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ORIGINAL ARTICLE

Downregulation of SRSF3 by antisense oligonucleotides sensitizes oral squamous cell carcinoma and breast cancer cells to paclitaxel treatment

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Abstract

Purpose Paclitaxel (PTX) is widely used in the chemotherapy of many cancers, including breast cancer and oral squamous cell carcinoma (OSCC). However, many patients respond poorly to PTX treatment. The SRSF3 oncogene and several splicing factors play important roles in OSCC tumorigenesis. This study aimed to understand the function of splicing factors in PTX treatment and improve the therapeutic effects of PTX treatment.

Methods Splicing factors regulated by PTX treatment were screened in CAL 27 cell by reverse transcription polymerase chain reaction. The function of SRSF3 in PTX treatment was analyzed by gain-of-function or loss-of-function assay in OSCC cell lines CAL 27 and SCC-9 and breast cancer cell line MCF-7. Alternative splicing of SRSF3 exon 4 in cancer tissues or cells was analyzed by RT-PCR and online program TSVdb. SRSF3-specific antisense oligonucleotide (ASO) SR-3 was used to downregulate SRSF3 expression and enhance the effect of PTX treatment.

Results PTX treatment decreased SRSF3 expression, and SRSF3 overexpression rescued the growth inhibition caused by PTX in both OSCC and breast cancer cells. Moreover, we found that PTX treatment could repress SRSF3 exon 4 (containing an in-frame stop codon) exclusion and then decrease the SRSF3 protein expression. Increased exclusion of SRSF3 exon 4 is correlated with poor survival in OSCC and breast cancer patients. SR-3 downregulated SRSF3 protein expression and significantly increased the sensitivity of cancer cells to PTX treatment.

Conclusions SRSF3 downregulation by ASO sensitizes cancer cells to PTX treatment.

Keywords SRSF3 · Paclitaxel · Cancer

Introduction

Paclitaxel (PTX) is widely used in the chemotherapy of many cancers such as breast cancer [1], non-small cell lung cancer, and oral squamous cell carcinoma (OSCC) [2]. PTX inhibits cancer cell mitosis through polymerizing tubulin and disrupting normal microtubule dynamics, thus leading to cell death eventually [3]. In spite of its effectiveness in clinical applications [4], the rate of pathological complete response to PTX treatment is merely 30% [5]. In recurrent or metastatic head and neck squamous cell carcinoma (HNSCC), the response rate was estimated at 40% when paclitaxel was applied alone [6]. Therefore, increasing the sensitivity of cancers to PTX is urgently required for improving cancer treatment. The PTX resistance of OSCC has been reported to be associated with the cleavage of HuR, an RNA-binding protein [7]. Cancer stem cells of OSCC cell lines show reduced sensitivity to PTX treatment [8]. However, the

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molecular mechanisms of PTX resistance in OSCC remain largely unknown.

Alternative splicing of pre-mRNA dramatically increased the proteomic diversity of the human genome and is increasingly associated with cancer and drug resistance [9]. Two families of splicing factors play important roles in regulating alternative splicing, namely serine/arginine (SR) proteins and heterogeneous ribonuclear proteins (hnRNPs). They are involved in cancer progression by regulating the alternative splicing of cancer-associated genes [10]. In our previous studies, splicing factor SRSF3 [11], hnRNP A1 [12], hnRNP L [13], and SRSF5 [14] have been found to be involved in OSCC tumorigenesis. To date, the function and expression of splicing factors in PTX treatment remain unclear.

SRSF3 (aliased SRp20, SFRS3) is a member of the SR protein family and participates in various biological processes including alternative splicing [15], alternative RNA polyadenylation [16], termination of transcription [17], RNA export [18], transcriptome integrity [19], microRNA biogenesis [20], and protein translation [21]. SRSF3 has been demonstrated as a proto-oncogene [22] and reported to be expressed aberrantly in many malignancies, including OSCC [11, 13], colon cancer [23], colorectal cancer [24], gastric cancer [20], and breast cancer [25]. SRSF3 regulates the alternative splicing of its own exon 4. Exon 4 contains an in-frame stop codon [11]. The exon 4-skipped isoform encodes full-length functional SRSF3 protein. Meanwhile, the exon 4-included isoform can be degraded by nonsense-mediated mRNA decay (NMD) or encode a truncated SRSF3 protein [26, 27]. To date, the function and expression of SRSF3 in PTX treatment is unknown.

This study aimed to understand the function of splicing factors in PTX treatment and improve the therapeutic effects of PTX treatment. This study analyzed the mRNA expression levels of splicing factors in the presence of PTX treatment, the function of SRSF3 in PTX treatment, alternative splicing of SRSF3 exon 4 in cancer tissues or cells, and the function of an anti-SRSF3 antisense oligonucleotide (ASO) SR-3 in the effect of PTX treatment.

Materials and methods

Cell cultures and reagents

CAL 27 (an OSCC cell line) cells were cultured in Dulbecco's modified Eagle medium (DMEM; HyClone, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, USA) and 1% antibiotic–antimycotic (Gibco, USA). OSCC cell line SCC-9 was cultured in DMEM:F12 (HyClone, USA) supplemented with 10% FBS, 1‰ hydrocortisone and 1% antibiotic–antimycotic. Breast cancer cell line MCF-7 was maintained in Minimum Essential Medium with Earle's Balanced Salts (HyClone, USA) supplemented with 10% FBS and 1% antibiotic–antimycotic. All cells were incubated at 37 °C in 5% CO_2 humidified air. PTX was purchased from MedChem Express (USA) and dissolved in dimethyl sulfoxide (DMSO).

Tissue samples

Human breast carcinoma tissue and adjacent normal breast tissue were obtained from Hubei Cancer Hospital. Five patients diagnosed with breast carcinoma were involved in this study. All histologic diagnoses were performed by the Pathology Department of Hubei Cancer Hospital. Informed consents were obtained from all participants. All experimental protocols were approved by the Ethics Committee at Hubei Cancer Hospital.

Data analysis of TCGA datasets

We selected TCGA datasets of head and neck squamous cell carcinoma (44 normal and 520 primary cancer cases) and breast invasive carcinoma (112 normal and 1093 primary cancer cases). The expression of genes in patients was measured by mRNA sequencing. The normalized expression levels of SRSF3 exon 4 exclusion or inclusion isoform in TCGA breast or head and neck cancer patients were collected from the online program TSVdb (http://www.tsvdb.com). The ratios of SRSF3 exon 4 exclusion isoform vs inclusion isoform were compared between normal and cancer tissues. Low or high level of the ratio of SRSF3 exon 4 exclusion vs inclusion was determined by the number of standard deviations from the mean. To analyze the correlation of SRSF3 exon 4 inclusion and the survival of patients, the overall survival data were downloaded and analyzed with GraphPad Prism software.

Antisense oligonucleotides and transfection

An anti-SRSF3 ASO modified by 2′-O-methoxyethyl-phosphorothioate (called SR-3, [28]) and a non-specific (NS) ASO were synthesized by Sangon Biotech (China). SR-3 or NS ASOs were transfected into cells using Lipofectamine 3000 reagent (Invitrogen, USA) in accordance with the manufacturer's protocol.

RNA extraction, reverse transcription polymerase chain reaction (RT-PCR), and quantitative RT-PCR (qRT-PCR)

Total RNA was extracted using the Total RNA Miniprep Kit (Axygen, USA). Then reverse transcription was performed using the Maxima H Minus cDNA Synthesis Master Mix (Thermo Scientific, USA) in accordance with the

manufacturer's protocol. PCR was performed using Green Taq Mix (Vazyme Biotech, China). qRT-PCR was performed in accordance with the protocol of Genecopoeia All-in-One qPCR Mix (Genecopoeia, USA). Primers involved in this experiment are listed in Table S1.

PCR conditions for SRSF3 were set as follows: 95 °C for 5 min (pre-denaturation), 26 cycles (long isoform) or 22 cycles (short isoforms) of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 20 s. A final extension at 72 °C for 7 min was also applied. For the detection of both long and short isoforms, PCR condition was modified as follows: 25 cycles, annealing at 58 °C for 2 min and 30 s, extension for 40 s. qRT-PCR was performed as follows: 95 °C for 10 min (pre-denaturation), 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 20 s, and extension at 72 °C for 15 s.

Western blot

Total cellular proteins were separated in 10% NuPAGE Bis–Tris gel (Invitrogen, USA), and transferred to nitrocellulose membrane (Pall Corporation, USA). The membrane was blocked by 5% milk for 1 h, and incubated with the primary antibody at 4 °C overnight. SRSF3 protein was detected using mouse monoclonal anti-SRSF3 antibody (3G271, sc-73059, Santa Cruz, USA and 7B4, 33-4200, Thermo Fisher, USA) or mouse anti-GAPDH (M20006, Abmart, China), followed by mouse IgG kappa-binding protein (m-IgGκ BP) conjugated to horseradish peroxidase (sc-516102, Santa Cruz, USA) at room temperature for 1 h. β-Actin protein was detected using a horseradish peroxidase-labeled mouse anti-β-actin antibody (A3854, Sigma-Aldrich, USA).

Apoptosis assay

Cell apoptosis was measured using the Annexin V-FITC/PI apoptosis assay kit (KGA108, Keygentec, China). Forty-eight hours after PTX or DMSO treatment, the cells were collected in 200 μL of binding buffer and incubated for 20 min with 2 μL of PI and 2 μL of Annexin V-FITC in dark conditions. Apoptosis was evaluated using flow cytometry.

Statistical analysis

The difference of SRSF3 isoform expression between the tumor and normal groups was compared using a nonparametric Mann–Whitney test. For cell growth assay, two-group statistical comparisons of means were calculated with Student's *t* test. Survival analysis of cancer patients was performed with a log-rank test. Survival curves were produced using the Kaplan and Meier method.

Results

PTX inhibits SRSF3 expression in cancer cells

To understand the association between splicing factors and PTX treatment, CAL 27 cells (an OSCC cell line) were treated with 7.8 or 15.6 nM PTX, and the mRNA expression levels of 10 members of SR protein family (Fig. 1a) and 16 members of hnRNP family (Figure S1) were analyzed. Among these splicing factors, the mRNA level of SRSF3 was the one that was the most significantly changed after PTX treatment (Fig. 1b, $P < 0.001$). Cells treated with 15.6 nM PTX showed significantly downregulated SRSF3 protein level compared with control (fold change > 1.5, $P < 0.01$) (Fig. 1d). SRSF3 downregulation by PTX treatment was confirmed in another OSCC cell line, SCC-9 (Figure S3A-B). Moreover, PTX treatment also significantly decreased SRSF3 mRNA and protein levels in MCF-7 cell, a breast cancer cell line, in a dose-dependent manner (Fig. 1c, d). These results suggested that PTX may inhibit cancers by downregulating SRSF3.

SRSF3 is overexpressed in breast cancer tissues

Previously, we have demonstrated that the expression levels of SRSF3 in OSCC tissues are significantly higher than those in normal oral mucosal tissues [29]. Given that PTX inhibits SRSF3 expression in breast cancer cells, we next aimed to determine whether SRSF3 is also overexpressed in breast cancer tissues. In line with OSCC, the transcription levels of SRSF3 in breast cancer tissues were higher than their adjacent normal tissues (Fig. 2a). By analyzing the expression of SRSF3 in a TCGA breast cancer database, we found that SRSF3 is significantly overexpressed in breast cancer tissue compared with normal controls (Fig. 2b). This phenomenon indicates SRSF3 may be associated with breast cancer carcinogenesis.

SRSF3 overexpression reduces the sensitivity to PTX in cancer cells

To explore the function of SRSF3 in PTX treatment, CAL 27 and MCF-7 cells with stably overexpressed SRSF3 were established. SRSF3 overexpression can partially rescue cancer cell proliferation inhibited by PTX in both CAL 27 and MCF-7 ($P < 0.001$, Fig. 2c, d) cells. These findings indicated that SRSF3 can reduce the sensitivity of cancer cells to PTX treatment.

PTX represses the exclusion of SRSF3 exon 4

SRSF3 is a splicing factor and also regulates the alternative splicing of its own exon 4. Exon 4 contains an in-frame stop

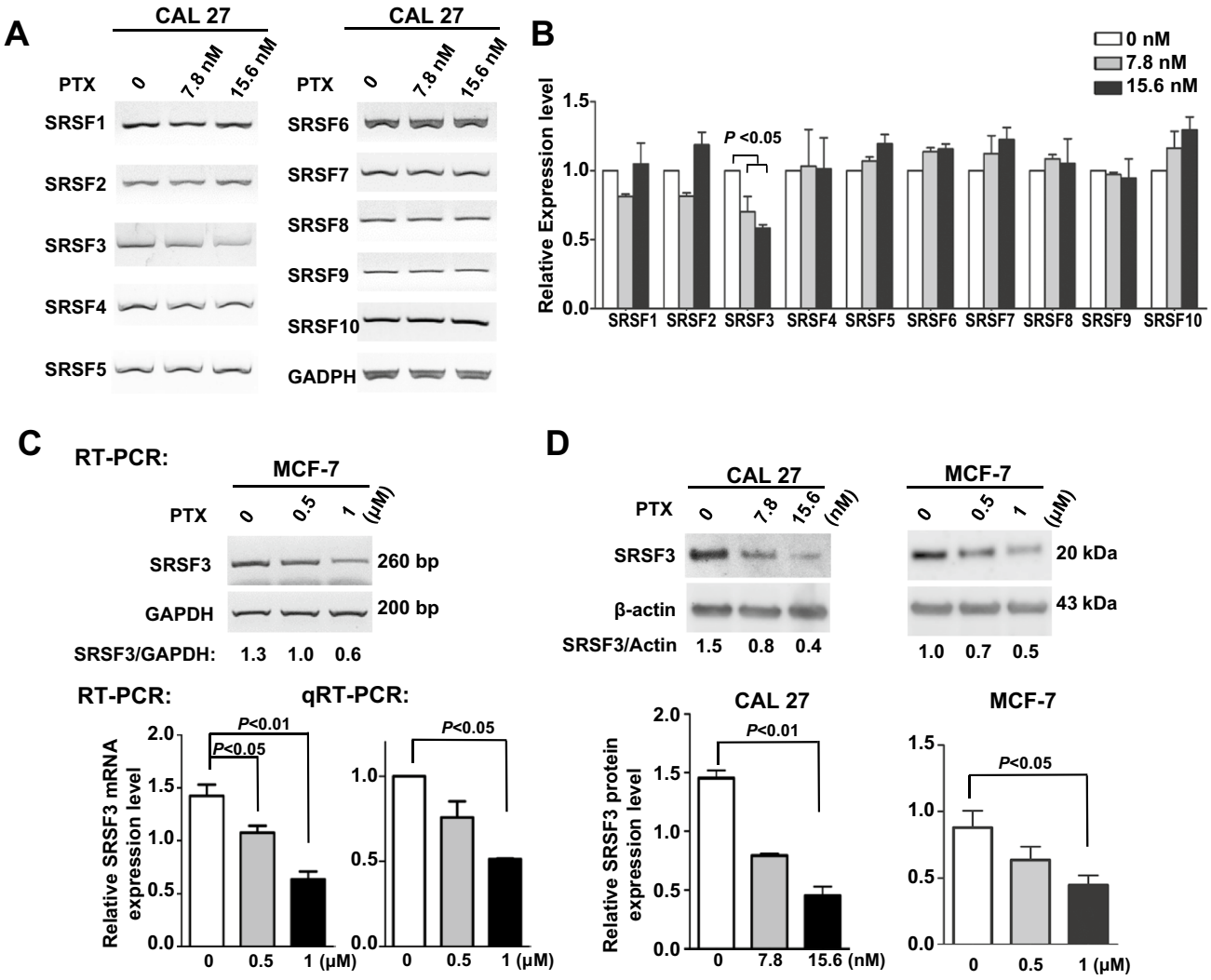

Fig. 1 PTX treatment significantly downregulated SRSF3 expression. **a** CAL 27 cell, an OSCC cell line, was treated with 0 nM, 7.8 nM, or 15.6 nM PTX for 48 h. The mRNA expression levels of SR proteins were analyzed by RT-PCR. **b** The histogram shows the relative expression levels of SR proteins. Data are mean ± SE, $n = 3$. **c**, **d** CAL 27 or MCF-7 (a breast cancer cell line) cell was treated with PTX at the indicated concentration. The expression of SRSF3 mRNA or protein was analyzed through RT-PCR, qRT-PCR (**c**) or Western blot (**d**). GADPH and β-actin served as loading controls. Histograms summarized the relative expression levels of SRSF3 mRNA or protein. Data are mean ± SE, $n = 3$

codon [11]. The short isoform without exon 4 encodes full-length SRSF3 protein. The long isoform with exon 4 can encode a truncated SRSF3 protein that lacks the vital RS functional domain, but it is also a target of nonsense-mediated mRNA decay (NMD) and is mainly degraded (Fig. 3a). Consequently, the mRNA expression level of long isoform is much lower than that of short isoform. To understand how PTX regulates the expression of SRSF3, we analyzed the effect of PTX treatment on the alternative splicing of SRSF3 exon 4 using a pair of primers amplifying both exon 4-included and -excluded isoforms. Compared with DMSO treatment control, PTX treatment significantly increased SRSF3 exon 4-included long isoform by relatively decreasing the exon 4-excluded short isoform in CAL 27, MCF-7 (Fig. 3b) and SCC-9 cells (Figure S3C). This phenomenon was further confirmed by qRT-PCR or RT-PCR using two pairs of isoform-specific primers in these cells (Fig. 3c; Suppl. Figs. S2A and S3D). These results suggest that PTX may downregulate full-length SRSF3 protein expression by increasing the inclusion of its exon 4.

Previously we demonstrated that an ASO, SR-3, could increase the inclusion of SRSF3 exon 4 and decrease the expression of full-length SRSF3 protein by blocking an exonic splicing suppressor in exon 4 in OSCC cells [28]. In line with this result, we found that SR-3 significantly increased the levels of long isoform of SRSF3 mRNA by relatively decreasing the exon 4-excluded short isoform (Fig. 3d; Suppl. Fig. S3E), and decreased the levels of

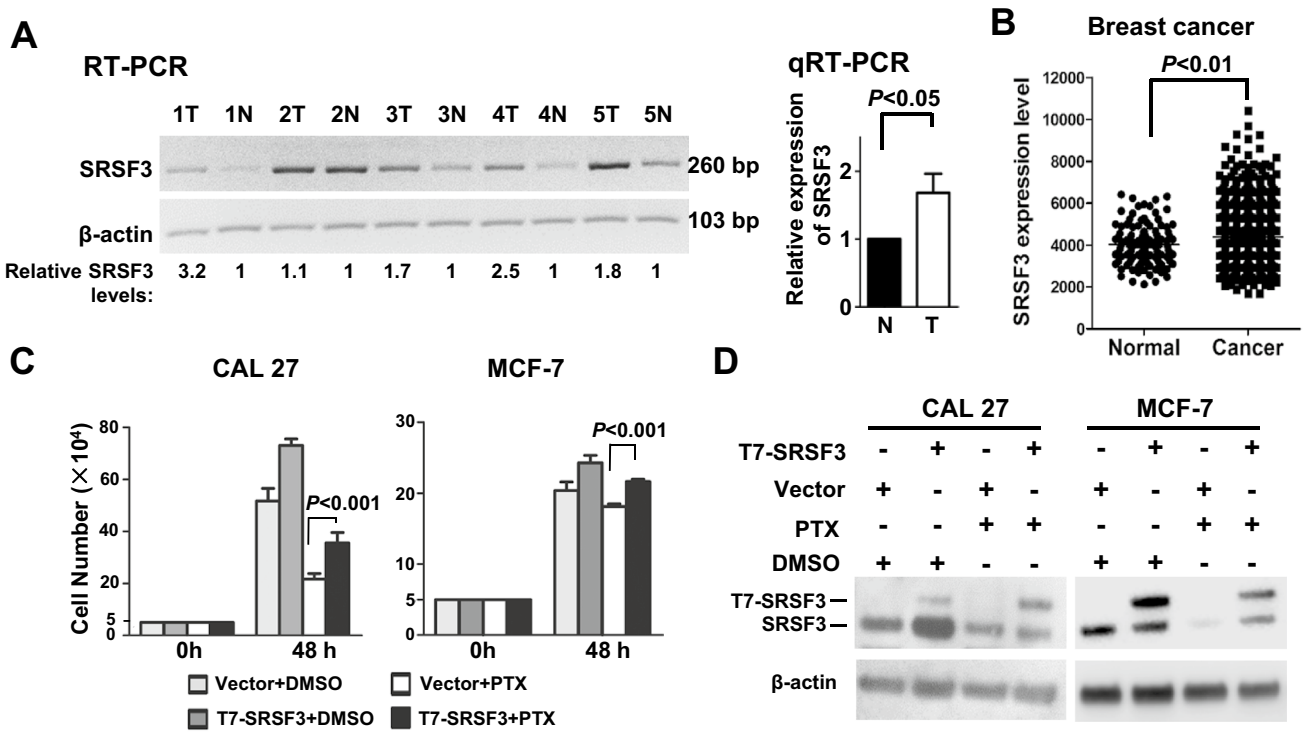

Fig. 2 SRSF3 is overexpressed in breast cancer tissues and can partially rescue cancer cell proliferation inhibited by PTX. **a** Expression of SRSF3 mRNA in breast cancer tissues and their adjacent normal tissues was analyzed by RT-PCR and qRT-PCR. GAPDH served as a loading control. The histogram shows relative SRSF3 mRNA expression levels (normalized to GAPDH). **b** The normalized SRSF3 (isoform encoding full-length SRSF3 protein) expression levels of patients in TCGA breast invasive carcinoma database were collected from the online program TSVdb (http://www.tsvdb.com). The expression levels of SRSF3 were compared between breast normal (112 cases) and cancer tissues (1093 cases). **c**, **d** CAL 27 or MCF-7 cells stably transfected by T7 tagged SRSF3 expression or vector control lentivirus. SRSF3 can partially rescue cancer cell proliferation inhibition by PTX. Cells were seeded for 24 h, and then treated with PTX for 48 h at 15.6 nM (for CAL 27) or 2 μM (for MCF-7). DMSO treatment was used as a control. Data are mean ± SE. $n = 3$. Overexpression of T7-tagged SRSF3 was confirmed by Western blot. β-Actin served as a loading control

full-length SRSF3 protein (Fig. 3f; Suppl. Fig. S3G) in CAL 27, MCF-7 and SCC-9 cells. This phenomenon was further confirmed by qRT-PCR or RT-PCR using two pairs of isoform-specific primers in these cells (Fig. 3e; Suppl. Figs. S2B and S3F). In addition, we confirmed that SR-3 could also downregulate the expression of known targets of SRSF3, PLK1 and CDC25B (Figure S4) in these cells. These results suggested that SR-3 ASO could be used to enhance the therapeutic effect of PTX treatment by decreasing SRSF3 expression.

Low ratio of exon 4 exclusion vs inclusion is associated with good prognosis

To date, the relationship between alternative splicing of SRSF3 exon 4 and cancer patients' clinical outcomes is unclear. By analyzing the alternative splicing of SRSF3 exon 4 in patients with breast or head and neck cancer (TCGA datasets), we found that cancer tissues showed significantly higher exclusion of exon 4 than normal controls (Fig. 4a, b). Moreover, low ratio of exon 4 exclusion vs inclusion isoform significantly correlates with good overall survival in patients with head and neck cancer or breast cancer (Fig. 4c, d). These results suggest that increasing the ratio of inclusion vs exclusion of SRSF3 exon 4 and decreasing SRSF3 full-length protein expression may improve the outcomes of cancers.

Anti-SRSF3 ASO sensitizes cancer cells to PTX treatment

Next, we aimed to determine whether SR-3 ASO could enhance the therapeutic effect of PTX treatment. Cancer cells were divided into four groups: PTX + SR-3, PTX + NS, DMSO + SR-3, and DMSO + NS. DMSO and non-specific (NS) served as controls. In MCF-7 cells, low PTX concentration (0.5 μM) in PTX + NS group could not inhibit cell proliferation (Fig. 5a). However, combined treatment with both 0.5 μM PTX and 20 nM SR-3 (PTX + SR-3 group) significantly inhibited MCF-7 cell proliferation. In CAL 27 cells, the PTX + SR-3 group (combined treatment with both 7.8 nM PTX and 20 nM

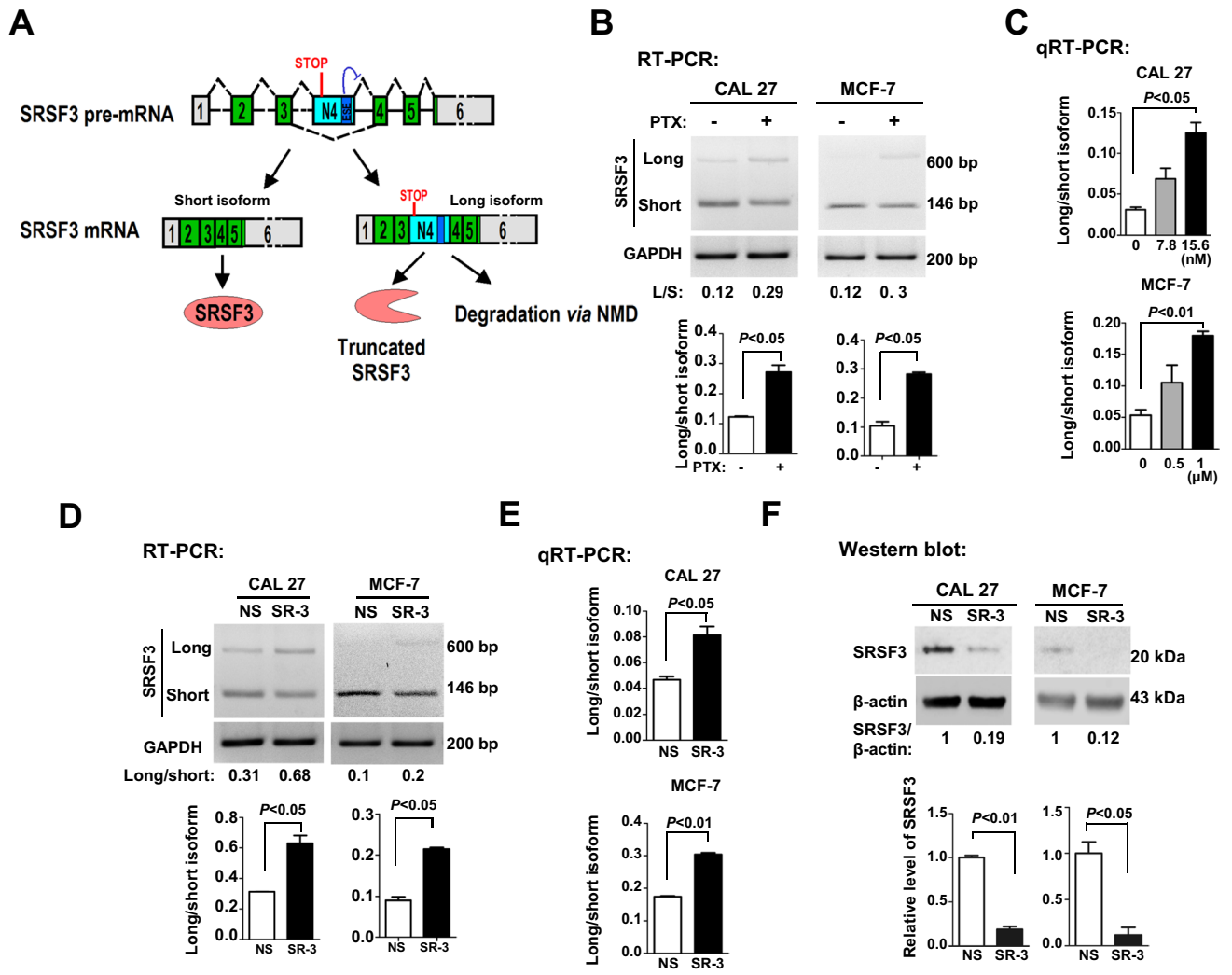

Fig. 3 PTX regulated the alternative splicing of SRSF3 exon 4. **a** Schematic diagram of the structures of human SRSF3 pre-mRNA and spliced products. The exon 4-excluded transcripts encode full-length functional SRSF3. The exon 4-included transcripts are degraded via the NMD pathway, or encode truncated SRSF3. RT-PCR (**b**) and qRT-PCR (**c**) was used to analyze the alternative splicing of exon 4 in CAL 27 or MCF-7 cells treated with PTX at the indicated concentration. **d**–**f** CAL 27 or MCF-7 cells were treated with 20 nM SR-3 (an anti-SRSF3 antisense oligonucleotide) or non-specific antisense oligonucleotide (NS). Alternative splicing of SRSF3 exon 4 was analyzed by RT-PCR (**d**) and qRT-PCR (**e**). The expression of full-length SRSF3 protein was analyzed by Western blot (**f**). Data are mean ± SE, $n = 3$. β-Actin served as a loading control

SR-3) also showed significantly less cell proliferation than the PTX + NS group (Fig. 5a). Similar results were observed in SCC-9 (Figure S5A). These results indicate that anti-SRSF3 ASO can sensitize cancer cells to PTX treatment. In line with the results of cell proliferation, the PTX + SR-3 group showed the lowest SRSF3 expression levels than other groups in both cell lines (Fig. 5b; Figure S5B). To determine the levels of truncated SRSF3 protein in cells treated individually or combined with PTX and SR-3 ASO, an antibody recognizing both full-length and truncated SRSF3 (clone 7B4, from Thermo Fisher) was used in Western blot assay. As shown in Figure S6, PTX or SR-3 ASO treatment increased the expression of truncated SRSF3 protein. As expected, the combination of both PTX and SR-3 ASO treatment showed the highest expression of SRSF3 truncated protein in both CAL 27 and MCF-7 cells.

To evaluate whether the inhibition of cancer cell growth was achieved by apoptosis induction, we next analyzed the apoptosis of cells treated individually or combined with PTX and SR-3. Flow cytometry showed that, in all three cell lines, either PTX or SR-3 treatment increased cell apoptosis significantly. However, combination of PTX and SR-3 induced the most percentage of apoptosis cells (Fig. 5c, d; Figure S5C and D). These results suggested that PTX and SR-3 can inhibit cancer cell growth via inducing cell apoptosis.

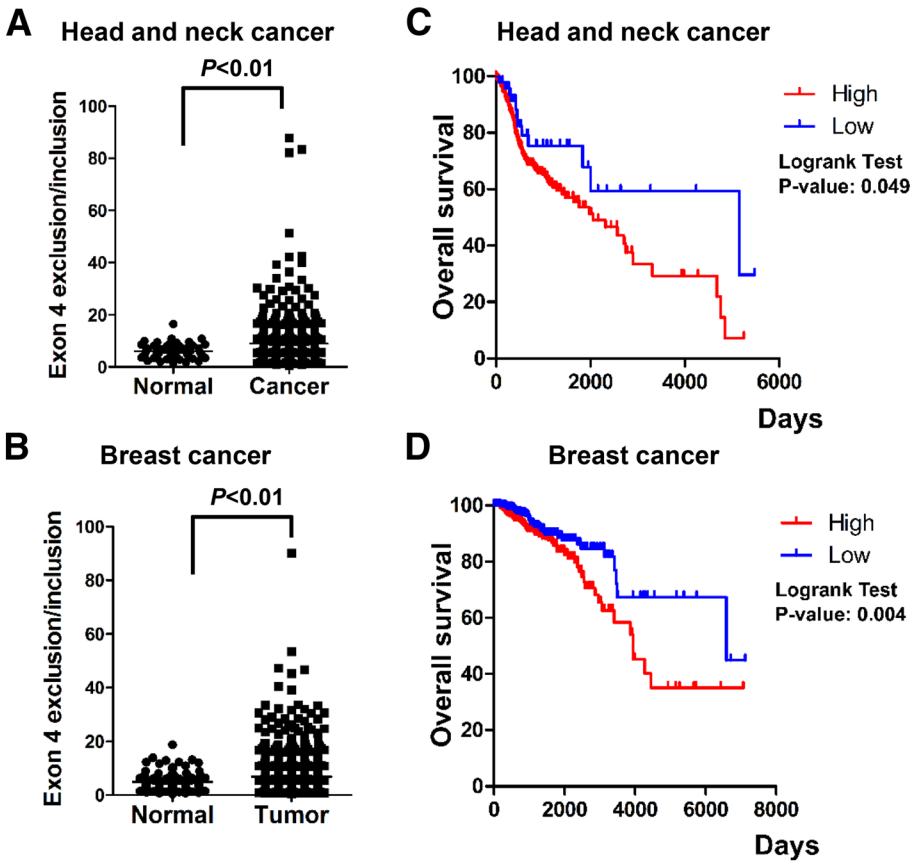

Fig. 4 Inclusion of SRSF3 exon 4 is associated with better prognosis. **a**, **b** Head and neck cancer or breast patients have higher ratios of SRSF3 exon 4 exclusion vs inclusion than normal controls. **a**, **b** The normalized expression levels of exon 4 exclusion or inclusion isoform of patients in TCGA head and neck cancer (**a**) or breast cancer (**b**) database were collected from the online program TSVdb (http://www.tsvdb.com). The ratios of SRSF3 exon 4 exclusion vs inclusion were compared between normal and cancer tissues. There were 44 normal cases and 520 cancer cases in head and neck cancer dataset, and 112 normal cases and 1093 cancer cases in breast cancer dataset. **c**, **d** Kaplan–Meier curves of overall survival for patients with lower ratio (49 cases in head and neck cancer, 474 cases in breast cancer) or higher ratio (391 cases in head and neck cancer, 517 cases in breast cancer) of exon 4 exclusion vs inclusion isoform. Low ratio of exon 4 exclusion vs inclusion isoform was defined as less than mean − 0.66SD (standard deviation) in head and neck cancer or mean − 0.25SD in breast cancer

Discussion

PTX is of great importance in clinical chemotherapy applied in many malignancies [4]. PTX exerts its effect via stabilizing microtubules, inducing cell cycle arrest in G2/M in breast cancer [30] or head and neck squamous cell carcinoma [31]. In the present study, we found that PTX treatment significantly decreased SRSF3 expression. Previously we have demonstrated that RNAi-mediated reduction of SRSF3 expression induces G2/M arrest, growth retardation, and apoptosis in cancer cells [22]. Therefore, PTX may inhibit cancer cell growth partially through the downregulation of SRSF3 expression.

PTX treatment can change the alternative splicing profile of global cellular RNA. Using RNA-seq analysis, Zhu et al. reported that PTX treatment controls the alternative splicing events of a series of genes that are involved in tumorigenesis including FMNL3, ZMIZ2, ECT2, PLD2, and DDIT3. For example, ECT2 is known to regulate rRNA synthesis that is essential for lung tumorigenesis. PTX treatment favors the production of ECT2-S, the short splicing isoforms of ECT2, which inhibits lung cancer cell proliferation [32]. However, the molecular regulatory mechanisms of these splicing events by PTX remain unknown. Determining whether these splicing events are regulated by SRSF3 will be interesting. In addition, Zhu et al. reported that PTX treatment decreases the expression of hnRNPUL1, a splicing factor, in lung cancer cell. However, we did not find the downregulated expression of hnRNPUL1 in OSCC in the present study (Figure S1).

To date, several splicing factors have been reported to be associated with the cancer cell's sensitivity to PTX or other anticancer drugs. For example, TRA2A is a member of transformer 2 (TRA2) proteins and regulate pre-mRNA

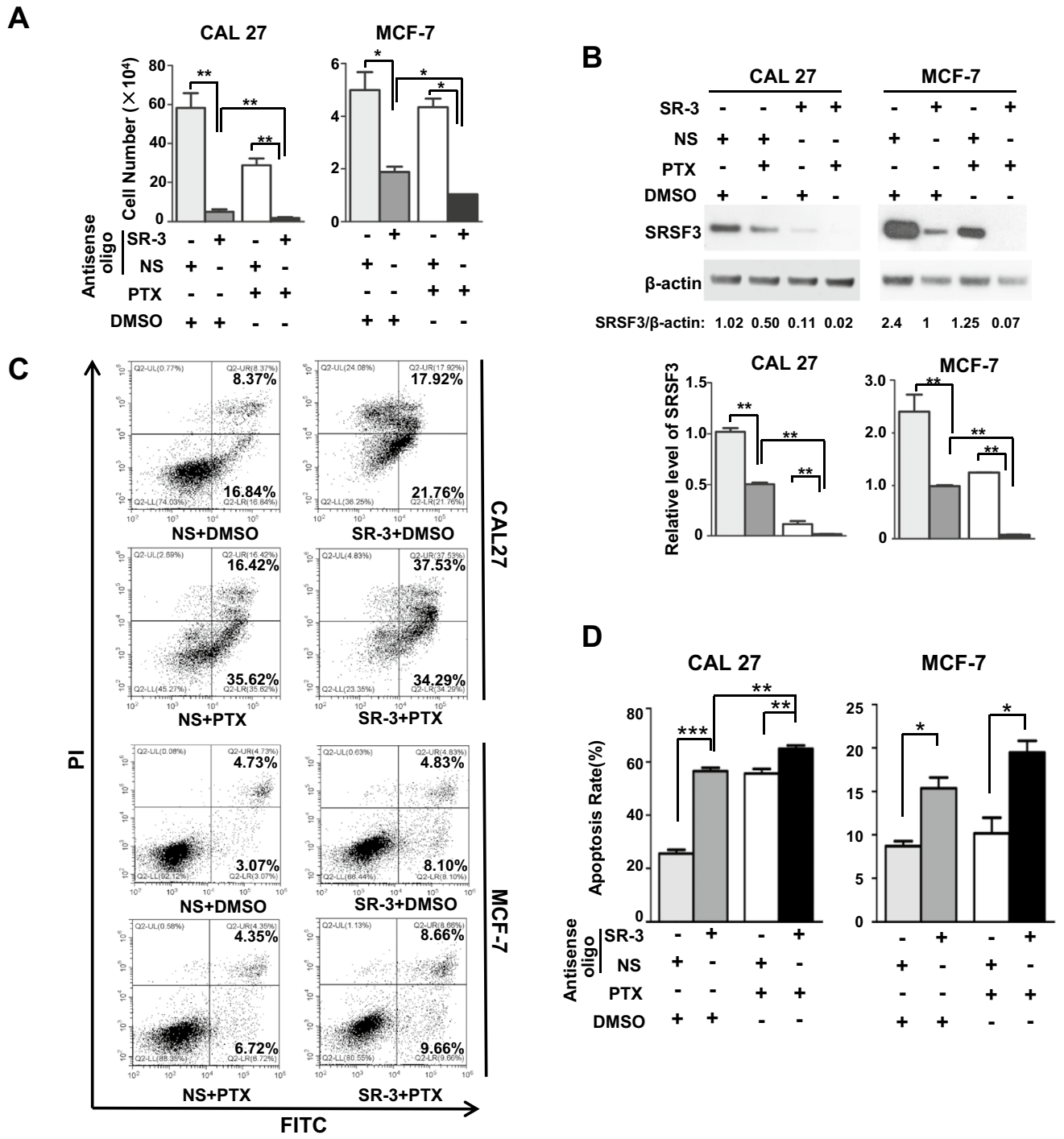

Fig. 5 Anti-SRSF3 antisense oligonucleotide (SR-3) sensitizes cancer cells to PTX treatment. Cells were divided into four groups: PTX + SR-3, PTX + NS, DMSO + SR-3, and DMSO + NS. DMSO and non-specific (NS) served as controls. **a** CAL 27 or MCF-7 cells were transfected with 20 nM SR-3 or non-specific (NS) antisense oligonucleotide on Day 0. Twenty-four hours after transfection, cells were treated with PTX (7.8 nM for CAL 27, 0.5 μM for MCF-7) or DMSO. Cell numbers were counted on Day 3. **b** SRSF3 expression in each group was detected through Western blot. β-Actin served as a loading control. Relative expression levels of SRSF3 protein were normalized to β-actin. **c**, **d** Cellular apoptosis were analyzed by flow cytometry on Day 3. **c** Annexin V-FITC/PI method was applied to detect the proportion of apoptotic cells. **d** The histograms summarized the cellular apoptotic rates in all groups. Data are mean ± SE, $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

splicing. TRA2A promotes PTX resistance of triple-negative breast cancer by promoting the isoform shift of RSRC2 from RSRC2 s to RSRC2 l [33]. Pre-mRNA processing factor-4 (PRP-4) was reported to be overexpressed in several PTX-resistant human ovarian cell lines, and transcriptional repression of PRP-4 reversed PTX resistance by five- to tenfold [34]. SR and hnRNP proteins are two families discovered as regulators of alternative splicing. SRSF1, a member of SR protein family, favors the splicing switch of caspase 9 from pro-apoptosis caspase 9a to anti-apoptosis caspase 9b, and decreases the cancer cell's sensitivity to PTX [35]. PTBP1, also known as hnRNP I, was reported to regulate the alternative splicing of pyruvate kinase gene (PKM) and promote the production of carcinogenic isoform PKM2. Knockdown of PTBP1 abolishes the pancreatic cancer cell's resistance to gemcitabine and cisplatin [36]. Interestingly, our previous works demonstrated that SRSF1 is regulated by SRSF3, and PTBP1 increases SRSF3 expression [11, 37]. All these studies suggest that splicing factors mainly reduce the sensitivity of cancer cells to anticancer drugs. Our present study demonstrated that oncogenic splicing factor SRSF3 plays similar role and reduces the cancer cell's sensitivity to PTX treatment.

Previously, we discovered that FOXM1, Cdc25B and PLK1 are targets of SRSF3 [22]. Interestingly, Zhu et al. also showed that the RNA levels of FOXM1 and PLK1 significantly decrease upon PTX treatment. In line with these studies, we found that PTX treatment downregulates SRSF3 in cancer cells in the present study, suggesting that PTX inhibits FOXM1 and PLK1 expression through downregulating SRSF3.

Apart from PTX, many clinical chemicals have negative effects on the expression of SRSF3. Saeki et al. showed that insulin treatment induced the degradation of SRSF3 via proteasome in HL-60, a human leukemia cell line [38]. Digitoxin is a member of the cardiotonic steroid class of drugs for the clinical treatment of heart failure. Interestingly, digitoxin can inhibit SRSF3 and TRA2B expression in HEK 293 cells [39]. Chang et al. found that amiloride, a common diuretic, reduced the expression of SRSF3 and altered oncogenic alternative splicing events in Huh-7, a human hepatocellular carcinoma cell [40]. Moreover, caffeine was demonstrated to downregulate SRSF3, and then influence the alternative splicing of p53 [41]. These studies suggested that SRSF3 is an important target for cancer therapy.

SRSF3 has an alternative exon 4. Long isoform with exon 4 can encode a truncated SRSF3 protein that lacks the vital RS functional domain, but it is also a target of NMD and is mainly degraded. Short isoform without exon 4 can encode full-length SRSF3 protein [42]. Consequently, the mRNA expression level of long isoform is much lower than that of short isoform. The truncated SRSF3 may be positively related to carcinogenesis. Kano et al. found that stable overexpression of truncated SRSF3 protein increases the expression of c-Jun, cyclin D1, cyclin D3, CDC25A and E2F1, and accelerates cell growth [43]. They also demonstrated that truncated SRSF3 is required for IL-8 expression in colon cancer cells [26]. However, Jimenez et al. demonstrated that truncated SRSF3 protein is a dominant negative form of SRSF3, and promotes the induction of DNA damage, genome instability, and cell cycle arrest. We have also demonstrated that increasing the inclusion of exon 4 by SR-3 ASO significantly decreased SRSF3 full-length protein expression and suppressed cancer cell growth [28]. Therefore, the function of truncated SRSF3 protein is opposite to that of oncogenic full-length SRSF3 protein. However, genome instability induced by truncated SRSF3 protein may contribute to early carcinogenesis by increasing the incidence of cellular mutations. Further studies are required to understand the function of truncated SRSF3 protein in carcinogenesis.

ASOs have been used to enhance the efficiency of anticancer drugs in cancer cells. An anti-Bcl2 ASO significantly enhances the cytotoxicity of cisplatin in bladder cancer cells [44]. An ASO targeting TNFR1-associated DD protein (TRADD) improves the chemosensitivity of HepG2 cancer cells to proteasome inhibitors MG132 or ALLN [45]. Recently, ASOs have also been applied to preclinical trial including amyotrophic lateral sclerosis (ALS) [46], Duchenne muscular dystrophy [47] and solid cancers [48]. Therefore, antisense oligonucleotides have been receiving increasing attention for cancer treatment. Combination of epidermal growth factor receptor ASO and docetaxel is more effective in the treatment of head and neck squamous cell carcinoma compared with standard chemotherapy [49]. In the present study, we showed that SR-3 ASO of SRSF3 sensitized cancer cells to PTX, indicating that ASOs against splicing factors may be a useful novel method for anticancer therapy.

In conclusion, our results demonstrated that SRSF3 is a target gene of PTX, and downregulation of SRSF3 by ASO is a promising method to sensitize cancer cells to PTX treatment.

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Data availability The data used to support the findings of this study are included within the article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Informed consent Informed consents were obtained from all participants. All experimental protocols were approved by the Ethics Committee at Hubei Cancer Hospital.

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