CADM1/TSLC1 is a Novel Cell Surface Marker for Adult T-Cell Leukemia/Lymphoma

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CADM1/TSLC1 (Cell adhesion molecule 1/Tumor suppressor in lung cancer 1) is a cell adhesion molecule that was originally identified as a tumor suppressor in lung cancer. CADM1/TSLC1 expression is reduced in a variety of cancers via promoter methylation, and this reduction is associated with poor prognosis and enhanced metastatic potential. In contrast, we observed that CADM1/TSLC1 is highly and ectopically expressed in all primary adult T-cell leukemia/lymphoma (ATLL) cells and in most human T-cell leukemia virus type (HTLV)-1-infected T-cell and ATLL cell lines. No expression, however, was detected in CD4+ T cells or in several other non-HTLV-1-infected leukemia cells. Moreover, we identified that high CADM1/TSLC1 expression plays an important role in enhanced cell-cell adhesion to the vascular endothelium, tumor growth and the ability of ATLL cells to infiltrate organs. We developed various antibodies as diagnostic tools to identify CADM1+ ATLL cells. Using flow cytometry, we determined that CADM1/TSLC1 is present on the surface of ATLL cells. The percentage of CD4+ CADM1+ cells in the peripheral blood of HTLV-1 carriers and ATLL patients was highly correlated with the DNA copy number of HTLV-1 in lymphocytes. In particular, we identified the soluble form of CADM1/TSLC1 in the peripheral blood of HTLV-1 carriers and ATLL patients. Therefore, measurements of soluble CADM1/TSLC1 serum levels and the detection of CD4+ CADM1+ cells in the blood, when combined with standard diagnostic methods, would be useful for identifying and monitoring disease progression in HTLV-1 carriers. Such tests would provide increased accuracy and may aid in early diagnosis and in determining the effects of ATLL treatments. (J Clin Exp Hematopathol 52(1) : 17-22, 2012)

Keywords: CADM1/TSLC1, cell adhesion molecule, cell surface marker, invasion, adult T-cell leukemia/lymphoma

INTRODUCTION

The CADM1 (Cell adhesion molecule 1, Tumor suppressor in lung cancer 1; TSLC1, IgSF4, Necl2, Syncam or SgIGSF) gene encodes an immunoglobulin (Ig) superfamily cell adhesion molecule (IgCAM). CADM1 is a well-known tumor suppressor gene in a variety of human cancers, particularly those of epithelial cell origin, including liver, pancreatic and prostate cancers. This gene is generally inactivated in these carcinomas via 2 mechanisms; promoter methylation and/or loss of heterozygosity at the gene locus. The CADM1 gene encodes a 442-amino acid class I membrane protein and contains three Ig loops in the extracellular domain, a transmembrane domain and a short cytoplasmic domain. This primary structure is also observed in IgCAM protein family members, which are referred to as neclins. Although neclins associate with afadin, CADM1/NecI2 (Nectin-like protein 2) does not. However, several CADM1/TSLC1-interacting proteins have been identified, such as DAL-1/4.1B of the protein 4.1 family, members of which are known to be spectrin-actin-binding proteins. CADM1/TSLC1 forms homodimers through cis interactions, and these interactions contribute to cell-cell interactions at the lateral membranes of polarized epithelial cells. Class I-restricted T cell-associated molecule (CRTAM), a two Ig domain-bearing surface receptor, was also identified as a CADM1/TSLC1 ligand. CADM1/TSLC1 interacts with CRTAM to promote natural killer (NK) cell cytotoxicity, interferon-γ secretion by CD8+ cells in vitro and NK cell-mediated rejection of tumors that express CADM1/TSLC1 in vivo. It is proposed that the disruption of cell adhesion via the loss of CADM1/TSLC1 leads to cancer cell invasion or metastasis. The tumorigenic potential of CADM1/TSLC1, which is located on chromosome 11q23, was first reported by Murakami et al. This gene was identified as a tumor suppressor in human non-small-cell lung cancers (NSCLCs) based on combinatorial analyses of yeast artificial chromosome transfers into human NSCLC cells and a tumorigenicity assay of these modified lines in

Received: January 26, 2012
Accepted: February 8, 2012

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Tax alters many transcriptional pathways, activating cellular genes by cooperating with cellular transcription factors. Tax activates the transcription of HTLV-1 and improves therapies, ATLL still has a very poor prognosis. In contrast, Tax expression is undetectable in approximately 60% of leukemias. Tax represses p53 and interferes with several cell cycle regulators, leading to the immortalization of HTLV-1-infected cells.

Potential oncoprotein Tax is encoded in the pX region of HTLV-1. Tax activates the transcription of HTLV-1 and cellular genes by cooperating with cellular transcription factors. Tax alters many transcriptional pathways, activating cyclic adenosine monophosphate response element binding protein, activator protein-1, and nuclear factor-κB. Tax also represses p53 and interferes with several cell cycle regulators, including cyclins and CDK inhibitors (p15 and p16). These multiple functions of Tax are believed to be involved in the immortalization of HTLV-1-infected cells.

In contrast, Tax expression is undetectable in approximately 60% of leukemias. There are 3 proposed mechanisms for the inactivation of Tax expression in ATLL cells. First, genetic changes (nonsense mutations and deletion) of the Tax gene have been described. Second, the deletion of the 5’ long terminal repeat (LTR) as a promoter has been implicated in the inactivation of Tax expression. Third, the 5’-LTR can be hypermethylated, leading to promoter inactivation. Because Tax is the major target of cytotoxic T-lymphocytes, a disruption or decrease in Tax expression can facilitate the escape of ATLL cells from the host cytotoxic T-lymphocytes, contributing to the development of ATLL. Alternatively, the 3’ LTR may remain unmethylated and intact in ATLL cells. HBZ is transcribed from the minus strand of the provirus using the 3’ LTR as a promoter. It has been reported that HBZ is expressed in ATLL cells and promotes ATLL cell proliferation. Considering the long latency period of ATLL, it has been proposed that at least five additional genetic or epigenetic events are required for the development of overt disease. Although no specific chromosomal abnormalities have been identified in ATLL, human leukemias are often associated with primary genetic alterations, usually non-random reciprocal chromosomal translocations. It has been reported that the tumor suppressor genes p53, p15, and p16 are disrupted in aggressive ATLL via chromosomal loss or promoter methylation. However, because ATLL-related genomic alterations are enormously diverse and complex, the molecular basis of the multistep process of leukemogenesis in ATLL remains unclear.

To identify specific genetic markers for ATLL, we previously described the gene expression profiles of ATLL cells and identified highly expressed genes therein. These analyses were performed using cells from patients with acute-type ATLL using a GeneChip microarray, which contained oligonucleotide hybridization probes for more than 12,000 genes. After determining the expression profiles from the panels of ATLL patients, we identified three genes that were up-regulated more than 30-fold in ATLL cells, including CADM1/TSLC1, caveolin 1 (CAV1), and prostaglandin D2 synthase. Unexpectedly, CADM1/TSLC1 was overexpressed in all primary ATLL cells and in most HTLV-1-infected T cells and ATLL cell lines. As described above, CADM1/TSLC1 is a member of the Ig superfamily of cell adhesion molecules and participates in cell-cell interactions. Previous reports have demonstrated that CADM1/TSLC1 is expressed in nearly all organs but not in lymphoid tissues, suggesting a functional role in epithelial cell adhesion. Our studies of a series of hematopoietic cells, CADM1/TSLC1 was weakly expressed in erythrocytes, to a lower degree by neutrophils, monocytes, and B cells and was not expressed in T cells. The activation of T cells with PHA or an anti-CD3 and anti-CD28 antibody mixture did not induce CADM1/TSLC1 expression, indicating that the CADM1/TSLC1 expression in ATLL cells did not result from the activation of normal T cells. Moreover, CADM1/TSLC1 expression was not detected in 34 HTLV-1-uninfected leukemia cell lines, consisting of 17 myelocytic/monocytic, 3 megakaryocytic, 2 erythrocytic, 4 B-lymphocytic, and 8 T-lymphocytic leukemia cell lines. These findings suggest that TSLC1 is specifically expressed in ATLL and HTLV-1-infected T cells.

**PHYSIOLOGICAL ROLES OF CADM1/TSLC1 IN ATLL CELLS**

One characteristic feature of ATLL cases is the invasion of the lymph nodes, skin, or various other organs by malignant cells. When the ED-ATLL cell line, which expresses very low CADM1/TSLC1 levels, was stably transfected with a CADM1 vector and assayed for self-aggregation ability, ED/CADM1 cells were found to form aggregates within 30 minutes. In contrast, parental ED cells or ED/neo cells exhibited little aggregation in this time period, suggesting that...
CADM1/TSLC1 mediates the intercellular adhesion of ATLL cells via homophilic interactions. However, the potential in vivo pathologic significance of this self-aggregation of ATLL cells is unclear. The initial step in the invasion of various human organs by ATLL cells is their interaction with vascular endothelial cells. Therefore, we examined the possible involvement of CADM1/TSLC1 in the adhesion of ATLL cells to human umbilical vein endothelial cells (HUVECs) in vitro.

When fluorescence-labeled ED cells and their derivatives were seeded onto HUVECs, incubated for 30 minutes, and then washed with medium, the numbers of attached ATLL cells was significantly (3-fold) higher in ED/CADM1 cells compared with parental ED or ED/Neo cells. These results suggest that ectopic CADM1/TSLC1 expression in ATLL cells may promote their invasion of various organs via an interaction with the surface molecules of vascular endothelial cells.

**CRITICAL ROLE OF CADM1/TSLC1 IN THE GROWTH AND ORGAN INFILTRATION OF ATLL CELLS**

We examined the role of CADM1/TSLC1 in the growth and infiltration of leukemia cells using C57BL/6j and NOD-SCID/c null (NOG) mice.

First, a murine IL-2-independent T-lymphoma cell line (EL4) was intraperitoneally injected into syngeneic C57BL/6j mice as a model for ATLL. The EL4/Neo cells did not develop a tumor. Alternatively, the ED/Neo cells exhibited liver invasion but did not develop a tumor. These results indicate that CADM1/TSLC1 overexpression in T-lymphoma cells aggressively promotes the development of leukemia/lymphoma. Next, ATLL-derived ED cells were subcutaneously injected into NOG mice. The ED/CADM1 cell lines caused greater formation of larger tumors than did the ED/Neo and parental cell lines. Clinical signs of being near death (e.g., piloerection, weight loss, and cachexia) at the time of sacrifice were more prevalent in those mice that were near death (e.g., piloerection, weight loss, and cachexia) 6 to 8 weeks following the inoculation. These mice also exhibited enlarged lymph nodes, spleens, lungs, and livers.

Microscopically, the ATLL cells invaded various organs to different degrees in all of the ATLL-bearing NOG mice. The dispersion diagram for the levels of invasion and the cellular CADM1/TSLC1 expression levels revealed a correlation coefficient of 0.714, suggesting that there was a moderate correlation between the invasive capability of the cells and the CADM1/TSLC1 expression level. Thus, CADM1/TSLC1 aided in the formation of a rapidly growing large tumor and massive infiltration of ATLL cells into various organs in NOG mice. Because CADM1/TSLC1 is expressed in various ATLL types, including smoldering and chronic types, this gene may be a promising target for the development of a novel ATLL therapy.

**CLINICAL SIGNIFICANCE OF CADM1/TSLC1 EXPRESSION IN ATLL**

The determination of clonal HTLV-1 proviral integration by Southern blot analysis is the gold standard for a definitive ATLL diagnosis. In addition, the presence of leukemia cells with multi-nucleated nuclei (referred to as “flower cells”) in the peripheral blood is a morphologic characteristic feature of ATLL. Hypercalcemia and high levels of either serum lactate dehydrogenase (LDH) or soluble IL-2Ra (sIL-2Ra) have been demonstrated to be unfavorable markers for ATLL; however, these markers are not specific for the diagnosis of ATLL. Recently, we generated a series of antibodies against CADM1/TSLC1 to be used as diagnostic tools for ATLL. These antibodies can be used for the identification and separation of ATLL and HTLV-1-infected cells, the detection of the soluble form of CADM1/TSLC1 in the peripheral blood and the pathological identification of lymphoma-type ATLL following formalin fixation.

**CADM1/TSLC1 expression in leukemia cells from ATLL patients and from HTLV-1-infected cells from viral carriers**

ATLL cells exhibit an activated helper T-cell profile (i.e.,
CD3+, CD4+, CD8+, and CD25+). It was reported that 10 of 17 ATLL cases (59%) expressed forward box P3, the expression of which is characteristic of CD4+ and CD25+ regulatory T (T-reg) cells.34 These data suggest that certain ATLL cases originate from T-reg cells. Interestingly, we observed, using flow cytometric analysis, that a subset of the T-reg fraction weakly expressed CADM1/TSLC1, suggesting that CADM1/TSLC1 is not a major marker for the T-reg fraction and that CADM1/TSLC1 expression on ATLL cells may reflect their T-reg cell origin.33 Among CD45+ cells in PBMCs from healthy volunteers, 7.3% of CD45+ cells also expressed CD4 and CADM1/TSLC1. These results indicate that the number of CD4+CADM1+ cells was significantly lower than the number of CD4+CD25+ cells in healthy volunteer PBMCs. The median percentages of CD4+CD25+ cells were 73.9% in acute cases, 72.4% in chronic cases, and 5.6% in lymphoma cases, indicating a major role for CD4+CD25+ T-reg cells in ATLL patients.34

The question of why CADM1/TSLC1 is strongly expressed on the surface of various ATLL types remains unclear. We previously examined whether CADM1/TSLC1 expression is induced by HTLV-1/Tax expression, demonstrating that Tax protein expression did not activate CADM1/TSLC1 expression in JPX-9 cells. We also introduced a Tax expression vector into MOLT4 and 293T cells and determined the subsequent CADM1/TSLC1 expression levels. With these analyses, we demonstrated that Tax was not able to induce CADM1/TSLC1 expression in these cells, suggesting that Tax expression is unrelated to high CADM1/TSLC1 expression. Because HBZ is known to be constitutively expressed in both HTLV-1-infected and ATLL cells and can modulate host transcription,22 it is possible that HBZ activates CADM1/TSLC1 expression. We also speculate that

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High CADM1/TSLC1 expression in ATLL-derived lymphomas

We examined immunohistochemical staining of 90 tissue samples from patients with various types of lymphoma, including 36 patients with ATLL and 54 with non-ATLL lymphomas. These latter cases included T- or NK cell lymphomas, B-cell lymphomas, and null-cell lymphomas. Using a four-grade scale to score CADM1 immunohistochemical staining (0 to 3+), we observed that 92% of ATLL lymphomas were positive for CADM1/TSLC1.33 Of these, 50% stained heavily for CADM1 and scored 2+ or higher. Specific membranous staining was typically observed in ATLL cells. Among the non-ATLL lymphomas, a small number of CADM1-positive cells (fewer than 5%; score 1+) were observed. These cells were small to medium-sized and contained non-atypical, normochromic round/ovoid nuclei. Based on these morphological and CADM1/TSLC1-staining analyses, the CADM1-positive cells in the non-ATLL lymphomas were not considered to be lymphoma cells but possibly histiocytes, including dendritic cells. This was suspected because these cells were similar to the CADM1-positive cells that were observed in reactive lymph nodes. Based on these results, a high degree of cell membrane staining for CADM1/TSLC1 with a score of 2+ may be highly specific for a diagnosis of ATLL. Furthermore, combined staining with CADM1/TSLC1 and other T-cell-specific markers may be necessary for a more accurate diagnosis of lymphoma-type ATLL.33

Detection of the soluble form of CADM1/TSLC1 in the serum of ATLL patients

A soluble CADM1/TSLC1 isoform consisting of the extracellular domain was recently isolated in murine mast cells. Using western blot analysis, we observed a 72-kDa soluble CADM1/TSLC1 protein in the sera of 5 patients with acute-type ATLL but not in the sera of 5 healthy volunteers. The analysis of the sera of 5 healthy controls and of 25 ATLL patients (14 acute-type, 7 lymphoma-type, 2 smoldering-type and 2 HTLV-1 carriers) revealed that high levels of soluble CADM1/TSLC1 were present in the serum of patients who had high numbers of CADM1+ cells in the peripheral blood. Furthermore, when comparing the soluble IL-2Ra and CADM1/TSLC1 serum levels in individual cases, significantly higher levels of soluble CADM1/TSLC1 were detected in the serum of ATLL patients who had increased levels of soluble IL-2Ra; thus, serum CADM1/TSLC1 levels may be predictive of disease progression in ATLL.31

Furthermore, when comparing the soluble IL-2Ra and CADM1/TSLC1 serum levels in individual cases, significantly higher levels of soluble CADM1/TSLC1 were detected in the serum of ATLL patients who had increased levels of soluble IL-2Ra; thus, serum CADM1/TSLC1 levels may be predictive of disease progression in ATLL.31
high CADM1 expression in ATLL cells may be associated with transcriptional abnormalities in ATLL cells via the accumulation of genomic or epigenomic alterations. The positive correlation between HTLV-1 copy number and the percentage of CD4+CADM1+ cells in the peripheral blood of HTLV-1 carriers suggests that, if they also exhibit high percentages of CD4+CADM1+ cells, these individuals may have developed more extensive genetic alterations and may be at high risk for developing ATLL.

Recent studies have shown that certain markers, such as CCR4 and CD70, are unique ATLL surface markers.36,37 Whereas the proportion of CD4+CCR4+ and CD4+CD70+ cells in the PBMCs of healthy individuals were observed to be approximately 5%,37,38 the proportion of CD4+CADM1+ cells in this population was less than 1%; therefore, the measurement of CADM1+ T cells is particularly efficient in the diagnosis of HTLV-1 infection in individuals who carry a small number of HTLV-1-infected cells. We have shown that CADM1/TSLC1 has important functions in increasing cell adhesion and in mediating cancer progression to organ invasion.29 In addition, we succeeded in using anti-CADM1-adhesion and in mediating cancer progression to organ invasion.29 In addition, we succeeded in using anti-CADM1-coated magnetic beads to isolate low percentages of HTLV-1-infected cells from PBMCs of HTLV-1 carriers with high HTLV-1 copy numbers, and ATLL cells from ATLL patients.33 Sorted HTLV-1-infected and ATLL cells may be useful tools for transcriptional and/or genomic analyses. The results of such tests could be compared between the PBMCs of healthy volunteers and peripheral leukemia cells from ATLL patients, potentially providing important information with respect to the expression pattern and genomic abnormalities that occur at the early stages of HTLV-1 infection and/or ATLL development. A recent study demonstrated that CADM1/TSLC1 directly associates with the PDZ domain of T-lymphoma invasion and metastasis 1 (Tiam1). This interaction induces the formation of lamellipodia by activating Rac in both HTLV-I-transformed cell lines and ATLL cell lines.39 These results indicate that Tiam1 integrates signals from CADM1/TSLC1 to regulate the actin cytoskeleton through Rac activation, potentially leading to tissue infiltration of leukemic cells in ATLL patients. The elucidation of the different downstream cascades that are activated by CADM1/TSLC1 in epithelial cells and T-lymphocytes would provide important insights into the roles of CADM1/TSLC1 in tumorigenesis.

CONCLUSION

In this manuscript, we describe how CADM1/TSLC1 is highly expressed in the majority of ATLL cells and in a subset of peripheral blood cells from HTLV-I carriers. These data suggest that CADM1/TSLC1 is potentially a prediagnostic indicator of ATLL development in high-risk HTLV-I carriers. Therefore, we are currently developing a diagnostic kit to detect soluble CADM1/TSLC1 protein in the peripheral blood. Furthermore, we are attempting to apply this diagnostic kit to various types of ATLL patient samples, including HTLV-I carriers. Moreover, CADM1/TSLC1 may be a novel molecular target for the treatment of ATLL. Dr. Kurosawa’s group (Fujita Health University, Japan) has already developed several types of anti-human CADM1/TSLC1 antibodies using the phage-display technique and has observed that certain clones exhibited cytotoxic activity against ATLL cells (manuscript in preparation). Moreover, recombinant shuttle viruses are being developed that will target ATLL cells by binding to CRTAM and/or CADM1/TSLC1. Finally, small molecules that interfere with the cell adhesion characteristics of ATLL cells will be valuable for blocking their organ-invading ability. Thus, CADM1/TSLC1 is a clinically useful molecule for detecting and for targeting ATLL cells.
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