Src inhibition enhances paclitaxel cytotoxicity in ovarian cancer cells by caspase-9-independent activation of caspase-3

Ting Chen, Yolande Pengetnze, and Christopher C. Taylor

Department of Cell Biology, Vincent T. Lombardi Comprehensive Cancer Center, Georgetown University School of Medicine, Washington, District of Columbia

Abstract
Src tyrosine kinase has been found to be overexpressed and activated in a high proportion of ovarian cancers and ovarian cancer cell lines. Furthermore, Src activation is associated with activation of growth and survival signaling pathways. The present study was conducted in order to determine the effects of Src inhibition on ovarian cancer cell survival in response to chemotherapeutic agents. Inhibition of Src, either pharmacologically or through expression of a Src dominant-negative fusion construct, enhanced the cytotoxicity of two different classes of chemotherapeutics: paclitaxel and cisplatinum, in both mouse and human ovarian cancer cells. Interestingly, Src inhibition also restored sensitivity to drug-resistant ovarian cancer cells. The increased cytotoxicity in response to Src inhibition was associated with a large increase in processing and activation of caspase-3. The activation of caspase-3 seems to be independent of cytochrome c release and caspase-9 activation. The present study indicates that Src tyrosine kinase may provide an important target for small molecule inhibition in ovarian cancer. [Mol Cancer Ther 2005;4(2):217–24]

Introduction
In the United States, ovarian cancer accounts for nearly 15,000 deaths per year (1). The prognosis for ovarian cancer is generally very poor with high mortality rates. This is largely the result of late presentation of patients due to clinically silent symptoms until disseminated and metastatic disease has been well established. Following tumor debulking, patients generally receive chemotherapy treatment with paclitaxel, platinum-based agents, or a combination of both (2, 3). These agents act via different mechanisms. Paclitaxel binds tubulin, enhancing tubulin polymerization and inhibiting microtubule disassembly, thus preventing completion of mitosis and producing a G2-M block (4, 5). Cisplatinum, in contrast, binds DNA causing DNA adducts, preventing DNA replication and elongation, inducing cell cycle arrest, and apoptosis (6, 7). Unfortunately, even in patients in whom there has been an initial positive clinical response to chemotherapy, development of recurrent chemoresistant disease is a common outcome (8). The development of multidrug resistance is multifactorial, but probably includes the activation of growth factor signal transduction cascades (9).

Src tyrosine kinase is the prototypical member of a family of cytoplasmic, membrane-associated, nonreceptor tyrosine kinases. Cellular Src is expressed in a wide variety of cell types, including several ovarian cancer cell lines (10), and has recently been identified to be overexpressed and activated in a high proportion of ovarian cancers examined (11). Cellular Src is involved in a variety of cell signaling events, regulating both cell proliferation (12, 13) and differentiation (14). We have recently described the expression and signaling of cellular Src in mouse and human ovarian cancer cell lines (15). Inhibition of Src was associated with decreased activation of cell growth (Ras-Raf-MEK-ERK) and survival (PI3-kinase-Akt-FOXO1) pathways. Furthermore, cellular Src inhibition in ID8 mouse ovarian cancer cells was found to enhance the cytotoxicity of paclitaxel and restore sensitivity in a paclitaxel-resistant mouse ovarian cancer cell line (15). The cell death pathways activated by paclitaxel in at least one ovarian cancer cell line has been reported to be caspase-9- and caspase-3-independent (16). In the current study, we investigated the cell death pathways activated by paclitaxel alone or in combination with Src inhibition. We find that enhancement of paclitaxel cytotoxicity by Src inhibition is mediated by markedly increased caspase-3 activity. Caspase-3 activation seems to be caspase-9-independent.

Materials and Methods
Reagents
All liquid media and LipofectAMINE 2000 transfection reagent were purchased from Invitrogen Life Technologies (Carlsbad, CA). Mitotracker red and 4',6-diamidino-2-phenylindole dihydrochloride were from Molecular Probes.
Cells were permeabilized with 0.1% NP40 in PBS for 20 minutes at room temperature. Coverslips were blocked and to serve as RNA interference controls.

Relative Cell Viability
Relative viable cell number was determined as previously described (18) by incubation of cells with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and measuring the production of formazan at an absorbance of 570 nm, which occurs only in the mitochondria of viable cells. Briefly, cells were seeded at 5,000 cells per well in 96-well plates and allowed to attach overnight. After the treatment period, cells were collected and 24 hours later, cells were collected in lysis buffer [50 mmol PIPES/NaOH (pH 8.5), 2 mmol EDTA, 0.1% CHAPS, 5 mmol DTT, 10 μg/mL of leupeptin, 10 μg/mL pepstatin, 10 μg/mL aprotinin, and 1 mmol phenylmethylsulfonyl fluoride]. Resuspended cells were frozen and thawed thrice to disrupt cell membranes. Insoluble material was cleared by centrifugation (14,000 × g for 20 minutes at 4°C). Protein concentration was determined by a modified Bradford protein assay and equalized across all samples. Soluble protein was mixed with an equal volume of 2× Laemmli sample buffer. Samples were heated to 95°C for 5 minutes and then subjected to SDS-PAGE. Protein was electrotransferred to polyvinylidene difluoride membranes. Membranes were blocked with TBST-5% milk [10 mmol Tris-HCl (pH 8.0), 150 mmol NaCl, 0.05% Tween 20, and 5% nonfat dry milk] for 1 hour at room temperature prior to overnight incubation with the appropriate specific antibody in TBST-5% milk (1:1,000 dilution) at 4°C. Membranes were washed extensively with TBST, incubated with the appropriate peroxidase-conjugated secondary antibody in TBST-5% milk for 1 hour and then washed with TBST. Proteins were visualized by enhanced chemiluminescence. For some experiments, bands were quantified by densitometric analysis using the NIH Image J software.

Cell Transfections and RNA Interference
Generation of a Src dominant-negative-EGFP fusion construct has been previously described (15). ID8 cells were transfected with expression vectors (Src dominant-negative-EGFP fusion construct or EGFP) with LipofectAMINE 2000 transfection reagent (Invitrogen Life Technologies) using the manufacturer’s protocol. For caspase-9 RNA interference, a pool of four different duplexes (SMART-pool, Dharmacon) targeted against caspase-9 was used. Twenty-four hours after plating, cells were transfected with RNA duplexes using LipofectAMINE 2000 transfection reagent. Twenty-four hours after transfection, treatments were initiated and 24 hours later, cells were collected in lysis buffer for subsequent analysis of protein expression. ID8 cells expressing EGFP were transfected with RNA duplexes against EGFP for determination of transfection efficiency and to serve as RNA interference controls.

Flow Cytometry
ID8 and ID8Src6 mouse ovarian cancer cells were treated as indicated in Results and collected by mild
trypsin digest. Cell number was determined and then adjusted to 1 to 2 x 10^6 cells per treatment group, washed in PBS and then resuspended in 0.1 mL of citrate/DMSO buffer. DNA cell cycle phase analysis was done by the Lombardi Comprehensive Cancer Center Flow Cytometry and Cell Sorting Shared Resource using a Becton Dickinson FACStar Plus dual laser system.

**Results**

**Inhibition of Src Enhances Cytotoxicity of Paclitaxel**

Because Src is involved in the activation of survival pathways, the effect of Src inhibition in combination with paclitaxel was investigated. Src inhibition with the Src-selective inhibitor PP2 enhanced the cytotoxic effects of paclitaxel in both mouse (ID8) and human (SKOV3 and CaOV3) ovarian cancer cell lines across a range of concentrations (Fig. 1A). Two other Src-selective inhibitors (SU6656 and herbimycin A) had similar effects (data not shown). In order to determine whether the effects of these pharmacologic inhibitors were a direct result of Src inhibition, ID8 mouse ovarian cancer cells were transfected with a Src dominant-negative EGFP fusion construct. These cells were used due to their high transfection efficiency. Cytotoxicity resulting from paclitaxel treatment was significantly greater in ID8 cells expressing the Src dominant-negative fusion construct compared with mock-transfected cells or cells transfected with EGFP (Fig. 1B), indicating that the increased cytotoxicity can be attributed to Src inhibition.

The human SKOV3 ovarian cancer cell line was less sensitive to paclitaxel than the CaOV3 human and ID8 mouse ovarian cancer cell lines. Interestingly, pharmacologic inhibition of Src with PP2 sensitized SKOV3 cells to paclitaxel (>10-fold increase; Fig. 1A). SKOV3 cells have been reported to be paclitaxel-resistant (19, 20). As previously reported (15), an ID8 paclitaxel-resistant cell line has been developed (ID8TaxR). These cells are completely resistant to up to 2 μmol/L paclitaxel. In addition, the ID8TaxR cells have cross-resistance to cisplatin. Inhibition of Src with either PP2 or SU6656 resensitizes ID8TaxR cells to both paclitaxel and cisplatin (Fig. 2A). A recently derived paclitaxel-resistant human CaOV3 cell line showed similar results (data not shown). Expression of the Src dominant-negative fusion construct also resensitizes ID8TaxR cells to paclitaxel, confirming the specificity of Src inhibition in the resensitization (Fig. 2B). Thus, Src inhibition seems to enhance the cytotoxicity of paclitaxel in both drug-sensitive and drug-resistant ovarian cancer cells.

**Src Inhibition and Paclitaxel Combinatorial Treatment Enhances Caspase-3 Activation**

As a first step in determining the mechanism by which Src inhibition enhances paclitaxel cytotoxicity, ID8 mouse ovarian cancer cells were exposed to paclitaxel (0.2 μmol/L), PP2 (10 μmol/L), or both overnight, after which caspase-3 processing was determined by immunoblot analysis. Treatment with either paclitaxel or PP2 alone resulted in little or no detectable cleaved caspase-3. In contrast, combination treatment resulted in accumulation of cleaved/processed caspase-3. Interestingly, similar results were obtained with colchicine (Fig. 3A), a microtubule-disrupting agent, indicating that Src inhibition sensitizes cells to the G2-M arrest rather than microtubule stabilization per se, or some other paclitaxel-specific effect. A time course experiment showed that cleaved caspase-3 began to accumulate by 12 hours of paclitaxel plus PP2 combination treatment, with even greater accumulation at 18 hours (Fig. 3B). Interestingly, paclitaxel alone induced caspase-3 processing and accumulation of cleaved caspase-3 at 18 hours of treatment, although accumulation was not as great as the combination treatment. The dose-response showed that paclitaxel plus PP2 combination treatment induced ~3- to 4-fold greater accumulation of cleaved caspase-3 compared with paclitaxel alone at all concentrations tested (Fig. 3C and D). Finally, combination treatment also resulted in increased cleaved poly-ADP ribose polymerase, a substrate of activated caspase-3 and marker of apoptosis, compared with paclitaxel alone (Fig. 3E).

**Caspase-3 Activation Is Caspase-9-Independent**

Caspase-3 can be activated by either intrinsic or extrinsic apoptotic stimuli. It seemed logical that combination treatment of Src inhibition and paclitaxel would activate...
caspase-3 by an intrinsic mechanism, via activation of caspase-9. Interestingly, treatment of ID8 mouse ovarian cancer cells with paclitaxel, PP2, colchicine, combinations of paclitaxel plus PP2, or colchicine plus PP2 all failed to consistently result in processing of caspase-9 as determined by immunoblot analysis (Fig. 4A). Even high concentrations of paclitaxel plus PP2, which induced large accumulations of cleaved caspase-3, did not result in processing of caspase-9 (Fig. 4B). Furthermore, ID8 cell lysates from combinatorial treated cells showed considerable caspase-3 activity compared with controls as determined by in vitro caspase-3 activity assays. In contrast, caspase-9 activity showed little change in response to treatments (Fig. 4C).

Pro-caspase-9 has a relatively high basal activity. In addition, in some experiments, some minor processing of caspase-9 was observed (see faint band in colchicine + PP2 treated cells in Fig. 4A), although this observation was not consistent. In order to rule out a role for caspase-9, several different approaches were undertaken. Caspase-9 is activated as a result of mitochondrial cytochrome c release. Immunoblot analysis for cytochrome c failed to show any increase in cytosolic cytochrome c resulting from any of the treatments (Fig. 5A). Furthermore, laser scanning confocal microscopy was utilized in order to colocalize cytochrome c and mitochondria. Functional mitochondria were labeled with a MitoTracker red dye. Cytochrome c localization was determined by immunofluorescence with a cytochrome c-specific antibody. Cytochrome c colocalized with mitochondria in both control and paclitaxel plus PP2 treated cells (Fig. 5B). In contrast, H2O2 treatment was associated with a dramatic decrease in functional mitochondria and release of cytochrome c to the cytosol.

Finally, small interfering RNA directed against caspase-9 effectively knocked-down caspase-9 protein expression with no concomitant decrease in caspase-3 processing in paclitaxel plus PP2 treated ID8 mouse ovarian cancer cells (Fig. 6). These results, taken in toto, suggest that mitochondrial release of cytochrome c and caspase-9 activation is not involved in caspase-3 activation in response to paclitaxel plus PP2.

Resensitization of Paclitaxel-Resistant Cells Also Involves Caspase-3

Microtubule disrupting agents such as paclitaxel and colchicine induce cell cycle arrest, which ultimately results in apoptosis. Paclitaxel-resistant cells presumably fail to respond to paclitaxel with cell cycle arrest. In order to determine whether the resensitization of ID8TaxR cells was the result of a restoration of cell cycle arrest in response to paclitaxel, cell cycle analysis was done under various treatment conditions. ID8 paclitaxel-sensitive ovarian cancer cells treated with paclitaxel (1 μmol/L) showed a dramatic decrease in G1 and massive apoptosis (Fig. 7A). In contrast, ID8TaxR cells treated with paclitaxel were completely unaffected by paclitaxel in comparison to control ID8TaxR cells (Fig. 7B). Treatment of ID8TaxR cells with the Src inhibitor PP2 (10 μmol/L) plus paclitaxel...
showed a decrease in the proportion of cells in G1 and massive apoptosis (Fig. 7B). Even more dramatically, treatment with colchicine (1 μmol/L) induced a large accumulation of ID8 cells in G2 and extensive apoptosis (Fig. 7A); however, a 5-fold increase in colchicine (5 μmol/L) had little effect on ID8TaxR cells (Fig. 7B). Src inhibition restored the accumulation of ID8TaxR cells in G2 and apoptosis in response to colchicine (Fig. 7B).

As outlined above, the human SKOV3 ovarian cancer cell line is relatively resistant to paclitaxel. It has also recently been reported that paclitaxel-induced cytotoxicity in SKOV3 cells is both caspase-9- and 3-independent (16). We have also produced CaOV3 human ovarian cancer cells that are completely resistant to paclitaxel (CaOV3TaxR cells) in a manner similar to ID8TaxR cells. Treatment of ID8TaxR and CaOV3TaxR (Fig. 7C) cells with paclitaxel alone was associated with little or no accumulation of cleaved caspase-3. However, treatment with paclitaxel plus pharmacologic inhibition of Src tyrosine kinase resulted in significant accumulation of cleaved caspase-3 in both resistant cell types. Similar results were observed with several different Src-specific inhibitors (PP2, SU6656; data not shown). Again, this was independent of caspase-9 processing (data not shown). Interestingly, treatment of SKOV3 cells with paclitaxel alone resulted in significant cleavage of caspase-3 (Fig. 7C), even at relatively lower concentrations (0.2 μmol/L), contrary to previous reports. Paclitaxel plus pharmacologic Src inhibition did increase the accumulation of cleaved caspase-3.

**Discussion**

Ovarian cancer is the leading gynecologic cause of death and the fifth leading cause of cancer deaths in women (1). Standard therapy includes tumor debulking followed by chemotherapy treatment with paclitaxel, platinum-based agents, or combinations of both (21). Unfortunately, the success of these treatment regimens in advanced disseminated disease is relatively poor, with 5-year survival rates <30% (1). Furthermore, development of recurrent drug-resistant disease is a common outcome in ovarian cancer. The data presented here shows that inhibition of Src tyrosine kinase, a proto-oncoprotein commonly overexpressed and constitutively activated in ovarian cancer and ovarian cancer cell lines (10, 11, 15), enhances the cytotoxicity of paclitaxel. Perhaps even more importantly, inhibition of Src tyrosine kinase resensitizes drug-resistant ovarian cancer cells to both paclitaxel and cisplatin. The question remains somewhat open concerning the Src family kinases involved in the resensitization. However, most evidence suggests that it is Src tyrosine kinase itself; expression of a Src dominant-negative or Src knockdown by RNA interference recapitulate the effects seen with pharmacologic Src kinase inhibitors (15).

The enhancement of cytotoxicity in paclitaxel-sensitive (ID8 and CaOV3), partially resistant (SkOV3), and resistant (ID8TaxR and CaOV3TaxR) cells seems to involve activation of caspase-3. Combination treatment of Src inhibition and paclitaxel resulted in a large accumulation of processed caspase-3, cleaved poly-ADP ribose polymerase, and increased caspase-3 activity. Interestingly, Src inhibition alone seems to induce some processing of caspase-3 in ID8, ID8TaxR, and Skov3 cells (see Figs. 6 and 7), suggesting that Src inhibition alone promotes some caspase-3 activation. Src inhibition has been linked to enhanced apoptosis under various different apoptotic stimuli (22–25).

---

**Figure 3.** Src inhibition and paclitaxel combination treatment induces processing of procaspase-3. ID8 mouse ovarian cancer cells were treated overnight with paclitaxel (P, 0.2 μmol/L), colchicine (Co, 10 μmol/L), the Src inhibitor PP2 (Si, 10 μmol/L), or the indicated combinations (A). Time course experiments were also done with paclitaxel (0.2 μmol/L), PP2 (10 μmol/L), or both for the indicated periods of time (B) and a dose-response to paclitaxel with or without PP2 (10 μmol/L) for 24 h was done (C). Processing and caspase-3 cleavage was determined by immunoblot analysis with an antibody that detects both procaspase-3 and cleaved/processed caspase-3 (A–C). Accumulation of cleaved caspase-3 was quantified by densitometric analysis and the dose-response presented in graphical form (D); numbers above bars (B) indicate relative densitometric values within a time period. Processing and poly-ADP ribose polymerase cleavage was determined by immunoblot analysis with an antibody that detects both intact and cleaved poly-ADP ribose polymerase (E). Blots are from representative experiments. Experiments were repeated at least thrice to ensure consistency of results.
Caspase-3 is a distal executioner caspase that can be activated by either an intrinsic mitochondrial-cytochrome c-caspase-9 activated pathway or via extrinsic ligand (e.g., Fas ligand) activated signals. At this point, the mechanism by which caspase-3 is activated in response to Src inhibition-paclitaxel treatment is not clear. It does not seem to include a mitochondrial-caspase-9–activated pathway, as there was no mitochondrial release of cytochrome c and caspase-9 processing and activity was minimal. Most convincingly, caspase-3 was activated in response to combination treatment even in cells in which caspase-9 expression had been knocked down by RNA interference. Some caspase-9 processing was observed in some experiments, however, this was an inconsistent observation and the cleaved product largely seemed to be the 39 kDa species, possibly the result of caspase-3-mediated cleavage of caspase-9 (26).

We have previously reported that Src inhibition is associated with decreased phosphorylation of Akt and the forkhead transcription factor, FOXO1 (15). Dephosphorylation of forkhead transcription factors allows for their translocation to the nucleus where they activate proapoptotic genes such as Fas ligand (27), resulting in activation of an autocrine extrinsic apoptotic pathway. The lack of activation of a mitochondrial-caspase-9 intrinsic apoptotic pathway in response to Src inhibition and paclitaxel combinatorial treatment might suggest that caspase-3 activation is the result of an autocrine extrinsic death signal, resulting in activation of an initiating caspase, such as caspase-8 or caspase-10. We have tried to identify the possible upstream caspase-3-activating caspases with specific caspase inhibitors; unfortunately, low concentrations were ineffective and higher concentrations have proven to be highly toxic in all of the cell lines we tested. However, a neutralizing antibody against Fas ligand failed to block the increased cytotoxicity and caspase-3 activation. Furthermore, no caspase-8 processing was detected (data not shown). Thus we have no compelling evidence for an autocrine loop activating an extrinsic apoptotic pathway.

**Figure 4.** Caspase-9 is not processed or activated in response to combination treatment. A, ID8 mouse ovarian cancer cells were treated overnight with paclitaxel (P, 0.2 μmol/L), colchicine (Co, 10 μmol/L), the Src inhibitor PP2 (Si, 10 μmol/L), or the indicated combinations. B, a dose-response to paclitaxel was done with or without PP2 (10 μmol/L) for 24 h. Caspase-9 processing was determined by immunoblot analysis with an antibody that detects both procaspase-9 and cleaved/processed caspase-9. C, caspase-3 and caspase-9 activation was determined in cell lysates by *in vitro* caspase activity assays.

**Figure 5.** Mitochondrial cytochrome c is not released in response to paclitaxel and Src inhibition combination treatment. ID8 mouse ovarian cancer cells were treated with paclitaxel (P, 1 μmol/L), the Src inhibitor PP2 (Si, 10 μmol/L), or both for 24 h. A, cytosolic cytochrome c was determined by immunoblot analysis. B, colocalization of cytochrome c and mitochondria was determined by confocal microscopy. At the end of the treatment period, functional mitochondria were labeled with MitoTracker red, followed by fixation of cells and immunofluorescence with an antibody specific for cytochrome c and a FITC-tagged secondary antibody (green). H2O2 (500 μmol/L)–treated cells served as a positive control for mitochondrial dysfunction and cytochrome c release. Note maintained colocalization of cytochrome c and mitochondria but fragmented nuclei (indicating apoptosis) in paclitaxel/PP2-treated cells.
Caspase-12, a caspase activated as a result of Ca^{2+} release from the endoplasmic reticulum (28, 29) can likewise be ruled out. Combinatorial treatment in fact decreased processed caspase-12 in comparison to control cells (data not shown).

It is possible that caspase-3 is being activated by a more direct route, perhaps by blocking the inhibition of an inhibitor of apoptosis. Inhibitors of apoptosis such as XIAP (30, 31) and survivin (32) have been implicated in protection from apoptotic stimuli in ovarian cancers cells. ID8 mouse ovarian cancer cells constitutively express high levels of survivin. Src or Akt inhibition is associated with decreased survivin expression (our observations). A recent report showed that SkOV3 cells have dysfunctional apoptosome activity which could not be explained by caspase-9 and APAF-1 or by expression of inhibitors of apoptosis, such as XIAP (33). However, XIAP was only examined in the context of apoptosome-caspase-9 activity. In the current study, combination treatment seems to bypass apoptosome formation and caspase-9 activation. It is possible that an inhibitor of apoptosis such as XIAP or survivin may act directly on caspase-3 (34), and that combination treatment interferes with this interaction or inhibition, promoting autoactivation of caspase-3. Further studies are required to test this possibility.

An important aspect of the present study is the demonstration that Src inhibition resensitizes drug-resistant CaOV3 TaxR and ID8 TaxR ovarian cancer cells to two different classes of chemotherapeutics, i.e. paclitaxel and cisplatinum. The mechanism of this resensitization is as yet unknown, however, it seems to be independent of multidrug resistance protein Mdr1 expression or function (our observations). The finding that Src inhibition and paclitaxel treatment results in caspase-3 processing in CaOV3TaxR and ID8TaxR cells, as well as partially resistant SkOV3 cells in a manner similar to that of sensitive cells suggests the resistance is proximal to caspase-3. Our results with SkOV3 cells are somewhat in conflict with a previous report demonstrating SkOV3 cell death to be independent of caspase-3 activity. However, other studies have shown a requirement for caspase-3 in adenoviral

Figure 6. Caspase-9 knockdown does not prevent caspase-3 processing in response to paclitaxel and Src inhibition combination treatment. ID8 cells were transfected with EGFP plasmid in combination with small interfering RNA specific for either caspase-9 or EGFP. Twenty-four hours posttransfection, cells were treated with paclitaxel (P, 1 μmol/L), the Src inhibitor PP2 (Si, 10 μmol/L), or both for a further 24 h. Cells were then lysed and caspase-9(A) and caspase-3(B) expression and processing was determined by immunoblot analysis. β-Actin expression was used as a loading control. Note cleaved caspase-3 even in cells in which caspase-9 has been efficiently knocked down.

Figure 7. Src inhibition results in cell cycle arrest, apoptosis, and processing of procaspase-3 in paclitaxel-resistant cells treated with paclitaxel. ID8 cells (A) or ID8TaxR cells (B) were treated with paclitaxel (1 and 5 μmol/L for ID8 and ID8TaxR cells, respectively), colchicine (1 and 5 μmol/L for ID8 and ID8TaxR cells, respectively), PP2 (10 μmol/L) or both paclitaxel and PP2 or colchicine and PP2 for 24 h. Cells were harvested and cell cycle analysis done by flow cytometry [A, B]. Shaded areas, sub-G1 and G0 phase apoptotic DNA. In separate experiments, mouse ID8TaxR, human CaOV3TaxR, and SkOV3 ovarian cancer cells were treated with paclitaxel (P, 2 μmol/L for ID8TaxR and CaOV3TaxR cells and 200 nmol/L for SkOV3 cells), Src inhibitor (Si, 10 μmol/L) or both for 24 h. At the end of the treatment period, cells were lysed and caspase-3 protein determined by immunoblot analysis. Numbers above bands indicate relative densitometric values (C).
Src Inhibition Enhances Paclitaxel Cytotoxicity

type 5 E1A-enhanced paclitaxel cytotoxicity in SkOV3 cells (19). The reasons for this discrepancy may involve several factors; different lots or passage numbers of the original SkOV3 cell line may contribute to the difference. The present study also used a higher concentration of paclitaxel (200 nmol/L), thus we believe caspase-3 to be activated in SkOV3 cells treated with paclitaxel.

In summary, we show that inhibition of Src tyrosine kinase enhances the cytotoxicity of chemotherapeutic agents such as paclitaxel and cisplatinum in drug-sensitive ovarian cancer cells and restores sensitivity in drug-resistant cells. The increased cytotoxicity seems to be the result of a caspase-9-independent increase in caspase-3 processing and activity. These results suggest that Src family kinase inhibitors may be useful agents in the treatment of drug-resistant ovarian cancers.

References

Molecular Cancer Therapeutics

Src inhibition enhances paclitaxel cytotoxicity in ovarian cancer cells by caspase-9-independent activation of caspase-3

Ting Chen, Yolande Pengetnze and Christopher C. Taylor


Updated version  Access the most recent version of this article at: http://mct.aacrjournals.org/content/4/2/217

Cited articles  This article cites 33 articles, 9 of which you can access for free at: http://mct.aacrjournals.org/content/4/2/217.full#ref-list-1

Citing articles  This article has been cited by 10 HighWire-hosted articles. Access the articles at: http://mct.aacrjournals.org/content/4/2/217.full#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.