A Src/Abl Kinase Inhibitor, SKI-606, Blocks Breast Cancer Invasion, Growth, and Metastasis In vitro and In vivo

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Abstract

The central role of Src in the development of several malignancies, including breast cancer, and the accumulating evidence of its interaction with receptor tyrosine kinases, integrins, and steroid receptors have identified it as an attractive therapeutic target. In the current study, we have evaluated the effect of a Src/Abl kinase inhibitor, SKI-606, on breast cancer growth, migration, invasion, and metastasis. Treatment of human breast cancer cells MDA-MB-231 with SKI-606 caused a marked inhibition of cell proliferation, invasion, and migration by inhibiting mitogen-activated protein kinase and Akt phosphorylation. For in vitro studies, MDA-MB-231 cells transfected with the plasmid encoding green fluorescent protein (GFP; MDA-MB-231-GFP) were inoculated into the mammary fat pads of female BALB/c nu/nu mice. Once tumor volume reached 30 to 50 mm³, animals were randomized and treated with vehicle alone or receiving SKI-606 at a dose that was well tolerated. Western blot analysis and immunohistochemical analysis of primary tumors showed that these effects were due to the ability of SKI-606 to block tumor cell proliferation, angiogenesis, growth factor expression, and inhibition of Src-mediated signaling pathways in vivo. Together, the results from these studies provide compelling evidence for the role of Src inhibitors as therapeutic agents for blocking breast cancer growth and metastasis.

Introduction

Src belongs to a non–receptor tyrosine kinase family that comprises nine members in vertebrates (1). It is overexpressed in several human cancers, including carcinomas of the breast, lung, colon, esophagus, skin, parotid, cervix, as well as gastric tissues (2–4). For breast cancer, increased Src activity is believed to play an important role in its development and progression. Furthermore, elevated expression of Src has been associated with poor prognosis (2). Src plays a role in several signaling pathways that are involved in cell proliferation and survival (5). Additionally, the ability of Src to promote tumor cell invasion can lead to the development of tumor metastasis (6). Collectively, Src plays a major role in regulating important mechanisms of specific receptor pathways where their activation can influence the biological activities of the tumor cell (1).

In the vascular endothelial growth factor (VEGF) signaling pathway, phosphorylation of VE-cadherin on Tyr663 by Src plays a pivotal role in regulating VE-cadherin adhesive activity (7). In addition, Src has been shown to regulate phosphatidylinositol 3-kinase and Akt pathways (8). Several growth factors and proteases, including parathyroid hormone-related protein (PTHrP) and urokinase-type plasminogen activator (uPA), can serve as downstream targets for Src kinase (9, 10). In previous studies, we have shown the role of PTHrP in tumor cell proliferation, invasion, and metastasis and in the development of hypercalcemia of malignancy in several common cancers, including breast cancer (9, 11–14). It is important to note that elevated PTHrP production, regulated by several oncogenes, such as Ras, Tpr-Met, and Src, can activate osteoclastic bone resorption, which leads to increased bone metastasis (11–14). uPA, production of which is activated by Src, has been shown to play a major role in the invasion, growth, and metastasis of several malignancies, including breast cancer, due to its ability to break down various components of the extracellular matrix (15–17). Src has also been shown to interact with integrins that are responsible for interaction between tumor cells and their surrounding stroma, which can alter tumor cell growth, differentiation, and migration (18, 19). Integrins can in turn activate extracellular matrix proteins, such as focal adhesion kinase (FAK). Autophosphorylation of FAK at Tyr927 enables the recruitment of Src family tyrosine kinases by engaging their Src homology 2 domains (20–22). Src can phosphorylate additional sites in FAK, leading to the recruitment of the Grb-2 adapter molecule and in turn activation of several signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway (23).

Due to the important role of Src in tumor progression, studies were carried out to evaluate the role of inhibition of Src in reducing tumor growth (24). Duxbury et al. (25) have used the well-characterized pyrazolopyrimidines (PP2) to show their ability to decrease pancreatic tumor growth and metastasis in an orthotopic xenograft model of pancreatic cancer. The purine analogue AP23846 can inhibit members of the Src kinase family but has not been advanced for clinical development. Nonetheless, both interleukin-8 and VEGF expression were inhibited by AP23846 in human solid tumor cell lines, lending further experimental support to a role for Src kinase in tumor growth and angiogenesis (26). The quinazoline compound AZM475271 has shown significant activity in orthotopic models for pancreatic cancer (27). Another Src inhibitor, SKI-606, was recently shown to be active in colon tumor
This compound is also an Abl kinase inhibitor and is active in chronic myelogenous leukemia (CML) models in vivo (34). Recently, Coluccia et al. (35) also showed that SKI-606 decreases growth and motility of colorectal cancer cells via inhibition of Src-mediated tyrosine phosphorylation of β-catenin. Nam et al. (36) showed that dasatinib (BMS-354825), a Src family kinase/Abl inhibitor, blocks the kinase activities of Lyn, Src, and FAK signaling in human prostate cancer cells. Collectively, these studies have shown the role of the Src oncogene in several malignancies. In the current study, we have evaluated the effect of Src kinase inhibition using SKI-606 on breast cancer growth, invasion, and migration in vitro and on tumor growth and metastasis in vivo.

**Materials and Methods**

**Cells and cell culture.** Human MDA-MB-231, MCF-7, and BT-474 breast cancer cells were obtained from the American Type Culture Collection (Rockville, MD). Cells transfected with GFP (MDA-MB-231-GFP) were prepared and maintained in culture as described previously (37–39).

**In vitro cell assay and Western blotting.** MDA-MB-231 cells were plated in triplicate in six-well plates in the presence of 2% fetal bovine serum with DMSO as vehicle alone or different concentrations (0.1–1 μmol/L) of SKI-606 (33–35). Triplicate wells were trypsinized and counted using a Coulter counter on alternate days (model ZF, Coulter Electronics, Harpenden, Hertfordshire, United Kingdom). Cell culture medium was replenished every 2nd day.

For cell migration assays, MDA-MB-231 cells (3 × 10⁵ per well) were plated in a six-well plate. Approximately 48 h later, when the cells were 100% confluent, the monolayer was scratched using a 1 mL pipette tip. Medium and nonadherent cells were aspirated, the adherent cells were washed once, and new medium containing various concentrations of SKI-606 (0.1–100 μmol/L) was added for 4 h. Cells were observed under the microscope at different times, and the inhibition of migration was assessed when the wound in the control was closed.

Tumor cell invasive capacity was examined as described previously (37, 38). After 18 h, the invading cells were then examined and counted in 10 randomly selected fields under a light microscope at ×400 magnification. The average number of invading cells was then calculated.

For DNA fragmentation, MDA-MB-231 cells were plated in six-well plates (Falcon Plastics, London, Ontario, Canada). Cells were treated with SKI-606 (0.1–1.0 μmol/L) for up to 72 h. DNA from cells treated with vehicle alone was isolated from vehicle-treated control cells and SKI-606–treated cells as described in Materials and Methods. Isolated DNA was subjected to electrophoresis on a 2% agarose gel and visualized under UV light. Columns, mean of three experiments; bars, SE. *, P < 0.05, significant inhibition in cell invasion from control cells was determined by ANOVA.

Figure 1. Effect of SKI-606 on MDA-MB-231 cell growth and invasion. A, MDA-MB-231 cells were seeded at a density of 5 × 10⁴ per well in six-well plates and treated with different doses (0.1 and 1.0 μmol/L) of SKI-606. Control (CTL) and experimental cells were trypsinized and counted with a Coulter counter as described in Materials and Methods. Change in cell number at various time points. B, MDA-MB-231 cells were grown in culture as described in Materials and Methods. The number of cells migrating to the lower aspect of the Boyden chamber filter 18 h after treatment with SKI-606 (0.1–1.0 μmol/L) was counted. The percentage of inhibition in cell invasion was calculated by taking the number of invading cells following treatment with vehicle alone as control as 100%. C, MDA-MB-231 cells were treated with different doses (0.1–1.0 μmol/L) of SKI-606 and change in cell morphology was observed. Representative photomicrograph of vehicle-treated control cells and cells treated with 1.0 μmol/L SKI-606. D, MDA-MB-231 cells were cultured in the presence of vehicle alone or SKI-606 (1.0 μmol/L) for 96 h. DNA was isolated from vehicle-treated control cells and SKI-606–treated cells as described in Materials and Methods. Isolated DNA was subjected to electrophoresis on a 2% agarose gel and visualized under UV light. Columns, mean of three experiments; bars, SE. *, P < 0.05, significant inhibition in cell invasion from control cells was determined by ANOVA.
and SKI-606 was collected using a phenol/chloroform/isoamyl alcohol solution (50:48:2). Equal amounts of DNA were subjected to gel electrophoresis on a 2% agarose gel. DNA fragmentation was visualized by UV light using a transilluminator.

For Western blotting, MDA-MB-231 cells (1 × 10⁶) were plated in 100-mm Petri dishes for 24 h. Cells were then washed with cold PBS and lysed with 200 μL of cold lysis buffer [150 mmol/L NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 50 mmol/L Tris-HCl (pH 7.2), 0.2 mmol/L sodium vanadate, 1% phenylmethylsulfonyl fluoride, 0.2% aprotinin]. Samples were kept on ice for 20 min and then spun at 13,000 × g for 20 min, and the protein concentrations of the supernatants were determined. Cell lysates were resolved on 10% SDS-PAGE, and protein was transferred onto nitrocellulose membranes. Expression of β-tubulin as control was determined using anti-β-tubulin (NeoMarkers, BD Biosciences, Mississauga, Ontario, Canada).

Animal protocols. For xenograft studies, 5-week-old (15–20 g) female BALB/c nu/nu mice (Charles River, St. Constant, Quebec, Canada) were used (37, 38). Before inoculation, MDA-MB-231-GFP cells grown in serum containing culture medium were washed with Hank's balanced buffer and centrifuged at 1,500 rpm for 5 min. Cell pellets (5 × 10⁵ per mouse) were resuspended in 100 μL Matrigel (Becton Dickinson Labware, Mississauga, Ontario, Canada) and saline mixture (20% Matrigel) and injected into the mammary fat pad of mice s.c. All animals were numbered and kept separately in a temperature-controlled room on a 12-h light/12-h dark schedule with food and water ad libitum. Tumors were allowed to grow to 30–50 mm³. At this stage, animals were randomized and treated with vehicle alone or different doses of SKI-606 (150 mg/kg/d) by oral gavage 5 days weekly for 5 weeks. Tumor volumes were determined from caliper measurements obtained weekly. At the end of the study, animals were sacrificed and their lung, liver, spleen, as well as the primary tumors were
removed for further analysis. Lung, liver, and spleen were sliced to 1-mm-thick slices of fresh tissue for direct examination under the fluorescent microscope for the presence of GFP-expressing tumor foci. The number of GFP-expressing tumor foci per field of examination was counted from 10 random sites of five different slides for each organ and calculated and graphed the average for each group. Total proteins from the primary tumors were extracted for Western blot analysis to examine levels of Src, phosphorylated Src, phosphorylated Akt, and phosphorylated MAPK in these tumors. All animal protocols were in accordance with and approved by the institutional review board.

Immunohistochemistry. Immunohistochemical reactions were carried out as previously described using the avidin-biotin-peroxidase complex method (40–43). The antibodies used were polyclonal antisera against PTEN (1–34) from rabbit (41), at 1:100, monoclonal antibodies against E-cadherin (1:100), and CD31 (1:50) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); rabbit polyclonal antibody against phosphorylated Akt (1:50) from Cell Signaling Technology (Beverly, MA); monoclonal antibody against Ki-67 (1:100) from DAKO (Carpinteria, CA); and monoclonal antibody against uPA (1:50) from American Diagnostica, Inc. (Stanford, CT) overnight at 4°C (39–41). Biotinylated goat anti-mouse or goat anti-mouse antibodies (Vector Laboratories, Inc., Burlingame, CA) were used as the secondary antibodies at 1:200 for 30 min at room temperature. The slides were treated with Vectastain ABC-AP kit (Vector Laboratories) diluted 1:200 for 30 min at room temperature. The signals were visualized with Fast Red TR/Naphthol AS-MX phosphate (Sigma-Aldrich, Oakville, Ontario, Canada) containing 1 mmol/L levamisole for 10 to 15 min. The sections were then lightly counterstained with hematoxylin (Fisher Scientific Ltd., Nepean, Ontario, Canada) and mounted with Kaiser’s glycerol jelly. All sections were washed thrice, 10 min each, with Tris buffer (pH 7.6) after each step. Negative controls included substitution of the primary antibody with PBS.

For terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay, tissue sections were dewaxed by heating at 60°C followed by washing in xylene and rehydrated through a graded series of ethanol and water. Tissues were incubated with protease K for 30 min at 37°C, fixed, blocked, and permeabilized. Apoptotic cells were detected by the TUNEL assay in situ cell death detection kit (Roche Molecular Biochemicals, Laval, Quebec, Canada) according to the manufacturer’s instructions. Positive TUNEL staining was visualized by fluorescence microscopy (40).

Computer-assisted image analysis. The immunostaining of all antibodies was quantitatively analyzed by using a computer-assisted image analysis system as described previously (42, 43). Briefly, images of stained sections were captured with a Leica digital camera (Richardson Hill, Ontario, Canada) and processed using BioQuant image analysis software version 6.50.10 (BioQuant Image Analysis Corp., Nashville, TN). The threshold was set by determining the positive staining of control cells and was used to automatically analyze all recorded images of all samples that were stained in the same session under identical conditions. The area of immunostained regions in each microscopic field was calculated automatically by the software. Pixel counts of the immunoreaction product were calculated automatically and were given as total density of the integrated immunostaining over a given area of the well. This reflects the relative amount of proteins detected by the antibodies on cells.

Statistical analysis. Results are expressed as the mean ± SE of at least triplicate determinations, and statistical comparisons are based on Student’s t test and ANOVA. A probability value of <0.05 was considered to be significant.

Results

Effects of SKI-606 on cell proliferation, invasion, and migration. SKI-606 is a potent dual Src/Abl kinase inhibitor that was shown to have antiproliferative effects on CML cells; however, the efficacy of SKI-606 in breast cancer is still unknown. Therefore, we determined the effect of SKI-606 on the growth of mesenchymal-like human breast cancer cells MDA-MB-231. Treatment of these breast cancer cells with SKI-606 caused a dose-dependent inhibition in tumor cell proliferation (Fig. 1A). The effect of SKI-606 on tumor cell invasive capacity was evaluated by Matrigel invasion assay. The results of these studies show that SKI-606 inhibits the invasive capacity of MBA-MB-231 cells in a dose-dependent manner.
manner (Fig. 1B). Anti-invasive effects of SKI-606 are independent of its effect on cell proliferation, which was observed following 48 h of treatment with SKI-606. Treatment of MDA-MB-231 cells with 0.1 to 1.0 μmol/L SKI-606 for 2 h caused a morphologic change from a spindle-like phenotype to an epithelial and condensed shape (Fig. 1C). We also examined the ability of SKI-606 to induce tumor cell apoptosis by DNA ladder formation assay. Treatment of MDA-MB-231 cells with 1.0 μmol/L SKI-606 at 96 h caused a significant induction of apoptosis in vitro (Fig. 1D).

To assess whether SKI-606 affects cell migration, a wound-healing assay was done. Following treatment with various concentrations of SKI-606 for 4 h, cells were allowed to migrate into the denuded area for 16, 24, and 48 h. SKI-606 at 1.0 μmol/L concentration caused a significant decrease in MDA-MB-231 cell migration (Fig. 2). Ability of SKI-606 to inhibit cell migration at all time points suggests that these effects are not due to its ability to inhibit cell proliferation, which was only seen after 48 h of treatment. We also examined the effect of SKI-606 on the invasion and migration of two additional human breast cancer cells (MCF-7 and BT-474). In these cells, which are low invasive, treatment with SKI-606 caused a statistically significant inhibition in cell invasion and migration compared with cells treated with vehicle alone. However, the ability of SKI-606 to inhibit tumor cell invasion and migration was significantly lower compared with the highly invasive MDA-MB-231 cells (data not shown).

**SKI-606 inhibits kinase activity of Src and its downstream signaling pathways in vitro.** Tyr 416 in the kinase domain plays a critical role in the regulation of Src tyrosine kinase activity. MDA-MB-231 cells were treated with 1.0 μmol/L SKI-606 for 2 h, a dose and time that is most effective in inhibiting cell proliferation, invasion, and migration as shown in Figs. 1 and 2. At the end of this incubation period, cell lysates were subjected to Western blot analysis to determine Src kinase activity. These studies showed that SKI-606 can inhibit kinase activity as measured by autophosphorylation of Tyr 416 in these human breast cancer cells (Fig. 3A).

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**Figure 4.** Effect of SKI-606 on MDA-MB-231-GFP tumor growth. A, MDA-MB-231-GFP cells (5 × 10⁵) were inoculated in the mammary fat pads of female BALB/c nu/nu mice. Once the tumor volume reached 30 to 50 mm³, animals were randomized and treated with vehicle alone as control or 150 mg/kg SKI-606 5 d weekly for 4 wks by daily oral gavage. Tumor volumes were determined by caliper measurements obtained weekly as described in Materials and Methods. B, to determine the effect of SKI-606 on tumor metastasis, at the end of these studies, control and experimental animals treated with SKI-606 were sacrificed. Various organs (lungs, liver, and spleen) were removed and evaluated for the presence of metastatic tumor cells. A number of tumor foci in 10 random fields per slide, five slides per organ, were counted to determine the average number of tumor foci in each of the organs as described in Materials and Methods. Columns, mean of at least 10 animals in each group in two separate experiments; bars, SE. *, P < 0.05, significant difference in tumor volume and tumor foci was determined by ANOVA (A) or Student’s t test (B).
To confirm the loss of Src activity, we examined the effect of Src inhibition on downstream signaling proteins, such as MAPK, FAK, and Akt. Results shown in Fig. 3B show that SKI-606 acts as an inhibitor of these signaling pathways by inhibiting phosphorylation of these proteins, findings which are consistent with the anti-invasive effect shown in Fig. 1B.

Role of SKI-606 in MDA-MB-231 tumor growth and metastasis in vivo. Previously, we had generated MDA-MB-231 cells stably transfected with a plasmid containing cDNA encoding GFP. These MDA-MB-231-GFP cells, which have similar cell proliferation and invasive capacity as the wild-type cells, allow for the determination of the number and size of microscopic GFP-positive tumor cells in various organs (lungs, liver, and spleen; refs. 37, 38). To test the effect of SKI-606 on tumor growth and metastasis, MDA-MB-231-GFP cells were inoculated into the mammary fat pad of female BALB/c nu/nu mice. Once the tumor volume reached 30 to 50 mm³, animals were randomized and treated with vehicle alone or SKI-606 (150 mg/kg) by daily oral gavage 5 days weekly for 4 weeks. Tumor volumes were determined by caliper measurements obtained weekly. The control group of animals treated with vehicle alone showed a progressive increase in their tumor volume; however, experimental animals treated with SKI-606 developed tumors of significantly smaller volume (P < 0.05) throughout the course of these studies (Fig. 4A).

To determine the efficacy of SKI-606 treatment on the ability of MDA-231-GFP cells to metastasize in vivo, at the end of these studies, control and experimental animals were sacrificed and various organs (lung, liver, and spleen) were removed and evaluated for the presence of GFP-positive tumor cells. Using fluorescent microscopy for the analysis of slices of fresh tissue from lung, liver, and spleen, we were able to show the presence of GFP-expressing tumor foci recognized by their green fluorescence. Results clearly show that animals inoculated with MDA-MB-231-GFP cells and treated with SKI-606 developed microscopic tumor metastases of significantly smaller number and size compared with control animals receiving vehicle alone (Fig. 4B).

Effects of SKI-606 on Src-mediated signaling pathway in vivo. To investigate the effect of Src inhibition on signaling pathways in vivo, control and experimental tumors were collected at the time of necropsy and snap frozen in liquid nitrogen. The mice were treated with SKI-606 at a concentration of 150 mg/kg via oral gavage following inoculation of MDA-MB-231-GFP cells in the mammary fat pad. Consistent with the results of our in vitro studies, SKI-606 markedly abolished phosphorylation of phosphorylated Src, phosphorylated MAPK, phosphorylated FAK, phosphorylated Akt, and phosphorylated Akt signaling proteins (Fig. 5).

Effects of SKI-606 on gene expression, tumor cell survival, proliferation, angiogenesis, and apoptosis in vivo. Because Src can regulate multiple signaling pathways and gene expression, control and experimental tumors were evaluated for the expression of genes involved in tumor progression. Immunohistochemical analysis of primary tumors treated with vehicle alone or SKI-606 showed a significant decrease in PTHrP and uPA expression, genes which are known to promote breast cancer progression. In contrast, treatment with SKI-606 showed increased expression of E-cadherin, which suggests the ability of SKI-606 to affect tumor cell adhesion (Fig. 6). Furthermore, as shown previously, SKI-606 inhibits Akt activity through the PI3K pathway, which results in...
decreased Akt phosphorylation. In addition, we evaluated the effect of SKI-606 on MDA-MB-231 proliferation, neovascularization, and apoptosis using antibodies against Ki-67 and CD31 and TUNEL assay, which are markers of cell proliferation, angiogenesis, and apoptosis, respectively. These studies showed a significant decrease in the levels of expression of Ki-67 and CD31 and increase in the number of TUNEL-positive cells in tumors from experimental animals treated with SKI-606 (Fig. 6).
Discussion

SKI-606 is an effective Src kinase inhibitor that blocks the activity of various signaling pathways, such as MAPK and Akt, at micromolar concentrations. This effect was tested on mesenchymal-like breast cancer cells MDA-MB-231, which exhibit high invasive capacity. SKI-606 showed antiproliferative effects and inhibited cell migration and invasion, which directly correlate with a significant (45–54%) reduction in tumor volume in experimental animals (Fig. 4A). Src phosphorylation at Tyr416 was significantly reduced in MDA-MB-231 tumors from animals treated with SKI-606. Furthermore, we showed that blockade of Src results in decreased expression of both PTHrP and uPA, which are known to promote the growth and metastasis of several common cancers, including breast cancer (12–17). Ability of SKI-606 to inhibit the expression of these genes is significant because they are regulated by Src oncogene and via the MAPK and Akt signaling pathways play an important role in breast cancer invasion and metastasis (12–17). Additionally, the ability of SKI-606 to affect tumor cell proliferation and angiogenesis as shown in Fig. 6 plays a significant role in antitumor and antimetastatic effects of SKI-606 as shown in this study.

MDA-MB-231 breast cancer cells are highly invasive and representative of cells in late-stage breast cancer. In agreement with previous studies with colorectal tumor xenografts where SKI-606 showed an antitumor effect, it showed a significant inhibition of MDA-MB-231 tumor growth and metastasis in the current study (27, 28). Therefore, this Src/Abl kinase inhibitor is effective in both types (colorectal and breast) of tumors. Furthermore, MDA-MB-231 invasive capacity was also diminished in a dose-dependent manner (Fig. 1B). Change in tumor cell morphology combined with increased expression of E-cadherin points to the potential role of Src inhibition in epithelial-mesenchymal transition, which needs in-depth investigation to fully understand the mode of action of these selective inhibitors. Cell migration is necessary in many physiologic processes, such as tissue development, wound healing, and tumor metastasis. Several studies suggested that Src might be involved in cell migration. Indeed, in src−/− mice fibroblasts, locomotion is reduced compared with rates displayed by wild-type fibroblasts (44). Consistent with those findings, SKI-606-mediated inhibition of Src activity impeded cell migration after 4 h of treatment (Fig. 2). These findings correlate with the reduction of uPA levels, which is associated with inhibition of cell adhesion and migration. Phosphorylation of Tyr116 in the kinase domain is a critical activation step in the regulation of Src tyrosine kinase activity (1, 45, 46). On addition of SKI-606, we show clearly that Src phosphorylation at Tyr116 is inhibited in MDA-MB-231 cells as well as in tumor lysates of experimental animals. These findings suggest that SKI-606 is acting through inhibition of Src kinase activity. In addition, Src regulates many cellular events through phosphorylation of multiple substrates. We investigated whether downstream signaling pathways would be altered through an inactivation of Src. Our data clearly show a decrease in phosphorylation of MAPK and Akt pathway. This change was observed at 2 h of treatment with SKI-606 and correlated with the findings in vivo.

More recently, using a xenograft model of breast cancer metastasis, Src (tyrosine kinase) activity was shown to be associated with tumor cell colonization in bone (47). Furthermore, Src (tyrosine kinase) stimulated the production of PTHrP at transcriptional levels in MDA-MB-231, which in turn can activate bone resorption by osteoclasts, leading to the progression of bone metastases (47).

In conclusion, we show in the study that SKI-606 inhibits metastasis and breast tumor growth in a xenograft mouse model. It is important to note that E-cadherin levels were increased in SKI-606-treated tumor. This is in accordance to the recent study where Nam et al. (36) showed that PP2 is also capable of restoring E-cadherin/β-catenin in various tumor cell lines. Collectively, results from these studies have provided compelling evidence for continued evaluation of the efficacy of SKI-606 in breast cancer.

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