

Silencing of synaptotagmin 7 regulates osteosarcoma cell proliferation, apoptosis, and migration

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Summary. Background. Synaptotagmin 7 (SYT7) is a component of the synaptotagmin family, which is essential in many physiological and pathological processes. In this study, we aimed to investigate the role of SYT7 in osteosarcoma.

Methods. We defined the expression levels of SYT7 in osteosarcoma tissues and para-sarcoma tissues by immunohistochemistry and analyzed the possible correlation between SYT7 expression and pathological characteristics via Mann-Whitney U analysis and Spearman correlation analysis. The effects of SYT7 silencing *in vitro* on cell growth were assessed by MTT assay. Cell cycle and cell apoptosis were assessed by flow cytometry analysis. Wound healing assay and transwell assay were applied to assess the migration and invasion capacity.

Results. The results showed that the expression levels of SYT7 were upregulated in osteosarcoma tissues compared with para-sarcoma tissues and positively correlated with the pathological characteristics of osteosarcoma. Functional experiments demonstrated that SYT7 silencing significantly inhibited cell proliferation and colony formation capacity ($P < 0.001$), induced cell cycle arrest which increased the proportion of G2 phase

and decreased the proportion of S phase, enhanced cell apoptosis ($P < 0.01$), and limited the capacity of migration and invasion ($P < 0.01$), compared with shCtrl group.

Conclusion. The results indicated that SYT7 plays a crucial role in the development of osteosarcoma. SYT7 can be applied as a new diagnostic and therapeutic target in osteosarcoma.

Key words: Osteosarcoma, SYT7, Proliferation, Apoptosis, Migration

Introduction

Osteosarcoma is one of the most common primary bone malignancies in children and adolescents (Fenger et al., 2014; Miller et al., 2016). In the last few years, scientists have attempted to reveal novel diagnosis and treatment strategies and have had some results, such as ganglioside GD2 (Roth et al., 2014) and phosphatidylinositol-3 kinases (PI3Ks) (Kuijjer et al., 2014) which were explored as potential therapeutic targets for therapy in patients with osteosarcoma. Although multi-agent chemotherapy combined with surgical techniques has improved the 5-year survival for patients, the current treatment strategies have limited efficacy in osteosarcoma with metastatic (Moore and Luu, 2014; Zhou et al., 2014). Hence, it is necessary to explore new strategies and innovative therapeutics to improve outcomes for these patients.

Synaptotagmin 7 (SYT7) is located in cardiac sympathetic nerve terminals and binds Ca^{2+} with high

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affinity and slow kinetics. SYT7 is one important member of the synaptotagmin family that consists of 17 human isoforms (Chapman, 2008; Gustavsson and Han, 2009). Synaptotagmin which was identified as a membrane trafficking protein is composed of a short N-terminal sequence and two functional C2 domains (C2A and C2B) (Gustavsson and Han, 2009). SYT7 belongs to Ca²⁺-dependent protein and controls certain types of Ca²⁺-dependent exocytosis (Fukuda et al., 2004). In addition, SYT7 has been identified to play an important role in many physiological processes. For instance, SYT7 can regulate hepatocellular carcinoma cell proliferation via Chk1-p53 signaling (Jin et al., 2017). In large secretory organelles, SYT7 linked fusion-activated Ca²⁺ entry and modulated fusion pore expansion to facilitate secretion during the exocytic post-fusion phase (Neuland et al., 2014). A previous study demonstrated that the downregulation of SYT7 expression inhibited glioblastoma growth by promoting cellular apoptosis (Xiao et al., 2017). There are no studies reporting the role of SYT7 in osteosarcoma.

Therefore, in this study, we aimed to investigate the role of SYT7 in osteosarcoma cell lines. Mann-Whitney U analysis and Spearman correlation analysis were applied to evaluate the relevance between clinicopathological characteristics and the expression of SYT7 in patients with osteosarcoma. Lentivirus-mediated specific shRNA targeting SYT7 was applied to identify the effect of SYT7 silencing on the growth, proliferation, cell cycle, and apoptosis in osteosarcoma cells.

Materials and methods

Tissue samples

40 pairs of human osteosarcoma tissue and paraneoplastic sarcoma were gathered from patients in the Fudan University Shanghai Cancer Center. All pathological diagnoses were confirmed and were analyzed following the criterion that was depicted in the 7th edition of the Union for International Cancer control TNM classification. This research was approved by Fudan University Ethics Committee. Written informed consent was acquired from all patients whose tissue samples were used in this study.

Tissue chips

Paraformaldehyde-fixed, paraffin-embedded human osteosarcoma tissue chip slides were purchased from US Biomax (Rockville, MD, USA). Each slide contained 40 duplicate samples of osteosarcoma tissues.

Briefly, the sections were incubated with a rabbit anti-SYT7 polyclonal antibody (1:200 dilution; Abcam, Cambridge, UK) overnight at 4°C. After washing with phosphate-buffered saline (PBS), biotinylated goat anti-rabbit secondary antibody was applied, and slides were incubated for 30 min at room temperature. SYT7

expression was visualized with 3,3'-diaminobenzidine (DAB) (MaiXin, Fuzhou, China). Tissue sections were examined under a microscope and evaluated for both staining intensity and percentage of positive cells. Staining intensity was classified as follows: 1 (weak), 2 (moderate), or 3 (strong). The percentage of positive cells was scored as 0 ($\leq 5\%$), 1 (6-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%). The staining intensity and percentage of stained cells were then multiplied to generate the immunoreactivity score for each case, ranging from 0 to 12. Tumor tissues with an immunoreactivity score of ≥ 4 were considered to have high expression, and those with a score of < 4 were considered to have low expression.

Cell culture

Human MNNG/HOS and U2OS cell lines were purchased from Shanghai cell bank of Chinese Academy of Sciences (Shanghai, China) and cultured in MEM medium (Gibco, Grand Island, NY, USA). All media were supplemented with 10% FBS, 100 U/mL penicillin G, and 100 $\mu\text{g}/\text{mL}$ streptomycin. Cells were maintained at 37°C in a humidified atmosphere with 5% CO₂.

Construction of lentivirus for RNAi

The lentiviruses expressing shRNA targeting the sequence of the SYT7 gene (5'-TCACCGTGAAGA TCATGAA-3') and negative control shRNA were purchased from Shanghai GeneChem Co., Ltd. The shRNAs were subsequently cloned into a pGCSIL-green fluorescent protein lentiviral vector with AgeI/EcoRI sites to generate recombinant the lentiviral shRNA expression vectors. Lentiviral vectors and packaging vectors were infected into 293T cells using Lipofectamine 2000, according to the manufacturer's instructions. Lentiviral particles were purified using ultracentrifugation, and an endpoint dilution assay was performed to determine the titer of the lentiviruses. The MNNG/HOS and U2OS osteosarcoma cells, seeded in 6-well plates at a density of 4.0×10^5 cells/well, were infected with shSYT7-lentivirus (4×10^8 TU/ml \times 2.5 μl) or negative control (8×10^8 TU/ml \times 1.25 μl) lentivirus. The cells were observed under a fluorescence microscope (MicroPublisher 3.3RTV; Olympus, Tokyo, Japan). Following 5 days of infection, the knockdown efficiency of the shRNA-SYT7 vectors was investigated via qPCR analysis.

Real-time quantitative polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted from the two osteosarcoma cell lines (MNNG/HOS and U2OS) using the TRIzol total RNA reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). cDNA synthesis was performed using 2 μg total RNA using the RevertAid™ H Minus First Strand cDNA Synthesis kit (Takara Bio,

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Inc., Otsu, Japan). The SYT7 primers were obtained from Shanghai GeneChem Co., Ltd. (Shanghai, China), and GAPDH was used as an internal control. The primers were as follows: SYT7 forward, 5'-ACTCCATCATCGTGAACATCATC-3' and reverse, 5'-TCGAAGGCGAAGGACTCATTG-3'; GAPDH forward, 5'-TGACTTCAACAGCGACACCCA-3' and reverse 5'-CACCTGTTGCTGTAGCCAAA-3'. qPCR was performed using the SYBR PrimeScript qPCR kit (Takara Bio, Inc.) on an Applied Biosystems 7300 Fluorescent Quantitative PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The reaction mixtures were incubated at 95°C for 30 sec, with 45 amplification cycles at 95°C for 5 sec, 60°C for 30 sec and 72°C for 60 sec, followed by a final extension of 72°C for 7 min. The PCR products of SYT7 and GAPDH were 178 and 121 bp, respectively. The relative expression of SYT7 mRNA was calculated via the $2^{-\Delta\Delta Cq}$ method, using the GAPDH mRNA expression level for normalization.

Western blot

Whole-cell protein extracts were prepared and quantified using a BCA Protein Assay kit (HyClone-Pierce, USA). Proteins were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and wet-transferred (Bio-Rad Laboratories Inc., Hercules, CA, USA) at 300 mA for 120 min onto PVDF membranes (Millipore, Billerica, MA, USA). The PVDF membrane was blocked using Tris-buffered saline/tween with 5% non-fat milk at room temperature for 2 h or at 4°C overnight and was incubated with primary antibody and subsequently with an appropriate secondary antibody. Protein bands formed were visualized using an ECL Western Blotting Substrate kit (Pierce, Thermo Fisher Scientific, Waltham, MA, USA). The primary Rabbit Anti-Flag antibody was purchased from Tianjin Saierbio and Rabbit Anti-GAPDH was purchased from Beijing bio needs biotechnology co., Ltd. The secondary antibody, Goat Anti-Mouse IgG, was purchased from Beyotime Institute of Biotechnology.

MTT cell proliferation assay

Lentivirus-infected MNNG/HOS cells or U2OS cells were seeded at a density of 2,000 cells/well into 96-well plates, cell viability was assessed using MTT (Glenview, Craigieburn, Australia). MTT was added to each well and incubated for 4 h at 37°C. Formazan crystals formed were then dissolved by adding 100 μ l dimethyl sulfoxide. After shaking for 2-5 min, the absorbance was measured at 490 nm using a microplate reader (Tecan Infinite, Tecan GmbH, Austria). Each experiment was performed in triplicate.

Flow cytometry assay

Cell cycle distribution was determined via flow

cytometry analysis. MNNG/HOS cells or U2OS cells infected with lentivirus were collected and washed once with D-Hanks solution. The cells were fixed in pre-chilled 75% ethanol for at least 1 h before washing once with D-Hanks solution. Samples were then stained with a solution containing propidium iodide (PI, 2 mg/mL; Sigma-Aldrich Co.), RNase concentrate (10 mg/mL), and D-Hanks solution at a ratio of 25:10:1,000, and the cells were analyzed on a flow cytometer (Guava easyCyte HT, Millipore). Each experiment was performed in triplicate.

To assess the apoptosis rate, MNNG/HOS or U2OS cells were starved in FBS-free culture medium for 48 h. Then, aliquots (100 μ l) of 10^6 cells/mL were incubated with Annexin-VAPC (allophycocyanin, BD Biosciences Pharmingen, San Diego, CA, USA) for 15 min in the dark. After incubation, 400 μ l of the binding buffer was added to the sample and then samples were analyzed by flow cytometry. The flow cytometric data were analyzed with the CELLQUEST software.

Wound healing assay and Transwell assay

The Wound healing assay was performed to determine the role of SYT7 in cell migration. MNNG/HOS cells and U2OS cells were plated on 60-mm plates at 5×10^4 cells, respectively. A scratch was made with a pipette tip when the plate was almost filled with cells. After 48 h, the image of cells that had migrated into the wounded area was obtained by a Zeiss Axiovert 200 inverted microscope. The wound area was analyzed by Metamorph version 7.5.6.0 software.

The transwell assay was used to determine the role of the SYT7 in cell invasion. Transwell kit (Corning, USA) was applied to monitor the cell invasion and the operation was carried out in accordance with the instructions.

Statistical analysis

For statistical analysis, the clinical relevance of the specified parameters was assessed via Mann-Whitney U analysis. Spearman correlation analysis was applied to evaluate the relevance between clinicopathological characteristics and the expression of SYT7. Statistically significant differences between studied groups were evaluated using the unpaired Student's t-test and Fisher's exact test. All analyses were performed using IBM SPSS Statistics software version 20.0 (Chicago, IL, USA). The results were determined to be statistically significant when $P < 0.05$ was obtained.

Results

SYT7 is highly expressed in osteosarcoma tissues

A tissue microarray analysis with 106 samples was utilized. According to the results of Mann-Whitney U analysis (Table 1) and Spearman analysis (Table 2), the

expression levels of SYT7 showed positive correlation with pathological characteristics of osteosarcoma, and the relevance between the expression of SYT7 and tumor infiltrate was statistically significant ($P < 0.05$). We determined the expression level of SYT7 in osteosarcoma tissues and para-sarcoma tissues by Immunohistochemistry (Fig. 1A). Our results showed that the SYT7 protein highly expressed in osteosarcoma tissues, compared with normal adjacent tissues (Table 3).

Construction of the ShSYT7 model in MNNG/HOS and U2OS cells

To study the role of SYT7 in the progression of osteosarcoma, we selected the MNNG/HOS cells and U2OS cells as typical osteosarcoma cells for subsequent studies. Lentivirus-mediated small RNA interference was performed and suppressed SYT7 expression levels. The cells were infected with lentivirus successfully and infection efficiency was above 80% by fluorometric analysis (Fig. 2A). RT-PCR was applied to assess the silencing efficiency of SYT7 in MNNG/HOS cells and U2OS cells, and the silencing efficiency of SYT7 reached 31.4% and 74% in comparison to the control group, respectively (Fig. 2B,C). The western blotting assay was performed to examine the expression of SYT7 in protein levels, and the results showed that SYT7 was significantly reduced in the shSYT7 group compared to the control group (Fig. 2D). The results suggested that the shSYT7 significantly suppressed the expression of SYT7 in MNNG/HOS cells and U2OS cells.

Table 1. Relationship between SYT7 expression and tumor characteristics in patients with osteosarcoma.

Features	No. of patients	SYT7 expression		p value
		low	high	
All patients	80	45	35	
Age (years)				0.823
<26	40	22	18	
≥26	40	23	17	
Gender				0.078
Male	52	33	19	
Female	28	12	16	
Grade malignancy				0.143
G2	44	28	16	
G3	36	17	19	
T infiltrate				0.015*
T1	14	12	2	
T2	66	33	33	
lymphatic metastasis (N)				0.858
N0	78	44	34	
N1	2	1	1	
Stage				0.150
1	44	28	16	
2	34	16	18	
4	2	1	1	

* $P < 0.05$, Tumor infiltrates vs SYT7 expression levels. T1 represents that tumor invades submucosa; T2 represents that tumor invades muscularis propria.

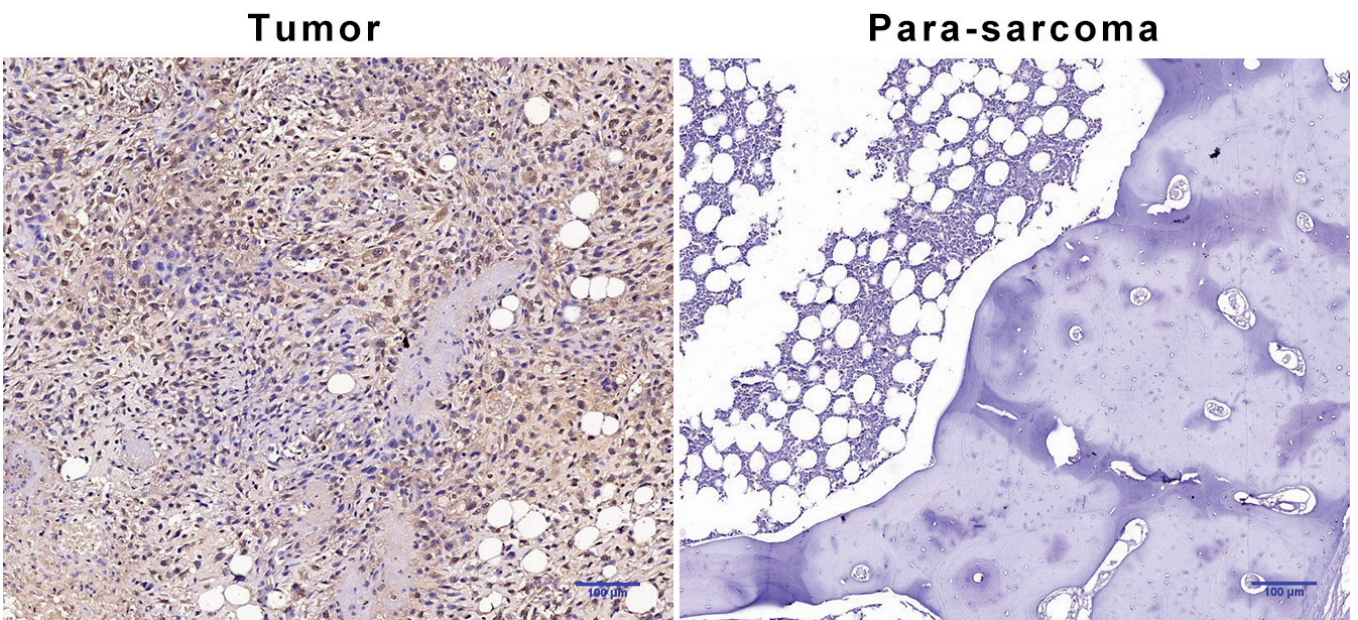


Fig. 1. Immunohistochemistry was used to determine the expression of SYT7. A pair of results of tissue chips in osteosarcoma tissue and para-sarcoma.

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ShSYT7 inhibited osteosarcoma cell proliferation and colony formation in vitro

To study the proliferation of MNNG/HOS cells and U2OS cells after silencing of SYT7, MTT assay was applied to determine the cell proliferation during five-day cultures. The results showed that shSYT7 significantly inhibited cell proliferation in comparison to the control group and with time-dependence, and the difference was statistically significant ($P < 0.001$) (Fig. 3A,B). Meanwhile, colony forming capability, as a character for malignant tumors, was assessed in MNNG/HOS cells and U2OS cells. Giemsa staining was applied to determine the impacts of SYT7 silencing on colony formation in U2OS cells. The cell colony numbers were quantified and results illustrated that shSYT7 was notably inhibiting colony formation in both MNNG/HOS cells and U2OS cells. (Fig. 3C,D). The results indicated that shSYT7 significantly inhibited the proliferation of MNNG/HOS and U2OS cells.

Table 2. Relationship between SYT7 expression and tumor characteristics in patients with osteosarcoma.

		SYT7
T Infiltrate	Pearson Correlation	0.274
	Sig. (2-tailed)	0.014*
	N	80

* $P < 0.05$, Tumor infiltrate vs SYT7 expression level.

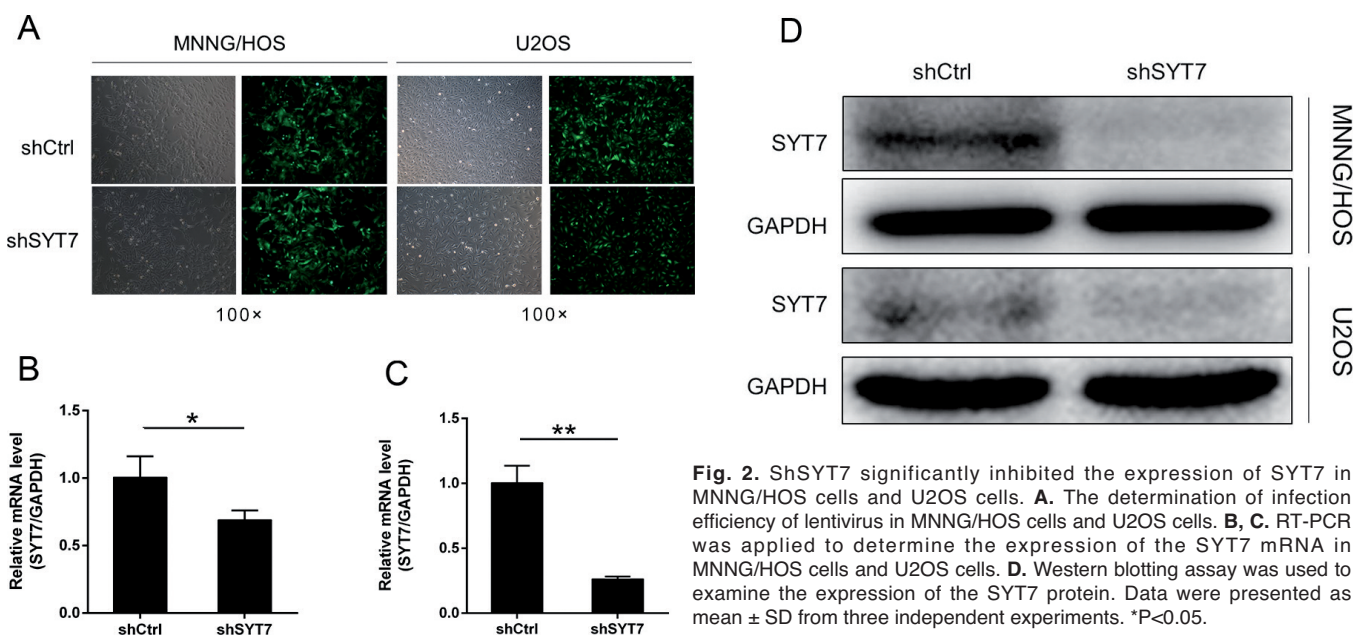
ShSYT7 enhanced cell apoptosis and affected cell cycle distribution

To explore alteration in the cell cycle and cell apoptosis, flow cytometry was used to analyze the effect of shSYT7 in MNNG/HOS cells and U2OS cells. The results showed that cell apoptosis rates significantly increased in the shSYT7 group compared to control groups in MNNG/HOS and U2OS cells, respectively, and the differences were statistically significant ($P < 0.01$) (Fig. 4A,B). Furthermore, the analysis of cell cycle showed that compared to the control group, SYT7 downregulation in MNNG/HOS cells significantly increased the proportion of G1 and G2 phase, while it decreased the proportion of S phase (Fig. 4C). In U2OS cells, shSYT7 significantly increased the proportion of G2 phase and decreased the proportion of S phase compared to the control group. There was no significant change between the groups of cells in G1 phase (Fig. 4D). The results revealed that shSYT7 can significantly

Table 3. Expression patterns in osteosarcoma tissues and para-sarcoma tissues revealed in immunohistochemistry analysis.

SYT7 expression	Tumor tissue		Para-sarcoma tissue		p value
	Cases	Percentage	Cases	Percentage	
Low	45	57.3%	26	100%	0.000**
High	35	42.7%	0	-	

* $P < 0.001$, Tumor tissue vs para-sarcoma.



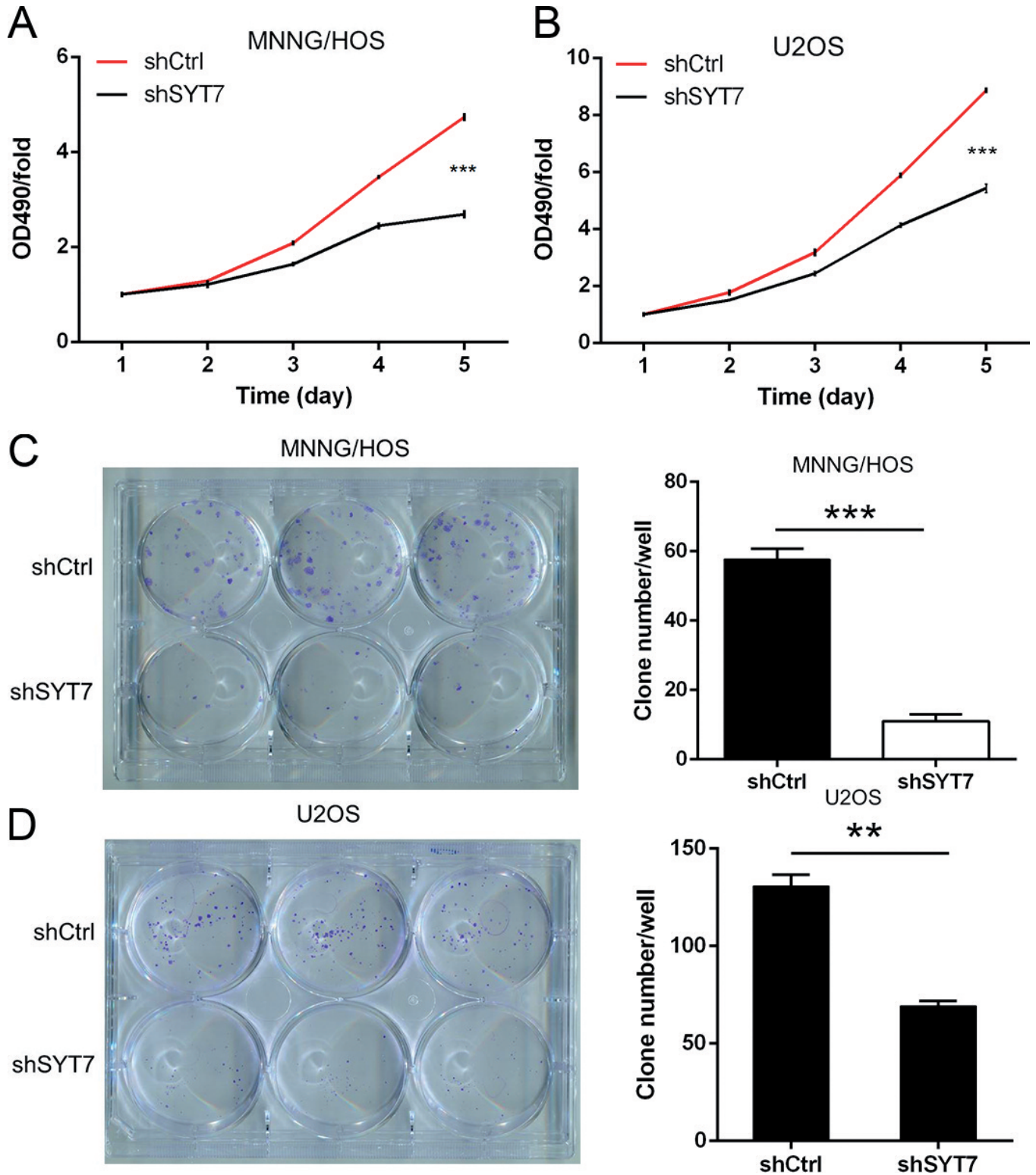


Fig. 3. ShSYT7 inhibited the proliferation of MNNG/HOS cells and U2OS cells. **A, B.** MTT assay was used to determine the proliferation of MNNG/HOS cells and U2OS cells. ShCtrl represented the normal control group, shSYT7 represented the silencing of SYT7 in MNNG/HOS cells and U2OS cells. **C, D.** Giemsa staining was used to evaluate the colony formation capacity in MNNG/HOS cells and U2OS cells. Data were presented as mean \pm SD from three independent experiments. ** $P < 0.01$, *** $P < 0.001$.

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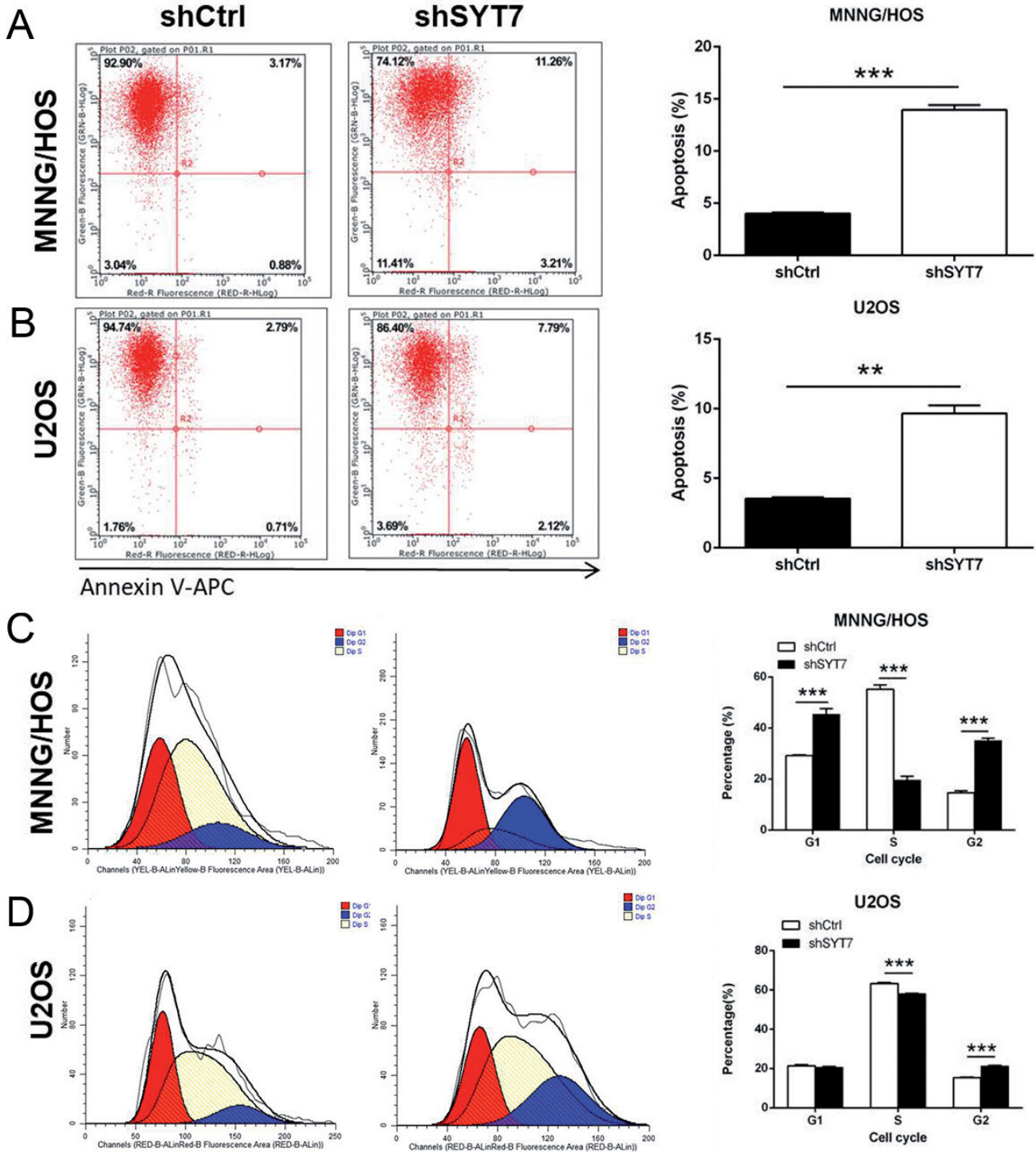


Fig. 4. ShSYT7 affected the cell cycle distribution and cell apoptosis. **A, B.** The apoptosis of MNNG/HOS cells and U2OS cells was determined by flow cytometry. **C, D.** Flow cytometry assay was used to analyze the cell cycle distribution. Data were presented as mean \pm SD from three independent experiments. ** $P < 0.01$, *** $P < 0.001$.

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accelerate the MNNG/HOS cells and U2OS cell apoptosis.

ShSYT7 decreased the invasion and migration capability of osteosarcoma cells

Invasion and migration are important characteristics of malignant tumors. We attempted to explore whether silencing SYT7 in osteosarcoma cells would have an effect on tumor migration and invasion. Transwell assay was applied to evaluate the invasion capacity of osteosarcoma cells, while wound healing assay was used to assess the migration capacity. The results revealed that silencing of SYT7 resulted in a lower invasion ability in comparison to the control group, and that the invasion fold change dropped about three quarters in comparison to control group in MNNG/HOS cells. Meanwhile, invasion fold change in U2OS cells also dropped about three quarters in comparison to control group (Fig. 5A-C). Furthermore, significantly reduced migration rate was also witnessed in MNNG/HOS and

U2OS cells silenced SYT7 as compared to the control group. The silencing of SYT7 reduced the migration rate of MNNG/HOS and U2OS cells by three quarters, respectively (Fig. 5D-F). Our data indicated that SYT7 was highly essential for invasion and migration capability in MNNG/HOS cells and U2OS cells.

Discussion

Previously, researchers have done much work on identifying potential biomarkers that can be used in the diagnosis and treatment of osteosarcoma (Botter et al., 2014; Zhou et al., 2014). There is abundant information about osteosarcoma at genetic and molecular levels. For instance, the mutations associated with retinoblastoma 1 (RB1) and tumor protein p53 play crucial roles in osteosarcoma pathogenesis (Fuchs and Winkler, 1993). The WW domain-containing oxidoreductase (WWOX) gene and lysyl oxidase (LOX) were regarded as tumor suppressor genes in human osteosarcoma, and overexpression of them can promote apoptosis of cells

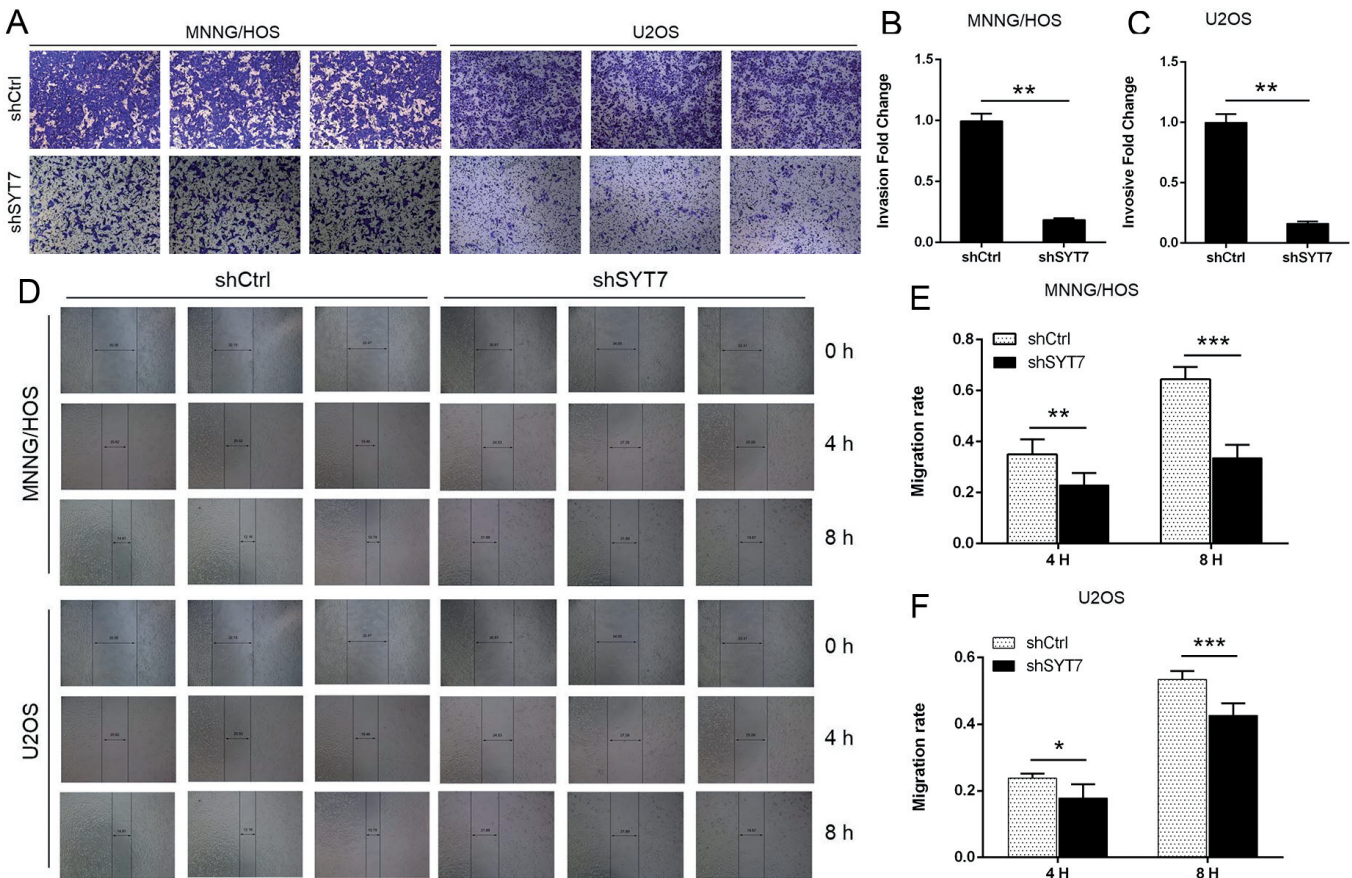


Fig. 5. Wound healing and Transwell assay. **A-C.** Transwell assay was used to determine the invasion capacity of MNNG/HOS cells and U2OS cells; ShSYT7 significantly decreased the invasion ability of MNNG/HOS cells compared to shCtrl group. **D-F.** Wound healing assay was performed to examine the migration capacity of MNNG/HOS cells and U2OS cells; ShSYT7 remarkably reduced the migration rate compared to shCtrl group. Data were presented as mean \pm SD from three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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and suppress proliferation and migration (Xu et al., 2013; Yang et al., 2013; Del Mare and Aqeilan, 2015). In addition, phosphatidylinositol-3 kinases (PI3Ks) have been defined to associate with cell cycle, apoptosis, angiogenesis and have been identified to inhibit proliferation in 2/3 osteosarcoma cell lines via active Akt signaling (Hou et al., 2014; Dong et al., 2015; Zhang et al., 2015). Many genes have been demonstrated to play crucial roles in the tumorigenesis and development of osteosarcoma. However, owing to the limited clinical value of past confirmed molecular markers, scientists have been encouraged to explore new genes.

SYT7 plays crucial roles in many processes of physiological and pathological processes in cells and is a ubiquitously expressed calcium sensor (Rao et al., 2014; Turecek et al., 2017). It has been reported that SYT7 interacts with cytoplasmic RNA-interacting protein which is a component of mRNA granules and is a prerequisite for synaptic plasticity (Tratnjek et al., 2017). Knockdown of endogenous SYT7 attenuated the fusion of dense core vesicles with the plasma membrane and blocked the fusion pore expansion (Li et al., 2009). Chemokines and chemoattractants are associated with leukocyte migration and are crucial for the inflammatory responses. SYT7 as a positive regulator of chemotaxis directs leukocyte migration (Colvin et al., 2010). One study showed that downregulation of SYT7 can suppress glioblastoma growth and enhance cellular apoptosis (Xiao et al., 2017). Hao et al confirmed that SYT7 knockdown in hepatocellular carcinoma cells increased phosphorylation of Chk1 and p53 and regulated HHC cell proliferation, as Chk1 is a cell cycle checkpoint (Jin et al., 2017). In addition, functional interaction network analysis revealed that SYT7 knockdown downregulated gene expression in DNA Damage Checkpoint Regulation pathways in colorectal cancer, which is an important cell cycle checkpoint (Wang et al., 2018). These may explain the reason for cell cycle phase arrest by SYT7 knockdown in cancer cells.

As far as we know, there are few reports investigating the role of SYT7 in tumors, particularly osteosarcoma. We initially investigated the expression of SYT7 in human osteosarcoma tissues and in normal tissues. Our results showed that osteosarcoma tissues expressed high levels of SYT7 compared to normal paraneoplastic tissues. Subsequently, lentivirus-mediated SYT7 knockdown significantly inhibited MNNG/HOS and U2OS cell proliferation, induced cell cycle arrest, and cell apoptosis. The results of MTT analysis revealed that the cell growth rate in the shSYT7 group was significantly decreased in comparison to those of the control group. SYT7 silencing also promoted cell cycle arrest at G1 and G2 phase in MNNG/HOS cells. In addition, the capacity of cell cycle regulation was confirmed in U2OS cells, that shSYT7 significantly decreased the distribution of S phase and increased the distribution of G2 phase. In further experiments, silencing of SYT7 significantly suppressed the ability of

migration and invasion of MNNG/HOS cells and U2OS cells. Compared to the control group, the migration rate was remarkably reduced. However, the molecular regulation mechanism of SYT7 in osteosarcoma is not demonstrated in this study and will be our future work.

In summary, our study preliminarily demonstrates the role of SYT7 in osteosarcoma. Silencing of SYT7 can inhibit the ability of osteosarcoma cell proliferation, migration, invasion, and induce cell apoptosis. Therefore, SYT7 may be a potential diagnostic and therapeutical target for osteosarcoma.

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Conflict of Interests. The authors declare that they have no conflict of interest.

References

- Botter S.M., Neri D. and Fuchs B. (2014). Recent advances in osteosarcoma. *Curr. Opin. Pharmacol.* 16, 15-23.
- Chapman E.R. (2008). How does synaptotagmin trigger neurotransmitter release? *Annu. Rev. Biochem.* 77, 615-641.
- Colvin R.A., Means T.K., Diefenbach T.J., Moita L.F., Friday R.P., Sever S., Campanella G.S., Abrazinski T., Manice L.A., Moita C., Andrews N.W., Wu D., Hacohen N. and Luster A.D. (2010). Synaptotagmin-mediated vesicle fusion regulates cell migration. *Nat. Immunol.* 11, 495-502.
- Del Mare S. and Aqeilan R.I. (2015). Tumor suppressor WWOX inhibits osteosarcoma metastasis by modulating RUNX2 function. *Sci. Rep.* 5, 12959.
- Dong Y., Liang G., Yuan B., Yang C., Gao R. and Zhou X. (2015). MALAT1 promotes the proliferation and metastasis of osteosarcoma cells by activating the PI3K/Akt pathway. *Tumour Biol.* 36, 1477-1486.
- Fenger J.M., London C.A. and Kisseberth W.C. (2014). Canine osteosarcoma: A naturally occurring disease to inform pediatric oncology. *ILAR J.* 55, 69-85.
- Fuchs N. and Winkler K. (1993). Osteosarcoma. *Curr. Opin. Oncol.* 5, 667-671.
- Fukuda M., Kanno E., Satoh M., Saegusa C. and Yamamoto A. (2004). Synaptotagmin VII is targeted to dense-core vesicles and regulates their Ca²⁺-dependent exocytosis in PC12 cells. *J. Biol. Chem.* 279, 52677-52684.
- Gustavsson N. and Han W. (2009). Calcium-sensing beyond neurotransmitters: Functions of synaptotagmins in neuroendocrine and endocrine secretion. *Biosci. Rep.* 29, 245-259.
- Hou C.H., Lin F.L., Tong K.B., Hou S.M. and Liu J.F. (2014). Transforming growth factor alpha promotes osteosarcoma metastasis by ICAM-1 and PI3K/Akt signaling pathway. *Biochem. Pharmacol.* 89, 453-463.
- Jin H., Xu G., Zhang Q., Pang Q. and Fang M. (2017). Synaptotagmin-7 is overexpressed in hepatocellular carcinoma and regulates hepatocellular carcinoma cell proliferation via chk1-p53 signaling. *Oncotargets Ther.* 10, 4283-4293.
- Kuijjer M.L., van den Akker B.E., Hilhorst R., Mommersteeg M., Buddingh E.P., Serra M., Burger H., Hogendoorn P.C. and Cleton-Jansen A.M. (2014). Kinome and mRNA expression profiling of high-grade osteosarcoma cell lines implies Akt signaling as possible

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- target for therapy. *BMC Med. Genomics* 7, 4.
- Li J., Xiao Y., Zhou W., Wu Z., Zhang R. and Xu T. (2009). Silence of synaptotagmin vii inhibits release of dense core vesicles in PC12 cells. *Sci. China C Life Sci.* 52, 1156-1163.
- Miller K.D., Siegel R.L., Lin C.C., Mariotto A.B., Kramer J.L., Rowland J.H., Stein K.D., Alteri R. and Jemal A. (2016). Cancer treatment and survivorship statistics, 2016. *CA: a Cancer J. Clin.* 66, 271-289.
- Moore D.D. and Luu H.H. (2014). Osteosarcoma. *Cancer Treat. Res.* 162, 65-92.
- Neuland K., Sharma N. and Frick M. (2014). Synaptotagmin-7 links fusion-activated Ca^{2+} entry and fusion pore dilation. *J. Cell Sci.* 127, 5218-5227.
- Rao T.C., Passmore D.R., Peleman A.R., Das M., Chapman E.R. and Anantharam A. (2014). Distinct fusion properties of synaptotagmin-1 and synaptotagmin-7 bearing dense core granules. *Mol. Biol. Cell* 25, 2416-2427.
- Roth M., Linkowski M., Tarim J., Piperdi S., Sowers R., Geller D., Gill J. and Gorlick R. (2014). Ganglioside GD2 as a therapeutic target for antibody-mediated therapy in patients with osteosarcoma. *Cancer* 120, 548-554.
- Tratnjek L., Zivin M. and Glavan G. (2017). Synaptotagmin 7 and SYNCRIP proteins are ubiquitously expressed in the rat brain and co-localize in purkinje neurons. *J. Chem. Neuroanat.* 79, 12-21.
- Turecek J., Jackman S.L. and Regehr W.G. (2017). Synaptotagmin 7 confers frequency invariance onto specialized depressing synapses. *Nature* 551, 503-506.
- Wang K., Xiao H., Zhang J. and Zhu D. (2018). Synaptotagmin7 is overexpressed in colorectal cancer and regulates colorectal cancer cell proliferation. *J. Cancer* 9, 2349-2356.
- Xiao B., Li J., Fan Y., Ye M., Lv S., Xu B., Chai Y., Zhou Z., Wu M. and Zhu X. (2017). Downregulation of SYT7 inhibits glioblastoma growth by promoting cellular apoptosis. *Mol. Med. Rep.* 16, 9017-9022.
- Xu X., Wang B. and Xu Y. (2013). Expression of lysyl oxidase in human osteosarcoma and its clinical significance: A tumor suppressive role of lox in human osteosarcoma cells. *Int. J. Oncol.* 43, 1578-1586.
- Yang J., Zhao L., Tian W., Liao Z., Zheng H., Wang G. and Chen K. (2013). Correlation of WWOX, RUNX2 and VEGFA protein expression in human osteosarcoma. *BMC Med. Genomics* 6, 56.
- Zhang J., Yu X.H., Yan Y.G., Wang C. and Wang W.J. (2015). PI3K/Akt signaling in osteosarcoma. *Clin. Chim. Acta* 444, 182-192.
- Zhou W., Hao M., Du X., Chen K., Wang G. and Yang J. (2014). Advances in targeted therapy for osteosarcoma. *Discov. Med.* 17, 301-307.

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