Stanniocalcin-1 mRNA expression in soft-tissue tumors

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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 increasingly 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

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INTRODUCTION

Stanniocalcin-1 (STC1) is a glycoprotein that was originally identified as a 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,
pancreas, and liver tumors. Furthermore, STC1 upregulation has been shown to be associated with disease relapse, lymphatic metastasis, increased tumor size, and advanced clinical stages in patients with breast cancer. 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% CO₂. (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 and determined to comprise 20 (seven benign, one intermediate, and 12 malignant) histological 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 absorbance 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'-TGGTGAAGACGC-CAGTGGGA-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 normalized 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.

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.
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 exhibited 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.

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.

| 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                            |
| GCTTTS                                | 1                                    | 1.86                            |
| Desmoid                               | 1                                    | 1.44                            |
| Ewing’s 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.
**Fig. 1** *STC1* expression in cell lines analyzed by real-time polymerase chain reaction. The expression was normalized 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 normalized 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 normalized 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.
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; however, to date no reports have investigated STC1 expression levels in soft-tissue tumors. A previous study by 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. (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. Notably, Law et al previously showed that STC1 mediates the angiogenic capacity of human umbilical vascular endothelial cells, and furthermore, that it promotes tumor angiogenesis in gastric cancer via vascular endothelial growth factor signaling. 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 significantly increased, and to be associated with high levels of JNK/c-Jun.
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signaling. 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 histologically 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 adipocyte maturation and increases resistance to apoptosis of mature fat cells. In this study, STC1 was expressed higher in myxoid liposarcoma and dedifferentiated liposarcoma than other malignant tumors. This indicates that STC1 may be involved in the oncogenesis of adipocytic tumor and contribute to the survival of the tumors. Thus, the results 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 limitation of this study is that STC1 protein is not detected by immunohistochemistry. We tried 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 of interest with this submission. No financial support was received.

REFERENCES

1) Yoshiko Y, Son A, Maeda S, et al. Evidence for stanniocalcin gene expression in mammalian bone. Endocrinology. 1999;140:1869–1874.
2) Yeung BH, Law AY, Wong CK. Evolution and roles of stanniocalcin. Mol Cell Endocrinol. 2012;349:272–280.
3) Joensuu K, Heikkila P, Andersson LC. Tumor dormancy: elevated expression of stanniocalcins in late relapsing breast cancer. Cancer Lett. 2008;265:76–83.
4) Wascher RA, Huynh KT, Giuliano AE, et al. Stanniocalcin-1: a novel molecular blood and bone marrow marker for human breast cancer. Clin Cancer Res. 2003;9:1427–1435.
5) Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F. WHO Classification of Tumours of Soft Tissue and Bone. 4th ed. Lyon, France: World Health Organization; 2013.
6) Chang AC, Doherty J, Huschtscha LJ, et al. STC1 expression is associated with tumor growth and metastasis in breast cancer. Clin Exp Metastasis. 2015;32:15–27.
7) Fujiwara Y, Sugita Y, Nakamori S, et al. Assessment of Stanniocalcin-1 mRNA as a molecular marker for micrometastases of various human cancers. Int J Oncol. 2000;16:799–804.
8) Tamura S, Oshima T, Yoshihara K, et al. Clinical significance of STC1 gene expression in patients with colorectal cancer. Anticancer Research. 2011;31:325–329.
9) Jellinek DA, Chang AC, Larsen MR, Wang X, Robinson PJ, Reddel RR. Stanniocalcin 1 and 2 are secreted as phosphoproteins from human fibrosarcoma cells. Biochem J. 2000;350(Pt 2):453–461.
10) Law AY, Wong CK. Stanniocalcin-1 and -2 promote angiogenic sprouting in HUVECs via VEGF/VEGFR2 and angiopoietin signaling pathways. Mol Cell Endocrinol. 2013;374:73–81.
11) He LF, Wang TT, Gao QY, et al. Stanniocalcin-1 promotes tumor angiogenesis through up-regulation of VEGF in gastric cancer cells. *J Biomed Sci.* 2011;18:39.

12) Han J, Jeon M, Shin I, Kim S. Elevated STC1 augments the invasiveness of triplenegative breast cancer cells through activation of the JNK/cJun signaling pathway. *Oncology Reports.* 2016;36:1764–1771.

13) Serlachius M, Andersson LC. Upregulated expression of stanniocalcin-1 during adipogenesis. *Express Cel Research.* 2004;296:256–264.