RelB-Dependent Differential Radiosensitization Effect of STI571 on Prostate Cancer Cells

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Abstract

Radiation therapy is an effective treatment for localized prostate cancer. However, when high-risk factors are present, such as increased prostate-specific antigen, elevated Gleason scores and advanced T stage, undetected spreading of the cancer, and development of radiation-resistant cancer cells are concerns. Thus, additional therapeutic agents that can selectively sensitize advanced prostate cancer to radiation therapy are needed. Imatinib mesylate (Gleevec, STI571), a tyrosine kinase inhibitor, was evaluated for its potential to enhance the efficacy of ionizing radiation (IR) against aggressive prostate cancer cells. STI571 significantly enhances the IR-induced cytotoxicity of androgen-independent prostate cancer cells but not of androgen-responsive prostate cancer cells. The differential cytotoxic effects due to STI571 are associated with the nuclear level of RelB in prostate cancer cells. STI571 inhibits IR-induced RelB nuclear translocation, leading to increased radiosensitivity in aggressive androgen-independent PC-3 and DU-145 cells. In contrast, STI571 enhances RelB nuclear translocation in androgen-responsive LNCaP cells. The different effects of STI571 on RelB nuclear translocation are consistent with RelB DNA binding activity and related target gene expression. STI571 inhibits the phosphoinositide 3-kinase-AKT-IκB kinase-a pathway in PC-3 cells by decreasing the phosphorylation levels of phosphoinositide 3-kinase (Tyr458) and AKT (Ser473), whereas STI571 increases NF-κB inducible kinase (Thr559) phosphorylation, leading to activation of IκB kinase-a in LNCaP cells. These results reveal that STI571 exhibits differential effects on the upstream kinases leading to different downstream effects on the NF-κB alternative pathway in prostate cancer cells and suggest that STI571 is effective for the treatment of androgen-independent prostate cancer in the context of high constitutive levels of RelB.

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Introduction

Prostate cancer is a common disease in North America and northwestern Europe, and it is the second leading cause of cancer deaths among males in the United States (1). In general, the majority of prostate cancer starts as hormone-dependent cancer. As the cancer progresses or after it is treated with androgen ablation, cancer develops androgen-independent features. As patients enter advanced stages of the disease, prostate cancer acquires several capabilities, such as self-sufficient growth signaling, reduced apoptosis, sustained angiogenesis, tumor invasion and metastasis, and insensitivity to antigrowth signals (2). Current treatment strategies for locally confined prostate cancer include radiation therapy and surgical prostatectomy. Radiation leads to lower disease-free survival rates when high-risk factors are present, such as increased prostate-specific antigen, elevated Gleason scores, and advanced T stage (3). We argue that available therapeutic strategies need to be improved to enhance the radiation sensitivity of advanced prostate cancer, especially localized prostate cancer with the possibility of metastasis.

The NF-κB signal pathway is thought to play a critical role in coordinating the innate and adaptive immune responses that maintain cellular defense systems (4). The high constitutive level of NF-κB in cancers has been implicated as a major protective mechanism against cancer therapeutics (5). Thus, the inhibition of NF-κB is considered to be a target for enhancing the efficacy of conventional chemotheraphy and radiation therapy. Indeed, inhibitors that target the p50:RelA-based NF-κB classic pathway have been shown to increase the sensitivity of cancer to treatment (6, 7). In addition, we and others have recently shown that the p52:RelB-based NF-κB alternative pathway plays a pivotal role in tumorigenesis of prostate cancer (8–10). Thus, a strategic combination of inhibiting both the classic and the alternative NF-κB pathways may be necessary to enhance the sensitivity of cancer cells to NF-κB–inducing therapeutic agents, including radiation.

STI571, an inhibitor of tyrosine kinase, is designed to treat chronic myelogenous leukemia and acute lymphoblastic leukemia, characterized by the Philadelphia bcr-abl translocation, which is driven by the Philadelphia chromosome, characterized by the Philadelphia bcr-abl translocation, which is driven by the Philadelphia chromosome.
chromosomal translocation that encodes BCR-ABL active tyrosine kinase (11-13). Because NF-κB is activated by several upstream kinases, we tested whether STI571 suppresses the survival of prostate cancer cells by enhancing its radiosensitivity. The effects of combining STI571 with IR on the survival of prostate cancer cells were investigated. We found that STI571 significantly suppresses the survival of aggressive androgen-independent prostate cancer cells, but it has marginally cytotoxic effects on androgen-responsive prostate cancer cells. Inactivation of the RelB-based NF-κB alternative pathway has been identified as a major mechanism in the cytotoxicity of STI571 to prostate cancer cells. Our finding that targeting the NF-κB alternative pathway enhances the radiosensitivity of prostate cancer cells suggests a promising approach for the selective use of STI571 in the treatment of advanced prostate cancer.

Materials and Methods

Cell culture and treatment

Human prostate carcinoma/adenocarcinoma LNCaP, PC-3, and DU-145 (American Type Culture Collection) were grown and maintained in RPMI (for LNCaP and PC-3) and EMEM (for DU-145) media (Invitrogen) with 10% fetal bovine serum (Hyclone). Cells were pretreated with STI571 (Novartis Pharmaceuticals) at concentrations of 0 to 40 μmol/L for 24 hours, followed by ionizing radiation (IR) treatment using a 250 kV X-ray machine (Faxitron X-ray Corp.) with the peak energy of 130 kV, a 0.05-mm Al filter, at a dose of 0 to 6 Gy. Cytotoxicity was quantified using cell survival fraction determined by an enhanced chemiluminescence detection system (Roche).

Cell transfection

To upregulate or downregulate the RelB-based NF-κB alternative pathway, expression constructs of RelB (American Type Culture Collection), RelB small interfering RNA (siRNA; ref. 14), and its inhibitor p100M (15) (American Type Culture Collection), RelB small interfering RNA (siRNA; ref. 14), and its inhibitor p100M (15) (American Type Culture Collection) were transfected into LNCaP and PC-3 cells using Lipofectamine in serum-reduced Opti-MEM medium (Invitrogen). After transfection, the cells were incubated with STI571 followed by IR exposure. The increased or decreased levels of RelB and its targets in the treated cells were confirmed by Western blot.

Immunocytochemistry

Approximately 1 × 10⁴ LNCaP and PC-3 cells were cultured overnight on BD Falcon Culture Slides (BD Biosciences) with 300 μL of medium. The cells were pretreated with STI571 at a concentration of 20 μmol/L for 24 hours and then treated with 6 Gy IR. After culturing the treated cells for 24 hours, the cells were fixed in 4% paraformaldehyde for 15 minutes and washed twice with 1× PBS. The cells were blocked with 3% Normal Donkey Serum (Jackson ImmunoResearch Laboratories, Inc.) for 30 min at 37°C and then incubated with the primary antibodies against RelA and RelB (Santa Cruz Biotech) at 2 μg/mL in the Donkey Serum for 1 hour at room temperature. After three washes with 1× PBS, the cells were further incubated with Donkey anti-rabbit Cy3 (Jackson ImmunoResearch Laboratories, Inc.) at 7.5 μg/mL in the Donkey Serum for 1 hour. After two washes with 1× PBS and one wash with dH₂O, the cells were stained with Hoechst (Invitrogen) at 20 μg/mL for 10 minutes and subsequently washed once with 1× PBS and once with dH₂O. The slides were allowed to dry and were viewed under Laser Scanning Confocal Microscopy (Leica Biosystems).

NF-κB binding assay

Nuclear proteins were extracted from LNCaP and PC-3 cells using a Nuclear Extraction kit (Active Motif). To determine the DNA binding activity of RelB versus RelA, the nuclear extracts were quantified using an enzyme-linked immunosorbent assay (ELISA)-based Trans NF-κB Family Members kit (Active Motif) according to the manufacturer’s instructions.

Real-time PCR

RNA was isolated from LNCaP and PC-3 cells by Trizol/chloroform extraction (Invitrogen) and treated with DNasease (Turbo DNA-free kit, Ambion). The RNA was used to synthesize cDNA using the SuperScript First-Strand Synthesis System for reverse transcription-PCR (Invitrogen), and the cDNA was quantified by reverse transcription-PCR with gene-specific primer probe sets using a LightCycler 480 Real-Time PCR System (Roche).

Western blot

To quantify the levels of the NF-κB members and the NF-κB targets, nuclear extracts or total cell extracts were separated using SDS-PAGE, 8% (w/v) polyacrylamide gel and then transferred onto a nitrocellulose membrane and blotted with primary antibodies to RelA, RelB, p52, BCL-xL, MnSOD, phosphoinositide 3-kinase (PI3K), AKT, NF-κB inducible kinase (NIK), proliferating cell nuclear antigen, and β-actin. Primary antibodies against the specific phosphorylated forms were used to detect the phosphorylation of PI3K, AKT, and NIK. With the exception of the MnSOD antibody from Upstate Biotech and p-PI3K (Tyr458) and p-AKT (Ser473) antibodies from Cell Signaling, all primary antibodies, including p-NIK (Thr559) and secondary antibodies, were purchased from Santa Cruz Biotech. Western blots were visualized by an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech).

Kinase assay

The treated and nontreated cells were lysed in a NP40 lysis buffer containing 50 mmol/L Tris-HCl (pH 7.5), 1% NP40, 1% glycerol, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL pepstatin, 10 μg/mL leupeptin, and 5 μg/mL aprotinin. After lysing the cells, 200 μg of cell...
extracts were immunoprecipitated using IκB kinase-α (IKKα), AKT, and NIK antibodies incorporated into protein A/G agarose beads (Santa Cruz Biotech). The beads were washed three times with the NP40 lysis buffer and twice with a kinase assay buffer containing 20 mmol/L HEPES/KOH (pH 7.4), 25 mmol/L β-glycerophosphate, 2 mmol/L DTT, and 20 mmol/L MgCl₂. The precipitated proteins were eluted from the beads in 100 μL of kinase buffer. To measure the amounts of precipitated IKKα, AKT, and NIK proteins, 50 μL of elution were analyzed by Western blot. For the kinase assay, 20 μL of elution were incubated with 5 μg of IκBα (Santa Cruz Biotech) or IKKα (Upstate) substrate and a supplement of 5 μCi [γ-32P] ATP/5 μmol/L ATP for 30 minutes at 37°C. The samples were separated on SDS-PAGE gel and scanned with the Typhoon 8600 system.

Statistical analyses

Multiple independent experiments were done for each set of data presented. Statistical significance between treatments and controls was analyzed using one-way ANOVA and Tukey’s Multiple Comparison Test, followed by data analysis with GraphPad Prism version 4.0. P < 0.05 was considered significant.

Results

**STI571 selectively enhances the radiosensitivity of androgen-independent prostate cancer cells**

To examine the radiosensitization effect of STI571, prostate cancer cells were pretreated with STI571 followed by IR treatment. Cytotoxicity was analyzed by colony survival assay. STI571 exerted a weak cytotoxic effect on androgen-responsive prostate cancer LNCaP cells (Fig. 1A), but it was very cytotoxic to androgen-independent prostate cancer PC-3 (Fig. 1B) and DU-145 (Fig. 1C). Importantly, STI571 combined with IR significantly enhanced the radiosensitivity of DU-145 and PC-3 cells, whereas it only exhibited a slight toxic effect on LNCaP cells. Our finding that STI571 is effective in enhancing IR-induced cell death in androgen-independent prostate cancer cells suggests that STI571 may be an efficient treatment of advanced prostate cancer. As both the androgen-independent PC-3 and DU-145 cells were similarly sensitive to STI571, in order to elucidate how STI571 differentially affects the survival of prostate cancer cells, LNCaP and PC-3 cells were used as models to investigate the signaling pathways associated with the radiosensitization effects of STI571.

Figure 1. Differential radiosensitization effects of STI571 on the survival of prostate cancer cells. The three human prostate cancer cell lines tested were as follows: A, androgen-responsive prostate carcinoma, LNCaP; B and C, androgen-independent prostate adenocarcinoma, PC-3, and prostate carcinoma, DU-145. Cells were incubated with 0 to 40 μmol/L STI571 for 24 hours followed by 0 to 6 Gy IR treatment. After 24 to 48 hours, cell survival was analyzed using colony formation assay. **, the combined treatments with the same STI571 doses have significantly reduced cell survival compared with both no STI571 and no IR controls.
STI571 affects RelB nuclear translocation in prostate cancer cells

Our previous studies suggest that the constitutive level of RelB in PC-3 and DU-145 cells is much higher than that in LNCaP cells. To examine whether STI571 regulates NF-κB nuclear translocation, nuclear proteins were extracted from STI571-treated cells, and the levels of RelA and RelB in nuclei were quantified by Western blot (Fig. 2A). IR increased the nuclear level of RelB more than it increased the nuclear level of RelA in both LNCaP and PC-3 cells. STI571 exhibited the same suppressive effect on RelA nuclear translocation in both PC-3 and LNCaP cells. Unexpectedly, STI571 exerted opposing effects on RelB nuclear translocation in PC-3 and LNCaP cells. In PC-3 cells, STI571 inhibited RelB nuclear translocation in both irradiated and nonirradiated cells. In contrast, STI571 enhanced RelB nuclear translocation in LNCaP cells. The changes in nuclear RelB levels were confirmed by immunocytochemistry (Fig. 2B). Furthermore, the effects of the treatment on RelA and RelB DNA binding activities were quantified using an ELISA NF-κB DNA binding system (Fig. 2C). Although the RelA DNA binding activity did not significantly change in STI571-treated cells, the RelB DNA binding activity increased in LNCaP cells but decreased in PC-3 cells.

STI571 alters the expression of NF-κB target genes

NF-κB-regulated genes, MnSOD, and BCL-xL have been previously shown to be activated by IR in PC-3 cells. Upregulation of the two proteins results in decreased cell radiosensitivity (9). To determine whether STI571 changes the radiosensitivity of prostate cancer cells due to the modulation of NF-κB target gene expression, the levels of mRNA and protein from the two target genes were quantified by reverse transcription-PCR and Western blot (Fig. 3). Consistent with its effect on the nuclear translocation of RelB (Fig. 2), STI571 eliminated IR-dependent increases in the levels of the NF-κB targets in PC-3 cells, whereas it enhanced the targets in LNCaP cells. Our results indicate that activation or inactivation of the RelB-based NF-κB alternative pathway by STI571 may be a major mechanism for the differential radiosensitization effects of STI571 on the survival of prostate cancer cells.

Figure 2. Effects of STI571 and IR on the nuclear translocation of RelA and RelB in LNCaP and PC-3 cells. A and B, the nuclear levels of RelA and RelB in the untreated and treated cells were measured by Western blot with a PCNA loading control (A) and confirmed by immunocytochemistry (B). C, the relative DNA binding activities of RelA and RelB were quantified using a NF-κB ELISA DNA binding kit. Significant differences were compared with the nontreated groups.
The effect of STI571 is validated by the manipulation of RelB

To verify the role of RelB in the radiosensitization effect of STI571, RelB was either inhibited in LNCaP or enhanced in PC-3. As the alternative pathway was activated, NIK or IKKα phosphorylated p100, leading to ubiquitin- and proteosome-mediated p100 degradation and the release of p52 (16). The p100M, in which the serine residues at 866/870 are mutated to alanine, was unresponsive to NIK-dependent phosphorylation (15). Knockdown of RelB by a specific siRNA or blockade of RelB nuclear translocation by the expression of p100M was used to reduce the nuclear level of RelB in LNCaP cells before treatment (Fig. 4A and B). The expression level of p100M and the level reduction of RelB and its targets were confirmed by Western blot. Cell survival analysis shows that the inhibition of RelB in LNCaP cells significantly increased the radiosensitization effect of STI571. In addition, ectopic expression of RelB in PC-3 cells was tested to protect the cells against the cytotoxic effect of the treatments (Fig. 4C). The increased levels of RelB and its targets in PC-3 cells are consistent with the increased cell survival rate after treatments. Our results indicate that the activation of the RelB-based NF-κB alternative pathway contributes to the resistance of prostate cancer cells to radiation.

STI571 inhibits AKT in PC-3 cells but activates NIK in LNCaP cells

To elucidate the mechanisms that underlie the observed differential effects of STI571 on the radiosensitization of prostate cancer cells, we examined the upstream signaling involved in the activation of the NF-κB alternative pathway. IKKα, a member of the IκB kinase family, is a key factor in the activation of both the NF-κB classic and alternative pathways (17–19). Thus, we assessed the effect of STI571 on IKKα activity in both LNCaP and PC-3 cells. IKKα was immunoprecipitated from the cells using a specific antibody. Phosphorylation of IκBα, an IKKα substrate, was determined using the precipitated samples with P32-labeled ATP (Fig. 5A). Consistent with the nuclear levels of RelB in the treated cells, STI571 inhibited IKKα activity in PC-3 cells but increased its activity in LNCaP cells. IKKα protein was subsequently used as a substrate to examine the activities of upstream kinases, AKT and NIK.
were used to immunoprecipitate cellular extracts from the treated cells followed by phosphorylation assays using IKKα as a substrate. IKKα was phosphorylated by both AKT and NIK. Interestingly, STI571 differentially regulated the phosphorylation of AKT and NIK in LNCaP and PC-3 cells. As shown in Fig. 5B and C, STI571 inhibited AKT phosphorylation in PC-3 cells but not in LNCaP cells. In contrast, STI571 enhanced NIK activation in LNCaP cells but not in PC-3 cells. These results indicate that the differential effects of STI571 on the survival of prostate cancer cells may be due, in part, to the AKT- or NIK-mediated activation of the RelB-based NF-κB alternative pathway.

**STI571 decreases PI3K tyrosine phosphorylation in PC-3 cells but increases NIK threonine phosphorylation in LNCaP cells**

To further elucidate the effects of STI571 on the phosphorylation of PI3K, a tyrosine kinase-dependent phosphorylation assay was done. IR slightly increased...
the tyrosine kinase activity of PI3K but STI571 significantly inhibited its activity in both irradiated and non-irradiated PC-3 cells, whereas it had no effect in LNCaP cells (Fig. 6A). To further verify the change in tyrosine kinase activity of PI3K, the cellular extracts were blotted with a specific antibody to p-PI3K Tyr485 (Fig. 6B). Tyrosine 485 phosphorylation was consistently decreased in STI571-treated PC-3 cells but not in LNCaP cells. Subsequently, the phosphorylation of downstream AKT serine 473 was also decreased in PC-3 cells, which is consistent with the decrease in PI3K tyrosine phosphorylation. Because STI571 had no effect on the PI3K-AKT pathway in LNCaP cells, a specific antibody against p-NIK Thr559 was used to determine the change in phosphorylation of NIK (Fig. 6C). In PC-3 cells, IR increased the phosphorylation of NIK Thr559, but STI571 slightly decreased its phosphorylation. In contrast, STI571 markedly increased NIK phosphorylation in LNCaP cells. Consistent with the increased NIK phosphorylation, STI571 increased the level of p100 and resulted in the release of more p52 in LNCaP cells compared with the slight decrease of p52 in PC-3 cells. Overall, these results suggest a basic mechanism that underlies the differential effects of STI571 on the NF-κB alternative pathway in androgen-responsive and androgen-independent prostate cancer cells (Fig. 6D).

**Discussion**

The constitutive activation of BCR-ABL tyrosine kinase has been identified as a consequence of chromosomal exchange mediated by genetic aberrations found in chronic myelogenous leukemia and acute lymphoblastic leukemia (20, 21). STI571 or its analogues, inhibitors of tyrosine kinases, have been designed to treat patients with BCR-ABL-positive leukemia (11, 22). Gain-of-function mutations in the c-KIT gene lead to increased tyrosine kinase receptor activation in gastrointestinal stromal tumors. STI571 is also used to block the activation of c-Kit and suppress gastrointestinal stromal tumors (23, 24). Mechanistically, STI571 was investigated for its effect on inhibiting other types of receptor tyrosine kinases, such as epidermal growth factor receptor, vascular endothelial growth factor receptor, and platelet-derived...
growth factor receptor (25–27). Clinically, STI571 is being tested for the treatment of multiple types of cancer including hormone refractory prostate cancer. The IC\textsubscript{50} of STI571 in prostate cancer cells was 10 to 20 μmol/L. A daily oral dose of 50 mg/kg was shown to significantly inhibit PC-3 metastasis to the bone of tumor-bearing mice (28, 29). The concentration of STI571 used to control prostate cancer seems to be higher than that used in patients receiving STI571 for the treatment of chronic myelogenous leukemia and acute lymphoblastic leukemia. Thus, it is possible that the effect of STI571 used for the treatment of prostate cancer may include off-target effects of STI571.

PI3K, an important kinase, activates the AKT-mediated prosurvival pathway in cancers (30, 31). Phosphorylation of tyrosine residues in PI3K has been identified as a major signal for PI3K activation (32, 33). Activation of the PI3K-AKT pathway has been shown to play a causal role in the progression of prostate cancer and the development of prostate cancer resistance to radiotherapy (34, 35). The present study was designed to test whether STI571 enhances the radiosensitivity of prostate cancer by inhibiting the phosphorylation of the tyrosine residues on PI3K. The results show that STI571 inactivates the PI3K-AKT pathway and enhances radiosensitization in androgen-independent PC-3 and DU-145 cells, but the radiosensitization effect is marginal in androgen-positive LNCaP cells. In fact, the constitutive level of PI3K was lower in LNCaP cells compared with its level in PC-3 cells. The finding that STI571 efficiently inhibits PI3K suggests the potential use of STI571 as a radiation adjuvant to treat prostate cancer with high levels of activated PI3K.

Tyrosine kinases are important mediators of signaling cascades. The enzymatic activity catalyzed by tyrosine kinases involves the transfer of the terminal phosphate from ATP to tyrosine residues on its substrates. STI571 inhibits protein tyrosine phosphorylation by binding to

![Diagram](image-url)
the ATP binding site in the BCR-ABL kinase. The present study shows that STI571 also inhibits PI3K tyrosine phosphorylation, resulting in the downregulation of the AKT/IKKα-activated NF-κB pathway in PC-3 cells. It has been reported that the inhibitor of class I PI3K, PI-103, reduces the phosphorylation of AKT at serine 473 leading to enhancement of radiosensitivity of tumor cells (36). Selective inhibition of the PI3K/AKT pathway using PI3K inhibitor or AKT inhibitor may be a strategic option to enhance radiosensitivity of advanced prostate cancers. The results from the present study also show that STI571 activates NIK, a different type of kinase, by inducing heroine phosphorylation in LNCaP cells. The diverse effects of STI571 on the phosphorylation of different kinases are strong indicators that the use of this kinase inhibitor in cancer therapy may include both target and off-target effects through multiple signaling pathways.

NF-κB is constitutively expressed at high levels in many types of cancers and can be activated through multiple signaling pathways including PI3K/AKT in response to chemotherapy and radiation therapy (37–39). An ongoing in-depth study of the RelA-based NF-κB classic pathway has led to the discovery of a series of inhibitors for cancer treatment (6, 7, 40). However, the role of the RelB-based NF-κB alternative pathway in cancer development and response to cancer treatment is poorly understood. We recently reported that RelB contributes to the tumorigenicity of prostate cancer and that a blockade of p52:RelB nuclear translocation results in the radiosensitization of prostate cancer (11, 12). It has been reported that STI571 prevents RelB nuclear translocation in dendritic cells, suggesting that STI571 also regulates the NF-κB alternative pathway (41). Therefore, combining STI571 with radiation may be a feasible strategy for treating advanced prostate cancer with high levels of RelB. Our present study suggests that the nuclear level of RelB in PC-3 cells is remarkably reduced by STI571, showing that STI571 is efficient for treating advanced prostate cancer when combined with IR. By contrast, STI571 can increase RelB nuclear translocation in LNCaP cells, possibly protecting the cells against radiation. The diverse effects of STI571 on RelB transactivation are consistent with its cytotoxic effects on various types of prostate cancer cells. Hence, the use of a tyrosine kinase inhibitor such as STI571 to treat prostate cancer may be only relevant for certain types of prostate cancer.

Although radiation therapy is an effective option based on the localized nature of prostate cancer, its therapeutic efficacy eventually decreases when prostate cancer develops radiation resistance. Therefore, a combination of growth inhibitors with traditional radiation treatment is considered to be a promising approach in the control of prostate cancer. Although multiple signaling pathways and many growth factors are involved in the progression of prostate cancer, the activation of NF-κB is thought to be one of the most important signaling events that contribute to resistance to radiation. Previously, we reported that inhibition of the RelB-based NF-κB alternative pathway is essential to enhancing IR efficiency in the treatment of aggressive prostate cancer. The results from our present study further show that upstream signaling pathways that activate RelB nuclear translocation are key factors in the enhancement of resistance to radiation in prostate cancer cells. Because multiple upstream signaling pathways that can modulate the nuclear translocation of RelB may be differentially affected by STI571, the use of STI571 is only effective in prostate cancer cases in which RelB is activated by the PI3K/AKT pathway but not by the NIK-mediated pathway. Thus, the use of therapeutic agents such as STI571 will be efficient in improving radiation therapy for advanced prostate cancer that is accompanied by high constitutive levels of RelB. Recently, Mikhailova et al. (42) have reported that although AKT upregulates prostate-specific antigen expression and androgen receptor–mediated growth, AKT-induced cell survival is largely androgen receptor independent. Consistently, our findings show that the PI3K/AKT-activated NF-κB classic pathway contributes to the radioresistance of androgen-independent prostate cancer cells. Additionally, NIK activates the NF-κB alternative pathway, which is responsible for cell survival in androgen-responsive prostate cancer cells, suggesting that activation of the RelB-based NF-κB alternative pathway may play an important role in androgen-dependent or androgen-independent cell growth in androgen receptor–positive prostate cancers. It should be noted that the present study shows a proof of concept that STI571 can enhance the radiosensitivity of androgen-independent prostate cancer cells through the inhibition of the NF-κB pathway. However, for clinical use of STI571 in combination with radiation therapy, additional studies will be needed to verify the observed effect using clinically relevant fractionation doses and radiation sources with effective energy appropriate for the treatment of human prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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