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Stanniocalcin-1 mRNA expression in soft-tissue tumors

Tetsuro Yamagishi[1], Hiroyuki Kawashima[1], Akira Ogose[2], Takashi Ariizumi[1], Naoki Oike[1], Taro Sasaki[3], Hiroshi Hatano[3] and Naoto Endo[1]

[1]*Division of Orthopedic Surgery, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan*
[2]*Department of Orthopedic Surgery, Uonuma Institute of Community Medicine, Niigata University Medical and Dental Hospital, Minami-Uonuma, Japan*
[3]*Department of Orthopedic Surgery, Niigata Cancer Center Hospital, Niigata, Japan*

ABSTRACT

Stanniocalcin-1 (*STC1*) is a glycoprotein that was originally identified as a calcium-regulating hormone in bony fish, and that has been shown to also critically mediate cell growth, proliferation and differentiation, etc. in humans. Increased *STC1* expression levels have been previously detected in different human cancer samples, such as those isolated from lung, breast, ovary, colon, pancreas, and liver tumors; thus, the present study evaluated *STC1* expression in various soft-tissue tumors. *STC1* mRNA isolated from 16 cell lines and 186 clinical soft-tissue tumor specimens were analyzed via quantitative real-time PCR, and the calculated expression levels were normalized to those exhibited by *STC1*-expressing MDA-MB-231 cells. The results of these analyses did not reveal any specific histological tumor types that displayed significantly increased *STC1* expression; however, they did not indicate that *STC1* expression was significantly higher in malignant compared to benign soft-tissue tumors. Furthermore, in adipocytic tumors, *STC1* expression in dedifferentiated liposarcomas was found to be highest and lowest in lipoma tissues, respectively, suggesting that adipocytic tumors may express increasely high levels of *STC1* mRNA as they become histologically more advanced. *STC1* expression correlates with the malignancy grade in soft-tissue tumors.

Keywords: Stanniocalcin-1, soft tissue tumor, expression

INTRODUCTION

Stanniocalcin-1 (*STC1*) is a glycoprotein that was originally identified as calcium-regulating hormone that is secreted by the corpuscles of Stannius in bony fish. The human STC homologue, which was discovered in somatic-cell line in 1995,[1] has since been shown to be expressed by various tissue types, including the kidney, small intestine, prostate, thyroid, and ovary, and to critically mediate cell growth, proliferation, and differentiation, as well as regulating calcium homeostasis.

Notably, *STC1* expression has been previously reported to be increased in human cancerous compared to normal tissue samples, including those isolated from lung, breast, ovary, colon,

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Corresponding Author: Hiroyuki Kawashima, MD, PhD
Division of Orthopedic Surgery, Niigata University Graduate School of Medical and Dental Sciences, Niigata 951-8510, Japan
Tel: +81-25-227-2272, E-mail: inskawa@med.niigata-u.ac.jp (HK)

pancreas, and liver tumors.[2] Furthermore, *STC1* upregulation has been shown to be associated with disease relapse,[3] lymphatic metastasis, increased tumor size, and advanced clinical stages in patients with breast cancer.[4] Thus, the present study investigated whether *STC1* expression could be used as a tumor marker in soft-tissue tumors.

MATERIALS AND METHODS

Cell Culture

Sixteen soft-tissue tumor cell lines, comprising HSSY2, SYO-1, NDDLS1, 402-92, SKNMC, NMS-2, HT1080, ST257, NEPS, U2OS, NOS-1, MG63, OST, HOS, SaOS2, NOS-10 were maintained at 37°C, in a incubator with 5% CO_2. (Table 1)

Specimens

Clinical specimens were obtained from 186 patients with soft-tissue tumors that were treated at our institutes between 2010 and 2014, via core-needle biopsy, incisional biopsy, or surgical resection. They were then independently histologically assessed by two experienced pathologists according to World Health Organization classification[5] and determined to comprise 20 (seven benign, one intermediate, and 12 malignant) histoligical tumor types (Table 1). All subjects provided written informed consent for their participation in the present study, which was approved by the Ethics Committee of the Niigata University School of Medicine.

Quantitative real-time PCR

Total RNA was extracted from each frozen clinical sample using an ISOGEN reagent (Nippon Gene, Tokyo, Japan), and assessed spectrophotometrically (i.e. via the 260/280 nm UV absorbamce ratio) to confirm its yield and purity. The extracted RNA was the reverse transcribed to cDNA using a PrimeScript RT Reagent Kit (TaKaRa Bio, Shiga, Japan), according to the manufacturer's instructions. Quantitative real-time PCR was performed using SYBR Premix EX Taq II (Tli RNaseH Plus; TaKaRa, Shiga, Japan), and primers targeted to human *STC1* (forward, 5'-ACGCTGCCTGCCAAAGTAAGTC-3'; reverse, 5'-CCATCTTGTAAACATCATGGCAGAA-3'), and GAPDH (forward, 5'-GCACCGTCAAGGCTGAGAAC-3'; reverse, 5'-TGGTGAAGACGCCAGTGGA-3'). The generated results were analyzed using the Thermal Cycler Dice Real Time System TP800 (TaKaRa, Shiga, Japan). Median relative *STC1* mRNA expression levels in the analyzed clinical specimens were nomalized to those exhibited by breast cancer cell line MDA-MB-231, and similarly, *STC1* copynumbers were calculated using a standard curve constructed using the same cell line. We selected MDA-MB-231 cells as calibrator, because high levels of *STC1* expression were reported in the MDA-MB-231.[6]

Statistical Analysis

All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS Inc. Chicago, Illi-nois, USA) version 21.0. The generated data were shown to be not normally distributed via a Shapiro-Wilk test; thus, they were further analyzed a post-hoc multiple comparison. A Mann-Whitney test was used to compare *STC1* expression in adipocytic tumors. A P value < 0.05 was considered to indicate statistical significance.

Table 1 Median *Staniocalcin-1 (STC1)* expression level exhibited by each of the analyzed histological tumor types

Histological tumor types	Analyzed clinical specimens (n = 186)	Median relative *STC1* expression
Lipoma	75	0.37
Schwannoma	10	0.82
ALT/WDL	30	0.81
UPS	23	0.97
Hemangioma	7	3.75
Nodular fasciitis	2	1.02
Myxoid liposarcoma	7	1.42
PVNS	1	0.42
GCTTS	1	1.86
Desmoid	1	1.44
Ewing' sarcoma	3	0.71
DFSP	6	0.89
Epithelioid sarcoma	2	0.13
Synovial sarcoma	4	0.19
Leiomyosarcoma	2	1.66
Angiosarcoma	2	0.13
Myxofibrosarcoma	1	7.88
Extraskeltal myxoidchondrosarcoma	3	3.13
Rhabdomyosarcoma	1	0.48
Dedifferentiated liposarcoma	4	1.83
Postradiation sarcoma	1	2.34

ALT/WDL: atypical lipomatous tumor/well-differentiated liposarcoma, UPS: undifferentiated pleomorphic sarcoma, PVNS: pigmented villonodular synovitis, GCTTS: giant cell tumor of tendon sheath, DFSP: dermatofibrosarcoma protuberans.

RESULTS

STC1 Expression Level

The *STC1* expression levels exhibited by the analyzed HT1080, NMS2, 402–92, and ST257 cell lines were 5.32, 1.21, 0.75, and 0.16 (Fig.1), while those exhibited by all other analyzed cell lines were < 0.05. Among the analyzed benign tumor types, the relative median *STC1* expression levels were limited to approximately ≤ 0.05, except for two hemangioma cases that exhibited *STC1* expression levels of 24.32 and 24.50, respectively (Fig. 2, Table 1).

In contrast, the median *STC1* expression levels exihibited by the various analyzed malignant types were 7.87, 3.13, 2.33, 1.83, 1.65, and 1.41 for myxofibrosarcoma, extraskeletal chondrosarcoma, postradiation sarcoma, dedifferentiated liposarcoma, leiomyosarcoma, and myxoid liposarcoma RNA extracted from each tumor tissue, respectively (Fig.3, Table1). Thus, overall the median *STC1* expression levels exhibited by the malignant tumors were higher than those displayed by the benign tumors (0.93 and 0.55, respectively; $p<0.05$, Mann-Whitney test) (Fig. 4). Atypical lipomatous tumor / well differentiated liposarcoma (ALT/WDL) is classified in intermediate type, so we did not include ALT/WDL cases in Fig. 4.

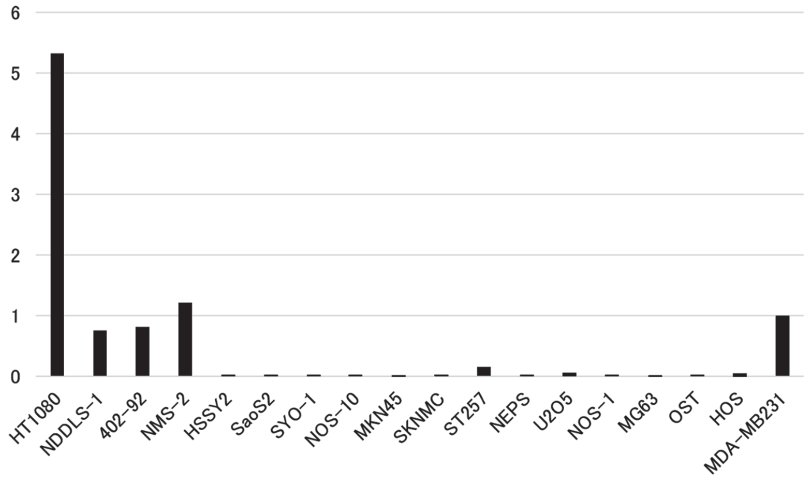

Fig. 1 *STC1* expression in cell lines analyzed by real-time polymerase chain reaction
The expression was were nomalized to those exhibited by *STC1*-expressing MDA-MB231 cells.

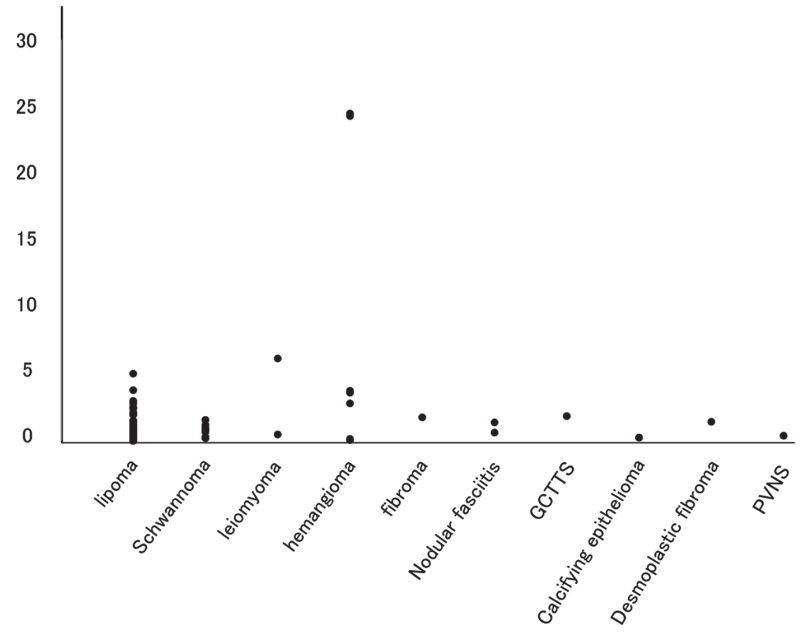

Fig. 2 *STC1* expression in benign tumors analyzed by real-time polymerase chain reaction
The expression was were nomalized to those exhibited by *STC1*-expressing MDA-MB231 cells.

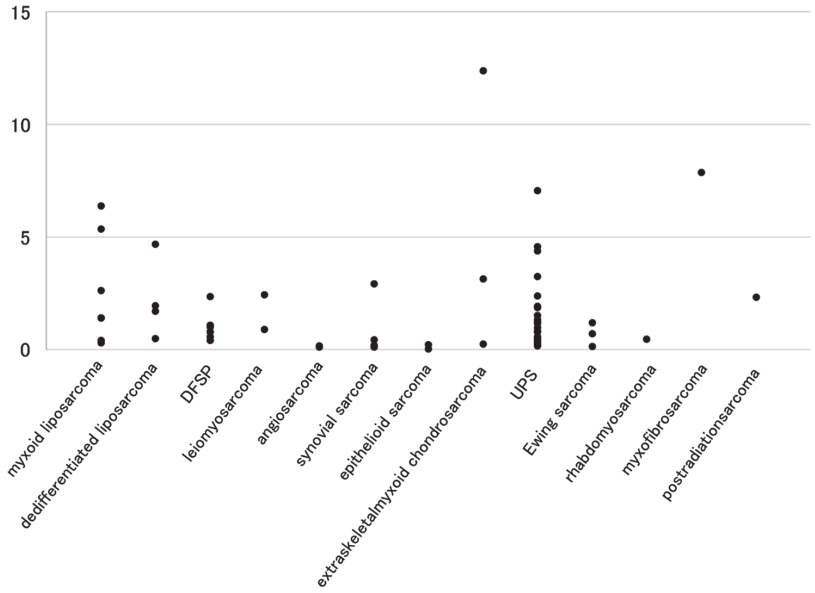

Fig. 3 *STC1* expression in malignant tumors analyzed by real-time polymerase chain reaction
The expression was were nomalized to those exhibited by *STC1*-expressing MDA-MB231 cells.

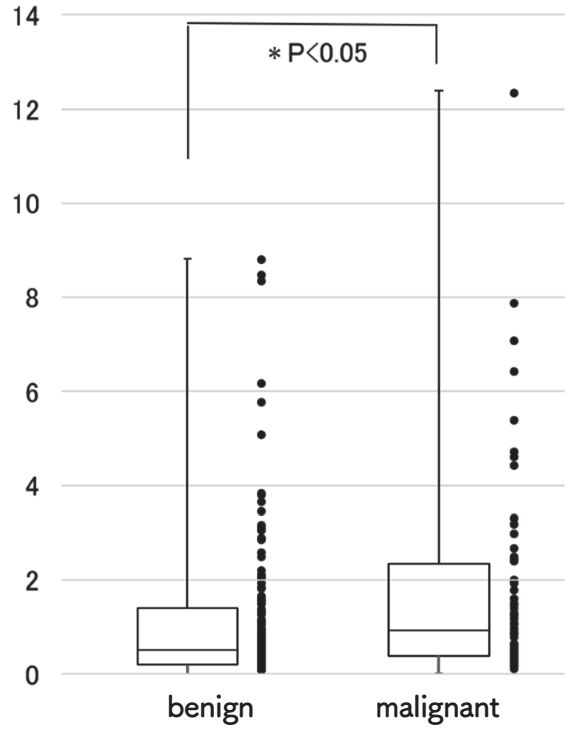

Fig. 4 *STC1* expression between benign and malignant tumor
The values were significantly increased in malignant tumor.

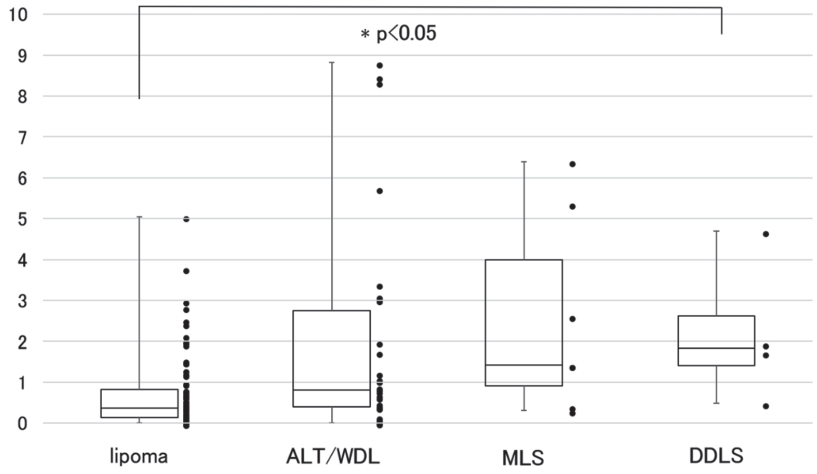

Fig. 5 *STC1* expression in adipocytic tumors
The weak correlation was observed between increasing *STC1* expression levels and advancing grades of adipocytic tumors.

The *STC1* expression levels exhibited by adipocytic tumors was next assessed, to ascertain whether they correlated with any particular histological type and/or tumor grade. The results of this analysis showed that the median *STC1* expression levels in lipoma, ALT/WDL, myxoid liposarcoma, and dedifferentiated liposarcoma tumor specimens were 0.36, 0.81, 1.41, and 1.82 respectively. These data generated a Spearman's rank correlation coefficient of 0.342, indicative of a weak correlation between increasing *STC1* expression levels and advancing grades of adipocytic tumors (Fig. 5).

DISCUSSION

STC1 has been shown to be produced by various cancer cell lines, such as breast, ovarian, and colorectal cancer[3,7,8]; however, to date no reports have investigated *STC1* expression levels in soft-tissue tumors. A previous study bu Jellinek et al did investigate *STC1* expression in HT1080 cells, and showed it to be high, consistent with the results of the present study, which showed HT1080 STC1 expression levels to be the highest of all the analyzed cell lines, and five times higher than those exhibited by the MDA-MB-231 cells.[9] (*STC1* mRNA was also found to be expressed by the NDDLS1, 402–92 and NMS-2 cells).

Among the analyzed clinical specimen types, *STC1* expressions was found to be highest in two cases of hemangioma; however, high *STC1* levels were not associated with any specific histological type overall. Notablly, Law et al previously showed that *STC1* mediates the angiogenic capacity of human umbilical vascular endothelial cells,[10] and furthermore, that it promotes tumor angiogenesis in gastric cancer via vascular endothelial groth factor aignaling.[11] In contrast, the present study identified only low levels of *STC1* expression in the analyzed angiosarcoma tissue.

The results of the present study did show that *STC1* expression was significantly increased in the analyzed malignant compared to benign tumors. This is consistent with the results of a previous study by Han et al, which reported the invasiveness of breast cancer cells expressing high levels of *STC1* to be significanctly increased, and to be associated with high levels of JNK/c-Jun

signaling.[12] The significant difference in *STC1* expression between malignant and benign tumors was further supported in the present study by the fact that among the analyzed adipocytic tumors, *STC1* expression in dedifferentiated liposarcoma, and lowest in lipoma-derived tissue. These results suggest that adipocytic tumors express increasing levels of *STC1* mRNA as they become histologicall more advanced, and support a correlation between the malignancy grade and *STC1* expression in adipocytic tumors. Serlachius has reported that *STC1* is strongly upregulated during terminal adipocite maturarion and increases resistance to apoptosis of mature fat cells.[13] In this study, *STC1* was expressed higher in myxoid liposarcoma and dedifferentiated liposarcoma than other malignant tumors. This indicates that *STC1* may involved in the oncogenesis of adipocytic tumor and contribute to the surval of the tumors. Thus, theresults of the present study provide the first insights into *STC1* expression in soft-tissue tumors. Further studies with larger cohorts are needed to confirm the presented results, and to elucidate the mechanisms underlying the demonstrated correlation between *STC1* expression and adipocytic tumor malignancy.

The limitaion of this study is that *STC1* protein is not detected by immunohistochemistry. We tryed the immunohistochemistry, but *STC1*-positive cells were not observed even in the specimens of positive *STC-1* mRNA cases.

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CONFLICT OF INTEREST

None of the authors has conflict fo interest with this submission. No financial support was received.

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