common to all 3 groups (Fig. 1B; Venn diagram). The top 19 genes with highest significance and fold change common to all GNAQ209L/P cells before and after treatment with selumetinib are displayed in a heatmap (Fig. 1D). A list of P values and fold change of representative genes for each group (GNAQ209L/P-only and overlap groups) are shown in Table 1. Of note, several of the genes that were shared between GNAQ209L/P and BRAFV600E cells were previously described ERK targets, such as CCND1, transcription factors ETV5, MYC, and genes involved in the feedback inhibition of MEK/ERK signaling, that is, dual specificity phosphatase 6 (DUSP6), and sprouty family members SPRY2, SPRY4. These genes are key components of “MEK signatures.”
described in other tumor cells (16, 17), and we confirmed their decline in expression (mRNA and protein levels) after selumetinib treatment, using immunoblotting and qRT-PCR (Fig. 2). Following exposure to selumetinib for up to 24 hours, pERK levels were determined by immunoblotting (Fig. 2A). In the less sensitive WT cell line, pERK was slightly downregulated at 2 hours and quickly rebounded at later time points, whereas its inhibition was more complete in cells with BRAFV600E and GNAQQ209L/P mutations. Basal expression levels of ETV5, DUSP6, and SPRY2 proteins were almost undetectable in the WT cell line, whereas they were highly expressed in the mutant cells (both BRAFV600E and GNAQQ209L/P), confirming elevated transcriptional output of MEK signaling in these cells (17). Cyclin D1 was durably downregulated in BRAFV600E cells, whereas its expression rebounded in GNAQQ209L/P and WT cells at later time points. The induction of pMEK did not occur in BRAFV600E cells, in accordance with the reported abrogation of negative feedback between ERK and RAF proteins by BRAFV600E (17, 20). The microarray results of these MEK-dependent genes were also confirmed by qRT-PCR in 3 GNAQQ209L/P cell lines, where these genes were suppressed (Fig. 2B–D). The high basal expression of these transcripts and their downregulation by selumetinib confirmed that the MEK/ERK pathway is active in GNAQQ209L/P cells and that the transcriptional events described in GNAQQ209L/P are at least, in part, similar to reported MEK functional activation signatures. For example, Pratilas and colleagues reported a signature of 52 MEK-dependent genes in BRAFV600E cells (17), of which 19 are represented among our 345 genes (5.5%) and are significantly overenriched ($P < 0.0001$).
MEK inhibition regulates unique genes in GNAQ<sup>G209L/P</sup> cells

The gene profile of MEK inhibition in GNAQ<sup>G209L/P</sup> cell lines also comprised uniquely regulated genes (Table 1). From this list, we selected 3 genes, 2 downregulated (DDX21, CDK5R1) and 1 upregulated (Jun) for validation by immunoblotting and qRT-PCR (Fig. 3). Immunoblot detection showed that DDX21 and CDK5R1 protein levels were downregulated in GNAQ<sup>G209L/P</sup> cells after MEK inhibition over time but not in the BRAF<sup>V600E</sup> and WT cell lines (Fig. 3A). Jun increased exclusively in the GNAQ<sup>G209L/P</sup> cells, whereas it was quickly downregulated in BRAF<sup>V600E</sup> cells. In contrast, Jun protein expression was high in WT cells and did not change upon treatment (Fig. 3A). Selumetinib also reduced DDX21 and CDK5R1 mRNA levels in GNAQ<sup>G209L/P</sup> cells, whereas they did not significantly change in WT cells (Fig. 3B and C). In the BRAF<sup>V600E</sup> cells there was a decrease in mRNA levels but generally to a lesser extent than the GNAQ<sup>G209L/P</sup> cells and this was associated with relatively minor changes in the protein levels of DDX21 and CDK5R1 (Fig. 3A–C). Also, Jun was induced upon treatment in GNAQ<sup>G209L/P</sup> cells, whereas it was profoundly decreased in BRAF<sup>V600E</sup> (Fig. 3D), in accordance with the protein levels. There was also a slight decrease in Jun mRNA in WT cells that did not affect the protein levels (Fig. 3D).

To prove specificity of MEK-dependent signaling downstream of GNAQ and to exclude drug-unrelated effects, the expression levels of these genes were analyzed in cells after GNAQ depletion. Phospho-MEK was markedly downregulated in the GNAQ<sup>G209L/P</sup> cells after GNAQ depletion (Fig. 3E). A slight decrease was seen in WT cell line possibly due to the disruption of WT GNAQ signaling. Consistent with the effects of selumetinib, CDK5R1 and DDX21 were downregulated in the GNAQ<sup>G209L/P</sup> cells, whereas c-Jun was increased, although less than was observed with drug alone (Fig. 3E). To determine whether the effects on GNAQ<sup>G209L/P</sup> cells were restricted to selumetinib, we treated uveal melanoma cells with a different MEK inhibitor, PD0325901. Both the GNAQ<sup>G209L/P</sup> and BRAF<sup>V600E</sup> cells were sensitive to increasing concentrations of the drug (Supplementary Fig. S3B). In terms of protein expression, the change in c-Jun (induction in GNAQ<sup>G209L/P</sup>, suppression in BRAF<sup>V600E</sup>, and no change in WT) was similar to selumetinib, but...
suppression of CDK5R1 and DDX21 was observed in both GNAQQ209L/P and BRAFV600E MEK-dependent signature. A, immunoblot analysis of cells treated with 250 nmol/L selumetinib over the time, using antibodies for the indicated proteins. Each blot is representative of at least 2 experiments showing same results. B–D, relative mRNA levels of selected ERK output genes before and after selumetinib in three GNAQQ209L/P cell lines. Cells were treated with selumetinib for 8 hours and RT-PCR was carried out using gene-specific primers for DUSP6, ETV5, SPRY2, and cyclin D1. Values were normalized with GADPH and HPRT as housekeeping genes using the ΔΔCt method. Each experiment was carried out in triplicates. Bars, ±SD.

Figure 2. Validation of MEK-regulated genes in GNAQQ209L/P cells. These genes were representative of those shared between GNAQQ209L/P and BRAFV600E MEK-dependent signature. A, immunoblot analysis of cells treated with 250 nmol/L selumetinib over the time, using antibodies for the indicated proteins. Each blot is representative of at least 2 experiments showing same results. B–D, relative mRNA levels of selected ERK output genes before and after selumetinib in three GNAQQ209L/P cell lines. Cells were treated with selumetinib for 8 hours and RT-PCR was carried out using gene-specific primers for DUSP6, ETV5, SPRY2, and cyclin D1. Values were normalized with GADPH and HPRT as housekeeping genes using the ΔΔCt method. Each experiment was carried out in triplicates. Bars, ±SD.
regulation compared with cells with BRAFV600E. Knockdown of c-Jun by 2 different siRNAs (Fig. 4C; Supplementary Fig. S7A) significantly increased the antiproliferative effects of selumetinib in GNAQQ209L/P cell lines (Fig. 4D; Supplementary Fig. S7B), suggesting that c-Jun induction may be involved in mechanisms of resistance to MEK inhibition. In contrast, c-Jun did not seem to play a role in WT and BRAFV600E cells as its knockdown did not alter the sensitivity to the selumetinib when compared with control siRNA (Fig. 4D; Supplementary Fig. S7B).

Validation of GNAQQ209L/P-specific ERK transcriptional output in tumor tissues

To assess the clinical efficacy of MEK inhibition on uveal melanoma, we are conducting a phase II clinical trial of selumetinib versus temozolomide in patients with metastatic uveal melanoma (clinicaltrials.gov; no. NCT01143402). Matched tumor biopsies are collected to examine target modulation between baseline and day 14 from patients with GNAQ/11 mutations receiving selumetinib. Results from 3 representative biopsy pairs are shown (Fig. 5A). The results of all the patients in the trial will be reported separately. Sustained inhibition of pERK and suppression of cyclin D1 were observed in patients A and B on day 14, but not in patient C. This correlated to best clinical response by Response Evaluation Criteria in Solid Tumors (RECIST; ref. 27): partial response in liver metastases in patient A (Fig. 5B), stable disease in patient B, and progression of disease in patient C. Furthermore, DDX21 was downregulated by selumetinib in patients A and C, CDK5R1 decreased in patient A and B, whereas they could not be detected in patients B and C, respectively. c-Jun expression increased in patient C (Fig. 5A). A similar trend has been seen in other patients treated on this clinical trial. These preliminary results are consistent with our in vitro studies indicating the existence of unique subset of genes in GNAQQ209L/P cells that are regulated by selumetinib and the expression of which could have an impact on clinical outcome.

Discussion

Uveal melanoma represents the most common intraocular malignancy. However, there are no effective treatments for this aggressive disease. Selumetinib is the only MEK inhibitor in clinical trials currently in the United States for patients with uveal melanoma. Here, we report that cells with GNAQQ209L/P mutations are sensitive to MEK inhibition by selumetinib, and sensitization was associated with a MEK-dependent gene expression profile. Some features of this profile are overlapping with that elicited in BRAFV600E uveal melanoma cells and other cell types (16), which
supports the MEK dependence of GNAQ<sup>Q209L/P</sup> cells. This gene profile includes the dual-specificity phosphatases (DUSP4/6), the sprouty homologues (SPRY1/2/4), which are known transcriptional targets of the ERK pathway involved in negative feedback regulation of ERK. The Ets variant transcription factor ETV5 was also regulated by MEK inhibition, along with cell-cycle division–associated protein 7 (CDCA7), the proto-oncogene MYC, and the solute carrier family 16, member 6 (SLC16A6).

Additional features of the MEK profile were identified as specific for GNAQ<sup>Q209L/P</sup> cells. A number of genes suppressed in GNAQ<sup>Q209L/P</sup> cells by selumetinib, such as LYAR, NOP58, GNL3, and PPAT, were reported as nuclear proteins involved in cell growth and tumorigenesis (28–31). DDX21 was recently identified as a novel biomarker for colorectal cancer (32), whereas CDK5R1 was involved in metastasis (24, 33) and associated with meningioma progression (34). Interestingly, it has been reported that mutant K-RAS regulates expression/stability of CDK5 and CDK5R1 (p35) to increase malignant progression and invasion of pancreatic cancer cells (35). It is plausible that mutant GNAQ acts similarly to mutant K-RAS, as CDK5R1 expression was in fact elevated in the GNAQ<sup>Q209L/P</sup> cells compared with cells with other genetic backgrounds. Furthermore, it has been reported that CDK5 negatively regulates JNK3 activity and its target c-Jun, to prevent apoptosis in developing neurons (36). This implicates a possible interaction between these proteins in promoting survival of uveal melanoma cells.

Jun was upregulated after selumetinib treatment in the GNAQ<sup>Q209L/P</sup> cells only. Depending on the cell type and drug treatment, c-Jun and Jun kinase have been implicated in both pro- and antiapoptotic responses (37). In cutaneous melanoma cells, active ERK induces c-Jun expression (38). In contrast, c-Jun was induced by MEK inhibition...
in GNAQ<sup>Q209L/P</sup> uveal melanoma cells, suggesting a differential regulation of the ERK/JNK pathway. G-protein-mediated signaling is complex and involves multiple downstream binding partners and various regulatory scaffolding/adaptor and effector proteins (12). For example, PKC is a target of GNAQ activation, and it might be involved in feedback regulation of c-Jun when ERK is inhibited. The upregulation of c-Jun could represent an alternative route to cell proliferation, which would explain the relative lower sensitivity to selumetinib in GNAQ<sup>Q209L/P</sup> cells as compared with BRAF<sup>V600E</sup> cells. Interestingly, increased expression of c-Jun has also been reported in colorectal cancer cells with K-RAS or BRAF mutations after acquired resistance to selumetinib (39). We showed that the antiproliferative effect of selumetinib can be enhanced by suppressing c-Jun in the presence of MEK inhibition would result in enhanced antitumor effects and may prevent selumetinib resistance. In conclusion, our findings define a unique molecular profile of MEK inhibition by selumetinib in uveal melanoma cells with mutant GNAQ and point to a set of transcriptionally modified genes that could have an impact on the activity of this agent in this disease.

References


event in uveal melanomas although it rarely occurs through mutation of BRAF or RAS. Br J Cancer. 2005;92:2032–8.


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