American Journal of Pathology, Vol. 163, No. 6, December 2003
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Short Communication

A Tetraspanin-Family Protein, T-Cell Acute Lymphoblastic Leukemia-Associated Antigen 1, Is Induced by the Ewing's Sarcoma-Wilms' Tumor 1 Fusion Protein of Desmoplastic Small Round-Cell Tumor

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Recurrent chromosomal translocations in neoplasms often generate hybrid genes that play critical roles in tumorigenesis. Desmoplastic small round-cell tumor (DSRCT) is an aggressive malignancy associated with the chromosomal translocation t(11;22)(p13;q12). This translocation generates a chimeric transcription factor, EWS-WT1, which consists of the transcriptional activation domain of the Ewing's sarcoma (EWS) protein and the DNA binding domain of the Wilms' tumor 1 (WT1) protein. One of the splice variants, EWS-WT1(-KTS) lacks three amino acid residues (Lys-Thr-Ser) in the DNA binding domain and transforms NIH3T3 cells. Therefore, it is likely that aberrant gene expression caused by EWS-WT1(-KTS) is involved in the malignant phenotype of DSRCT. Microarray analysis of 9600 human genes revealed that a gene encoding a tetraspanin-family protein, T-cell acute lymphoblastic leukemia-associated antigen 1 (TALLA-1), was induced in EWS-WT1(-KTS)-expressing cell clones. This induction was EWS-WT1(-KTS)-specific, and more importantly, TALLA-1 protein was expressed in the three independent cases of DSRCT. Tetraspanin-family genes encode transmembrane proteins that regulate various cell processes such as cell adhesion, migration and metastasis. Our findings provide a novel insight into the malignant phenotype of DSRCT, suggesting that TALLA-1 is a useful marker for diagnosis and a potential target for the therapy of DSRCT. *(Am J Pathol 2003, 163:2165–2172)*

Specific recurrent chromosomal translocation in malignant tumors is thought to play a causative role in tumorigenesis. Translocation frequently generates a chimeric gene, and the functions of such chimeric genes have been studied extensively to clarify the molecular mechanisms underlying tumorigenesis. Desmoplastic small round-cell tumor (DSRCT) is an aggressive neoplasm with distinctive histological and immunophenotypic features that suggest a multilineage origin.[1–3] The tumor often develops in the abdominal and pelvic peritoneum of adolescent males. Recent integrated strategies including surgery, multiagent chemotherapy, and radiation therapy have improved the prognosis considerably, although progression-free survival remains poor.[3] Thus, identification of tumor-associated antigens for DSRCT would be useful for the development of anti-tumor drugs. DSRCT has a unique chromosomal translocation t(11;22)(p13;q12) that

Supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by a grant from the New Energy and Industrial Technology Development Organization (NEDO).

Accepted for publication August 28, 2003.

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generates a chimeric gene comprising part of the *Ewing's sarcoma* (*EWS*) gene on chromosome 22 and the *Wilms' tumor 1* (*WT1*) gene on chromosome 11.[4,5] The EWS-WT1 chimeric protein consists of the N-terminal transcriptional regulatory domain of EWS and the C-terminal three zinc-finger motifs of WT1 as the DNA binding domain. Therefore, the fusion protein acts as a transcriptional activator. Alternative splicing in the *WT1* region generates two types of EWS-WT1 protein. One isoform, EWS-WT1(-KTS), which lacks three amino acid residues (Lys-Thr-Ser) between the third and the fourth zinc fingers of WT1, confers NIH3T3 cells with anchorage-independent growth and tumorigenicity in nude mice.[6] In contrast, EWS-WT1(+KTS), which contains the three amino acid residues, does not have such transforming activity. Thus, the EWS-WT1 protein is a molecular marker for DSRCT and is believed to be involved in tumorigenesis.

Recent reports have shown that EWS-WT1(-KTS) activates a number of genes, including those encoding platelet-derived growth factor-A,[7] insulin-like growth factor-1 receptor,[8,9] interleukin-2/15 receptor β-chain (IL2/15Rβ),[10] and brain-specific angiogenesis inhibitor 1-associated protein 3 (BAIAP3).[11] In the present study, we used DNA microarrays to carry out a comprehensive analysis of the downstream genes that are up-regulated by EWS-WT1(-KTS). We found that a tetraspanin-family protein, T-cell acute lymphoblastic leukemia-associated antigen 1 (TALLA-1, also referred to as A15,[12] CCG-B7,[13] and TM4SF2), was induced specifically by EWS-WT1(-KTS) and TALLA1 protein was expressed in the three independent cases of DSRCT. Tetraspanin-family proteins contain four transmembrane domains and two extracellular loops. This evolutionally conserved gene family contains more than 30 known members in mammals, 37 in *Drosophila* and 20 in *Caenorhabditis elegans*.[14] They regulate a variety of normal and pathological processes such as cell adhesion, motility, egg-sperm fusion, virus-induced syncytium formation, and cancer metastasis through formation of a network of multimolecular complexes via tetraspanin-tetraspanin and tetraspanin-protein interactions.[14,15] We discuss the significance of TALLA-1 in the malignant phenotype of tumors.

Materials and Methods

Microarray Analysis

Synthetic polynucleotides (80-mers) representing 9600 human genes (MicroDiagnostic, Tokyo, Japan) were arrayed with a custom-made arrayer. Poly(A)$^+$ RNA was prepared from cells with TRIzol reagent (Invitrogen, Carlsbad, CA) and Poly(A)Purist Kit (Ambion, Austin, TX). Two micrograms of poly(A)$^+$ RNA were labeled with Cyanine 5-dUTP or Cyanine 3-dUTP. Hybridization and subsequent washes of arrays were performed with a Labeling & Hybridization Kit (MicroDiagnostic). Hybridization signals were measured with a GenePix 400A scanner (Axon Instruments, Union City, CA) and then processed into primary expression ratios (ratios of Cyanine 5-labeled to Cyanine 3-labeled samples) by the GenePix Pro software (Axon Instruments). A secondary ratio of expression of each gene was calculated by averaging the primary expression ratio obtained from an experiment with Cyanine 5-labeled target and Cyanine 3-labeled control sample and the reciprocal of the primary expression ratio obtained from an experiment with Cyanine 5-labeled control and Cyanine 3-labeled target. The secondary expression ratios calculated from the pair of experiments were converted into $\log_2$ values as the final expression ratios.

Cells

A human osteosarcoma cell line, U2OS, was maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS). A human DSRCT cell line, JN-DSRCT-1,[16] was maintained in DMEM:Nutrient Mixture F12 (1:1) mixture with 10% FBS. To establish clones with stable expression of EWS-WT1(-KTS), U2OS cells (1×10^5) were transfected with 2 μg of an EWS-WT1(-KTS) expression vector, pcDNA-EWS-WT1(-KTS), and 6 μl of Lipofectamine (Invitrogen). Clones were isolated in the presence of 800 μg/ml of G418 and used for subsequent microarray analysis. A mixture of mock-transfected cells was maintained in the presence of G418 and used as control.

Transfection

A nucleofector device (Amaxa Biosystems, Cologne, Germany) was used for highly efficient gene transfer. This device delivers DNA directly into nuclei, allowing exogenous gene expression after a short incubation period of only 2 to 8 hours. U2OS cells (2×10^6) were transfected with 2 μg of an expression vector with R solution and the pre-set U-16 program. The transfection efficiency was monitored by flow cytometric analysis of pCMV-GFP transfected cells.

Western Blotting

Proteins solubilized in Laemmli sample buffer were resolved on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels and blotted onto Immobilon membranes (Millipore Co., Bedford, MA). Filters were probed for WT1 and EWS-WT1 with anti-WT1 antibody (C19; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and for EWS-FLI1 with anti-FLI1 antibody (C19; Santa Cruz Biotechnology). Bands were visualized with Renaissance Chemiluminescence Reagent Plus (NEN Life Sci Products, Boston, MA).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

RNA was prepared with TRIzol reagent from cultured cells or tumor tissues. First-strand cDNA was synthesized using SuperScript first-strand synthesis system for RT-PCR (Invitrogen). *Taq* polymerase was purchased from Sigma (St. Louis, MO). Primers are 5′-TACACGGACGCTATG-CAGAC-3′ and 5′-GATTCCAAACGCCACTCCAG-3′ for

Talla-1, 5′-TACCCCATGCAGCCAGTCAC-3′ and 5′-TTTGAGCTGGTCTGAACGAG-3′ for *EWS-WT1*, and 5′-CCAGCCGAGCCACATCGCTC-3′ and 5′-ATGAGCCCCAGCCTTCTCCAT-3′ for *GAPDH*.

Northern Blotting

Northern blotting was done with the NorthernMax Kit (Ambion). ^{32}P-labeled probe was prepared with a Multiprime DNA Labeling System (Amersham Biosciences, Arlington Heights, IL).

Flow Cytometry

Cells were incubated with ascites diluted 100-fold containing monoclonal anti-TALLA-1 antibody (B2D) and fluorescent anti-mouse IgG (Alexa Fluor 488; Molecular Probes, Eugene, OR), and then subjected to flow cytometric analysis (Epics XL; Beckman Coulter, Fullerton, CA).

Immunofluorescence

Cells seeded on cover glasses were fixed with 4% paraformaldehyde for 15 minutes at 37°C, and permeabilized with 0.2% Triton X-100/PBS for 5 minutes at room temperature. EWS-WT1 and TALLA-1 were detected with the same antibodies diluted 100-fold as used for Western blotting and flow cytometry. Proteins were visualized with fluorescent secondary antibody (Molecular Probes).

Immunohistochemistry

Frozen blocks and sections of DSRCT were kindly provided by the Pediatric Division of Cooperative Human Tissue Network (Columbus, OH). Diagnosis of DSRCT was confirmed on hematoxylin and eosin-stained frozen sections by pathologists T. K. and S. M. For detection of TALLA-1, frozen sections were incubated with 800-fold diluted B2D and then subjected to color reaction with the indirect immunoperoxidase method (Histofine Simple Stain, Nichirei, Japan).

Results

To explore comprehensively downstream genes with expression induced by EWS-WT1(-KTS), we established three clonal lines of U2OS cells that stably expressed EWS-WT1(-KTS). Microarray analysis of 9600 human genes identified a group of genes whose expression was reproducibly induced or repressed in these clones (Table 1). These genes included those encoding IL2/15Rβ, adrenomedullin, and BAIAP3, which were reported previously.[10,11] We also found that expression of desmin, an established molecular marker for DSRCT, was induced by EWS-WT1(-KTS). We confirmed the change in expression of desmin by immunoblotting (data not shown).

We also found that expression of *Talla-1*, a member of the tetraspanin family and a marker of T-cell acute lymphoblastic leukemia (T-ALL),[17] was up-regulated in clones expressing EWS-WT1(-KTS). Northern blotting confirmed the induction of *Talla-1* mRNA in the clones (C9 and D9; Figure 1A). Flow cytometric analysis showed that TALLA-1 protein was expressed on the plasma membranes of EWS-WT1(-KTS)-expressing cells (Figure 1B). To exclude the possibility that these changes reflected clonal variation, we analyzed *Talla-1* expression in cells that transiently expressed EWS-WT1(-KTS). For this purpose, we used a Nucleofector device, which enabled us to achieve approximately 80% transfection efficiency. EWS-WT1(-KTS) protein was detected at 8 hours after transfection, was sustained at 24 hours, and then gradually decreased (Figure 2A). With transient transfection, *Talla-1* mRNA was initially detected at 8 hours, and was expressed at high levels at 24 hours after transfection of vector encoding EWS-WT1(-KTS) (Figure 2A). To examine if the induction of *Talla-1* expression is specific to EWS-WT1(-KTS), we transfected vectors encoding EWS-WT1(+KTS), EWS-FLI1, WT1(-KTS), and WT1(+KTS) into U2OS cells. EWS-FLI1 is generated by chromosomal translocation t(11;22)(q24;q12), and is found in 85% of cases of Ewing's sarcoma. The fusion protein consists of the N-terminal transcriptional activation domain of EWS and the C-terminal ETS DNA binding domain of FLI1. Thus, the primary target genes of Ewing's sarcoma are thought to be different from those of DSRCT because of the difference in DNA recognition. Northern blot analysis revealed that only EWS-WT1(-KTS) induced *Talla-1* expression (Figure 2B).

Recently a DSRCT cell line, JN-DSRCT-1, was established from the pleural effusion of a patient with pulmonary metastasis from a typical intraabdominal DSRCT.[16] We confirmed that *Talla-1* mRNA and protein were expressed in this cell line (data not shown). Because some tetraspanins are localized not only on the plasma membrane but also at the intracellular compartments such as lysosome and exosome, we analyzed subcellular localization of TALLA-1 by immunohistochemistry. Figure 2C shows that TALLA-1 protein in U2OS cells was also localized in the cytoplasm when cells were permeabilized. This dot-like localization pattern is quite similar to that of endogenous TALLA-1 in JN-DSRCT-1 cells. Induction of *Talla-1* mRNA was also observed when the EWS-WT1(-KTS) expression vector was transfected into human 293T cells, indicating that induction of *Talla-1* expression by EWS-WT1(-KTS) was not specific to U2OS cells (data not shown). Taken together, these data suggest that EWS-WT1(-KTS) induces expression of *Talla-1* mRNA and protein in human cells without cell type-specific cofactors.

We then examined expression of TALLA-1 in DSRCT specimens. Because the supply and amount of tumor tissues were limited, we used RT-PCR analysis to detect *Talla-1* mRNA. Under the condition we used, *Talla-1* mRNA was detected in three of three independent DSRCT tissues (Figure 3A). Immunohistochemical analysis showed localization of TALLA-1 protein in the nest of tumor cells in all three specimens (representative data shown in Figure 3B). Less intense reaction was noted on the stromal endothelial cells. Other cellular components including fibroblasts, lymphocytes, fat cells, myothelial cells, or adventitial cells were basically unstained.

Table 1. Microarray Identification of Genes Activated or Repressed Downstream of EWS-WT1(-KTS)

Gene symbol	Accession number	B12	C9	D9	Average
RGS5	NM_003617	4.8069	4.5771	4.9195	4.7678
DKFZp434B227	NM_032263	4.3451	4.6415	4.9425	4.6430
DES (desmin)	NM_001927	4.8052	3.3748	5.1433	4.4411
APOA1	NM_000039	3.4595	5.0025	3.7444	4.0688
CHI3L1	NM_001276	3.4901	4.4559	3.8189	3.9216
WT1	NM_024424	3.9531	3.8653	3.8197	3.8794
NK4	NM_004221	2.7691	4.3032	4.1529	3.7417
APOL2	NM_030882	3.5625	4.3115	2.9113	3.5951
FLJ20245	NM_017723	3.4295	3.6399	2.9387	3.3360
ADM (adrenomedullin)	NM_001124	3.2513	4.1579	2.4834	3.2975
LOC135562	XM_069429	3.0812	4.5899	2.0095	3.2269
BAIAP3	NM_003933	2.6486	4.0179	2.6717	3.1127
BIK	NM_001197	2.3391	3.4097	3.3323	3.0270
LOC90189	XM_029748	2.9116	2.7470	2.8376	2.8321
FLJ22671	NM_024861	2.4854	2.7474	3.1525	2.7951
KLK6	NM_002774	2.8500	2.5292	2.7768	2.7187
LCK	NM_005356	1.7054	2.2152	4.1992	2.7066
IL8	NM_000584	1.9838	3.7999	2.2517	2.6785
DRP2	NM_001939	1.9726	2.7053	3.2519	2.6433
KREMEN2	NM_024507	2.3643	2.9575	2.3405	2.5541
TNFRSF21	NM_014452	2.3449	2.9221	2.2719	2.5130
SSTR3	NM_001051	2.7267	2.5529	2.1279	2.4692
FLJ20154	NM_017787	1.8621	2.3683	2.9680	2.3995
NFATC1	NM_006162	2.3952	2.8337	1.9534	2.3941
GAGE7	NM_021123	3.0143	2.0046	2.1603	2.3931
SNX9	NM_016224	2.3430	2.3643	2.2830	2.3301
TM4SF2 (Talla-1)	NM_004615	1.8004	2.4611	2.6285	2.2967
LOC139728	XM_060051	2.2622	2.3246	2.1933	2.2600
TRIM29	NM_012101	2.0576	2.8573	1.8001	2.2383
SERPINB2	NM_002575	2.1313	2.2954	2.2759	2.2342
RELB	NM_006509	1.9808	2.8510	1.8384	2.2234
FLJ23058	NM_024696	2.1799	1.9497	2.5029	2.2108
IL2RB (IL2 receptor β)	NM_000878	2.4299	2.5900	1.5083	2.1761
HSPC022	NM_014029	2.2705	2.4120	1.8273	2.1699
MSLN	NM_005823	1.9397	2.2261	2.2971	2.1543
OPRL1	NM_000913	1.9394	2.7325	1.5796	2.0838
MAGP2	NM_003480	2.3341	2.0496	1.6075	1.9971
NEDD4L	NM_015277	1.7847	2.4666	1.7302	1.9938
SLC2A3	NM_006931	1.6088	2.2489	2.1073	1.9883
BGN	NM_001711	1.5921	1.9808	2.3423	1.9717
COL6A3	NM_004369	1.8971	2.3618	1.5310	1.9300
S100A4	NM_002961	1.8033	2.2082	1.6691	1.8935
KRT17	NM_000422	1.6139	2.2038	1.6792	1.8323
MS4A6A	NM_022349	1.7096	2.0112	1.5267	1.7492
C18orf1	NM_004338	−1.5796	−1.5385	−1.6386	−1.5856
H1F2	NM_005319	−1.9450	−1.7604	−1.8163	−1.8406
OSBPL10	NM_017784	−1.6272	−2.2958	−1.6578	−1.8603
FXYD6	NM_022003	−1.5180	−2.1517	−1.9351	−1.8683
HNOEL-iso	NM_020190	−1.8806	−2.7950	−1.5057	−2.0604
P311	NM_004772	−2.0767	−2.2441	−1.9064	−2.0757
NECL1	NM_021189	−1.5862	−3.3417	−1.9093	−2.2791
FLJ20716	NM_017938	−2.0441	−2.7866	−2.2385	−2.3564
H2AFL	NM_003512	−2.4452	−2.2226	−2.4156	−2.3611
BRAK	NM_004887	−2.2034	−2.7810	−2.4533	−2.4792
CRTL1	NM_001884	−1.9105	−3.5013	−2.0543	−2.4887
SERPINF1	NM_002615	−1.8785	−3.0730	−2.6775	−2.5430
MMP2	NM_004530	−2.4785	−2.8093	−2.4158	−2.5679
LOC51316	NM_016619	−2.7566	−3.4258	−2.2395	−2.8073
SRCRB4D	NM_080744	−3.4141	−3.1876	−3.4979	−3.3665
MVD	NM_002461	−3.1784	−3.7718	−3.9392	−3.6298

Expression levels ($\log_2$ values) of 9,600 human genes in the three EWS-WT1(-KTS)-expressing U2OS clones (B12, C9, and D9) relative to a mixture of control mock-transfected clones are calculated as expression ratios processed from the raw data of two individual experiments labeled reciprocally with two different fluors. Genes listed in the table show more or less than 1.5 of expression level ($\log_2$ values) in all three clones. A more detailed description of each gene is available on the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/).

Discussion

In the present study, we screened for downstream genes with expression induced by EWS-WT1(-KTS), which is thought to be involved in the tumorigenesis of DSRCT. The overall profile of gene expression by EWS-WT1(-KTS)-expressing cells was quite different from that of WT1(-KTS)-expressing cells (Ito E, unpublished data). In addition to the replacement of transcriptional regulatory domain of WT1 with that of EWS, the N-terminal first zinc finger that contributes to the binding specificity of WT1 is deleted

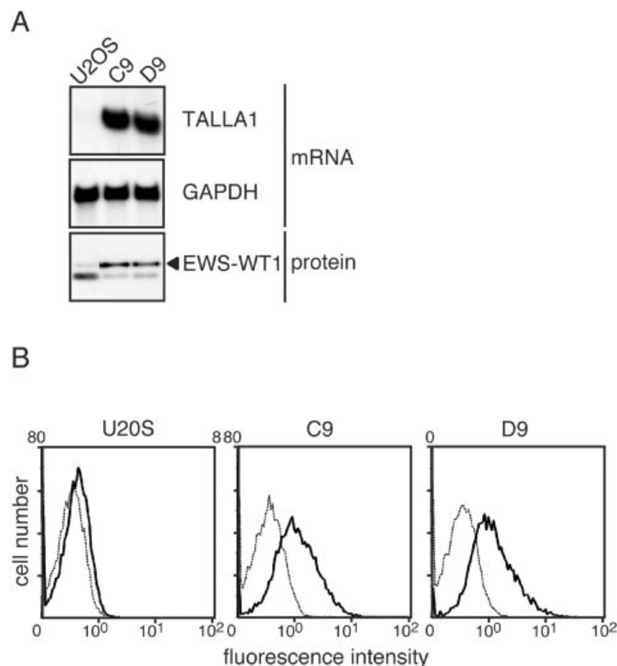

Figure 1. Expression of *Talla-1* in cell clones stably expressing EWS-WT1(-KTS). **A:** Northern blotting analysis of *Talla-1* mRNA. Total RNA (5 μg) was loaded in each lane. EWS-WT1(-KTS) protein (**filled triangle**) was detected by Western blotting with anti-WT1 antibody. **B:** Flow cytometric analysis of TALLA-1 protein. Living cells were incubated with anti-TALLA-1 (B2D) monoclonal antibody and Alexa-Fluor 488-labeled anti-mouse IgG and then subjected to flow cytometric analysis (**solid line**). Background profiles (**dotted line**) were measured by staining with Alexa-Fluor 488-labeled anti-mouse IgG only.

during the generation of the EWS-WT1 fusion protein.[18,19] Moreover, EWS-WT1 has 10-times higher binding affinity for WT1-binding sequence than WT1.[20] Such differences may account for the unique expression profile of EWS-WT1.

We found that *Talla-1*, a member of the tetraspanin family, was expressed in a DSRCT cell line and tumor specimens, and was induced specifically by EWS-WT1(-KTS). Transcriptional regulatory sequence of the *Talla-1* gene has not been identified so far; thus, mechanism of *Talla-1* induction by EWS-WT1(-KTS) remains to be analyzed. *Talla-1* was initially identified due to its preferential expression in T-ALL and neuroblastoma cell lines[12,17] In addition, *Talla-1* is expressed at high levels in neurons of the brain,[21] and intriguingly its inactivation by chromosomal translocation (X;2) or by point mutations (Gly218 to stop codon, Pro172 to His) is associated with X-linked forms of nonsyndromic mental retardation (MRX).[22] Aberrant expression of *Talla-1* in T-ALL is mediated cooperatively by two transcription factors, TAL1 and RBTN1, both of which are ectopically activated by chromosomal translocation in T-ALL,[23] thus *Talla-1* may be a common target that is involved in oncogenesis. The biochemical and biological function of TALLA-1 is largely unknown except that TALLA-1 is associated with phosphoinositide 4-kinase.[24] Accumulating evidence indicate that tetraspanin proteins regulate integrin-extracellular matrix (ECM) interaction,[25] which includes migration,[26] invasion into collagen gels,[27] and morphology on Matrigel.[28] Furthermore, ligation of tetraspanin-$\alpha3\beta1$ integrin

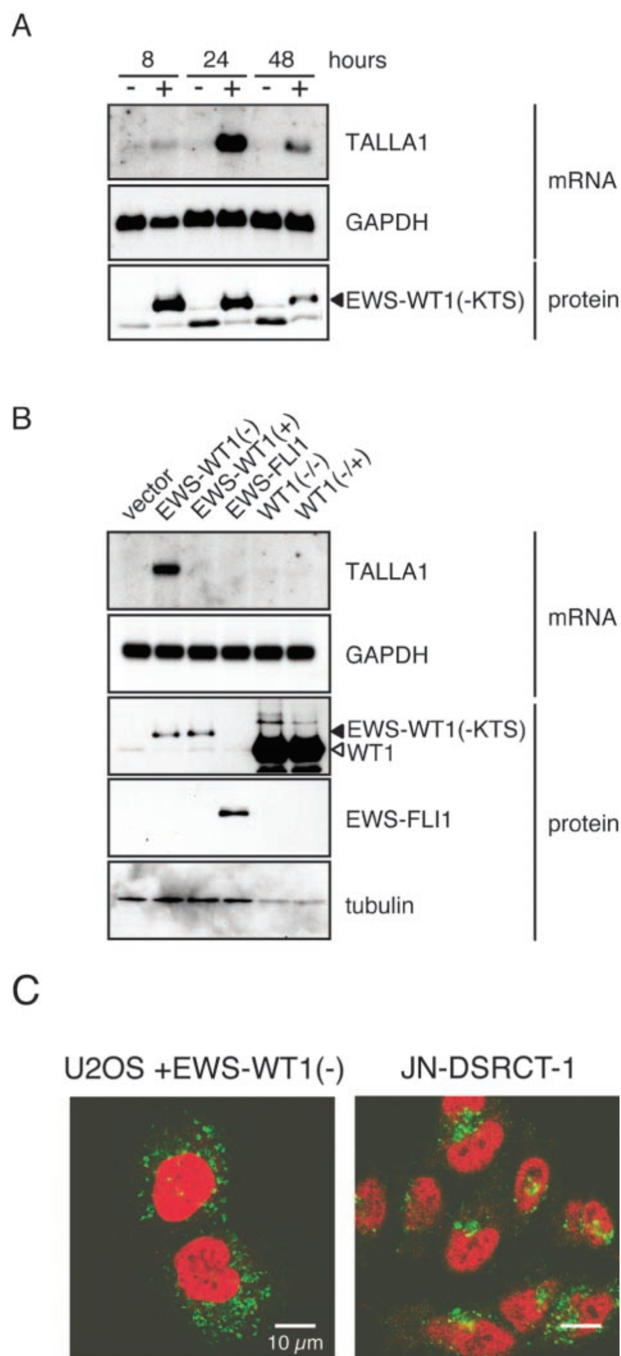

Figure 2. Specific expression of *Talla-1* mRNA and subcellular localization of TALLA-1 protein in cells transiently expressing EWS-WT1(-KTS). **A:** Time course of *Talla-1* mRNA induction after transfection of vector encoding EWS-WT1(-KTS). RNA and protein were prepared at the indicated times after transfection. **B:** Specific induction of *Talla-1* expression by EWS-WT1(-KTS). Each expression vector was introduced into U2OS cells and then RNA and protein were prepared 24 hours after transfection. **C:** Immunofluorescent staining of EWS-WT1(-KTS)-transfected cells and JN-DSRCT-1. TALLA-1 and EWS-WT1 were stained with Alexa Fluor 488 (green) and Alexa Fluor 568 (red), respectively.

complex by anti-tetraspanin or anti-integrin antibodies induces phosphoinositide 3-kinase-dependent production of matrix metalloproteinase 2 (MMP2) and long invasive protrusions within Matrigel, which is thought to increase invasive potential of tumor cells in the three

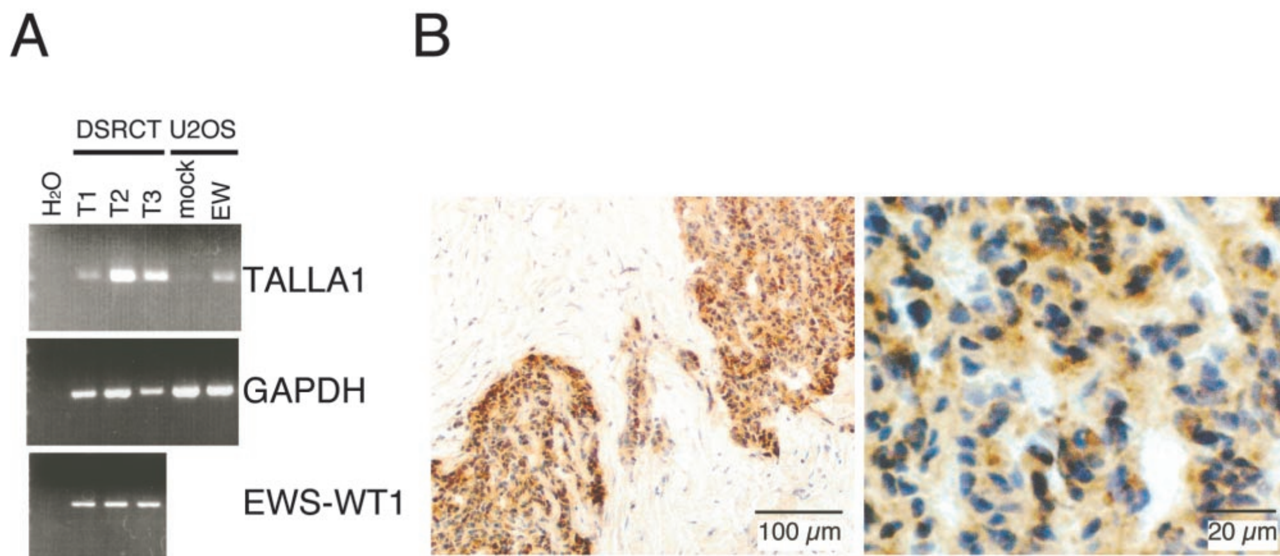

Figure 3. Expression of TALLA-1 in DSRCT specimens. **A:** RT-PCR analysis of the *Talla-1* mRNA in DSRCT. The *Talla-1* mRNA was amplified from various sources by RT-PCR and then separated by electrophoresis on agarose gels. Sources are DSRCT tissues (T1, T2, T3), cells transiently expressing EWS-WT1(-KTS) (EW) or its negative control (mock), and various human tissues. Expression of *EWS-WT1* fusion transcript was also detected in the three DSRCT tumors. PCR products were visualized by staining with ethidium bromide and UV transillumination. **B:** Immunohistochemical staining of a DSRCT specimen (T3). TALLA-1-positive cells are stained brown. Sections were counter-stained with hematoxylin.

dimensional extracellular matrix environment.[29] Recent biomechanical analysis directly showed that CD151, a member of the tetraspanin family, selectively strengthens $\alpha 6\beta 1$ integrin-mediated adhesion to laminin-1.[30] Exogenous expression of tetraspanin proteins, Co-029 and CD151, increases the metastatic potentials of tumor cell lines[31,32] and anti-CD151 antibody inhibits metastasis and migration of a CD151-expressing tumor cell line.[31] Taken together, TALLA-1 may be involved in cellular processes, including cell adhesion and regulation of cytoskeleton through a phosphoinositide-dependent signaling pathway, which may reflect infiltration and desmoplastic character of DSRCT. This hypothesis is also attractive for pathogenesis of MRX because genes encoding Oligophrenin-1,[33] PAK3,[34] and ARHGEF6,[35] all of which are thought to regulate actin cytoskeleton, and are mutated in the cases of MRX. It should be noted that not all tetraspanin play a positive role in tumorigenesis or cancer metastasis. Some tetraspanins such as CD9, CD63, and CD82 are thought to function as negative regulators of metastasis.[15] In the case of CD82, a tetraspanin-associated protein, EWI-2 synergizes CD82 in inhibiting prostate cancer cell migration.[36] This discrepancy is not surprising, however, given that the function of the tetraspanin family depends on the components of tetraspanin-containing protein complex in individual cells.

TALLA-1 protein shows dot-like localization pattern in cytoplasm[37] (Figure 2C). Tetraspanin proteins are localized in various types of intracellular vesicles such as late endosomes and Weibel-Palade bodies of endothelial cells,[38,39] multivesicular major histocompatibility class II-enriched compartments of B-lymphocytes,[40] and exosomes of dendritic cells.[41,42] These data point to a possible role of tetraspanin proteins in turnover and/or sorting of tetraspanin-associated proteins. A metastatic suppressor, CD82, attenuates the epidermal growth factor (EGF) signaling by accelerating EGF receptor endocytosis via its association with the EGF receptor.[43] TALLA-1 has a tyrosine-based sorting motif at the C terminus that might recruit clathrin adapter proteins to direct the TALLA-1-containing protein complex along various trafficking routes.[44] Thus TALLA-1 may perturb normal turnover of cell surface proteins in DSRCT cells.

To elucidate the function of TALLA-1, it is necessary to characterize the TALLA-1-containing protein complex on the plasma membrane and intracellular compartments. Using recent proteomics technology, tetraspanin-associated proteins have been isolated.[36,45,46] Our preliminary analysis shows that a number of cell surface proteins are associated with TALLA-1 in Jurkat (T-ALL), IMR32 (neuroblastoma), and JN-DSRCT-1 cells. Characterization of these proteins may help in understanding the functions of TALLA-1 in tumor cells.

Acknowledgments

We thank S. Kobayashi for secretarial assistance and A. Aono, N. Hoshina, and T. Kimura for technical assistance.

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