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ORIGINAL ARTICLE

Suppression of STAT3 by chemically modified siRNAs increases the chemotherapeutic sensitivity of parental and cisplatin-resistant non-small cell lung cancer cells

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Summary

Purpose: *Increased activation of the JAK-STAT signaling pathway is frequently observed in several primary cancers as well as cancer cell lines. Thus, targeting JAK-STAT pathway components by different molecular-biologic approaches in the search for new anticancer therapies has become widespread and resulted in encouraging outcomes. In this study, the effects of chemically modified anti-STAT3 small interfering (si)RNAs on cell viability, proliferation and apoptosis of parental and cisplatin resistant non-small cell lung cancer (NSCLC) cells were investigated with the aim to provide a new therapeutic strategy for overcoming cisplatin resistance in lung cancer.*

Methods: *The parental NSCLC cell line Calu1 and its cisplatin-resistant subline CR-Calu1 were used to study the effects of STAT3 suppression with chemically modified anti-STAT3 siRNAs. STAT3 gene and protein expressions were analyzed by real-time (RT) quantitative (q) PCR and Western blot, respectively. Apoptosis was evaluated by Caspase-3 activity and cell death assays.*

Results: *STAT3 messenger (m)RNA and protein expression were significantly increased in CR-Calu1 cells and suppressing its expression with specific siRNAs increased the rate of apoptosis through Caspase-3 activation. STAT3 suppression also significantly increased cisplatin sensitivity of Calu1 and CR-Calu1 cells after transfection with STAT3 siRNAs.*

Conclusions: *NSCLC cells could be sensitized to cisplatin by targeting STAT3 with chemically modified siRNAs together, a fact which was accompanied with increased apoptosis.*

Key words: *Calu1, chemically modified siRNA, cisplatin resistance, non-small cell lung cancer, STAT3*

Introduction

Lung cancer is the most common malignancy and leading cause of cancer-related mortality worldwide in both men and women [1]. Successful treatment of NSCLC, which represents 80-85% of all lung cancers, is limited in late stages mostly due to intrinsic resistance to cytotoxic chemotherapeutic agents [2]. Cisplatin is widely used in the treatment of lung cancer and exerts its cytotoxic effects by DNA damage. Nevertheless, its efficacy gets limited by extended use due to acquired drug-resistance [3-6].

Signal transducer and activator of transcription (STAT) proteins are latent cytoplasmic transcription factors that can be activated through the JAK-STAT signaling pathway by cytokines, growth factors and other chemical messengers. Activated STATs form homo- or heterodimers, translocate into the nucleus and regulate the expression of various genes by binding to their promoter regions [7,8]. In this way, STATs can control many important signaling cascades that are also involved in the process of carcinogenesis like cell cycle, angiogenesis, apoptosis, invasion and metastasis [9,10]. Among STATs (STAT1 to 6), while STAT1 exerts a pro-apoptotic function, STAT3 and STAT5 (5A and 5B) take part in the activation of malignant transformations. Although STAT5 is widely known to be related with hematological

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malignancies [10], there is evidence that its increased expression may serve as favorable prognostic biomarker for lung cancer patients treated with surgery [11]. Yet, STAT3 is found upregulated in some cisplatin resistant cell lines [12]. Since STAT3 has been shown as an apoptotic pathway inhibitor [13], it has become one of the most crucial anti-lung cancer agent thereafter [13].

Gene silencing by RNA interference mechanism has become a very promising process in biomedical and life sciences area during the past few years. In this mechanism, siRNA molecules specifically bind mRNA and then cleave transcripts of target genes when introduced into cells, thereby causing full or partial loss of gene function [14]. Because varying expression profiles affect cellular fateful events, siRNAs have become good therapeutic targets [15]. Chemically modified siRNAs can manage RNA interference mechanism stably and efficiently for longer periods rather than unmodified ones because they are more stable to endonucleases [16]. Owing to these facts, chemically modified siRNA usage is commonly preferred in gene silencing experiments for achieving higher suppression rates both at mRNA and protein levels [17,18]

In this study, we first aimed to evaluate the mRNA expression values of target STAT3, STAT5A and STAT5B genes in both parental and cisplatin-resistant Calu1 cells, in order to identify the one that exhibited maximum upregulated expression pattern in the gain of resistance. Since STAT3 displayed the most dysregulated expression level in the resistant cells, we focused on it in the subsequent experiments. Second, we examined whether chemotherapeutic sensitivity could be provided in the parental and cisplatin-resistant Calu1 cells by suppressing STAT3 expression with chemically modified siRNAs. Finally, we aimed to identify whether this application provokes suppressive regulation on apoptosis which leads to increased cell death.

Methods

Cell lines and culture conditions

The parental human NSCLC epidermoid carcinoma cell line Calu1 was purchased from the American Type Culture Collection (ATCC, Manassas, USA) and its cisplatin-resistant subline CR-Calu1 was established as previously described [19]. Soluble cisplatin (10 mg/20 ml) was obtained from EBEWE Pharma (Attersee, Austria). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) heat inactivated fetal calf serum, 100 U of penicillin and 100 µg streptomycin/ml, and 1% L-glutamine at 37 ^{0}C in humidified air containing 5% CO_2. Before each experiment, the acquired cisplatin-resistance of CR-Calu1 cells was confirmed with the WST-1 cytotoxicity assay.

Determination of STAT3-STAT5A-STAT5B gene expression in parental and cisplatin-resistant cells by real-time quantitative RT-PCR

The mRNA expression level of STAT3 (NM_003150), STAT5A (NM_003152) and STAT5B (NM_012447) in Calu1 and CR-Calu1 cells was analyzed by RT-qPCR. For this assessment, first total RNA was isolated from the cells by using the MagNAPure LC RNA Isolation Kit (Roche Applied Science, Mannheim, Germany), and then was reverse-transcribed into cDNA by using the Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Science, Mannheim, Germany). RT-qPCR was performed with gene-specific primer and probe pairs by using the LightCycler Fast Start DNA Master Hybridization Probes Kit and the LightCycler® 2.0 Instrument (Roche Applied Science, Mannheim, Germany). Relative expression levels of the target genes were determined by dividing their mRNA copy number to the mRNA copy number of the housekeeping gene glucose-6-phosphate dehydrogenase (G6PDH). For these calculations a standard curve was obtained with reference standards ranging from 5×10^2 to 5×10^6 mRNA copy numbers of G6PDH and were included into each run [20].

Gene silencing by siRNA transfection

For gene silencing, the Dharmafect-1 Transfection Reagent DF-1 (Dharmacon, Chicago, IL, USA) was used in combination with chemically modified anti-STAT3 siRNAs (100 nM final concentration; Dharmacon, Chicago, IL, USA). The sugar modification on anti-STAT3 siRNAs, obtained by a FluoroUridine substation with FluoroCytidine and applied to the 2'-ribose region of all nucleotides (STAT3/2'-FU/FC), increases the binding affinity to target STAT3 mRNAs and reduces off-target effects. The reason for its preferred use was also that siRNAs with this modification are more stable than endonucleases and thus gene silencing is achieved for longer periods without the need of extra transfections. Non-targeting siRNA treated (NT) and untransfected (UT) cells were used as controls in each experiment. The sequences of used anti-STAT3 siRNAs were as follows: STAT3-S: 2'-F-C.2'-F-C.A.A.2'-F-C.A.A.2'-F-U.2'-F-C.2'-F-C.2'-F-.A.A.G.A.A.2'-F-U.G.2'-F-U.2'-F-U.2'-F-U; and STAT3-AS: A.2'-F-C.A.2'-F-U.2'-F-U.2'-F-C.2'-F-U.2'-F-U.G.G.G.A.2'-F-U.2'-F-U.G.2'-F-U.2'-F-U.G.G.2'-F-U.2'-F-U. Optimal silencing effects were reached when cells were incubated for 72 h at 37 ^{0}C and 5% CO_2. Untransfected control and anti-STAT3 siRNA transfected Calu1 and CR-Calu1 cells were used for the RT-qPCR, Western blot, WST-1 cell proliferation, and apoptosis assays.

Assessment of cell viability and proliferation by the WST-1 cytotoxicity assay

Cell viability and proliferation of untransfected controls and of anti-STAT3 siRNA transfected Calu1 and CR-Calu1 cells were determined by using the colorimetric WST-1 assay. Metabolically active cells cleave the tetrazolium salts to formazan dye by cellular enzymes. Quantification of the formazan produced by metabolically active cells by a scanning multiwell spectrophotometer, correlates to the number of metabolically active cells in the culture.

Briefly, 1×10^5 cells were plated onto each well of a 96-well plate with 100 µl medium containing 4% fetal bovine serum (FBS) for 24 h. Then, they were either not treated or treated with increased doses of cisplatin for 24, 48 and 72 h. Afterwards, performing the proliferation assay, the absorbance of each well was measured spectrophotometrically at 450 nm with an ELISA reader (Thermo, Vantaa, Finland). All experiments were performed in triplicate. The obtained data were evaluated with the GraphPad Prism 5.01, cell proliferation curves were generated and IC_{50} concentrations were calculated for both Calu1 and CR-Calu1 cells.

Apoptosis assay

Apoptosis of cisplatin-treated and untreated, anti STAT3 siRNA transfected Calu1 and CR-Calu1 cells was assessed by measuring Caspase-3 activity and the relative quantification of histone-complexed DNA fragments. Four different groups for each of the Calu1 and CR-Calu1 cells were generated for analyzing apoptosis: 1) untransfected control group; 2) anti-STAT3 siRNA transfected group; 3) cisplatin-treated group (10 µM for Calu1, 100 µM for CR-Calu1); and 4) anti-STAT3 siRNA transfected (72 h) and cisplatin-treated (24 h) group.

Caspase-3 activity was measured in 1×10^6 cells by using the Caspase-3 Colorimetric Assay Kit (BioVision Research Products, Mountain View, CA, USA). This assay allows for spectrophotometric detection of the chromophore p-nitroaniline (pNA) after cleavage from the labeled substrate DEVD-pNA. The pNA light emission can be quantified using a spectrophotometer at 405-nm. Comparison of the absorbance of pNA from untreated and treated samples allows for determination of the fold increase in Caspase-3 activity.

Briefly, cells were collected and resuspended in chilled lysis buffer. After centrifugation the supernatant was aliquoted and the protein concentrations were measured. For reaching equal amounts of protein (40 µg/ml), samples were diluted with cell lysis buffer. Before samples were incubated at 37 ^{0}C for 2 h, reaction buffer and substrate were added. Finally, absorbance was measured spectrophotometrically at 405 nm with an ELISA reader (Thermo, Vantaa, Finland).

Apoptotic cell rate was also quantified with a photometric enzyme immunoassay by using the Cell Death Detection ELISA Kit (Roche Applied Science, Mannheim, Germany) which allows for the detection of mono- and oligonucleosomes and measures apoptotic cell death. For this purpose, 5×10^4 cells were collected and resuspended in incubation buffer. After 30 min of incubation, the lysate was centrifuged and 100 µl of the supernatant was 1:10 pre-diluted with incubation buffer. Coating solution and incubation buffer were added into the wells of the MP-modules but rinsed with washing buffer before adding the sample solution. After incubation for 90 min at room temperature, conjugate and substrate solutions were added. The MP-modules were put on a shaker and incubated at 300 rpm for 10 min. Finally, absorbance was measured spectrophotometrically at 405 nm with an ELISA reader (Thermo, Vantaa, Finland). Apoptotic cell rates were calculated after the average values of duplicated absorbance measurements of samples were taken and the background value was subtracted from each of these average values.

Determination of STAT3 protein expression in parental and cisplatin-resistant cells by Western blot analysis

STAT3 protein expression in untransfected controls and of anti-STAT3 siRNA transfected Calu1 and CR-Calu1 cells was determined by Western blot analysis. For this purpose, cells were lysed in complete Lysis-M buffer containing Protease Inhibitor Cocktail Tablets (Roche Applied Science, Mannheim, Germany). Protein concentrations were measured with the Bradford method by using bovine serum albumin (BSA) standards in concentrations between 0.25 and 2 mg/ml. Forty µg of protein extract per sample were loaded and run on 8% SDS-PAGE gels and afterwards transferred to PVDF membranes by using the I-Blot System (Invitrogen Corporation, Carlsbad, CA, USA). Primary antibodies used were polyclonal STAT3 (1:500 diluted; Upstate), phospho-STAT3 Tyr705 (1:500 diluted; Cell Signaling Technology, USA), and β-Actin (1:2500 diluted; Cell Signaling Technology, USA). For chemiluminescent detection of the proteins the iBlot® Western Detection Chemiluminescent Kit (Anti Rabbit) [(Invitrogen Corporation Carlsbad, CA, USA)] was used together with the Kodak Gel Imaging System (KODAK Gel Logic 440 Digital Imaging System, Leander, Texas, United States).

Statistics

Gene silencing experiments were triplicated and the average of relative expression values was taken at the end of RT-qPCR analyses. Statistical analyses were performed by using the Student's t test and a p value of <0.05 was defined as statistically significant with the SPSS 15.0 Software. Cisplatin IC_{50} concentrations were calculated with the GraphPad Prism Software 5.01.

Results

To examine the relationship between STAT3, STAT5A and STAT5B expression pattern and cis-

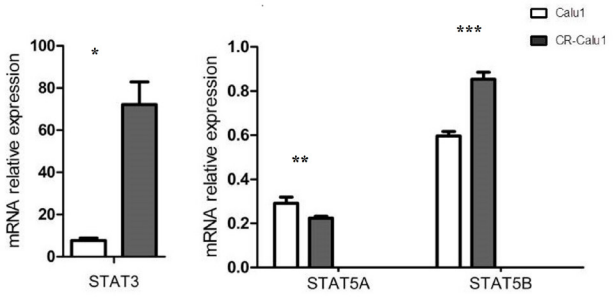

Figure 1. STAT3, STAT5A and STAT5B messenger RNA expressions in parental Calu1 and cisplatin resistant CR-Calu1 cells. (*p=0.0005; **p=0.173, ***p=0.09).

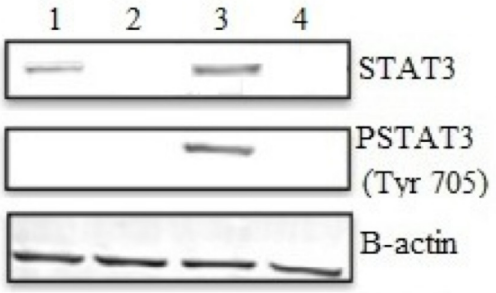

Figure 2. STAT3 protein expression in parental Calu1 and cisplatin resistant CR-Calu1 cells before and after suppression with chemically modified siRNAs. Line 1: Parental Calu1; Line 2: STAT3 siRNA treated parental Calu1; Line 3: Cisplatin resistant CR-Calu1; Line 4: STAT3 siRNA treated CR-Calu1.

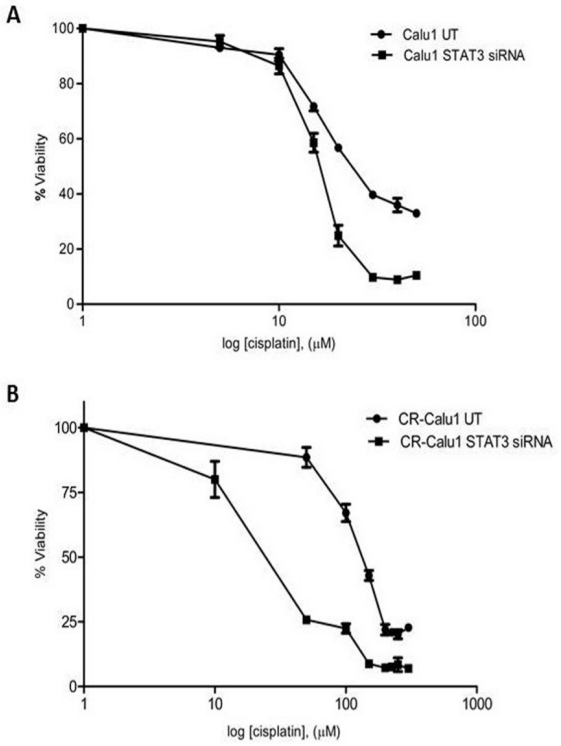

Figure 4. Cell viability and proliferation of parental Calu1 and cisplatin resistant CR-Calu1 cells before and after STAT3 suppression with chemically modified siRNAs. Cell viability and proliferation were determined by using the WST-1 cytotoxicity assay at 24, 48 and 72h time-intervals. Data represent the mean±SD of 3×wells at 72h. **A:** Cisplatin IC_{50} values for untransfected Calu1 (Calu1-UT) and anti-STAT3 siRNA transfected Calu1 (Calu1-STAT3-siRNA) cells were 17.23 μM and 15.50 μM, respectively (p<0.0001); and for **B:** untransfected CR-Calu1 (CR-Calu1-UT) and anti-STAT3 siRNA transfected CR-Calu1 (CR-Calu1-STAT3-siRNA) cells 116.80 μM and 17.52 μM, respectively (p<0.0001).

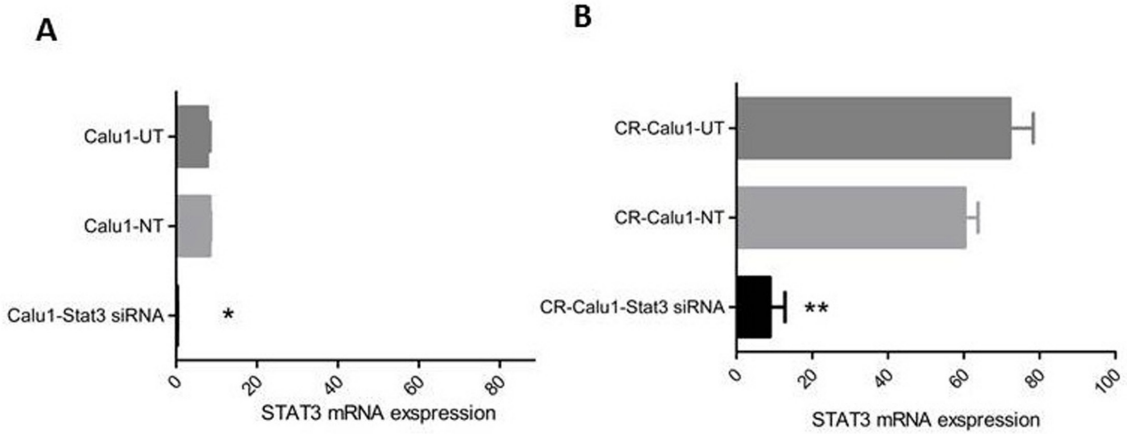

Figure 3. STAT3 gene expression in parental Calu1 and cisplatin resistant CR-Calu1 cells before and after suppression with chemically modified siRNAs. Results are based on 3 separate experiments and are expressed as the mean ratios to the G6PDH housekeeping gene. **A:** STAT3 messenger RNA expression in cisplatin sensitive Calu1 cells (*p=0.0004); and **B:** cisplatin resistant CR-Calu1 cells (**p=0.001).The p value between Calu1-UT and CR-Calu1-UT cells was equal to 0.0005 that stands for differing expression of STAT3 in parental and resistant cells. UT: Untransfected cells; NT: Non-targeting siRNA transfected cells.

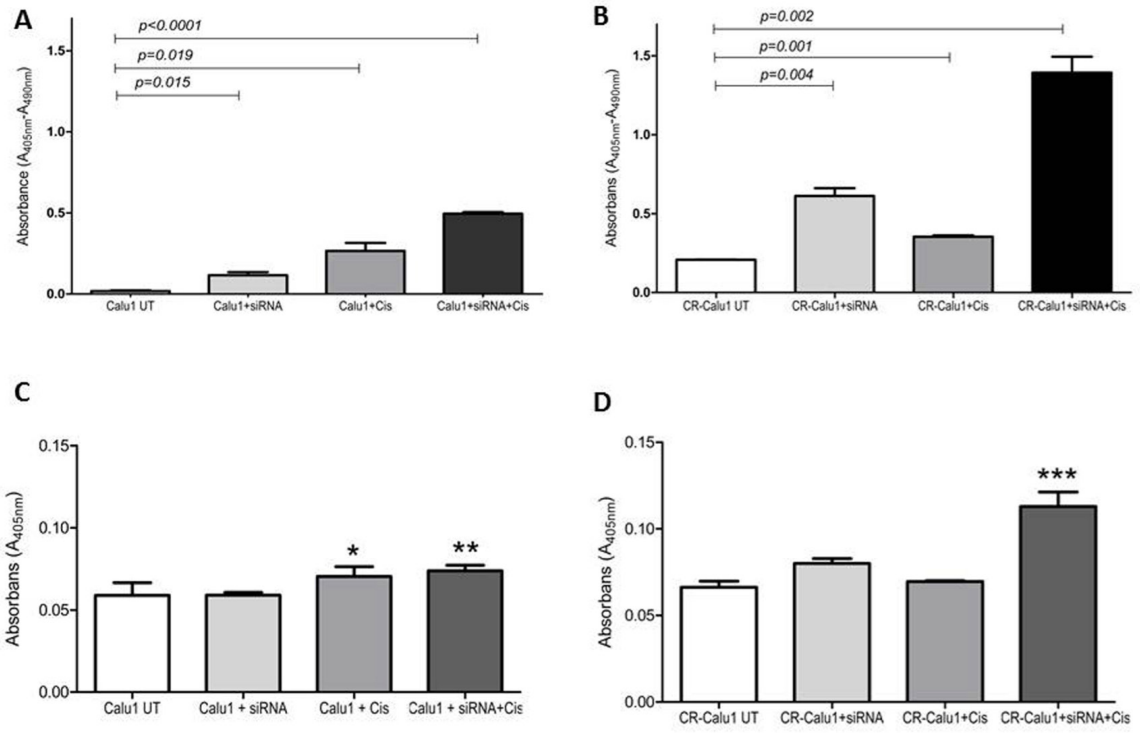

Figure 5. Apoptosis of parental Calu1 and cisplatin resistant CR-Calu1 cells before and after STAT3 suppression with chemically modified siRNAs. **A and B:** Histone-complexed DNA fragments: Detection of mono- and oligonucleosomes in the cytoplasm of untransfected, anti-STAT3 siRNA transfected, cisplatin treated, and anti-STAT3 siRNA transfected and cisplatin treated cells. Statistical significance was determined by using the Student's t test; **C and D:** Caspase-3 activity: Comparison of Caspase-3 enzyme activity in untransfected, anti-STAT3 siRNA transfected, cisplatin treated, and anti-STAT3 siRNA transfected and cisplatin treated cells (significant comparisons of Bonferroni test, * and ** p=0.0021, ***p=0.0019).

platin response in lung cancer cells, their relative expressions were analyzed in parental and cisplatin-resistant Calu1 cells. Our initial results showed that, while Calu1 and CR-Calu1 cells did not have different mRNA expression patterns for STAT5A and STAT5B (p=0.173 and p=0.09, respectively), STAT3 mRNA expression was upregulated significantly in CR-Calu1 cells with 9.5-fold significant increase compared to parental cells (p=0.0005; Figure 1). An increase in total STAT3 protein expression could also be detected in CR-Calu1 cells when compared with parental Calu1 cells by Western Blot analysis (Figure 2). Elevated phosphorylated STAT3 protein expression levels also showed that activated STAT3 accounts for this increase in protein expression in cisplatin-resistant CR-Calu1 cells (Figure 2).

Since, STAT3 gene and protein expressions levels were found to be increased in cisplatin-resistant CR-Calu1 cells, we wanted to find out if - by suppressing STAT3 with chemically modified siRNAs - chemotherapeutic sensitivity to cisplatin could be achieved in these cells. The efficacy of siRNA transfection was determined by the suppression rates of STAT3 at mRNA and protein levels by RT-qPCR and Western blot analysis, respectively. As shown in Figure 3, after siRNA transfection, STAT3 gene expression levels were suppressed by 96.7% (30.8-fold; p=0.0004) and 88.7% (8.8-fold; p=0.001) in Calu1 and CR-Calu1 cells, respectively. In accordance to this, decreased levels of phosphorylated STAT3 protein following STAT3 siRNA treatment implied that this suppression was actually affecting the active form of STAT3.

Next, cell viability and proliferation of Calu1 and CR-Calu1 cells after 72 h of anti-STAT3 siRNA transfection was assessed by using the WST-1 cytotoxicity assay. This showed that cisplatin sensitivity was significantly increased in both Calu1 and CR-Calu1 cells. As shown in Figure 4, after siRNA transfection cisplatin IC_{50} values decreased from 17.23 to 15.50 μM in Calu1, and from 116.80 to 17.52 μM in CR-Calu1 cells. These results indicated that, after suppression of STAT3 by siRNA transfection, parental Calu1 cells showed different inhibition curve and became 1.12-fold more sensitive to cisplatin than untransfected Calu1 cells

(p<0.0001), and that cisplatin-resistant CR-Calu1 cells became 6.66-fold more sensitive to cisplatin than untransfected CR-Calu1 cells (p < 0.0001).

In order to find out whether suppression of STAT3 by chemically modified siRNAs in parental Calu1 and cisplatin-resistant CR-Calu1 cells also increases cell death by apoptosis, histone-complexed DNA fragments and Caspase-3 activity were assessed. For this purpose, 4 experimental cell groups were created: Calu1-UT and CR-Calu1-UT constituted the untransfected control group; Calu1+siRNA and CR-Calu+siRNA the anti-STAT3 siRNA transfected group (72 h); Calu1+cisplatin and CR-Calu1+cisplatin the cisplatin treated group (24 h); and Calu1+siRNA+cisplatin and CR-Calu1+siRNA+cisplatin the anti-STAT3 siRNA transfected (72 h) and then cisplatin-treated (24 h) group.

Compared with the Calu1-UT cells the absorbance levels obtained from cell death analysis were significantly increased in the Calu1+siRNA (p=0.015), Calu1+cisplatin (p=0.019) and Calu1+siRNA+cisplatin (p<0.0001) cells (Figure 5A). Increased absorbance levels were interpreted as apoptotic nucleosomes increased in the Calu1+siRNA (6.47-fold), Calu1+cisplatin (14.82-fold) and Calu1+siRNA+cisplatin (27.67-fold) cells. Similarly, the apoptotic rate and nucleosomes were significantly increased in CR-Calu1+siRNA (p=0.004; 2.95-fold), CR-Calu1+cisplatin (p=0.002;1.70-fold), and CR-Calu1+siRNA+cisplatin (p=0.001; 6.71-fold) when compared with the CR-Calu1-UT cells (Figure 5B). These results implied that, after anti-STAT3 siRNA transfection followed by cisplatin treatment, the apoptotic rate in parental Calu1 and cisplatin-resistant CR-Calu1 cells was increased, hence raising cisplatin response, i.e. achieving chemotherapeutic sensitivity.

Apoptosis by Caspase-3 activity was significantly increased in Calu1+cisplatin (1.2-fold) and CR-Calu1+cisplatin (1.25-fold) cells when compared with their corresponding UT-cells (Figure 5C). Besides, Caspase-3 activity was also increased in CR-Calu1+siRNA+cisplatin (1.71-fold; Figure 5D) and CR-Calu+siRNA (1.20-fold) cells, but this increase was not statistically significant.

Discussion

Increased expression of STAT3, a member of the JAK-STAT signaling pathway, is frequently observed in different types of malignancies like leukemia, breast, ovarian and lung cancer. In NSCLC the increase of STAT3 expression is usually associated with poor tumor differentiation [18,21-23]. Recent studies have also indicated that increased STAT3 expression is involved in the development of chemotherapeutic drug-resistance [20,24]. Thus, suppressing STAT3 expression might provide a convenient situation for overcoming these problems. In the present study we showed that STAT3 expression was seriously increased in CR-Calu1 cells and thus, suppression of STAT3 provided enhanced cisplatin response in parental Calu1 and CR-Calu1 cells. However, increased response rate was more pronounced in CR-Calu1 cells which possess activated form of STAT3 protein. The possible reason of not detecting any protein after siRNA application could be due to the very high transfection efficiency levels and the success of siRNA process in our experiments. Although some authors reported that STAT3 protein is not fully inhibited following siRNA application [25], we believe that modified siRNA usage provides extra efficiency and causes translational inhibition. In our study, translational inhibition of STAT3 pointed out that the siRNA machinery succeeded in this gene silencing mission by degrading STAT3 at protein level. Besides by transfecting parental and cisplatin-resistant NSCLC cells with siRNAs apoptosis was induced due to the suppression of STAT3. Similar observations had been made for prostate and gastric cancer cells; i.e., that suppression of STAT3 gene expression induced apoptosis [26,27].

Silencing of the Caveolin-1 gene, which encodes a scaffolding protein, can suppress the proliferation of metastatic lung cancer cells through the inhibition of the JAK-STAT signaling pathway [28]. On the other hand, inhibition of the JAK-STAT signaling pathway with Cucurbitacin-B can enhance the chemosensitivity of laryngeal squamous cell carcinoma cells to cisplatin [29]. Similarly, when targeting specific JAK-STAT signaling pathway components like STAT3 with siRNAs, radiosensitization of lung adenocarcinoma cells A549 and SPC-A1 can be achieved [30]. Double-stranded decoy oligodeoxynucleotide targeted blocking of STAT3 in A549 lung adenocarcinoma cells and in xenograft nude mice also induced apoptosis [31,32]. In our study, we used the epidermoid lung carcinoma cell line Calu1 and its cisplatin-resistant subline CR-Calu-1. When we specifically suppressed STAT3 expression with chemically modified siRNAs, chemotherapeutic sensitivity to cisplatin could be achieved and apoptosis induced, especially in CR-Calu1 cells.

STAT3-expressing tumors are often impaired in their intrinsic and extrinsic apoptotic pathways

because activated STAT3 upregulates apoptosis inhibitory proteins, acts as endogenous inhibitor of Caspases and provides escape from apoptosis and the immune system [33,34]. Induction of apoptosis by activated, Caspase-3 can be achieved for instance, by inhibition of STAT3 phosphorylation [35]. At late stages of the apoptotic process oligonucleosomal DNA fragmentation occurs through to the site-specific proteolytic cleavage by Caspase-3. Our findings support a previous study that Caspase-3 activity and the rate of apoptotic nucleosomes were increased significantly when STAT3 expression was inhibited [36].

The epidermal growth factor receptor (EGFR) and STATs are commonly expressed and activated in many tumors and there are several different mechanisms by which STAT proteins can mediate intracellular EGFR signaling. Therefore, defects in these routes might result in chemoresistance of cancer cells; for instance, hyperactive EGFR signaling through STAT3 and the activated JAK-STAT signaling pathway together promote ovarian cancer progression to cisplatin resistance [37]. Besides, STAT3 mediates the oncogenic effects of somatic EGFR mutations in lung cancer [38]. Since, EGFR is activated in 50% of all lung cancer cases, the importance of this pathway in the treatment of lung cancer might get more obvious in the future [8].

Lung cancer is still the most common fatal cancer in the world and unfortunately many cases are diagnosed when the disease is already at a locally advanced or metastatic stage [39]. Chemotherapy is the most important option in the treatment of lung cancers, but resistance to cisplatin is the major obstacle that limits its use. Our results show that, when anti-STAT3 siRNAs and cisplatin are applied together, cisplatin response and apoptosis can be significantly increased in parental and cisplatin-resistant NSCLC cells. We therefore propose that the combined treatment of the conventional chemotherapeutic drug cisplatin with STAT3 inhibitors can help overcome the chemotherapeutic resistance in NSCLC.

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