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Cross-Talk between Integrins and Oncogenes Modulates Chemosensitivity

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ABSTRACT

Chemotherapy often relies on cancer cell death resulting from DNA damage. The p53 tumor suppressor pathway that is an important player in DNA damage response is frequently inactivated in cancer. Genotoxicants also activate DNA damage-independent stress pathways and activity of oncogenic signaling and adhesive interactions with the cancer microenvironment can have a strong impact on chemosensitivity. Here, we have investigated how two different oncogenes modulate the response to genotoxicants in the context of two classes of integrin adhesion receptors. Epithelial cells expressing either β1 or β3 integrins, in which p53 activity is suppressed, undergo G_2 arrest but show little apoptosis after treatment with cisplatin or other genotoxicants. The apoptotic response is strongly enhanced by the c-Src[Y530F] oncogene in cells expressing β1 integrins, whereas such sensitization is reduced when these cells are engineered to express β3 integrins instead. The H-Ras[G12V] oncogene fails to sensitize, regardless of the integrin expression profile. The enhanced sensitivity induced by c-Src[Y530F] in the context of β1 integrins does not rely on p53-mediated DNA damage signaling but instead involves increased endoplasmic reticulum stress and caspase-3 activation. Our data implicate that the expression profiles of oncogenes and integrins strongly affect the response to chemotherapeutics and may thus determine the efficacy of chemotherapy.

Most chemotherapeutic treatments rely on cancer cell death in response to DNA damage, although many genotoxic compounds also activate stress pathways independently of DNA damage, for example by inducing reactive oxygen species or binding to proteins within cells. In general, the induction of DNA damage by genotoxic agents triggers apoptosis initiated by DNA checkpoint proteins such as ataxia telangiectasia mutated (ATM), ataxia telangiectasia mutated and Rad3-related (ATR), and p53 (Roos and Kaina, 2006). However, the majority of cancers harbor (epi)genetic changes that inactivate the Rb and p53 tumor suppressor pathways. Loss of p53 may suppress the apoptotic response to DNA damage and cause resistance to therapy. Conversely, accelerated cell cycle progression and compromised repair in the absence of Rb and p53 may lead to accumulation of DNA damage, causing sensitization to therapy. The final outcome of treatment with genotoxicants will also be determined to a large extent by other oncogenic pathways present in the tumor cells as well as by interactions with the cancer microenvironment.

The cancer microenvironment, including the extracellular matrix (ECM), is known to critically modulate the apoptotic response to treatment with genotoxic compounds (Bissell and Radisky, 2001; Morin, 2003). For example, ovarian cancer cells were shown to be able to remodel the ECM, thereby favoring survival in the presence of the genotoxic compound cisplatin (Sherman-Baust et al., 2003). In addition, increased deposition of the ECM proteins fibronectin, collagen IV, and laminin was shown to confer resistance of small cell lung cancer to chemotherapeutic agents (Sethi et al., 1999). (Cancer) cells interact with the ECM through a family of receptors called integrins. These heterodimeric transmembrane receptors couple the ECM microenvironment to the cytoskeleton and are able to recruit multiple adaptor and signaling proteins to sites of adhesion (Hynes, 2002). Besides their essential role in cell adhesion, integrins are important for providing survival and proliferative signals through extensive cross

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ABBREVIATIONS: ECM, extracellular matrix; ER, endoplasmic reticulum; FACS, fluorescence-activated cell sorting; eIF2α, eukaryotic initiation factor 2α; z-VAD-fmk, z-Val-Ala-DL-Asp-fluoromethylketone; siRNA, small interfering RNA; DMSO, dimethyl sulfoxide; FITC, fluorescein isothiocyanate; ECL, enhanced chemiluminescence; DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; SID, substance accession identifier (PubChem database).

talk with growth factor receptors (Yamada and Even-Ram, 2002).

Changes in integrin expression levels are frequently associated with tumor growth and progression (Mizejewski, 1999), and it has been suggested that the tumor microenvironment directly modulates integrin signaling, thereby promoting malignancy (Paszek et al., 2005). It is noteworthy that changes in expression levels of certain integrins have also been reported to either promote or reduce sensitivity to genotoxic compounds (Cordes, 2006). Indeed, drugs targeting integrin function are considered promising adjuvants to sensitize tumor cells to chemotherapeutics (Damiano, 2002; Huveneers et al., 2007a). However, it remains largely unclear how such perturbations modulate drug sensitivity and how the pattern of mutations in tumor suppressors and oncogenes modulates these effects.

To address these issues, we made use of a panel of cell lines derived from poorly adhesive, integrin β1 knockout cells that were immortalized using simian virus 40 Large T (inactivation of p53 and Rb pathways). In the absence or presence of oncogenes (activated H-Ras or c-Src), we restored adhesion by either re-expressing β1 or by enhancing β3 expression to compensate for the absence of β1. When this panel of cell lines was subjected to treatment with a variety of DNA-damaging and non–DNA-damaging cytotoxicants, the apoptotic response to these compounds revealed a remarkably strong dependence on both the oncogene and the integrin profile. Our data indicate that activated c-Src but not H-Ras sensitizes cells to p53-independent, caspase-3-dependent, endoplasmic reticulum (ER) stress-related apoptosis, particularly in the context of β1 integrins. These results delineate a novel oncogene- and integrin-controlled signaling pathway that determines (cancer) cell sensitivity to chemotherapeutic agents.

Materials and Methods

Cell Lines, Plasmids, Antibodies, and Compounds. The β1-deficient GE11 cells were described previously (Gimond et al., 1999). Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics. LZRS bicistronic retroviral expression plasmids encoding Src^{YF}, Ras^{GV}, and human integrins β1 and β3 were described previously (Danen et al., 2002; Huveneers et al., 2007b). To ensure identical expression of Src^{YF} (c-Src[Y530F]) or Ras^{GV} (H-Ras[G12V]) in cell lines, we first generated $GESrc^{YF}$ and $GERas^{GV}$ single cell clones and subsequently expressed β1 or β3 integrin subunits using retroviral transduction and bulk sorting by FACS.

The following antibodies were used: mouse monoclonal anti-p53 (Pab240; Santa Cruz Biotechnology) and anti-α-tubulin (DM1A, Sigma); rabbit polyclonal anti-p21 (C-19, Santa Cruz Biotechnology, Santa Cruz, CA), anti-phospho-p53 (Ser15; Cell Signaling Technology Inc., Danvers, MA), anti-caspase-3 (Cell Signaling Technology Inc.), anti-phospho-Ser139-H2A.X (Cell Signaling Technology Inc.), and anti-phospho-Ser51-eIF2α (Cell Signaling Technology Inc.). Cisplatin [*cis*-diammineplatinum(II) dichloride, $Pt(NH_3)_2Cl_2$, SID: 24278632], etoposide (4-*O*-β-D-galactopyranoside, $C_{35}H_{42}O_{18}$), menadione (2-methyl-1,4-naphthoquinone sodium bisulfite, $C_{11}H_9NaO_5S$, SID: 24896741), mitomycin C ($C_{15}H_{18}N_4O_5$, SID: 24896554), and thapsigargin ($C_{34}H_{50}O_{12}$, SID: 24278762) were all purchased from Sigma-Aldrich (St. Louis, MO). Salubrinal (eIF2α inhibitor, $C_{21}H_{17}Cl_3N_4OS$, SID: 26758665) was from Calbiochem (San Diego, CA), vincristine (22-oxovincaleukoblastine sulfate salt, $C_{46}H_{56}N_4O_{10}{\cdot}H_2SO_4$, SID: 24278774) was from TEVA Pharmachemie (Utrecht, The Netherlands), and z-Val-Ala-DL-Asp-fluoromethylketone (z-VAD-fmk; $C_{21}H_{28}FN_3O_7$, SID: 26758420) was from Bachem (Bubendorf, Switzerland). *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC; $C_5H_7Cl_2NO_2S$, SID: 49865984) was synthesized as described previously (Hayden and Stevens, 1990). Compounds were diluted in complete medium and supplied to the cells as indicated in the text.

siRNA Transfections. Cells were plated at approximately 40% confluence and were transfected the following day using DharmaFECT 1 reagent (Dharmacon RNA Technologies, Lafayette, CO) and a final concentration of 50 nM p53 SMARTpool siRNA or siCONTROL nontargeting siRNA 2 purchased from Dharmacon RNA Technologies. Twenty-four hours after transfection, cells were replated and used for subsequent experiments.

Cell Cycle Analysis and Apoptosis Assay. Floating and trypsinized adherent cells were pooled and fixed in 80% ethanol overnight at −20°C. Cell pellets were subsequently washed with phosphate-buffered saline-EDTA (1 mM) twice and resuspended in phosphate-buffered saline-EDTA containing 7.5 μM propidium iodide and 40 μg/ml RNase A. After 45-min incubation at room temperature, the cell cycle was analyzed by flow cytometry on a FACSCalibur (BD Biosciences, San Jose, CA). Cell cycle analysis and the percentage of apoptotic cells were determined using CellQuest software (BD Biosciences).

Immunofluorescence. Cells were plated at approximately 40% confluence overnight, treated with cisplatin or dimethyl sulfoxide (DMSO, vehicle control, C_2H_6OS, SID: 67640) for various time points, and fixed with 4% paraformaldehyde. After permeabilization with 0.5% Triton X-100 and blocking with 1% bovine serum albumin, cells were stained for phosphorylated H2A.X followed by a FITC-labeled secondary antibody and analyzed by fluorescence microscopy.

Time-Lapse Imaging of Phosphatidyl-Serine Translocation and Caspase-3 Activation. Cells were plated in multiwell plates at approximately 40% confluence overnight, treated with cisplatin or DMSO control, and imaged 24 h in the presence of FITC-labeled Annexin-V (ImmunoTools, Friesoythe, Germany) to detect translocation of phosphatidyl-serine to the outer membrane leaflet or in the presence of the "Nucview" Alexa Fluor-488-labeled caspase-3 substrate (Biotium, Hayward, CA) to detect caspase-3 activation. Transmission and fluorescence imaging was performed on a "BD-Pathway" system (BD Biosciences) under temperature- and CO_2-controlled conditions.

Western Blot Analysis. For Westerns, total cell lysates were prepared in ice-cold TSE (10 mM Tris-HCl, 250 mM sucrose, 1 mM EGTA, pH 7.4, and protease inhibitors), sonicated, separated by SDS-polyacrylamide gel electrophoresis, and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA). Blots were blocked in 5% bovine serum albumin Tris-buffered saline/Tween 20 and incubated with primary and secondary antibodies, followed by ECL or ECL Plus reaction (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK). ECL signal was detected using films or a Typhoon 9400 scanner (GE Healthcare).

Results

β1 integrin-deficient GE11 cells were used to investigate the role of integrins in the cellular response to genotoxic agents. These cells adhere weakly, and we used retroviral transduction to restore adhesion by re-expressing β1 (GEβ1) or compensate for the absence of β1 by enhancing β3 expression (GEβ3) (Danen et al., 2002). Exposure of GEβ1 and GEβ3 cells to the DNA-damaging agents cisplatin, mitomycin C, etoposide, or doxorubicin caused a cell cycle arrest at the G_2 phase in both β1- and β3-expressing cell types (Fig. 1A, top two panels; data not shown). Induction of apoptosis was minimal after treatment with these genotoxic agents,

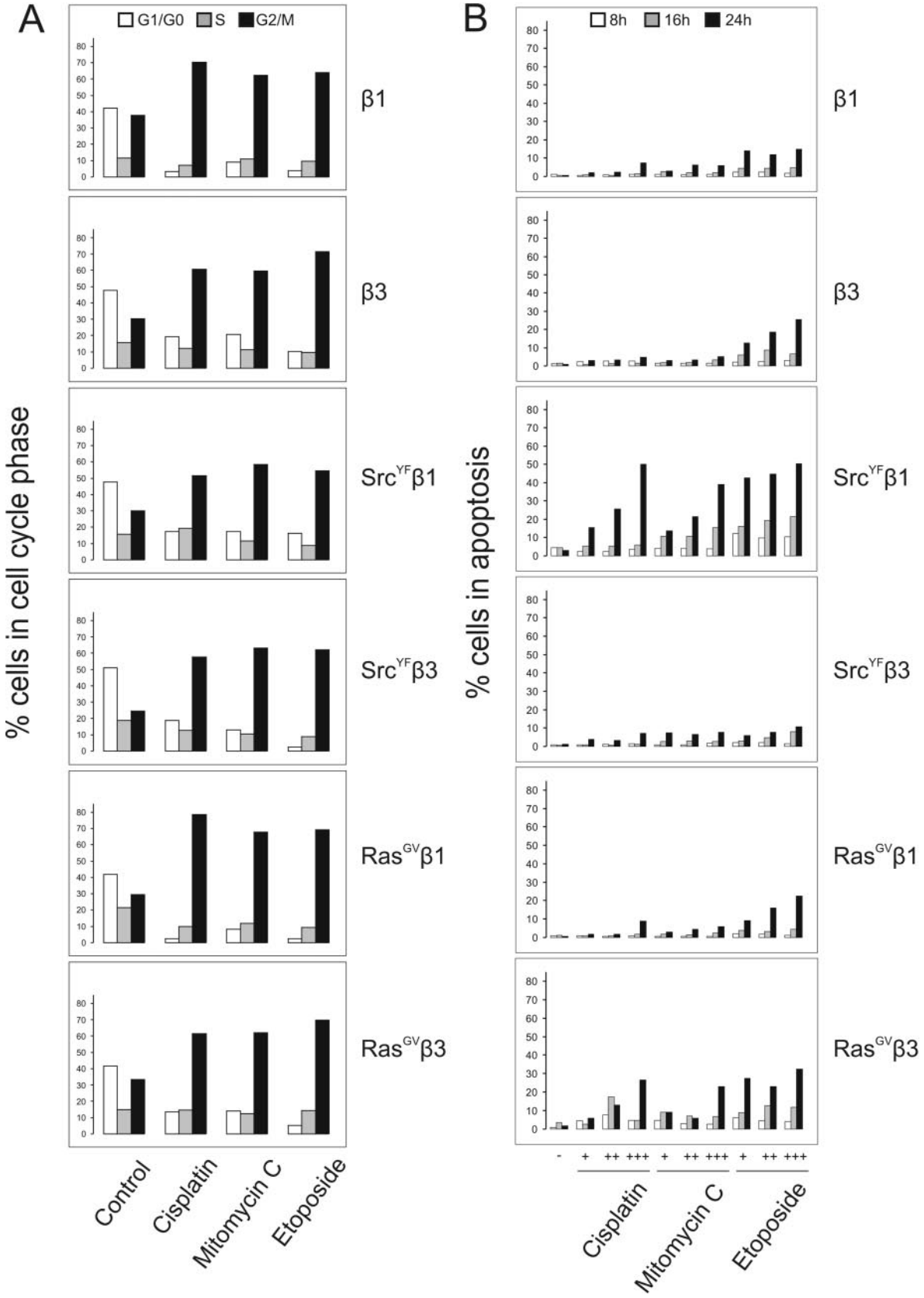


Fig. 1. β1 integrins sensitize cells to DNA-damaging compounds in presence of the Src oncogene. A, percentage of GE11 cell types in G_1/G_0, S, or G_2/M phase of the cell cycle after treatment with DNA-damaging compounds. Ectopic expression of integrin β1, integrin β3, c-Src[Y530F], or Ras[G12V] is indicated. Treatments were performed during 8 h in complete culture medium supplied with cisplatin (5 μM), mitomycin C (1 μM), or etoposide (10 μM). Control indicates treatment with DMSO. Cell cycle was determined by flow cytometry analysis of DNA content. B, percentage of apoptosis of GE11 cell types induced by DNA-damaging compounds. Treatments were performed during 8, 16, or 24 h in complete culture medium supplied with DNA-damaging compounds used at different concentrations: cisplatin (5, 10, and 20 μM; indicated by +, ++, and +++, respectively), mitomycin C (1, 5, and 10 μg/ml), or etoposide (10, 20, and 40 μM). Apoptosis was determined by flow cytometry analysis of DNA content.

which can be explained by Large T-mediated inactivation of the p53 tumor suppressor pathway, mimicking the situation in cancer cells (Fig. 1B, top two panels). We next generated the following cell types by retroviral transduction: GESrc^{YF}β1 and GESrc^{YF}β3 cells that express a constitutively primed form of the proto-oncogene c-Src[Y530F] together with β1 or β3 integrins and GERas^{GV}β1 and GERas^{GV}β3 cells that express oncogenic H-Ras[G12V] in the context of these integrin classes (Huveneers et al., 2007b). Regardless of the integrin expression profile, genotoxic treatment of

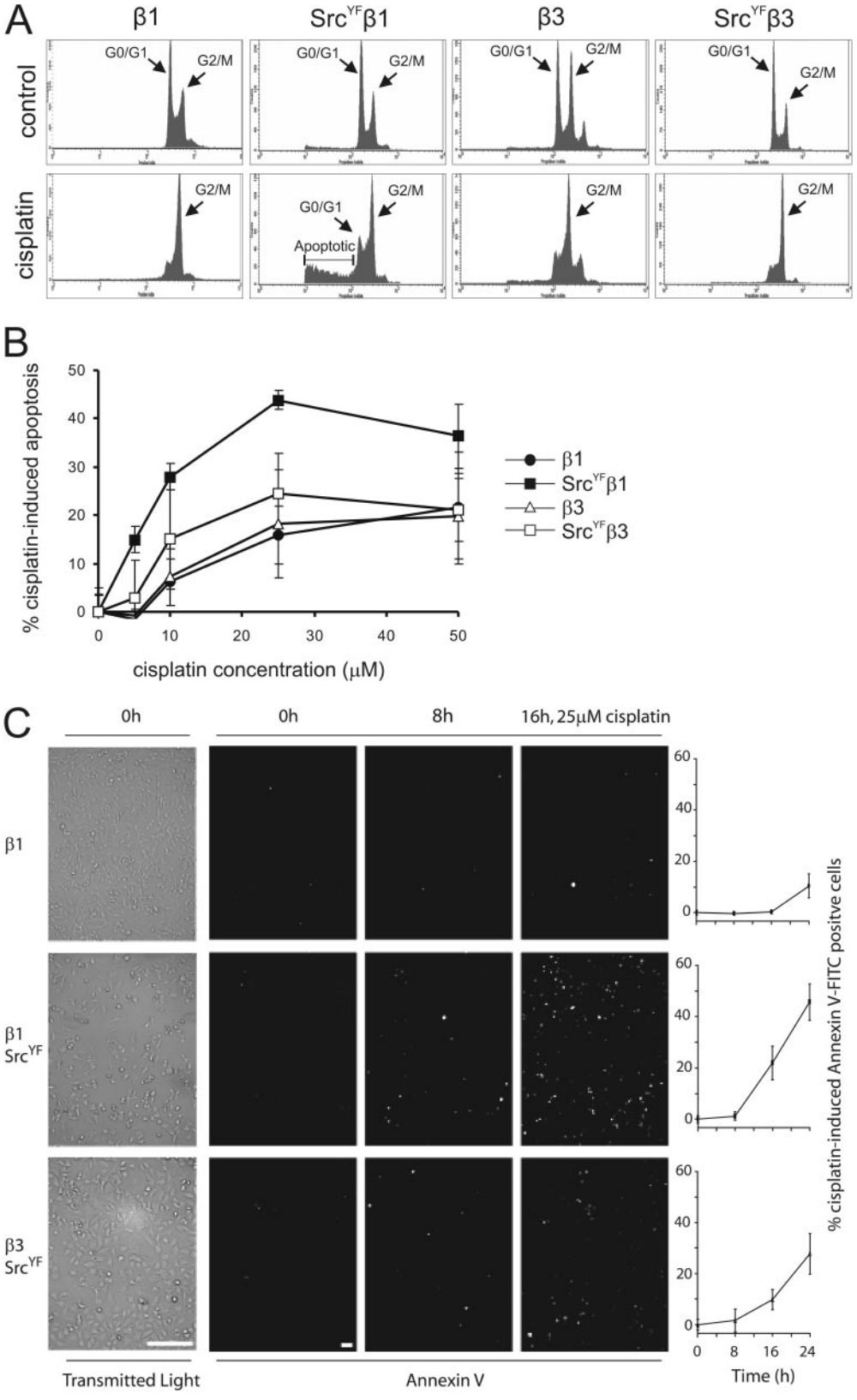


Fig. 2. Increased cisplatin-induced apoptosis in Src^{YF}-transformed cells expressing β1 integrins. A, analysis of DNA content by flow cytometry using propidium iodide in GE11 cells expressing indicated constructs after treatment with DMSO (control) or 10 μM cisplatin for 24 h. B, dose-response curves, indicating the increase in percentage of apoptotic (sub-G_0/G_1) cells, induced by treatment with various concentrations of cisplatin for 24 h (average ± S.D. of three independent experiments). C, FITC-labeled Annexin-V staining of phosphatidyl-serine exposed in apoptotic cells in response to cisplatin. Images show results for one representative experiment of three. Scale bar, 20 μm. Note that the immunofluorescence images consist of four stitched images obtained from the BD pathway, whereas for clarity, a single, enlarged image is shown for transmission light. Threshold for the FITC signal was based on the unexposed cells. Graphs indicate percentage of Annexin-V-labeled cells (average ± S.D. of 12 images taken from three independent experiments).

these oncogene-transformed cells led to a similar G_2 cell cycle arrest as observed in the absence of oncogenes (Fig. 1A). However, a remarkably strong induction of apoptosis was observed specifically in GESrc^{YF}β1 cells (Fig. 1B). By contrast, Src^{YF} did not enhance apoptosis in β3-expressing cells. It is noteworthy that these differences could not be explained by different levels of Src^{YF} expression; the GESrc^{YF} variant was made first, followed by expression and bulk FACS sorting for the integrin subunits, ensuring identical expression levels of Src^{YF} in the GESrc^{YF}β1 and GESrc^{YF}β3 lines (Huveneers et al., 2007b). Moreover, this effect was specific for Src^{YF}: transformation by Ras^{GV} only weakly affected the

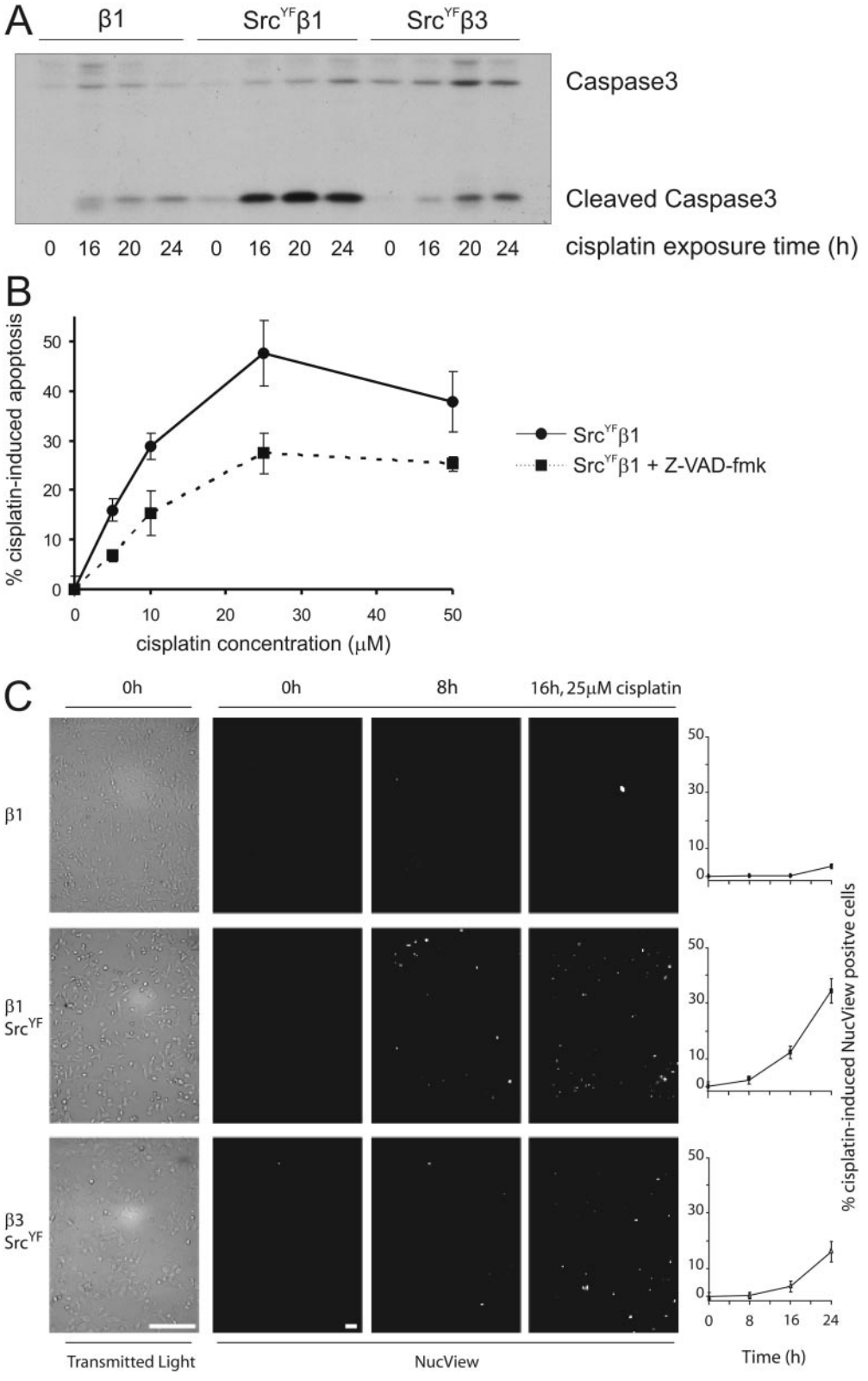


Fig. 3. Increased caspase activity mediates cisplatin-induced apoptosis of GESrc^{YF}β1 cells. A, Western blot analysis of caspase-3 cleavage in lysates of GE11 cells expressing indicated constructs treated with 10 μM cisplatin for indicated exposure times. B, dose-response curves indicating the increase in percentage of apoptotic (sub-G_0/G_1) cells, induced by treatment with various concentrations of cisplatin for 24 h in the absence or presence of z-VAD-fmk caspase inhibitor (average ± S.D. of three independent experiments). C, nuclear accumulation of Alexa Fluor-488-labeled caspase-3 substrate in apoptotic cells in response to cisplatin. Images show results for one representative experiment of three. Threshold for Alexa Fluor-488 was set based on the signal in unexposed cells. Scale bar, 20 μm. Note that the immunofluorescence images consist of four stitched images obtained from the BD pathway, whereas for clarity, a single, enlarged image is shown for transmission light. Graphs indicate percentage of cells with nuclear caspase-3 activity (average ± S.D. of 12 images taken from three independent experiments).

response to DNA-damaging agents, regardless of the integrin expression profile (Fig. 1). Together, these results indicate that expression of an oncogenic c-Src mutant, but not of H-Ras, leads to a strong sensitization to genotoxic compounds, especially in the context of β1 integrins.

For further studies, we compared GE11 cells expressing both Src^{YF} and β1 ($GESrc^{YF}β1$), only β1 (GEβ1), or Src^{YF} in the absence of β1 ($GESrc^{YF}β3$). We focused on cisplatin as a marker compound that induces prominent apoptosis in $GESrc^{YF}β1$ cells, but not in other oncogene/integrin combinations (Fig. 2A). The amount of apoptotic $GESrc^{YF}β1$ cells after cisplatin exposure as detected by sub-G_0/G_1 DNA content was significantly increased compared with the other cell types tested over a cisplatin concentration range from 0 to 25 μM ($p < 0.01$, Student's t test; parenthetically, at higher cisplatin concentrations apoptosis seemed to decrease in these FACS assays, although visual inspection clearly showed massive cell death at 50 μM. This can be explained by loss of highly fragmented cells during sample preparation; Fig. 2B). We used fluorescent Annexin-V to visualize translocation of phosphatidyl-serine to the outer leaflet of the cell membrane as an alternative method to detect apoptotic cells. Again, $GESrc^{YF}β1$ cells showed enhanced signs of apoptosis after treatment with 25 μM cisplatin compared with all other cell types tested ($p < 0.001$, Student's t test) (Fig. 2C). We investigated whether the enhanced apoptosis in the presence of Src^{YF} in combination with β1 integrins occurred at the level of caspase-3 activation. Indeed, cisplatin readily induced caspase-3 cleavage in $GESrc^{YF}β1$ cells, whereas caspase-3 cleavage in cells lacking Src^{YF} or in cells expressing Src^{YF} in the context of β3 was much weaker (Fig. 3A). In agreement, time-lapse experiments using a fluorescent caspase-3 substrate showed that accumulation of cleaved caspase-3 in response to cisplatin was enhanced in $GESrc^{YF}β1$ cells compared with the other cell types tested ($p < 0.01$, Student's t test) (Fig. 3B). Finally, a pan-caspase inhibitor, z-VAD-fmk, strongly reduced cisplatin-induced apoptosis of $GESrc^{YF}β1$ cells at each cisplatin concentration tested ($p < 0.0001$; Student's t test) (Fig. 3C). These results indicate that the increased sensitivity of $GESrc^{YF}β1$ cells to cisplatin occurs through a caspase-mediated apoptosis program.

We analyzed whether the increased apoptotic response observed in $GESrc^{YF}β1$ cells was reflected by increased accumulation of double-strand DNA breaks and early recognition of such lesions. Immunostaining for histone H2A.X phosphorylation at Ser139, which represents an early response to double-strand breaks, showed that all cell lines accumulated and sensed cisplatin-induced DNA lesions over time. At 24-h cisplatin exposure, $GESrc^{YF}β1$ cells did not show significantly more γH2A.X staining compared with GEβ1 or $GESrc^{YF}β3$ cells ($p > 0.1$, Student's test) (Fig. 4). One of the major pathways that couples DNA-damage sensing to apoptosis involves the transcription factor p53 (Jordan and Carmo-Fonseca, 2000; Roos and Kaina, 2006). Large T expression in GE11 cells mimics inactivation of the p53 pathway seen in the majority of cancers, but we examined whether there was a potential residual role for p53 in the increased cisplatin-induced apoptosis of $GESrc^{YF}β1$ cells. Irrespective of the integrin/oncogene expression status, cisplatin treatment induced phosphorylation of p53 on Ser15 and induced an increase in the expression of the p53 target gene p21, thus revealing a fraction of p53 that is not sequestered by Large T (Fig. 5A). To directly test whether p53-mediated effects were involved in cisplatin-induced apoptosis of $GESrc^{YF}β1$ cells, we silenced p53 gene expression. Introduction of si-p53 only weakly reduced total p53 protein expression after 72 h (pre-

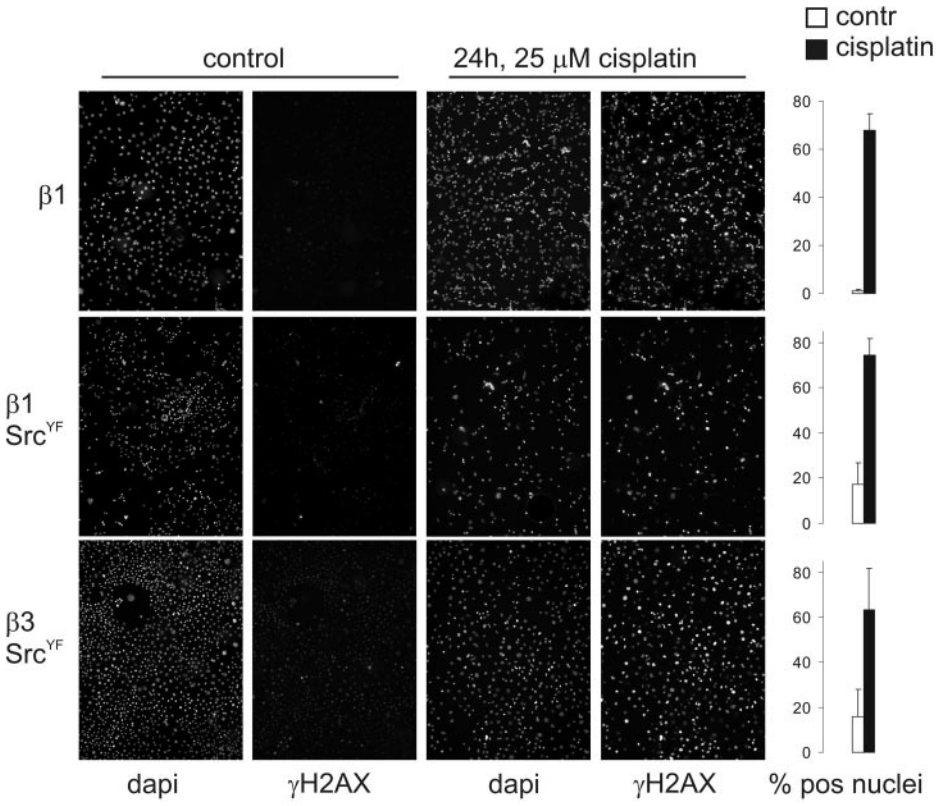


Fig. 4. Cisplatin-induced accumulation of phosphorylated H2A.X is not enhanced in $GESrc^{YF}β1$ cells. The indicated cell types were incubated for 24 h with 25 μM cisplatin or DMSO control and stained for phosphorylated H2A.X. Images show results for one representative experiment of two. Graphs indicate percentage of cells with phospho-H2A.X accumulation (average ± S.D. of at least six images taken from two independent experiments).

sumably because of the high stability of the major fraction of Large T-sequestered, inactive p53), but it abolished the induction of phosphorylated (active and presumably less stable) p53 and its target p21 upon cisplatin treatment (Fig. 5B). However, despite this elimination of the p53 response, si-p53 failed to inhibit cisplatin-induced apoptosis in GESrc^{YF}β1 cells ($p > 0.4$, Student's t test) (Fig. 5C). These results indicate that Src^{YF} sensitizes cells to cisplatin, especially in the context of β1 integrins, through a p53-independent pathway.

Besides causing DNA damage, genotoxic agents can react with cellular proteins and induce other cytotoxic stresses such as oxidative, mitochondrial, or ER stress that may contribute to the clearance of tumor cells (Asakura and Ohkawa, 2004; Linder and Shoshan, 2005; Chen et al., 2007). Because accumulation and sensing of DNA damage was similar for the various cell lines and we ruled out a role for the classic p53-mediated DNA damage response pathway in GESrc^{YF}β1, we wondered whether activation of any of the DNA damage-independent pathways could underlie the enhanced sensitivity to cisplatin upon Src^{YF} expression in the context of β1 integrins. We first tested whether the response to a variety of different cytotoxic agents mimicked the pattern of response to cisplatin in our panel of cell lines. Oxidative stress inducers (H_2O_2 and menadione) readily induced cell death as judged by cell rounding or detachment, but this response was not related to integrin expression profiles or Src^{YF} expression (Fig. 6A). Vincristine, a disruptor of the microtubule network, only weakly affected viability of these cells and DCVC, a nephrotoxicant inducing oxidative and mitochondrial stress (van de Water et al., 1999), caused cell death in β1-expressing cells, irrespective of the expression of Src^{YF}. In contrast, the response to treatment with thapsigargin, a specific inducer of ER stress (Linder and Shoshan, 2005), mimicked the response to cisplatin: thapsigargin readily triggered cell death of GESrc^{YF}β1 cells, whereas it did not or only weakly affected GEβ1 or GESrc^{YF}β3 cell survival (Fig. 6A). FACS analysis of DNA content confirmed this effect and indicated that thapsigargin, like cisplatin, selectively induced high levels of apoptosis in GESrc^{YF}β1 cells at each concentration tested ($p < 0.0001$, Student's t test) (Fig. 6B). In line with an induction of ER stress in cisplatin-treated cells, a small increase in phosphorylation of eIF2α was observed (Boyce et al., 2005) (Fig. 6C). To examine whether ER stress could underlie the enhanced sensitivity of GESrc^{YF}β1 cells to cisplatin, we made use of salubrinal, a specific inhibitor of the protein phosphatase 1-GADD34 phosphatase that induces increased phosphorylation of eIF2α and protects against ER stress-induced apoptosis (Boyce et al., 2005). As expected, at all concentrations tested, salubrinal inhibited thapsigargin-induced apoptosis in GESrc^{YF}β1 ($p < 0.001$, Student's t test), reaching a level that was close to that observed in the much less sensitive GESrc^{YF}β3 cells (Fig. 6B). In line with a role for ER stress in the enhanced sensitivity of GESrc^{YF}β1 to cisplatin, salubrinal readily suppressed apoptosis of these cells upon treatment with 10, 25, or 50 μM cisplatin ($p < 0.05$, Student's t test) (Fig. 6D). Altogether, these findings demonstrate that depending on the integrin expression profile, oncogenic c-Src but not H-Ras can sensitize cells to cisplatin-induced apoptosis and indicate that ER stress may underlie such sensitization.

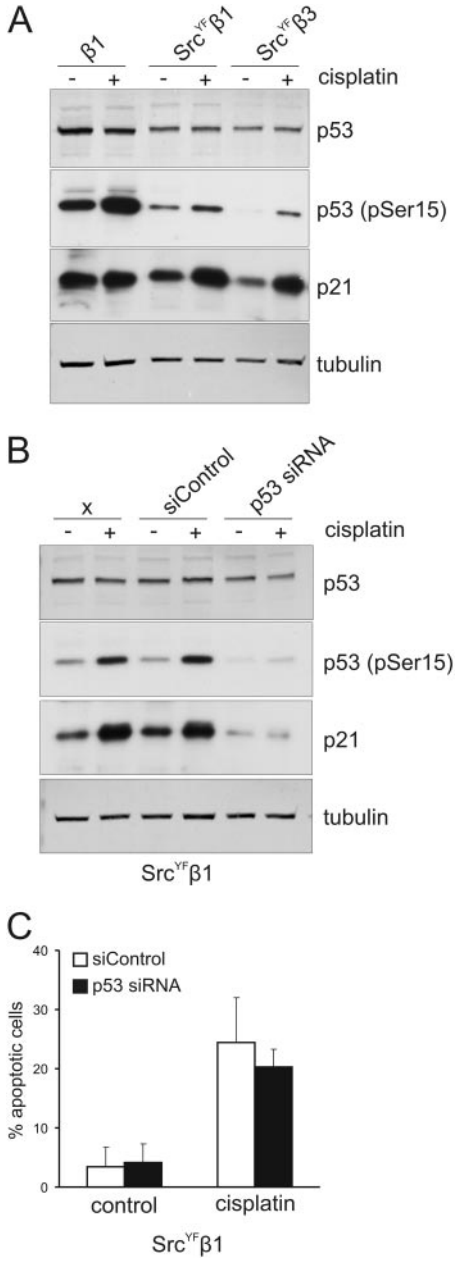


Fig. 5. β1 integrins sensitize Src^{YF}-transformed cells to cisplatin independently of p53 signaling. A, Western blot analysis of total p53, p-p53 (phospho-Ser15), p21, and tubulin (loading control) in lysates from GE11 cells expressing indicated constructs with or without 25 μM cisplatin treatment (24-h exposure). B, Western blot analysis of lysates from GESrc^{YF}β1 cells treated with 25 μM cisplatin (24-h exposure) transfected with p53 or control siRNAs. C, average percentage of apoptosis induced by cisplatin (25 μM; 24-h exposure) in GESrc^{YF}β1 cells transfected with si-p53 or control siRNA ± S.D. of three independent experiments.

Discussion

The development and application of specific integrin-blocking strategies as treatment against cancer is promising. Interactions with the extracellular matrix have been described to provide survival and proliferation signals and integrins can mediate resistance of tumor cells to genotoxic injury caused by chemotherapeutic agents, a process referred to as cell adhesion-mediated drug resistance (Damiano et al., 1999). Little is known about roles for distinct integrins in these processes or how the genetic make-up of the tumor influences the impact

that integrins can have. Previous experiments using β1-deficient cells indicate that in the absence of oncogenic mutations, the loss of β1 integrins sensitizes to radiation-induced genotoxic injury (Cordes et al., 2006). In our current study, we have compensated for the weak adhesion in the absence of β1 by enhancing the expression of β3, and we rule out a specific requirement for β1 integrins in protection against genotoxicants. We have investigated how two different classes of integrins affect chemosensitivity in the absence or presence of two different oncogenes. Our results reveal extensive cross-talk between the integrin expression profile and active oncogenic pathways in determining chemosensitivity. Moreover, we find that it is not the classic DNA damage response that is controlled by these parameters. Rather, the ability of genotoxic compounds to induce ER stress seems to underlie different levels of apoptosis.

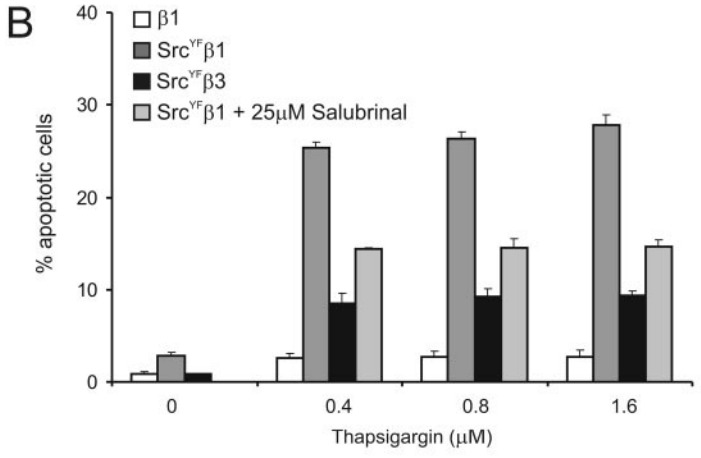
Our findings indicate that the increased levels of Src activity that are often seen in various types of cancer may enhance their chemosensitivity. However, the role of Src in regulating the balance between survival and apoptosis in cancer cells is complex. Cisplatin-induced elevation of the activities of epidermal growth factor receptor and c-Src in cancer cells has been reported to act as a survival response, and inhibition of these activities increased cisplatin-induced cell death (Benhar et al., 2002; Pengetnze et al., 2003). In contrast, cisplatin-induced apoptosis has been shown to depend on epidermal growth factor receptor and c-Src activity in other systems, and expression of v-Src can induce sensitivity to cisplatin analogs, pointing to a proapoptotic and drug-sensitizing effect of enhanced Src activity (Webb et al., 2000; Arany et al., 2004; Turkson et al., 2004). Our current findings indicate that integrin-mediated interactions with the microenvironent can determine how Src affects chemosensitivity.

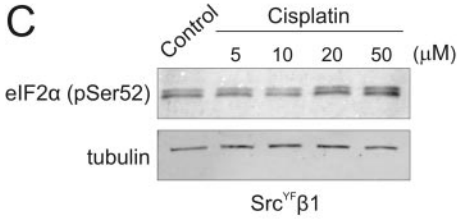
Rather than the classic DNA damage response, our findings suggest that Src and integrins act as regulators of the ER stress response that can also be induced by genotoxic compounds. This is based on the fact that 1) cells expressing

A

	H_2O_2	DCVC	Menadione	Vincristine	Thapsigargin
β1	+++	++	+++	-	-
Src^{YF}β1	++	+++	+++	+	+++
Src^{YF}β3	++	-	++	-	+

-	no cell death
+	at least 10% cell death
++	at least 25% cell death
+++	at least 50% cell death





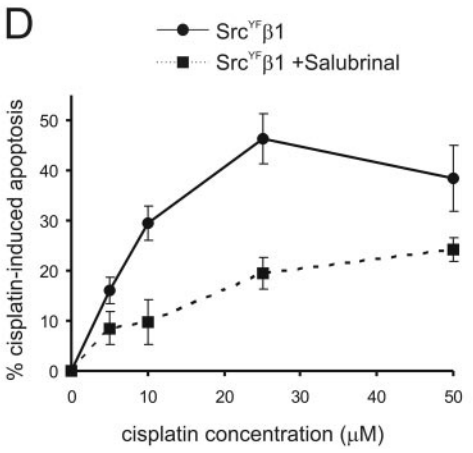


Fig. 6. Cisplatin induces apoptosis of GESrc^{YF}β1 cells through an elevated ER stress response. A, summary of the approximate amount of cell death (judged by cell rounding and detachment) induced by various cytotoxic compounds. GE11 cells expressing indicated constructs were treated with 100 μM H_2O_2 (oxidative stress inducer), 250 μM DCVC (nephrotoxicant inducing oxidative and mitochondrial stress), 100 μM menadione (oxidative stress inducer), 20 nM vincristine (microtubule disruptor), or 5 μM thapsigargin (ER stress inducer). B, percentage of apoptosis in GE11 cells expressing the indicated constructs after treatment with the ER stress inducer thapsigargin at indicated concentrations (24-h exposure). Salubrinal was used to inhibit ER stress induced in GESrc^{YF}β1 cells. Average ± S.D. of three independent experiments is shown. C, Western blot analysis of phosphorylated eIF2α and tubulin in lysates of cisplatin treated GESrc^{YF}β1 cells (8-h exposure). D, percentage of apoptosis (sub-G_0/G_1) after treatment with the indicated concentrations of cisplatin in GESrc^{YF}β1 cells in the presence or absence of the ER stress inhibitor salubrinal. Average ± S.D. of three independent experiments is shown.

mutant c-Src and β1 integrins also show specifically increased sensitivity to non–DNA-damaging compounds that activate ER stress; 2) compounds that activate oxidative or mitochondrial stress clearly behave differently from cisplatin in the panel of cell lines tested; and 3) salubrinal, a specific inhibitor of the protein phosphatase 1-GADD34 phosphatase that induces increased phosphorylation of eIF2α and protects against ER stress-induced apoptosis (Boyce et al., 2005; Wiseman and Balch, 2005) suppresses the enhanced cisplatin-induced apoptosis in the context of mutant c-Src and β1 integrins. It has been previously observed that cisplatin can induce apoptosis by stimulating ER stress (Mandic et al., 2003). Moreover, Src activation has also been implicated in p53-independent pathways, including those leading to apoptosis (Webb et al., 2000; Ulianich et al., 2008). Our results extend these findings and indicate that Src activity can enhance cisplatin-induced apoptosis under conditions where p53-dependent pathways are suppressed by inducing ER stress.

Given the strong attention that $\alpha_v\beta_3$ currently receives as a potential target for cancer therapy in combination with chemo- or radiotherapy (Huveneers et al., 2007a), it is intriguing that enhanced Src activity indeed fails to sensitize cells to cisplatin in the context of $\alpha_v\beta_3$. We have shown that Src-mediated tumor growth is strongly supported by an interaction with $\alpha_v\beta_3$ (Huveneers et al., 2007b). Our current study extends these findings and demonstrates that survival/apoptosis signaling by Src in the presence of chemotherapeutic compounds is similarly subject to tight regulation by the integrin expression profile. To some extent, differential regulation of Src-induced cytoskeletal organization and effects on adhesion/spreading may underlie the strong effect of the integrin expression profile: high levels of $\alpha_v\beta_3$ protect against the inhibition of cell spreading that is a hallmark of Src-transformed cells (Huveneers et al., 2008). Nevertheless, no differences in proliferation are observed between GESrc^{YF}β1 cells and any of the other cell lines tested in the absence of drugs, indicating that potential effects through adhesion signaling only become evident upon drug exposure.

Altogether, our findings indicate that cross-talk between integrins and oncogenes can have dramatic effects on chemosensitivity of cancer cells. Expanding studies along these lines could provide the information that will enable us to predict for which tumors integrin antagonists may be successfully used in combination with classic chemotherapy to stop cancer growth and progression.

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