Research Article

Solamargine upregulation of Fas, downregulation of HER2, and enhancement of cytotoxicity using epirubicin in NSCLC cells

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Nonsmall-cell lung cancer (NSCLC) is not generally a chemosensitive tumor, and the mechanism of resistance to the relevant anticancer drugs has not been fully elucidated. Solamargine (SM), the major steroidal glycoalkaloids extracted from the Chinese herb *Solanum*, inhibits the growth of human tumor cells. We have previously demonstrated that SM regulates tumor necrosis factor receptors (TNFRs)- and mitochondria-mediated pathways and sensitizes NSCLC cells to initiate apoptosis. Interestingly, this investigation reveals that SM up-regulated Fas expression and down-regulated the expression of HER2, whose overexpression is associated with resistance to drugs, and promotes chemotherapy-induced apoptosis in NSCLC A549 and H441 cells. After treatment with SM, the expression of HER2 mRNA was correlated with the expression of topoisomerase IIα (TOP2A) mRNA. The combinatory use of low concentrations of SM with low-toxic topoisomerase II inhibitor epirubicin accelerated apoptotic cell death. Therefore, the downregulation of the HER2 and TOP2A expression by SM with epirubicin may partially explain the SM and epirubicin cytotoxicity synergy effect in NSCLC. Results of this study suggest that SM induces Fas and TNFR-induced NSCLC cell apoptosis and reduces HER2 expression. These findings provide the synergistic therapeutic interaction between SM and epirubicin, suggesting that such combinations may be effectively exploited in future human cancer clinical trials.

Keywords: Epirubicin / Fas / HER2 / NSCLC / Solamargine

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1 Introduction

Lung cancer is the leading cause of death by cancer worldwide and its incidence continues to rise. Instances are divided into two groups by histological features – nonsmall cell lung cancer (NSCLC) and small cell lung cancer (SCLC). The sensitivities of these two types to treatment differ, perhaps because of their susceptibility to apoptosis [1]. NSCLC, the most prevalent subtype, is less sensitive to apoptosis, making it more resistant to cytotoxic chemotherapy and causing the treatment of patients to fail, whereas SCLC is more susceptible to apoptosis [2]. Adenocarcinoma is the most common of the NSCLC subtypes, and its frequency is rising in the world [3]. Therefore, NSCLC of the human adenocarcinoma A549 and H441 cells were studied and the actions of chemotherapy were also illustrated.

Aberrations in the apoptotic signaling promote tumorigenesis and underlie resistance of human tumors to anticancer agents. CD95/Fas, a member of the tumor necrosis factor receptor (TNFR) superfamily, is critical in apoptotic caspase cascade signaling in various cells [4]. Fas-signaling may be caused by the ligation of the Fas receptors by the Fas ligand (FasL) or by anti-Fas antibodies. Several tumor cells can escape Fas-mediated cell death by modifying the Fas receptor associated with FasL expression including

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Abbreviations: FasL, Fas ligand; **MTS,** [3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium]; **NSCLC,** nonsmall-cell lung cancer; **SCLC,** small cell lung cancer; **SM,** solamargine; **TNF,** tumor necrosis factor; **TNFR,** TNF-receptor; **TOP2A,** topoisomerase IIα

lung cancer cells [5]. We have previously established that solamargine (SM), a purified compound from *Solanum* plants, regulates the expressions of TNFRs and the mitochondrial-mediated pathway, and sensitizes lung cancer cells to the initiation of apoptosis [6]. The binding of death ligands to their receptors, including Fas (for FasL) and TNFRs (for TNF), triggers apoptosis by the recruitment of the adaptor protein Fas-associated death domain (FADD)/TNFR-1-associated death domain (TRADD), which promotes the recruitment of caspase-8 and -3, activating the cell death pathway [7]. These results support the hypothesis that SM may activate the Fas pathway, which may affect the chemosensitivity of NSCLC A549 and H441 cells to anticancer agents.

The HER2 oncogene (also known as c-erbB2) encodes a 185 kDa transmembrane tyrosine kinase with homology to epidermal growth factor receptor [8]. The amplification/overexpression of the human HER2 gene is commonly observed in human cancer, including NSCLC. It has been shown to be associated with poor patient survival in NSCLC [9]. Cellular and animal experiments have shown that promoting the expression of the HER2 gene can increase tumorigenicity and experimental metastasis in mouse embryo fibroblasts and human cancer cells [10]. Furthermore, the overexpression of HER2 in NSCLC has been recently reported to induce resistance to chemotherapeutic drugs such as cisplatin, doxorubicin, and epirubicin [11]. The association of HER2 overexpression in cancer cells with malignant phenotypes and chemoresistance provides a plausible explanation for the poor clinical outcome of patients with HER2-overexpressing tumors [12], indicating that the downregulation of HER2 in lung cancers may prevent drug resistance.

Epirubicin is a topoisomerase II inhibitor, and is employed as a base for single or combination chemotherapy to treat solid tumors, including NSCLC [13]; however, the development of drug resistance reduces its effectiveness. Factors that have been reported to affect resistance to epirubicin include the altered expression of topoisomerase II and integrins, changes in glutathione levels [14] and the expression of membrane-associated pumps such as *P*-glycoprotein encoded by the multidrug resistance gene MDR1 [15].

This study elucidates the success of chemotherapy that utilizes SM and epirubicin for NSCLC cells. (i) SM inhibits the growth of A549 and H441 cells; upregulates the expression of Fas, and downregulates the oncogene HER2. (ii) After treatment with SM, the mRNA expression of HER2 is greatly significantly correlated with topoisomerase IIα (TOP2A), and low concentrations of SM combination epirubicin caused significantly more apoptosis than either drug treatment alone. Therefore, the presented results suggest that SM upregulation of Fas and downregulation of HER2 expressions simultaneously downregulate TOP2A expression, the fact which may partially explain the synergy of SM with epirubicin cytotoxicity in NSCLC.

2 Materials and methods

2.1 Agents and cell culture

Epirubicin was purchased from Sigma Chemical Company (St. Louis, MO). SM was isolated from berries of *Solanum incanum*. SM was dissolved at a concentration of 60 μM in 100% DMSO as stock solution. Epirubicin (200 μM) was prepared with ddH_2O as stock solution. Stock solution was diluted to the desired final concentrations with growth medium just before use. The final DMSO or ddH_2O concentration did not exceed 0.1%. The human adenocarcinoma cell lines A549 and H441 were cultured in medium supplemented with 10% fetal bovine serum (Hazelton Product, Denver, PA, USA) and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin) at 37°C in 5% CO_2; specifically A549 cells in F-12 medium (GIBCO, Grand Island, NY) and H441 cells in RPMI 1640 medium (GIBCO).

2.2 Assessment of cell viability and apoptosis

Cells (1×10^4 cells/well) were seeded in each 100 μL of 96-well multidishes for at least 24 h prior to use. The cells were treated with serial concentrations of agents for 18 h. The effects on cell growth were examined by [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] (MTS) assay according to the manufacturer's procedure (CellTiter 96™ AQ, Promega, Madison, WI). Absorbance at 490 nm (A_{490}) was measured with an automated plate reader (Dydatech, Alexandria, VA). Values are expressed as the percentage of mean cell viability relative to the untreated cultures. The IC_{25} and IC_{50} were calculated from the drug concentration that induced 25 and 50% of cell survival rate. All determinations were performed in triplicate and statistically analyzed by Student's *t*-test. To assess specific apoptosis, cells were treated with SM (IC_{50}), epirubicin (9.2 μM) alone or in combination for 18 h. Cells were then fixed in 70% ethanol/PBS, pelleted, and resuspended in buffer containing 200 μg/mL RNase A and 0.01 mg/mL propidium iodide. $SubG_1$-peak was determined by flow cytometry analysis. The percentages of cells were determined using the WinMDI software. For fluorescent staining, after incubation with SM (IC_{50}) for 18 h, cells were fixed in 4% paraformaldehyde and permeabilized in saponin (0.1% v/v in PBS-BSA). The cell nuclei were stained with 0.1 lg/mL of 4,6-diamidino-2-phenylindole (DAPI) (Sigma) and inspected using a fluorescent microscope.

2.3 RT-PCR and Southern hybridization

RNA preparations were extracted form the cells treated with SM (IC_{50}) for serial times using the Qiagen RNeasy kit (Hilden, Germany), and RT-PCR was performed using 1 μg of total cellular RNA and the Superscript cDNA Preampli-

fication System (Clontech, Palo Alto, CA) in a Perkin-Elmer amplification cycler (Weiterstadt, Germany). Primers used were as follows: (i) Fas (264 bp), 5′-AGCTTGGTCTAGAGTGAAAA-3′, 5′-GAGGCAGAATCATGAGATAT-3′; (ii) HER2 (420 bp), 5′-TGCGGCTCGTACACAGGGACTT-3′, 5′-TGCGGAGAATTCAGACACCAACT-3′; (iii) TOP2A (596 bp), 5′-GCCCTCCTGCTACACATTTC-3′, 5′-AACACTTGGGCTTTACTTCACTT-3′. The RNA used for reverse transcription was controlled by the RT-PCR of human β-actin (286 bp) in the same conditions. The PCR conditions included an initial denaturation for 2 min at 95°C, 29 cycles consisting of (i) 1 min denaturation at 94°C; (ii) 1 min primer annealing at 60°C for Fas and HER2, 54°C for TOP2A and β-actin, respectively; (iii) 1 min elongation at 72°C; and (d) one final step of 5 min at 72°C. The amplified RT-PCR products were analyzed in 2% agarose gels and were visualized by ethidium bromide staining and photographed under UV illumination. For Southern hybridization assay, cDNA products were electrophoresed on agarose gel and transferred onto nylon membrane (BioRad, Richmond, USA). After prehybridization, the blot was hybridized with human monoclonal HER2 probes labeled with [^{35}S]-dCTP using randomly primed labeling procedure (Promega).

2.4 Detection of Fas and HER2 expressions

Cells were treated with SM (IC_{50}) for 18 h and the expressions of Fas and HER2 were studied after trypsinization. The cell surface expression of HER2 receptor was measured as the amount of selective binding sites for trastuzumab (1:100) (DAKO, Carpinteria, CA), a humanized anti-HER2 antibody containing the murine antigen binding loops and human variable region framework residues plus human IgG_1 constant domains, followed by secondary staining with an antibody reacting specifically with the Fc portion of the heavy chain of human IgG_1 (Molecular Probes). For immunoblotting, cells were harvested, washed with PBS, and incubated for 20 min at 4°C in 1 mL of lysis buffer containing 50 mM Tris-HCl, pH 7.5, 1% Triton X-100, 5 mM EGTA (ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid), 150 mM NaCl, and 1 mM PMSF. Cell lysates were cleared by centrifugation, separated by SDS-PAGE under reducing or nonreducing conditions, and immunoblotted with anti-Fas mAb (R&D Systems, MN, USA), trastuzumab and anti-β-actin (Sigma). For immunofluorescence analysis, cell surface expressions of HER2 were analyzed by fluorescent staining and flow cytometry. After incubation with SM (IC_{50}) for 18 h, cells were stained with trastuzumab in 1' PBS containing 0.5% BSA (PBS-BSA) and 0.1% sodium azide (Sigma–Aldrich) for 45 min at 4°C. Cells were then washed twice with cold PBS and incubated with FITC-conjugated antihuman IgG1 at 4°C for 30 min. Cells were washed with cold PBS and fixed in 4% formalin. The cell nuclei were stained with 0.1 μg/mL of DAPI and inspected using a fluorescent microscope. Two additional washing steps with cold PBS were performed before the cells were analyzed by FACScan flow cytometer (Becton–Dickinson) using WinMDI software.

2.5 Statistical analysis

To evaluate the statistical significance of the difference of all the values, statistical analysis was performed on the means of the triplicates of at least three independent experiments using a Student's *t*-test. *p* values less than 0.05 was considered significant for all tests.

3 Results

3.1 Dose dependence of SM-induced apoptosis in NSCLC A549 and H441 cells

SM was extracted from a Chinese herb, as reported above [16]. Figure 1A plots the effect of SM on the cell growth of A549 and H441 cells, as determined by the MTS assay. The treatment of A549 and H441 cells with serial concentrations of SM for 18 h inhibited cell proliferation, which was dose-dependent. The concentrations of SM that caused 50% cell death (IC_{50}) were approximately 5.46 μM for A549 and 5.85 μM for H441 cells. After incubation with SM (IC_{50}), the nuclei of A549 and H441 cells were stained with DAPI and exhibited typical nuclear condensation and apoptotic bodies (Fig. 1B).

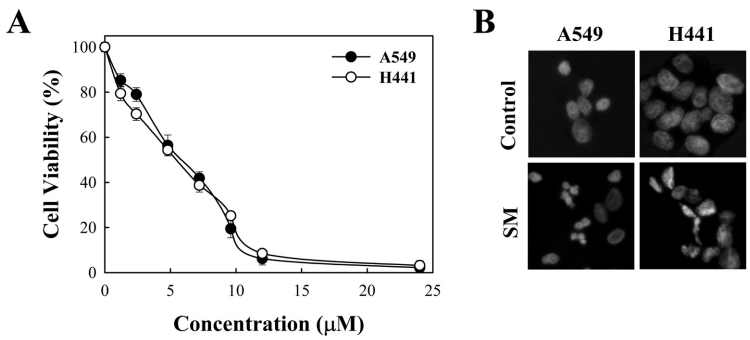

Figure 1. Dose dependence of SM-induced apoptosis in NSCLC cells. (A) Percentage of viable in A549 (•) and H441 (○) cells treated with serial concentrations of SM for 18 h. Cell viability was determined by MTS assay. Data are means ± SD from three independent experiments. (B) Cells were grown on coverslips and treated with SM (IC_{50}) for 18 h. The cells were then fixed in formaldehyde and DNA stained with DAPI. The nuclei of the cells were visualized using a fluorescent microscope (200 ×).

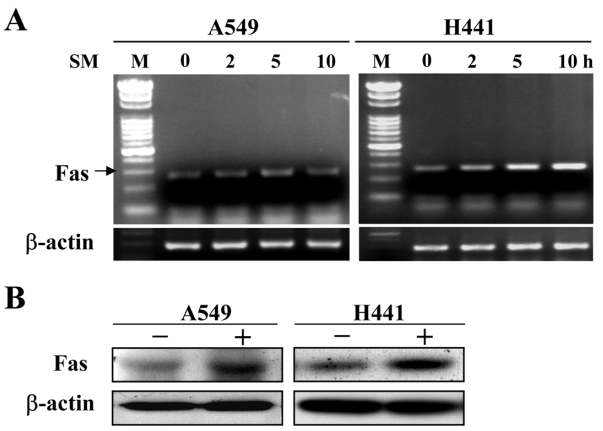

Figure 2. Expression of Fas on NSCLC cells after SM treatment. A549 and H441 cells were treated with SM (IC_{50}) for various periods. The expressions of Fas and β-actin on the cells were determined by RT-PCR (A) and immunoblot analyses (B) using specific cDNA primers and immunoblotting with anti-Fas mAb and anti-β-actin, as described in Section 2.

3.2 Apoptosis of SM-induced NSCLC cells required Fas expression

The apoptosis caused by chemotherapeutic agents can be mediated by Fas/FasL signaling pathway in numerous lung cancers [7]. The modulation of Fas expression in SM-treated A549 and H441 cells was investigated. As presented in Fig. 2A, adding SM (IC_{50}) up-regulated the gene expression of Fas in A549 and H441 cells according to RT-PCR. The results of immunoblot analysis of Fas were consistent with the levels of Fas mRNA and protein expression (Fig. 2B). These results demonstrate that SM might predominantly induce the NSCLC cells apoptosis through upregulation of Fas expression.

3.3 Effect of HER2 downregulation on growth inhibition by SM in NSCLC cells

Previous reports have demonstrated that HER2 overexpression is correlated with resistance to drugs [11], indicating that the down-regulation of HER2 in tumors may cause such resistance. The gene expression of HER2 in A549 and H441 cells with IC_{50} doses of SM was tested by RT-PCR analysis followed by Southern hybridization, to examine the effect of SM on HER2 that has been correlated with metastatic potential. As displayed in Figs. 3A and B, the ratios of HER2 to β-actin gene expressions revealed that cells treated with SM exhibited a level of significance of HER2 expression lower than the untreated control. The results were confirmed by immunoblot (Fig. 3C) and the binding of humanized anti-HER2 antibodies (trastuzumab) to the receptors on SM-treated cells by immunofluorescent analysis and flow cytomerty (Figs. 3D and E). Similar results for the downregulation of HER2 receptor expressions upon exposure to SM in A549 and H441 cells were obtained. Taken together, these results indicated that SM can reduce the gene and protein expressions of HER2 in NSCLC cells.

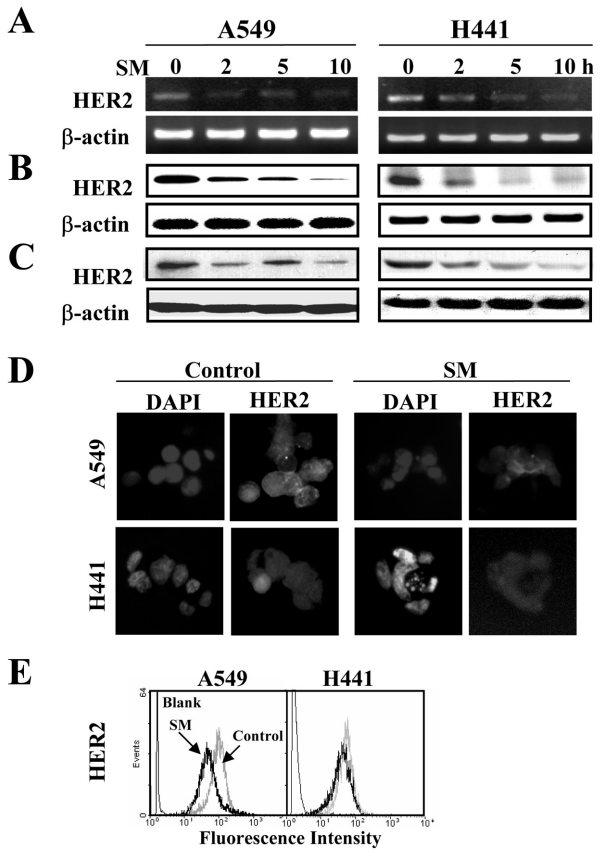

Figure 3. Downregulation of HER2 expression in NSCLC cells following SM treatment. (A) Cells were treated with SM (IC_{50}) at various times. The expressions of HER2 on cells were studied by RT-PCR (A), Southern hybridization (B), immunoblot (C), immunofluorescent analysis (D), and flow cytometry (E), as described in Section 2. The blank, the primary antibodies, and the secondary antibodies were replaced with 1 × PBS under the same conditions. Data are representative of three independent experiments.

3.4 SM-enhancement of epirubicin cytotoxicity is associated with downregulation of TOP2A expression

One of several mechanisms that may explain the resistance of HER2-overexpressing tumors to epirubicin is coregulation or coamplification of HER2 and TOP2A [17]. Following treatment with SM (IC_{50}) to study a possible correlation between mRNA expression of HER2 and TOP2A, an RT-PCR approach, using primers specific for HER2 and TOP2A (Fig. 4A), was performed. SM down-regulated the gene expression of TOP2A in A549 and H441 cells, as determined by comparison with the control, and a consider-

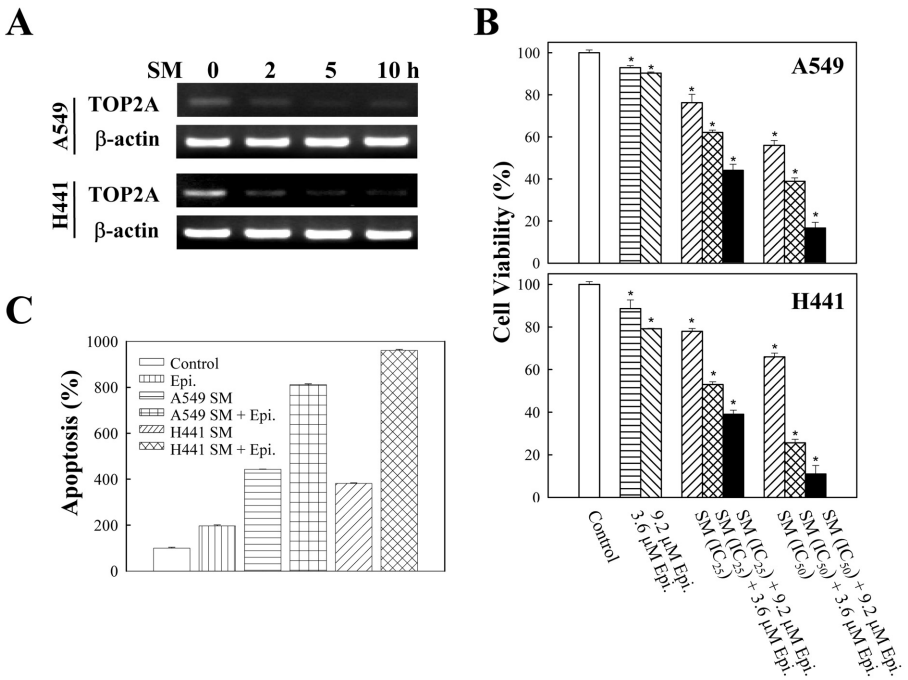

Figure 4. SM-enhanced epirubicin-induced cell cytotoxicity in NSCLC cells. (A) Expression of TOP2A on A549 and H441 cells after SM treatment. A constant amount of SM (IC_{50}) was added to the cells for various intervals. The expressions of TOP2A on cells were determined by RT-PCR. (B) The susceptibility of A549 and H441 cells to epirubicin was improved by adding SM. Constant concentrations of SM (IC_{25} and IC_{50}) and epirubicin (3.6 and 9.2 μM) alone or in combination were added to A549 and H441 cells for 18 h. The cytotoxicity was determined by MTS assay. Each value is the mean (SD of the percentage cell viability from triplicate determinations. *p <0.05 compared to the control values. (C) Percentage of cells in which apoptosis induced by SM (IC_{50}) and epirubicin (9.2 μM) alone or in combination for 18 h, as analyzed by flow cytometry for DNA fragmentation using nuclear staining with propidium iodide. The percentage of the specific apoptotic subG$_1$-peak was calculated using WinMDI software. The results are obtained from a representative experiment of three independent experiments.

able correlation was obtained between the mRNA expression of TOP2A and HER2.

The treatment of A549 and H441 cells with SM alone or in combination with epirubicin was observed to elucidate whether chemosensitivity to epirubicin can be influenced by the inhibition of HER2 and/or TOP2A (Fig. 4B). Surprisingly, low concentrations of SM plus epirubicin inhibited the proliferation of A549 cells as potently as did drug treatment alone. Similar results were observed with H441 cells. These results were confirmed by the appearance of a subG$_1$ population, revealed by flow cytometry; the combination of SM with epirubicin resulted in more beneficial apoptotic cell death than with individual agents alone (Fig. 4C). Hence, the results herein suggest that the downregulation of HER2 and TOP2A expressions by SM with epirubicin may partially explain the synergistic cytotoxicity effect in NSCLC.

4 Discussion

Using herbs to treat malignant diseases has been dramatically rising in recent years. Members of the *Solanaceae* family of plants synthesize secondary metabolites such as glycoalkaloids and polypenols, presumably to protect themselves against phytopathogens. These plants include potatoes, tomatoes, and eggplants. SM is a major glycoalkaloid that is found in eggplants and at least 100 other *Solanum* species [18]. This investigation presents the action mechanism of SM in NSCLC A549 and H441 cells.

A role of the Fas/FasL signaling system in apoptosis induced by chemotherapy has been proposed for some lung cancer cell types. The expression of Fas and FasL on the surface of NSCLC cells is up-regulated after exposure to anticancer agents, and apoptosis is induced in some cell lines upon exposure to the Fas agonistic mAb [19]. The overexpression of the HER2 oncogene is commonly associated with several cancers, including lung cancer. A large body of evidence indicates that HER2 overexpression is associated with elevated tumorigenicity in nude mice xenograft models, enhanced metastatic potential, increased resistance to ligation of death receptors by TNF, FasL, and TRAIL, and in particular circumstances, resistance to chemotherapy [20]. Accordingly, cancer cells that overexpress HER2 are therefore an excellent target for the development of anticancer therapies. For instance, an anti-HER2 anti-

body (trastuzumab, Herceptin™) has been used clinically as a potent growth inhibitor of such breast and lung cancer cells [21], and previous research has demonstrated that the overexpression of HER2 upregulates $p21^{Waf1}$ and leads to resistance by these cancer cells to Taxol [22]. The DNA-binding protein PEA3, which is encoded by a previously isolated gene of the *ets* family, specifically targeted a DNA sequence on the HER2 promoter and down-regulated the promoter activity. The expression of PEA3 resulted in the preferential inhibition of cell growth and the development of a tumor by HER2-overexpressing cancer cells [23]. Antisense oligonucleotides have been adopted to suppress the *in vitro* proliferation of human cancer cells in which HER2 is amplified and tumorigenicity is inhibited *in vivo* [24]. Moreover, previous studies have shown that Akt was constitutively activated in HER2-overexpressing cancer cells and that Akt/NF-κB activity was required to establish the resistance of these cells to TNF-induced apoptosis [25]. Hence, HER2-overexpressing cancer cells are more resistant to TNF-induced apoptosis, leading to poor prognosis and shortening the survival time of patients. Our laboratory has recently established that SM can modulate TNFRs- and mitochondria-related Bcl-2/Bcl-x_L and Bax expression, and overcome the resistance to TNFs and cisplatin in lung cancer cells [26]. Interestingly, in this study, SM's upregulation of Fas and its downregulation of HER2 expression inhibited cell growth, according to RT-PCR, Southern hybridization, immunoblot, immunofluorescent analysis, and flow cytometry. These findings suggest that SM-enhanced sensitization to TNFR- and Fas-mediated pathway was associated with the suppression of HER2 expression and the induction of cell death in NSCLC A549 and H441 cells.

Cytotoxic chemotherapy remains an important part of optimal therapy for patients in all stages of cancers, but its use is limited by toxicity, nonspecificity, inevitable development of resistance, and serious consequences on the patients' quality of life [27]. Current chemotherapeutic agents can not only kill cancer cells, but also express toxicity for normal cells [28]. Recent strategies including coadministered modulating agents, hammerhead ribozymes and antisense oligonucleotides may increase the specificity in cell targeting. The strategies may be used to reduce drug resistance and increase drug bioavailability to improve the profile of chemotherapeutic efficacy *versus* toxicity [29]. Chemotherapeutic agents can compromise the survival of the graft. Drugs that are effective at low doses could therefore, not only give fewer side effects, but also provide a better graft survival [30]. In this regard, this study uses SM to upregulate the Fas death receptor and downregulate the HER2 resistant gene, and consequently enhanced the efficacy of epirubicin in NSCLC cells. Thus, the effective dose of epirubicin in the treatment of NSCLC may be reduced.

The mechanism by which HER2 may be associated with resistance to chemotherapy is coampliferation or coregulation with TOP2A. The chromosomal location of both HER2 and TOP2A is 17q21–22. Amplifications occur over large segments of the chromosome and may comprise many mega bases. TOP2A was found to be coamplified in 12% of HER2; other investigators have obtained similar results [31]. Therefore, a subgroup of patients with HER2 overexpression is likely to exhibit a high TOP2A activity. A previous investigation reported that tumor cell lines that are resistant to topoisomerase-targeting drugs, such as doxorubicin with down-regulated TOP2A can simultaneously downregulate the oncogene HER2 and thus become sensitive to TNF and FasL [32]. This study demonstrated that low concentrations of SM augment epirubicin cytotoxicity, achieving a greater therapeutic effect than that expected by the simple addition of the effects of the component drugs. This result is associated with the downregulation of coregulation and the coamplification of HER2 and TOP2A expression, which potentially result in prominent synergistic effects on the A549 and H441 cells. These findings provide additional insight into the synergistic therapeutic interaction between SM and epirubicin, suggesting that such combinations can be successfully exploited in future human clinical trials.

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5 References

[1] Bergqvist, M., Brattstrom, D., Gullbo, J., Hesselius, P. *et al.*, p53 status and its in vitro relationship to radiosensitivity and chemosensitivity in lung cancer, *Anticancer Res.* 2003, *23*, 1207 – 1212.

[2] Frasci, G., On behalf of the Southern Italy Cooperative Oncology Group (SICOG), Chemotherapy of lung cancer in the elderly, *Crit. Rev. Oncol. Hematol.* 2002, *41*, 349 – 361.

[3] Shivapurkar, N., Reddy, J., Chaudhary, P.-M., Gazdar, A.-F., Apoptosis and lung cancer: A review, *J. Cell. Biochem.* 2003, *88*, 885 – 898.

[4] Sharov, A.-A., Siebenhaar, F., Sharova, T.-Y., Botchkareva, N.-V. *et al.*, Fas signaling is involved in the control of hair follicle response to chemotherapy, *Cancer Res.* 2004, *64*, 6266 – 6270.

[5] Du, A., Zhao, B., Miao, J., Yin, D. *et al.*, Safrole oxide induces apoptosis by activating caspase-3, -8, and -9 in A549 human lung cancer cells, *Bioorg. Med. Chem.* 2006, *14*, 2438 – 2445.

[6] Liang, C.-H., Liu, L.-F., Shiu, L.-Y., Huang, Y.-S. *et al.*, Action of solamargine on TNFs and cisplatin-resistant human lung cancer cells, *Biochem. Biophys. Res. Commun.* 2004, *24*, 751 – 758.

[7] Zheng, L., Bidere, N., Staudt, D., Cubre, A. *et al.*, Competitive control of independent programs of tumor necrosis factor receptor-induced cell death by TRADD and RIP1, *Mol. Cell. Biol.* 2006, *26*, 505 – 3513.

[8] Knowlden, J.-M., Hutcheson, I.-R., Jones, H.-E., Madden, T. *et al.*, Elevated levels of epidermal growth factor receptor/c-erbB2 heterodimers mediate and autocrine growth regulatory pathway in tamoxifen-resistant MCF-7 cells, *Endocrinology* 2003, *144*, 1032 – 1044.

[9] Kristiansen, G., Yu, Y., Petersen, S., Kaufmann, O. *et al.*, Overexpression of c-erbB2 protein correlates with disease-stage and chromosomal gain at the c-erbB2 locus in nonsmall cell lung cancer, *Eur. J. Cancer* 2001, *37*, 1089 – 1095.

[10] Andrechek, E.-R., Hardy, W.-R., Siegel, P.-M., Rudnicki, M.-A. *et al*., Amplification of the neu/erbB-2 oncogene in a mouse model of mammary tumorigenesis, *Proc. Natl. Acad. Sci. USA* 2000, *97*, 3444 – 3449.

[11] Campiglio, M., Somenzi, G., Olgiati, C., Beretta, G. *et al.*, Role of proliferation in HER2 status predicted response to doxorubicin, *Int. J. Cancer* 2003, *105*, 568 – 573.

[12] Azzoli, C.-G., Krug, L.-M., Miller, V.-A., Kris, M.-G. *et al.*, Trastuzumab in the treatment of nonsmall cell lung cancer, *Semin. Oncol.* 2002, *29*, 59 – 65.

[13] Behnam, M.-P., Henriksson, R., Grankvist, K., Interaction of the antiemetics ondansetron and granisetron with the cytotoxicity induced by irradiation, epirubicin, bleomycin, estramustine, and cisplatin in vitro, *Oncologica* 1995, *34*, 871 – 875.

[14] Yu, D.-S., Ma, C.-P., Chang, S.-Y., Establishment and characterization of renal cell carcinoma cell lines with multidrug resistance, *Urol. Res.* 2000, *28*, 86 – 92.

[15] Huesker, M., Folmer, Y., Schneider, M., Fulda, C. *et al.*, Reversal of drug resistance of hepatocellular carcinoma cells by adenoviral delivery of anti-MDR1 ribozymes, *Hepatology* 2002, *36*, 874 – 884.

[16] Liu, L.-F., Liang, C.-H., Shiu, L.-Y., Lin, W.-L. *et al.*, Action of solamargine on human lung cancer cells – enhancement of the susceptibility of cancer cells to TNFs, *FEBS Lett.* 2004, *577*, 67 – 74.

[17] Jarvinen, T.-A., Liu, E.-T., HER-2/neu and topoisomerase IIalpha – simultaneous drug targets in cancer, *Comb. Chem. High Throughput Screen.* 2003, *6*, 455 – 470.

[18] Roddick, J.-G., Weissenberg, M., Leonard, A.-L., Membrane disruption and enzyme inhibition by naturally-occurring and modified chacotriose-containing Solanum steroidal glycoalkaloids, *Phytochemistry* 2001, *56*, 603 – 610.

[19] Odoux, C., Albers, A., Amoscato, A.-A., Lotze, M.-T. *et al.*, TRAIL, FasL and a blocking anti-DR5 antibody augment paclitaxel-induced apoptosis in human nonsmall-cell lung cancer, *Int. J. Cancer* 2002, *97*, 458 – 465.

[20] Ueno, N.-T., Bartholomeusz, C., Herrmann, J.-L., Estrov, Z. *et al.*, E1A-mediated paclitaxel sensitization in HER-2/neu-overexpressing ovarian cancer SKOV3.ip1 through apoptosis involving the caspase-3 pathway, *Clin. Cancer Res.* 2000, *6*, 250 – 259.

[21] Nahta, R., Trent, S., Yang, C., Schmidt, E.-V., Epidermal growth factor receptor expression is a candidate target of the synergistic combination of trastuzumab and flavopiridol in breast cancer, *Cancer Res.* 2003, *63*, 3626 – 3631.

[22] Bacus, S.-S., Gudkov, A.-V., Lowe, M., Lyass, L. *et al.*, Taxol-induced apoptosis depends on MAP kinase pathways (ERK and p38) and is independent of p53, *Oncogene* 2001, *11*, 147 – 155.

[23] Xing, X., Wang, S.-C., Xia, W., Zou, Y. *et al.*, The ets protein PEA3 suppresses HER-2/neu overexpression and inhibits tumorigenesis, *Nat. Med.* 2000, *6*, 189 – 195.

[24] Casalini, P., Menard, S., Malamdrin, S.-M., Rigo, C.-M. *et al.*, Inhibition of tumorigenicity in lung adenocarcinoma cells by c-erbB-2 antisense expression, *Int. J. Cancer* 1997, *72*, 631 – 636.

[25] Zhou, B.-P., Hu, M.-C., Miller, S.-A., Yu, Z. *et al.*, HER-2/neu blocks tumor necrosis factor-induced apoptosis via the Akt/NF-kappaB pathway, *J. Biol. Chem.* 2000, *275*, 8027 – 8031.

[26] Barbashina, V., Benevenia, J., Aviv, H., Tsai, J. *et al.*, Oncoproteins and proliferation markers in synovial sarcomas: A clinicopathologic study of 19 cases, *J. Cancer Res. Clin. Oncol.* 2002, *128*, 610 – 616.

[27] Tonini, G., Schiavon, G., Silletta, M., Vincenzi, B. *et al.*, Antiangiogenic properties of metronomic chemotherapy in breast cancer, *Future Oncol.* 2007, *3*, 183 – 190.

[28] Galderisi, M., Marra, F., Esposito, R., Lomoriello, V.-S. *et al.*, Cancer therapy and cardiotoxicity: The need of serial Doppler echocardiography, *Cardiovasc. Ultrasound* 2007, *5*, 4.

[29] Hardwick, L.-J., Velamakanni, S., van Veen, H.-W., The emerging pharmacotherapeutic significance of the breast cancer resistance protein (ABCG2), *Br. J. Pharmacol.* 2007, *151*, 163 – 174.

[30] Markasz, L., Stuber, G., Flaberg, E., Jernberg, A.-G. *et al.*, Cytotoxic drug sensitivity of Epstein-Barr virus transformed lymphoblastoid B-cells, *BMC Cancer* 2006, *6*, 265.

[31] Park, K., Kim, J., Lim, S., Han, S., Topoisomerase II-alpha (topoII) and HER2 amplification in breast cancers and response to preoperative doxorubicin chemotherapy, *Eur. J. Cancer* 2003, *39*, 631 – 634.

[32] Sleijfer, S., Asschert, J.-G., Timmer-Bosscha, H., Mulder, N.-H., Enhanced sensitivity to tumor necrosis factor-alpha in doxorubicin-resistant tumor cell lines due to down-regulated c-erbB2, *Int. J. Cancer* 1998, *77*, 101 – 106.

