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Endometrial cancers with activating KRas mutations have activated estrogen signaling and paradoxical response to MEK inhibition

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Abstract

Objective—To determine if activating KRas mutation alters estrogen signaling in endometrial cancer (EC) and to explore the potential therapeutic impact of these alterations.

Methods—The Cancer Genome Atlas (TCGA) was queried for changes in estrogen-regulated genes in EC based on KRas mutation status. In vitro studies were conducted to evaluate ER α phosphorylation changes and related kinase changes in KRas mutant EC cells. The resulting effect on response to MEK inhibition, using trametinib, was evaluated. Immunohistochemistry was performed on KRas mutant and wild-type EC tumors to test estrogen signaling differences.

Results—KRas mutant tumors in TCGA showed decreased progesterone receptor expression ($p=0.047$). Protein analysis in KRas mutant EC cells also showed decreased expression of ER α ($p<0.001$) and progesterone receptor ($p=0.001$). Although total ER α is decreased in KRas mutant cells, phospho-ER α S118 was increased compared to wild-type. Treatment with trametinib in KRas mutant cells increased phospho-ER α S167 and increased expression of estrogen-regulated genes. While MEK inhibition blocked estradiol-stimulated phosphorylation of ERK1/2 and p90RSK in wild-type cells, phospho-ERK1/2 and phospho-p90RSK were substantially increased in KRas mutants. KRas mutant EC tumor specimens showed similar changes, with increased phospho-ER α S118 and phospho-ER α S167 compared to wild-type EC tumors.

Conclusions—MEK inhibition in KRas mutant cells results in activation of ER signaling and prevents the abrogation of signaling through ERK1/2 and p90RSK that is achieved in KRas wild-type EC cells. Combination therapy with MEK inhibition plus anti-estrogen therapy may be necessary to improve response rates in patients with KRas mutant EC.

Keywords

endometrial cancer; KRas; MEK inhibitor; estrogen signaling

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Introduction

Endometrial cancer (EC) remains the most common gynecologic cancer in the United States with an estimated 60,050 new cases and 10,470 deaths in 2016¹. The majority of women who present with EC are diagnosed with early stage disease and have a favorable overall survival of 80–90%². Unfortunately, women who experience recurrence have poor response to current therapies with an overall survival of 12 months³.

Unopposed estrogen is a well-established risk factor for the development of EC⁴ and is known to drive proliferative signals. The majority of endometrioid EC express the estrogen receptor (ER), emphasizing the role for estrogen in the development and progression of EC^{5–7}. While patients with low grade, hormone receptor-positive EC generally have a favorable prognosis, the majority of recurrences occur in this subset because 80% of EC fit into this subtype². First line treatment for recurrent EC includes anti-estrogen hormonal therapies; yet, low response rates of only 10–30% have been observed in recurrent EC^{8–11}. Recent studies from The Cancer Genome Atlas (TCGA) have shown that uterine cancer is heterogeneous at the molecular level and can be further subdivided into several clusters, which include aberrations beyond the ER pathway and in pathways that have crosstalk with ER signaling^{12,13}.

Therapeutic resistance is not well understood in EC. However, previous studies in breast cancer have found that activation of the Ras/MAPK pathway is associated with resistance to anti-estrogen treatment^{14–16}. Oncogenic alterations in Ras/MAPK pathway occur in the form of activating KRas mutations in 10–30% of endometrioid EC. Unfortunately, similar to responses to anti-hormone therapy alone, single agent activity of MEK inhibitors has been very limited and an improved understanding of interactions through these signaling pathways will be critical to overcoming resistance¹⁷.

Our previous EC studies have characterized a panel of estrogen-regulated genes as a readout of ER activity¹⁸. ER signaling action occurs through not only the classical ligand-dependent genomic mechanisms via direct or tethered binding to estrogen response elements (EREs) but also non-genomic (non-transcriptional) rapid signaling¹⁹. The exact mechanisms of non-genomic signaling are less clear, but result in activation of various kinases to induce phosphorylation of ER α and lead to rapid signaling activation²⁰. At least five serine residues of the N-terminal domain of ER α have been identified as phosphorylation sites, of which S118 and S167 are the best characterized. Phosphorylation of both of these sites occurs through several different mechanisms. Ligand-dependent phosphorylation, as a result of estrogen binding, occurs at S118²¹. Activation of ER α can also be accomplished through ligand-independent phosphorylation at both S118 and S167 by several member kinases of receptor tyrosine kinase pathways, such as the PI3K/AKT and Ras/MAPK pathways. ERK1/2 phosphorylates S118 and is independent from the phosphorylation that occurs as a result of estrogen binding^{22–24}. In addition, AKT, p70S6K, and p90RSK have been found to phosphorylate and activate ER α at S167^{25–30}.

Based on previous studies of hormone resistance in breast cancer, we hypothesized that ECs with activating KRas mutations would have aberrant signaling through ER α . We further

hypothesized that targeted treatment against Ras/MAPK pathway would result in differential changes in ER signaling in KRas mutant EC cells compared to wild-type. Modified signaling through ER based on KRas mutation status could have important implications in the treatment of EC and inform the design of improved combination therapeutic strategies.

Methods

The Cancer Genome Atlas

In collaboration with our institution's Bioinformatics and Biostatistics Resource Group, The Cancer Genome Atlas (TCGA) was queried to identify endometrioid adenocarcinomas of the uterus. Cases lacking PI3K pathway aberrations were excluded to focus on the effect of KRas mutation status. RNA sequencing (RNAseq) version 2 data were downloaded from <http://cancergenome.broadinstitute.org>, log2 transformed, and feature-by-feature two-sample t-tests were performed to compare profiles between samples with and without KRas mutations. Beta-uniform (BUM) models were used to fit the p-value distributions to adjust for multiple comparisons.

Cell Culture and Reagents

β -estradiol was purchased from Sigma (St. Louis, MO). MEK1/2 inhibitor, trametinib, was provided by Dr. Kwong Wong at University of Texas MD Anderson Cancer Center. Ishikawa cells, well-differentiated human endometrial carcinoma cells with wild-type KRas, loss of PTEN, and positive ER α expression was purchased from the European Collection of Cell Cultures (EACC, Porton Down, United Kingdom). Ishikawa cells were cultured in RPMI 1640 with L-glutamine and 10% fetal bovine serum (FBS), penicillin and streptomycin. Ishikawa cells were transfected to stably express oncogenic KRasG12V mutant using a pMEV-2HA plasmid vector (Biomyx Technology, San Diego, CA). Plasmid DNA transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) per manufacturer's protocol. For *in vitro* studies, cells were cultured in phenol-free Dulbecco's Modified Eagle Medium (DMEM) with L-glutamine and 10% charcoal-stripped FBS, penicillin and streptomycin.

Reverse Phase Protein Array

Reverse phase protein array (RPPA) was performed by the Functional Proteomics Core. Ishikawa cells expressing wild-type or mutant KRas (G12V) were serum starved overnight for 12 hours in phenol-free DMEM with L-glutamine containing vehicle (DMSO) or 10nM trametinib. Cells were then stimulated with 0.01 μ M estradiol and cell lysates were harvested at 30 minutes.

Serially diluted lysates were arrayed on nitrocellulose-coated slides (Grace Biolab, Bend, Oregon) and then probed, analyzed, and quantitated as previously described³¹. Each dilution curve was fitted with a logistic model ("Supercurve Fitting" developed by the Department of Bioinformatics and Computational Biology at MD Anderson, "<http://bioinformatics.mdanderson.org/OOMPA>"). The protein concentrations of each set of slides were then normalized by median polish, which was corrected across samples by the linear

expression values using the median expression levels of all antibody experiments to calculate a loading correction factor for each sample.

Data were analyzed using Ingenuity Pathway Analysis (Ingenuity Systems, Redwood City, CA) to evaluate changes in molecular functions and canonical signaling pathways.

Western Blot

Ishikawa cells stably transfected with wild-type KRas and mutant KRas (G12V) were treated with vehicle (DMSO) or 10nM trametinib, and stimulated with estradiol, as described above. Primary antibody (1:1000–1:2000 dilution) incubations were performed overnight at 4°C. Phospho-ER α (Ser167) (D1A3) Rabbit mAb, phospho-ER α (Ser118) (16J4) Mouse mAb, ER α (D8H8) Rabbit mAb, phospho-AKT (Ser473) (D9E) XP Rabbit mAb, AKT Rabbit mAb, phospho-p70 S6 Kinase (Thr389) Rabbit mAb, phospho-p44/42 MAPK (ERK1/2) Rabbit mAb, p44/42 MAPK (ERK1/2) Rabbit mAb, and phospho-p90RSK (Ser380) Rabbit mAb were purchased from Cell Signaling Technology (Danvers, MA). Blots were re-probed using anti- β -actin mouse mAb (Sigma, St. Louis, MO). Relative densities were calculated using Image J software (National Institutes of Health, Bethesda, MD). All experiments were performed in triplicate.

Gene Expression

Quantitative real time polymerase chain reaction (qRT-PCR) was performed for estrogen-regulated genes¹⁸ through the Quantitative Genomics and Microarray Core at the University of Texas Medical School at Houston. Ishikawa cells stably transfected with wild-type KRas or mutant KRas (G12V) were treated with vehicle or trametinib, followed by estradiol, as described above and harvested 16 hours later. Total RNA was extracted using RNeasy Mini Kit (Qiagen, Valencia, CA) with DNAase treatment.

Patient Specimens and Immunohistochemistry

Endometrial tumors were obtained from archived specimens in the Gynecologic Oncology Tumor Bank. KRas status was determined from clinical mutation analysis (as reported in the medical record) or via PCR-based Sanger sequencing at the Sequencing and Microarray Core³². All specimens were endometrioid endometrial cancer, low grade, and obtained from primary hysterectomy. Primary antibody against Phospho-ER α (S167), Phospho-ER α (S118), PR (Cell Signaling Technology) and total ER (Dako, Carpinteria, CA) were used to stain KRas wild-type (n=10) and KRas G12V mutant (n=5) specimens. Negative controls were run in parallel, with primary antibody replaced with antibody dilution buffer. Staining was imaged with Vectra[®] 3 automated quantitative pathology system, and analyzed with inForm[®] software (PerkinElmer, Waltham, MA) in collaboration with the Flow Cytometry and Cellular Imaging Facility. Expression was quantified using H-scores, determined by percentage and staining strength (negative, 1+, 2+, 3+) of positive nuclear staining.

Statistical Analysis

Data were analyzed with independent t-test for pairwise comparisons using GraphPad Prism 7 (GraphPad Software, La Jolla, CA). For multiple comparisons, analysis of variance (ANOVA) was used with the Tukey test.

Results

Endometrial tumors with KRas mutations express decreased progesterone receptor

Two hundred and forty-one patients with EC had both mutation and RNASeq data available in the TCGA. Only endometrioid tumors were included in our analysis resulting in 194 cases. Of these, 9 tumors lacked PI3K pathway alterations and were excluded to focus on the effects of KRas mutation status. Of 185 endometrioid EC cases with PI3K pathway aberrations, 49 tumors (26.5%) had a confirmed KRas mutation (specific mutations listed in Table 1, Supplemental Digital Content 1) and 136 tumors (73.5%) had wild-type KRas. Decreased expression of progesterone receptor, one downstream readout of ER signaling, was noted in KRas mutant tumors compared to wild-type (Figure 1). IGF1, another gene modified by ER signaling was also down-regulated in KRas mutant endometrioid EC.

KRas mutant EC cells have altered response to estradiol stimulation

In vitro studies were then used to evaluate rapid molecular changes due to activating KRas mutation following estradiol stimulation in EC cells. Changes at the protein level were evaluated using RPPA and then Ingenuity Pathway Analysis. At 30 minutes following estradiol stimulation, KRas mutant Ishikawa cells had decreased expression of ER α and decreased expression of PR compared to Ishikawa cells expressing wild-type KRas (Figure 2). Top canonical pathways significantly activated were ErbB2-ErbB3 Signaling ($p=1.30e-21$), ErbB Signaling ($p=4.33e-19$), and EGF Signaling ($p=1.24e-17$). Proteins included in these pathways overlapped significantly, and included NRG1, EGFR, HER2, HER3, SHC, c-RAF, p27-Kip1, MEK1/2, p38MAPK, JNK, PI3K, mTOR, and STAT3/5. ERK/MAPK Signaling was also identified as significantly activated. In addition, Upstream Regulators analysis indicated the top two regulators to be ER α and PTEN. This global pathway analysis confirms our observations in the TCGA analysis, which is that KRas mutation status not only drives activation of Ras/MAPK signaling, but also indicated alterations in growth factor signaling and interactions with estrogen signaling.

KRas mutant Ishikawa cells exhibit altered rapid estrogen signaling and altered response to MEK inhibition

Further analysis of ER was needed to capture alterations in activity through phosphorylation states, as well as signaling changes in intersecting pathways. To evaluate activation of estrogen signaling, phosphorylated-ER quantitation was normalized to total ER α , which was decreased in the KRas mutant cells (Figure 3). Differences in phosphorylation of S118 and S167 in response to estradiol stimulation compared to no stimulation were observed based on KRas mutation status (vehicle versus 30 minute time point). As shown in Figure 3A, phosphorylation at S118 was increased by estradiol stimulation at 30 minutes compared to vehicle in both wild-type and KRasG12V. However, the magnitude of induction is reduced in KRasG12V. Phosphorylation at S167 is increased by estradiol stimulation compared to vehicle only in wild-type cells.

We then evaluated phosphorylation of S167 and S118 at the 30 minute time point to evaluate differences in phosphorylated states between KRas wild type and mutant cells. KRas wild type cells had increased phosphorylation at S167 compared to KRas mutant cells. However,

cells with mutant KRas had increased phosphorylation at S118 after estradiol stimulation compared to cells with wild-type KRas (Figure 3).

To evaluate the direct influence of the Ras/MAPK pathway in differential estrogen signaling, Ishikawa cells were treated with 10nM trametinib, a MEK inhibitor, prior to estradiol stimulation and western blots were performed to evaluate signaling through ER α (Figure 3). MEK inhibition did not create a large change in total ER α for wild-type or KRas mutant cells. Following treatment with trametinib, cells with wild-type KRas maintained a similar response to estradiol stimulation, with little change to phosphorylation at S118, but perhaps a slightly modified time course for S167, with decreased phosphorylation at 30 min and increased phosphorylation of S167 at the 1 hour time point. MEK inhibition in mutant KRas cells resulted in minimal changes to levels of phosphorylation at S118 and an increase in phosphorylation at S167 after estradiol stimulation, indicating activation of rapid estrogen signaling. Interestingly, phosphorylation of ER α S167 without estrogen stimulation (vehicle) is increased in the presence of MEKi, suggesting activation of ligand independent estrogen signaling.

KRas mutation decreases estradiol-stimulated signaling through Ras/MAPK pathway and causes differential response to MEK inhibition

AKT, p70S6K, ERK1/2, and p90RSK are able to phosphorylate ER α to activate rapid non-genomic signaling. For this reason, phosphorylation at these points in the PI3K/AKT and Ras/MAPK pathways was evaluated.

No differences were observed for AKT phosphorylation or p70S6K phosphorylation between wild-type and mutant KRas cells. Treatment with trametinib resulted in minimal change in phosphorylation of either AKT or p70S6K in either cell type (Figure 4). Cells with mutant KRas demonstrated a decrease in phosphorylation of ERK1/2 and p90RSK after estradiol stimulation compared to wild-type. While MEK inhibition blocked estradiol-stimulated phosphorylation of ERK1/2 in wild-type KRas cells, phospho-ERK1/2 was substantially increased in mutant KRas cells. Similar to the pattern of phospho-ERK1/2, MEK inhibition decreased phospho-p90RSK in wild-type cells but led to increased phospho-p90RSK in KRas mutant cells (Figure 4).

MEK inhibition in mutant KRas cells results in upregulation of estrogen-regulated genes

The functional effect of KRas mutation and MEK inhibition was evaluated at the gene expression level. Expression of sFRP1 was decreased -1.85 fold (p=0.002) and RALDH2 was decreased -3.87 fold (p=0.006) in KRas mutant cells compared to wild-type. These genes contribute to the inhibition of estrogen-driven proliferation. Relative expression of these genes was not significantly changed when cells with wild-type KRas were treated with trametinib. However, MEK inhibition in KRas mutant cells increased relative expression of four estrogen-regulated genes, EIG121 (p=0.001), sFRP1 (p<0.0001), PR (p=0.0003), and HOXA10 (p=0.002) (Figure 5).

Immunohistochemistry shows altered ER signaling in KRas mutant tumors

To test these observations in human endometrial tumors, markers of ER signaling were evaluated in untreated endometrioid ECs with wild-type (n=10) or mutant KRas (n=5). Staining showed that total ER was not significantly different, but phospho-ER S167 and phospho-ER S118 were significantly increased in KRas mutant tumors compared to wild-type ($p<0.001$ and $p=0.033$, respectively). PR was significantly reduced in KRas mutant tumors ($p=0.044$, Figure 6).

Discussion

We investigated ER α signaling alterations associated with KRas mutations across large scale genomic data from the TCGA, using in vitro studies, and using human tumor specimens to evaluate the role of Ras/MAPK signaling in relation to estrogen signaling in EC.

Understanding these intersecting pathways has important implications for rational therapeutic strategies. In evaluating rapid estrogen signaling, we demonstrated that KRas mutant EC cells have decreased expression of estradiol-stimulated total ER α compared to cells with wild-type KRas. However, levels of total ER α do not tell the full story of ER signaling in these cells; KRas mutant cells show activation of estrogen signaling through phosphorylation of ER α at S118 in response to estradiol stimulation. Further, MEK inhibition in KRas mutant cells results in additional activation of ER α signaling through increased phosphorylation at S167. Consistent with this increased activation through phosphorylation, MEK inhibition in KRas mutant EC also resulted in increased expression of estrogen-regulated genes. While MEK inhibition in wild-type cells blocks rapid estradiol-induced phosphorylation of ERK1/2 and phospho-p90RSK, this effect is abrogated in KRas mutant cells. In fact, MEK inhibition in KRas mutant cells results in increased phospho-ERK1/2 compared to vehicle-treated cells. Overall, these results suggest that while KRas mutant endometrial cancer cells have decreased total ER α , these cells readily activate estradiol-induced rapid ER signaling following MEK inhibition via trametinib. Further, KRas mutant cells are able to circumvent the downstream effects of MEK inhibition, resulting in activation of ERK and p90RSK. These findings highlight the importance of KRas mutation status in predicting potential clinical response to MEK inhibitors and rational combination therapies. Evaluation of ER signaling in KRas wild-type and mutant endometrial tumor specimens confirmed baseline activation of ER signaling through phosphorylation at S118 and S167 with a paradoxical decrease in PR.

Although further questions remain, this study provides two particularly potent points of relevance to clinical treatment of EC and future study design. First, KRas mutant ECs have increased activation of estrogen signaling that is mediated through Ras/MAPK pathway and provides a route to circumvent MEK inhibition with trametinib to ultimately upregulate MAPK signaling. This suggests that combination treatment with anti-estrogen therapy will be required for efficacy in patients with KRas mutant tumors. Second, both in vitro studies and immunohistochemistry of human tumor samples highlight that the traditional evaluation of total ER α and PR are not definitive indicators of ER signaling activity. While total levels may be reduced, phosphorylation and subsequent activation is increased. Evaluation of phospho-specific ER α markers can provide critical information about baseline tumor

biology, but even more importantly, help identify appropriate therapeutic combinations and better understand response to molecularly targeted agents.

This study focuses on changes in ER signaling at short time points (from 10 minutes to 4 hours). This approach enabled evaluation of rapid activation of the Ras/MAPK and PI3K/AKT pathways and corresponding alterations in phosphorylation of ER α . Yet, additional intersecting signaling pathways are likely and there remains the possibility that genomic ER signaling could also be altered even at these short time points. However, this study provides a critical snapshot of rapid changes in ER signaling related to activating KRas mutations in EC and identifies related differential response to MEK inhibition. This information is of interest in predicting sensitivity to treatment with various therapeutic combinations.

One limitation of our study is that we used Ishikawa EC cells to transfect wild-type and mutant KRas for the *in vitro* model, as Ishikawa cells exhibit loss of PTEN expression at baseline. Indeed, activation of the PI3K/AKT pathway has been shown to be a predictive marker for nonresponse to MEK inhibition in KRas mutant cancers³³. However, this is an important characteristic of our *in vitro* studies, as activation of the PI3K/AKT and Ras/MAPK pathways co-occurs in a proportion of endometrial tumors^{34–36}. In addition, as activation of PI3K/AKT has been implicated as a mechanism of acquired resistance, this suggests that our findings are relevant both for advanced cancers with these aberrations and for tumors that have acquired resistance following treatment with MEK inhibitors. In the context of these *in vitro* studies, both the wild-type and mutant KRas cells exhibited loss of PTEN expression, so this molecular aberration was controlled for in our *in vitro* model.

While KRas has proven difficult to target, MEK inhibitors are currently being investigated in phase I and II trials in various cancer types. MEK inhibitors bind adjacent to the ATP binding site on MEK, leading to non-competitive interference with MEK function. As a result, MEK inhibitors are highly specific. This specificity is heightened as ERK is the only known downstream effector of MEK³⁷. In preclinical studies, MEK inhibitors have been found to be cytostatic but not cytotoxic, supporting the notion that additional agents are needed in addition to MEK inhibitors to affect tumor regression³⁸. In addition, while BRAF mutant tumors are sensitive to MEK inhibition, tumors with activating KRas mutations have shown more variable responses^{33,39}. A single arm phase II GOG trial of AZD6244, a MEK1/2 inhibitor, was performed in patients with advanced or recurrent EC. The objective response rate was only 6%; however, 13 patients had stable disease⁴⁰. In addition, multiple hormonal therapies have been evaluated in the treatment of recurrent EC with response rates ranging from 10–30%^{8–11}. Single agent letrozole, an aromatase inhibitor, showed a response rate of only 9.4% in this patient population. The addition of anti-hormonal therapy to MEK inhibition may provide added benefit for patients with recurrent EC compared to either therapy alone. These findings provide preclinical support for the combination of endocrine therapy and MEK inhibition in the treatment of KRas mutant endometrial tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Analysis of gene expression changes between KRas mutant and wild-type uterine cancers.

A) Sample selection from Uterine Cancer TCGA data to include endometrioid tumors for analysis of gene expression changes and KRas mutations. Of 185 endometrioid tumors with PI3K pathway aberrations, 26.5% had KRas mutations and 73.5% had normal KRas. **B)** A significant decrease in progesterone receptor ($p=0.047$) and IGF1 ($p=0.044$) expression was found between KRas mutant (red) and KRas wild-type (green) tumors.

Figure 2.

Analysis of protein expression levels after estradiol stimulation in Ishikawa cells using Reverse Phase Protein Array (RPPA) indicates that cells transfected to express mutant KRas G12V have reduced expression of both Progesterone Receptor (PGR) and Estrogen Receptor alpha (ESR1) compared to cells expressing wild-type (WT) KRas. Cells were stimulated with 0.01 μ M estradiol and cell lysates were harvested at 30 minutes. Experiments were performed in triplicate. Graphs represent mean $\pm$ standard deviation.

Figure 3.

A) Total levels of ERα are decreased in KRas mutant cells compared to wild-type (WT) and this is maintained following treatment with trametinib (MEK inhibitor, MEKi). Differences in phosphorylation of S118 and S167 in response to estradiol stimulation were observed based on KRas mutation status (vehicle versus 30 minute time point). **A.** Phosphorylation at S118 was increased by estradiol stimulation at 30 minutes in both wild-type and KRasG12V. However, the magnitude of induction is reduced in KRasG12V. Baseline phosphorylation at S167 is increased by estradiol stimulation only in wild-type cells. We then evaluated phosphorylation of S167 and S118 at the 30 minute time point to evaluate differences in phosphorylated states between KRas wild type and mutant cells. KRas wild type cells had increased phosphorylation at S167 compared to KRas mutant cells. Cells with mutant KRas had increased phosphorylation at S118 after estradiol stimulation compared to cells with wild-type KRas. Following treatment with a MEK inhibitor, KRas mutant cells have increased phosphorylation at S167. The triangle at left indicates the band of interest for phosphorylation of S167. **B)** Graphical depiction of immunoblot results at the 30 minute time point expressed as normalized relative density. Phospho-ERs are normalized to total ERα; ERα is normalized to β-actin control. V, vehicle. Cells were stimulated with 0.01μM estradiol and cell lysates were harvested at 10minutes, 30 minutes, 1 hour, and 4 hours. All experiments were performed in triplicate.

Figure 4.

No differences in levels of phospho-AKT or phospho-p70S6K were seen in response to estradiol stimulation. Both had a slight decrease in expression following treatment with trametinib (MEKi). Wild-type (WT) KRas cells had increased phosphorylation of ERK1/2 and p90RSK in response to estradiol stimulation compared to mutant KRas cells. Following treatment with trametinib, wild-type cells exhibited decreased phosphorylation at ERK1/2 and p90RSK, while KRas mutant cells had increased phosphorylation of ERK1/2 and p90RSK. Cells were stimulated with 0.01 μ M estradiol and cell lysates were harvested at 10minutes, 30 minutes, 1 hour, and 4 hours. All experiments were performed in triplicate.

Figure 5.

Ishikawa cells expressing mutant KRas had increased expression of estrogen-induced genes following treatment with trametinib (MEKi), while cells with wild-type KRas had stable or slightly decreased expression. Gene expression changes are presented as fold-change compared to the vehicle control. Specifically, wild-type with trametinib treatment are normalized to wild-type with vehicle control and KRas mutant cells treated with trametinib are normalized to KRas mutant cells with vehicle control. * $p < 0.05$ comparing the difference between genotypes. All experiments were performed in triplicate. Graphs represent mean $\pm$ standard error.

Figure 6.

Immunohistochemical staining was conducted on endometrioid endometrial tumor specimens to compare ER and PR expression in KRas wild-type and KRas mutant tumors. PR expression was lower in KRas mutants ($p=0.044$). KRas mutants showed increased expression of phospho- ER α S118 ($p=0.033$) and phospho- ER α S167 ($P<0.001$).