Original Article
The interaction of SS18 and β-catenin promotes tumorigenesis of synovial sarcoma

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Abstract: To clarify the clinicopathological and biological role of SS18 and β-catenin in synovial sarcoma, our results showed that SS18 and β-catenin expression were upregulated in synovial sarcoma cells and tissues. SS18 positive expression was significantly associated with primary tumor location (P<0.05). The 5-year OS rate (22.0%) for patients with SS18 positive expression was less than that (38.4%) for patients with SS18 negative expression. Significant positive correlation between SS18 expression and β-catenin abnormal expression was found in synovial sarcoma tissues (P=0.004). SS18 knockdown dramatically inhibited cellular proliferation, colony formation, and migration, but induced G1 phase arrest and apoptosis in SW982 cells. Further study showed that SS18 knock-down dramatically suppressed β-catenin and its down-stream genes expression in SW982 cells. Interestingly, β-catenin knock-down dramatically suppressed SS18 expression in SW982 cells. Wnt-3a and DKK1 increased and suppressed nuclear and cytoplasmic SS18 expression in SW982 cells, respectively. Most importantly, a direct interaction of SS18 and β-catenin was found in SW982 cells. β-catenin transcriptional activity significantly increased in SW982 cells transfected with SS18 expression plasmid. In conclusion, our findings first indicate that the interaction of SS18 and β-catenin might play a crucial role in tumorigenesis and progression of synovial sarcoma.

Keywords: SS18, β-catenin, synovial sarcoma

Introduction
Synovial sarcoma is a high-grade soft tissue malignancy and has a specific chromosomal translocation t(X; 18), which leads to the fusion of the SS18 gene to one of three SSX genes (SSX1, SSX2 or SSX4). The 5- and 10-year disease-specific survival is 62% and 52% in adults, respectively [1]. β-catenin is a key player in Wnt-signaling pathway. Oncogenic activation of Wnt-signaling pathway is mandatory for the initial neoplastic transformation of intestinal epithelium [2]. Nuclear accumulation of β-catenin as a cell-signaling event may play an important role in the progression of synovial sarcoma [3]. Abnormal levels of β-catenin could contribute to the development and progression of synovial sarcoma, through increasing the proliferative activity of the tumour cells [4]. More importantly, SYT-SSX2 recruits β-catenin to the nucleus and associates with it in an active complex [5]. Upregulation of the Wnt/β-catenin cascade by SYT-SSX2 correlates with its nuclear reprogramming function in synovial sarcoma [6]. SS18-SSX-induced Wnt/β-catenin signaling appears to be of crucial biological importance in synovial sarcoma tumorigenesis and progression [7]. Most recently, Barrott et al. have reported that β-catenin stabilization enhances SS18-SSX2-driven synovial sarcomagenesis and blocks the mesenchymal to epithelial transition [8].

Whether β-catenin regulates SS18 in synovial sarcoma has not been reported. Our current study is to investigate the clinicopathological and biological significance of SS18 and β-catenin in synovial sarcoma.

Materials and methods
Cell lines and small interfering RNA (siRNA) sequences
The human synovial sarcoma cell line SW982 was maintained in Leibovitz’s L-15 Medium
SS18 and β-catenin in synovial sarcoma

Table 1. The relationship between SS18 expression and clinicopathological features of synovial sarcoma

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SS18 expression</th>
<th>Overall survival analysis</th>
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<tbody>
<tr>
<td></td>
<td>Positive (%)</td>
<td>Negative (%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22 (34.9)</td>
<td>7 (11.1)</td>
</tr>
<tr>
<td>Female</td>
<td>28 (44.4)</td>
<td>6 (9.6)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥34</td>
<td>25 (39.6)</td>
<td>3 (5.0)</td>
</tr>
<tr>
<td>&lt;34</td>
<td>25 (39.6)</td>
<td>10 (15.8)</td>
</tr>
<tr>
<td>Primary location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axial</td>
<td>19 (30.0)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Peripheral</td>
<td>31 (49.2)</td>
<td>12 (19.5)</td>
</tr>
<tr>
<td>Histological type</td>
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<td></td>
</tr>
<tr>
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</tr>
<tr>
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<td>2 (3.3)</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4.8</td>
<td>22 (34.9)</td>
<td>8 (12.6)</td>
</tr>
<tr>
<td>&gt;4.8</td>
<td>14 (26.4)</td>
<td>2 (3.3)</td>
</tr>
<tr>
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<td>14 (22.2)</td>
<td>3 (4.7)</td>
</tr>
<tr>
<td>Metastasis</td>
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<td>9 (14.2)</td>
</tr>
<tr>
<td>Yes</td>
<td>22 (34.9)</td>
<td>4 (6.5)</td>
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<td>Relapse</td>
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</tr>
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<td>37 (58.5)</td>
<td>6 (9.6)</td>
</tr>
<tr>
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<td>1 (1.5)</td>
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<td>TNM</td>
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<td></td>
</tr>
<tr>
<td>I-II</td>
<td>25 (39.6)</td>
<td>9 (14.2)</td>
</tr>
<tr>
<td>III-IV</td>
<td>25 (39.6)</td>
<td>4 (6.6)</td>
</tr>
<tr>
<td>SS18 expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>50 (79.4)</td>
<td>-</td>
</tr>
<tr>
<td>Negative</td>
<td>13 (20.6)</td>
<td>-</td>
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</table>

(Invitrogen, Carlsbad, CA), human fibroblast-like synoviocyte (FLS) was cultured in high-glucose Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen, Carlsbad, CA). All medium were supplemented with 10% (v/v) fetal bovine serum (Invitrogen), 1× antibiotic/antimycotic (100 units/mL streptomycin, 100 units/mL penicillin, and 0.25 mg/mL amphotericin B). All cell lines were cultured in humidified incubator at 37°C with 5% CO₂.

The targeted SS18 sequences were: sense, 5'-GAU GAC AAU AAC CUU AdTdT-3'. The targeted β-catenin sequences were: sense, 5'-GCC ACA AGA UUA CAA GAA AdTdT-3'. The siRNA duplexes were chemically synthesized and purified by Ribobio Co. Ltd (Guangzhou, China) and transfected using Lipofectamine RNAiMAX transfection reagent (Invitrogen). Lipofectamine RNAiMAX alone (Mock) and scrambled siRNA (NC-siRNA) were used as negative control groups.

Patient information and tissue specimens
A total of 63 samples of paraffin-embedded synovial sarcoma tissues and one pair of fresh synovial sarcoma tissues and their respective adjacent non-tumor (ANT) samples between 1994 and 2013 were collected from our Department of Pathology. Prior patient consent and approval from the Institutional Research Ethics Committee were obtained. No patients had received chemotherapy or radiotherapy before operation. The histopathology of the disease was determined by two pathologists according to WHO classification of tumours of soft tissue and bone. Thirty two samples of synovial sarcoma collected between 2008 and 2013 were also confirmed by fluorescent in situ hybridization (FISH) analysis using a LSI SYT (18q11.2) Dual Color, Break-Apart Rearrangement Probe kit (Vysis, Downers Grove, IL). Clinical staging was done according to UICC classification. Pertinent follow-up information was available for all patients. Detailed clinical information is summarized in Table 1.

Cell proliferation assay
1×10⁵ SW982 cells were plated onto 96-well plates with medium containing 10% FBS and incubated overnight. After transfection with 100 nM SS18-siRNA, cell proliferation was
SS18 and β-catenin in synovial sarcoma

Figure 1. SS18 and β-catenin expression in synovial sarcoma cells and tissues. (A) SS18 and β-catenin protein expression in synovial cell line SW982 and human fibroblast-like synoviocyte (FLS); (B) SS18 (negative: a, c; positive: b, d) and β-catenin (normal expression: e, g; abnormal expression: f, h) and expression in monophasic (a, b, e, f) and biphasic (c, d, g, h) synovial sarcoma tissues by immunohistochemistry staining, ×200; (C) Overall survival (OS) of synovial sarcoma patients with SS18 positive expression and negative expression by Kaplan-Meier analysis.

Colony formation assay

After 48 hours 100nM SS18-siRNA transfection, 600 SW982 cells were plated onto 6-well plates and incubated at 37°C in a 5% CO₂ incubator for 2 weeks. Fresh medium was added every 4 days. NC-siRNA was used as the control group. At the end-point, the cells were washed with cold phosphate buffered saline (PBS), fixed with 4% paraformaldehyde for 30 minutes and stained with 1% crystal violet solution for 20 minutes at room temperature. The visible colony numbers were counted. This experiment was performed in triplicate.

Cell cycle and apoptosis assay

3×10⁶ SW982 cells were seeded in 6-well plates and incubated overnight until 50% confluent, then transfected with 100 nM SS18-siRNA for 48 hours, washed in cold PBS, fixed with 70% cold ethanol for 24 hours at 4°C, then stained with propidium iodide buffer (50 mg/ml propidium iodide, 0.1% sodium citrate and 0.1% Triton X-100) for 30 minutes at room temperature. 2×10⁴ cells were analyzed for cell cycle and apoptosis using a Becton Dickinson FACScan (Becton Dickinson Immunocytometry Systems, San Jose, CA). The percentage of cells in each phase of the cell cycle and apoptotic cells was quantified using Cell Quest software, respectively. This experiment was performed in triplicate.

Determination of mitochondrial membrane potential

Mitochondria were isolated from SW982 cells and incubated overnight until 30%-50% confluence, then transfected with 100 nM SS18-siRNA. NC-siRNA was used as the control group. Both floating and adherent cells were collected at 24 hours. 1×10⁶ cells were incubated with 500 μl JC-1 working solution containing JC-1 stock solution and assay buffer (1:100, Keygene, China) at 37°C for 20 minutes. Cells were washed with the assay buffer twice. The depolarization of mitochondrial membrane potential was determined using a Becton Dickinson FACScan. This experiment was performed in triplicate.
Table 2. The correlation between SS18 expression and β-catenin expression in synovial sarcoma

<table>
<thead>
<tr>
<th>Variable</th>
<th>SS18 expression</th>
<th>$P$ value</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (%)</td>
<td>Negative (%)</td>
<td></td>
</tr>
<tr>
<td>β-catenin expression</td>
<td>Normal</td>
<td>18 (28.6)</td>
<td>11 (17.4)</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>32 (50.8)</td>
<td>2 (3.2)</td>
</tr>
</tbody>
</table>

Determination of intracellular free calcium concentration

After SS18 siRNA transfection for 48 hours, SW982 cells were serum-starved and incubated for 24 hours. Both floating and adherent cells were collected and washed twice with cold PBS, then resuspended in 4 μM Fluo-2AM and 0.05% Pruronic F-127 (Keygene, China). The cells were incubated for 45 min in the dark room at 37°C. Calcium fluorescence intensity was determined using a Becton Dickinson FACScan. This experiment was triplicated independently.

Scratch wound assay

SW982 cells were plated in 6-well plates and incubated overnight until 30%-50% confluent, then transfected with 100 nM SS18-siRNA. Vertical scratches were then made using a 100 μl plastic filter tip to create a 'wound' of approximately 100 μm in diameter. To eliminate dislodged cells, culture medium was removed and wells were washed with PBS. 'Wound closure' was observed at 0, 12, 24, 48 hours and digital images were taken under an inverted microscope.

Transwell migration assay

Transwell migration assay was carried out in Transwell chambers containing polycarbonate filters (8 μm pore size; Corning Incorporated, Life Sciences, NY). After transfected with 100 nM SS18-siRNA for 48 hours, 2×10⁴ SW982 cells in a 500 μl volume of serum-free medium were placed in the upper chambers and incubated at 37°C with 5% CO₂ for 24 hours, respectively. While a 200 μl volume of medium containing 15% FBS was added to the lower chamber as chemoattractant. Cells were allowed to migrate for 24 hours at 37°C with 5% CO₂. Following migration, cells were fixed with 4% formaldehyde and stained with 1% crystal violet. Cells on the upper surface of the filters were removed by wiping with a cotton swab. Cells counts were the mean number of cells per fields of view. Three independent experiments were performed and the data were presented as mean ± standard deviation (SD).

Cellular fractionation

Cultured cells were collected and resuspended in 500 μl 1× hypotonic buffer on ice. 25 μl detergent was added and vortexed for 10 seconds at highest settings. Suspension was centrifuged for 30 seconds at 14000 g in a microcentrifuge tube and precooled at 4°C. The supernatant (cytoplasmic fraction) was transferred into a prechilled microcentrifuge tube. Nuclear pellet was resuspended in 50 μl complete lysis buffer and centrifuged for 10 minutes at 14000 g in a microcentrifuge tube and precooled at 4°C. Supernatant (nuclear fraction) was transferred into a prechilled microcentrifuge tube.

Western blot analysis

As our previously described [9], SS18 (Abcam), β-catenin, cyclin D1, c-Myc, phosphorylated-glycogen synthase kinase 3β (phos-GSK-3β) (Ser9), MMP9 (Cell Signaling Technology, Danvers, MA) antibodies were used to probe expression of proteins at 4°C for 12 hours. Signal was detected by enhanced chemiluminescent techniques (Amersham Life Science, Piscataway, NJ). GAPDH (Sigma) and Lamin B1 (Cell Signaling Technology) were used as the loading control.

Co-immunoprecipitation and immunoblotting analysis

For co-immunoprecipitation analysis, cells lysates were incubated with 5 μg antibody on a rotator overnight at 4°C. The protein-antibody-protein A/G agarose complexes were prepared by adding protein A/G agarose beads (Invitrogen) for an hour at 4°C. After extensive washing with Radio-Immunoprecipitation Assay (RIPA) lysis buffer, the immunoprecipitated complexes were resuspended in reducing sample buffer and boiled for 10 minutes. After centrifugation to pellet the agarose beads, supernatants were subjected to SDS-PAGE and immunoblotting.
Luciferase reporter assay

1x10^4 SW982 cells were seeded in triplicates in 96-well plates. 0.45 ug luciferase reporter plasmid containing SS18 plasmid and 0.45 ug pRL-TK Renilla plasmid (Promega) were transfected into cells using the Lipofectamine 2000 reagent (Invitrogen). Empty vector was used as the control group. After 48 hours transfection, Luciferase and Renilla signals were measured using the Dual-Luciferase Reporter Assay kit (Promega). Three independent experiments were done and the data were presented as the mean ± SD.

Immunohistochemistry and evaluation

As our previously described [9], the working concentrations of primary antibody for the detection of β-catenin and SS18 (Abcam) was 1:100 and 1:200, respectively. The staining of β-catenin was scored as following. When more than 70% of synovial sarcoma cells were positively stained for membranous β-catenin, the cells were classified as β-catenin normal expression; if more than 10% of sarcoma cells were positively stained for cytoplasm or nuclei was regarded as β-catenin abnormal expression. The expression of SS18 was located in the cell nucleus. SS18 expression was considered as positive when nuclear staining was found in ≥10% of tumour cells.

Statistical analyses

Chi-square test was used to compare the levels of SS18 and β-catenin expression with different groups and various clinicopathological parameters. The Kaplan-Meier survival curves were used to estimate overall survival (OS). The significance of predictor variables for OS was evaluated by the long-rank test. Prognostic factors associated with OS were investigated according to the Cox proportional hazards regression model in a stepwise manner. Only those factors that were statistically significant (P<0.05) in the univariate survival analysis were included in the multivariate analyses. Groups from cell culture experiments were compared using an unpaired, two-tailed Student’s tests and results were presented as mean ± SD. For cell proliferation assay, comparison was done by univariate variance analysis (two-way ANOVA). Statistical analyses were performed using SPSS 16.0 statistical software. P<0.05 was considered to be statistically significant.

Results

SS18 and β-catenin expression in synovial sarcoma cell line and tissues

As shown in Figure 1, SS18 and β-catenin protein expressions were significantly higher in synovial sarcoma cell line SW982 compared with FLS cell line by Western blot analysis. In our series, 50 samples (79.4%, 50/63) of synovial sarcoma were positive for SS18 by immunohistochemistry staining. The positive rate of SS18 ranged from 80% to 90% of the tumor cells. However, SS18 expression was not found in ANT tissues. SS18 positive expression was significantly higher in synovial sarcoma tissues than that in ANT tissues (P<0.01). SS18 positive expression was significantly associated with primary tumor location (P<0.05). No significant relationship between SS18 positive expression and gender, age, histological type, tumor size, tumor metastasis, relapse, and TNM stage was found. The 5-year OS rate was 22.0% for patients with SS18 positive expression, which was less than 38.4% for patients with SS18 negative expression, but the difference did not reach a level of statistical significance by Kaplan-Meier analysis (P=0.47). Univariate Cox regression analysis showed that age, metastasis and TNM stage were independent prognostic factors for OS in synovial sarcoma patients. Further multivariate Cox regression analysis demonstrated that tumor metastasis (HR=4.086, 95% CI 0.625~1.545; P=0.043) was the independent prognostic factors for OS in synovial sarcoma patients (Table 1).

Thirty four samples of synovial sarcoma (54.0%, 34/63) were β-catenin abnormal expression, 29 samples (46.0%, 29/63) were β-catenin normal expression. However, β-catenin abnormal expression was found in 21 ANT tissues (33%). β-catenin abnormal expression was higher in synovial sarcoma tissues than that in ANT tissues (P<0.01). There was a positive correlation between SS18 positive expression and β-catenin abnormal expression in synovial sarcoma (r^2=0.395, P=0.004, Table 2).

SS18 knock-down reduced synovial sarcoma cell growth

SS18 protein level in SW982 cells transfected with SS18-siRNA at 48 hours was significantly decreased compared with the control group. CCK8 assay showed that SS18 knockdown sig-
SS18 and β-catenin in synovial sarcoma

Significantly suppressed SW982 proliferation compared with NC-siRNA control group (P<0.0001). Furthermore, the mean number of colony formation in SW982 cells transfected with SS18-siRNA (mean number=150) was significantly less than that in NC-siRNA transfection group (mean number=277) (P<0.0001). The cell colony formation rate was also significantly suppressed about 40% in SW982 cells transfected with SS18-siRNA compared with NC-siRNA group (P<0.0001) (Figure 2).

SS18 knock-down resulted in cell cycle arrest and induced apoptosis in synovial sarcoma cells

As shown in Figure 2, SS18 knock-down significantly resulted in G1-S phase arrest in SW982 cells compared with NC-siRNA transfection group (P<0.001). Furthermore, the proportion of early and late apoptotic cells were 4.84% and 8.18% in SW982 transfectected with SS18-siRNA compared with about 2.86% and 3.39% in the control group, respectively. The percentage of apoptotic cells in SW982 upon SS18 knock-down increased by 100% compared with the control group (P<0.05).

To further investigate the molecular events triggered by SS18 knockdown, we determined the depolarization of mitochondrial membrane potential and intracellular free calcium concentration in SW982 cells. The depolarization of mitochondrial membrane potential is considered as a symbolic event of early cellular apoptotic. Our results showed that the depolarization rate of mitochondrial membrane potential (16.0 ± 1.386%) was dramatically higher in SW982 transfected with SS18-siRNA than that in NC-siRNA group (5.2 ± 1.815%, P<0.01). The calcium fluorescence intensity (13.47×10^4) was significantly higher in SW982 upon SS18 knock-down than that in NC-siRNA group (4.68×10^4, P<0.01).

SS18 knock-down suppressed migration in synovial sarcoma cell

Scratch wound assay showed that cell migration was dramatically suppressed in SW982
SS18 knockdown inhibited SW982 cell migration by scratch wound assay (A) and transwell migration assay (B), respectively.

SS18 knockdown dramatically suppressed β-catenin protein expression in SW982 cells compared with NC-siRNA control group by Western blot analysis. Further study showed that decreased nuclear and cytoplasmic β-catenin and its down-stream gene including c-Myc, cyclin D1, MMP9 and up-stream gene phos-GSK3β (Ser9) expression were found in SW982 cells transfected with β-catenin-siRNA compared with the control group (Figure 4).

β-catenin knockdown suppressed SS18 expression in synovial sarcoma cells

To determine the effect of β-catenin on SS18 expression in synovial sarcoma, our data showed that β-catenin knockdown dramatically suppressed SS18 and β-catenin down-stream gene including c-Myc, cyclin D1, MMP9 and up-stream gene phos-GSK3β (Ser9) expression in SW982 cells compared with NC-siRNA control group by Western blot analysis. Further study showed that decreased nuclear and cytoplasmic SS18, β-catenin down-stream gene including c-Myc, cyclin D1, MMP9 and up-stream gene phos-GSK3β (Ser9) expression were found in SW982 cells transfected with β-catenin-siRNA compared with the control group (Figure 4).
SS18 and β-catenin in synovial sarcoma

Figure 4. SS18 knock-down inhibited β-catenin signaling pathway in synovial sarcoma (A, B). β-catenin knockdown inhibited SS18 expression in synovial sarcoma (C, D). (A) SS18 knockdown inhibited β-catenin expression in SW982 cells; (B) Nuclear and cytoplasmic β-catenin, c-Myc, cyclin D1, MMP9 and phos-GSK-3β (Ser9) expression decreased in SW982 cells upon SS18 knock-down; (C) β-catenin knockdown inhibited SS18 expression in SW982 cells; (D) Nuclear and cytoplasmic SS18 and β-catenin, c-Myc, cyclin D1, MMP9 and phos-GSK-3β (Ser9) protein expression decreased in SW982 cells upon β-catenin knock-down. GAPDH was considered as loading control. Lamin B1 was considered as nuclear loading control.

showed that β-catenin transcriptional activity significantly increased in SW982 cells transfected with SS18 expression plasmid compared with the empty vector control group at 48 hours (P<0.0001).

Discussion

Synovial sarcoma is characterized by chromosomal translocation t(X; 18), which leads to the fusion of the SS18 gene to one of three SSX genes (SSX1, SSX2 or SSX4). Disease stage at presentation, tumor size, and tumor grade were significant predictors of survival in synovial sarcoma. SYT-SSX fusion type was not correlated with survival [10]. There was no significant difference in OS or disease-specific survival between SS18-SSX1 and SS18-SSX2, but there were indications of SS18-SSX1 being an unfa-
SS18 and β-catenin in synovial sarcoma

Figure 5. Interaction of SS18 and β-catenin in synovial sarcoma. (A, B) Recombinant Wnt3a (A) and DKK1 (B) increased and decreased nuclear and cytoplasmic SS18, β-catenin, and c-Myc, cyclin D1, MMP9 and phos-GSK-3β (Ser9) expression in SW982 cells, respectively; (C) Recombinant Wnt3a reversed SS18, β-catenin, and c-Myc, cyclin D1, MMP9 and phos-GSK-3β (Ser9) expression suppression in SW982 cells upon SS18 knock-down; (D) Direct interaction of SS18 protein and β-catenin protein in SW982 cells by co-immunoprecipitation assay; (E) β-catenin transcriptional activity significantly increased in SW982 cells transfected with SS18 expression plasmid by dual-luciferase reporter assay compared with the empty vector control group.

Favorable prognostic factor of progression-free survival or metastasis-free survival [11]. He et al. have reported forty-one out of 47 (87%) synovial sarcoma displayed strong positive nuclear staining for SYT antibody by immunohistochemistry staining [12]. Consistent with He’s findings, our data showed that 79.4% (50/63) of synovial sarcoma were positive for SS18 by immunohistochemistry staining and synovial sarcoma patients with SS18 positive expression had lower OS compared with the patients with SS18 negative expression although the difference did not reach a level of statistical significance. This issue needs further study in large series.

As for the biological role of SS18 in synovial sarcoma, SS18-SSX1 knock-down inhibits cell growth and induces apoptosis in synovial sarcoma [13, 14]. Downregulation of SS18-SSX1 expression in synovial sarcoma by small interfering RNA enhances the focal adhesion pathway and inhibits anchorage-independent growth in vitro and tumor growth in vivo [15]. Jones et al. show in human and murine synovial sarcoma cells that SS18-SSX increases BCL2 expres-
SS18 and β-catenin in synovial sarcoma

...expression, but represses other anti-apoptotic genes, including MCL1 and BCL2A1 [16]. Truncated SSX protein suppresses synovial sarcoma cell proliferation by inhibiting the localization of SS18-SSX fusion protein [17]. Our results demonstrated that SS18 knockdown dramatically inhibited cellular proliferation, migration, colony formation, but induced G1 phase arrest and apoptosis in synovial sarcoma cells. Further study showed that SS18 knock-down increased the depolarization rate of mitochondrial membrane potential and calcium fluorescence intensity in synovial sarcoma cells.

Pretto et al. have reported that SYT-SSX2 recruits β-catenin to the nucleus [5]. SS18-SSX fusion protein-induced Wnt/beta-catenin signaling is a therapeutic target in synovial sarcoma [7]. Consistent with these findings, our data showed that there was a positive correlation between SS18 positive expression and β-catenin abnormal expression in our series. Further study showed that SS18 knock-down suppressed nuclear and cytoplasmic β-catenin and its down-stream gene including c-Myc, cyclin D1, MMP9 and up-stream gene phos-GSK3β (Ser9) expression in SW982 cells. Most importantly, a direct interaction of SS18 protein and β-catenin protein was found in SW982 cells by co-immunoprecipitation assay. β-catenin transcriptional activity significantly increased in SW982 cells transfected with SS18 expression plasmid. To further study whether β-catenin regulates SS18 in synovial sarcoma, our data first showed that β-catenin knockdown dramatically suppressed SS18 expression in SW982 cells. Wnt-3a and DKK1 increased and suppressed nuclear and cytoplasmic SS18 expression in SW982 cells, respectively. Moreover, suppression of nuclear and cytoplasmic SS18, β-catenin and its down-stream gene including c-Myc, cyclin D1, MMP9 and up-stream gene phos-GSK3β (Ser9) expression in SW982 cells upon SS18 knock-down was attenuated by recombinant Wnt3a treatment. The results suggest that there might be a positive feedback of SS18 and β-catenin in synovial sarcoma. Whether are there other pathways of SS18 regulating in synovial sarcoma besides β-catenin? Wakamatsu et al. show deflection of vascular endothelial growth factor action by SS18-SSX and composite vascular endothelial growth factor- and chemokine (C-X-C motif) receptor 4-targeted therapy in synovial sarcoma [18]. Minami et al. report that SS18-SSX-regulated miR-17 promotes tumor growth of synovial sarcoma by inhibiting p21WAF1/CIP1 [19].

In summary, our findings indicate that the interaction of SS18 and β-catenin might play a crucial role in tumorigenesis and progression of synovial sarcoma.

Disclosure of conflict of interest

None.

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References


SS18 and β-catenin in synovial sarcoma


