Inhibition of Activated Fibroblast Growth Factor Receptor 2 in Endometrial Cancer Cells Induces Cell Death Despite PTEN Abrogation

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Abstract

***KRAS* activation and *PTEN* inactivation are frequent events in endometrial tumorigenesis, occurring in 10% to 30% and 26% to 80% of endometrial cancers, respectively. Because we have recently shown activating mutations in fibroblast growth factor receptor 2 (FGFR2) in 16% of endometrioid endometrial cancers, we sought to determine the genetic context in which FGFR2 mutations occur. Analysis of 116 primary endometrioid endometrial cancers revealed that *FGFR2* and *KRAS* mutations were mutually exclusive, whereas *FGFR2* mutations were seen concomitantly with *PTEN* mutations. Here, we show that shRNA knockdown of FGFR2 or treatment with a pan-FGFR inhibitor, PD173074, resulted in cell cycle arrest and induction of cell death in endometrial cancer cells with activating mutations in *FGFR2*. This cell death in response to FGFR2 inhibition occurred within the context of loss-of-function mutations in *PTEN* and constitutive AKT phosphorylation, and was associated with a marked reduction in extracellular signal-regulated kinase 1/2 activation. Together, these data suggest that inhibition of FGFR2 may be a viable therapeutic option in endometrial tumors possessing activating mutations in *FGFR2*, despite the frequent abrogation of *PTEN* in this cancer type.** [Cancer Res 2008;68(17):6902–7]

Introduction

Endometrial cancer is the most commonly diagnosed malignancy of the female reproductive tract in the United States. It was estimated that 39,080 new cases of cancer of the uterine corpus would be diagnosed and 7,400 women would die of this disease in the United States in 2007 (1). The majority of women presenting with endometrial cancer are surgically cured with a hysterectomy; however, ~15% of women show persistent or recurrent tumors that are refractory to current chemotherapies. For those women with advanced stage, progressive, or recurrent disease, survival is poor as there are no adjuvant therapies proven to be effective. The 5-year survival for patients who have recurred is only 13% (2).

A variety of somatic gene defects have been reported in endometrial carcinoma. Well or moderately differentiated endometrioid endometrial carcinomas account for ~80% of uterine cancers and are characterized by a high frequency of inactivating mutations in *PTEN* (26–80%), activating *KRAS* mutations (10–30%), and gain-of-function *CTNNB1* (β-catenin) mutations (25–38%) (3). Our laboratory recently reported mutations in fibroblast growth factor receptor (*FGFR*)2 in 19 of 187 (10%) primary uterine tumor samples (4). Mutations were seen primarily in tumors of the endometrioid histologic subtype (18 of 115, 16%). The majority of the somatic mutations identified were identical to germline-activating mutations in *FGFR2* and *FGFR3* that cause a variety of craniosynostosis and skeletal dysplasia syndromes (4).

The discovery of activating *FGFR2* mutations in endometrial carcinoma raises the possibility of using anti-FGFR molecularly targeted therapies in patients with advanced or recurrent endometrial carcinoma. Indeed, recent studies have indicated FGFRs hold promise as targets for anticancer therapy. The efficacy of FGFR inhibitors to inhibit cancer cell growth *in vitro* and *in vivo* has been investigated in a variety of malignancies, including myeloma (5) and bladder cancer (6), with significant inhibition of cell growth observed after FGFR inhibition.

Here, we present evidence that activating mutations in *FGFR2* occur within the context of *PTEN* inactivation but are mutually exclusive with *KRAS* mutations in endometrioid endometrial tumors. Using endometrial cancer cell lines expressing mutationally activated FGFR2 and WT or mutant PTEN, we show inhibition of activated FGFR2, either through shRNA knockdown or treatment with a pan-FGFR inhibitor, PD173074, results in cell death, even within the context of *PTEN* inactivation. Together, these data suggest that FGFR2 may be a viable therapeutic target in endometrial cancer, despite the high frequency of *PTEN* abrogation in this tumor type.

Materials and Methods

Study subjects and clinical data. Tumor and matched normal tissue samples were collected from hysterectomy specimens from patients being treated for suspected uterine cancer over the period 1993 to 2005. All participants consented to molecular analyses and follow-up as part of a Washington University Medical Center Human Studies Committee approved protocol (HSC 93-0828). Tissue specimens collected for research were evaluated and diagnoses confirmed by experienced gynecologic pathologists. Given our previous observation that *FGFR2* mutations are largely restricted to the endometrioid subtype of endometrial cancer (4), our studies were limited to this histologic subtype. The 116 patient specimens analyzed were originally selected to overrepresent cases with tumor microsatellite instability (MSI) and those patients with advanced stage disease (Supplementary Table S1), to assess whether *FGFR2* mutations were more common in MSI-positive tumors and whether *FGFR2* mutations were restricted to early stage cancer, as has been reported for *FGFR3* mutations in bladder cancer (4). Clinical data were extracted from clinic charts, hospital records, and Barnes-Jewish Hospital Oncology Data Services.

MSI typing. Tissue specimens and blood were obtained at the time of surgery, snap frozen, and stored at −70°C. DNA was prepared from

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi:10.1158/0008-5472.CAN-08-0770

neoplastic cellularity (>70%) using proteinase K and phenol extraction or with the DNeasy Tissue kit (Qiagen, Inc.). Matched normal DNA was extracted from peripheral blood leukocytes as previously described (7). Microsatellite analysis was performed as previously described (7) using five National Cancer Institute consensus microsatellite markers (*BAT25, BAT26, D2S123, D5S346*, and *D17S250*). Tumors were classified as MSI+ if novel PCR bands were identified in at least two of the five consensus panel markers. Tumors were considered microsatellite stable (MSI−) if there was no evidence of MSI in any marker.

Sequencing analysis. Mutation analysis was performed as previously described (4). PCR primer sequences, listed in Supplementary Table S2, were M13 tailed and sequencing performed in two directions.

Cell culture and reagents. The MFE296 cell line was purchased from the European Collection of Cell Cultures (Salisbury). AN3CA, HEC1A, Ishikawa, RL952, and KLE were provided by Dr. Paul Goodfellow (Washington University, St. Louis, MO). PD173074 was purchased from Sigma-Aldrich. The KH1-LV lentivector plasmid was kindly provided by Dr. Maria S. Soengas (University of Michigan, Ann Arbor, MI), and lentiviral packaging plasmids were kindly provided by Dr. Matthew Huentelman (Translational Genomics Research Institute, Phoenix, AZ).

Lentiviral transduction of shRNA. Two independent shRNA constructs, targeting two different exons of *FGFR2* (exon 2 and exon 15), were designed against the following sequences: shRNA targeting exon 2, 5′-TTAGTTGAGGATACCACATTA-3′ (nucleotides 79-99, NM_022970); shRNA targeting exon 15, 5′-ATGTATTCATCGAGATTTA-3′ (nucleotides 1866–1884, NM_022970). A nonsilencing shRNA construct was also designed based on a nonsilencing siRNA sequence from Qiagen (5′-AATTCTCCGAACGTGTCACGT-3′), and was used as a negative control. The corresponding oligonucleotides were annealed and cloned into a self-inactivating lentiviral vector (8). Virus production and cell transduction was performed as described (9). Greater than 90% transduction efficiency was achieved in each shRNA experiment, as determined by eGFP visualization (data not shown).

Growth inhibition assay. Twenty-four hours after infection, cells were plated in 96-well plates at a density of 5,000 cells per well and proliferation assessed on multiple days using the Sulforhodamine B (SRB) assay (Sigma-Aldrich).

Fluorescence-activated cell sorting analysis. Cells were transfected with 25 nmol/L nonsilencing siRNA or FGFR2 siRNA X2 using Lipofectamine 2000 (Invitrogen). Forty-eight hours after transfection, floating and attached cells were collected and analyzed for Annexin staining according to the manufacturer's instructions (BioVision, Inc.) using a CyAn ADP flow cytometer and Summit software, version 4.3 (Dako Cytomation). For PD173074 studies, cells were treated with 1 μmol/L PD173074 and Annexin staining evaluated at the indicated time points. For UO126 studies, cells

Table 1. Mutation status of endometroid endometrial tumors

Case ID	FGFR2 Mutation	KRAS Mutation	PTEN Mutation	Stage	Grade	MSI
AN3CA	p.[Lys310Arg(+)Asn550Lys]	WT	p.Arg130fsX4			+
MFE296	p.Asn550Lys	WT	WT			−
1359	p.Ser252Trp	WT	WT	I	2	+
1574	p.Ser252Trp	WT	p.[Gly44AlafsX7(+)Y68X]	I	2	+
1492	p.[Ser252Trp(+)Tyr376Cys]	WT	p.Arg130Gly	I	1	−
1484	p.Ser252Trp	WT	p.[Arg130Gly(+)F56V]	III	3	−
1316	p.Ser252Trp	WT	p.Leu112Val	III	1	−
1792	p.Ser252Trp	WT	wt	III	1	+
1482	p.Ser252Trp	WT	p.Thr319X	IV	2	+
1267	p.Asn550Lys	WT	p.AlaA126Asp	II	2	+
1391	p.Asn550Lys	WT	p.Q245X	III	2	+
1528	p.Asn550Lys	WT	p.Arg130Gly	IV	2	−
1655	p.Tyr376Cys	WT	p.Arg308llefsX5	III	2	+
1684	p.Ser373Cys	WT	p.Arg130Gly	I	1	+
1094	p.Cys383Arg	WT	p.Leu108-Asp109	I	1	+
1361	p.Met392Arg	WT	p.Thr319X	I	1	+
1744	p.lle548Val	WT	p.[Phe21SerfsX2(+)K66N]	III	2	+
1717	p.Lys660Glu	WT	p.Ser59X	I	2	−
1272	c.1287+2A>C	WT	WT	I	1	−
1289	p.Thr762fsX3	p.Gly12Asp	WT	I	3	+
1284	WT	p.Gly12Asp	p.[Arg130Gly(+)Gly165Arg]	II	1	+
1606	WT	p.Gly12Asp	p.Val191GlyfsX7	I	1	−
1856	WT	p.Gly12Asp	wt	I	2	+
1411	WT	p.Gly12Ala	p.[Arg47Gly(+)Gly165Arg]	III	2	+
1966	WT	p.Gly12Ala	p.[Arg130X(+)Ala148LysfsX3]	III	2	+
1393	WT	p.Gly12Cys	p.lle4HisfsX5	III	2	+
1609	WT	p.Gly12Cys	p.Lys267ArgfsX8	I	3	+
1044	WT	p.Gly12Val	p.Arg130Gln	III	3	+
1599	WT	p.Gly12Val	p.[Arg130Gly(+)Gln171X]	III	2	+
1873	WT	p.Gly12Val	p.V290X	I	1	+
1656	WT	p.Gly12Val	p.Gly251ValfsX5	I	I	−
1664	WT	p.Gly12Asp	p.Tyr16LeufsX27	III	I	−
1287	WT	p.Gly13Asp	p.[H123Y(+)Ala126Ser]	III	1	−
1576	WT	p.Gly13Asp	p.Arg130Gln	I	2	+

NOTE: Numbering relative to FGFR2 protein sequence NP_075259.2, KRAS protein sequence NP_203524.1, and PTEN protein sequence NP_000305.3.

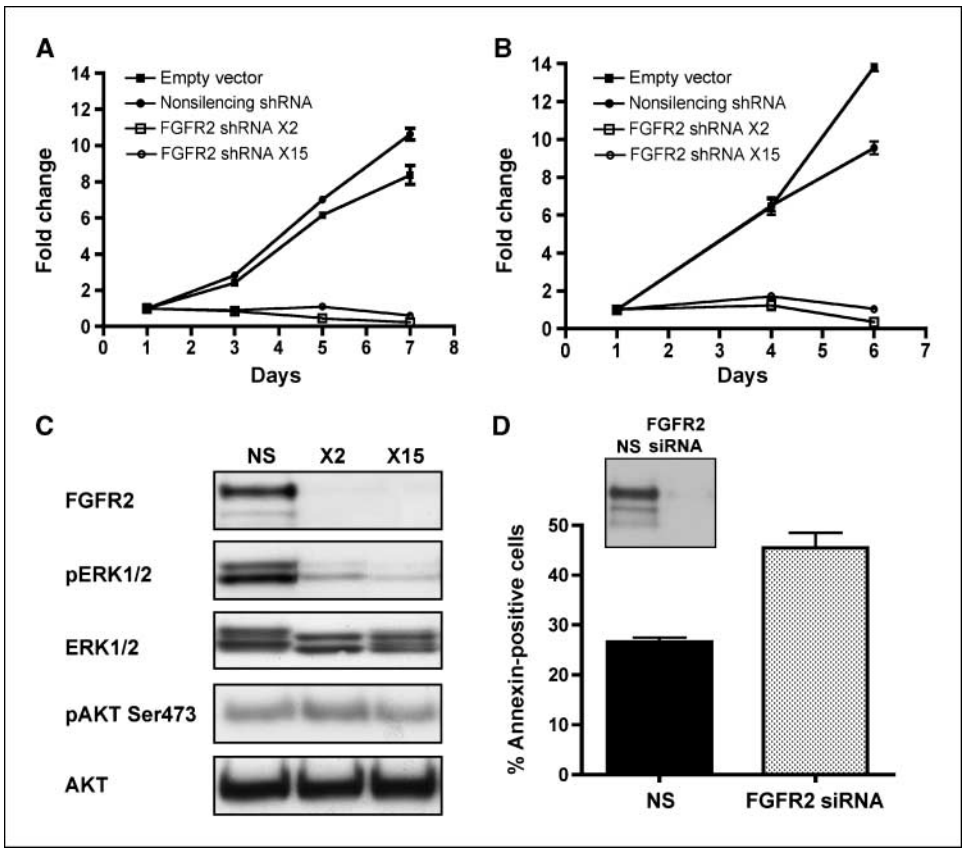

Figure 1. shRNA-mediated knockdown of FGFR2 induces cell death in endometrial cancer cells with activated FGFR2. *A* and *B,* effect of FGFR2 shRNA on cell proliferation of endometrial cancer cells with activating mutations in *FGFR2*. AN3CA (*A*) or MFE296 (*B*) cells were transduced with empty vector, nonsilencing (*NS*) shRNA, or two independent FGFR2 shRNA constructs, and the effect on cell proliferation was assessed using the SRB assay. Treatment with FGFR2 shRNA suppressed proliferation of both cell lines. Nonsilencing control shRNA had no effect on cell proliferation. *C,* effect of FGFR2 knockdown on activation of ERK1/2 and AKT. Twenty-four hours after shRNA transduction, AN3CA cells were serum starved in 0.2% FBS for 18 h. Lysates were collected and analyzed by Western blot for FGFR2 expression and activation of ERK1/2 and AKT. Knockdown of FGFR2 resulted in reduced ERK1/2 phosphorylation, and had no effect on AKT phosphorylation. *D,* cell death after knockdown of FGFR2. After siRNA transfection, AN3CA cells were analyzed for Annexin V-FITC–positive cells by flow cytometry. Knockdown of FGFR2 resulted in an increase in Annexin V–positive cells, indicative of apoptosis. *Inset,* protein lysates were analyzed by Western blot analysis to confirm FGFR2 knockdown. This knockdown was achieved with siRNAs rather than the shRNA constructs as the latter also expressed green fluorescent protein, which has an overlapping emission spectra with FITC.

were treated with 25 μmol/L UO126 (Sigma-Aldrich) and Annexin staining evaluated 72 h after treatment. For cell cycle analysis, cells were treated with 1 μmol/L PD173074 and, 72 h later, were stained with propidium iodide as described (10). Cell cycle analysis was performed using ModFit software (Verity Software House, Inc.).

Immunoprecipitation and Western blot analysis. For PD173074 studies, cells were starved overnight in 0.2% fetal bovine serum (FBS) and then incubated with 1 μmol/L PD173074 for 0 to 72 h. For shRNA studies, 24 h after lentiviral transduction, cells were starved overnight in 0.2% FBS and then lysates collected. For p-FGFR2 and p-FRS2α studies, AN3CA cells were starved overnight in 0.2% FBS, pretreated with 1 μmol/L PD173074 for 1 h, and then stimulated with 1 nmol/L FGF7 and 10 μg/mL heparin for 5 min. Five hundred micrograms of AN3CA lysate were immunoprecipitated with 2 μg of a FGFR2-specific antibody (Bek C-17; Santa Cruz Biotechnology, Inc.), and Western blot analysis was performed using a phospho-specific FGFR antibody (pFGFR Tyr653/654; Cell Signaling Technology). The Western blot was then stripped and reprobed for total FGFR2 (Bek C-17). pFGFR (Tyr653/654), pFRS2α (Tyr197), AKT, and extracellular signal-regulated kinase (ERK)1/2 antibodies were purchased from Cell Signaling Technology, and total FGFR2 (BekC17) and FRS2α antibodies were from Santa Cruz Biotechnology, Inc.

Statistical analysis. Statistical analyses were performed using GraphPad Prism version 4.0 for Macintosh (GraphPad Software). IC_{50} values were calculated by dose-response analysis using nonlinear regression of sigmoidal dose response with variable slope. All *P* values were considered significant when *P* value is <0.05. Data were expressed as mean ± SE.

Results

Patterns of *FGFR2, PTEN,* and *KRAS* mutations in primary endometrial cancers. Given that *PTEN* and *KRAS* mutations are common in endometrioid endometrial cancer, and as FGFRs signal through the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-OH kinase (PI3K) pathways, we first sought to determine whether *FGFR2* mutations occurred in tumors that harbor gain-of-function mutations in *KRAS* and/or loss-of-function mutations in *PTEN.* We sequenced all 9 exons of *PTEN* and exon one of *KRAS* in 116 endometrioid endometrial tumors for which we knew the *FGFR2* mutation status. Due to the limiting amount of DNA available, we only sequenced exon one of *KRAS,* as mutations in exon one account for >96% of *KRAS* mutations in endometrioid endometrial cancer.[4] *KRAS* mutations were identified in 12% (15 of 116) of tumors (Supplementary Table S1). Activating mutations in *FGFR2* and *KRAS* were mutually exclusive (Table 1). Of note, one tumor possessed a frameshift mutation in *FGFR2* (2290-91 del CT) and contained a *KRAS* mutation. However, as the pathogenic nature of this *FGFR2* mutation is unknown, we concluded that activating mutations in *FGFR2* were mutually exclusive with activating mutations in *KRAS.* Mutation analysis revealed *PTEN* mutations in 70% (82 of 116) of tumors (Supplementary Table S1). Of those tumors with *FGFR2* mutations, 77% (14 of 18) also carried a *PTEN* mutation (Table 1), demonstrating that mutations in *FGFR2* frequently occur alongside *PTEN* mutations in endometrioid endometrial tumors. As previously reported (11), *PTEN* mutations also occur alongside *KRAS* mutations (Table 1).

shRNA knockdown of FGFR2 induces cell death in endometrial cancer cells, despite PTEN inactivation. Given the occurrence of activating *FGFR2* mutations within the context of *PTEN* inactivation in endometrial cancer and the known role of

[4] http://www.sanger.ac.uk/genetics/CGP/cosmic

the PI3K/AKT pathway in promoting cell survival, we sought to determine whether inhibition of FGFR2 could induce cell death in the presence of *PTEN* inactivation. AN3CA and MFE296 endometrial cancer cells were selected as models for these studies. AN3CA cells reflect the majority (~82%) of primary tumors with activating *FGFR2* mutations, as these cells carry an activating mutation in the kinase domain of FGFR2 (N550K) and exhibit PTEN abrogation. AN3CA cells have mutations in both *PTEN* alleles and do not express PTEN (Supplementary Fig. S1). MFE296 cells model the ~18% of primary tumors with *FGFR2* mutations that were WT for *PTEN,* as these cells express mutationally activated FGFR2 (N550K) and are WT for *PTEN.*[4]

Knockdown of FGFR2 with two independent shRNAs inhibited cell proliferation in both AN3CA and MFE296 cells (Fig. 1*A* and *B*), demonstrating the effectiveness of targeting activated FGFR2 even in the presence of PTEN inactivation. As shown in Fig. 1*C*, shRNA knockdown of FGFR2 resulted in >90% reduction in FGFR2 protein in AN3CA cells and a marked decrease in ERK1/2 phosphorylation. No change in AKT phosphorylation was evident (Fig. 1*C*).

To investigate whether knockdown of FGFR2 induced apoptosis, AN3CA cells were transfected with siRNA targeted toward FGFR2 and labeled with Annexin V-FITC to detect exposed phosphatidylserine by flow cytometry. An increase in Annexin-positive

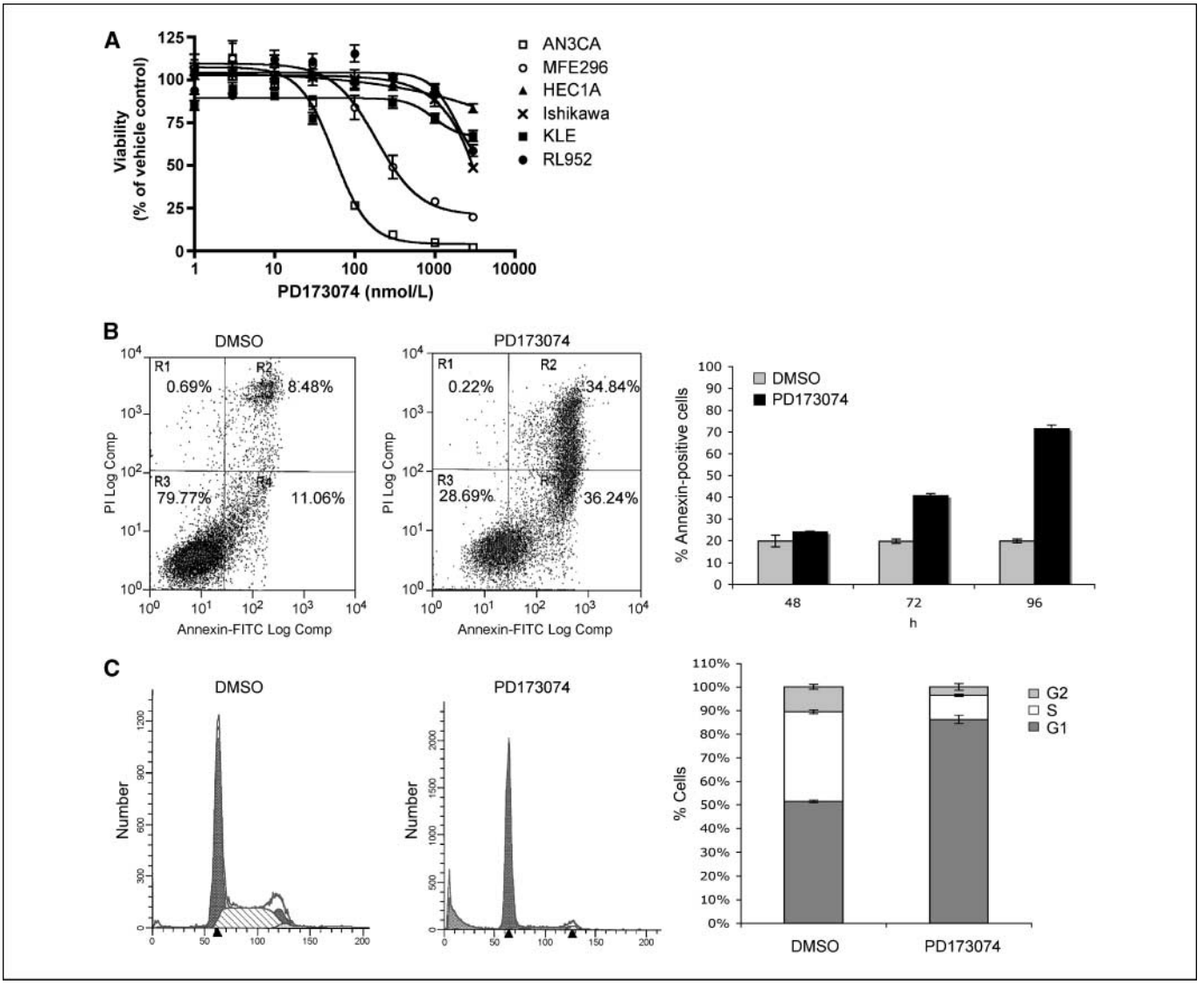

Figure 2. Endometrial cancer cells with activating *FGFR2* mutations are sensitive to PD173074, a pan-FGFR inhibitor. *A,* PD173074 dose-response curves for six endometrial cancer cell lines. Cell viability was measured with the SRB assay 72 h after addition of PD173074 and data normalized to DMSO (vehicle control) for each cell line. AN3CA and MFE296 cells carry the N550K FGFR2 mutation. HEC1A, Ishikawa, KLE, and RL952 are WT for FGFR2. PD173074 treatment had a profound negative effect on cell viability of cell lines expressing mutant FGFR2 compared with those expressing WT FGFR2. PD173074 IC50 values are as follows: AN3CA, 61.8 nmol/L; MFE296, 284.3 nmol/L; HEC1A, >3000 nmol/L; Ishikawa, 2,920.7 nmol/L; KLE, 3,000 nmol/L; RL952, 3,000 nmol/L. *B,* Annexin staining reveals cell death of AN3CA cells after treatment with PD173074. AN3CA cells were treated with 0.1% DMSO (vehicle control) or 1 μmol/L PD173074. Forty-eight, 72, or 96 h later, cells were collected and analyzed for Annexin-positive cells by flow cytometry. The dot plots show representative samples after 96 h of PD173074 treatment. PD173074-treated cells showed an increase in Annexin V staining compared with cells treated with DMSO alone, indicative of apoptosis. *C,* PD173074 leads to G_1 cell cycle arrest in AN3CA cells. Cells were treated with 1 μmol/L PD173074, and cell cycle analysis was performed by propidium iodide staining and flow cytometry 72 h after addition of PD173074. PD173074 treatment resulted in an accumulation of cells in G_1 phase of the cell cycle.

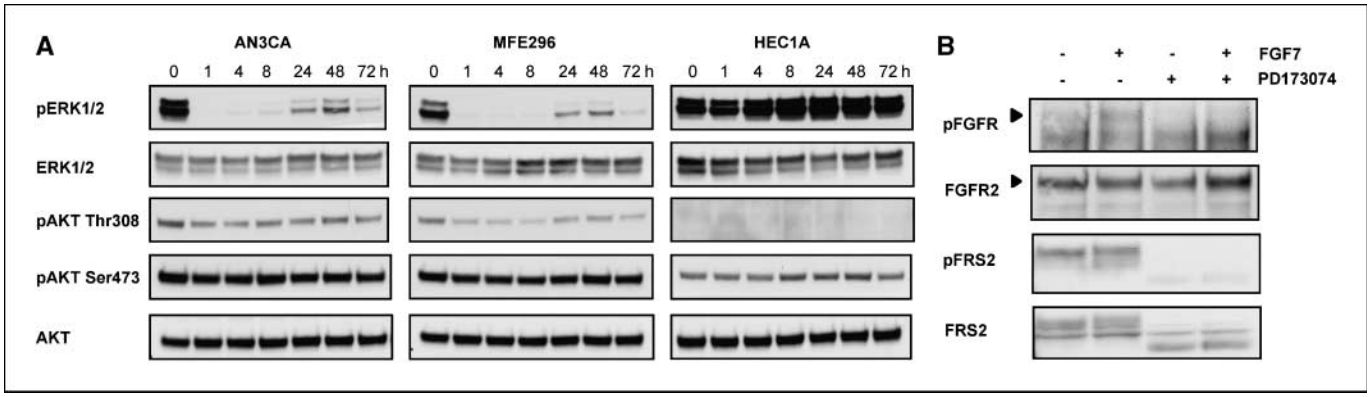

Figure 3. PD173074 treatment markedly inhibits ERK1/2 but not AKT phosphorylation in endometrial cancer cells with activated FGFR2. *A,* cells were starved in 0.2% FBS overnight and then treated with 1 μmol/L PD173074 in 0.2% FBS for the indicated times of 0 to 72 h. Lysates were collected and evaluated by Western blot analysis for activation of ERK1/2 and AKT. PD173074 treatment resulted in marked suppression of ERK1/2 activation and modest suppression of AKT phosphorylation in AN3CA and MFE296 cells. PD173074 had no effect on ERK1/2 or AKT activation in HEC1A cells. Due to the low level of phosphorylated AKT (Threonine 308) in HEC1A cells (13), the image shown here is an overexposure relative to the other pAKT blots presented. *B,* AN3CA cells were starved in 0.2% FBS overnight, pretreated with or without 1 μmol/L PD173074 for 1 h, and then stimulated with 1 nmol/L FGF7 and 10 μg/mL heparin for 5 min. Phosphorylation status of FGFR2 was assessed by immunoprecipitating with a FGFR2-specific antibody and immunoblotting with a phosphorylation-specific FGFR antibody. The blot was then stripped and reprobed for total FGFR2. *Arrow,* fully glycosylated, cell surface–expressed, mature receptor. *Bottom band,* partially glycosylated, immature intracellular receptor. In cells not pretreated with PD173074, phosphorylation of the mature receptor (*top band, solid arrow*) is evident, with an increase in receptor phosphorylation upon FGF7 stimulation. Phosphorylation of the receptor is abrogated upon pretreatment with PD173074. Total levels of receptor are similar in all treatments. The phosphorylation status of FRS2α was detected using a phosphorylation-specific FRS2α antibody. The blot was then stripped and reprobed for total FRS2α. PD173074 treatment blocked basal and FGF7-stimulated phosphorylation of FRS2α. Total FRS2α was detected in all treatment conditions. The change in mobility of FRS2α is due to the phosphorylation status of the protein, with phosphorylated FRS2α migrating slower than unphosphorylated FRS2α (14).

staining was evident following transfection with FGFR2 siRNA compared with the nonsilencing siRNA control, indicating that these cells were undergoing apoptosis (Fig. 1*D*).

Endometrial cancer cells expressing activated FGFR2 are sensitive to PD173074, a pan-FGFR inhibitor. Six endometrial cancer cell lines (two mutant N550K FGFR2 and four WT FGFR2) were treated with increasing concentrations of PD173074, a pan-FGFR tyrosine kinase inhibitor. PD173074 was highly selective for FGFRs in a screen of 224 kinases, inhibiting FGFR1, FGFR2, and FGFR3 at low nanomolar concentrations (12). As shown in Fig. 2*A*, the two endometrial cancer cell lines with mutant *FGFR2* (AN3CA and MFE296) were 10 to 40 times more sensitive to inhibition with PD173074 than cell lines with WT *FGFR2.* The AN3CA line, which has loss-of-function mutations on both *PTEN* alleles, was the most sensitive cell line. Annexin V-FITC labeling indicated that ~70% of AN3CA cells were undergoing apoptosis 96 hours after drug treatment (Fig. 2*B*). In addition, cell cycle analysis revealed that PD173074 treatment induced G_1 arrest of AN3CA cells (Fig. 2*C*).

Activation status of ERK1/2 and AKT after PD173074 treatment. We next evaluated ERK1/2 and AKT activation at various time points after PD173074 treatment. FGFR inhibition resulted in a marked reduction in ERK1/2 activation in AN3CA and MFE296 cells (Fig. 3*A*). Phosphorylated ERK1/2 began to return 24 to 48 hours after PD173074 treatment but was still below baseline activation at 72 hours. No change in ERK1/2 activation was detectable in HEC1A cells (Fig. 3*A*), which are WT for *FGFR2.* PD173074 treatment resulted in a modest reduction in phosphorylated AKT at Threonine 308 and had no effect on phosphorylation at Serine 473 in AN3CA and MFE296 cells (Fig. 3*A*). The constitutive activation of AKT in the AN3CA cell line in 0.2% FBS is likely due to inactivation of both *PTEN* alleles; the mechanism of constitutive AKT activation is unknown in MFE296 cells as they express WT PTEN and PIK3CA.[4] No change in phosphorylation of Serine 473 was detected in HEC1A cells. As previously reported (13), HEC1A cells showed minimal activation of pAKT at Threonine 308 (Fig. 3*A*).

To confirm that the observed effects are due to FGFR2 inhibition, AN3CA cells were pretreated for 1 hour with 1 μmol/L PD173074, stimulated with 1 nmol/L FGF7 for 5 minutes, and phosphorylation of FGFR2 and FRS2α, an adaptor molecule downstream of FGFRs (14), assessed. As shown in Fig. 3*B*, PD173074 pretreatment efficiently blocked both basal and FGF7-stimulated phosphorylation of FGFR2 and FRS2α.

Discussion

Understanding the molecular basis of tumor progression has led to the development and success of targeted therapies in a variety of cancer types. There is increasing evidence that activating mutations in genes involved in various signaling pathways can result in "addiction" of tumor cells to these pathways (15). Furthermore, these activating mutations serve not only to identify potential therapeutic targets, but their presence can also predict clinical response to pathway inhibition (16). However, it has become increasingly clear that the response to target inhibition is also influenced by the molecular context wherein these mutations occur. As we have previously identified activating mutations in *FGFR2* in ~16% of endometrioid endometrial tumors (4), here, we sought to investigate the genetic context in which *FGFR2* mutations occur in endometrial cancer. We also sought to evaluate the therapeutic potential of targeting activated *FGFR2* by investigating the biological consequence of inhibiting FGFR2 in endometrial cancer cells possessing activating mutations in *FGFR2.*

In the present study, we evaluated the *KRAS* and *PTEN* mutation status of endometrioid endometrial tumors with known *FGFR2* mutation status. Activating *KRAS* and *FGFR2* mutations did not occur together in the same tumor, consistent with FGFR2 driving

tumorigenesis through the MAPK pathway. *FGFR2* activation occurred alongside *PTEN* inactivation, suggesting that, at least in endometrial cells, FGFR2 does not mediate its biological effect through PI3K/AKT. This is supported by one previous report where FGF7 or FGF10 stimulation of endometrial cells resulted in ERK1/2, but not AKT, activation (17). The role of MAPK in FGFR2-mediated effects is further supported by our signaling studies, where inhibition of FGFRs resulted in a rapid and robust decrease in ERK1/2 activation but had a very modest effect on AKT phosphorylation (Figs. 1 and 3).

We have also shown that FGFR2 signaling is essential for survival and proliferation of endometrial cancer cell lines with activating *FGFR2* mutations. This is supported by the IC_{50} studies in which we showed the two cell lines with mutationally activated FGFR2 were selectively sensitive to the pan-FGFR inhibitor, PD173074. It is noteworthy that the AN3CA cells, which show abrogation of *PTEN,* were the most sensitive to PD173074. This is of particular importance given the high incidence of *PTEN* mutation in endometrioid endometrial cancer and the suggestion that abrogation of PTEN may be a common mechanism involved in resistance to targeted therapies across multiple cancer types. Indeed, ErbB2-overexpressing breast tumors with reduced or absent PTEN are relatively resistant to trastuzumab-containing chemotherapy regimens (18). Abrogated *PTEN* has also been associated with resistance to the epidermal growth factor receptor inhibitor, gefitinib, in endometrial cancer cell lines (19) and resistance to imatinib in acute lymphoblastic leukemia (20). Importantly, PD173074 induced cell death and cell cycle arrest in the AN3CA cell line, despite loss of PTEN in this cell line. These data suggest that loss of PTEN may not predict resistance to FGFR inhibitors in endometrial cancer. Interestingly, PD173074 treatment induced cell cycle arrest but did not result in enhanced Annexin V staining in MFE296 cells (data not shown).

Although the mechanism of PD173074-induced cell cycle arrest and apoptosis remains to be fully elucidated, it is intriguing to speculate that these phenotypes may be dependent on inhibition of FGFR-mediated MAPK activation. Consistent with this hypothesis, AN3CA cells are sensitive to MAP/ERK kinase inhibition, with UO126 treatment resulting in Annexin-positive staining similar to that observed with PD173074 (Supplementary Fig. S2).

In summary, we have shown that *FGFR2* mutations are coincident with *PTEN* inactivation and mutually exclusive with *KRAS* mutations in primary endometrioid endometrial cancers. Blockade of FGFR2 signaling by shRNA knockdown or treatment with a pan-FGFR inhibitor, PD173074, resulted in cell death and cell cycle arrest of endometrial cancer cell lines expressing mutationally activated FGFR2. Together, these data suggest inhibition of constitutively active mutant FGFR2 may be therapeutically beneficial for endometrial cancer patients despite the frequent inactivation of PTEN in this tumor type.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 2/29/2008; revised 6/11/2008; accepted 7/2/2008.

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Correction: Inhibition of Activated FGFR2 in Endometrial Cancer

In the article on inhibition of activated FGFR2 in endometrial cancer in the September 1, 2008 issue of *Cancer Research* (1), the following grant support information should have been included:

Grant support: A V Scholar Grant from The V Foundation for Cancer Research (P.M. Pollock), a Postdoctoral Fellowship PF-07-215-01-TBE from the American Cancer Society (S.A. Byron), and an R01 CA071754 from the NIH (P.J. Goodfellow).

1. Byron SA, Gartside MG, Wellens CJ, Mallon MA, Keenan JB, Powell MA, Goodfellow PJ, Pollock PM. Inhibition of activated fibroblast growth factor receptor 2 in endometrial cancer cells induces cell death despite PTEN abrogation. Cancer Res 2008;68:6902–7.

doi:10.1158/0008-5472.CAN-68-23-COR2

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Cancer Res 2008;68:6902-6907.