Introduction

The Polo-like kinases (Plk) are a family of serine-threonine kinases involved in cell-cycle regulation and cellular response to stresses, for example, DNA damage. Plk1 is the best characterized kinase and positively regulates progression through G2 (1). Polo-like kinase 2/Snk (Plk2) and the best characterized kinase and positively regulates pro-

Abstract

Resistance to platinum- and taxane-based chemotherapy remains a major clinical impediment to effective management of epithelial ovarian cancer (EOC). To gain insights into resistance mechanisms, we compared gene and confirmed expression patterns of novel EOC cell lines selected for paclitaxel and carboplatin resistance. Here, we report that resistance can be conferred by downregulation of the Polo-like kinase Plk2. Mechanistic investigations revealed that downregulation occurred at the level of transcription via associated DNA methylation of the CpG island in the Plk2 gene promoter in cell lines, primary tumors, and patient sera. Inhibitory RNA (RNAi)-mediated knockdown and ectopic overexpression established a critical functional role for Plk2 in determining apoptotic sensitivity to paclitaxel and carboplatin. In drug-resistant human EOC cell lines, Plk2 promoter methylation varied with the degree of drug resistance and transcriptional silencing of the promoter. RNAi-dependent knockdown of Plk2 abrogated G2-M cell-cycle blockade by paclitaxel, conferring resistance to both paclitaxel and platinum. Conversely, ectopic expression of Plk2 restored sensitivity to G2-M cell-cycle blockade and cytotoxicity triggered by paclitaxel. In clinical cases, DNA methylation of the Plk2 CpG island in tumor tissue was associated with a higher risk of relapse in patients treated postoperatively with carboplatin and paclitaxel (P = 0.003). This trend was also reflected in the analysis of matched serum samples. Taken together, our results implicate Plk2 as a clinically important determinant of chemosensitivity, in support of the candidacy of Plk2 as a theranostic marker to inform EOC management. Cancer Res; 71(9): 3317-27. ©2011 AACR.
Materials and Methods

Drugs used
Carboplatin, cisplatin, paclitaxel, 5’-azacytidine, and tri-chostatin A were obtained from Sigma Aldrich.

Cell lines and plasmids
Primary normal ovarian surface epithelial cells (OSE) were prepared as described (22). A2780 cells were obtained from European Collection of Cell Cultures (ECACC). SKOV-3 cells were obtained from American Type Culture Collection (ATCC; LGC Promochem). Cell lines were authenticated at source by short tandem repeat profiling, morphology (ATCC), and DNA profiling (ECACC). Sublines of A2780 and SKOV-3 ovarian carcinoma cell lines with acquired resistance to paclitaxel and carboplatin (SKOV-3TaxR and SKOV-3CR, respectively) were derived by sequential, pulsed exposure to increasing concentrations of drug. Maintenance doses for the drug-resistant cell lines were generally sub-IC50 doses and representative of clinically achievable levels.

Transfection (knock-in) and silencing (knockdown) of Plk2
Stable knockdown was achieved using plasmid-based inhibitory RNA (RNAi). Sequences (from Ambion) were ligated into pSilencer. A2780 cells were transfected with pSilencer plasmids using Lipofectamine (Invitrogen) and inhibitory RNA (RNAi). Sequences (from Ambion) were selected in hygromycin B. Cells were then challenged with paclitaxel and subjected to flow cytometry. The plasmid for ectopic expression of Plk2 was described previously (15). For transient, RNAi-mediated knockdown of Plk2, we used reagents provided by SMARTpool (Dharmacon; Thermo-Scientific) according to the manufacturer’s protocol. Following 72 hours of silencing, SKOV-3 cells were subcultured into T250 tissue culture flasks at approximately 30% to 50% confluence and exposed to paclitaxel (10 mol/L) or carboplatin (100 mol/L; both used at approximately twice the IC50 dose) for a period of 48 hours, harvested, and then subjected to the Annexin assay.

Clinical samples
Samples of EOC were obtained from the Tissue Bank of the Charité Hospital, Berlin, Germany. Tissues obtained at initial debulking surgery and at first relapse (obtained with informed consent and local ethical committee approval) were collected as snap-frozen biopsies at surgery. Serum was collected at the time of diagnosis and relapse and stored at −80°C. Genomic DNA was obtained from tissues using proteinase K/phenol and from serum using the Qiagen system. Cases were predominantly serous adenocarcinomas (n = 43) but also included endometrioid carcinomas (n = 5), mucinous (n = 1), clear cell (n = 1), mixed histology (n = 2), and 2 unclassified cases (Supplementary Table S2). Postoperative chemotherapy comprised carboplatin and paclitaxel for all patients, given on either a 21-day (q21) or 7-day (q7) schedule. In some cases, gemcitabine was added. Response was assessed by standard criteria including clinical examination, serum CA125, and computerized tomographic (CT) imaging.

mRNA analysis
Analysis of Plk2 expression by reverse transcriptase PCR (RT-PCR) and quantitative PCR (qPCR) was as described previously (15).

Methylation analysis
Genomic DNA was purified from cell pellets by proteinase K digestion and from serum, using a commercially available system (Qiagen). For methylation analysis, genomic DNA (1 μg) was modified by sodium bisulfite as described previously (15). The location of primers for bisulfite sequencing and methylation-specific PCR (MSP) was shown in Supplementary Figure S1. Quantitative MSP (qMSP) of the Plk2 CpG island was carried out using primer pair 4. PCR was conducted in an ABI PRISM 7700 Sequence Detection system (Applied Biosystems) using SYBRgreen (Qiagen).

Western blotting
Cell pellets were prepared from attached and floating cell populations, subjected to osmotic rupture in hypotonic detergent-based buffer (1 mmol/L phenylmethylsulfonylfluoride, NaVO4, aprotinin, and leupeptin as protease inhibitors, 150 mmol/L NaCl, in 50 mmol/L Tris buffer, 0.2% SDS, and 1% NP-40, pH 7.5), and then 40 to 50 μg of protein per sample electrophoresed on SDS-PAGE gels. Membranes were incubated overnight at 4°C with primary antibody of choice, the membrane was stripped and reprobed using a monoclonal antibody against Plk2 antibody (sc-25421, H-90 rabbit polyclonal antibody; Insight Biotechnology) or Plk3, 4, and 4 (AbCam). In some experiments, the anti-Plk2 antibody used was as described previously (13, 15). Following the probing of each membrane with the primary antibody of choice, the membrane was stripped and reprobed using a monoclonal antibody against Plk2 antibody (sc-25421, H-90 rabbit polyclonal antibody; Insight Biotechnology) or Plk3, 4, and 4 (AbCam).

Cytotoxicity assays
A2780 and SKOV-3 parental and resistant cells for cytotoxicity testing were seeded into 96-well plates at a density of 3 × 104 to 4 × 104 cells/mL to allow approximately 3 doublings (approximately 10- to 20-fold increase in control cell number) for the duration of the drug exposure. Cells were allowed to attach and equilibrate for 24 hours and then treated with various concentrations of drug more than 2 to 3 log orders of concentration. Results were not materially affected by initial plating densities. Drugs were diluted in tissue culture medium containing 10% fetal calf serum. Control cell wells were treated with drug-free tissue culture medium. Drug-treated cells were then returned to the incubator for a period of 72 hours. Cell viability was determined by addition of 0.1 mg MTT to each well for 4 hours at 37°C. Wells were aspirated to remove the medium, the resulting formazan crystals were solubilized in 200 μL dimethyl sulfoxide, and the absorbance was read at 570 nm. The absorbance of the formazan product obtained for drug-treated cells was
calculated as a fraction of that for the untreated control wells. Growth curves were constructed by using PRISM software and results expressed as IC50 values, that is, dose of drug causing a 50% reduction in cell viability.

Annexin V assay for assessment of apoptosis

The effect of drugs on cell-cycle distribution was assessed by flow cytometry as described previously (15). Cell death was assessed by measurement of apoptosis by using Annexin V. Briefly, cells were seeded into tissue culture flasks to give a density approximately 30% to 50% confluence (for SKOV-3 and A2780 cell lines, this corresponded to 4 × 105 cells/T25 flask), allowed to attach for 2 to 3 hours, and then treated for 48 hours with the appropriate compound at various concentrations. In our hands, initial plating densities between 20% and 50% did not significantly affect apoptosis induction in this assay. An Annexin V–FITC (fluorescein isothiocyanate)-conjugated apoptosis detection kit incorporating propidium iodide (PI) was used according to the manufacturer’s protocol (OncoGene; supplied by CN Biosciences). Harvesting of cells included collection of attached cells (following trypsinization) and floating cells. Samples were analyzed by flow cytometry, using the FL1 (FITC) and FL3 (PI) laser lines, and each sample was assessed by using a collection of 10,000 events. All analyses were carried out in triplicate.

Statistical analysis

Statistical analysis was carried out using SPSS (version 14) and Minitab programs. Tissue and serum methylation results were assessed in the groups of responders and nonresponders in terms of the utility of DNA methylation as a potential marker of chemoresponsiveness.

Results

Ovarian carcinoma cell lines with primary resistance to paclitaxel show cross-resistance to platinum and vice versa

We derived, de novo, a series of novel A2780 EOC cell lines with increasing degrees of acquired resistance to paclitaxel (Supplementary Table S1). Calculating the relative resistance (as a resistance factor) by using the ratio of IC50-resistant variant/IC50 of parental cell line (1.5 nmol/L), we derived the nomenclature for the A2780TaxR panel of cell lines; hence, A2780TaxR354 is 354-fold resistant to paclitaxel. We also developed independent SKOV-3 cell lines with primary resistance to paclitaxel and carboplatin; SKOV-3TaxR was 52-fold resistant compared with the parental SKOV-3 cell line (IC50 = 5.7 nmol/L) and SKOV-3CR cell lines were 3-fold resistant to carboplatin (IC50 = 298.0 μmol/L) and 2-fold resistant to cisplatin (IC50 = 21.7 μmol/L; Supplementary Table S1). We tested whether cell lines with acquired resistance to paclitaxel were cross-resistant to platinum compounds and vice versa. Paclitaxel-resistant cell lines were challenged with carboplatin or cisplatin, and cytotoxicity was evaluated. In each cell line in our series, paclitaxel-resistant cell lines showed decreased sensitivity to cisplatin and carboplatin (Fig. 1, Supplementary Table S1). Conversely, SKOV-3CR cells showed decreased sensitivity to paclitaxel (Fig. 1, Supplementary Table S1).

Drug resistance is associated with loss of G2-M checkpoint

Using Annexin V staining and flow cytometry, we asked whether resistance to paclitaxel was associated with changes in drug-induced apoptosis and/or cell-cycle distribution. In A2780TaxR354 paclitaxel-resistant cell lines, there was a significant reduction in apoptosis on exposure to paclitaxel relative to parental cells (Fig. 2A). The apoptotic effects of drug treatment could be seen by the emergence of Annexin V–positive (quadrants top right and bottom right) and PI-positive populations (emergence toward top left/right quadrants). Summation of the apoptotic quadrants revealed approximately 49% and 25% cell death for A2780 parent and A2780TaxR354 cells, respectively, following treatment with paclitaxel. Likewise, following treatment with cisplatin, a clear reduction in the extent of cell death is also seen by comparing A2780 parent and A2780TaxR354 resistant cells. Next, we analyzed perturbations in cell cycle following drug exposure. Following 24-hour paclitaxel exposure, A2780 parent cells, sub-G1 (apoptotic)- and G2-M–arrested populations were clearly increased (Fig. 2B), but in paclitaxel-resistant A2780TaxR354 cells, there was no increase in the sub-G1 population. Furthermore, the G2-M population was greatly reduced in A2780TaxR354 cells relative to A2780 parental cells (Fig. 2B). Together, these results imply that acquired resistance to paclitaxel involves loss of G2-M cell-cycle checkpoint function and abrogation of apoptosis. In SKOV-3 parent cells, paclitaxel exposure resulted in clear increase in the apoptotic cell population which was completely absent in SKOV-3TaxR cells (Fig. 2C). In carboplatin-resistant SKOV-3CR cells, there was reduced apoptosis on exposure to paclitaxel (Fig. 2C).

Transcriptional silencing of Plk2 in cell lines with acquired drug resistance

Next, we analyzed the expression and regulation of Plk2. Plk2 mRNA was abundantly expressed in normal OSE and in the parent A2780 and SKOV-3 lines (Fig. 3A and B). There was, however, downregulation of Plk2 mRNA in A2780TaxR354, SKOV-3TaxR (paclitaxel-resistant), and SKOV-3CR (carboplatin-resistant) cells relative to the respective parent lines (Fig. 3A and B). Western blot analysis confirmed reduced levels of Plk2 protein in paclitaxel-resistant A2780 and SKOV-3 cell lines (Fig. 3C). In contrast, there was no change in expression of Plk1 or Plk3 between parent and paclitaxel-resistant A2780 and SKOV-3 cell lines (Fig. 3D). Plk4 levels were higher in A2780TaxR354 cells but not in SKOV-3TaxR. To investigate whether downregulation of Plk2 mRNA is associated with methylation changes, we carried out bisulfite sequencing of the Plk2 CpG island. The CpG island was unmethylated in OSE, in the parent A2780 and SKOV-3 EOC cell lines, and in other EOC cell lines. However, in A2780TaxR354 and SKOV-3TaxR cells, there was acquisition of methylation within a defined region of the CpG island.
We tested whether demethylating agents reactivate expression of Plk2 mRNA and observed an increase in Plk2 mRNA after exposure to 5′-aza-cytidine (aza-C; Fig. 4B), consistent with methylation-dependent silencing. Using both conventional MSP and qMSP, we confirmed that methylation was detectable in the paclitaxel-resistant derivatives A2780TaxR354 and SKOV-3TaxR but not in the respective parent cell lines (Fig. 4C and D).

**Methylation and expression of Plk2 vary quantitatively with drug resistance**

We analyzed expression and methylation of Plk2 in our panel of paclitaxel-resistant variants of A2780. Bisulfite sequencing showed a progressive, quantitative increase in methylation with increasing drug resistance (Fig. 5A). qMSP analysis confirmed that methylation increased with increasing paclitaxel resistance (Fig. 5B). Expression of Plk2 mRNA (Fig. 5C) and protein (Fig. 5D) decreased with increasing paclitaxel resistance, consistent with changes in CpG methylation.

**Paclitaxel resistance correlates with epigenetic status of the Plk2 CpG island**

We next tested the stability of the drug-resistant phenotype. Clones of the A2780 paclitaxel-resistant cell line panel, with increasing levels of primary resistance to paclitaxel, were transferred to drug-free medium and grown continuously in the absence of paclitaxel. After 6-week growth in drug-free medium, A2780TaxR354 cells grown in the absence of paclitaxel (−) had substantially reverted to a paclitaxel-sensitive phenotype (Fig. 6A). In A2780TaxR615 (−) cells, there was a clear, but less complete, restoration of the paclitaxel-induced G2-M block (Fig. 6A). Plk2 expression in A2780TaxR354 (−) had returned to almost 75% of the level of parental cells and that of A2780TaxR615 (−) to approximately 33% of parent A2780 cells.
Plk2 expression modulates cytotoxicity of carboplatin and paclitaxel  

We used plasmid-mediated RNAi to stably downregulate Plk2 in the drug-sensitive A2780 parent cell line. Knockdown was confirmed by RT-PCR ("KD" in Fig. 7A). Clonal cell lines with "knocked down" expression of Plk2 (A2780KD) were challenged with a cytotoxic concentration of paclitaxel (Fig. 7B). G2-M block and increase in the sub-G1 population were greatly reduced in the knocked down cells (Fig. 7B). To exclude the so-called "off-target" effects, A2780 cell lines with knocked down Plk2 (A2780KD) were retransfected with a Plk2 expression plasmid (A2780KD + KI). The effect of Plk2 reexpression was increased paclitaxel-induced G2-M blocked and sub-G1 populations, relative to the A2780KD cells (Fig. 7B). Next, we ectopically expressed Plk2 in A2780TaxR354 cells which have undetectable levels of endogenous Plk2. Flow cytometry revealed that in A2780TaxR354 KI cell lines engineered to ectopically express Plk2, there was restoration of the paclitaxel-dependent G2-M checkpoint, which was lost in the A2780TaxR354 cells (Fig. 7C). Finally, we used transient transfection of Plk2 siRNA to silence expression in SKOV-3 parental cells. Western blotting confirmed downregulation of Plk2 expression (Fig. 7D). The silenced cells were exposed to paclitaxel and carboplatin for 48 hours. There was a reproducible reduction in apoptosis induced by both paclitaxel and carboplatin in cells silenced for Plk2 expression compared with control cells (Fig. 7E).
**Plk2 is a candidate predictor of chemotherapy resistance in EOC**

We used qMSP to analyze methylation in the *Plk2* CpG island in a test series of 54 cases of EOC, treated in a single center with postoperative carboplatin/paclitaxel chemotherapy. Thirty-four cases had relapsed clinically at the time of censor (as evidenced by rising serum CA125 levels and/or CT evidence of progressive disease), whereas 20 cases remained disease free at the time of censor. Full clinical details of the cases are given in Supplementary Table S2. Methylation in tumor tissues in the *Plk2* CpG island was detected by using qMSP in 19 of 54 (31%) cases at diagnosis. Cases with methylation in tumor tissue at diagnosis were more likely to have relapsed at the time of censor than those with undetectable methylation in tumor tissue at diagnosis ($P = 0.0032$, Fisher’s exact test). We also carried out qMSP for *Plk2* CpG methylation in serum (where available). Thirty-nine of 50 sera were concordant with the methylation status of their matched tumor tissue ($P = 0.00216$, Fisher’s exact test). We asked whether the presence of methylated *Plk2* DNA in serum was more common at relapse than at diagnosis. Paired sera at diagnosis and relapse were available for 32 cases. Twenty-three of 32 sera were concordant with the matched tissue ($P = 0.011$, Fisher’s exact test). The proportion of sera with detectable methylation was higher at relapse than at diagnosis but because of small sample numbers, this did not reach statistical significance (0.47 vs. 0.62, $P = 0.204$). Although there was a clear trend for shorter relapse-free survival in cases with...
detectable methylated DNA from the Plk2 CpG island, both in tumor tissue and serum, this did not reach statistical significance with the small numbers of cases available for study ($P = 0.142$ for tissue and 0.054 for serum). See Supplementary Figure 3 for additional data and Kaplan–Meier survival curves.

These data suggest that detection of methylated Plk2 DNA may have utility in prediction of clinical outcome in EOC.

Discussion

We have shown that Plk2 is a determinant of cellular sensitivity to paclitaxel and carboplatin and via a mechanism of methylation-dependent transcriptional silencing, Plk2 is associated with drug resistance. Paclitaxel resistance in A2780 cell lines was associated with reduction or complete loss of the drug-induced G2-M checkpoint and reduced apoptosis. Similarly, in paclitaxel- or carboplatin-resistant SKOV-3 cells, there was reduced apoptosis relative to drug-sensitive parental cells. Importantly, in A2780 and SKOV-3 cell lines with acquired resistance to paclitaxel, there was significantly reduced apoptosis when challenged with platinum compounds. Moreover, carboplatin-resistant SKOV-3 cells exhibited reduced apoptosis when exposed to paclitaxel.

Plk2 has both G2-M checkpoint (16) and proapoptotic (15) function(s), prompting us to ask whether changes in expression of Plk2 occur with acquisition of drug resistance. Plk2 expression was reduced relative to parent cell lines in A2780 and SKOV-3 cell lines with acquired resistance to paclitaxel and also in a SKOV-3 cell line resistant to carboplatin. The specificity of these effects was emphasized by analysis of the related genes Plk1 and Plk3/Fnk/Prk in which there were no changes in expression with acquisition of resistance to paclitaxel. Three other lines of experimental evidence support an important role for Plk2 as a determinant of drug sensitivity. First, ectopic expression of Plk2 in cells in which endogenous Plk2 was epigenetically downregulated substantially restored
sensitivity to paclitaxel. Second, RNAi-mediated "knockdown" of Plk2 in drug-sensitive A2780 and SKOV-3 parental cell lines conferred reduced sensitivity to paclitaxel and platinum compounds, accompanied by reduced G2-M block and apoptosis, an effect reversible by reexpression of Plk2. Third, low to moderate degrees of paclitaxel resistance were reversible when cells were allowed a drug-free "holiday." In such cell lines, with reacquired drug sensitivity, expression of Plk2 increased with increasing drug sensitivity. Our results are compatible with studies showing that decreased sensitivity to paclitaxel is associated with compromised checkpoint function or spindle assembly (23–25). Our data are also consistent with microarray analyses of platinum-resistant cell lines in which Plk2 is downregulated (21, 26). Together, these results afford further support for the candidacy of Plk2 as a determinant of chemotherapy sensitivity and for a predominantly epigenetic basis for acquired drug resistance (27). Our data also imply that Plk2 expression and Plk2-associated kinase activity may be required, at least in some cell types, for sensitivity to chemotherapy. This is supported by a recent study in chronic lymphocytic leukemia in which failure of Plk2 upregulation was associated with chemotherapy resistance (28).

Our hypothesis that dynamic changes in the epigenetic status of Plk2 influence the drug sensitivity of cancer cells is supported by various lines of evidence from other authors. For example, changes in DNA methylation within the CpG islands of specific genes are detectable very rapidly after exposure to cytotoxic agents (29). Also, increasing cisplatin resistance in vivo is associated with elevated expression of methyltransferases and quantitative changes in CpG island methylation (21). In vivo supportive evidence comes from studies of the DNA mismatch repair gene hMLH1. Inactivation of hMLH1 is associated with increased resistance to cisplatin in vitro. Analysis of methylated DNA in serum of individuals receiving platinum-based adjuvant chemotherapy for EOC...
revealed that methylation levels changed during chemotherapy and had utility in predicting clinical outcome (30). A profiling study of global changes in the methylome of drug-sensitive and -resistant MCF-7 cells supports a general model of acquired drug resistance in which opposing and ongoing hypo- and hypermethylation in multiple genes constitute a major mechanism driving the process of drug resistance (31). In initial clinical studies, we have shown that cases of EOC with \textit{Plk2} methylation at diagnosis were at significantly increased risk of early clinical relapse with a less favorable response to postoperative chemotherapy than cases lacking methylation. Also, methylated \textit{Plk2} DNA in serum was more common at clinical relapse than at initial diagnosis. Our results imply that analysis of \textit{Plk2} methylation may have utility as a biomarker of both disease activity and chemosensitivity in EOC. In the context of our results, \textit{Plk2} has been identified as a significantly downregulated gene in chemotherapy-resistant primary ovarian carcinomas, affording independent validation of our hypothesis for it being a determinant of chemotherapy sensitivity and candidate biomarker in EOC (32). An important feature of the taxane-resistant cell line models we used in the current study is their cross-resistance with platinum agents. In support of this, previous reports in the literature cite decreased platinum sensitivity with cross-resistance to chemotherapeutic agents, such as taxanes, in EOC cell lines (33). Clinically, a proportion of EOC patients show platinum–taxane non–cross-resistance (34). Both our cell line panel data and our EOC patient cohort used in this study are representative of platinum and taxane cross-resistance.

We previously reported that \textit{Plk2} is subject to transcriptional downregulation in B lymphomas, and expression profiling has shown that \textit{Plk2} downregulation is a predictor of poor clinical outcome in B lymphomas (35). Our data are in support of clinical trials of epigenetic therapies in EOC patients and other cancer patients (36) and suggest that detection of

---

**Figure 6.** Reversal of paclitaxel resistance by growth in the absence of drug. A, flow cytometry analysis of A2780 parent cells and paclitaxel-resistant A2780TaxR354 and A2780TaxR615 sublines. Parent A2780 cells and derivative cell lines allowed a 6-week period of growth in drug-free medium (–) were exposed to paclitaxel and then subjected to flow cytometry. B, expression of \textit{Plk2} mRNA in A2780 cell lines grown with (dark gray boxes) or without maintenance paclitaxel (light gray boxes). Expression is relative to parent A2780 cells. C, MSP analysis of the \textit{Plk2} Cpg island in A2780 cell lines grown with (+) or without (–) maintenance paclitaxel. Controls are: CU (control unmethylated DNA) and CM (control methylated DNA). MSP reactions for unmethylated (U) and methylated (M) alleles are shown.
methylated Plk2 DNA warrants independent evaluation in larger clinical series as a candidate predictor of response of EOC patients to chemotherapy and as a serum biomarker of drug-resistant clinical relapse.

**Disclosure of Potential Conflicts of Interest**

The authors declare they have no competing financial interests in relation to the work in this study.

**References**

Inactivation of Plk2 in Ovarian Cancer


Polo-like Kinase Plk2 Is an Epigenetic Determinant of Chemosensitivity and Clinical Outcomes in Ovarian Cancer


Cancer Res 2011;71:3317-3327. Published OnlineFirst March 14, 2011.

Updated version  Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-10-2048

Supplementary Material  Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2011/03/14/0008-5472.CAN-10-2048.DC1

Cited articles  This article cites 35 articles, 16 of which you can access for free at: http://cancerres.aacrjournals.org/content/71/9/3317.full#ref-list-1

Citing articles  This article has been cited by 1 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/71/9/3317.full#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.