Sorafenib Inhibits STAT3 Activation to Enhance TRAIL-Mediated Apoptosis in Human Pancreatic Cancer Cells

Shengbing Huang and Frank A. Sinicrope

Abstract

Signal transducers and activators of transcription 3 (STAT3) is constitutively active in human pancreatic cancer cells and can promote cell growth and apoptosis resistance that contribute to tumorigenesis. We determined if sorafenib, a multikinase inhibitor, can induce apoptosis by targeting STAT3 signaling to enhance apoptosis induction by tumor necrosis factor–related apoptosis-inducing ligand (TRAIL). Human pancreatic cancer cell lines (PANC-1 and BxPC-3) were preincubated with sorafenib (Nexavar) alone or followed by TRAIL. Apoptosis was determined by Annexin V labeling, caspase cleavage, and Bax/Bak activation. Protein expression was analyzed by immunoblotting. Knockdown of STAT3, Mcl-1, and Bim were achieved by lentiviral small hairpin RNA. Adenoviral dominant-negative or retroviral constitutively active (CA) STAT3 were also used. Sorafenib inhibited constitutive STAT3 phosphorylation (Tyr705) and suppressed Mcl-1 and Bcl-xL proteins in a dose- and time-dependent manner. CA-STAT3 overexpression was shown to attenuate caspase-3 cleavage and suppression of Mcl-1 by sorafenib. STAT3 knockdown or a DN STAT3 was shown to downregulate Mcl-1 and Bcl-xL, and to sensitize cells to TRAIL-mediated apoptosis. Treatment with sorafenib enhanced TRAIL-induced Annexin V staining and release of mitochondrial cytochrome c and AIF. Because the BH3-only Bim protein is a potent inducer of mitochondrial apoptosis, Bim knockout was shown to attenuate caspase-3, caspase-9 cleavage, and Bax/Bak activation by sorafenib plus TRAIL. The suppression of STAT3 by genetic means or using sorafenib was shown to downregulate Mcl-1 and Bcl-xL, and to sensitize cells to TRAIL-mediated apoptosis. These data indicate that targeting STAT3 may enhance treatment efficacy against pancreatic cancer. Mol Cancer Ther; 9(3); 742–50. ©2010 AACR.

Introduction

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States (1). The intrinsic resistance of this malignancy to chemotherapy and radiation is due, in large part, to defects in apoptotic signaling pathways (2). Pancreatic cancer and other tumor types show constitutively active (CA) signal transducer and activator of transcription 3 (STAT3; refs. 3, 4). STAT3 is a member of a family of transcription factors that convey signals from the cell surface to the nucleus upon activation by cytokines and growth factors (4, 5). STAT3 can also be activated by nonreceptor tyrosine kinases, including Src in human pancreatic cancer cells (6). The engagement of cell surface receptors by polypeptide ligands, such as interleukin-6, induces tyrosine phosphorylation (Tyr705) of the STAT3 protein that then translocates to the nucleus and regulates the expression of genes harboring STAT3-binding sites in their promoters, including genes that govern cell cycle progression, apoptosis, and angiogenesis that contribute to oncogenesis (4, 5). Specifically, STAT3 can transcriptionally regulate prosurvival Bcl-2 proteins such as Bcl-xL, Mcl-1, and survivin, and the disruption of STAT3 signaling induces apoptosis and decreases their expression in diverse human tumor cell types (7–10). Together, these data suggest that STAT3 may represent an important therapeutic target in pancreatic cancer.

Sorafenib (Nexavar, BAY43-9006) is an oral multikinase inhibitor that can block the Ras/Raf/mitogen-activated protein/extracellular signal-regulated kinase (ERK) kinase (MEK)/ERK signaling cascade that is important for the growth of solid tumors (11, 12). The MEK-ERK signaling pathway is a downstream target of oncogenic Ras mutations (12) that occur in ~90% of human pancreatic carcinomas (13). Sorafenib also targets several other receptor tyrosine kinases, including vascular endothelial growth factor (VEGF) receptor 2, platelet-derived growth factor receptor, FLT3, Ret, and c-Kit (11). Sorafenib has shown preclinical activity against a variety of tumor types and is a standard treatment for hepatocellular and renal cell carcinomas (14, 15). Sorafenib can induce apoptosis and has been shown to downregulate the prosurvival Mcl-1 protein to enhance mitochondrial apoptotic signaling (16, 17). However, Mcl-1 expression is regulated by multiple
mechanisms (18) including STAT3 (9). Mcl-1 can inhibit apoptosis by sequestering proapoptotic BH3-only proteins including Bim and Noxa, as well as the multidomain Bak protein that regulates the permeability of the outer mitochondrial membrane (19). Sorafenib has been shown to induce Bim expression in human leukemia cells (20). Bim can bind to all prosurvival Bcl-2 proteins to neutralize them and is, therefore, a potent inducer of apoptosis (19, 21). Sorafenib has been shown to enhance tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)–mediated apoptosis in human leukemia and colon cancer cell lines (22–24). TRAIL is a proapoptotic cytokine and a promising anticancer drug that is currently undergoing phase I/II evaluation in cancer patients (25). TRAIL triggers death receptor–mediated apoptosis that requires the mitochondrial amplification of a membrane death receptor signal in most human cancer cells (26, 27). We and others have shown that TRAIL-mediated apoptosis can be negatively regulated by Mcl-1 (28, 29) and Bcl-2 (30, 31) proteins.

We hypothesized that sorafenib can downregulate Mcl-1 expression by inhibiting STAT3 activation and thereby enhance TRAIL-mediated apoptosis. We show that inhibition of STAT3 activation by sorafenib or, alternatively, by STAT3 knockdown or a dominant-negative (DN) STAT3 can downregulate Mcl-1 and Bcl-xL proteins to enhance TRAIL-induced apoptosis. Furthermore, overexpression of CA-STAT3 was shown to attenuate the sorafenib-induced downregulation of Mcl-1 and to inhibit caspase-3 cleavage by TRAIL and/or sorafenib in human pancreatic cancer cells.

Materials and Methods

Cell Culture, Drugs, and Reagents

Human pancreatic cancer cell lines BxPC-3 and PANC-1 cells, previously purchased from the American Type Culture Collection, were cultured in RPMI 1640 (Invitrogen) supplemented with 10% fetal bovine serum and with 1% penicillin/streptomycin, 10 mmol/L L-HEPES, and 1% sodium pyruvate. Human embryonic kidney cell line 293T and 293A cells were maintained in high-glucose DMEM (Sigma) with 10% fetal bovine serum. For lentiviral production, 293T cells were cultured in high-glucose DMEM containing 2% fetal bovine serum as per the manufacturer’s manual. Briefly, an equal number (10^6) of cells was pelleted and lysed in 200 μL of lysis buffer by incubating for 30 min at room temperature. The lysates were centrifuged at 200 × g for 10 min and 20 μL of the supernatant was transferred into the microplate well. Then, 80 μL of immunoreagent was added into each well. The plate was incubated at room temperature for 2 h while being gently shaken. After washing the cells thrice, ABTS solution was added and the absorbance at 405 nm was measured (reference wavelength at 490 nm) using a VERSAmax Microplate Reader (Molecular Devices, Inc.). Samples were run in duplicate and the average values are shown.

Cell Viability Assay

Cell viability was determined in the presence or absence of drug treatment using the MTS reduction assay per the manufacturer’s protocol (Promega) as previously described (32).

Western Blotting

Protein samples were prepared in a lysis buffer [5 mmol/L MgCl₂, 137 mmol/L KCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% CHAPS, 10 mmol/L HEPES (pH 7.5)] containing a protease inhibitor cocktail and/or a phosphatase inhibitor cocktail 2 (both from Sigma), normalized using nanodrop measurement (NanoDrop Technologies), boiled in LDS sample buffer (Invitrogen), and loaded onto 14% SDS-PAGE gels with electrophoretic transfer onto a polyvinylidene difluoride membrane (Bio-Rad). Western blotting was done, as previously described (32), using anti-mouse antibodies against Mcl-1, caspase-8 (BD Biosciences), Bcl-xL, Bak (EMD Chemicals), and HA (Roche Diagnostics). Anti-rabbit antibodies were used against BirB, VEGF (both from Santa Cruz Biotechnology), Bid, Bax, STAT3, and p-STAT3, p-Erk1/2, p-AKT, caspase-9, cleaved caspase-3, anti-FLAG (all from Cell Signaling Technology), and ERK1/2 (EMD). p-ERK1/2 was quantified relative to total ERK1/2 with the ratio arbitrarily set to 1 in vehicle-treated cells. Quantitation was done using Image J software (NIH).

Knockdown of Bim, STAT3, and Mcl-1 Using Lentiviral Small Hairpin RNA

Target sequences for Bim and Mcl-1 were selected and the top and bottom strands of the template were synthesized by the Mayo Clinic Molecular Biology Core Facility. The targeting sequences for Mcl-1 was GG-CAGTCGCTG-GAGATTAT (si1) and GATTGTGACTCTCATTTCTGAGATTAT (si2). Specific apoptosis was assessed by this formula: % specific apoptosis = (test − control) × 100/(100 − control; ref. 32).

DNA Fragmentation Assay

After treatment, cells were harvested, resuspended in cold PBS, and then counted. DNA fragmentation was then quantified by a Cell Death Detection ELISA plus kit (Roche Applied Science) as per the manufacturer’s manual. Briefly, an equal number (10^6) of cells was pelleted and lysed in 200 μL of lysis buffer by incubating for 30 min at room temperature. The lysates were centrifuged at 200 × g for 10 min and 20 μL of the supernatant was transferred into the microplate well. Then, 80 μL of immunoreagent was added into each well. The plate was incubated at room temperature for 2 h while being gently shaken. After washing the cells thrice, ABTS solution was added and the absorbance at 405 nm was measured (reference wavelength at 490 nm) using a VERSAmax Microplate Reader (Molecular Devices, Inc.). Samples were run in duplicate and the average values are shown.
(si8; ref. 33), and the sequence for Bim was GACCGAGAGCTACCTG (34). The targeting sequence for STAT3 was CATCGTACATCGGCTA (35). Cloning of small hairpin RNA (shRNA) and generation of lentivirus in the producer cells and transduction of lentivirus into pancreatic cancer cell lines were done as previously described (32).

**Bak and Bax Conformational Change**

After drug treatment, cells were lysed in lysis buffer [5 mmol/L MgCl2, 137 mmol/L KCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% CHAPS, 10 mmol/L HEPES (pH 7.5)] in the presence of protease inhibitor cocktail. Bak and Bax conformational change was then determined by immunoprecipitation followed by immunoblotting using anti-mouse antibodies against Bax 6A7 (Sigma) and Bak (Ab-1, EMD Chemicals), as previously described (32).

**Adenovirus Construction, Production, Amplification, Purification, and Transduction**

The replication-deficient adenovirus expressing DN STAT3, in which Tyr705 was substituted with a Phe residue (36), was obtained by sequential steps using the

---

**Figure 1.** Sorafenib inhibits constitutive STAT3 phosphorylation at Tyr705 and downregulates Mcl-1 and Bcl-xL expression in PANC-1 (A) and BxPC-3 (B) cell lines. Cells were incubated with sorafenib at the indicated doses for 6, 12, or 24 h, and the whole-cell lysate (WCL) was analyzed by Western blotting using highly specific antibodies. The level of p-ERK1/2 was normalized to total ERK1/2 in the same blots and the relative ratio is shown (see Materials and Methods). C, BxPC-3 cells were stably transduced with a retroviral CA-STAT3-FLAG or an empty vector. Overexpression of STAT3 was confirmed by detecting STAT3 and the FLAG tag using Western blotting. Cells were treated with sorafenib (0 and 16 μmol/L) for 12 h and Mcl-1 and cleaved caspase-3 were probed. The relative intensity of the Mcl-1 protein bands was determined by densitometry. D, PANC-1 cells containing a lentiviral STAT3 shRNA or control were probed for Mcl-1, Bcl-xL, VEGF, or cleaved caspase-3 by Western blotting (left). Cleaved caspase-3 was detected after a prolonged exposure. Alternatively, PANC-1 cells were transduced with an adenoviral DN STAT3 containing a 2HA tag at the NH2 terminus at an increasing multiplicity of infection (MOI). The WCL was prepared 48 h posttransduction and subjected to Western blotting for STAT3, Mcl-1, Bcl-xL, or HA tag (*, a nonspecific band).
ViraPower Adenoviral Expression System (Invitrogen). Briefly, DN STAT3 expression cassette was made by PCR-mediated mutagenesis using the wild-type STAT3 cDNA (Origene) as the template and cloned into the entry vector pENTR4 (Invitrogen). The DN STAT3 cassette in the vector pENTR4 was then transferred into the adenovirus expression vector pAD/CVM/V5-DEST by recombination using the LR Clonase II enzyme mix (Invitrogen). The DN STAT3 cassette was verified at each step by sequencing. The vector pAD/CVM/V5-DEST containing the DN STAT3 cassette was digested with PacI (NEB) and transfected into 293A cells using Lipofectamine 2000 according to the protocol (Invitrogen). The amplification and purification of adenovirus was done with an Adeno-X Maxi purification kit (Clontech). The viral titer of the purified adenovirus was estimated based on the absorbance at 260 nm and the titer was calculated and expressed as optical particle unit (opu) per milliliter unit/mL. Viral titer (opu/mL) was calculated as absorbance260 × viral dilution × 1.1 × 1012. One pfu is estimated as 25 opu (37). Multiplicity of infection is expressed as pfu per cell.

Ectopic Expression of a CA-STAT3 Mediated by Retrovirus

A retrovirus-based expression vector pBabe-puro containing the CA-STAT3 fused with a COOH-terminal FLAG, or the empty vector (a gift from Dr. J. Bromberg, Memorial Sloan Kettering Cancer Center, New York, NY), was mixed with pMD.MLV and pMD.G plasmids and transfected into 293T cells using Lipofectamine and Plus reagent (Invitrogen). Retroviral-containing medium was collected 48 h posttransfection and was concentrated by incubating with 10% (final) PEG-8000 (Sigma) at 4°C overnight, then centrifuged at 1,500 × g for 15 min with the collection of the pellet. BxPC-3 cells that were then incubated with sorafenib. Ectopic CA-STAT3 was shown to potently inhibit caspase-3 cleavage by sorafenib and to attenuate sorafenib-induced Mcl-1 downregulation consistent with a STAT3-mediated proapoptotic effect of this drug. A retroviral vector containing a constitutively active (CA) STAT3 was introduced into BxPC-3 cells that were then incubated with sorafenib. Ectopic CA-STAT3 was shown to potentiate caspase-3 cleavage by sorafenib and to attenuate sorafenib-induced Mcl-1 downregulation consistent with a STAT3-mediated proapoptotic effect (Fig. 1C). Furthermore, knockdown of STAT3 using short hairpin RNA (shRNA) was shown to reduce Mcl-1 and Bcl-xL expression associated with an increase of basal caspase-3 cleavage, whereas no change in peptidyl-prolyl cis/trans isomerase 1 (Pin1), included as a control, was found (Fig. 1D). Pin1 interacts with STAT3 upon cytokine/growth factor stimulation and its overexpression promotes STAT3 transcriptional activity and target gene expression (40). In addition, STAT3 shRNA was

Figure 2. Sorafenib enhances TRAIL-mediated apoptosis. PANC-1 (A) or BxPC-3 (B) cells were pretreated with sorafenib for 24 or 12 h, respectively, followed by TRAIL for 24 h. Apoptosis was analyzed by Annexin V labeling and fluorescence-activated cell sorting analysis. Annexin V-positive cells were quantified (see Materials and Methods) and results shown in a bar graph. Columns, mean of experiments conducted in triplicate; bars, SD.

Results

Sorafenib Inhibits STAT3 Phosphorylation (Tyr270) and Downregulates Mcl-1 and Bcl-xL Expression

We determined the effect of sorafenib on key mediators of the Ras/Raf/MEK/ERK signaling pathway and its potential downstream effector STAT3 that are frequently activated in pancreatic cancers. STAT3 is activated by phosphorylation at Tyr270, which induces dimerization, nuclear translocation, and DNA binding (38). Sorafenib treatment (6, 12, and 24 hours) was shown to potently inhibit the constitutive phosphorylation at Tyr270 in a dose- and time-dependent manner in PANC-1 (Fig. 1A) and BxPC-3 (Fig. 1B) cell lines. Sorafenib inhibited ERK1/2 but not AKT phosphorylation consistent with its ability to inhibit the mitogen-activated protein kinase pathway (11). The inhibition of STAT3 Tyr270 phosphorylation (pSTAT3) by sorafenib occurred coincidentally with the suppression of prosurvival Mcl-1 or Bcl-xL proteins (Fig. 1A and B). The suppression of Mcl-1, but not Bcl-xL by sorafenib, was detected after a 6-hour incubation (Fig. 1A and B), potentially due to the shorter half-life (t1/2) of Mcl-1 compared with Bcl-xL (39).

Because sorafenib can suppress p-STAT3, we determined whether the inhibition of p-STAT3 contributes to the proapoptotic effect of this drug. A retroviral vector containing a constitutively active (CA) STAT3 was introduced into BxPC-3 cells that were then incubated with sorafenib. Ectopic CA-STAT3 was shown to potently inhibit caspase-3 cleavage by sorafenib and to attenuate sorafenib-induced Mcl-1 downregulation consistent with a STAT3-mediated proapoptotic effect (Fig. 1C). Furthermore, knockdown of STAT3 using short hairpin RNA (shRNA) was shown to reduce Mcl-1 and Bcl-xL expression associated with an increase of basal caspase-3 cleavage, whereas no change in peptidyl-prolyl cis/trans isomerase 1 (Pin1), included as a control, was found (Fig. 1D). Pin1 interacts with STAT3 upon cytokine/growth factor stimulation and its overexpression promotes STAT3 transcriptional activity and target gene expression (40). In addition, STAT3 shRNA was

The statistical significance of the differences between experimental variables was determined using the Student’s t test. The values shown represent the mean ± SD for triplicate experiments.

Statistical Analysis

The statistical significance of the differences between experimental variables was determined using the Student’s t test. The values shown represent the mean ± SD for triplicate experiments.
shown to inhibit downstream VEGF expression (Fig. 1D), a known transcriptional target of STAT3 (6, 41). To further support these data, STAT3 suppression was performed using an adenovirus-expressing construct containing a DN STAT3 whose phosphorylation site at Tyr705 was substituted with a phosphorylation defective residue Phe (36). In PANC-1 cells transduced with the DN STAT3 construct, the expression of Mcl-1 and to a lesser extent Bcl-xL were attenuated (Fig. 1D).

**Sorafenib Potentiates TRAIL-Induced Apoptosis That Is Negatively Regulated by STAT3**

Sorafenib monotherapy was shown to induce a dose-dependent apoptosis in pancreatic cancer cell lines, as shown by Annexin V (+) FITC staining (Fig. 2). Using TRAIL-sensitive (BxPC-3) and TRAIL-resistant (PANC-1) cell lines (42), sorafenib treatment was shown to significantly enhance TRAIL-induced apoptosis (Fig. 2). Apoptosis induction was associated with increased caspase cleavage, Bid truncation, and release of the mitochondrial cytochrome c and AIF compared with either drug alone (Fig. 3A and B), indicating enhanced cross-talk between the death receptor- and mitochondria-mediated apoptotic pathways (26, 27). The ability of sorafenib to enhance TRAIL-mediated apoptotic signaling could be blocked by a pan-caspase inhibitor (z-VAD-fmk; data not shown). We then determined whether targeting STAT3 can modulate TRAIL-mediated apoptotic signaling. Stable STAT3 knockdown by shRNA was shown to enhance TRAIL-induced caspase-3 cleavage (Fig. 4A). Similarly, cells transduced with a DN STAT3 showed enhanced caspase cleavage and reduced full-length Bid when treated with TRAIL compared with empty vector-alone cells (Fig. 4B). DN STAT3 was also shown to downregulate Mcl-1 and Bcl-xL proteins in TRAIL-treated cells (Fig. 4B). Conversely, cells transduced with CA-STAT3 were protected from caspase-3 cleavage triggered by sorafenib plus TRAIL (Fig. 4C). Mcl-1 knockdown by shRNA was shown to sensitize PANC-1 cells to TRAIL-mediated cytotoxicity and caspase-3 cleavage compared with shRNA control cells (Fig. 4D). Together, these data show that the suppression of p-STAT3 can downregulate Mcl-1 and Bcl-xL proteins to enhance TRAIL-induced apoptosis.

**Proapoptotic Bim Regulates Apoptotic Signaling by Sorafenib Plus TRAIL**

The BH3-only Bim protein can bind to and neutralize all prosurvival Bcl-2 family proteins and is, therefore, a potent inducer of apoptosis (19). We determined the role

![Figure 3. Sorafenib enhances TRAIL-mediated apoptotic signaling. PANC-1 (A) and BxPC-3 cells (B) cells were pretreated with sorafenib (16 μmol/L) for 24 or 12 h, respectively, followed by TRAIL (10 ng/mL for PANC-1; 2.5 ng/mL for BxPC-3) for 5 h. Then, the WCLs (left) were analyzed by Western blotting for caspase-8, caspase-9, and Bid. The cytosolic fractions (right) were analyzed for mitochondrial cytochrome c and AIF release. COX IV was used as a mitochondrial marker and β-tubulin served as a loading control.](https://example.com/figure3)
of Bim in the apoptotic response to sorafenib and TRAIL. In contrast to acute myelogenous leukemia cells (20), sorafenib treatment did not alter Bim expression (Fig. 5A). The knockdown of Bim using a lentiviral shRNA in BxPC-3 cells was shown to significantly attenuate apoptosis induction by TRAIL alone and combined with sorafenib (Fig. 5B and C), and reduced caspase-9 and caspase-3 cleavage (Fig. 5D). Sorafenib potentiated a TRAIL-mediated activation of Bak, but not Bax, as shown by its conformational change that was attenuated by Bim knockdown (Fig. 5D).

Discussion

STAT3 can promote tumor cell growth and apoptosis resistance that contribute to tumor progression and treatment failure (38). We found that sorafenib can suppress p-STAT3 that was associated with the downregulation of
Mcl-1 and Bcl-xL proteins, apoptosis induction, and sensitization to TRAIL-mediated apoptosis. Phosphorylation of STAT3 on Tyr^{705} has been shown to control its dimerization, nuclear translocation, DNA binding, and transcriptional activation of target genes, including Mcl-1 (9, 38). Mcl-1 and Bcl-xL are known to play a critical role in tumor cell survival and sorafenib has been consistently shown to downregulate Mcl-1 expression in diverse tumor cell types (16, 17). To determine whether STAT3 can regulate Mcl-1 or Bcl-xL expression in pancreatic cancer cells, we suppressed STAT3 using shRNA or a DN STAT3 that resulted in the suppression of Mcl-1 and Bcl-xL expression. STAT3 knockdown was also shown to inhibit expression of its known transcriptional target, VEGF (6, 41). In stable STAT3 knockdown cells, an enhanced basal caspase-3 cleavage was observed compared with control knockdown cells. In addition, overexpression of CA-STAT3 was shown to attenuate sorafenib-mediated Mcl-1 downregulation and to potently inhibit caspase-3 cleavage. Although sorafenib was shown to inhibit STAT3 activation and to downregulate Mcl-1, STAT3-independent pathways may also contribute to its proapoptotic effect. In this regard, other mechanisms of sorafenib-induced Mcl-1 downregulation have been reported and include alterations in NF-κB-mediated transcription, inhibition of eIF4E-associated translation, and accelerated proteosomal degradation (16, 17, 22). Sorafenib inhibited p-ERK, but not p-AKT, expression consistent with its ability to block Ras/Raf/MEK/ERK activation (11, 12). Both ERK and Src/Jak signaling have been shown to activate STAT3 (6). Other studies have also shown that sorafenib or sunitinib can reduce cellular levels of p-STAT3 Tyr^{705} in cancers of the bile duct (43) and kidney (44) as well as in medulloblastoma cells (45). Together, our data indicate that sorafenib induces apoptosis and inhibits Mcl-1 through blockade of the

Figure 5. BH3-only Bim protein mediates sorafenib and/or TRAIL-induced apoptosis. A, BxPC-3 cells were treated with vehicle or sorafenib (16 μmol/L) for 24 h and WCLs were probed for the indicated proteins by Western blotting. B, BxPC-3 cells were transduced with shRNA to Bim or control and the WCL was analyzed for Bim proteins by Western blotting (inset). Cells with Bim knockdown or control shRNA were then incubated with TRAIL for 48 h, and cell viability was determined using the MTS assay. Columns, mean of experiments conducted in triplicate; bars, SD. C, BxPC-3 cells were pretreated with sorafenib (8 μmol/L; 12 h) alone and in combination with TRAIL (2.5 ng/mL; 2 h). Caspase activation was analyzed in WCL by Western blotting. In addition, a treatment-induced Bak or Bax conformational change was analyzed by immunoprecipitation (IP) using conformation-specific antibodies. β-Tubulin was used as a control for protein loading.
STAT3 signaling pathway in human pancreatic cancer cells.

Suppression of p-STAT3 and Mcl-1 by sorafenib enhanced TRAIL-induced apoptosis that was associated with Bid truncation and release of mitochondrial cytochrome c and AIF, consistent with cross-talk between the death receptor-mediated and mitochondrial apoptotic pathways. STAT3 was shown to negatively regulate TRAIL-mediated apoptosis in that STAT3 knockdown or a DN STAT3 sensitized cells to TRAIL-induced caspase-3 cleavage. In contrast, a CA-STAT3 attenuated caspase-3 cleavage induced by sorafenib alone and combined with TRAIL. Given that STAT3 can downregulate Mcl-1, we confirmed the prosurvival role of Mcl-1 in our pancreatic cancer cell lines. Specifically, cells with Mcl-1 knockdown showed increased TRAIL-mediated apoptosis in accordance with prior studies by our laboratory and others showing that ectopic Mcl-1 or Bcl-2 expression can confer TRAIL resistance (28–31). Sorafenib potentiated a TRAIL-mediated Bak, but not Bax, conformational change consistent with its activation. Similarly, the combination of sorafenib and TRAIL activated Bak in acute myelogenous leukemia cells (23). Mcl-1 has been shown to preferentially inhibit Bak versus Bax activation (24), and this finding along with the release of Bak from Mcl-1 may be important contributors to the ability of sorafenib to enhance TRAIL-mediated apoptosis. Sorafenib has been shown to enhance TRAIL-mediated apoptosis in other tumor cell types in association with the suppression of Mcl-1, NF-κB (22), or c-FLIP (24), indicating that sorafenib exerts its antitumor effects via multiple mechanisms.

Studies in human leukemia cells have shown that sorafenib can induce Bim expression and that Bim can regulate sorafenib-mediated apoptosis (20). Bim can mediate a mitochondrial apoptotic response when Mcl-1 is suppressed (46). We examined the ability of Bim to regulate apoptosis induction by sorafenib plus TRAIL. In contrast to leukemic cells, sorafenib did not modulate Bim expression in pancreatic cancer cells. In cells treated with sorafenib and/or TRAIL, Bim knockdown attenuated caspase cleavage and reduced Bak/Bax activation, indicating that apoptosis induction by sorafenib plus TRAIL is partially dependent on Bim. Bim may play a key role in Bak/Bax activation given that Bim, tbid, or Bad can displace the mitochondrial outer membranes protein VDAC2 from Bak to enable its homooligomerization and resultant apoptosis (47).

In conclusion, sorafenib treatment can suppress STAT3 phosphorylation at Tyr705 and inhibit STAT3-regulated Mcl-1 and Bcl-xL expression to enhance TRAIL-mediated apoptosis in pancreatic cancer cell lines. These findings show that targeting STAT3 using sorafenib, or potentially with a STAT3 inhibitor, can enhance apoptotic susceptibility and suggest a strategy to increase therapeutic efficacy against pancreatic cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Terri Johnson and Jonelle Morales for their very capable secretarial assistance. We appreciate the input of Dr. S.K. Srivastava (Texas Tech University, Amarillo, TX) regarding CA-STAT3 expression.

Grant Support

Pilot Award from the Mayo Clinic Pancreatic Cancer Specialized Programs of Research Excellence (NCl P50 CA10270) and a Fraternal Order of Eagles Foundation Award (both to F.A. Sinicrope). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 10/28/2009; revised 12/30/2009; accepted 01/19/2010; published OnlineFirst 03/02/2010.

References


23. Meng XW, Lee SH, Dai H, et al. Mcl-1 as a buffer for proapoptotic Bcl-2 family members during TRAIL-induced apoptosis: a mecha-
nistic basis for sorafenib (Bay 43-9006)-induced TRAIL sensitiza-

24. Rosato RR, Almenara JA, Cee S, Grant S. The multikinase inhibitor sorafenib potentiates TRAIL lethality in human leukemia cells in asso-


27. Scalfi C, Fulda S, Brinivasan A, et al. Two CD96 (APO-1/Fas) signal-


29. Wang X, Chen W, Zeng W, et al. Akt-mediated eminent expression of c-FLIP and Mcl-1 confers acquired resistance to TRAIL-


33. Lin X, Morgan-Lappe S, Huang X, et al. “Seed” analysis of off-

34. Huang S, Sinicrope FA. BH3 mimetic ABT-737 potentiates TRAIL-
mediated apoptotic signaling by unsequestering Bim and Bak in hu-

35. Ling X, Arlinghaus RB. Knockdown of STAT3 expression by RNA inter-


37. Behrend L, Mohr A, Dick T, Zwicka RM. Manganese superoxide dis-

38. Buetner R, Mora LB, Jove R. Activated STAT signaling in human tu-


43. Blechacz BR, Smoot RL, Bronk SF, Werneburg NW, Sirica AE, Gores GJ. Sorafenib inhibits signal transducer and activator of transcription-3 signaling in cholangiocarcinoma cells by activating the phos-

44. Xin H, Zhang C, Herrmann A, Du Y, Figlin R, Yu H. Sunitinib inhibition of Stat3 induces renal cell carcinoma tumor cell apoptosis and re-


Molecular Cancer Therapeutics

Sorafenib Inhibits STAT3 Activation to Enhance TRAIL-Mediated Apoptosis in Human Pancreatic Cancer Cells

Shengbing Huang and Frank A. Sinicrope

*Mol Cancer Ther* 2010;9:742-750. Published OnlineFirst March 9, 2010.

Updated version

Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-09-1004

Cited articles

This article cites 46 articles, 24 of which you can access for free at:
http://mct.aacrjournals.org/content/9/3/742.full.html#ref-list-1

Citing articles

This article has been cited by 11 HighWire-hosted articles. Access the articles at:
/content/9/3/742.full.html#related-urls

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.