Cross-talk between EphA2 and BRaf/CRaf Is a Key Determinant of Response to Dasatinib

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Abstract

Purpose: EphA2 is an attractive therapeutic target because of its diverse roles in cancer growth and progression. Dasatinib is a multikinase inhibitor that targets EphA2 and other kinases. However, reliable predictive markers and a better understanding of the mechanisms of response to this agent are needed.

Experimental design: The effects of dasatinib on human uterine cancer cell lines were examined using a series of in vitro experiments, including MTT, Western blot analysis, and plasmid transfection. In vivo, an orthotopic mouse model of uterine cancer was utilized to identify the biologic effects of dasatinib. Molecular markers for response prediction and the mechanisms relevant to response to dasatinib were identified by using reverse phase protein array (RPPA), immunoprecipitation, and double immunofluorescence staining.

Results: We show that high levels of CAV-1, EphA2 phosphorylation at S897, and the status of PTEN are key determinants of dasatinib response in uterine carcinoma. A set of markers essential for dasatinib response was also identified and includes CRaf, pCRafS338, pMAPKT202/Y204 (mitogen-activated protein kinase [MAPK] pathway), pS6S240/244, p70S6kT389 (mTOR pathway), and pAKTS473. A novel mechanism for response was discovered whereby high expression level of CAV-1 at the plasma membrane disrupts the BRaf/CRaf heterodimer and thus inhibits the activation of MAPK pathway during dasatinib treatment.

Conclusions: Our in vitro and in vivo results provide a new understanding of EphA2 targeting by dasatinib and identify key predictors of therapeutic response. These findings have implications for ongoing dasatinib-based clinical trials. Clin Cancer Res; 20(7); 1–10. ©2014 AACR.
Translational Relevance

EphA2 is considered an important therapeutic target as it is involved in many processes crucial to malignant progression. Among the various therapeutic strategies targeting EphA2, dasatinib is the farthest along with regard to clinical development. However, reliable predictors of response and the mechanisms relevant to dasatinib response have been largely lacking. We have identified a set of molecular predictors of dasatinib sensitivity in uterine cancer that may have significant implications for ongoing dasatinib-based clinical trials. Our key findings are that CAV-1, EphA2 phosphorylation at S897, and the status of PTEN are key determinants of dasatinib response. Moreover, we have identified a previously unknown mechanism involving the association between CAV-1, EphA2, and the heterodimer BRAF/CRAF, which has implications for designing new therapeutic combinations.

Materials and Methods

Cell cultures

We selected uterine cancer cell lines HEC1-A, Ishikawa, SKUT-2, RL95-2 and KLE (all EphA2 positive) and SPEC-2, AN3CA, and HEC-265 (all EphA2 negative). Cells were purchased from the MD Anderson Characterized Cell Line Core Facility, which supplies authenticated cell lines. The cell lines were routinely tested to confirm the absence of mycoplasma, and all experiments were performed with cell lines at 60% to 80% confluence. Cells were maintained in specific culture medium as described previously (6). The molecular features of cell lines (EphA2 status, CAV-1 status, RAS mutation, and PTEN mutation) are listed in Fig. 1A (left).

MTT assay

Inhibition of cell growth by dasatinib was detected using the MTT cell-proliferation assay, a tetrazolium-based colorimetric assay performed in quadruplicate. Additional details about the treatment and MTT assay are provided in the Supplementary Methods (available online).

Western blot analysis and immunoprecipitation analyses of dasatinib targets in vitro

The expression levels of pEphA2S897, pSrcY416, pFAKY397, pFAK1925, pPaxillinV118, pAKTV397, pp130CasY410, CAV-1, and pSE5240/244 in the uterine cells lines were determined using Western blot analysis. Additional details about the Western blot analysis are provided in the Supplementary Methods (available online). To test the interaction between EphA2, BRAF, and CAV-1, whole-cell lysates of related cells before and after dasatinib treatment were immunoprecipitated with antibodies against EphA2 (Millipore and Santa Cruz Biotechnology), BRAF, or CAV-1 (CST, Danvers, and Santa Cruz Biotechnology), and blots were detected with antibodies against them. Additional details about the immunoprecipitation assays are provided in the Supplementary Methods (available online).

Site-directed mutagenesis of EphA2 at serine 897

We designed 2 types of point mutations at S897 of EphA2, 1 for inactivated pEphA2S897 (serine-897 to alanine; 5’-TCACCCCCTCCGAGCCGCTCGT-3’ and 5’-CGGGGAGCCGGATAGACACGC-3’) and 1 for constitutively activated pEphA2S897 (serine-897 to glutamine; 5’-GCCGTGTCTATCCGGCTCCCC-3’ and 5’-CACCCCCCTCCGAGCCGGATAGACACGC-3’). The human cDNA open reading frame Myc-DDK-tagged clone of EphA2 (NM_004431; Origene) was amplified using these primers containing the desired mutation by polymerase chain reaction. The product was digested with DPN1 for 1 hour to eliminate methylated DNA. The final mutant EphA2 was confirmed by sequencing (SeqWright).

Double immunofluorescence staining

To test the dynamic changes in the localization of EphA2, CAV-1, and BRAF in SKUT-2 cells treated with dasatinib at 100 nmol/L for 16 hours, double staining was carried out using the rabbit monoclonal antibody against CAV-1 (red) and mouse monoclonal antibody for BRAF (green) or EphA2 (red or green). The stained cells were visualized by confocal microscopy at ×200 magnification.

In situ proximity ligation assay

In situ PLA was performed according to manufacturer’s instructions (Olink Bioscience). Briefly, after incubation with primary antibodies, the cells were incubated with a combination of corresponding PLA probes, secondary antibodies conjugated to oligonucleotides (mouse MINUS and rabbit PLUS). Subsequently, ligase was added forming circular DNA strands when PLA probes were bound in close proximity, along with polymerase and oligonucleotides to allow rolling circle amplification. Fluorescently labeled probes complementary in sequence to the rolling circle amplification product was hybridized to the rolling circle amplification product (Duolink Detection Kit 563; Olink Bioscience). Thus, each individual pair of proteins generated a spot (blob) that could be visualized using fluorescent microscopy at ×200 magnification.

Transfection of siRNA

siRNA was purchased from Sigma-Aldrich. A non-silencing siRNA that did not share sequence homology with any known human mRNA based on a BLAST search was used as control for target siRNA. For in vitro delivery, siRNA (5 μg) was incubated with 30 μL RNAiFect transfection reagent (Qiagen) for 10 minutes at room temperature and added to cells in culture at 80% confluence in 35 mm culture plates. The medium was changed 6 hours later, and cells collected after 48 hours as lysate for Western blot analysis.
Detection of multiple signaling pathways by RPPA

**SKUT-2, SPEC-2, HEC1-A, and Ishikawa cells were treated with 1 μmol/L dasatinib and 5 nmol/L paclitaxel for 16 hours. Samples were probed with 176 validated primary antibodies by RPPA at the MD Anderson Cancer Center RPPA Core Facility (see Supplementary Methods [available online] for additional details.**

**Orthotopic in vivo model of uterine cancer and tissue processing**

All animal studies were approved and supervised by the MD Anderson Institutional Animal Care and Use Committee. Two uterine cancer cell lines, SPEC-2 (no EphA2 expression) and SKUT-2 (high EphA2 expression) were used for in vivo experiments as previously described (6). For in vivo therapy experiments, 10 mice were randomly allocated into 4 treatment groups: control, dasatinib (15 mg/kg oral, daily), paclitaxel (100 μg in 200 μL of PBS intraperitoneally, weekly), or dasatinib plus paclitaxel. Therapy was initiated 2 weeks after cell injection. Additional details are provided in the Supplementary Methods (available online).

**Immunohistochemical staining in the mouse uterine tumor samples**

Paraffin-embedded tissues were used to detect cell proliferation (with Ki67) and apoptosis (with cleaved caspase-3). The sections were incubated with the Ki67 antibody (1:400, Dako) and monoclonal mouse antibody against cleaved caspase-3 (1:100; Biocare Medical). CD31 staining with rat monoclonal anti-mouse CD31 (1:800, PharMingen) was used as a loading control (right, bottom).

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**Figure 1. In vitro effects of dasatinib in uterine cancer cell lines. A. expression level of EphA2 and CAV-1 and expression of wild-type or mutant RAS and PTEN for each cell line. --, no expression; -, low expression; +++, high expression (left). Median inhibitory concentration (IC50) after treatment with dasatinib (middle). Cell viability after treatment with dasatinib at 0, 10, 50, 100, 1,500, 150, 1,000, or 10,000 nmol/L for 72 hours (right). Data represent means of triplicate measurements with error bars to represent SEM. B, immunoblot analysis of proteins associated with the Src/FAK/EphA2 pathway in cells treated with (+) or without (-) dasatinib at 100 nmol/L for 16 hours (left). Quantification of band intensity relative to actin intensity is shown graphically. Black bars, no treatment. White bars, treatment (right). C, immunoprecipitation (IP) and Western blot (WB) analysis of tyrosine-phosphorylated EphA2 in HEC1-A, Ishikawa, and SKUT-2 cells treated with (+) or without (-) dasatinib at 100 nmol/L for 16 hours. D, effect of wild-type (WT) EphA2 and pEphA2S897 status on the sensitivity of SKUT-2 cells to dasatinib at 0, 10, 50, 100, 1,500, 150, 1,000, or 10,000 nmol/L for 72 hours. Cell viability assay was performed with SKUT-2 cells stably transfected with Myc-DDK-tagged plasmid with WT EphA2, inactivated pEphA2S897, or constitutively activated pEphA2S897 (left). Median inhibitory concentration (IC50) after treatment with dasatinib (right, top) at 100 nmol/L for 16 hours. Western blot results showing status of EphA2 and pEphA2S897 in SKUT-2 cells after transfection. Anti-Myc antibody was used as a marker for efficiency of transfection. β-Actin was used as a loading control (right, bottom). EV, empty vector.
performed on frozen sections. Additional details about immunohistochemistry (IHC) method are provided in the Supplementary Methods (available online).

Immunohistochemical staining of human uterine cancer specimens
After approval by the MD Anderson Institutional Review Board, slides from 40 cases of uterine cancer were obtained from the surgical pathology files of MD Anderson. For human uterine cancer samples, immunohistochemical analysis for CAV-1 (1:200 dilution; CST), pAKT\(^{5473}\) (1:100 dilution; CST), and pEphA2\(^{5974}\) and pEphA2\(^{5897}\) (1:200 dilution; Cell Applications) was performed as described previously (18). See Supplementary Methods (available online) for additional details.

Statistical analysis
For in vivo therapy experiments, 10 mice were used in each group, which provided the power to detect a 50% reduction in tumor size (\(\beta\) error = 0.2). Continuous variables were compared using Student t test (2 groups) or analysis of variance (all groups) if the data were normally distributed. For nonparametric distributions, the Mann–Whitney test (2 groups) or analysis of variance (all groups) was used. A value of \(P<0.05\) with 2-tailed testing was deemed statistically significant.

Results

**In vitro effect of dasatinib on EphA2-positive or -negative uterine cancer cell lines**
We first assessed the effect of dasatinib on a panel of 6 uterine cancer cell lines with known EphA2 expression levels (Fig. 1A, left). The median inhibitory concentration (IC\(_{50}\)) of these cell lines ranged from 0.03 to 17.9 μmol/L (Fig. 1A, middle). Among these, EphA2-positive SKUT-2 cells were the most sensitive to dasatinib, whereas EphA2-negative SPEC-2 cells were the most resistant (Fig. 1A, right), suggesting that EphA2 status is a potential determinant of dasatinib sensitivity. The exception to this pattern was the EphA2-positive HEC1-A cell line (harbors a RAS mutation), with a higher IC\(_{50}\) value than other EphA2-negative cells. These observations were further supported by EphA2-negative HEC-265 and EphA2-positive KLE uterine cancer cells, and by ectopic expression of EphA2 in the EphA2-negative A2780 ovarian cancer cells (Supplementary Fig. S1A and S1B).

Wild-type PTEN increased sensitivity to dasatinib in EphA2-positive cell lines in vitro
We found that dasatinib exhibited less growth inhibition in EphA2-positive RL95-2 cells, which harbors high basal level of pAKT\(^{5473}\). Therefore, next we addressed whether PTEN, which is frequently altered in uterine carcinoma (19), influences response to dasatinib through its effects on pAKT\(^{5473}\). AKT activation and PTEN mutational status in a panel of uterine cancer cell lines is shown in Fig. 1A and B. Next, we transfected wild-type or mutant PTEN into Ishikawa cells carrying mutant PTEN. The dasatinib sensitivity of Ishikawa cells transfected with wild-type PTEN was significantly enhanced by 5-fold, compared with parental cells carrying PTEN mutation (Supplementary Fig. S2), suggesting that wild-type PTEN is also a determinant of dasatinib sensitivity in uterine cancer cells.

**In vitro effects of dasatinib on Src/FAK/EphA2 signaling pathway**
Next, we examined signaling events in the Src/FAK/EphA2 pathway known to be perturbed by dasatinib. The expression level of the traditional targets of dasatinib, including pAKT\(^{5473}\), pSrc\(^{416}\), pFAK\(^{3925}\), pPaxillin\(^{118}\), and pp130Cas\(^{1410}\), were reduced in all 6 uterine cancer cell lines after dasatinib treatment, whereas pFAK\(^{3925}\) expression level was not notably affected in any of the cell lines (Fig. 1B). These results support the known effects of dasatinib, but they do not explain the sensitivity of specific uterine cancer cell lines to it.

pEphA2\(^{5897}\) status is an important determinant of response to dasatinib in uterine cancer cells
Given the differential expression of EphA2 in dasatinib-sensitive versus dasatinib-resistant cell lines and the role of ligand-independent pEphA2\(^{5897}\) in many oncogenic functions (20), we next examined the effects of dasatinib on pEphA2\(^{5897}\). The expression of pEphA2\(^{5897}\) was significantly decreased in SKUT-2 cells, but moderately so in HEC1-A and Ishikawa cells (Fig. 1B and Supplementary Fig. S4). Furthermore, because the expression levels of pEphA2\(^{5897}\) and pEphA2\(^{594}\) are inversely related (20), EphA2 tyrosine phosphorylation was decreased in HEC1-A and Ishikawa cells, but increased in SKUT-2 cells, after dasatinib treatment (Fig. 1C). The extent of inhibition was directly related to dasatinib response. To further test whether phosphorylation at S897 is critical for dasatinib response, we performed site-directed mutagenesis of pEphA2 at S897 and transfected SKUT-2 cells with plasmids for wild-type EphA2, inactivated pEphA2\(^{5974}\), or constitutively activated pEphA2\(^{5897}\). The cells with constitutively activated pEphA2\(^{5897}\) had reduced sensitivity to dasatinib, whereas the cells with inactivated pEphA2\(^{5897}\) remained highly sensitive to dasatinib (Fig. 1D). These results confirmed that pEphA2\(^{5897}\) status is an important determinant of response to dasatinib in uterine cancer cells (Supplementary Table S1).

Identification of predictive molecular markers
To explore other potential markers of response to dasatinib, we used RPPA to quantify protein expression of genes involved in cell cycle, apoptosis, angiogenesis, and adhesion that are modulated by dasatinib in the SPEC-2, SKUT-2, HEC1-A, and Ishikawa cell lines (Fig. 2 and Supplementary Figs. S3, S4, and Tables S3–S10). The expression levels of total CRaf, pCRaf\(^{338}\), pS6\(^{240/244}\), pB70S6\(^{225}\), pAKT\(^{5473}\), total EphA2, pEphA2\(^{5897}\), pMAPK\(^{202/204}\), mTOR, and pMEK\(^{1517/221}\) were significantly decreased in the dasatinib-sensitive SKUT-2 cells but not in the dasatinib-resistant SPEC-2, HEC1-A, or Ishikawa cells. Moreover, KRAS and PI3K expression levels did not change significantly in
SKUT-2 cells. Interestingly, a high basal level of CAV-1 was detected in SKUT-2 cells compared with the 3 other cell lines. We next carried out a series of experiments to identify potential mechanistic explanations for response to dasatinib-based therapy. In SKUT-2 cells, Western blot analysis validated the changes in the levels of pS6S240/244 and CRAf following dasatinib therapy; a high basal level of CAV-1 was detected only in SKUT-2 cells and CAV-1 and BRAf had no notable changes (Fig. 3A and B). Next, we carried out immunoprecipitation studies with antibodies against EphA2, CAV-1, and BRAf. Immunoprecipitation analysis showed direct binding between EphA2 and CAV-1. EphA2 and BRAf, and CAV-1 and BRAf in SKUT-2 cells treated with dasatinib (Fig. 3C), but not in HEC1-A and Ishikawa cells (data not shown), or in the absence of dasatinib. Meanwhile, our immunoprecipitation analysis showed that dasatinib induced the heterodimer of BRAf/CRAf in Ishikawa cells, but not in SKUT-2 cells (Fig. 3D), indicating that
colocalization between BRaf and CAV-1 induced by dasatinib in SKUT-2 cells interferes with the formation of the BRaf/CRaf heterodimer and therefore can inactivate signaling downstream of BRaf/CRaf (mitogen-activated protein kinase [MAPK] pathway). The heterodimer of BRaf/CRaf was found in HEC1-A cells treated with or without dasatinib (Fig. 3D), likely because of the fact that HEC1-A bears a Ras mutation and is more resistant to dasatinib treatment (21). To further explore the association between EphA2, CAV-1, and BRaf, we immunostained SKUT-2 cells for these proteins with or without dasatinib treatment. Immunofluorescence staining analysis showed that BRaf was translocated from the cytoplasm to the plasma membrane and colocalized with CAV-1 after dasatinib treatment (Fig. 4A, left and B, left). EphA2 was also translocated and colocalized with CAV-1 after dasatinib treatment (Fig. 4A, right, top), and EphA2 and BRaf showed colocalization as well (Fig. 4A, right, bottom). No colocalization between BRaf and CAV-1 was observed following dasatinib treatment in SPEC-2 cells (Fig. 4B, right) or in HEC1-A and Ishikawa cells (data not shown). Furthermore, following CAV-1 gene silencing, the growth-inhibitory effects of dasatinib on SKUT-2 cells were attenuated (Fig. 4C). There was no colocalization between BRaf and CAV-1 (Fig. 4B, right) or between BRaf and EphA2 (data not shown) in SKUT-2 cells following CAV-1 silencing, indicating that a high level of CAV-1 is important for the response to dasatinib in tumor cells and required for recruiting BRaf in response to dasatinib therapy. EphA2 silencing resulted in partially increased sensitivity to dasatinib in SKUT-2 cells, but it did not alter the response to dasatinib in HEC1-A cells (Supplementary Fig. S5), which suggests that EphA2 is also important for the response to dasatinib in tumor cells.

To further investigate whether BRaf or EphA2 have the capability to interact with CAV-1, we used in situ proximity ligation assay (PLA), which is a unique method developed to visualize subcellular localization and protein–protein interactions in situ (22). SKUT-2 and HEC1-A cells treated with or without dasatinib for 8 hours were incubated overnight with primary antibody pair of different species directed to BRaf or EphA2 (mouse monoclonal antibody) and to CAV-1 (rabbit monoclonal antibody), respectively. The secondary antibodies were modified by addition of complementary oligonucleotides capable of interacting when in close proximity, an event that was detected by PCR amplification using a fluorochrome-based detection method. We observed that the colocalization of CAV-1 with BRaf (Fig. 4D, top) and EphA2 (Fig. 4D, bottom) was increased substantially in SKUT-2 cells after dasatinib treatment, and that the majority of binding interactions were localized on the plasma membrane. No significant increase in the colocalization of CAV-1 with BRaf and EphA2 was observed following dasatinib treatment in HEC1-A cells. Notably, CAV-1 gene silencing by siRNA significantly inhibited the colocalization of CAV-1 with BRaf and EphA2 (Fig. 4D), indicating that high expression of CAV-1 in the cells is required for CAV-1 binding to BRaf or EphA2.

**In vivo effects of dasatinib in orthotopic models of uterine cancers**

Next, we tested the effects of dasatinib-based therapy in orthotopic mouse models of EphA2-positive and -negative uterine carcinoma. In the EphA2-positive SKUT-2 model, dasatinib treatment resulted in significant antitumor activity (79% [P < 0.01] and 63% [P < 0.01], respectively; Fig. 5A). In contrast, in the EphA2-negative SPEC-2 model, dasatinib demonstrated only minimal antitumor activity compared with the untreated (control) group (15% reduction of tumor weight vs. control [P < 0.05] and 10% decrease in number tumor nodules vs. control [P > 0.05]; Fig. 5B). Given the role of paclitaxel in combination with biologically targeted agents, we also tested dasatinib with paclitaxel. The addition of paclitaxel resulted in a large reduction in the SKUT-2 model (95% [P < 0.001] and 68% [P < 0.01], respectively; Fig. 5A), but minimal antitumor activity in the SPEC-2 model (37% [P < 0.05] and 30% [P > 0.05], respectively; Fig. 5B). No obvious toxicity was observed in the various groups in that the mean body weight was similar in all groups (Supplementary Fig. S6A and S6B).

To examine the biologic effects of dasatinib-based therapy, we examined tumors from the SKUT-2 and SPEC-2 models for markers of tumor cell proliferation (Ki67), angiogenesis (CD31), and apoptosis (cleaved caspase-3). Changes in these markers mirrored the antitumor activity in response to dasatinib. Specifically, significant reductions in proliferation and microvessel density and increased apoptosis were noted in the SKUT-2 model (Fig. 5C), whereas more modest changes were noted with the SPEC-2 model (Fig. 5D). In addition, the expression level of pEphA2S897 was significantly decreased in the SKUT-2 model (79% [P < 0.01]; Fig. 5C, bottom).

**Expression of predictive markers in human samples**

We next examined the expression of the putative dasatinib response markers (CAV-1, pEphA2Y594, pEphA2S897, and pAKTS473) in 30 human uterine cancer samples and 10 normal uterine samples by IHC. Representative pictures of immunohistochemical staining are presented in Fig. 6A. CAV-1 was not highly expressed in the normal uterine samples by IHC. Representative pictures of immunohistochemical staining are presented in Fig. 6A. CAV-1 expression was detected in 90% of normal samples and 10% of tumor samples. Changes in these markers mirrored the antitumor activity in response to dasatinib. Specifically, significant reductions in proliferation and microvessel density and increased apoptosis were noted in the SKUT-2 model (Fig. 5C), whereas more modest changes were noted with the SPEC-2 model (Fig. 5D). In addition, the expression level of pEphA2S897 was overexpressed in none of the normal samples but 70% of tumors, whereas high pEphA2Y594 expression was detected in 90% of normal samples and 10% of tumor samples.

**Discussion**

The focus of this work is on the multitargeted tyrosine kinase dasatinib, which is used in the clinic for several diseases. Unfortunately, predictive markers guiding its use and subsequent response are largely absent, resulting in “hit or miss” clinical application. Herein, we present evidence from *in vitro* and *in vivo* experiments for a set of markers essential for dasatinib response. Among these, CAV-1, EphA2 phosphorylation at S897 and the status of...
PTEN were key determinants of dasatinib response. In addition, CAV-1-mediated cross-talk between EphA2 and BRAf is required for response to dasatinib whereby dasatinib drives BRAf and EphA2 to CAV-1 at the plasma membrane, disrupting the BRAf/CRAf heterodimer and thus downmodulation of the MAPK pathway in dasatinib-sensitive cells, but not in dasatinib-resistant cells (Fig. 6B).
The biologic functions of CAV-1 on cancer cells have been controversial. CAV-1 is known to directly interact via its scaffolding domain with multiple signaling proteins and function as preorganized signalosomes by sequestering and regulating proteins localized in caveolae, including receptor tyrosine kinases and their signaling effectors (23). In general, caveolins bind to and inactivate signaling molecules (24), including those of the MAPK signaling pathway (e.g., Ras, RAF-1, and MAPK; ref. 25). Loss of CAV-1 expression confers a significant growth advantage that is associated with constitutive hyperactivation of the p42/44 MAPK pathway (26). In addition, interaction between Eph families, including EphA2 and CAV-1, has been reported (27). It has been reported that the Eph feedback loop in the activation of the HRAS/RAF/ERK pathway also increases EphA2 expression (28). We found that dasatinib inhibited EphA2 signaling, but unexpectedly stimulated Braf recruitment to the cell membrane, potentially promoting changes in the phosphorylation status and triggering its kinase activity (29). This resulted in increased heterodimerization with CRAF, which resulted in increased MEK/ERK activation (30). Furthermore, we demonstrated the mechanistic basis for high CAV-1 at the plasma membrane holding BRaf, resulting in disruption of the BRAF/CRAF heterodimer and inhibiting MAPK activation following dasatinib treatment. Importantly, this finding could provide new approaches to enhance and extend the activity of dasatinib (e.g., CAV-1 peptide and RAF or MEK inhibitors). The role of estrogen (ER) and progesterone (PR) receptor status could also be relevant for therapeutic considerations.

Figure 5. In vivo effects of therapy with dasatinib and paclitaxel in uterine cancer models. A and B, in vivo effects of dasatinib (15 mg/kg oral, daily), paclitaxel (100 μg in 200 μL of PBS intraperitoneally, weekly), or both in an EphA2-positive model (SKUT-2; A) and an EphA2-negative model (SPEC-2; B). Error bars indicate SEM. *, P < 0.05; **, P < 0.01; and ***, P < 0.001. C and D, immunohistochemical staining showing the effect of dasatinib, paclitaxel, or both on uterine cancer cells angiogenesis (CD31), proliferation (Ki67), and apoptosis (caspase-3) in the SKUT-2 model (C) and the SPEC-2 model (D). Immunohistochemical staining of pEphA2S897 expression in SKUT-2 cells (C, bottom). *, P < 0.05; **, P < 0.01; and ***, P < 0.001 compared with control group. Original magnification, ×100 or ×200.
previously we have reported that tumors with EphA2 overexpression are more likely to be ER/PR negative. Thus, our findings may have therapeutic implications for patients with poorly differentiated endometrial cancers that are steroid hormone receptor deficient (8).

Although our findings may have clinical implications for ongoing dasatinib-based clinical trials, some potential limitations should be considered. Whether the identified markers are indeed predictive of dasatinib sensitivity in human clinical trials is not known. A pilot and translational study (NSC #732517) of dasatinib, paclitaxel, and carboplatin for women with advanced-stage and recurrent uterine cancer is ongoing. This trial is evaluating pharmacodynamic variances of EphA2 signaling in response to both dasatinib alone (in the lead-in phase) and in combination with chemotherapy. Validation of these biomarkers will be important for the next generation of clinical investigation involving treatment allocation based on specific biomarkers (31). The diversity of biologic characterization that represents "uterine cancer" is only just being described (32) and presents "uterine cancer" is only just being described (32) and highlights the need for target prevalence description and prospective determination of expression to effectively test our portfolio of targeted therapeutics (33).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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