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Human β-galactoside α-2,3-sialyltransferase (ST3Gal III) attenuated Taxol-induced apoptosis in ovarian cancer cells by downregulating caspase-8 activity

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Abstract

Taxol triggers apoptosis in a variety of cancer cells, but it also upregulates cytoprotective proteins and/or pathways that compromise its therapeutic efficacy. In this report, we found that Taxol treatment resulted in caspase-8-dependent apoptosis in SKOV3 human ovarian cancer cells. Moreover, Taxol-induced apoptosis was associated with caspase-3 activation. Interestingly, Taxol treatment upregulated α-2,3-sialyltransferase (ST3Gal III) expression and forced expression of ST3Gal III attenuated Taxol-induced apoptosis. Furthermore, ST3Gal III overexpression inhibited Taxol-ttiggered caspase-8 activation, indicating that ST3Gal III upregulation produces cellular resistance to Taxol and hence reduces the efficacy of Taxol therapy.

Keywords

Taxol; ST3Gal III; Sialyltransferases; Apoptosis; Caspase-8; Sialylation; Ovarian cancer

Introduction

The glycosylation of proteins is important for their biological activity, conformation, and stability. Sialic acids are terminal sugars on oligosaccharides that are attached to glycoproteins or lipid moieties; the transfer of sialic acids from GMP-sialic acid to an acceptor carbohydrate is catalyzed by sialyltransferases [1-5]. Sialic acids of cell surface glycoconjugates play a pivotal role in cellular structure and function. Sialyltransferases play a role in a variety of biological processes, including cell–cell communication, cell-matrix interaction, adhesion, and protein targeting [1, 6–8]. A large body of evidence indicates that tumor cells have different surface properties compared to their nonnal counterparts, and that these differences are partially due to aberrant glycosylation (altered sialo-glycoconjugates expressed on the plasma membrane) [1, 9, 10]. These altered surface glycosylations change the ability of cancer cells to invade and metastasize [11–14]. Altered expression of α-2,3-sialyltransferase mRNA has been shown in ovarian cancer [15]. The most expressed sialyltransferase in breast tumors was ST3Gal III, which is involved in Sialyl-Lewis X

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(SLX) synthesis, and its expression was positively correlated with tumor size and the number of axillary nodes involved [16]. High levels of ST3Gal III expression in human tumors are associated with poor prognosis and metastasis [12, 15, 17].

Taxol (paclitaxel) is one of the most active cancer chemotherapeutic agents known and is effective against several human malignancies, including ovarian, breast, and non-small cell lung cancer (NSCLC) [18–23]. Taxol promotes microtubule (MT) assembly, which produces a change in MT dynamics that disrupts the reorganization of the microtubule network required for mitosis and cell proliferation [24]. As a result, cells treated with Taxol are arrested in the G_1 and G_1/M phases of the cell cycle [25]. Other critical actions of Taxol include the induction of apoptosis and necrosis [26, 27]. However, Taxol can also upregulate cytoprotective pathways in cancer cells that compromise its therapeutic efficacy [28-31]. Therefore, targeted therapeutics against the Taxol-induced cytoprotective signals may offer a rational strategy to improve the anticancer efficacy of Taxol. In this report, we find that Taxol treatment results in upregulation of ST3Gal III expression in SKOV3 human ovarian cancer cells.

Moreover, forced ST3Gal JJI expression attenuated Taxol-induced apoptosis by inhibiting caspase-8 activity, indicating that ST3Gal III upregulation produces cellular resistance to Taxol and hence reduces the efficacy of Taxol therapy. Therefore, ST3Gal III could serve as a therapeutic target of Taxol therapy.

Materials and methods

Cell culture and Taxol treatment

The human SKOV3 cell line was purchased from American Type Culture Collection (ATCC, Rockville, MD). Cells were maintained in RPMI media (Mediatec Inc., Herndon, VA) supplemented with 10% fetal bovine serum (FBS) at 37°C in 5% CO_2. Generally, 2 × 105 cells/well were plated in six-well plate and incubated overnight. The next day, 100 nM Taxol dissolved in dimethylsulfoxide (DMSO) were added into the indicated wells, and an equal amount of DMSO was added into control wells. The final concentration of DMSO was less than 0.1%. After 48 h treatment, cells were collected for apoptosis analysis.

Identification of ST3Gal JJI in Taxol-treated cells

To identify potential cytoprotective signaling molecules upregulated in cancer cells by the toxic insult of Taxol, we conducted an unbiased proteomic analysis. Briefly, lysates of Taxol-treated versus untreated CCRF-HSB-2 leukemia cells were resolved on 2D-SDS-PAGE gels, and protein spots were visualized by silver staining with a Silver Stain Plus kit (Bio-Rad, Hercules, CA). Taxol-upregulated protein species were identified, excised, and in-gel digested with trypsin. Protein identification was performed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS). One of these proteins was identified as ST3Gal III, an enzyme important in several cancer types including ovarian cancer [15–17]. To confirm our MS results and to establish whether ST3Gal III is involved in Taxol-induced apoptosis, we treated SKOV3 ovarian cancer cells with Taxol and confirmed that Taxol increases expression of ST3Gal III.

Cloning of ST3GAL III

To clone the full-length ST3Gal III-B1 gene, we used the ST3Gal III-B1-specific sense primer 5′-GCGATGGGAC TCTTGGTATTTG-3′ and the ST3Gal III-B1-specific anti-sense primer 5′-GATGCCACTGCTTAGATCAGTG-3′ to amplify the coding region using the template provided by Grahn et al. [32]. The full-length ST3Gal JJI DNA fragment was purified and subcloned into the pEF6/V5-His TOPO expression vector (lnvitrogen Corp., Carlsbad, CA) to produce ST3Gal III-EF6. The human ovarian cell line SKOV3 was transfected with ST3Gal III-EF6 using Fu-GENE 6 transfection reagent according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN), and stable clones were obtained by adding 10 μg/ml Blastacidin (Invitrogen) to the growth media. Cells transfected with ST3Gal III-EF6 or the empty plasmid pEF/V5-His were grown in a 6-well plate (5–100 cells/well), and after 2 weeks of growth, two cell clones from each sample were isolated and expanded for further studies.

RNA isolation and RT-PCR

Total RNA from Taxol-treated and untreated cells was isolated using the RNeasy Mini Kit (Qiagen, Valencia, CA) as described by the manufacturer. Two micrograms of total RNA was used in reverse transcription reactions with M-MLV reverse transcriptase and oligo (dT) 15 primer (Promega, Madison, WI) as described by the manufacturer. Four microliters of the resulting total cDNA were then used as the template in PCR to measure the mRNA level of interest using the designed primers: (forward, 5′-GCGAT GGGACTCTTGGTATTTG-3′; reverse, 5′-GATGCCACT GCTTAGATCAGTG-3′). The reactions were performed for denaturation at 95°C for 55 s, annealing at 55°C for 55 s, and extension at 72°C for 2 min for a total of 30 cycles. β-actin mRNA levels were used as internal controls. The amplified fragments (1128 bp) were separated on 1.5% agarose gels and visualized by ethidium bromide staining.

Annexin V binding assay for detecting apoptotic cells

Following treatment as stated above, the cells (5×10^5 cells/treatment) were used to dete1mine the translocation of phosphatidylserine to the outer surface of the plasma membrane during apoptosis using annexin V binding assay. For this assay, the cells were incubated with Taxol as described above, harvested, and stained with fluorescein isothiocyanate-labeled annexin V (BO Biosciences, San Jose, CA) and propidium iodide according to the manufacturer's protocol. Apoptosis and necrosis were analyzed by quadrant statistics on the propidium iodide (PI)-negative, Ruorescein-positive cells, and PI-positive cells, respectively.

Western blot analysis

In this study, the following primary antibodies were used: anti-caspase-8, anti-caspase-3 (Cell Signaling Technology, Danvers, MA), anti-V5 antibody (lnvitrogen Corp., Carlsbad, CA), anti-β-actin clone AC-74 (Sigma-Aldrich, St. Louis, MO), and the human ST3Gal-III antibody (gift from Dr. Göran Larson, Institute of Laboratory Medicine, Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, SE 413 45 Götcborg, Sweden). Protein concentrations were determined by using the $BCNCu_2SO_4$

protein (Sigma-Aldrich) assay as described by the manufacturer. For Western blot analysis, 50 μg protein/lane were separated by 12% SDS-PAGE, blotted onto a PVDF Immobilon membrane, and then the protein levels were detected using peroxidase-conjugated secondary antibodies as described by the manufacturer. The primary antibody was used al a concentration of 1:2000 (v/v). The membranes were then exposed to Kodak X-Omat film for various times. The mouse monoclonal anti-*β*-actin (clone AC-74) was purchased from Sigma-Aldrich (St. Louis, MO) and used al 1:5000 (v/v).

siRNA preparation and transfection

The ON-TARGET plus SMARTpool caspase-8 siRNA was synthesized by Dharmacon and siCONTROL non-targeting siRNA (catalog number D-001210-03) was purchased from Dharmacon Research, Inc. (Lafayette, CO). SKOV3 cells were seeded at a density of 2×10^5 cells/ml in antibiotic-free medium I day prior to transfection. For transfection, 100 nM of siRNA were mixed with DharmaFECT 1 transfection reagent (Dharmacon) according to the manufacturer's instructions. The cells were incubated with the siRNA-DharmaFECT 1 complexes for 48 h and 100 nM of Taxol were subsequently added.

Results

ST3Gal III Is induced during Taxol-induced apoptosis

We have found that Taxol at 100–500 nM induces robust apoptosis in cell lines from differenl. types of cancer. As shown in Fig. 1a and b, Taxol induced apoptosis in 35% of the SKOV3 cells, and immunoblot blot analysis of cell lysates showed that while SKOV3 cells express little ST3Gal III protein, its level of expression was significantly increased after Taxol treatment. Next, we determined whether the Taxol-mediated induction of ST3Gal III expression occurred at the transcriptional or post-transcriptional level. RT-PCR analysis showed that Taxol treatment did not affect the ST3Gal III transcript level, indicating that Taxol increases ST3Gal III by a post-transcriptional mechanism (Fig. 1e). Similar results were obtained when CCRF-HSB-2 leukemia and MCF-7 breast cancer cells were treated with Taxol (data not shown). These results reveal that Taxol induces the expression of ST3Gal III by a post-transcriptional mechanism in SKOV3 cells.

Expression of ST3Gal III decreases Taxol-induced apoptosis in SKOV3 cells

To determine the role of ST3Gal III in Taxol-induced apoptosis, we constmcted a ST3Gal III-EF6 plasmid and transfected SKOV3 cells. Stable SKOV3 transfected populations harboring the ST3Gal III-EF6 plasmid contained elevated levels of ST3Gal III as determined by immunoblot analysis (Fig. 2a). Treatment of SKOV3 cells with 100 nM Taxol for 48 h induced apoptosis in 38% of the cells, as compared to 22% in transfectants expressing ST3Gal III (Fig. 2b). These results show that the overexpression of ST3Gal III reduces Taxol-induced apoptosis of SKOV3 cells ($P < 0.05$). To prove that the antiapoplotic effect of ST3Gal III on Taxol-triggered apoptosis is not due to clonal differences between the blasticidin-resistant cell clones, we compared two ST3Gal III expressing cell clones with two cell clones containing the empty expression vector. Western blot analysis showed overexpression of ST3Gal III in both the cell clones transfected with the ST3Gal III expression vector (Fig. 3a). The results shown in Fig. 3b clearly show that Taxol-induced

apoptosis was significantly reduced in both of the cell clones overexpressing ST3Gal III compared with the cell clones containing the empty plasmid ($P < 0.05$).

Taxol triggers caspase-8-mediated apoptosis in SKOV3 cells

We next examined the role of caspase-8 in Taxol-induced apoptosis, since it is a central caspase in the death receptor and mitochondrial apoptotic signaling pathways. SKOV3 cells were treated with Taxol and caspase-8 levels were analyzed by immunoblotting. As shown in Fig. 4a, Taxol induced the processing of caspase-8 to its active pl8 sub-unit, revealing that caspase-8 is involved in mediating Taxol's death signals. To directly assess the function of caspase-8 in Taxol-triggered apoptosis in SKOV3 cells, we silenced the caspase-8 gene using a caspase-8-specific siRNA (Fig. 4b). Moreover, the knockdown of caspase-8 decreased Taxol-induced apoptosis from 35% to 25% in SKOV3 cells (Fig. 4c). These data reveal that Taxol induces apoptosis in SKOV3 cells by a caspase-8-mediated mechanism ($P < 0.05$).

Overexpression of ST3Gal III inhibits Taxol-induced activation of caspase-8 in SKOV3 cells

While the results shown in Fig. 5 demonstrate that Taxol induced processing of pro-caspase-8 to its active form in the two cell clones expressing the empty expression vector, the ectopic expression of ST3Gal III inhibited this process, and significantly less active caspase-8 was formed in the two cell clones expressing ST3Gal III. Moreover, Taxolinduced apoptosis was associated with significantly reduced caspase-8, -9, and -3 activation in the two cell clones expressing ST3Gal III compared to the cell clones with the empty vector. These data clearly show that ST3Gal III functions as an endogenous caspase-8 inhibitor in SKOV3 cells, and decreasing ST3Gal III expression may be a useful strategy to sensitize ovarian cancers to Taxol.

Discussion

In this study, for the first time we showed that Taxol triggers caspase-8-mediated apoptosis in SKOV3 ovarian cancer cells. A previous study showed that treatment of SKOV3 cells with Taxol did not lead to caspase-3 and -9 activation [27]. Moreover, another report indicated that Taxol induces caspase-independent apoptosis [33]. However, our results show that Taxol-induced apoptosis is associated with caspase-8, -9, and -3 activation in these cells. We previously demonstrated that low Taxol concentrations triggered caspase-8- and caspase-10-dependent apoptosis in the CCRF-HSB-2 human lymphoblastic leukemia cell line, and induced downregulation of the FLICE inhibitory protein variants, c-FLIP8 and c-FLIPc [34]. Our results also corroborate previous reports showing that incubation with Taxol leads to caspase-8 activation in the human colon cancer cell line HT29-D4 [35, 36] and in the lung adenocarcinoma LC-2-AD cell line [37]. Furthermore, it was reported that during Taxol treatment in BJAB Burkitt-like lymphoma cells, both caspases-3 and -8 are part of a mitochondrial feedback amplification loop of apoptosis [38]. We [39] and others [40] have shown that overexpression of a dominant negative FADD (dnFADD) is unable to recruit caspase-8/10, and this protects against Taxol-induced apoptosis in Jurkat cells and CCRF-HSB-2 human lymphoblastic leukemia cells [39, 40]; however; other studies revealed that FADD is not involved in this process in B-lymphoma cells [38, 41]. Several sludies

have demonstrated caspase-9 activation during Taxol-induced apoptosis [38, 42, 43]; however, we [39] and others could not detect cleavage of caspase-9 [27]. It appears that the effects of Taxol on caspase-9 activation are cell type specific. For instance, Taxol-induced cleavage of caspase-9 was shown in the HL-60 human leukemia cell line [44]. Therefore, the data collectively indicate that Taxol uses varying apoptosis signaling pathways in different cell types.

Epithelial ovarian cancer is the leading cause of death in women with gynecological tumors. Usually, after an initial high response rate, the patient relapses and the tumor acquires resistance to chemotherapy. Defects in apoptotic signaling pathways contribute to drug resistance in ovarian cancer [45, 46]. In the search to identify novel cytoprotective signaling molecules, we found for the first time that ST3Gal III expression is upregulated in Taxol-treated SKOV3 cells. Similar results were obtained in MCF-7 breast cancer and CCRF-HSB-2 human lymphoblastic leukemia cell lines (data not shown). Furthermore, our data revealed that increased expression of ST3Gal III serves a cytoprotective function and prevents Taxol-induced apoptosis by inhibiting caspase-8 activation.

ST3GALIII is a member of sialytransferases family which is mostly resident in the Golgi apparatus. Sialytransferases specifically catalyze the transfer of sialic acid from CMP-sialic acid to oligosaccharides. Each sialytransferase is unique for a pa1ticular sugar substrate. Sialytransferases add sialic acid to the terminal portions of sialylated glycolipids or to the *N*- or *O*-linked sugar chains of glycoproteins. These sialytransferases are distinguished from each other based on the different substrate structures and the different sugar linkages they form [47]. Some catalyze an a-2,3 linkage to add sialic acid to a galactose residue in the glycoprotein; others catalyze an α-2,6 linkage adding sialic acid to galactose residues [48, 49]. ST3GAL III is normally found in the Golgi apparatus, but can be proteolytically processed to a soluble form. Human ST3GAL III (Galβ1-3(4)GlcNAc α-2,3-sialytransferase) preferentially acts on the chain Galβ-3GlcNAc, and is therefore involved in synthesizing the sialyl-Lewis epitope in vivo [50].

To our knowledge, this is the first report describing the role of ST3Gal III as an anti-apoptotic protein. It is tempting to speculate that ST3Gal III may alter the sialylation of glycoproteins involved in apoptosis and/or interact with proteins in the death-inducing apoptosis signaling complex.

Identification of the critical apoptosis regulatory molecular targets of Taxol in cancer cells may lead to improved cancer therapy regimens that utilize a lower dose of Taxol, thereby limiting systemic toxicities. Moreover, identifying novel cytoprotective molecules such as ST3Gal III capable of preventing Taxol-triggered apoptosis validates these molecules as rational targets to sensitize cancers to Taxol's death signals. Investigating these targets and their roles in Taxol-induced apoptosis will also provide novel information on the specific signaling pathways involved in this process, which could be useful for discovering drugs with greater selectivity and efficacy. The molecular mechanism of upregulating ST3Gal III by Taxol and how it decreases caspase-8 activation are being pursued in our laboratory. Whether Taxol-triggered upregulation of ST3Gal III results in increased sialylation of specific target proteins involved in preventing apoptosis in cancer cells remains to be found.

However, strategies to silence the expression of ST3Gal III and/or inhibit its activity by small molecule inhibitors are likely to render cancer cells more sensitive to Taxol-induced apoptosis.

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References

1. Fukuda M. Possible roles of tumor-associated carbohydrate antigens. Cancer Res. 1996; 56:2237–2244. [PubMed: 8625291]
2. Haltiwanger RS, Lowe JB. Role of glycosylation in development. Annu Rev Biochem. 2004; 73:491–537. doi:10.1146/annurev.biochem.73.011303.074043. [PubMed: 15189151]
3. Pinho S, Marcos NT, Ferreira B, Carvalho AS, Oliveira MJ, Santos-Silva F, Harduin-Lepers A, Reis CA. Biological significance of cancer-associated sialyl-Tn antigen: modulation of malignant phenotype in gastric carcinoma cells. Cancer Lett. 2007; 249:157–170. (Epub 2006 Sep 11). [PubMed: 16965854]
4. Sperandio M, Frommhold D, Babushkina I, Ellies LG, Olson TS, Smith ML, Fritzsching B, Pauly E, Smith DF, Nobiling R, Linderkamp O, Marth JD, Ley K. Alpha 2,3-sialyltransferase-IV is essential for L-selectin ligand function in inflammation. Eur J Immunol. 2006; 36:3207–3215. doi:10.1002/eji.200636157. [PubMed: 17111351]
5. Mandal C, Srinivasan GV, Chowdhury S, Chandra S, Mandal C, Schauer R, Mandal C. High level of sialate-O-acetyltransferase activity in lymphoblasts of childhood acute lymphoblastic leukaemia (ALL): enzyme characterization and correlation with disease status. Glycoconj J. 2008; 3:3.
6. Kean EL, Munster-Kuhnel AK, Gerardy-Schahn R. CMP-sialic acid synthetase of the nucleus. Biochim Biophys Acta. 2004; 1673:56–65. [PubMed: 15238249]
7. Dall'Olio F, Chiricolo M. Sialyltrdnsferases in cancer. Glycoconj J. 2001; 18:841–850. doi:10.1023/A:1022288022969. [PubMed: 12820717]
8. Chen CL, Lee WL, Tsai YC, Yuan CC, Ng HT, Wang PH. Sialyltransferase family members and cervix squamous cell carcinoma. Eur J Gynaccol Oncol. 2002; 23:514–518.
9. Hakomori S. Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism. Cancer Res. 1996; 56:5309–5318. [PubMed: 8968075]
10. Saldova R, Royle L, Radcliffe CM, Abd Hamid UM, Evans R, Arnold JN, Banks RE, Hutson R, Harvey DJ, Antrobus R, Petrescu SM, Dwek RA, Rudd PM. Ovarian cancer is associated with changes in glycosylation in both acute-phase proteins and IgG. Glycobiology. 2007; 17:1344–1356. (Epub 2007 Sep 20). [PubMed: 17884841]
11. Gorelik E, Galili U, Raz A. On the role of cell surface carbohydrates and their binding proteins (lectins) in tumor metastasis. Cancer Metastasis Rev. 2001; 20:245–277. doi:10.1023/A:1015535427597. [PubMed: 12085965]
12. Hsu CC, Lin TW, Chang WW, Wu CY, Lo WH, Wang PH, Tsai YC. Soyasaponin-I-modified invasive behavior of cancer by changing cell surface sialic acids. Gynecol Oncol. 2005; 96:415–422. doi:10.1016/j.ygyno.2004.10.010. [PubMed: 15661230]
13. Brooks SA, Carter TM, Royle L, Harvey DJ, Fry SA, Kinch C, Dwek RA, Rudd PM. Altered glycosylation of proteins in cancer; what is the potential for new anti-tumour strategies. Anticancer Agents Med Chem. 2008; 8:2–21. doi:10.2174/187152008783330860. [PubMed: 18220502]
14. Oei AL, Moreno M, Verheijen RH, Sweep FC, Thomas CM, Massuger LF, von Mensdorff-Pouilly S. Induction of IgG antibodies to MUC1 and survival in patients with epithelial ovarian cancer. Int J Cancer. 2008; 123:1848–1853. doi:10.1002/ijc.23725. [PubMed: 18661524]

15. Wang PH, Li YF, Juang CM, Lee YR, Chao HT, Ng HT, Tsai YC, Yuan CC. Expression of sialyltransferase family members in cervix squmnous cell carcinoma correlates with lymph node metastasis. Gynecol Oncol. 2002; 86:45–52. doi:10.1006/gyno.2002.6714. [PubMed: 12079299]
16. Recchi MA, Hebbar M, Hornez L, Harduin-Lepers A, Peyrat JP, Delannoy P. Multiplex reverse transcription polymerase chain reaction assessment of sialyltransferase expression in human breast cancer. Cancer Res. 1998; 58:4066–4070. [PubMed: 9751611]
17. Gretschel S, Haensch W, Schlag PM, Kemmner W. Clinical relevance of sialyltransferases ST6GAL-I and ST3 GAL-III in gastric cancer. Oncology. 2003; 65:139–145. doi: 10.1159/000072339. [PubMed: 12931020]
18. Crown J, O'Leary M, Ooi WS. Docetaxel and paclitaxel in the treatment of breast cancer: a review of clinical experience. Oncologist. 2004; 9:24–32. doi:10.1634/theoncologist.9-suppl_2-24. [PubMed: 15161988]
19. Fields AL, Runowicz CD. Current therapies in ovarian cancer. Cancer Invest. 2003; 21:148–156. doi:10.1081/CNV-120016409. [PubMed: 12643016]
20. Runowicz CD, Wiernik PH, Einzig AI, Goldberg GL, Horwitz SB. Taxol in ovarian cancer. Cancer. 1993; 71:1591–1596. [PubMed: 8094322]
21. Simon GR, Bunn PA Jr. Taxanes in the treatment of advanced (stage III and IV) non-small cell lung cancer (NSCLC): recent developments. Cancer Invest. 2003; 21:87–104. doi:10.1081/CNV-120005919. [PubMed: 12643013]
22. Amadori D, Cecconetto L. Gemcitabine and taxanes in metastatic breast cancer. Ann Oncol. 2006; 17:v173–v176. doi:10.1093/annonc/mdj976. [PubMed: 16807451]
23. Rossi D, Dennetta D, Ugolini M, Alessandroni P, Catalano V, Fedeli SL, Giordani P, Casadei V, Baldelli AM, Graziano F, Catalano G. Weekly paclitaxel in elderly patients (aged > or = 70 years) with advanced non-small-cell lung cancer: an alternative choice? Results of a phase II study. Clin Lung Cancer. 2008; 9:280–284. doi:10.3816/CLC.2008.n.043. [PubMed: 18824450]
24. Jordan MA, Wilson L. Microtubules as a target for anti-cancer drugs. Nat Rev Cancer. 2004; 4:253–265. doi:10.1038/nrc1317. [PubMed: 15057285]
25. Liao PC, Lieu CH. Cell cycle specific induction of apoptosis and necrosis by paclitaxel in the leukemic U937 cells. Life Sci. 2005; 76:1623–1639. (Epub 2005 Jan 20). [PubMed: 15680171]
26. Huisman C, Ferreira CG, Broker LE, Rodriguez JA, Smit EF, Postmus PE, Kruyt FA, Giaccone G. Paclitaxel triggers cell death primarily via caspase-independent routes in the non-small cell lung cancer cell line NCI-H460. Clin Cancer Res. 2002; 8:596–606. [PubMed: 11839682]
27. Ofir R, Seidman R, Rabinski T, Krup M, Yavelsky V, Weinstein Y, Wolfson M. Taxol-induced apoptosis in human SKOV3 ovarian and MCF7 breast carcinoma cells is caspase-3 and caspase-9 independent. Cell Death Differ. 2002; 9:636–642. doi:10.1038/sj.cdd.4401012. [PubMed: 12032672]
28. Landen CN, Kim TJ, Lin YG, Merritt WM, Kamat AA, Han LY, Spannuth WA, Nick AM, Jennnings NB, Kinch MS, Tice D, Sood AK. Tumor-selective response to antibody-mediated targeting of alphavbeta3 integrin in ovarian cancer. Neoplasia. 2008; 10:1259–1267. [PubMed: 18953435]
29. Cao C, Lu S, Sowa A, Kivlin R, Amaral A, Chu W, Yang H, Di W, Wan Y. Priming with EGFR tyrosine kinase inhibitor and EGF sensitizes ovarian cancer cells to respond to chemotherapeutical drugs. Cancer Lett. 2008; 266:249–262. (Epub 2008 Apr 8). [PubMed: 18400375]
30. Engels K, Knauer SK, Loibl S, Fetz V, Harter P, Schweitzer A, Fisseler-Eckhoff A, Kommoss F, Hanker L, Nekljudova V, Hermanns I, Kleinert H, Mann W, du Bois A, Stauber RH. NO signaling confers cytoprotectivity through the surviving network in ovarian carcinomas. Cancer Res. 2008; 68:5159–5166. doi:10.1158/0008-5472.CAN-08-0406. [PubMed: 18593915]
31. Duan Z, Weinstein EJ, Ji O, Ames RY, Choy E, Mankin H, Homicek FJ. Lentiviral short hairpin RNA screen of genes associated with multidrug resistance identifies PRP-4 as a new regulator of chemoresistance in human ovarian cancer. Mol Cancer Ther. 2008; 7:2377–2385. (Epub 2008 Aug 7). [PubMed: 18687998]
32. Grahn A, Barkhordar GS, Larson G. Cloning and sequencing of nineteen transcript isoforms of the human alpha2,3-sialyltransferase gene, ST3Gal III; its genomic organisation and expression in

human tissues. Glycoconj J. 2002; 19:197–210. doi:10.1023/A:1024253808424. [PubMed: 12815231]

33. Ahn HJ, Kim YS, Kim JU, Han SM, Shin JW, Yang HO. Mechanism of taxol-induced apoptosis in human SKOV3 ovarian carcinoma cells. J Cell Biochem. 2004; 91:1043–1052. doi:10.1002/jcb.20006. [PubMed: 15034938]
34. Day TW, Najafi F, Wu CH, Safa AR. Cellular FLICE-like inhibitory protein (c-FLIP): a novel target for Taxol-induced apoptosis. Biochem Phannacol. 2006; 71:1551–1561. (Epub 2006 Mar 31).
35. Motwani M, Delohery TM, Schwartz GK. Sequential dependent enhancement of caspase activation and apoptosis by flavopiridol on paclitaxel-treated human gastric and breast cancer cells. Clin Cancer Res. 1999; 5:1876–1883. [PubMed: 10430095]
36. Goncalves A, Braguer D, Carles G, Andre N, Prevot C, Briand C. Caspase-8 activation independent of CD95/CD95-L interaction during paclitaxel-induced apoptosis in human colon cancer cells (HT29-D4). Biochem Pharmacol. 2000; 60:1579–1584. doi:10.1016/S0006-2952(00)00481-0. [PubMed: 11077039]
37. Oyaizu H, Adachi Y, Taketani S, Tokunaga R, Fukuhara S, Ikehara S. A crucial role of caspase 3 and caspase 8 in paclitaxel-induced apoptosis. Mol Cell Biol Res Commun. 1999; 2:36–41. doi:10.1006/mcbr.1999.0146. [PubMed: 10527889]
38. van Haefen C, Wieder T, Essmann F, Schulze-Osthoff K, Darken B, Daniel PT. Paclitaxel-induced apoptosis in BJAB cells proceeds via a death receptor-independent, caspases-3/-8-driven mitochondrial amplification loop. Oncogene. 2003; 22:2236–2247. doi:10.1038/sj.onc.1206280. [PubMed: 12700660]
39. Park SJ, Wu CH, Gordon JD, Zhong X, Emami A, Safa AR. Taxol induces caspase-10-dependent apoptosis. J Biol Chem. 2004; 279:51057–51067. doi:10.1074/jbc.M406543200. [PubMed: 15452117]
40. Biswas RS, Cha HJ, Hardwick JM, Srivastava RK. Inhibition of drug-induced Fas ligand transcription and apoptosis by Bcl-XL. Mol Cell Biochem. 2001; 225:7–20. doi:10.1023/A:1012203110027. [PubMed: 11716366]
41. Wieder T, Essmann F, Prokop A, Schmelz K, Schulze-Osthoff K, Beyaert R, Dorken B, Daniel PT. Activation of caspase-8 in drug-induced apoptosis of B-lymphoid cells is independent of CD95/Fas receptor-ligand interaction and occurs downstream of caspase-3. Blood. 2001; 97:1378–1387. doi:10.1182/blood.V97.5.1378. [PubMed: 11222383]
42. Perkins CL, Fang G, Kim CN, Bhalla KN. The role of Apaf-1, caspase-9, and bid proteins in etoposide- or paclitaxel-induced mitochondrial events during apoptosis. Cancer Res. 2000; 60:1645–1653. [PubMed: 10749135]
43. Razandi M, Pedrain A, Levin ER. Plasma membrane estrogen receptors signal to antiapoptosis in breast cancer. Mol Endocrinol. 2000; 14:1434–1447. doi:10.1210/me.14.9.1434. [PubMed: 10976921]
44. Perkins C, Kim CN, Fang G, Bhalla KN. Overexpression of Apaf-1 promotes apoptosis of untreated and paclitaxel- or etoposide-treated HL-60 cells. Cancer Res. 1998; 58:4561–4566. [PubMed: 9788601]
45. Fraser M, Leung B, Jahani-Asl A, Yan X, Thompson WE, Tsang BK. Chcmoresistance in human ovarian cancer: the role of apoptotic regulators. Reprod Biol Endocrinol. 2003; 1:66. doi:10.1186/1477-7827-1-66. [PubMed: 14609433]
46. Richardson A, Kaye SB. Drug resistance in ovarian cancer: the emerging importance of gene transcription and spatio-temporal regulation of resistance. Drug Resist Updat. 2005; 8:311–321. (Epub 2005 Oct 17). [PubMed: 16233989]
47. Harduin-Lepers A, Vallejo-Ruiz V, Krzewinski-Recchi MA, Samyn-Petit B, Julien S, Delannoy P. The human sialyltransferase family. Biochimie. 2001; 83:727–737. doi:10.1016/S0300-9084(01)01301-3. [PubMed: 11530204]
48. Harduin-Lepers, A. Recchi MA; Delannoy, P. 1994, the year of sialyltransferases. Glycobiology. 1995; 5:741–758. doi:10.1093/glycob/5.8.741. [PubMed: 8720072]
49. Tsuji S, Datta AK, Paulson JC. Systematic nomenclature for sialyltransfcrascs. Glycobiology. 1996; 6:5–7. doi:10.1093/glycob/6.7.647.

50. Kitagawa H, Paulson JC. Cloning and expression of human Gal beta 1,3(4)GlcNAc alpha 2,3 - sialyltransferase. Biochem Biophys Res Commun. 1993; 194:375–382. doi:10.1006/bbrc.1993.1830. [PubMed: 8333853]

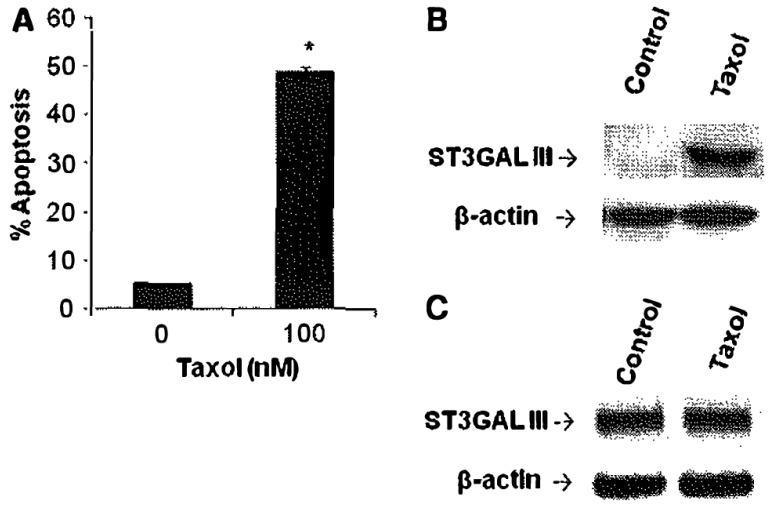

Fig. 1.

Taxol-induced apoptosis and ST3Gal III expression in SKOV3 cells. **a** Cells were treated with 100 nM taxol for 48 h, harvested, and apoptosis was determined by FACS analysis as described in section “Matrial and Methods”. Compensation was executed for each experiment using untreated cells and cells stained only with Annexin V or propidium iodide, respectively. Error bars show standard deviation from triplicate measurements. * $p < 0.05$. **b** Immunoblot analysis of ST3Gal III and β-actin in cell lysates following the treatment with 100 nM Taxol. **c** Total RNA from SKOV3 cells was extracted and the levels of ST3Gal III mRNA were measured by RT-PCR. β-actin was used as an internal control in the RT-PCR reactions

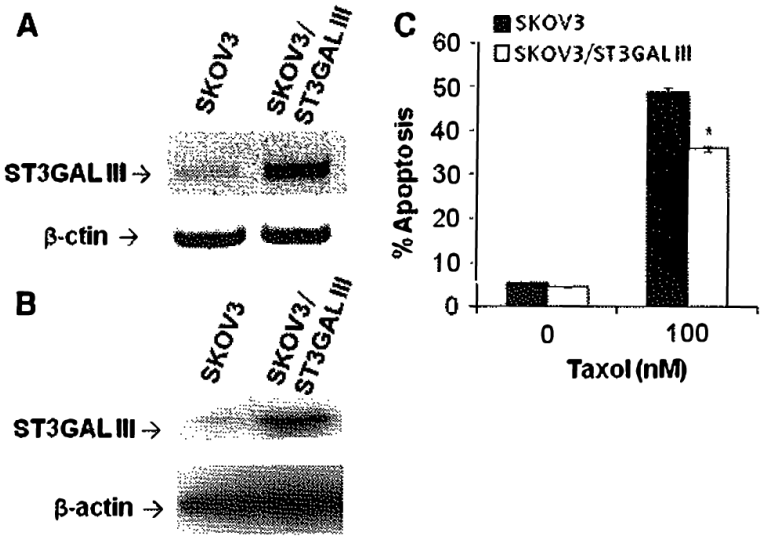

Fig. 2.

Overexpression of ST3Gal III inhibits the Taxol-induced apoptosis of SKOV3 cells. **a** Total RNA was isolated from SKOV3 cells or the SKOV3 stable cell line transfected with the ST3GAL III gene, and the expression of ST3Gal III was measured by RT-PCR. **b** Cell lysates from SKOV3 and SKOV3 cells stably transfected with the ST3Gal III gene were collected. Immunoblot analysis of ST3Gal III expression was conducted. **c** SKOV3 and SKOV3/ST3Gal III cells were treated with 100 nM Taxol for 48 h, harvested, and apoptosis was determined by FACS analysis as described in the section “Material and Methods”. Compensation was executed for each experiment using untreated cells and cells stained only with annexin V or propidium iodide, respectively. Error bars show standard deviation from triplicate measurements. * $P < 0.05$

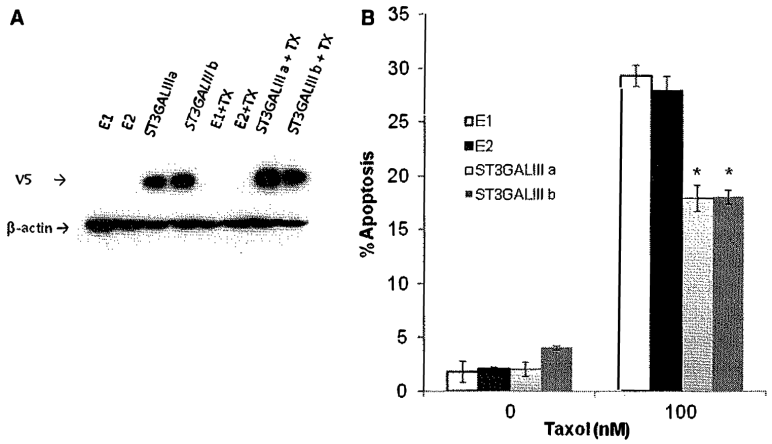

Fig. 3.

ST3Gal III attenuates the Taxol-induced apoptosis. **a** Western biot analysis of ST3Gal III expression in two SKOV3 cell colonies using V5 antibody. Two SKOV3 cell clones expressing the empty vector (E1 and E2) and two cell clones expressing ST3Gal III transfectants (ST3Ga1 III a and ST3Gal III **b**) were treated with 100 nM Taxol for 48 h, respectively, harvested, and apoptosis was determined as described in the section “Material and Methods”. Compensation was executed for each experiment using untreated cells and cells stained only with Annexin V or propidium iodide, respectively. Error bars show standard deviation fro1n triplicate measurements. * $P < 0.05$

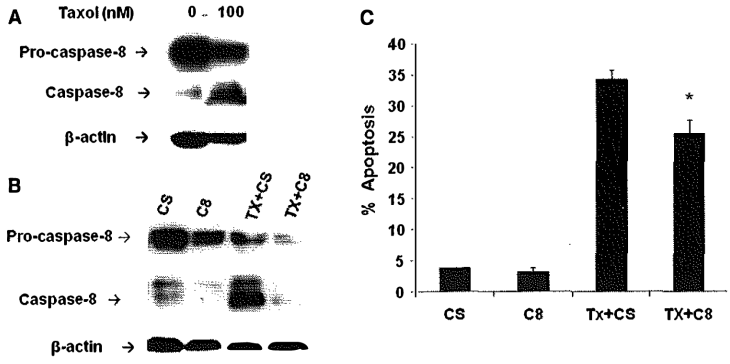

Fig. 4.

Taxol triggers caspase-8 mediated apoptosis in SKOV3 cells. **a** Cells were treated with 100 nM Taxol for 48 h, harvested, and immunoblot analysis of caspase-8 and *β*-actin was conducted. **b** Immunoblot analysis of caspase-8 and *β*-actin in cell lysates following treatment with 100 nM of non-targeting siRNA (CS) or caspase-8-specific siRNA (CS) with or without Taxol for 48 h. **c** Cells were transfected with 100 nM non-targeting siRNA (CS) or caspase-8-specific siRNA (CS) for 48 h, and treated with or without 100 nM Taxol at the same time, harvested, and apoptosis was detennined by FACS analysis as described in the section “Material and Methods”. Error bars show standard deviation from triplicate measurements. * $P < 0.05$. Immunoblot analysis of caspase-8 was conducted

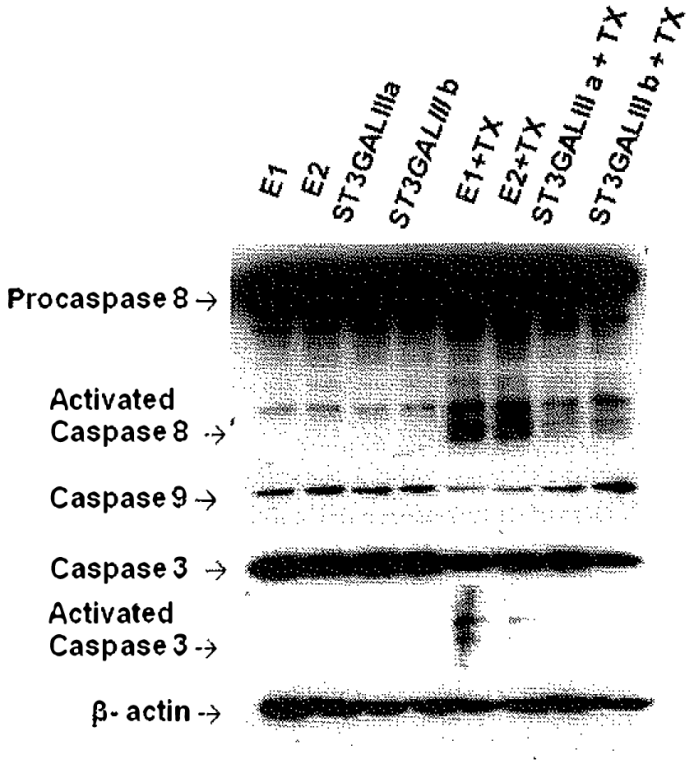

Fig. 5.

ST3Gal III inhibits Taxol-induced activation of caspases-8, -9, and -3. Two SKOV3 cell clones with the empty vector and two cell clones expressing ST3Gal-III transfectants were treated with 100 nM Taxol for 48 h, respectively, harvested, and caspases-8, -9, and -3 were detected by Western blot analysis as described in the section “Material and Methods”. Note that forced expression of ST3Gal III attenuates Taxol-induced apoptosis and caspase-8, -9, and -3 activation