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SOCS3 overexpression enhances ADM resistance in bladder cancer T24 cells

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Abstract. – OBJECTIVE: JAK-STAT3 signaling pathway widely participates in cell proliferation and apoptosis. Suppressor of cytokine signaling 3 (SOCS3) is a negative regulator of JAK-STAT3. SOCS3 downregulation is associated with drug resistance in breast cancer and leukemia. However, its role in bladder cancer drug resistance is still unclear. This study established ADM resistant bladder cancer cell model to investigate the role of SOCS3-JAK/STAT3 signaling pathway ADM resistance.

MATERIALS AND METHODS: ADM drug-resistant cell line T24/ADM was established. SOCS3, p-JAK2, p-JAK3, and Bcl-2 expressions in ADM, T24, and HBEC cells were compared. Cell proliferation and apoptosis were evaluated by flow cytometry. T24/ADM cells were divided into to five groups, including control, pSicoR-blank, pSicoR-SOCS3, FLLL32 and pSicoR-SOCS3 + FLLL32 groups. Cell proliferation was determined by EdU staining.

RESULTS: SOCS3 was reduced while p-JAK2, p-STAT3, and Bcl-2 expressions were elevated in T24 cells compared with HBEC cells. T24/ADM cells exhibited lower SOCS3, higher p-JAK2, p-STAT3, and Bcl-2 levels than T24 cells. Cell apoptosis was higher, while cell proliferation was weaker in T24 cells compared with T24/ADM cells. SOCS3 overexpression and/or FLLL32 treatment significantly downregulated p-JAK2, p-STAT3, Bcl-2 expressions, attenuated cell proliferation, and elevated sensitivity to ADM in ADM cells.

CONCLUSIONS: SOCS3 reduction was associated with bladder cancer sensitivity to ADM. SOCS3 overexpression decreased JAK-STAT3 signaling pathway activity, declined Bcl-2 expression, inhibited cell proliferation, elevated cell apoptosis, and enhanced ADM sensitivity in T24 cells.

Key Words:
SOCS3, JAK-STAT3, T24, ADM, Drug resistance.

Introduction

Bladder cancer is the most common urinary system tumor, bladder cancer exhibits the leading morbidity and mortality in China. Chemotherapy is an important method for its postoperative treatment. However, some patients in advanced stage show poor sensitivity to chemotherapy, leading to poor therapeutic effect[1]. Adriamycin (ADM) is the first line drug for bladder cancer. Long-term application of ADM may cause drug resistance in bladder cancer[2]. Therefore, exploring the mechanism of bladder cancer drug resistance is of great significance to decrease drug resistance, elevate chemotherapy effect, and improve the prognosis of bladder cancer. Multiple mechanisms are involved in mediating chemotherapy resistance, such as cell survival, proliferation, and apoptosis. Regulation of cell survival, proliferation, and apoptosis may change the sensitivity to chemotherapy drugs. Janus kinase (JAK) - signal transducer and activator of transcription (STAT) signaling pathway widely exists in various tissues and cells. It participates in the regulation of cell proliferation, cycle, and apoptosis[3,4]. It was showed that JAK-STAT3 activity enhancement was related to drug resistance in lung cancer[3], leukemia[5], and lymphoma[6]. Suppressor of cytokine signaling 3 (SOCS3) is a member of SOCS family with the strongest activity. It suppresses JAK-STAT signaling pathway activation and transduction[7]. It was revealed that SOCS3 downregulation was associated with drug sensitivity reduction in breast cancer[8], suggesting its potential role in alleviating cancer cell drug resistance. However, its impact in bladder cancer drug resistance is still unclear. This study established ADM

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resistant bladder cancer cell model to investigate the role of SOCS3-JAK/STAT3 signaling pathway ADM resistance.

Materials and Methods

Main Reagents and Materials

Human bladder cancer cell T24 and normal bladder epithelial cell HBEC were purchased from Shanghai Cell Bank of Chinese Academy of Sciences. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were got from Gibco (Rockville, MD, USA). Trizol was bought from Invitrogen (Carlsbad, CA, USA). ReverTra Ace qPCR RT Kit and SYBR were obtained from Toyobo (Tokyo, Japan). Rabbit anti-human SOCS3 and p-JAK2 primary antibodies were purchased from Abcam (Cambridge, MA, USA). Mouse anti-human p-STAT3 and Bcl-2 primary antibodies were purchased from CST (Danvers, MA, USA). Mouse anti-human β-actin primary antibody was got from Santa Cruz Biotechnology (Santa Cruz, CA, USA). HRP conjugated secondary antibody was bought from Bio-Rad (Hercules, CA, USA). ADM was derived from Hengrui Medicine Co., Ltd. (Jiangsu, China). EdU staining kit was obtained from RiboBio (Guangzhou, China). Annexin V-FITC/PI cell apoptosis detection kit, caspase-3 activity detection kit, and RIPA were purchased from Beyotime (Jiangsu, China). JAK/STAT inhibitor FLLL32 was bought from Selleck (Houston, TX, USA). pSicoR-GFP was obtained from Addgene (Cambridge, MA, USA). CCK-8 reagent was derived from Dojindo (Kumamoto, Japan).

Cell Culture

T24 and HBEC cells were cultured in DMEM containing 10% FBS and 1% penicillin-streptomycin. Cells were passaged at 1:4.

T24/ADM Drug Resistant Cell Line Establishment

Cells in logarithmic phase were treated by ADM from 0.2 mg/L for 24 h. Then cells kept growing and were treated by increased concentration of ADM up to 2.0 mg/L when the cells can stable grow in ADM. At last, cells can be stable passaged in ADM to obtain ADM resistant bladder cancer cell line T24/ADM. T24 and T24/ADM cells were treated by different concentrations of ADM for 48 h. Next, cells were added with CCK-8 to measure the absorbance value (A450). Inhibition rate = (1-A450 in drug group)/A450 in control × 100%. IC50 was calculated using SPSS18.0 software (SPSS Inc., Chicago, IL, USA). Resistance index (RI) = IC50 of drug resistant cell/IC50 of parent cell.

Cell Treatment and Grouping

T24/ADM cells were divided into groups, including control, pSicoR-blank, pSicoR-SOCS3, FLLL32 (1.0 μM), and pSicoR-SOCS3 + FLLL32 (1.0 μM) groups. Cells were treated by ADM 2.0 mg/L for 72 h before following experiments.

qRT-PCR

Total RNA was extracted by Trizol and reverse transcribed to cDNA using ReverTra Ace qPCR RT Kit. The qPCR reaction was performed under the effect of Taq DNA polymerase. The primers were as follows:

SOCS3P$_F$: 5′-CCTGCGCCTCAAGACCTTC-3′,
SOCS3P$_R$: 5′-GTGCTGCGCTCCAGTAGAA-3′;
JAK2P$_F$: 5′-GCGGGTCATGTGTGTGG-3′,
JAK2P$_R$: 5′-CCTCAGGTACTCAGTCATCC-3′;
β-actin$_F$: 5′-CCCTAAGGCCAAC-3′;
β-actin$_R$: 5′-TCACGCACGATTTCC-3′.

Western Blot

The protein was extracted by radioimmunoprecipitation assay (RIPA) on ice for 150 min. After centrifuged at 12000 rpm for 10 min, a total of 50 μg protein was separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to membrane. Next, the membrane was blocked by 5% skim milk and was incubated in primary antibody at 4°C overnight (SOCS3, p-JAK2, p-STAT3, Bcl-2, and β-actin at 1:400, 1:200, 1:200, 1:200, and 1:600, respectively). Then the membrane was incubated in secondary antibody (1:8000) for 60 min and then it was washed by PBST for three times. At last, the protein expression was detected by enhanced chemiluminescence (ECL).

EdU Staining

Cells were added to EdU solution in logarithmic phase. After incubated for 48 h, cells were digested by 0.125% trypsin and collected. After they were fixed in paraformaldehyde, and were neutralized in glycine. Next, cells were incubated in 0.1% Triton X-100 and resuspended in PBS. At last, cells were stained by 500 μl Apollo at room temperature for 10 min and they were tested on EPICS XL-MCL flow cytometry (Beckman, Brea, CA, USA).

Flow Cytometry

Cells were re-suspended in 500 µl binding buffer and were incubated in 5 µl Annexin V-FITC and 5 µl PI. Next, cells were tested on EPICS XL-MCL flow cytometry to evaluate cell apoptosis.

Caspase-3 Activity Detection

According to the manual, pNA standard substance was established and tested at A405 to draw the standard curve. Cells were digested by trypsin and cracked on ice. Then the 96-well plate was incubated with buffer, sample, Ac-DEVD-pNA at 37°C for 2 h. A405 was tested to reflect caspase-3 activity.

Statistical Analysis

All data analyses were performed by SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). The measurement data were depicted as mean ± standard deviation and compared by *t*-test. $p < 0.05$ was considered as statistical significance.

Results

IC50 Increased in T24/ADM Cells

SPSS software revealed that IC50 of T2 was 0.69 mg/L, while it was 7.82 mg/L in 4/ ADM cells. The RI of T24/ADM was 11.33. spase-3 activity (Figure 1 A) and cell apopto (Figure 1 B) were higher, while liferatio (Figure 1 C) was significantl er 24 cells compared with T24/ADM s after ated by ADM with concentration 24 IC5

SOCS3 Level Re ion, J TAT3 Activity and B Expressio vation were Relate drug Resi ce

qRT-PCR monstr that SOCS3 mRNA markedly ined, while mRNA apparently enha d in T24 cells co red with HBEC cells /ADM ls exhibited lower SOCS3 and hig Bcl-2 s than T24 cells (Figure 2 A). Weste owed SOCS3 protein significantly de ed, w as p-JAK2, p-STAT3, and B prote ly upregulated in T24 cells co red wit EC cells. T24/ADM cells re- ve er SOCS3 protein, and higher p-JAK2, STA Bcl-2 protein levels than T24 cells igure 2 B).

S 3 Upregulation Declined T24/ADM Drug Resistance

SOCS3 mRNA and protein levels significantly increased in pSicoR-SOCS3 transfection group compared with pSicoR-blank group, suggesting successful SOCS3 overexpression in T24/ADM cells (Figure 3 A-B). SOCS3 mRNA and protein were elevated, while p-JAK2, p-STAT3, a Bcl- 2 protein levels markedly declined in cells transfected by pSicoR-SOCS ompared with control (Figure 3 C-D). Ca -3 activity (Figure 3 E) and cell apoptosis (Fig E) apparently increased, while cell liferat igure 3 G) significantly attenua n T24/AD treated by 2.0 mg/L AD FLLL treatm 1.0 µM also downre ed p- 2, p-STAT3, and Bcl-2 express ns, h cell p fera-

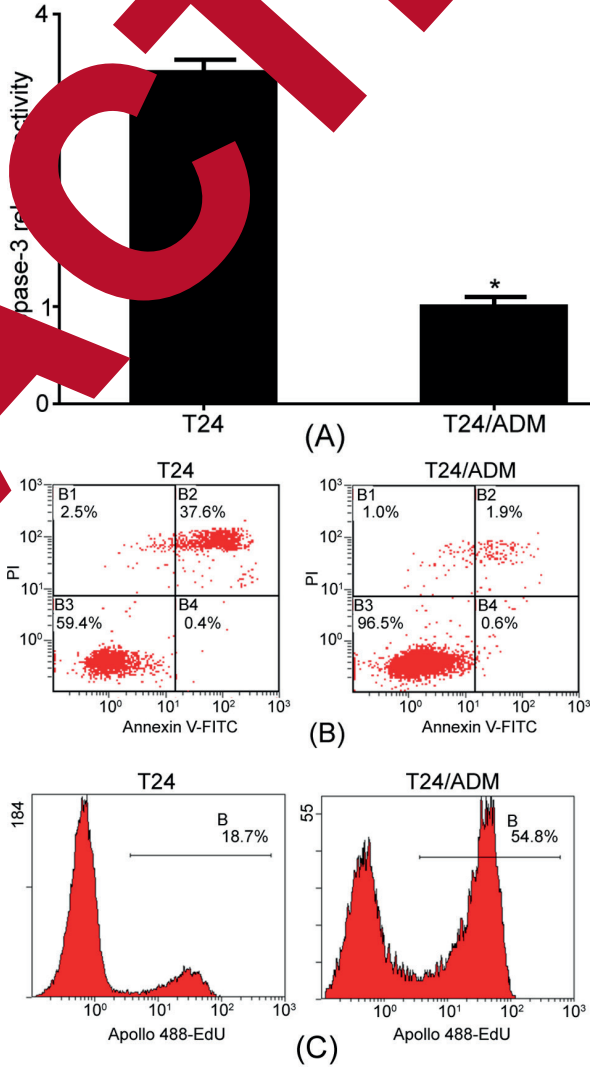

Figure 1. T24/ADM decreased sensitivity to ADM. ***(A)*** Spectrophotometry detection of caspase-3 activity; ***(B)*** Flow cytometry detection of cell apoptosis; ***(C)*** Flow cytometry detection of cell proliferation. $^{*}p < 0.05$, compared with T24 cells.

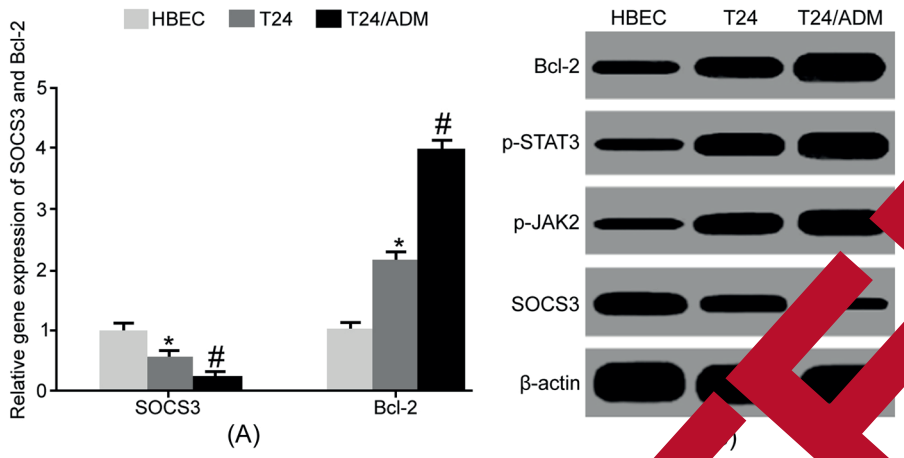

Figure 2. SOCS3 level reduction, JAK-STAT3 activity and Bcl-2 expression el[illegible] were related t[illegible]g resistance. ***(A)*** qRT-PCR detection of mRNA expression; ***(B)*** Western blot detection of [illegible]ssion. $^{*}p < 0$[illegible] compared with HBEC cells. # $p < 0.05$, compared with T24 cells.

tion, and promoted cell apoptosis in T24/ADM cells. pSicoR-SOCS3 transfection combined FLLL32 treatment exhibited the strongest impact.

Discussion

B-cell lymphoma 2 (Bcl-2) is an anti-apo[illegible]totic factor depending on mi[illegible]drial pa[illegible]thway[9]. It plays an anti-apop[illegible]e[illegible]through multiple mechanisms, su[illegible]s chan[illegible]g mitochondrial membrane pe[illegible]bility [illegible]ting cytochrome C r[illegible]ase[illegible]regula[illegible]n is closely related [illegible]cell ap[illegible]s reduction and drug resist[illegible]in various [illegible]r cells[10], such as cervi[illegible]a[illegible]11, gastric c[illegible]er[12], and lung cancer[13]. JAK-ST[illegible]ignaling pathway is found to [illegible]an importa[illegible]in cell proliferation, c[illegible]and apoptosis. [illegible]ated JAK phospho[illegible]es me[illegible]ane receptor, leading to confor[illegible]n, c[illegible]ges to promote STAT, covering the ty[illegible]hospho[illegible]tion loci on membra[illegible]ecep[illegible]mpl[illegible]t this time, JAK kinase p[illegible]horyl[illegible]to let it form dimer with and[illegible]r STAT[illegible]s entering the nucleus to regu[illegible]e transcription and expression[14]. Nu[illegible]es demonstrated that Bcl-2 is one [illegible]the important target genes of JAK-STAT3 si[illegible]ling pathway[15,16]. JAK-STAT3 mediated Bcl-2[illegible]gulation and cell apoptosis reduction are associated with drug resistance in cancer cells, including lung cancer[17] and colorectal cancer[16]. SOCS3 is the strongest member in SOCS family [illegible]can inhibit[illegible]AK phosphorylation and the a[illegible]ity of JAK[illegible]ase[7]. It is a critical negative r[illegible]tor of JA[illegible]TAT3[18]. It was showed that SO[illegible]decre[illegible]was related to chemotherapeutic dru[illegible]ivity reduction in breast cancer [illegible]ells[8], indicating that SOCS3 may have the ef[illegible]f lowering drug resistance. However, its [illegible]bladder cancer drug resistance is still unclear. This study established ADM resistant bladder cancer cell model to investigate the role of SOCS3-JAK/STAT3 signaling pathway ADM resistance. We revealed that IC50 in T24/ADM cells was significantly higher than that in T24 cells. Cell apoptosis was higher, while cell proliferation was significantly lower in T24 cells compared with T24/ADM cells after treated by ADM. It suggested the successful establishment of ADM resistant T24/ADM cells. SOCS3 reduced, while p-JAK2, p-STAT3, and Bcl-2 expressions upregulated in T24 cells compared with HBEC cells. It indicated that SOCS3 downregulation mediated JAK-STAT3 activity enhancement and Bcl-2 elevation were associated with bladder cancer occurrence. Gaballah et al[19] revealed that SOCS3 expression markedly declined in bladder cancer tissue compared with normal control. Its expression was related to pathological grading, as it declined in patients with higher pathological grade. We demonstrated that SOCS3 expression in bladder cancer cells was markedly lower than that in normal bladder epithelial cells, revealing that SOCS3 downregulation was related to bladder cancer occurrence, which was similar to Gabal-

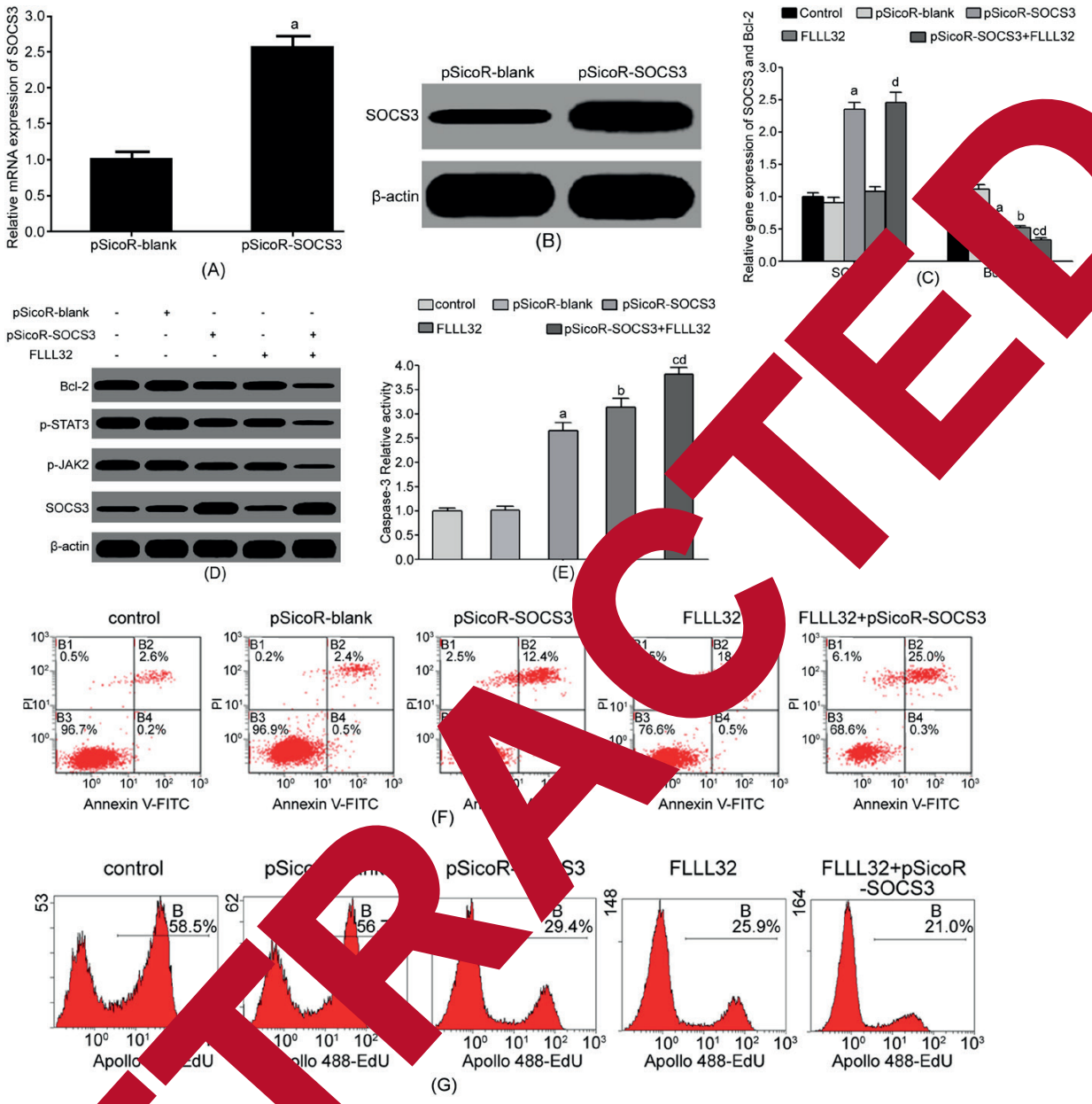

Figure OCS3 upregulation de ed T24/ADM drug resistance. ***(A)*** qRT-PCR detection of SOCS3 mRNA expression in T2 M cells; Western blot detection of SOCS3 protein expression in T24/ADM cells; ***(C)*** qRT-PCR detection of mR pressi T24/ADM cells; ***(D)*** Western blot detection of protein expression in T24/ADM cells; ***(E)*** Spectrophotometr of caspa ctivity; ***(F)*** Flow cytometry detection of cell apoptosis; ***(G)*** Flow cytometry detection of cell proliferat $p < 0.05$ pared with pSicoR-blank; (b) $p < 0.05$, compared with control; (c) $p <$ pSi-OCS3, 0 mpared with FLLL32.

ings[19]. Moreover, T24/ADM cells vealed lower SOCS3 protein, higher p-JAK2, TAT3, and Bcl-2 protein levels than T24 c It showed that SOCS3 downregulation mediated JAK-STAT3 activity enhancement and Bcl-2 elevation may play a regulatory role in bladder cancer drug resistance. Souckova et al[8] reported that SOCS3 lower expression and dysfunction was related to chemotherapeutic drug sensitivity decrease in breast cancer cells. Liu et al[20] revealed that SOCS3 expression was closely associated with leukemia cell K562 and KU812 sensitivity to imatinib. However, the relation between SOCS3 expression and

bladder cancer cell drug resistance was still unreported. Our results indicated that SOCS3 downregulation may play a promoting role in bladder cancer cell ADM resistance. Thus, this work further explored the regulatory role of SOCS3-JAK-STAT3 in bladder cancer cell ADM resistance through intervening SOCS3 expression and downstream JAK-STAT3 pathway activity. SOCS3 overexpression and/or FLLL32 treatment significantly downregulated Bcl-2 expressions, attenuated cell proliferation, and elevated sensitivity to ADM induced cell apoptosis. Yang et al[21] reported that JAK-STAT3 signaling pathway activation markedly enhanced bladder cancer cell migration and invasion. Zhang et al[22] showed that reducing STAT3 expression and function inhibit bladder cancer T24 cell proliferation and survival. Bednarek et al[23] revealed that reducing STAT3 may attenuate T24 cell proliferation and accelerate cell apoptosis. Joung et al[24] demonstrated that JAK2 kinase inhibitor AG490 markedly declined JAK-STAT3 signaling pathway, weakened cell proliferation and migration, and restrained cell grow[illegible] *in vivo*. In this study, SOCS3 overexp[illegible] and/or JAK-STAT3 signaling pathway b[illegible]a-ge significantly weakened bladder cancer[illegible] malignancy, which was similar to Zhang et a[illegible] and Joung et al[24]. Ojha et al[25][illegible]vered th[illegible] JAK-STAT3 signaling path[illegible] cement facilitated autophagy to [illegible]ngthe[illegible]isplatin resistance in bladder ca[illegible]cells[illegible] trary, inhibiting JAK[illegible]na[illegible]obvi[illegible]y declined stemnes[illegible]ne and[illegible] resistance gene expression[illegible]trained cel[illegible]iferation, and enhanced[illegible]sensitivity[illegible]his study confirmed[illegible]hat JA[illegible]TAT3 signaling pathway pla[illegible] regulatory[illegible]in T24 cell ADM resista[illegible]which was in ac[illegible]ance with Ojha et al[illegible]e also[illegible]vealed that SOCS3 downregu[illegible] pla[illegible] regulatory role in enhancing JAK-[illegible]ignalin[illegible]thway and drug resi-[illegible]ce i[illegible]der [illegible]er cells.

Conclusions

SOCS3 reduction was associated with bladder [illegible]er sensitivity to ADM. SOCS3 overexpres-si[illegible]ecreased JAK-STAT3 signaling pathway activity, declined Bcl-2 expression, inhibited cell proliferation, elevated cell apoptosis, and enhanced ADM sensitivity in T24 cells.

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Conflict of interest

The authors declare no conflicts of interes[illegible]

Refe[illegible]es

1) Massari F, Santo[illegible] M[illegible]re[illegible] Brunel[illegible] Conti A, Santini D[illegible]ntiro[illegible]scinu S[illegible]ora G. Emerging c[illegible]pts on dr[illegible]stan[illegible] bladder cancer: In[illegible]ions for futu[illegible]es. Crit Rev Oncol [illegible]15; 96: 81-90.

2) Meng [illegible] Lei T, Z[illegible]M, Zhao J, Zhao XH. Identification of protei[illegible]fferentially expressed in [illegible]cin-resistant [illegible]91/ADM) and parental (pumc-91) human b[illegible]dder cancer cell lines by proteome analysis. J Cancer Res Clin Oncol 2013; 139: 5[illegible]9.

3) [illegible]u Y, Hong Y[illegible]Y, Liu P, Guo DH, Chen Y. Inhibition of the J[illegible]TAT pathway with ruxolitinib overcomes resistance in non-small-cell lung cancer[illegible]SCLC. Apoptosis 2014; 19: 1627-1636.

4) Yang S, Luo C, Gu Q, Xu Q, Wang G, Sun H, Qian Z, Tan [illegible]Y, Shen Y, Xu X, Chen SH, Chan CC, Wang H, Mao [illegible]ang DD. Activating JAK1 mutation may predict the sensitivity of JAK-STAT inhibition in hepatocellular carcinoma. Oncotarget 2016; 7: 5461-5469.

5) Guo A, Lu P, Coffey G, Conley P, Pandey A, Wang YL. Dual SYK/JAK inhibition overcomes ibrutinib resistance in chronic lymphocytic leukemia: Cerdulatinib, but not ibrutinib, induces apoptosis of tumor cells protected by the microenvironment. Oncotarget 2017; 8: 12953-12967.

6) Ruchatz H, Coluccia AM, Stano P, Marchesi E, Gambacorti-Passerini C. Constitutive activation of Jak2 contributes to proliferation and resistance to apoptosis in NPM/ALK-transformed cells. Exp Hematol 2003; 31: 309-315.

7) Tamiya T, Kashiwagi I, Takahashi R, Yasukawa H, Yoshimura A. Suppressors of cytokine signaling (SOCS) proteins and JAK/STAT pathways: regulation of T-cell inflammation by SOCS1 and SOCS3. Arterioscler Thromb Vasc Biol 2011; 31: 980-985.

8) Souckova K, Kovarik A, Dusek L, Humpolikova-Adamkova L, Lauerova L, Krejci E, Matouskova E, Bursikova E, Fojtova M, Kovarik J. Reduced inducibility of SOCS3 by interferon gamma associates with higher resistance of human breast cancer lines as compared to normal mammary epithelial cells. Neoplasma 2009; 56: 379-386.

9) Gahl RF, Dwivedi P, Tjandra N. Bcl-2 proteins bid and bax form a network to permeabilize the mitochondria at the onset of apoptosis. Cell Death Dis 2016; 7: e2424.

10) Han J, Chen Q. MiR-16 modulate temozolomide resistance by regulating BCL-2 in human glioma cells. Int J Clin Exp Pathol 2015; 8: 12698-12707.

11) Leisching G, Loos B, Botha M, Engelbrecht AM. Bcl-2 confers survival in cisplatin treated cervical cancer cells: circumventing cisplatin dose-dependent toxicity and resistance. J Transl Med 2015; 13: 328.

12) Han Z, Hong L, Han Y, Wu K, Han S, Shen H, Li C, Yao L, Qiao T, Fan D. Phospho Akt mediates multidrug resistance of gastric cancer cells through regulation of P-gp, Bcl-2 and Bax. J Exp Clin Cancer Res 2007; 26: 261-268.

13) Yang TM, Barbone D, Fennell DA, Broaddus VC. Bcl-2 family proteins contribute to apoptotic resistance in lung cancer multicellular spheroids. Am J Respir Cell Mol Biol 2009; 41: 14-23.

14) Swierkot J, Nowak B, Czarny A, Zaczynska E, Sokolik R, Madej M, Korman L, Sebastian A, Wojtala P, Lubinski L, Wiland P. The activity of JAK/STAT and NF-kappaB in patients with rheumatoid arthritis. Adv Clin Exp Med 2016; 25: 709-717.

15) Sepulveda P, Encabo A, Carbonell-Uberos F, Minana MD. BCL-2 expression is mainly regulated by JAK/STAT3 pathway in human CD34+ hematopoietic cells. Cell Death Differ 2007; 14: 378-380.

16) Zhu Z, Li E, Liu Y, Gao Y, Sun H, Ma G, Wang Z, Liu X, Wang Q, Qu X, Yu Y. Inhibition of Jak-STAT3 pathway enhances bufalin-induced apopto in colon cancer SW620 cells. World J Sur 2012; 10: 228.

17) Zhu F, Dai C, Fu Y, Loo JF, Xia D, Gao SP, Ma Z, Z. Physalin A exerts anti-tumor activity in non-s cell lung cancer cell lines by suppressing J STAT3 signaling. Oncotarget 20 62-9476.

18) Collins AS, McCoy CE, Llo OT ly C, Stevenson NJ. miR-19a: a ective ulator of SOCS3 and enhancer of JAK-STAT signalling. PLoS One 2013; 8: e69090.

19) Gaballah HH, Shafik NM, Wasfy RE, Abou Farha MO. Significance of suppressor of cytokine signaling-3 expression in bladder urothelial carcin lation to proinflammatory cytokines a stopathological grading. Asian Pac ncer Prev 2015; 16: 307-314.

20) Liu YX, Wang L, Liu WJ, Zhang H H, Zhang ZW, Gao CJ. MiR-124-3p/P ALT1 a s an important role in SOCS3- ated growt he mo-sensitivity of CML. matol Oncol 201

21) Yang C, Zhang W, L, Kaz G, Han B, Hou T. Musashi- ote ration a inva- sion in bladder ce ation of AK2/ STAT3 path ab Inv 16; 96 958.

22) Zhang B, ou Y, Hu J, e effects of STAT3 silencing o growth of human ma cells. Tumour Biol 2014; 35: 5401-5407.

23) l, Sypniewski D k N, Galilejczyk A, Go- s K. The efficiency o ilencing expression of he gene codin STAT3 transcriptional factor and susceptibilit ladder cancer cells to apoptosis. Contemp On Pozn) 2012; 16: 316-321.

24) ng YH, N M, Yoo YB, Darvin P, Sp N, Kang HS, Choi YH, Lee HK, Park KD, Cho BW, Yang YM. Combination of AG490, a Jak2 inhibitor, and methylsulfonylmethane synergistically suppresses bladder tumor growth e Jak2/STAT3 pathway. Int J Oncol 2014; 4 883-895.

25) Ojha R, Singh SK, Bhattacharyya S. JAK-mediated autophagy regulates stemness and cell survival in cisplatin resistant bladder cancer cells. Biochim Biophys Acta 2016; 1860: 2484-2497.