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Microtubule-Targeted Chemotherapeutic Agents Inhibit Signal Transducer and Activator of Transcription 3 (STAT3) Signaling[S]

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ABSTRACT

The transcription factor signal transducer and activator of transcription 3 (STAT3) is inappropriately activated in the majority of breast tumors, especially in aggressive and invasive ones. In addition to driving the expression of genes promoting malignancy, STAT3 associates with tubulin and can promote cell migration. Because microtubule-targeted drugs are among the most active agents used in the treatment of breast cancer, we examined whether microtubule-based chemotherapy modulates STAT3 activity. When treated with paclitaxel or vinorelbine, breast cancer cells with constitutive activation of STAT3 display a loss of STAT3 phosphorylation, and paclitaxel disrupts the interaction of STAT3 with tubulin. Paclitaxel also inhibits cytokine-induced STAT3 activation. This effect is specific for microtubule-targeted agents, because other chemotherapeutic drugs, such as doxorubicin, have no effect on STAT3. The loss of STAT3 tyrosine phosphorylation is also reflected in an inhibition of expression of STAT3 target genes. This effect is not restricted to breast cancer, because similar effects are also seen in ovarian cancer and prostate cancer cells. Thus, in addition to their role in disrupting microtubule function, microtubule-targeted agents also suppress STAT3 signaling. This may be an important component of their activity, raising the possibility that microtubule targeted therapy may be particularly effective in tumors characterized by STAT3 activation.

Introduction

Breast cancer is the second leading cause of cancer-related deaths in women in the United States and often arises as a result of aberrant activation of transcription factors that play important roles in normal mammary function (Visvader and Lindeman, 2003). Important among these are the signal transducers and activators of transcription (STAT) family of transcription factors.

STATs are latent transcription factors that reside in the cytoplasm until activated by tyrosine phosphorylation. STATs can be phosphorylated via the JAK family of kinases and by other receptor and nonreceptor tyrosine kinases. Tyrosine phosphorylated STAT dimers translocate to the nucleus, bind DNA, and modulate transcription of target genes involved in many cellular processes. In normal cells, STAT signaling is rapid and transient; in cancer cells, by contrast, STATs are often activated constitutively, leading to enhanced expression of genes promoting proliferation, survival, and invasion. One STAT family member in particular, STAT3, is constitutively activated in a majority of breast cancers. Whereas tyrosine phosphorylation is critical for STAT3 function, STAT3 can also be phosphorylated on a specific serine residue, Ser727. STAT3 serine phosphorylation may enhance STAT3-mediated transcription (Zhang et al., 1995), but it can also inhibit the tyrosine phosphorylation of STAT3 (Chung et al., 1997). In normal cells, after modulating gene expression, STATs become dephosphorylated by tyrosine phosphatases and are thus free for subsequent rounds of stimulation (David et al., 1993; Haque et al., 1995; Chen et al., 2003). STAT signaling is also inhibited via two additional pathways: SOCS family members are STAT target genes that bind to receptors and block further STAT activation, thereby turning off the initial signal (Morita et al., 2000; Tonko-Geymayer et al., 2002; Denson et al., 2003; Galm et al., 2003); and PIAS proteins inhibit STATs through direct interaction (Chung et al., 1997; Liu et al., 1998). Many of

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ABBREVIATIONS: STAT, signal transducer and activator of transcription; IFNγ, interferon-γ; JAK, Janus tyrosine kinase; IL-6, interleukin-6; LIF, leukemia inhibitory factor; NFκB, nuclear factor-κB; SOCS, suppressors of cytokine signaling; PIAS, protein inhibitors of signal transducer and activator of transcription; DFCI, Dana-Farber Cancer Institute; MAPK, mitogen-activated protein kinase; U0126, 1,4-diamino-2,3-dicyano-1,4-bis(methylthio)butadiene.

these inhibitory pathways are inactivated in cancer cells, resulting in constitutively activated STATs.

In addition to the canonical role of STATs in regulating transcription, STAT3 has other non–transcription-based roles. Tyrosine phosphorylated STAT3 may be located at the leading edge of migrating cells, specifically at focal adhesions, in which it promotes migration (Silver et al., 2004). Moreover, both JAKs and STATs can be associated with microtubules (Lopez-Perez and Salazar, 2006; Ma and Sayeski, 2007), and the interaction between STAT3 and microtubules promotes migration by competing with the binding of the microtubule associated protein stathmin (Ng et al., 2006).

STAT3 is activated in 70% of breast tumors and is often associated with aggressive and invasive tumors (Alvarez et al., 2005; Walker et al., 2009). Furthermore, inhibition of STAT3 leads to a reversion of the malignant phenotype of these cells, indicating that it is a key mediator of breast cancer pathogenesis. Elucidating the role of STAT3 in breast cancer, and identifying methods to inhibit STAT3, would be beneficial for developing rational therapies for cancer. Among the most active drugs used in the treatment of breast cancer are microtubule-targeting agents. Two types are used: microtubule stabilizers, such as paclitaxel (Taxol; Bristol-Myers Squibb Co., Stamford, CT), and microtubule destabilizers such as vinorelbine (Navelbine; GlaxoSmithKline, Uxbridge, Middlesex, UK). Because STAT3 is activated in the majority of breast cancers and associates with microtubules, we wanted to determine whether microtubule-targeted therapy modulates STAT3 signaling and function in breast cancer cells.

Materials and Methods

Cell Lines. MDA-MB-468 (American Type Culture Collection, Manassas, VA), MDA-MB-231 (American Type Culture Collection), MCF-7 (kindly provided by Francis Kern, Southern Research Institute, Birmingham, AL), NIH-3T3 STAT1-luc (Lynch et al., 2007), and U3A STAT3-luc (Lynch et al., 2007) were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. SKOV3 and OVCAR8 cell lines (both kindly provided by Ronny Drapkin, DFCI, Boston, MA), BT549 cells (kindly provided by Kornelia Polyak, DFCI), and DU145 cells (kindly provided by Max Loda, DFCI) were maintained in RPMI 1640 medium containing 10% fetal calf serum. Cells were treated with paclitaxel, vinorelbine, or doxorubicin (NOVAPLUS, Irving, TX), with 10 mg/ml cycloheximide (Sigma-Aldrich, St. Louis, MO), 20 μM 1,4-diamino-2,3-dicyano-1,4-bis(methylthio)butadiene (U0126) (Cell Signaling Technology, Danvers, MA), or with Jak inhibitor 1 at the concentration indicated (Calbiochem, San Diego, CA), 10 ng/ml leukemia inhibitory factor (LIF) (Millipore Bioscience Research Reagents, Temecula, CA), 10 ng/ml oncostatin M, 10 ng/ml interleukin 6 (IL-6), or 500 U/ml interferon γ (IFNγ) (R&D Systems, Minneapolis, MN).

Immunoblots and Immunoprecipitations. Immunoblots were performed as described previously (Nelson et al., 2008); the following antibodies were used: phospho-tyrosine-STAT3, phospho-akt, akt, phospho-src, phospho-p44/42 MAPK, and p44/42 MAPK, all from Cell Signaling Technology; phospho-serine STAT3 (Frank et al., 1997), STAT3, and src were from Santa Cruz Biotechnology (Santa Cruz, CA); and α-tubulin was from Sigma-Aldrich. Immunoprecipitations were performed as described previously (Nelson et al., 2008) using anti-Flag antibodies (Sigma-Aldrich) or NP-40 buffer (1% NP-40 alternative, 0.15 M NaCl, 0.01 M sodium phosphate, 10% glycerol, and 2.5 mM EDTA) with α-tubulin antibodies. After immunoprecipitation, immunoblots were performed with anti-STAT3 antibodies. The density of the bands was quantified by ImageJ (available at http://rsbweb.nih.gov/ij/). Error bars represent the S.E.M. of at least two different experiments. *P* values were generated using Student's *t* test.

mRNA Quantitation. RNA was harvested using the RNeasy Mini kit from QIAGEN (Valencia, CA), according to the manufacturer's protocol. cDNA was generated using a TaqMan first strand kit from Applied Biosystems (Foster City, CA), and qualitative polymerase chain reaction was performed in triplicate using SYBR green master mix on a real-time polymerase chain reaction system (both from Applied Biosystems) with the following parameters: 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s, and then a dissociation stage. Data are expressed as the mean fold change ± S.E.M. Primer sequences are provided in Supplemental Table 1.

Luciferase Reporter Assays. MDA-MB-468 cells (5×10^4 cells) were transfected in duplicate with 1 μg of a STAT-responsive firefly luciferase reporter plasmid (M67-luc; kindly provided by J. Bromberg, Memorial Sloan-Kettering, New York, NY) or with NFκB-responsive NFκB-luc (Stratagene, La Jolla, CA) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Cells were also transfected with 0.1 μg of a plasmid that constitutively expresses *Renilla reniformis* luciferase (phRL-TK; Promega, Madison, WI) as a transfection control. Six hours after transfection, medium was replaced, and drug or vehicle was added. Luciferase activity was quantitated 22 h later as described previously (Walker et al., 2007). To analyze the specificity of paclitaxel on particular STAT family members, cell lines that stably express the M67-luc construct were used in which luciferase expression is driven by a specific STAT in isolation. STAT1 (and not STAT3) is activated in response to IFNγ in NIH-3T3-luc cells; therefore, only STAT1 activity is measured (Lynch et al., 2007). In U3A-luc fibrosarcoma cells, STAT1 is not expressed, and therefore, STAT3 is the only STAT activated in response to IL-6 (Lynch et al., 2007). Cells were pretreated with 7 μM paclitaxel for 1 h followed by stimulation with IFNγ (STAT1 NIH3T3-luc cells) or IL-6 (STAT3 U3A-luc cells) for 6 h. Data are expressed as mean ± S.D. and are representative of at least two different experiments.

Viability Measurement. Viability was assayed in MDA-MB-468 and MDA-MB-231 cells (5×10^3) and DU145 cells (3×10^3) plated in duplicate in 96-well dishes. Twenty-four hours after plating, drug was added at the indicated doses. Viability was measured 48 h after drug treatment by ATP-dependent bioluminescence (CellTiter-Glo; Promega). All values were normalized to the vehicle treated. Each data point is representative of at least two different experiments.

Results

Microtubule-Based Chemotherapy Inhibits STAT3 Tyrosine Phosphorylation. To determine the effect of microtubule-targeted agents on STAT3 signaling, we used MDA-MB-468 cells, which contain high levels of phosphorylated STAT3 and are sensitive to paclitaxel treatment (Supplemental Fig. 1). Treatment with paclitaxel resulted in a dose- and time-dependent decrease in tyrosine-phosphorylated STAT3 in MDA-MB-468 cells (Fig. 1, a and b). Paclitaxel also inhibited STAT3 tyrosine phosphorylation in BT549 cells, another breast cancer cell line containing constitutively active STAT3 (Supplemental Fig. 2).

To determine whether paclitaxel only inhibits constitutively active STAT3, we used MCF-7 cells, which do not contain phosphorylated STAT3, but in which STAT3 becomes activated in response to LIF. In cells pretreated with paclitaxel, the induction of STAT3 phosphorylation was reduced by 26% (Supplemental Fig. 3). Similar results were obtained in mouse embryonic fibroblasts activated by LIF (data not shown). Therefore, paclitaxel not only inhib-

its STAT3 in breast cancer cells that contain constitutively active STAT3, but paclitaxel also inhibits the induction of STAT3 phosphorylation.

Inhibition of STAT3 phosphorylation was not restricted to breast cancer cells, because paclitaxel also inhibited STAT3 phosphorylation in the ovarian cancer cell lines OVCAR8 and SKOV3 (Supplemental Fig. 4), and the prostate cancer cell line DU145 (Supplemental Fig. 5). The inhibition of STAT3 tyrosine phosphorylation did not arise from cytotoxicity, because any changes in cellular viability occurred much later then changes in STAT3 tyrosine phosphorylation (data not shown).

To determine whether paclitaxel inhibited STAT3 specifically or whether paclitaxel affected multiple pathways, signaling molecules known to be activated in breast cancer cells were analyzed. Paclitaxel treatment of MDA-MB-468 cells inhibited STAT3 tyrosine phosphorylation (by 30% in this experiment) but did not inhibit the phosphorylation of SRC, MAPK, or AKT (Fig. 1c). This suggests that the inhibition of STAT3 by paclitaxel is not due to global inhibition of cellular signaling.

To determine whether STAT3 inhibition was specific to microtubule stabilizers such as paclitaxel or whether it was due to modulation of microtubule function in general, we next examined the effect of an equitoxic dose of the microtubule destabilizer vinorelbine, which is also an active agent in breast cancer therapy. Similar to paclitaxel, vinorelbine decreased STAT3 tyrosine phosphorylation by approximately 50% in MDA-MB-468 cells (Fig. 1d). Thus, STAT3 tyrosine phosphorylation is inhibited by both microtubule stabilizers and destabilizers. To exclude the possibility that all cytotoxic agents lead to a loss of STAT3 function, we treated MDA-MB-468 cells with an equitoxic dose of doxorubicin, a DNA intercalating agent, and topoisomerase II inhibitor, which is a potent chemotherapy drug used in the treatment of breast cancer (Supplemental Fig. 6). Treatment of MDA-MB-468 cells with doxorubicin at equitoxic doses had no effect on STAT3 tyrosine phosphorylation (Fig. 1d). These results indicate that the loss of STAT3 tyrosine phosphorylation is not a nonspecific effect of chemotherapy drug treatment but is specific to microtubule-targeted therapy.

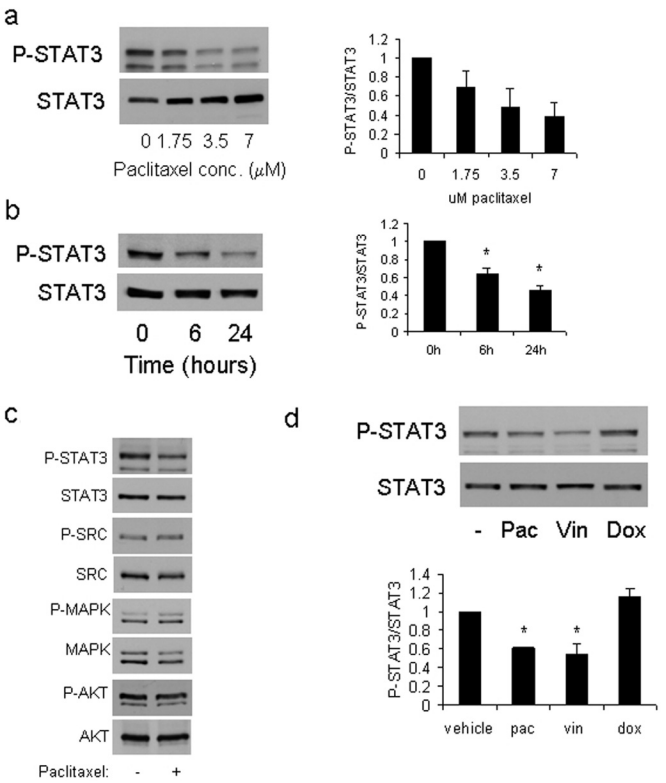

Fig. 1. Paclitaxel inhibits STAT3 tyrosine phosphorylation. Tyrosine phosphorylated and total STAT3 was analyzed by immunoblot in MDA-MB-468 cells treated with paclitaxel at the indicated doses for 6 h (a) or treated with 7 μM paclitaxel for the indicated times (b). MDA-MB-468 cells were treated with 7 μM paclitaxel for 6 h and analyzed by immunoblot for the total and phosphorylated forms of the indicated signaling molecules (c). MDA-MB-468 cells were treated with 7 μM paclitaxel, 9.26 μM vinorelbine, or 5 μM doxorubicin for 6 h and phosphorylated and total STAT3 was analyzed by immunoblot (d). Where indicated, the density of the bands was quantitated from at least two experiments, and the ratio of phosphorylated STAT3 to total STAT3 is shown. Error bars represent the S.E.M. *, P value <0.05.

Paclitaxel Inhibits STAT3-Dependent Reporter Gene Expression. Given that paclitaxel decreases STAT3 phosphorylation, we next considered whether paclitaxel inhibited STAT3-dependent gene regulation. We first examined how paclitaxel affected the expression of a luciferase reporter gene driven by a STAT-dependent promoter. MDA-MB-468 cells contain constitutive activation of STAT3 but not STAT1 (data not shown), so the STAT-dependent M67-luc reporter construct used only measures STAT3 activity in these cells. MDA-MB-468 cells were transfected with M67-luc and then treated with paclitaxel. STAT3-dependent luciferase expression was reduced by 40 to 60% in paclitaxel-treated cells compared with those treated with vehicle control (Fig. 2a). The reduction in levels of luciferase was similar to that after treatment with Jak inhibitor 1 (Fig. 2a), which inhibits STAT3 tyrosine phosphorylation in these cells (data not shown). To determine whether this effect was specific to STAT3 or whether paclitaxel also influences other transcription factors, an NFκB-dependent reporter was transfected into MDA-MB-468 cells, which also have high levels of constitutive NFκB activity (Newton et al., 1999). In contrast to its reduction of STAT3-dependent luciferase activity, paclitaxel treatment had no effect on NFκB-dependent luciferase activity (Fig. 2a); this indicates that paclitaxel specifically inhibits STAT3- but not NFκB-dependent gene expression.

To determine whether paclitaxel also inhibits cytokine-induced STAT3-dependent reporter expression, we used U3A cells that were engineered to stably express a STAT3-dependent luciferase construct. Cells were pretreated with paclitaxel and then stimulated with IL-6 to activate STAT3. IL-6 stimulation resulted in a prominent increase of luciferase expression in cells pretreated with vehicle; however, after paclitaxel pretreatment, the induction by IL-6 was decreased by approximately 50% (Fig. 2b). Similar results were also obtained in mouse embryonic fibroblasts transfected with a STAT3-dependent luciferase reporter gene (data not shown), indicating that paclitaxel inhibits both constitutive and cytokine-induced STAT3-dependent gene expression. To determine whether this effect of paclitaxel is specific to STAT3 or whether all STATs are similarly affected, NIH-3T3 cells stably expressing a STAT1-dependent luciferase construct were stimulated with IFNγ, which activates STAT1. Compared with vehicle, paclitaxel pretreatment had no effect on IFNγ

induction of luciferase (Fig. 2b), indicating that paclitaxel specifically targets STAT3-dependent gene regulation.

Paclitaxel Inhibits Endogenous STAT3-Dependent Gene Expression. Given that paclitaxel specifically inhibited STAT3-dependent reporter gene expression, we next examined whether this drug also inhibits endogenous STAT3 target genes. MDA-MB-468 cells were treated with paclitaxel or vinorelbine for 6 h and mRNA expression was analyzed by quantitative reverse-transcriptase polymerase chain reaction for *BCL6*, *BCL3*, and *SMAD7*, all of which are known to be STAT3 target genes. Paclitaxel and vinorelbine treatment inhibited the expression of each of these STAT3 target genes by 80 to 90% (Fig. 2c), indicating that paclitaxel and vinorelbine functionally inhibit STAT3 signaling.

Paclitaxel Induces STAT3 Serine Phosphorylation. It has been reported that inhibition of STAT3 tyrosine phosphorylation can be mediated by increased phosphorylation of STAT3 on serine 727 (Chung et al., 1997). We thus considered the possibility that paclitaxel and vinorelbine inhibit STAT3 tyrosine phosphorylation through induction of STAT3 serine phosphorylation. Treatment of MDA-MB-468 cells with paclitaxel (Fig. 3a) or vinorelbine (data not shown) enhanced STAT3 serine phosphorylation, concurrent with the loss of STAT3 tyrosine phosphorylation. Given this finding, we next tested the possibility that serine phosphorylation is necessary to mediate the inhibition of tyrosine phosphorylation. A mutant form of STAT3, in which serine 727 has been changed to alanine (S→A), was stably expressed in MDA-MB-468 cells; these cells were treated with paclitaxel, followed by immunoprecipitation and immunoblotting. The S→A mutant of STAT3 had no effect on the inhibition of STAT3 tyrosine phosphorylation by paclitaxel (Fig. 3b), demonstrating that induction of serine phosphorylation is not a prerequisite for the inhibition of STAT3 tyrosine phosphorylation by paclitaxel.

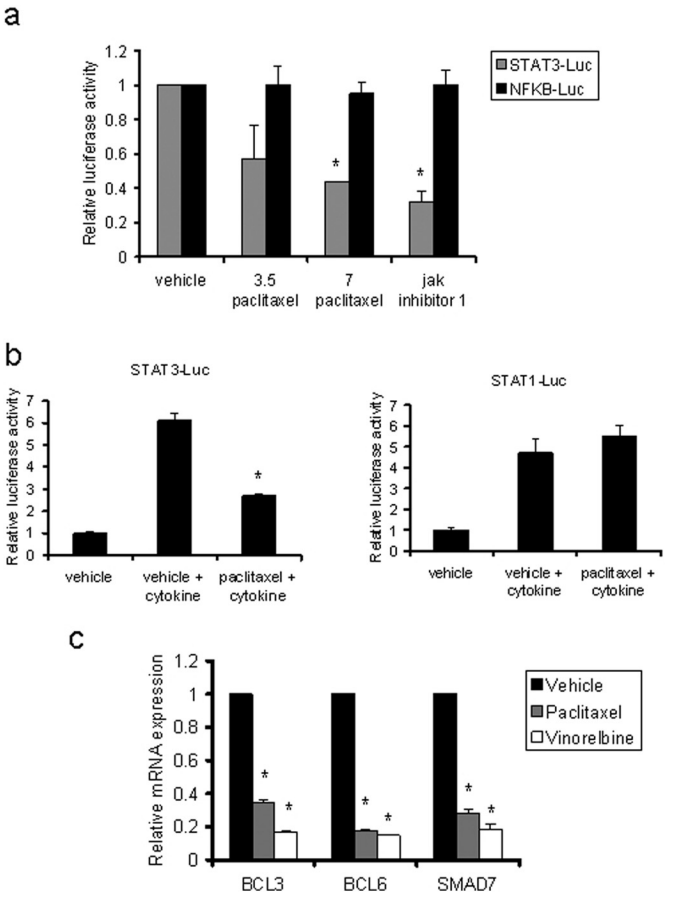

Fig. 2. Paclitaxel inhibits STAT3 dependent gene expression. a, MDA-MB-468 cells were transfected with either a STAT-dependent luciferase reporter gene or an NFκB-dependent luciferase reporter. Cells were then treated with the indicated doses of paclitaxel (in micromoles) or 0.1 μM Jak inhibitor 1 for 22 h, and normalized luciferase activity was measured. b, STAT3-responsive U3A-STAT3-luc and STAT1-responsive NIH-3T3-STAT1-luc cells were pretreated with vehicle or 7 μM paclitaxel for 1 h followed by stimulation with IL-6 or IFNγ, respectively, for 6 h, and luciferase activity was measured. c, MDA-MB-468 cells were treated with paclitaxel or vinorelbine for 6 h and analyzed for expression of the indicated STAT3 target genes (normalized to β-actin).

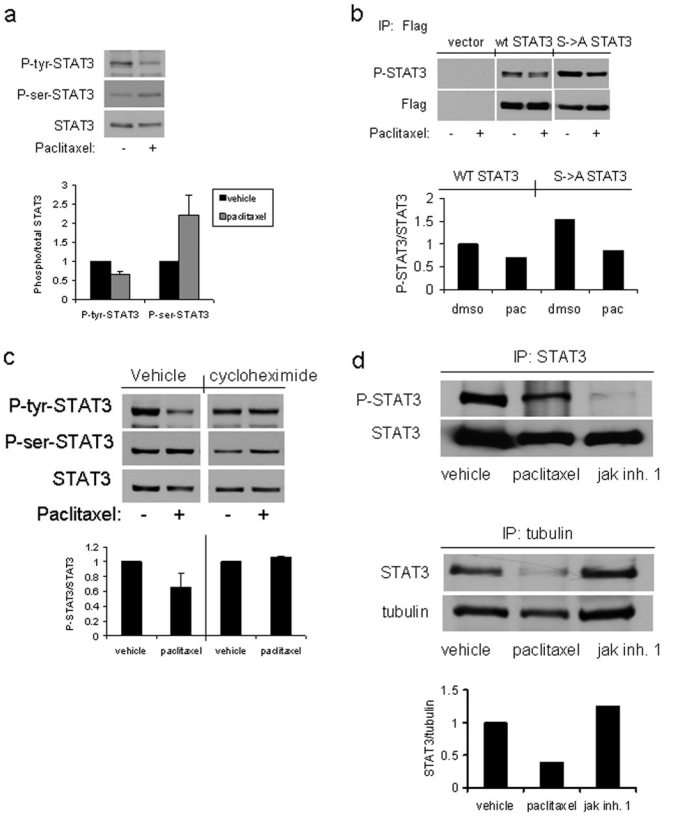

Fig. 3. Paclitaxel induces serine phosphorylation of STAT3 and prevents the interaction of STAT3 with tubulin. a, MDA-MB-468 cells were treated with 7 μM paclitaxel for 6 h and analyzed by immunoblot for tyrosine and serine phosphorylation of STAT3. The density of the bands was quantitated and the ratio of phosphorylated to total STAT3 is shown. Error bars represent the S.E.M. for at least two experiments. b, MDA-MB-468 cells stably expressing epitope (Flag)-tagged constructs of STAT3 were treated with 7 μM paclitaxel for 6 h, followed by anti-Flag immunoprecipitation. Immunoblots were then performed to tyrosine-phosphorylated STAT3 and total (Flag) STAT3. The density of the bands was quantitated, and tyrosine-phosphorylated STAT3 was compared with total (Flag) STAT3. c, paclitaxel inhibition of STAT3 is dependent on protein synthesis. MDA-MB-468 cells were pretreated with vehicle or cycloheximide for 1 h followed by treatment with paclitaxel for 5 h. Immunoblots were then performed with the indicated antibodies. The density of the bands was quantitated, and tyrosine-phosphorylated STAT3 was compared with total STAT3 for two separate experiments. d, paclitaxel disrupts the interaction between STAT3 and tubulin. MDA-MB-468 cells were treated with 7 μM paclitaxel or 1 μM JAK inhibitor 1 for 5 h, followed by immunoprecipitation with antibodies to STAT3 or tubulin. Immunoblots were performed with the indicated antibodies. The density of the bands was quantitated, and STAT3 was normalized to tubulin as a loading control.

Paclitaxel Inhibition of STAT3 Tyrosine Phosphorylation Requires Protein Synthesis. Because the inhibition of STAT3 tyrosine phosphorylation by paclitaxel was not an immediate effect (Fig. 1b), we considered the possibility that this inhibition requires gene transcription and translation. To assess this, MDA-MB-468 cells were treated with cycloheximide to prevent protein synthesis, before treatment with paclitaxel. Cycloheximide treatment prevented the inhibition of STAT3 tyrosine phosphorylation by paclitaxel but did not affect serine phosphorylation of STAT3 (Fig. 3c), showing a specific need for protein synthesis in order for paclitaxel to inhibit STAT3 signaling.

Given this finding, we hypothesized that paclitaxel induces the expression of a negative regulator of STAT3 signaling. However, paclitaxel did not significantly up-regulate the mRNA expression of any of the five phosphatases (protein phosphatase 2, Protein tyrosine phosphatases 1B and 1C, and Src homology region 2 domain-containing phosphatases 1 and 2), eight SOCS family members (SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6, SOCS7, and cytokine-inducible SH2 protein), or four PIAS family members (PIAS1, PIAS2, PIAS3, and PIAS4) analyzed (Supplemental Fig. 7). Therefore, the inhibition of STAT3 signaling induced by paclitaxel does not seem to be mediated through increased expression of one of these negative regulators.

Paclitaxel Inhibits the Association of STAT3 with Tubulin. Because STAT3 has been reported to interact directly with tubulin, we considered the possibility that paclitaxel was inhibiting STAT3 function by interfering with its association with this protein. MDA-MB-468 cells were treated with vehicle, paclitaxel, or Jak inhibitor 1, and STAT3 phosphorylation was analyzed. Both paclitaxel and Jak inhibitor 1 inhibited STAT3 tyrosine phosphorylation (Fig. 3d). Immunoprecipitation was then performed with antibodies to tubulin, and associated STAT3 was determined by immunoblot. The association of STAT3 with tubulin was lost upon paclitaxel treatment (Fig. 3d); however, inhibiting STAT3 tyrosine phosphorylation with Jak Inhibitor 1 did not modify the association between STAT3 and tubulin. Thus, paclitaxel disrupts the association of STAT3 with tubulin but does so independent of an effect on STAT3 phosphorylation.

Inhibition of STAT3 Phosphorylation by Paclitaxel Correlates with Cytotoxic Effect. To determine whether the inhibition of STAT3 tyrosine phosphorylation by paclitaxel and vinorelbine correlate with the cytotoxic effects of these drugs, we analyzed the sensitivity of additional breast cancer cell lines to these agents. MDA-MB-231 cells are less sensitive to the cytotoxic effects of paclitaxel and vinorelbine than MDA-MB-468 cells (Fig. 4a and Supplemental Fig. 8). Likewise, neither microtubule-targeted therapy was effective in inhibiting STAT3 tyrosine phosphorylation or inducing STAT3 serine phosphorylation (Fig. 4b). Furthermore, paclitaxel had no effect on the interaction of STAT3 with tubulin in MDA-MB-231 cells (Fig. 4c). These findings suggest that the cytotoxic effects of paclitaxel and vinorelbine correlate with the ability of these drugs to inhibit STAT3 tyrosine phosphorylation.

Discussion

Microtubule-targeted chemotherapeutic drugs are among the most active agents in the treatment of breast cancer. We have shown that microtubule stabilizers and destabilizers inhibit STAT3 signaling, which results in a loss of STAT3 tyrosine phosphorylation and function. These drugs also disrupt the interaction of STAT3 with tubulin, thereby further blocking STAT3 function.

Both paclitaxel and vinorelbine inhibit STAT3 signaling, and this ability correlates with the cytotoxic effects of these agents (Fig. 4). This raises the possibility that inhibition of STAT3 is an important event in the cell death induced by these agents. To address this, we had hoped to rescue cells with a constitutively active STAT3 mutant (Bromberg et al., 1999); however, microtubule-targeted agents decrease the tyrosine phosphorylation that is necessary for the activity of this mutant (Liddle et al., 2006), and thus we were unable to assess this possibility (data not shown). Through their effects on microtubules, drugs such as paclitaxel can undoubtedly kill cells that lack STAT3 activation. However, the results presented here suggest that inhibition of STAT3 may be an important component of the effects of microtubule-targeted therapy on tumor cells that contain activated STAT3.

Several mechanistic questions remain as to how the microtubule targeting agents inhibit STAT3 activation. Although we examined 17 potential negative regulators of STAT3 signaling, we were not able to identify one whose expression is

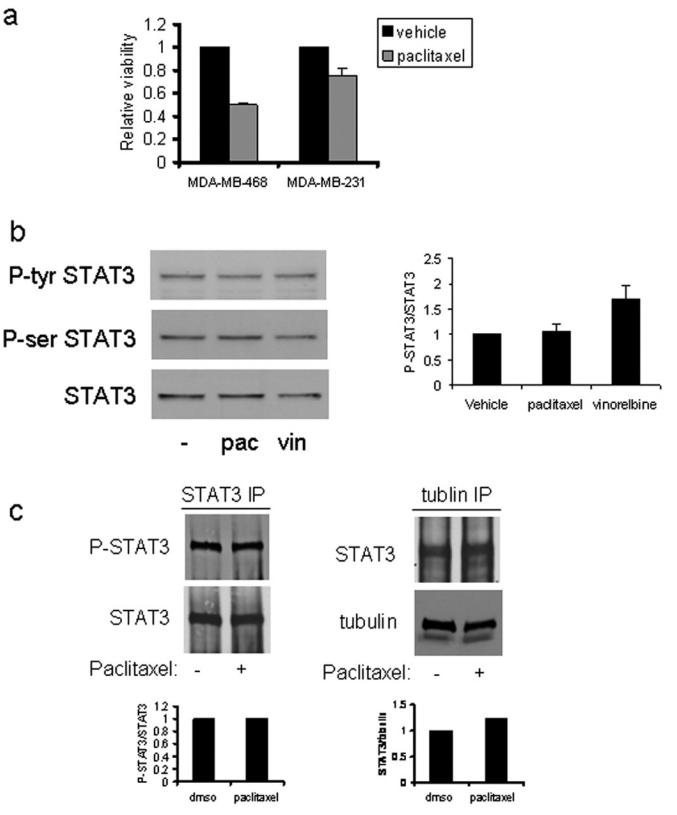

Fig. 4. Inhibition of STAT3 phosphorylation correlates with sensitivity of cells to paclitaxel cytotoxicity. a, MDA-MB-468 and MDA-MB-231 cells were treated with 7 μM paclitaxel for 48 h and relative viability was measured using ATP-dependent bioluminescence. b, MDA-MB-231 cells were treated with paclitaxel and vinorelbine for 6 h and analyzed by immunoblot with the indicated antibodies. The density of the bands was quantitated, and the ratio of tyrosine-phosphorylated STAT3 to total STAT3 is shown. Error bars represent the S.E.M. for at least two experiments. c, MDA-MB-231 cells were treated with paclitaxel for 6 h, after which STAT3 and tubulin were immunoprecipitated and analyzed by immunoblot with the indicated antibodies.

increased significantly after treatment with paclitaxel. However, analysis of signaling pathways and transcription factor activation upon paclitaxel treatment may provide insight into the mechanism of paclitaxel-induced STAT3 inhibition. We did find that the interaction between STAT3 and microtubules was disrupted by paclitaxel, and this may be an important component of the effects of microtubule-targeting agents on this pathway. Paclitaxel may also function on nonphosphorylated forms of STAT3, thereby preventing their translocation to the nucleus (Lopez-Perez and Salazar, 2006), or it may prevent their binding to focal adhesions (Silver et al., 2004), thus, inhibiting many forms of STAT3 signaling.

In conclusion, microtubule-targeted therapies specifically target STAT3 signaling via inhibition of STAT3 tyrosine phosphorylation and loss of interaction of STAT3 with tubulin. Because STAT3 activation is associated with high-grade cancers, targeting STAT3 is a potentially important approach to the treatment of breast cancer. These results suggest that screening for the STAT3 activation status of tumors may help to identify those patients most likely to benefit from microtubule-targeted therapies. In addition, a number of STAT3 inhibitors have been identified recently (Turkson et al., 2004; Song et al., 2005; Nelson et al., 2008). This raises the possibility that the combination of a novel STAT3 inhibitor with a microtubule-targeted therapy will work in synergy to provide a better therapeutic effect, with fewer side effects, in breast cancers with activated STAT3.

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