Using an integrative genomics approach called amplification breakpoint ranking and assembly analysis, we nominated KRAS as a gene fusion with the ubiquitin-conjugating enzyme UBE2L3 in the DU145 cell line, originally derived from prostate cancer metastasis to the brain. Interestingly, analysis of tissues revealed that 2 of 62 metastatic prostate cancers harbored aberrations at the KRAS locus. In DU145 cells, UBE2L3-KRAS produces a fusion protein, a specific knockdown of which attenuates cell invasion and xenograft growth. Ectopic expression of the UBE2L3-KRAS fusion protein exhibits transforming activity in NIH 3T3 fibroblasts and RWPE prostate epithelial cells in vitro and in vivo. In NIH 3T3 cells, UBE2L3-KRAS attenuates MEK/ERK signaling, commonly engaged by oncogenic mutant KRAS, and instead signals via AKT and p38 mitogen-activated protein kinase (MAPK) pathways. This is the first report of a gene fusion involving the Ras family, suggesting that this aberration may drive metastatic progression in a rare subset of prostate cancers.

**SIGNIFICANCE:** This is the first description of an oncogenic gene fusion of KRAS, one of the most studied proto-oncogenes. KRAS rearrangement may represent the driving mutation in a rare subset of metastatic prostate cancers, emphasizing the importance of RAS-RAF-MAPK signaling in this disease. Cancer Discovery; 1(1); 35–43. © 2011 AACR.

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**INTRODUCTION**

To understand the characteristic features of driving gene fusions in cancer, we previously carried out a large-scale integrative analysis of cancer genomic datasets matched with gene rearrangement data (1). As part of this analysis, we observed that in many instances a small subset of tumors or cancer cell lines harboring an oncogenic gene fusion displays characteristic amplification at the site of genomic rearrangement (refs. 2–6; Supplementary Fig. S1A and B). Such amplifications usually affect a portion of the fusion gene and are generally considered secondary genetic lesions associated with disease progression, drug resistance, and poor prognosis (2, 4–8). In contrast, high-level copy number changes that result in the marked overexpression of oncogenes usually encompass the target genes at the center of overlapping amplifications across a panel of tumor samples. Thus, a “partially” amplified cancer gene may suggest that this gene participates...
in a genomic fusion event important in cancer progression. A partially amplified gene could arise from several independent genetic accidents, including the formation of the gene fusion and subsequent amplification, suggesting possible selective pressure in cancer cells for this aberration. Toward this end, we developed an integrative genomic approach called amplification breakpoint ranking and assembly (ABRA) to discover causal gene fusions from cancer genomic datasets.

To uncover driving gene fusions contributing to prostate cancer progression, we applied ABRA analysis to genomic data from 10 prostate cancer cell lines. Of most interest, this led to the identification of a KRAS gene fusion in a rare subset of metastatic prostate cancer. RAS proteins play a critical role in cellular physiology, development, and tumorigenesis (9, 10). Mutations in RAS have been identified in a wide spectrum of cancers (9), but rarely in prostate cancer (11). To date, oncogenic alterations in the Ras pathway have been exclusively restricted to activating point mutations, including the most commonly studied Gly-to-Val substitution at codon 12 and substitutions at codons 13 and 61 of the different RAS isoforms (9, 12, 13). Gene fusions involving RAS genes have thus far not been described as a class of cancer-related mutations. This is the first description of a mutant chimeric version of KRAS and thus may represent a new class of cancer-related alterations.

RESULTS

On the basis of the fusion breakpoint principle previously described (1), amplifications associated with gene fusions usually involve the 5′ region of 5′ partners and the 3′ region of 3′ partners. Further, the amplification levels of 5′ and 3′ fusion genes will be identical owing to their coamplification as a single fusion gene. This observation provided the rationale to assemble putative gene fusions from amplification breakpoints by matching the amplification levels of candidate 5′ and 3′ partners. We therefore developed ABRA analysis, which leverages the in vivo amplification and breakpoint analysis in cancer cells to assemble novel gene fusions and predict their tumorigenicity. Concept signature analysis (ConSig) was developed in a previous study (1) and provides a ConSig score, which is helpful in ranking biologically relevant candidates based on prior knowledge and has been incorporated into ABRA analysis.

The detailed methodology of ABRA analysis is depicted in Supplementary Fig. S1C and discussed in the Supplementary Data. We initially focused this analysis on cancer cell lines because breakpoint analyses are more reliable in uniform cellular populations, as opposed to tumors, which comprise multiple cell types, many of which are not malignant.

Next, we tested the ABRA approach using a published single-nucleotide polymorphism microarray (aSNP) dataset (2) generated from 36 leukemia cell lines, including the K-562 chronic myeloid leukemia cell line known to harbor the amplified BCR-ABL1 fusion (14). We inferred the relative DNA copy number data and identified all 5′ and 3′ amplified genes from the 36 cell lines (≥2 copies). In this dataset, ABL1 was the top-ranking gene, with a 3′ copy number increase (Supplementary Fig. S1D, left; Supplementary Table S1). The amplification levels of all 5′ amplified genes in K-562 cells were then matched with ABL1 to nominate potential 5′ partners. In total, six 5′ amplified genes were found in K-562, and 5 matched the level of ABL1 3′ amplification. After curation of the amplification breakpoints, BCR and NUP214 were nominated as ABL1 fusion partner candidates (Supplementary Fig. S1D, right; see Methods and Supplementary Fig. S2 for criteria of candidate selection). These findings demonstrated the feasibility of this method in nominating driver gene fusions from genomic datasets.

To nominate novel gene fusions contributing to the progression of advanced prostate cancer, we applied this method to an array comparative genomic hybridization (aCGH) dataset of 10 prostate cancer cell lines. Interestingly, the top candidate nominated in the ETS gene fusion-negative prostate cancer cell line, DU145, was the KRAS locus, which exhibited a clear breakpoint accompanied by a 3′ amplification of KRAS (Fig. S1A, left; Supplementary Table S2). This result was particularly intriguing owing to the well-known role of KRAS as an oncogene (9) and the observation that activating point mutations of KRAS are found in a number of tumor types but are rarely seen in prostate cancer (11). Interestingly, the activation of downstream signaling intermediaries of the RAS–mitogen-activated protein kinase (MAPK) pathway has been observed in prostate cancer by a number of studies (15–17).

To assemble amplification breakpoints in the KRAS gene with more confidence, we carried out replicate aCGHs for DU145 cells. Matching the amplification level of KRAS with the 5′ amplified genes from DU145 cells, we identified 10 potential 5′ partner candidates that were suggested by either of the 2 aCGHs. After curation, C14orf166, SOX5, and UBE2L3 remained as the top 5′ partner candidates for KRAS (Fig. 1A, right; Supplementary Table S3), based on the criteria detailed in Supplementary Fig. S2.

To experimentally validate the predicted fusions of C14orf166-KRAS, SOX5-KRAS, and UBE2L3-KRAS, we designed primer pairs from the first exons of candidate 5′ partners and the last exon of KRAS, as well as the exons next to the breakpoints (Supplementary Table S3). Reverse transcriptase (RT)–PCR analysis of DU145 cells identified a specific fusion band for UBE2L3-KRAS, but not for the others. Subsequent sequencing of the RT-PCR product confirmed fusion of UBE2L3 exon 3 to KRAS exon 2, as schematically depicted in Fig. 1B.

To assess the abundance of the UBE2L3-KRAS fusion transcripts, we analyzed a panel of prostate cell lines by SYBR green quantitative PCR (Fig. 1C) and RNase protection assay (Supplementary Fig. S3). UBE2L3-KRAS was highly expressed in DU145 cells, but not in the other prostate cancer cell lines tested; this result was confirmed by subsequent paired-end transcriptome sequencing of DU145 cells (Supplementary Fig. S4). Moreover, mutation analysis of the fusion sequences from DU145 did not reveal canonical point mutations in the fusion allele of KRAS (Supplementary Fig. S5).

To examine the chromosomal rearrangements involving UBE2L3 and KRAS loci in DU145 cells, we carried out FISH analysis. By both KRAS split probe and UBE2L3-KRAS fusion probe FISH analysis, DU145 cells clearly showed a rearrangement at the KRAS genomic loci and fusion with UBE2L3 (Fig. 1D). In addition, we observed low-level amplification (3 copies) of the UBE2L3-KRAS fusion consistent with its nomination by the ABRA approach. To extend our studies to prostate tumors, we carried out a combination
Figure 1. Identification and characterization of a novel KRAS rearrangement in metastatic prostate cancer. A, left, amplification breakpoint analysis and ConSig scoring (yellow line) of 3' amplified genes from a panel of advanced prostate cancer cell lines nominating KRAS as a fusion gene candidate with 3' amplification (red columns) in the DU145 prostate cancer cell line. Right, matching the amplification level of 5' amplified genes of KRAS with red and green bars indicating the location of bacterial artificial chromosome clones. For genes, the direction of transcription is indicated by gray arrows. B, sequencing results from RT-PCR, revealing fusion of UBE2L3 with KRAS in DU145. Structures for the UBE2L3 and KRAS genes have their basis in the Genbank reference sequences. Numbers above the exons (boxes) indicate the last base of each exon. Open reading frames are shown in darker shades. Exons of UBE2L3-KRAS fusion are numbered from the original reference sequences. Line graphs show the position and DNA sequencing of the fusion junction. C, panel of prostate cancer cell lines analyzed for UBE2L3-KRAS mRNA expression by SYBR assay with the fusion primers. NPP, normal prostate pool. D, left, genomic organizations of UBE2L3 and KRAS loci, with red and green bars indicating the location of bacterial artificial chromosome clones. For genes, the direction of transcription is indicated by arrows and exons, by bars. Right, FISH assay (top) and copy number data analysis (bottom) confirm the fusion of UBE2L3 to KRAS in DU145 cells and recurrent rearrangements at the KRAS locus. The left FISH image shows 3 copies of fusion signals (yellow arrows), using colocalizing probes for the fusion. The FISH image on the right shows triplicate KRAS 3' signals in DU145 and 3' deletion of KRAS in a metastatic prostate tumor, PCA0216, using probes that tightly encompass the KRAS locus. The middle image shows relative quantification of copy number aCGH data at the KRAS locus in DU145 and metastatic prostate tumors PCA0211 and PCA0216. At bottom is a summary of KRAS rearrangements revealed by FISH and copy number analysis of a series of prostate cancer tissues from the University of Michigan (UM) and Memorial Sloan-Kettering Cancer Center (MSKCC); table indicates number of positive cases divided by total number of cases evaluated. Positive cases: PCA0211 (spine metastasis), 3' amplification; PCA0216 (bladder metastasis), 3' deletion.
by aCGH; FISH analysis was attempted, but because of decalcification, adequate hybridization was not possible (Fig. 1D; Supplementary Fig. 6A). Further investigation of the available gene and exon expression data for case PCA0211 suggested that this case did not overexpress ETS family genes but instead exhibited high expression of KRAS exons 2 to 6 (except exon 1), similar to the DU145 cell line (Supplementary Fig. 6B and C).

We next examined expression of the UBE2L3-KRAS protein. The predicted 296 amino acid fusion protein trims 17 amino acids from the C-terminus of UBE2L3 (Fig. 2A). The full length of the KRAS protein is preserved, with a 4–amino acid insertion between UBE2L3 and KRAS. Using both a monoclonal antibody raised against the Ras family and a polyclonal antibody raised against KRAS specifically, we detected a 33-kDa fusion protein in addition to the 21-kDa band corresponding to wild-type KRAS in DU145 cells (Fig. 2B). Specificity of the band attributed to the UBE2L3-KRAS protein was shown by knocking down expression using RNA interference against KRAS, UBE2L3, and the chimeric junction of UBE2L3-KRAS (Supplementary Fig. S7A). The UBE2L3-KRAS protein was found specifically in DU145 cells and not in a panel of other prostate cell lines (Fig. 2C). Specific expression of the protein was independently confirmed by mass spectrometry assessment of DU145 cells, using a multiple reaction monitoring (MRM) assay, which does not require antibody-based detection (Fig. 2D). Overexpression of UBE2L3-KRAS in HEK 293 cells, however, did not result in detectable fusion protein. Interestingly, in the presence of the proteosomal inhibitor bortezomib, expression of the fusion protein was clearly apparent, suggesting decreased stability of the fusion protein in the overexpression system (Fig. 2C).

One area that needs to be explored and may explain the protein stability issues is the function of the UBE2L3 ubiquitin-conjugating (E2) enzyme portion of the fusion protein. Using both a full-length and a truncated version of the UBE2L3-KRAS protein in HEK 293 cells, however, did not result in detectable fusion protein. Interestingly, in the presence of the proteosomal inhibitor bortezomib, expression of the fusion protein was clearly apparent, suggesting decreased stability of the fusion protein in the overexpression system (Fig. 2C).

We therefore attempted to detect ubiquitination of the UBE2L3-KRAS protein. We identified a rat anti-Ras monoclonal antibody and an anti-UBE2L3 rabbit polyclonal antibody detects a 33-kDa fusion protein specific to DU145 cells. Small interfering RNA (siRNA) duplexes are indicated.

**Figure 2.** Characterization of the UBE2L3-KRAS fusion protein. A, UBE2L3, KRAS, and the predicted UBE2L3-KRAS fusion protein. B, expression of the UBE2L3-KRAS fusion protein in DU145 cells. Immunoblot analysis of DU145 cells using an anti-RAS rabbit polyclonal antibody and an anti-KRAS mouse monoclonal antibody and an anti-UBE2L3 monoclonal antibody detects a 33-kDa fusion protein specific to DU145 cells. Small interfering RNA (siRNA) duplexes are indicated.

β-Actin was used to demonstrate equal loading. C, survey of the UBE2L3-KRAS fusion protein in a panel of prostate cancer cell lines and stabilization of protein expression with a proteosome inhibitor, bortezomib (Bort). Cell lines are indicated and treated in the presence or absence of 500 nM bortezomib for 24 hours. HEK293 cells were transfected with an expression construct encoding UBE2L3-KRAS. Immunoblot analysis was carried out using KRAS polyclonal and RAS monoclonal antibodies. D, MS assay for detection of UBE2L3-KRAS protein in DU145 cells. An MRM-MS assay was developed to detect the UBE2L3-KRAS fusion protein. Top, sequence of the UBE2L3-KRAS fusion protein with amino acids from UBE2L3 (red) and from KRAS (green). Trypic peptides used for MRM-MS analysis are underlined. Bottom, matrix represents positive measurement (red) of peptides from corresponding gel fractions of DU145, LNCaP, and VCaP whole-cell lysates.
monoclonal antibody that precipitated the 33-kDa UBE2L3-KRAS protein as well as additional bands in the 40- to 55-kDa region, which were specific to HEK 293T cells expressing the fusion (Supplementary Fig. S8A). These shifted bands are recognized by both anti-Ras and anti-HA tagged ubiquitin antibodies, and their molecular weights match the predictions for ubiquitinated fusion proteins. We further validated these ubiquitinated UBE2L3-KRAS proteins in DU145 cells (Supplementary Fig. S8B). These data suggest that the UBE2L3-KRAS protein is ubiquitinated, which may contribute to its decreased stability.

To determine the function of the UBE2L3-KRAS fusion, we overexpressed the fusion protein in NIH 3T3 cells (Supplementary Fig. S7B), a system classically used to study RAS biology (12, 19). Of note, enforced expression of UBE2L3-KRAS induced loss of fibroblast morphology and increased cell proliferation and focus formation (Supplementary Fig. S9A; Fig. 3A and B). Cell-cycle analysis revealed an increase in the S-phase fraction of cells (Supplementary Fig. S9B). To determine the effects of UBE2L3-KRAS expression on tumor growth in vivo, we implanted nude mice with stable NIH 3T3 vector control cells or NIH 3T3 UBE2L3-KRAS fusion-expressing cells. We observed robust tumor formation by the UBE2L3-KRAS–expressing cells, but not the vector-transfected cells (Fig. 3C; Supplementary Fig. S10). To interrogate the potential RAS-related signaling pathways engaged

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**Figure 3.** Transforming activities of the UBE2L3-KRAS fusion in NIH 3T3 cells. A, overexpression of UBE2L3-KRAS in NIH 3T3 cells, increasing cellular proliferation. pDEST40 represents an empty vector. B, focus formation from overexpression of UBE2L3-KRAS in NIH 3T3 cells. Oncogenic KRAS G12V was used as a positive control with respective empty vectors as negative controls (pDEST40 and pBABE). Top, photographs of representative plates. Bottom, quantification of focus formation shown by bar graph. C, UBE2L3-KRAS–transfected NIH 3T3 cells form tumors in nude mice. Stable polyclonal populations of NIH 3T3 cells expressing either the vector or the UBE2L3-KRAS fusion gene were injected s.c. into nude mice. Tumor growth was monitored from day 7 to 15, as indicated. The inset shows fusion protein in the stably transfected NIH 3T3 cells, which is further stabilized upon bortezomib treatment. D, downstream signaling pathways engaged by UBE2L3-KRAS fusion in NIH 3T3 cells. Lysates prepared from stably transfected NIH 3T3 polyclonal populations and vector controls were subject to immunoblot analysis for phospho- and total MEK, ERK, AKT, and p38 MAPK. Oncogenic BRAFV600E and KRASG12V were controls, with β-actin used as a loading control.
by UBE2L3-KRAS in NIH 3T3 cells, we carried out a series of immunoblot analyses on key signaling intermediaries. As reported in the literature for NIH 3T3 cells, KRAS is a stronger inducer of the MEK/ERK cascade, whereas HRAS is a stronger activator of the PI3K/AKT pathway (20). Of note, UBE2L3-KRAS overexpression resulted in attenuated endogenous MEK and ERK phosphorylation (Fig. 3D) in NIH 3T3 cells; the signaling was instead directed to AKT and p38 MAPK cascades, both of which have been implicated in prostate cancer (15, 17).

As activation of the MEK/ERK pathway is dependent on membrane attachment of Ras proteins, we investigated Ras subcellular localization, using immunofluorescence assays. Interestingly, Ras proteins, which are normally distributed in the cytoplasm, were found to be highly enriched in the late endosome after ectopic expression of UBE2L3-KRAS fusion in NIH 3T3 cells (Supplementary Fig. S11). We speculate that this relocation of Ras proteins may decrease their association with the cellular membrane and possibly enhance growth factor receptor signaling in the endosome.

To investigate the role of UBE2L3-KRAS fusion in a prostate background, we overexpressed the fusion in RWPE prostate epithelial cells (Supplementary Fig. S7C). The expression of the fusion protein was enhanced by incubation with bortezomib (Fig. 4A, inset). Overexpression of the UBE2L3-KRAS fusion in RWPE cells led to increased cell invasion, proliferation, and a transient increase in tumor growth in nude mice (Fig. 4A; Supplementary Fig. S12). Of note, in the RWPE model, signaling pathway analysis did not reveal inhibition of the MEK/ERK pathway or activation of AKT/p38 MAPK (data not shown). Although the MEK inhibitor U0126 inhibits the invasion of RWPE cells overexpressing either wild-type or mutant KRAS, treated RWPE cells overexpressing the fusion continued to exhibit invasive properties in the presence of U0126 (Supplementary Fig. S13), suggesting that downstream effectors other than MEK/ERK may be engaged by the fusion in the prostate context.

To further confirm the function of endogenous UBE2L3-KRAS in DU145 cells, we performed stable knockdown of UBE2L3-KRAS fusion and generated chicken embryo chorioallantoic membrane and mouse xenograft models. This resulted in decreased cell invasion and proliferation in vitro, as well as inhibition of tumor formation in the in vivo models (Fig. 4B and C; Supplementary Fig. S14).

**DISCUSSION**

The addiction of cancer cells to causal gene fusions often results in amplification in vivo, which may be exploited to reveal unidentified recurrent gene rearrangements. On the basis of this observation, we developed an integrative genomic-based approach called ABRA to explore driving oncogenic activating point mutations of KRAS have been identified, this is the first description of a mutant chimeric version of KRAS that is oncogenic and thus may represent a new class of cancer-related alterations. Consistent with this finding, we recently described gene fusions of BRAF and RAF1 in 1% to 2% of prostate tumors, further implicating the RAS-RAF-MAPK signaling pathway in subsets of prostate cancer (21). A summary of gene fusions involving this pathway that have been discovered in prostate cancer is shown in Fig. 4D. The rarity of KRAS rearrangement will limit its clinical relevance to a small subset of patients with metastatic prostate cancer.

Although both KRAS G12V and UBE2L3-KRAS exhibit an oncogenic phenotype in vitro and in vivo, UBE2L3-KRAS overexpression leads to attenuation, rather than activation, of the MEK/ERK pathway in NIH 3T3 cells. Instead, it appears that the KRAS fusion enriches Ras proteins in the endosome and switches signaling to the AKT and p38 MAPK cascades. This observation may have important implications in understanding the biological nature of this most studied proto-oncogene. These signaling switches were not observed in RWPE cells expressing the fusion, which bypasses the ERK pathway (unlike the KRAS G12V mutant), indicating that other downstream effectors are being engaged in the prostate background. KRAS rearrangements, although rare in the prostate cancer setting, could modulate metastasis, using signaling pathways that have thus far not been associated with KRAS. Future studies will be needed to elucidate the details of how chimeric KRAS engages endogenous RAS-related signaling pathways in the context of prostate cancer.

**METHODS**

**Amplification Breakpoint Ranking and Assembly**

Cell lines used for aCGH analysis were obtained from either American Type Culture Collection or collaborators and authenticated by providers (detailed in the Supplementary Data). The aCGH data from prostate cancer cell lines were segmented by the circular binary segmentation algorithm (22), and the genomic position of each amplification breakpoint was mapped with the genomic regions of all human genes. The 3′ amplified genes were rated by their ConSig score (1), which identified KRAS as the top candidate. Matching the amplification level of 3′ KRAS with 5′ amplified genes from DU145 cells nominated UBE2L3, SOX5, and C14orf166 as 5′ partner candidates. The aCGH data used in this study have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus with the accession number GSE26447.

**RT-PCR, Nuclease Protection Assay, and FISH**

RT-PCR with the fusion primers UBE2L3-q1 and KRAS-q2 (Supplementary Table S3) confirmed the UBE2L3-KRAS fusion in DU145 cells. Fusion qPCR was performed on a panel of prostate cancer cell lines using primers UBE2L3-q1 and KRAS-q2 (StepOne Real-Time PCR System; Applied Biosystems). Ribonuclease protection...
Figure 4. The oncogenicity of UBE2L3-KRAS fusion in the prostate context. A, expression of the UBE2L3-KRAS fusion in RWPE benign prostate epithelial cells, leading to increased cellular proliferation and invasion. Left, results of cell proliferation assays using stable RWPE cell clones infected with either pLenti-6 vector or UBE2L3-KRAS. Inset shows the 33-kDa fusion protein detected only in the fusion-transfected cells treated with bortezomib to enhance protein stability [data from a representative clone are shown (Clone 2)]. Right, modified Boyden chamber–Matrigel assays using pLenti-6 vector and fusion-expressing cells (Clone 2). Invading cells were stained with crystal violet and quantified. DU145 prostate cancer cells were used as a positive control. B, knockdown of the UBE2L3-KRAS fusion, reducing cell proliferation and invasion in DU145 cells. Left, cell growth relative to the control shRNA was monitored using WST-1 assay for 6 days. Inset shows immunoblot analysis for the 33-kDa fusion protein detected using Ras monoclonal antibody. Right, results of Matrigel invasion assay for DU145 pool and clone with UBE2L3-KRAS knockdown. Scrambled short hairpin RNA (shRNA) duplexes are used as control. C, knockdown of the UBE2L3-KRAS fusion, attenuating prostate tumor growth in mouse xenograft models; plot shows mean tumor volume trajectories over time for mice inoculated with DU145 pool (red) or single clone (green) after UBE2L3-KRAS stable knock-down. Error bars represent SEM at each time point. D, summary of RAS-RAF signaling pathways in relation to recurrent gene fusions characterized in prostate cancer. Genes that participate in fusion events are denoted in red. Within parentheses are percentages of prostate cancers harboring aberrations in the ETS family, RAF family, and KRAS gene locus. *ETS family members involved in gene fusions include ERG, ETV1, 4, and 5. Adapted from Gioeli et al. (16).
assays were performed using a 230-bp fragment spanning the UBE2L3-KRAS fusion junction. Interphase FISH was done on cell lines, paraffin-embedded tissue sections, and tissue microarrays, using bacterial artificial chromosome probes. The GenBank accession number for UBE2L3-KRAS fusion sequence is JF343812.

Western Blotting and MRM-MS

Lysates from DU145, PrEC, RWPE, 22RV1, VCaP, and PC3 cells, either untreated or treated with 500 nM of bortezomib for 12 hours, were probed with anti-RAS monoclonal (Millipore) and anti-KRAS rabbit polyclonal antibodies (Proteintech Group Inc.). Cell lysates from DU145 and LnCaP cells treated with bortezomib were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subjected to trypsin digestion. Transitions of tryptic digested peptides were compared with those of labeled internal standard peptides (spanning the fusion junction) by MRM-MS to identify the fusion peptides; see the Supplementary Data for more details.

In vitro Overexpression and Stable Knockdown of UBE2L3-KRAS Fusion

Expression plasmids for UBE2L3-KRAS were generated with the pDEST40 (with or without 5′ FLAG) and plenti-6 vectors (without 5′ FLAG). The expression plasmids were introduced into HEK (5′ FLAG-UBE2L3-KRAS pDEST40 vector), NIH 3T3 (UBE2L3-KRAS pDEST40 vector), and RWPE cells (UBE2L3-KRAS plenti-6 vector) using standard protocols, detailed in the Supplementary Data. The prostate cancer cell line DU145 was infected with lentiviruses with scrambled shRNA or UBE2L3-KRAS shRNA, and stable cell lines were generated by selection with Puromycin (Invitrogen).

Cell Proliferation, Invasion and Pathway Analysis, Xenograft Mouse Model

Cell counting analysis and basement membrane matrix invasion assays were performed as described previously (23, 24). Protein lysates from NIH 3T3 stable cell lines expressing UBE2L3-KRAS, V600E mutant BRAF, G12V mutant KRAS, and vector controls were probed with phospho- and total MEK1/2, p38 MAPK, Akt, and ERK antibodies (Cell Signaling Technologies). The stable NIH 3T3 and RWPE cells expressing UBE2L3-KRAS, as well as the pooled or single-common population of DU145 cells with the stable knockdown of UBE2L3-KRAS, were implanted subcutaneously into nude mice. Additional details on these methods can be found in the Supplementary Data.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed. The Editor-in-Chief of Cancer Discovery is an author of this article. In keeping with the AACR’s Editorial Policy, the paper was peer reviewed and a member of the AACR’s Publications Committee rendered the decision concerning acceptability.

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