Tandutinib Inhibits the Akt/mTOR Signaling Pathway to Inhibit Colon Cancer Growth

Sivapriya Ponnurangam1, David Standing1, Parthasarathy Rangarajan1, and Dharmalingam Subramaniam1,2

Abstract

The c-Kit receptor can activate distinct signaling pathways including phosphoinositide 3-kinase (PI3K)/Akt and mTOR. Aberrant c-Kit activation protects cells from apoptosis and enhances invasion of colon carcinoma cells. Tandutinib is a novel quinazoline-based inhibitor of the type III receptor tyrosine kinases including c-Kit. We determined the effect of tandutinib on colon cancer growth and identified a mechanism of action. Tandutinib inhibited phosphorylation of c-Kit, Akt, mTOR, and p70S6 kinase. In addition, tandutinib significantly inhibited the proliferation and colony formation ability of colon cancer cell lines but did not affect normal colonic epithelial cells. There were increased levels of activated caspase-3 and Bax/Bcl2 ratio, coupled with a reduction in cyclin D1, suggesting apoptosis. There was also a downregulation of COX-2, VEGF, and interleukin-8 expression, suggesting effects on cancer-promoting genes. In addition, overexpressing constitutively active Akt partially suppressed tandutinib-mediated colon cancer cell growth. In vivo, intraperitoneal administration of tandutinib significantly suppressed growth of colon cancer xenografts. There was a reduction in CD31-positive blood vessels, suggesting that there was an effect on angiogenesis. Tandutinib treatment also inhibited the expression of cancer-promoting genes COX-2 and VEGF and suppressed the activation of Akt/mTOR signaling proteins in the xenograft tissues. Together, these data suggest that tandutinib is a novel potent therapeutic agent that can target the Akt/mTOR/p70S6K signaling pathway to inhibit tumor growth and angiogenesis. Mol Cancer Ther; 12(5): 598–609. ©2013 AACR.
The c-Kit proto-oncogene encodes a transmembrane tyrosine kinase receptor. Aberrant c-Kit expression, described in subgroups of patients with colorectal cancer, correlates with dismal prognosis (10, 11). c-Kit mRNA expression has been detected in various human colonic adenocarcinoma cell lines (9, 12). Furthermore, activating mutations of c-Kit protects human colon adenocarcinoma cells against apoptosis and enhance their invasive potential (12). The c-Kit ligand stem cell factor (SCF) has been also detected in normal intestinal epithelial cells (13), suggesting autocrine and paracrine control of transforming functions by SCF in human colon cancer (9). Therefore, c-Kit is a therapeutic target for colorectal carcinoma.

Upon binding with its ligand SCF, c-Kit undergoes dimerization and autophosphorylation at specific tyrosine residues Tyr567 and Tyr719. This activated receptor then phosphorylates various signaling pathways: the phosphoinositide 3-kinase (PI3K)/Akt/mTOR signaling axis plays a critical role in the proliferation, resistance to apoptosis, angiogenesis, and metastasis that is central to the development and maintenance of colorectal cancers (15). PI3K is activated upon growth factors binding to their cognate receptors. Activated PI3K leads to the activation of Akt by phosphorylation at Ser473 and Thr308 (16). Akt activates several downstream targets including mTOR. Deregulation of mTOR signaling occurs in several human tumors including colon cancer (15). mTOR associates with Raptor (mTORC1 complex) to phosphorylate p70S6K, which in turn phosphorylates 4E-BP1, leading to increased cell proliferation (17). In addition, mTOR associates with Rictor (mTORC2 complex) and functions in a feedback loop to phosphorylate and activate Akt at Ser473 (16).

In this article, we are the first to show the effect of tandutinib on colon cancer cells and have identified at least one mechanism of action to be through the inhibition of the Akt/mTOR signaling pathway.

Materials and Methods

Cells and reagents

HCT-116, HT-29, and SW480 human cancer cells (all obtained from American Type Culture Collection, at passage 4) were grown in Dulbecco’s Modified Eagle’s Media (DMEM) containing 10% heat-inactivated FBS (Sigma-Aldrich), 1% antibiotic–antimycotic solution (Mediatech Inc.) at 37°C in a humidified atmosphere of 5% CO2. All the cell lines used in this study were within 20 passages after receipt or resuscitation (~3 months of noncontinuous culturing). The cell lines were not authenticated as they came from national repositories. Tandutinib was purchased from LKT Laboratories.

Proliferation and apoptosis assays

Cells were seeded on to 96-well plates and grown overnight. Then, the cells were treated with increasing doses of tandutinib (0–50 μmol/L) in 10% FBS containing DMEM. The cell proliferation was analyzed by hexosaminidase assay as described previously (18). For apoptosis, caspase-3/7 activity was measured using the Apo-one Homogeneous Caspase-3/7 Assay Kit (Promega).

Colony formation assay

Briefly, 6-well dishes were seeded with 500 viable cells per well, treated with tandutinib (25 μmol/L) in 10% FBS containing DMEM for 48 hours, the tandutinib-containing medium was removed, and the cells were incubated for an additional 10 days. Treatments were conducted in triplicate. The colonies obtained were fixed in formalin and stained with crystal violet. The colonies were compared with untreated cells.

Cell-cycle analyses

Cells were treated with tandutinib for 24- and 48-hours and subsequently trypsinized and suspended in PBS. Single-cell suspensions were fixed using 70% ethanol for 2 hours and subsequently permeabilized with PBS containing 1 mg/mL propidium iodide (Sigma-Aldrich), 0.1% Triton X-100 (Sigma-Aldrich), and 2 μg DNase-free RNase (Sigma-Aldrich) at room temperature. Flow cytometry was done with a FACScalibur analyzer (Becton Dickinson) capturing 10,000 events for each sample. Histograms were analyzed for cell-cycle compartments, and the percentage of cells at each phase of the cell cycle was calculated using CellQuest (Becton Dickinson) analysis software.

Real time reverse-transcription PCR analysis

Total RNA isolated from HCT-116 cells treated with TRLzol reagent and reverse-transcribed with Superscript II reverse transcriptase in the presence of random hexanucleotide primers (Invitrogen). CDNAs were then used for real-time PCR using Jumpstart Taq DNA polymerase (Sigma-Aldrich) and SYBR Green Nucleic Acid Stain (Molecular Probes). Crossing threshold values for individual genes were normalized to β-actin. Changes in mRNA expression were expressed as fold change relative to control. Primers used in this study were as follows: β-actin: 5'-GCTGATCCACATCTGCCTGG-3' and 5'-ATCATGTCCTCCTCAGCGG-3'; COX-2: 5'-GAATCATTACACGGAAATTG-3' and 5'-TCGTACTCCGGTTGGAACA-3'; VEGF: 5'-AGCGCAAGAAGTTCCCGGTA-3' and 5'-TGCTTTCTCCAGCTGACG-3'; insulin, 0.005 mg/mL transferrin, 100 ng/mL hydrocortisone, 10% FBS (Sigma-Aldrich), and 1% antibiotic–antimycotic solution (Mediatech Inc.) at 37°C in a humidified atmosphere of 5% CO2. All the cell lines used in this study were within 20 passages after receipt or resuscitation (~3 months of noncontinuous culturing). The cell lines were not authenticated as they came from national repositories. Tandutinib was purchased from LKT Laboratories.
interleukin (IL-8: 5'-CTCTTGGCAGCCTTCTGTATT-3' and 5'-TATGCACTGACATCAAAGTCTCTTTAAG-3'; cyclin D1: 5'-AATGCCCGACCGATTTTC-3' and 5'-TCAGGTTCAGGCCTTGAC-3'; and p53: 5'-CACATCAGCACAACTACGCA-3').

Plasmids and transfections

HCT-116 and SW480 cells were transfected with plasmid 9008: 1036 pcDNA3 Myr-HA-Akt1 or the empty vector pcDNA3 (Addgene Inc.) and subsequently treated with 25 mol/L tandutinib (50 mg/kg body weight) was administered intraperitoneally daily for 21 days. Tumors were allowed to form xenograft. One week following implantation, 106 HCT-116 cells in the left and right flank and SW480, and HT-29 cells in a dose- and time-dependent manner (Fig. 1B). This antiproliferation effect on tumor cells was seen within 24 hours at a dose of 25 mol/L, which continued to significantly increase over the next 72 hours (Fig. 1B). In contrast, tandutinib did not affect proliferation of FHC normal colon cells even at doses of 30 μmol/L (Fig. 1C). To determine the long-term effect of tandutinib treatment, cells were treated with tandutinib for 48 hours before it was allowed to grow in normal medium. There were lower numbers of colonies in all 3 colon cancer lines following drug treatment than in untreated control (Fig. 1D). These data suggest that tandutinib treatment suppresses growth of colon cancer cells in culture.

Statistical analysis

All values are expressed as the mean ± SEM. Data were analyzed using an unpaired 2-tailed t test. *P < 0.05 was considered statistically significant.

Results

Tandutinib inhibits colon cancer cell proliferation

Previous studies have shown that tandutinib blocks growth of leukemia cells (5). To determine whether tandutinib affects colon cancer cells, we first determined the effect of the compound on the proliferation of 3 different colon cancer cell lines and normal colon epithelial cells. Tandutinib significantly suppressed proliferation of HCT-116, SW480, and HT-29 cells in a dose- and time-dependent manner (Fig. 1B). This antiproliferation effect on tumor cells was seen within 24 hours at a dose of 25 mol/L, which continued to significantly increase over the next 72 hours (Fig. 1B). In contrast, tandutinib did not affect proliferation of FHC normal colon cells even at doses of 30 μmol/L (Fig. 1C). To determine the long-term effect of tandutinib treatment, cells were treated with tandutinib for 48 hours before it was allowed to grow in normal medium. There were lower numbers of colonies in all 3 colon cancer lines following drug treatment than in untreated control (Fig. 1D). These data suggest that tandutinib treatment suppresses growth of colon cancer cells in culture.

Tandutinib induces apoptosis

Given the effects of tandutinib on suppression of proliferation and colony formation, we next determined whether the compound affects cell-cycle progression. After 24 hours of tandutinib treatment, there was a significantly higher number of cells arrested in the G2–M phase (Fig. 2A). In addition, there was a greater number of dead cells in both HCT-116 and SW480 cell lines (Fig. 2A). At 48 hours, while tandutinib induced G0–G1 arrest in HCT-116 cells, there was G2–M arrest in SW480 cells. We also determined how the cells died following tandutinib treatment. Caspases are a family of proteins known for the execution of apoptosis. Caspase-3/7 are key effector molecules of apoptosis in variety of cancer cells that amplify signals from initiator caspases, such as caspase-8 or caspase-9 (19). Increased caspase-3/7 activity was observed within 48 hours in all 3 cell lines HCT-116, SW480, and HT-29 treated with tandutinib suggesting the induction of apoptosis (Fig. 2B). This was further confirmed by Western blot analyses where treatment with tandutinib showed a significant increase in the levels of cleaved caspase-3 (Fig. 2C). In addition, tandutinib inhibited the expression of antiapoptotic proteins.
genes Bcl2 and Bcl-XL proteins while increasing the expression of apoptosis-promoting Bax protein (Fig. 2D). Moreover, we have also determined the effect of tandutinib on caspase-3/7 activity in FHC normal colonic epithelial cells. There was no significant increase in caspase-3/7 activity in normal colonic epithelial treated with 25 μmol/L of tandutinib (data not shown). These data suggest that tandutinib is a potent inducer of apoptosis of colon cancer cells.

**Tandutinib affects expression of cell-cycle–related proteins**

To further characterize the mechanism of cell death, we examined the level of expression of several known cell-cycle regulatory factors (20). Cyclin D1 overexpression has been linked to the development and progression of cancer (21). It is a cell-cycle regulatory protein that regulates the G1 to S-phase transition of the cell cycle and functions as a cofactor for several transcription factors. Consistent with cell-cycle arrest, the expression of cyclin D1 was decreased at both the mRNA and protein levels in the 2 cell lines (Fig. 3A and B). Furthermore, cyclin A1 and B1 also reduced following tandutinib treatment in both HCT-116 and SW480 cells (Fig. 3B). In addition, c-Myc is upregulated in cancers. In both HCT-116 and SW480 cells, tandutinib treatment resulted in reduced levels of c-Myc expression (Fig. 3A and B).
Tandutinib inhibits expression of cancer-promoting genes

COX-2, a key rate-limiting enzyme in prostaglandin synthesis, is overexpressed in many cancers. Previous studies have shown increased COX-2 levels in colorectal cancer (22, 23). COX-2 plays a significant role in increased invasiveness, promotion of angiogenesis, and resistance to apoptosis (24). Therefore, we next determined the effect of tandutinib on COX-2 expression. Tandutinib reduced COX-2 mRNA and protein levels in both HCT-116 and SW480 cells (Fig. 3C and D). Prostaglandins and the other tumor promoters are known to induce the expression of VEGF and IL-8 in epithelial cells, thereby promoting angiogenesis and resistance to apoptosis (24). Therefore, we determined the effect of tandutinib on COX-2 expression. Tandutinib reduced COX-2 mRNA and protein levels in both HCT-116 and SW480 cells (Fig. 3C and D). Prostaglandins and the other tumor promoters are known to induce the expression of VEGF and IL-8 in epithelial cells, thereby promoting angiogenesis and resistance to apoptosis (24).

Tandutinib affects c-Kit and Akt/mTOR signaling

Tandutinib is a known inhibitor of PDGFR and c-Kit in AML cells lines (5). Aberrant activation of c-Kit protects colon carcinoma cells from apoptosis and enhances their invasive potential (12). To further show the effect of tandutinib on c-Kit in colon cancer cells, a Western blot analysis was conducted. Tandutinib treatment significantly downregulated the phosphorylation of c-Kit in both HCT-116 and SW480 cells (Fig. 4A). Similar result was obtained in HT-29 cells (data not shown). Previous studies have shown that tandutinib selectively blocks
FLT3-ITD phosphorylation, leading to an inhibition of Akt and Erk signaling and to induction of apoptosis in FLT3-ITD-positive human AML cell lines (5). To determine whether a similar mechanism was occurring in colon cancer cells, we determined the effect of tandutinib on the Akt/mTOR pathway. Western blot analyses showed that tandutinib treatment downregulated the phosphorylation of both Akt and mTOR in the 2 cell lines (Fig. 4B). Furthermore, tandutinib also further downregulated the phosphorylation of downstream target protein P70S6K and 4EBP1 (Fig. 4B). In addition, tandutinib treatment increased PTEN expression in both HCT-116 and SW480 cells. We also determined the effect of tandutinib on Akt phosphorylation in normal colonic epithelial cells. There was no significant difference observed in the phosphorylation of Akt in normal FHC cells (data not shown).

We next determined the effect of overexpressing a constitutively active form of Akt on tandutinib-mediated cell growth. We overexpressed myristoylated Akt in the 2 cell lines, HCT-116 and SW480, and subsequently treated the cells with tandutinib. As before, in control cells, tandutinib treatment significantly suppressed cell proliferation. However, this tandutinib-mediated suppression was reduced in the presence of myristoylated Akt (Fig. 4C, right). We confirmed this by determining the levels of apoptosis by caspase-3/7 assay. While tandutinib alone increased caspase-3/7 activity, this was significantly reduced in cells expressing constitutively active Akt in both HCT-116 and SW480 cells (Fig. 4C, left). Additional confirmation was obtained by Western blot analyses. Expression of constitutively active Akt resulted in increased expression of Bcl2 and reduced expression of Bax proteins, even in the presence of tandutinib in the 2 cell lines (Fig. 4D, right). These data suggest that tandutinib-mediated suppression of cell growth occurs, in part, through suppression of Akt phosphorylation.

**Figure 3.** Tandutinib affects expression of cell-cycle–related proteins and cancer-promoting genes. A, real-time PCR analyses for cell-cycle genes. Total RNA from HCT-116 cells treated with 25 μmol/L of tandutinib for 48 hours was subjected to real-time PCR analyses. Tandutinib treatment significantly reduced the expression of cyclin D1 and c-Myc mRNA (*, P < 0.05). B, Western blotting for cell-cycle genes. Lysates from HCT-116 and SW480 cells were incubated with 25 μmol/L of tandutinib and analyzed by Western blotting for cyclin A1, B1, D1, and c-Myc proteins. Expression of all 4 proteins was suppressed following tandutinib treatment. C, real-time PCR analyses for tumor-promoting genes. Total RNA subjected to real-time reverse transcription analyses showed that tandutinib treatment significantly reduced the expression of COX-2, VEGF, and IL-8 mRNA (*, P < 0.05). D, Western blotting for tumor-promoting genes. Tandutinib treatment suppressed the levels of cancer-promoting genes COX-2, VEGF, and IL-8.

Tandutinib Inhibits Colon Cancer Growth

To evaluate the role of tandutinib on tumor growth in vivo, we next examined its effects on HCT-116 colon cancer cell xenografts. HCT-116 were injected into the flanks of athymic nude mice and allowed to develop into a xenograft. One week following injection of the cells, tandutinib mixed with 5% sodium bicarbonate was administered intraperitoneally daily for 3 weeks. Tandutinib inhibited the growth of the tumor xenografts (Fig. 5C). The excised tumors from control animals weighed about 1,200 mg, whereas those treated with tandutinib weighed about 400 mg (Fig. 5B, top and bottom). In addition, tumor volume was significantly decreased (Fig. 5C). There was no apparent change in liver, spleen, or body weight in the animals (data not shown). These data imply that the tandutinib is a potential therapeutic agent for treating colon cancers. We also determined the effect of the compound on tumor vascularization by staining for endothelial-specific antigen CD31. As shown in Fig. 5D, the treatment with the tandutinib leads to a significant reduction in CD31 staining and to the obliteration of the normal vasculature (Fig. 5D).

**Tandutinib inhibits colon tumor xenograft growth and angiogenesis**

To determine whether a similar mechanism was occurring in colon cancer cells, we determined the effect of tandutinib on the Akt/mTOR pathway. Western blot analyses showed that tandutinib treatment downregulated the phosphorylation of both Akt and mTOR in the 2 cell lines (Fig. 4B). Furthermore, tandutinib also further downregulated the phosphorylation of downstream target protein P70S6K and 4EBP1 (Fig. 4B). In addition, tandutinib treatment increased PTEN expression in both HCT-116 and SW480 cells. We also determined the effect of tandutinib on Akt phosphorylation in normal colonic epithelial cells. There was no significant difference observed in the phosphorylation of Akt in normal FHC cells (data not shown).

We next determined the effect of overexpressing a constitutively active form of Akt on tandutinib-mediated cell growth. We overexpressed myristoylated Akt in the 2 cell lines, HCT-116 and SW480, and subsequently treated the cells with tandutinib. As before, in control cells, tandutinib treatment significantly suppressed cell proliferation. However, this tandutinib-mediated suppression was reduced in the presence of myristoylated Akt (Fig. 4C, right). We confirmed this by determining the levels of apoptosis by caspase-3/7 assay. While tandutinib alone increased caspase-3/7 activity, this was significantly reduced in cells expressing constitutively active Akt in both HCT-116 and SW480 cells (Fig. 4C, left). Additional confirmation was obtained by Western blot analyses. Expression of constitutively active Akt resulted in increased expression of Bcl2 and reduced expression of Bax proteins, even in the presence of tandutinib in the 2 cell lines (Fig. 4D, right). These data suggest that tandutinib-mediated suppression of cell growth occurs, in part, through suppression of Akt phosphorylation.
investigated whether the treatment affects the COX-2 and VEGF in the tissues. Western blot analyses showed that the tandutinib significantly reduced the expression of COX-2 and VEGF when compared with controls in the tumor xenograft tissues (Fig. 6A). Cyclin D1 protein levels were also significantly lower in tandutinib-treated tumor xenografts (Fig. 6A). These data were confirmed by immunohistochemical analyses (Fig. 6C, left). We also quantified the number of cells expressing COX-2, VEGF, and cyclin D1 in the immunohistochemistry and found all 3 proteins to be significantly decreased following tandutinib treatment (Fig. 6D, left). These data suggest that tandutinib affects cancer-promoting genes in vivo.

We also examined the effects on Akt/mTOR signaling proteins in the tumor tissues obtained from control and tandutinib-treated mice. Treatment with tandutinib again resulted in significantly lower levels of phosphorylation of Akt, mTOR, p70S6K, and 4E-BP1 than control untreated tumors (Fig. 6B). Again, further confirmation of the downregulation was obtained by
immunohistochemistry for the proteins in the xenograft tissues (Fig. 6C, middle and right). We also determined the number of cells expressing phospho-Akt, phospho-mTOR, phospho-P70S6K, and phospho-4EBP1 in the tumor sections and found them to be significantly decreased following tandutinib treatment (Fig. 6D, right). These data suggest that tandutinib significantly affects expression of Akt/mTOR signaling–related proteins, which might contribute to the inhibitory effects of this treatment.

Discussion

Given the high mortality rate due to colon cancer and the significant morbidity, apparent toxicity and poor response rates of current chemotherapy regimens there has been a big push to identify novel therapeutic modalities that have fewer toxicity profiles. The PI3K/Akt/mTOR signaling axis plays a critical role in the proliferation, resistance to apoptosis, angiogenesis, and metastasis that is central to the development and maintenance of colorectal cancer cells (27–29). Therefore, therapeutic targeting of the PI3K pathway with its downstream targets Akt and mTOR at multiple molecular levels may provide better antitumor effects than selective inhibition of only one component of the pathway. Our results indicate that tandutinib possesses potential as a promising therapeutic agent against colon cancer.

Previous studies showed that c-kit overexpression in patients with colorectal cancer resulted in poor prognosis (10, 11). c-Kit plays an important role in cell growth differentiation, motility, and cell survival in a variety of cancers including colorectal cancer (9). Furthermore, c-kit protects the cells from undergoing apoptosis while also increasing their invasiveness in colon cancer cells (12). In fact, the c-kit tyrosine kinase inhibitor STI571 significantly inhibits cells survival and invasive potential of these cells and hence may be appropriate for colorectal cancer therapy (9, 30). Another study also reported that BAY43-9006 inhibits c-Kit, along with several receptor tyrosine kinases. Moreover, BAY43-9006 inhibits tumor growth,
angiogenesis, and MAPK pathway in colon, pancreatic, and breast tumor cell lines, in part, through inhibiting the expression of VEGFR-2 and -3 (31). In addition, c-Kit receptor signaling enhanced the proliferation and invasion of colorectal cancer cells through the PI3K/Akt pathway (32). Similar to these studies, tandutinib treatment effectively downregulated the c-kit phosphorylation in colon cancer cells. Moreover, tandutinib inhibited cell proliferation and induced apoptosis in colon cancer cells, in part, through inhibiting PI3K/Akt signaling.

Aberrant PI3K/Akt signaling is common in colon cancer. Recent studies have reported the potential for therapeutic targeting of the PI3K/Akt/mTOR network at multiple molecular levels (33, 34). mTOR resides in 2 distinct multiprotein complexes called mTORC1 and mTORC2 (36). mTORC1 directly phosphorylates ribosomal protein S6 kinase 1 (S6K1) and the eukaryotic translation initiation factor elf4E-binding protein 1 (4EBP1), both involved in protein translation (37, 38). Moreover, mTORC2 can phosphorylate Akt on Ser 473, thereby leading to activation of the Akt pathway and thus several cancer-related cellular responses, including increased cell growth, proliferation, and survival (39). A recent preclinical study with OSI-027, a potent inhibitor of Akt/mTOR suppressed the growth of colon cancer (39). In addition, Akt and mTOR inhibitors can downregulate leptin-mediated PI3K/Akt/mTOR signaling affecting colon cancer cell proliferation and inducing apoptosis (40). Our studies in the manuscript had
similar results with tandutinib effectively downregulating phosphorylation of both Akt and mTOR phosphorylation and also mTOR downstream target proteins phospho-p70S6K (Thr389) and phospho-4EBP1 (Thr37/46). These findings suggest that tandutinib downregulates the functions of Akt and mTOR. Moreover, tandutinib inhibited proliferation and induced apoptosis in colon cancer cell lines and suppressed formation of microvessels in the xenografts indicating that the in vivo anticancer effects of tandutinib may be due to induction of apoptosis, in addition it also inhibits angiogenesis.

In our in vivo studies, we observed marked decrease in tumor xenograft growth in mice treated with tandutinib. Moreover, there was no apparent toxicity observed in liver, kidney, and spleen. A significant body of evidence suggest that constitutive COX-2 expression is a contributing factor for promoting colorectal cancer (41). In addition, there are various genetic, epigenetic, and inflammatory pathways involved in the etiology and development of colorectal cancer (41). Tandutinib treatment seems to mediate its actions through multiple molecular targets including COX-2. Given the high levels of resistance to apoptosis due to COX-2 overexpression (42, 43), treating cells with tandutinib could potentially restore apoptotic susceptibility of colon cancer cells, in part, through the downregulation of this gene.

Recent studies showed that IL-8 and its receptors are significantly upregulated in the tumor and act as key regulators of proliferation, angiogenesis, and metastasis. Although IL-8 overexpression has been correlated with the inflammation-related risk of sporadic colorectal cancer (44), it is also a potent inducer of proliferation of colorectal cancer cells (45). Hence, IL-8 has been considered a therapeutic target (46). In this regard, the IL-8 receptor antagonist SCH-527123 was shown to inhibit tumor growth and sensitize cells to oxaliplatin in preclinical colon cancer models (47). Tandutinib treatment inhibits IL-8 expression, but it remains to be seen if this inhibition also correlates with changes in inflammatory responses that are associated with the development of cancer.

VEGF and its receptors are overexpressed in colon cancer and play an important in angiogenesis and promotion of tumor growth (48, 49). Pharmacologic inhibition with STI571 (Gleevec) of VEGF expression also suppresses VEGF- and basic fibroblast growth factor (bFGF)-induced angiogenesis in vivo (50). The compound also inhibits angiogenesis and tumor growth in an experimental bone metastasis model (50). Our studies with tandutinib significantly downregulating VEGF expression in both in vitro and in vivo tumor tissues suggest that tandutinib can also affect angiogenesis. Future studies are warranted to determine whether the combination of tandutinib with known angiogenesis inhibitors such as STI571 has greater effect than either compound alone.

In conclusion, our studies show that treatment of colon cancer cells with tandutinib results in significant inhibition of tumor cell growth, both in vitro and in vivo. Our studies show that tandutinib affects Akt/mTOR and c-kit pathways. One of the biggest problems with therapeutic compounds is the toxicity to normal cells. One mechanism to reduce toxicity is to use a combination of compounds that have synergistic activity. Hence, identifying a compound or compounds that can act synergistically with tandutinib may have value in reducing toxicity associated with the amount of compound needed to have therapeutic efficacy. Nevertheless, in our studies, we observed that tandutinib does not affect proliferation of normal colonic epithelial cells, strongly suggesting that the compound has promising potential for use as a therapeutic option for colon cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Disclaimer
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' Contributions
Conception and design: D. Subramaniam
Development of methodology: S. Ponnurangam, P. Rangarajan, D. Subramaniam
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Standing, D. Subramaniam
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Ponnurangam, P. Rangarajan, D. Subramaniam
Writing, review, and/or revision of the manuscript: D. Subramaniam
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Ponnurangam
Study supervision: D. Subramaniam

Acknowledgments
The authors thank Dr. Shrikant Anant for supporting the studies and with helpful suggestions and for critically reading the manuscript. They also thank members of Dr. Shrikant Anant’s laboratory and the cancer center for their discussion during the course of this study.

Grant Support
This work was supported by Thomas O’Sullivan Foundation and a pilot grant from the NCI-designated University of Kansas Cancer Center (P30CA168524-01; D. Subramaniam) and the Flow Cytometry Core Laboratory, which is sponsored, in part, by the Cancer Center.

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Received September 14, 2012; revised January 29, 2013; accepted February 8, 2013; published OnlineFirst February 20, 2013.

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Molecular Cancer Therapeutics

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