

Evaluation of expression of cancer stem cell markers and fusion gene in synovial sarcoma: Insights into histogenesis and pathogenesis

YANG ZHOU^{1*}, DONGDONG CHEN^{1*}, YAN QI^{1*}, RUIXUE LIU¹, SHUGANG LI³,
HONG ZOU¹, JIAOJIAO LAN¹, XINXIN JU¹, JINFANG JIANG¹,
WEIHUA LIANG¹, YAOYUAN SHEN⁴, LIJUAN PANG¹ and FENG LI^{1,2}

¹Department of Pathology and Key Laboratory of Xinjiang Endemic and Ethnic Diseases (Ministry of Education), Shihezi University School of Medicine; Department of Pathology, First Affiliated Hospital to Shihezi University School of Medicine, Shihezi, Xinjiang 832002; ²Department of Pathology, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020; ³Department of Public Health, Shihezi University School of Medicine, Shihezi, Xinjiang 832000; ⁴Department of Pathology, People's Hospital of Xinjiang Autonomous Region, Urumqi, Xinjiang 830000, P.R. China

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Abstract. Synovial sarcoma (SS) is an aggressive soft tissue tumor, with uncertain histological and cellular origin. SYT-SSX is considered to be responsible for sarcoma initiation and progression. The histogenesis and pathogenesis of this tumor are poorly understood, and prognosis of patients of SS is unsatisfactory. Recent studies have shown an association of cancer stem cells with the initiation and development of tumors. We explored immunohistochemical expression level of stem cell associated markers to determine the possible histogenesis and pathogenesis of SS. Fusion gene SYT-SSX was tested to assess diagnostic value and the molecular pathological features. We obtained the clinicopathological data of 20 SS patients, immunohistochemical staining were used to evaluate stem cell-associated markers included CD133, CD29, CD44, nestin, and ALDH1. Fusion gene SYT-SSX was tested by reverse transcriptase-polymerase chain reaction (RT-PCR). Twenty

SS cases were observed and the positive immunoexpression results showed CD133 (17/20), CD29 (11/20), CD44 (11/20), nestin (6/20), and ALDH1 (5/20). Fusion gene SYT-SSX was successfully detected by RT-PCR from 18 available samples. The expression of stem cell-associated markers (CD133, CD29, CD44, Nestin, and ALDH1) and clinical data (age, gender, sites, tumor size, histological type, tumor stage, and distant metastases) did not show statistically significant relationship ($P>0.05$), whereas, statistically significance between ALDH1 and metastases was observed ($P<0.01$). The ALDH1 positive synovial sarcoma (ALDH1⁺ SS) cases had significantly poor prognosis compared to ALDH1 negative synovial sarcoma (ALDH1⁻ SS) cases ($P<0.05$). Immunohistochemical results indicated different expression levels of the five cancer stem cell markers in SS suggesting that SS may arise from cancer stem cells. Fusion gene SYT-SSX may play a critical role in the molecular pathological of SS.

Correspondence to: Professor Lijuan Pang, Department of Pathology and Key Laboratory for Xinjiang Endemic and Ethnic Diseases, Shihezi University School of Medicine, North 2nd Road, Shihezi, Xinjiang 832002, P.R. China
E-mail: ocean123456@163.com

Professor Feng Li, Department of Pathology, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, P.R. China
E-mail: lifeng7855@126.com

*Contributed equally

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Introduction

Synovial sarcoma (SS) is a malignant tumor that accounts for 7-10% of soft tissue sarcomas, which arises mostly in young adults with high risk of metastasis and recurrence (1). SS occurs in a wide variety of organs, with higher incidence near the joints of the lower extremities (2). The histological types of SS include biphasic type synovial sarcoma (BSS), monophasic type synovial sarcoma (MSS), and poorly differential type synovial sarcomas (PDSS) (1). Immunohistochemistry plays an important role in the identification and diagnosis of SS (3). EMA and CD99 are the commonly used markers for diagnosis of epithelial and mesenchymal tumors, recently, they have also been used in the diagnosis of SS. Approximately 60% of CD99 expression was positive and may be related to the histological type of SS. Most of the studies reported that CD99 showed high levels of expression

in single phase synovial sarcoma and poorly differentiated synovial sarcoma. The positive expression rate of CD99 in the poorly differentiated SS was >90% (2).

The cellular origin of SS remains unknown, SS is currently classified as a miscellaneous tumor of uncertain histological origin, which is considered to arise from undifferentiated mesenchymal cells (2). Studies have supported the histogenesis and initiation of tumor may involve cancer stem-like cells (CSLCs) that may derive from a small population of pluripotent stem cells (4-6). These stem-like cells initiate and sustain tumor growth in epithelial tumors including various soft tissue sarcoma such as synovial sarcoma (7-12). Specific surface markers such as CD133, CD44 and nestin have been identified to isolate the cancer stem cells in breast cancer, pancreatic cancer and gastric cancer (13-15). However, the relationship between SS and tumor stem cells are still poorly understood. Studies have indicated that CD133 and other tumor stem cell-related markers are highly expressed in SS. The identification of cancer stem cell marker expression level has contributed to the understanding of the pathogenesis and origin of SS, and provides a new perspective for the diagnosis and treatment of SS.

Recent studies showed that >90% of synovial sarcoma with chromosomal translocation t(X;18) (p11.2;q11.2) leads to formation of SYT-SSX fusion protein (16). It is generally believed that the SYT-SSX fusion gene is an important event in the early stage of synovial sarcoma. SYT-SSX is thought to be responsible for sarcoma initiation and development. The value of SYT-SSX fusion gene in the diagnosis of synovial sarcoma has been widely recognized, however, the mechanism remains elusive (17).

This study explored immunohistochemical expression level of stem cell-associated markers to determine the possible histogenesis and pathogenesis of SS. Fusion gene SYT-SSX was evaluated to assess diagnostic value and the molecular pathological features.

Materials and methods

Patients and tissue specimens. A total of 20 SS patients were included from the Department of Pathology, the First Affiliated Hospital, Shihezi University School of Medicine between 1978 and 2016. Histopathological diagnosis was evaluated independently by two certified pathologists from the Department of Pathology, the First Affiliated Hospital, Shihezi University School of Medicine. The collected data included age, gender, sites, tumor size, histological type, tumor stage, molecular pathology, metastases, and the follow-up surveys (Table I). The study was approved by the institutional ethics committee at the First Affiliated Hospital of Shihezi University School of Medicine. Consent was obtained from the subjects for participation in the study and the use of their tissue.

Immunohistochemistry. Sections (4 μ m) prepared from formalin-fixed and paraffin-embedded tissue were obtained for immunohistochemical analysis. EnVisions two-step immunohistochemical kit (EnVision; Dako, Glostrup, Denmark) were used to detect specific target proteins. Briefly, the baked sections were deparaffinized with xylene and rehydrated in graded ethanol. Then sections were performed by microwave

in citrate buffer (pH 6.0), heated at 100°C and quenched with 3% hydrogen peroxide. The samples were incubated with specific target antibodies at 4°C overnight. The information of stem cell markers (including CD133, CD29, CD44, nestin and ALDH1) are shown in Table II. Sections were then washed with PBS and incubated with secondary antibodies at 37°C. 3, 3'-Diaminobenzidine (DAB) was used as a chromogen. Finally, slides were counterstained with hematoxylin, gradient alcohol and xylene dehydration, and mounted. The expression of stem cell markers was scored semi-quantitatively according to the percentage of positive cells and staining intensity. The samples were scored according to the staining intensity and the ratio of positive cells as follows: distribution (0, 0%; 1, $\leq 10\%$; 2, 10-50%; 3, $\geq 50\%$) and intensity (0, negative; 1, weak; 2, moderate; and 3, strong). A score of 0 was given for sections with no staining, and 1 was the lowest positive score. The product of the two scores determined the final score values; a score of 0 indicated negative expression (-), whereas a score of 1-3 represented weak positive expression (+). Similarly, a score of 4-12 was considered as strong positive expression (++). All of the results were confirmed by at least two senior pathologists independently.

Reverse transcription polymerase chain reaction (RT-PCR). Total RNA was extracted from 18 of 20 paraffin-embedded tissues of SS using TRIzol, the other 2 cases were from consultations which did not provide paraffin tissue. Since 2 of 18 paraffin tissues could not be evaluated by RT-PCR because of the low RNA quality, 16 cases of RNA were successfully tested. To detect SYT-SSX transcript and positive control β -actin transcript, the design of the primers is shown in Table III. One-step RT-PCR was conducted using RNA PCR kit. The PCR products were visualized by electrophoresis on 2% agarose gels.

Statistical analysis. Data were analyzed by SPSS software 17.0, and Fisher exact test was used to compare the relationship between the stem cell associated markers expression and clinicopathological data. Two-tailed P-values at <0.05 were considered statistically significant.

Results

Clinical findings. The SS patients comprised of 10 males and 10 females, with a median age of 37 years (range: 10-64 years). All samples were primary tumors located in the limbs (17 cases) and extra-limb (oral, kidney, and neck). According to the follow-up survey, 7 patients had a poor prognosis with distant metastases (5 patients with lung metastasis, 1 patient with liver metastasis, and 1 patient with bone metastasis). Three patients still live with disease after presentation. Three patients dropped out, the other 14 patients died of their disease (Table I). ALDH1⁺ SS cases had good prognosis with higher survival rate than ALDH1⁻ SS cases ($P < 0.05$) (Fig. 1, Table IV).

Pathological findings. According to the fourth WHO diagnostic criteria SS can be divided into biphasic synovial sarcoma (BSS), monophasic fibrous synovial sarcoma (MFSS) and poorly differentiated synovial sarcoma (PDSS). Histologically, the main characteristic of synovial sarcoma is

Table I. Clinical data in 20 cases of synovial sarcomas.

| Patient ID | Gender/Age | Site | Size (cm) | Diagnosis | TNM | Fusion gene | Metastases | Outcome |
|------------|------------|-----------------------|-----------|-----------|-----|-------------|--------------------|---------|
| 1 | M/14 | Left elbow fossa | 6 | MFSS | IIB | SYT-SSX2 | NM | DOD |
| 2 | F/19 | Left thigh | 7.5 | MFSS | III | SYT-SSX | NM | NA |
| 3 | M/32 | Oral | 3 | MFSS | I | SYT-SSX1 | Lung | DOD |
| 4 | M/37 | Right elbow | 2 | MFSS | III | SYT-SSX2 | NM | DOD |
| 5 | M/40 | Left hip | 22 | MFSS | IV | SYT-SSX2 | Liver | Alive |
| 6 | M/47 | Left leg | 5 | MFSS | IV | SYT-SSX2 | Lung | DOD |
| 7 | F/40 | Left bone | 7.5 | PDSS | III | SYT-SSX | NM | DOD |
| 8 | F/10 | Right elbow | 5 | BSS | IV | SYT-SSX1 | Bone marrow cavity | DOD |
| 9 | F/15 | Right neck | 4 | BSS | IIA | NA | NM | NA |
| 10 | F/21 | Right thigh | 7.5 | BSS | I | SYT-SSX1 | NM | Alive |
| 11 | F/22 | Left heel | 3 | BSS | IIA | SYT-SSX1 | NM | NA |
| 12 | F/32 | Left groin | NA | BSS | IIA | NA | NM | DOD |
| 13 | M/36 | Left forearm | 11 | BSS | III | SYT-SSX1 | NM | DOD |
| 14 | F/37 | Left hand and forearm | 6 | BSS | III | SYT-SSX1 | NM | DOD |
| 15 | M/40 | Right femoral | 2.8 | BSS | IV | SYT-SSX1 | NM | DOD |
| 16 | F/43 | Right kidney | 13 | BSS | III | SYT-SSX1 | NM | DOD |
| 17 | M/52 | Left ilium | 5 | BSS | III | SYT-SSX2 | NM | Alive |
| 18 | F/55 | Right foot | 10 | BSS | IV | SYT-SSX1 | Lung | DOD |
| 19 | M/55 | Left thigh | 5.2 | BSS | I | SYT-SSX1 | Lung | DOD |
| 20 | M/64 | Left hip | 11.5 | BSS | IV | SYT-SSX1 | Lung | DOD |

BSS, biphasic synovial sarcoma; MFSS, monophasic fibrous synovial sarcoma; PDSS, poorly differentiated synovial sarcoma; F, female; M, male; NM, not metastasizing; DOD, died of disease; NA, not available.

Table II. Primary antibodies used in the immunohistochemistry staining.

| Antigen | Antibody species | Location | Company | Clone number | Dilution |
|---------|-------------------|-----------|-------------------------|--------------|----------|
| CD133 | Rabbit Polyclonal | Cytoplasm | ARP, Waltham, MA, USA | 05-PA1021 | 1:200 |
| CD29 | Rabbit monoclonal | Cytoplasm | Abcam, Cambridge, UK | EP1041Y | 1:800 |
| CD44 | Mouse monoclonal | Cytoplasm | Dako, Glostrup, Denmark | DF1485 | 1:300 |
| Nestin | Rabbit monoclonal | Cytoplasm | Abcam, Cambridge, UK | SP103 | 1:200 |
| ALDH1 | Rabbit monoclonal | Cytoplasm | Abcam, Cambridge, UK | EP1933Y | 1:200 |

Table III. The primers of SYT-SSX and β -actin.

| Primer | Sequence |
|------------------|------------------------------|
| SYT | 5'-CCAGCAGAGGCCCTTATGGATA-3' |
| SSX | 5'-TTTGTGGGCCAGATGCTTC-3' |
| SSX1 | 5'-GTGCAGTTGTTTCCCATCG-3' |
| SSX2 | 5'-GCACAGCTCTTTCCCATCA-3' |
| β -actin-F | 5'-CAGTTTGGAGCTCCTGGAAG-3' |
| β -actin-R | 5'-TGCAAATCCAGGGTGCACTG-3' |

epithelial and mesenchymal biphasic differentiation. In our study, the median tumor size was 7.2 cm, with a range from 2 to 22 cm. The cases were composed of 13 biphasic synovial sarcoma (BSS), 6 monophasic fibrous synovial sarcoma (MFSS), and 1 poorly differentiated synovial sarcoma (PDSS). Biphasic SS contained epithelial cells and spindle cells. The variable biphasic differentiation presented glands with a tubular or papillary architecture or solid nests with a population of oval or rounded cells. Epithelioid cells formed glands. Glands are irregular and covered by columnar or cuboidal cells. Tumor cells had eosinophilic cytoplasm, nucleus was round or oval and hyperchromatic (Fig. 2A and B). The spindle

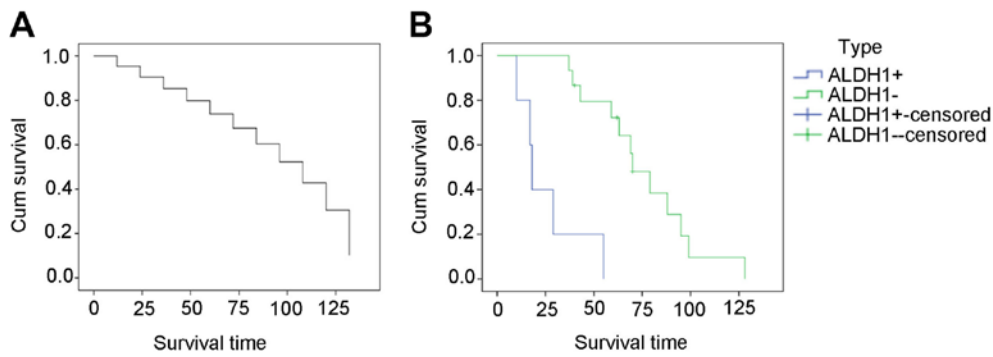


Figure 1. The cumulative survival rate of all synovial sarcoma patients. All patients died at follow-up in 128 months, excepting lost to follow-up patients (A). The cumulative survival rate of ALDH1+ and ALDH1- synovial sarcoma patients. Patients who ALDH1-positive had a worse event-free survival than patients ALDH1-negative (B).

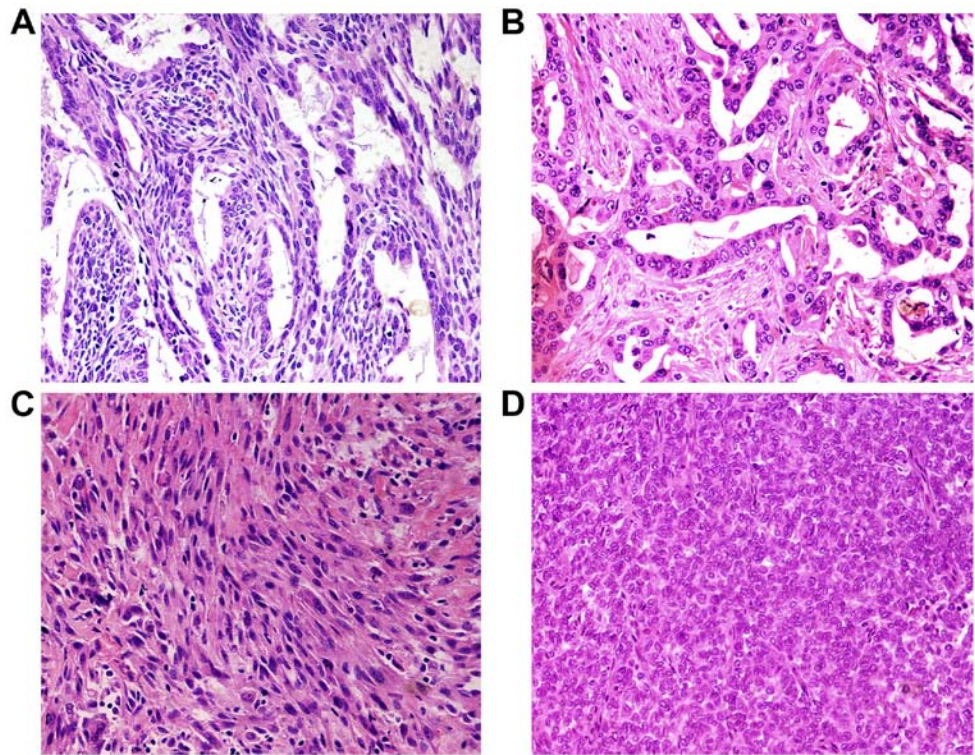


Figure 2. Histologic features of synovial sarcoma (SS). SS displaying biphasic type synovial sarcoma (BSS) of glandular structure (A, x200) or solid nests (B, x200). MFSS composed of highly cellular short fascicles of uniform spindle cells (C, x200). PDSS composed of round tumor cells with nuclear atypia (D, x200).

Table IV. P-value of the cumulate survival of ALDH1⁺ and ALDH1⁻ synovial sarcoma patients.

| Test | Overall comparisons | | |
|-----------------------|---------------------|----|--------|
| | Chi-square | df | Sig |
| Log Rank (Mantel-Cox) | 18.190 | 1 | <0.001 |

Test of survival distributions for the different level of type.

cells resemble histologically the monophasic fibrous synovial sarcoma and were ovoid to uniform spindle-shaped with

elongated nuclear features forming dense cellular sheets and fascicles. The cell atypia was inconspicuous with pale-stained or eosinophilic cytoplasm. Nucleus showed short spindle shape, granular chromatin, mesenchymal vascular slightly enriched, slit-shaped thin wall (Fig. 2C). Poorly differentiated type synovial sarcoma was composed of round hyperchromatic atypical tumor cells with diffuse distribution. The cell nucleus was large, obviously atypical and eosinophilic (Fig. 2D).

Immunohistochemical findings of stem cell marker expression in SS. In 20 specimens, 17 were stained positive for CD133, 11 of 20 were positive for CD29 and CD44, 6 of 20 were positive for nestin (Tables V and VI). CD133 was detected in epithelial cells (Figs. 3A and B, and 4A and B). CD29 were also detected in the vascular endothelial cells

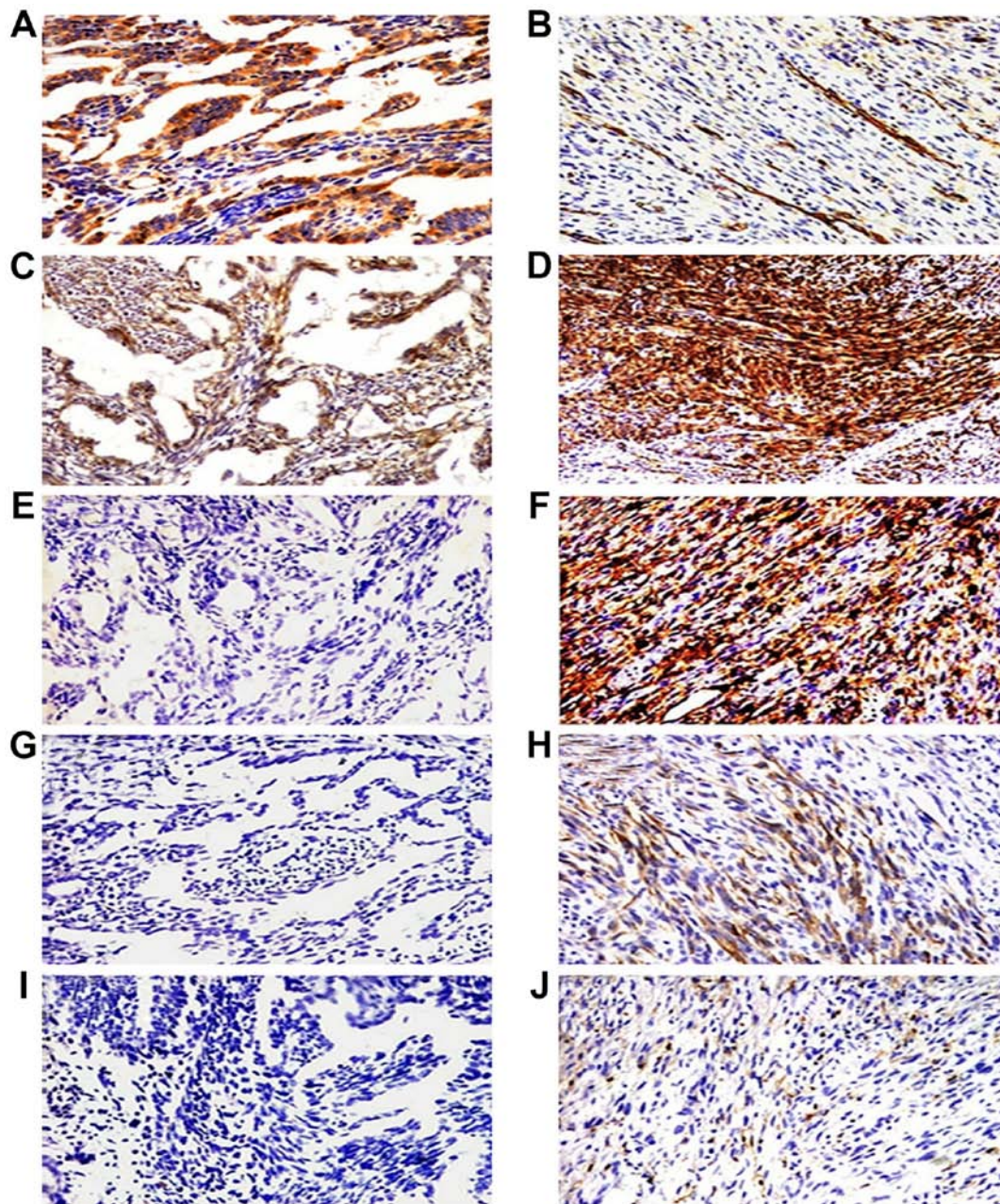


Figure 3. Immunohistochemical staining of the five cancer stem marker expression including CD133, CD29, CD44, nestin and ALDH1 in epithelial cells and spindle cells of biphasic type synovial sarcoma (BSS). Epithelial cells of BSS positive expression for CD133 and CD29 (A and C, x200) and negative expression for CD44, nestin and ALDH1 (E, G and I, x200). Spindle cells of BSS overexpressed CD29 and CD44 (D and F, x400), scattered expression of CD133, nestin and ALDH1 (B, H and J, x400).

(Figs. 3C and D, and 4C and D). CD44 and nestin displayed focal immunoreactivity in spindle cells (Figs. 3E-H; 4E-H). Of the 20 specimens, 5 were ALDH1-positive in scattered tumor cells (Figs. 3J and 4J) and negative in 15 cases (Figs. 3I and 4I). There was no statistically significant relationship between the expression of stem cell-associated markers (CD133, CD29, CD44, nestin, and ALDH1) and clinical data (age, gender, sites, tumor size, histological type, tumor stage, and metastases) ($P>0.05$), the expression of ALDH1 was significantly related to the metastases of SS ($P<0.05$) (Tables VII and VIII).

RT-PCR. Total RNA was extracted and evaluated successfully from 18 cases of SS. The β -actin was tested in 247 bp. The

results of RT-PCR showed that fusion gene SYT-SSX (98 bp) was available in all the cases, SYT-SSX1 and SYT-SSX2 fusion gene were tested in 118 bp. SYT-SSX1 and SYT-SSX2 transcript were detected in 11 cases and 5 cases respectively. Among 11 cases of SYT-SSX1, 10 cases were BSS and 1 case was MFSS, the 5 cases of SYT-SSX2 included 4 cases of MFSS and 1 case of BSS. Two cases showed no explicit typing (Table I; Figs. 5 and 6).

Discussion

Synovial sarcoma (SS) is classified as uncertain differentiation tumor in the 4th WHO Classification of soft tissue tumors that occur in a widely variety of organs, especially near the joints

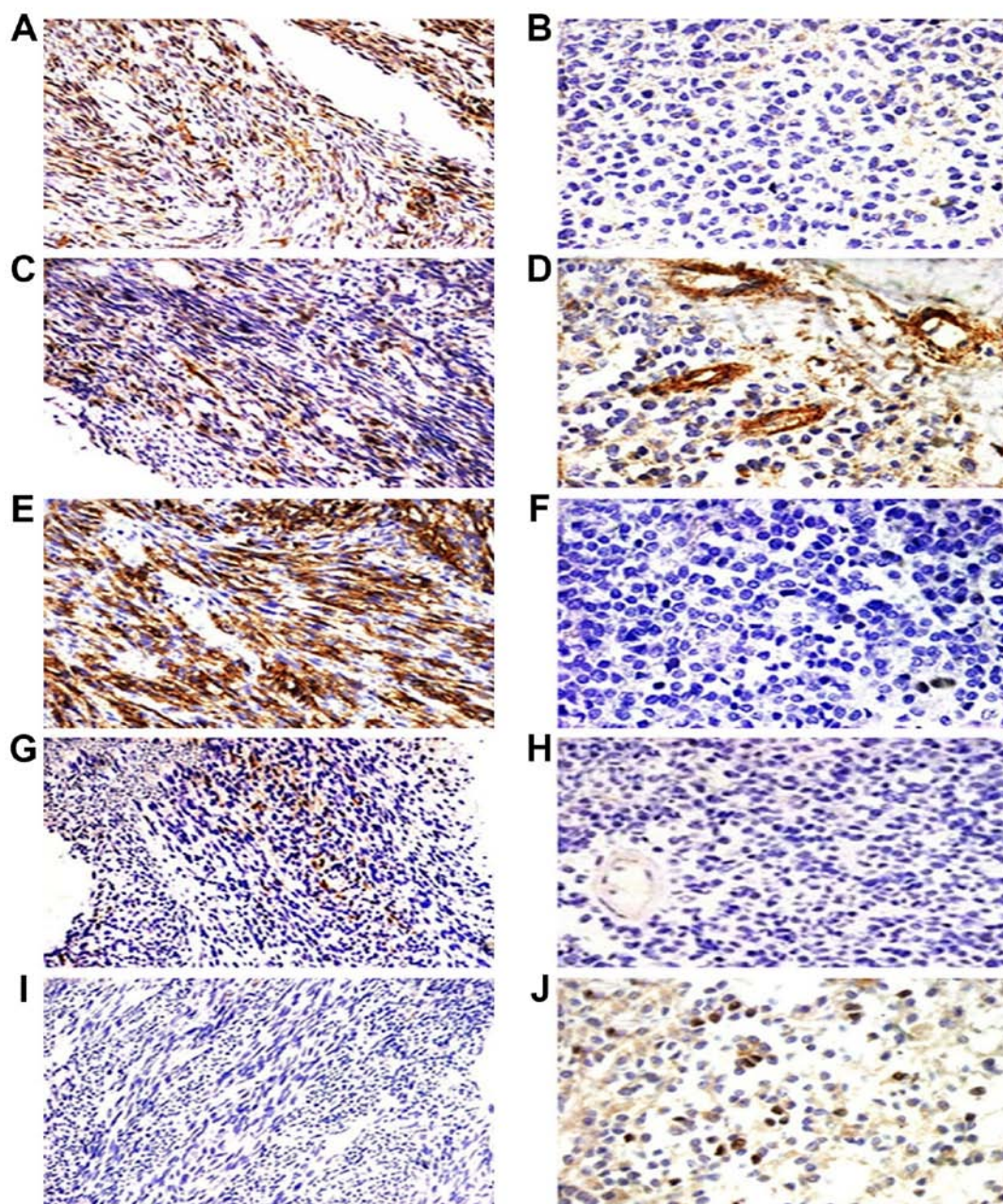


Figure 4. Immunohistochemical staining of the five cancer stem marker expression including CD133, CD29, CD44, nestin and ALDH1 in monophasic fibrous synovial sarcoma (MFSS) and poorly differential synovial sarcoma (PDSS). MFSS positive expression of CD133, CD29 and CD44 (A, C and E, x200) and scattered expression of ALDH1 (J, x200), scattered expressed of nestin (G, x200), negative expression of CD133, CD44 and nestin (B, F, and H, x400). PDSS scattered expression of CD29 and ALDH1 (D and J, x400), and with positive expression of CD29 in vascular (D, x400), negative expression of ALDH1 (I, x200).

of the limbs. SS occurs more frequently in young adults, in males more common than females. SS is a highly malignant soft tissue tumor with poor prognosis, the recurrence rate was approximately 50% within two years after operation and metastasis occurred in 40% of patients. The 5-year survival rate was <30%, lung and lymph node metastasis may be negative prognostic factors of long-term survival. Thus, confirming the tumor cellular and tissue origin is of great significance in the process of making a correct diagnosis and differential diagnosis of SS (1,16,17).

In the present study, distant metastasis occurred in 7 patients, in addition to 3 patients lost to follow-up and 3 patients still alive, the other 14 patients all died of a tumor. Recent studies supported that cancer is initiated by a popula-

tion of tumor cells with stem cell characteristics (18-20). The cancer stem cell hypothesis suggests that cancer stem cells may contribute to the initiation, progression and recurrence of cancer (21,22). Expression of cancer stem cell makers have been reported in leukemia, breast cancer, colon cancer and sarcoma (7,8,10,11,23,24). CD133 was first described as a surface antigen specific to human hematopoietic stem and progenitor cells, but has also recently been recognized as a common stem cell marker (14,25-28). CD133 may be responsible for tumorigenesis and represents a pool of tumor progenitor cells or tumor stem cells (12). Terry *et al* isolated a small amount of CD133⁺ cells from SS cell line for the first time, this suggested the relationship between SS and cancer stem-like cells (25).

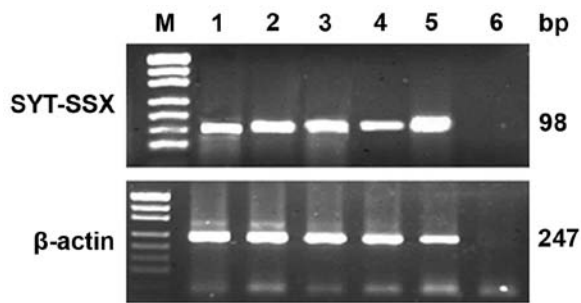


Figure 5. Bio-Rad gel image of SYT-SSX and β -actin. Lanes 1-5, positive for SYT-SSX in 98 bp and β -actin in 247 bp; lane 6, blank control.

Table V. Immunohistochemical expression for 5 stem cell markers in synovial sarcoma.

| Patient ID | Stem cell markers | | | | |
|------------|-------------------|------|------|--------|-------|
| | CD133 | CD29 | CD44 | Nestin | ALDH1 |
| 1 | ++ | - | ++ | - | - |
| 2 | + | - | + | - | - |
| 3 | + | - | ++ | - | - |
| 4 | + | + | + | + | - |
| 5 | ++ | + | + | - | - |
| 6 | + | - | - | - | + |
| 7 | - | - | - | - | + |
| 8 | ++ | - | - | - | + |
| 9 | ++ | ++ | - | - | - |
| 10 | ++ | ++ | + | - | - |
| 11 | + | + | - | - | - |
| 12 | + | ++ | - | - | - |
| 13 | - | ++ | ++ | + | - |
| 14 | ++ | + | ++ | - | - |
| 15 | + | - | - | - | - |
| 16 | - | ++ | ++ | - | - |
| 17 | ++ | ++ | ++ | + | + |
| 18 | ++ | - | - | + | - |
| 19 | ++ | - | - | ++ | - |
| 20 | + | ++ | ++ | ++ | + |

-, negative; +, weak positive; ++, positive.

In our immunohistochemical study, CD133 was positively expressed in 85% SS cases. It implies SS may be derived from stem cells and progenitor cells. ALDH1 has been considered to be cancer stem cell marker in lung cancer, liver cancer, colon cancer, prostate cancer, and breast cancer. Recently, some evidence proved that ALDH1 staining was observed as stem cell marker in soft tissue tumors, such as solitary fibrous tumor (29,30). Bouvier *et al* (29) reported that ALDH1 expression may play an important role in distinguishing SS from soft tissue tumors such as solitary fibrous tumors. Many studies have found that the expression of ALDH1 protein is related to the histologic subtypes, recurrence, metastasis and invasion of

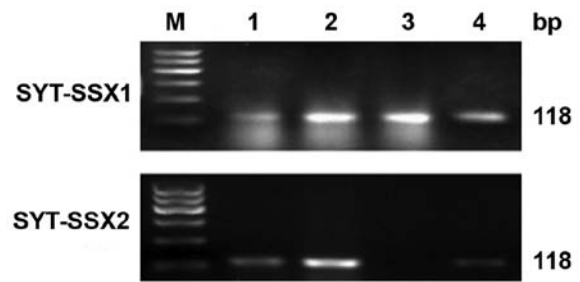


Figure 6. Bio-Rad gel image of SYT-SSX1 and SYT-SSX2: positive for SYT-SSX1 and SYT-SSX2 in 118 bp.

Table VI. Five stem cell marker expression in synovial sarcoma.

| Markers | Negative (-) | Positive | | Positive rate (%) ^a (n) |
|---------|--------------|----------|------|---------------------------------------|
| | | (+) | (++) | |
| CD133 | 3 | 9 | 8 | 85 (17) |
| CD29 | 9 | 4 | 7 | 55 (11) |
| CD44 | 9 | 4 | 7 | 55 (11) |
| Nestin | 14 | 4 | 2 | 30 (6) |
| ALDH1 | 15 | 4 | 1 | 25 (5) |

^aIncluded all weak positive (+) and positive (++) . Positive expression: CD133 (17/20), CD29 (11/20), CD44 (11/20), nestin (6/20) and ALDH1 (5/20).

the tumor. Increasing evidence has demonstrated ALDH1 as therapeutic target and a malignant tumor stem cell marker that indicates poor prognosis for tumors, decreased ALDH1 may suggest a good prognosis (31,32).

In our study, only 5 cases were positive for ALDH1, while ALDH1-positive cases are characteristic of poorly differentiated, tumors ≥ 5 cm, and TNM III/IV, especially in metastases. ALDH1⁻ SS cases had better survival rate than ALDH1⁺ SS cases. This suggested that the SS may originate from cancer stem like cells. The high expression of ALDH1 may suggest an immature undifferentiated state and high malignancy, which leads to poor prognosis. The high expression of ALDH1 may be responsible for the failure of traditional chemotherapy of some malignancies including colon cancer (33), prostate cancer (34) and breast cancer (35).

More studies need to be done to explore the role of ALDH1 in the development of SS. SS occur in multiple sites of the body, the cellular origin may be cells with multiple differentiation ability, such as stem cells. Studies show high expression of MSC surface markers in SS. CD29, nestin, and CD44 are surface proteins associated with mesenchymal stem cells (MSCs) (14,15,36). MSC markers CD29 and CD44 displayed high expression (55%). The mesenchymal stem/progenitor cells markers CD29 or CD44 could be identified and separated from SS, this suggested that SS may originate from mesenchymal stem/progenitor cells (37). Recently, nestin was considered as a stem cell or progenitor cell surface marker. Nestin was detected in 6 SS cases in this investiga-

Table VII. Association between expression of 5 stem cell markers, fusion gene and clinicopathological features.

| Factors | CD133 | | χ^2 value | P-value | CD29 | | χ^2 value | P-value | CD44 | | χ^2 value | P-value |
|-------------|----------|----------|----------------|---------|----------|----------|----------------|---------|----------|----------|----------------|---------|
| | Negative | Positive | | | Negative | Positive | | | Negative | Positive | | |
| Age | | | 0.131 | >0.05 | | | 0.067 | >0.05 | | | 0.067 | >0.05 |
| <50 | 2 | 13 | | | 7 | 8 | | | 7 | 8 | | |
| ≥50 | 1 | 4 | | | 2 | 3 | | | 2 | 3 | | |
| Gender | | | 0.392 | >0.05 | | | 1.181 | >0.05 | | | 0.202 | >0.05 |
| F | 2 | 8 | | | 3 | 7 | | | 5 | 5 | | |
| M | 1 | 9 | | | 6 | 4 | | | 4 | 6 | | |
| Size (cm) | | | 1.644 | >0.05 | | | 0.024 | >0.05 | | | 2.170 | >0.05 |
| <5 | 0 | 6 | | | 3 | 3 | | | 4 | 2 | | |
| ≥5 | 3 | 10 | | | 6 | 7 | | | 4 | 9 | | |
| TNM | | | 0.669 | >0.05 | | | 0.900 | >0.05 | | | 3.104 | >0.05 |
| I, II | 1 | 10 | | | 3 | 6 | | | 3 | 8 | | |
| III, IV | 2 | 7 | | | 6 | 5 | | | 6 | 3 | | |
| Metastases | | | 1.900 | >0.05 | | | 0.642 | >0.05 | | | 0.020 | >0.05 |
| + | 0 | 7 | | | 4 | 3 | | | 3 | 4 | | |
| - | 3 | 10 | | | 5 | 8 | | | 6 | 7 | | |
| Fusion gene | | | 1.039 | >0.05 | | | 0.042 | >0.05 | | | 0.95 | >0.05 |
| SYT-SSX1 | 2 | 9 | | | 5 | 6 | | | 5 | 6 | | |
| SYT-SSX2 | 0 | 5 | | | 2 | 3 | | | 1 | 4 | | |
| Histology | | | 1.644 | >0.05 | | | 1.310 | >0.05 | | | 2.328 | >0.05 |
| MFSS | 0 | 6 | | | 4 | 2 | | | 1 | 5 | | |
| BSS | 3 | 10 | | | 5 | 8 | | | 7 | 6 | | |

BSS, biphasic synovial sarcoma; MFSS, monophasic fibrous synovial sarcoma; PDSS, poorly differentiated synovial sarcoma.

tion, it was positive in MFSS or spindle cells of BSS in our study. This suggested spindle cells of SS may originate from MSCs. However, the relationship between stem cells and SS is poorly understood, a larger number of SS patients needs to be explored for the histogenesis of SS.

SS is characterized by a chromosomal translocation t(X;18) (p11.2;q11.2), and this molecular genetic feature does not appear in other tumors (38-40). SYT-SSX was tested positive in 93% cases of SS. The function of SYT-SSX fusion protein is not clear, but Naka *et al* reported that SS may be a stem cell malignancy because of the dysregulation of self-renewal and differentiation driven by SYT-SSX fusion protein (37). Garcia *et al* (41) reported that mesenchymal stem cells may be reprogrammed by SS-associated protein SYT-SSX, the aberrant differentiation of human mesenchymal stem cells were caused by SYT-SSX2. However, more studies need to be done to explore the relationship between stem cells and SYT-SSX fusion gene. Saito (42) reported that the tumor phenotype may be determined by the SYT-SSX fusion gene. Our study showed that 77% of BSS had the SYT-SSX1 fusion gene (10/13) and 67% MFSS the SYT-SSX2 fusion gene (4/6). SYT-SSX can be used as an excellent diagnostic marker for SS. In our study, SYT-SSX was detected in 18 cases, of these, 7 had distant metastasis (1 case metastasis to bone, 1 case metastasis to liver, 5 cases metastasis to lung), 14 patients died

of the disease (3 patients were not available, 3 patients are still alive). SYT-SSX1 may be related to the high proliferation rate of tumor cells, tumor distant metastasis and poor prognosis (38-40). SYT-SSX1 fusion gene was detected in 71.4% (5/7) of cases of SS with distant metastasis. Gene therapy for SYT-SSX will be of great importance for the diagnostics, treatment and prognosis of SS.

In conclusion, we detected stem cell marker (CD133, CD29, CD44, nestin, and ALDH1) expression to explore the histogenesis and cellular origin of SS. The immunohistochemical findings for the stem cell markers indicated that SS may originate from MSCs. The RT-PCR results showed fusion gene SYT-SSX may play an important role in the tumor initiation and progression of SS. Due to the characteristic expression of stem cell markers and SYT-SSX for SS, these may provide new efficient drug design and therapy strategy in future. Our results need to be confirmed by further exploration to study the function of cancer stem cell markers and fusion gene SYT-SSX for histogenesis and pathogenesis of SS.

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Table VIII. Association between expression of 5 stem cell markers and clinicopathological features.

| Factors | Nestin | | χ^2 value | P-value | ALDH1 | | χ^2 value | P-value | Fusion gene | | χ^2 value | P-value |
|-------------|----------|----------|----------------|---------|----------|----------|----------------|---------|-------------|----------|----------------|---------|
| | Negative | Positive | | | Negative | Positive | | | SYT-SSX1 | SYT-SSX2 | | |
| Age | | | 2.260 | >0.05 | | | 0.800 | >0.05 | | | 0.097 | >0.05 |
| <50 | 13 | 4 | | | 12 | 3 | | | 8 | 4 | | |
| ≥50 | 1 | 2 | | | 3 | 2 | | | 3 | 1 | | |
| Gender | | | 0.952 | >0.05 | | | 0.267 | >0.05 | | | 4.364 | >0.05 |
| F | 8 | 2 | | | 7 | 3 | | | 6 | 0 | | |
| M | 6 | 4 | | | 8 | 2 | | | 5 | 5 | | |
| Size (cm) | | | 0.012 | >0.05 | | | 3.132 | >0.05 | | | 0.097 | >0.05 |
| <5 | 4 | 2 | | | 6 | 0 | | | 3 | 1 | | |
| ≥5 | 9 | 4 | | | 8 | 5 | | | 8 | 4 | | |
| TNM | | | 0.087 | >0.05 | | | 0 | >0.05 | | | 0.428 | >0.05 |
| I, II | 8 | 3 | | | 9 | 3 | | | 4 | 1 | | |
| III, IV | 6 | 3 | | | 6 | 2 | | | 7 | 4 | | |
| Metastases | | | 0.848 | >0.05 | | | 12.381 | 0.001 | | | 0.042 | >0.05 |
| + | 4 | 3 | | | 13 | 0 | | | 5 | 2 | | |
| - | 10 | 3 | | | 2 | 5 | | | 6 | 3 | | |
| Fusion gene | | | 0.019 | >0.05 | | | 0.873 | >0.05 | | | - | - |
| SYT-SSX1 | 7 | 4 | | | 9 | 2 | | | - | - | | |
| SYT-SSX2 | 3 | 2 | | | 3 | 2 | | | | | | |
| Histology | | | 0.903 | >0.05 | | | 3.041 | >0.05 | | | 8.045 | 0.013 |
| MFSS | 5 | 1 | | | 9 | 4 | | | 1 | 4 | | |
| BSS | 8 | 5 | | | 8 | 0 | | | 10 | 1 | | |

BSS, biphasic synovial sarcoma; MFSS, monophasic fibrous synovial sarcoma; PDSS, poorly differentiated synovial sarcoma.

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