Overexpression of *LAMP3/TSC403/DC-LAMP* Promotes Metastasis in Uterine Cervical Cancer

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Abstract

***LAMP3* (*DC-LAMP, TSC403, CD208*) was originally isolated as a gene specifically expressed in lung tissues. *LAMP3* is located on a chromosome 3q segment that is frequently amplified in some human cancers, including uterine cervical cancer. Because two other members of the *LAMP* family of lysosomal membrane glycoproteins, *LAMP1* and *LAMP2*, were previously implicated in potentially modulating the interaction of vascular endothelial and cancer cells, we hypothesized that *LAMP3* might also play an important part in metastasis. To clarify the metastatic potential of LAMP3 in cervical cancers, we transfected a *LAMP3* expression vector into a human uterine cervical cancer cell line, TCS. In an *in vitro* invasion assay, the migration of *LAMP3*-overexpressing TCS cells was significantly higher than in control TCS cells. In an *in vivo* metastasis assay, distant metastasis was detected in 9 of 11 *LAMP3*-overexpressing TCS cell–injected mice and in only 1 of 11 control mice. Histologic study showed that *LAMP3*-overexpressing cells readily invaded into the lymph-vascular space. In clinical samples, quantitative real-time reverse transcription-PCR (RT-PCR) analyses showed that *LAMP3* mRNA was significantly up-regulated in 47 of 47 (100%) cervical cancers and in 2 of 15 (13%) cervical intraepithelial neoplasias, compared with a low level of *LAMP3* mRNA expressed in normal uterine cervixes. Interestingly, high *LAMP3* expression was significantly correlated with the overall survival of patients with stage I/II cervical cancers. These findings indicate that *LAMP3* overexpression is associated with an enhanced metastatic potential and may be a prognostic factor for cervical cancer.** (Cancer Res 2005; 65(19): 8640-5)

Introduction

LAMP3 was first cloned as a gene specifically expressed in lung tissues, which was called *TSC403* that was subsequently found to be overexpressed in primary cancers of the esophagus, colon, fallopian tube, ovary, breast, and liver, although its expression was barely detectable in the corresponding normal tissues (1). *TSC403* is now better known as the cell surface marker gene *DC-LAMP* (2) or *CD208*, but it codes for a 416-amino-acid protein that is the third member of the lysosome-associated membrane glycoprotein (*LAMP*) family; thus, the HUGO Gene Nomenclature Committee has designated this gene and its protein *LAMP3*/LAMP3, respectively. We will use the LAMP3 nomenclature for the protein and *LAMP3* for the gene throughout, although *DC-LAMP* has been used more frequently in the literature. LAMP3 is a member of the *LAMP* family. LAMP1 and LAMP2 are located primarily in the lysosomal membrane and are rarely present on the surface of normal cells (3). They are the major carriers for poly-*N*-acetyllactosamines, including those with sialyl-Le^x termini (4, 5), which are critical ligands for the E-selectin present on endothelial cells and platelets (6–9). Cancer cells show increased levels of sialyl-Le^x determinants and adhere to vascular endothelial cells through their interaction with E-selectin (10, 11). LAMP1 and LAMP2 are constitutively expressed on the surface of some colon cancer cell lines, being much more associated with highly metastatic than less metastatic colon carcinoma cell lines (12). These observations suggest that cancer cells can modulate the display of E-selectin ligands on their cell surface in part by regulating expression of LAMP1 and LAMP2, and that up-regulation of surface-localized LAMP1 and LAMP2 may contribute significantly to the metastatic process. Although a definitive function of *LAMP3* has not been clarified yet, *LAMP3* may promote metastasis in the same way as *LAMP1* and *LAMP2*.

LAMP3 is located on chromosome 3q27, a region that is often amplified in several types of cancer, especially squamous cell carcinomas, including uterine cervical cancers (13–15). Heselmeyer et al. (16) found a gain of 3q in 9 of 10 cases of invasive cervical carcinomas, but in only 1 of 13 cases of severe dysplasia. They suggested that a functionally important gene for cervical carcinogenesis might exist at 3q24-27. *LAMP3* is a suitable candidate gene and its altered expression may play a role in cervical carcinogenesis, especially during late events such as tumor cell migration into lymph vessels.

To date, several candidates have already been suggested as being the potentially critical, frequently amplified, 3q oncogene. For example, *PIK3CA* at 3q26 (17), which encodes the p110α catalytic subunit of phosphatidylinositol 3-kinase, is an oncogene in ovarian and uterine cervical cancers (18, 19). Another possible 3q gene is *hTR*, which encodes the RNA component of human telomerase (20). Telomerase plays an essential role in stabilizing telomere length and, consequently, contributes to the processes of cellular immortality and tumorigenesis. Differential expression of telomerase activity has been reported during cervical cancer development (21). Other possible 3q locus genes are *p63* at 3q28 (22) and *eIF-5A2* at 3q26 (23).

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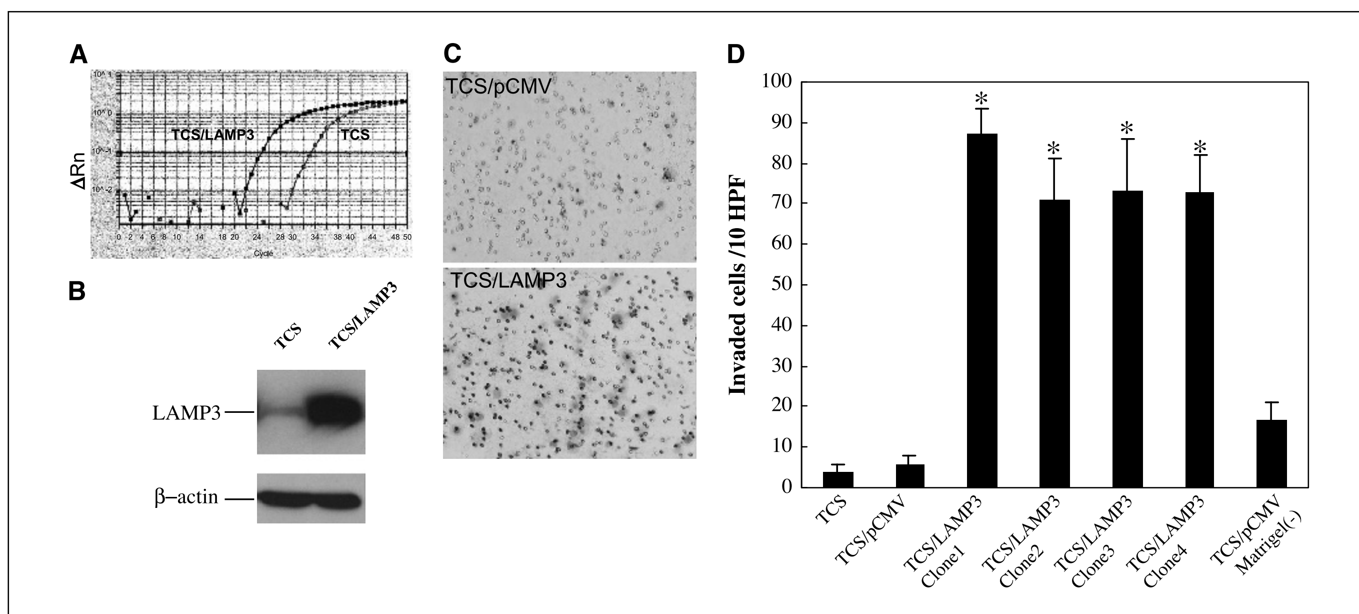

Figure 1. Overexpression of *LAMP3* in TCS cells. *A,* quantitative real-time RT-PCR shows that TCS cells transfected with a *LAMP3* expression vector expressed ~250-fold more *LAMP3* mRNA than parental TCS cells. *B,* a single 70 kDa band was detected by Western blot analysis. An increase of LAMP3 protein in TCS/*LAMP3* cells was confirmed. *C,* TCS/*LAMP3* cells had increased migration in an *in vitro* invasion assay. *Top,* control cells (TCS/pCMV); *bottom,* TCS/*LAMP3*-overexpressing cells. *D,* numbers of invaded cells per 10 high power fields. *Columns,* mean; *bars,* SD. ΔR_n, normalized fluorescence emission with subtracted baseline fluorescence. $^*P < 0.05$, Student's *t* test.

In this article, we propose that *LAMP3* should now also be considered as one of the relevant oncogene candidates at 3q.

Materials and Methods

Overexpression of *LAMP3* in TCS cells. The complete open reading frame of the *LAMP3* gene was cloned into a pCMV-script vector (Stratagene, La Jolla, CA). The human uterine cervical cancer cell line TCS was purchased from the Riken Gene Bank (Tokyo, Japan). Stable transfection of the construct into TCS cells was done using LipofectAMINE-Plus Reagent (Invitrogen Corp., Carlsbad, CA). *LAMP3*-overexpressing clones were selected with G418 (600 μg/mL) and were maintained in DMEM supplemented with 10% fetal bovine serum, penicillin, and streptomycin at 37°C in a 5% CO_2 incubator.

***In vitro* invasion assay.** An *in vitro* invasion assay was done using a 24-well transwell unit with Chemotaxicell (8 μm pores on polycarbonate filters; Kurabo, Osaka, Japan). The upper side of the filter was coated with 25 μg Matrigel/100 μL; the lower chamber was filled with serum-free medium containing 0.1% bovine serum albumin. Cells (1×10^5/200 μL) were placed in the upper part of the transwell plate, incubated for 24 hours, fixed with 10% buffered formamide, and stained with trypan blue. The invasive phenotype was determined by counting the cells that migrated to the lower side of the filter, with microscopy at ×200. Assay was done on four isolated clones of LAMP3-overexpressing cells. Ten fields were counted for each filter and each sample was assayed five times.

Severe combined immunodeficient mice xenografts and *in vivo* metastasis assay. Female severe combined immunodeficient (SCID) mice (CB-17/Icr-SCID) of between 6 and 8 weeks of age were maintained in a barrier facility on HEPA-filtered racks. The animals were fed with autoclaved laboratory rodent diet.

To generate xenografts, 10^7 *LAMP3*-overexpressing TCS cells, or 10^7 TCS/pCMV cells, in 0.1 mL saline were s.c. injected into the back of 11 SCID mice. S.c. tumors were measured at days 0, 3, 7, 12, 15, 18, 20, and 28, and then metastatic tumors were checked and removed from the 28-day-old mice after euthanasia. Tumors were fixed in 10% formaldehyde, paraffin-embedded, and sectioned.

Clinical samples. Forty-seven cervical cancers (41 squamous cell carcinomas and 6 adenocarcinomas), 15 cervical intraepithelial neoplasias (5 CIN grade 1, 5 CIN grade 2, and 5 CIN grade 3), and 5 normal uterine cervixes were obtained from patients of the Osaka University Hospital. Surgically removed tissues were sampled for histologic diagnosis and the remainders were quick frozen for subsequent extraction of DNA and RNA. Normal uterine cervixes were sampled from the patients who had undergone surgery due to leiomyoma of the uterus. The CIN lesions were biopsied subsequent to an abnormal cervical smear and abnormal colposcopic findings. None of the patients had been given treatment before analysis. Written informed consents were obtained from all patients.

DNA and RNA extraction. Total DNA and RNA were extracted from frozen tissues using the TRIzol LS Reagent (Life Technologies, Inc., Rockville, MD). For CINs and normal uterine cervixes, epithelial lesions were collected using a laser microdissection system (Leica Microsystems, Wetzlar, Germany) to avoid contamination with stromal cells.

Quantitative reverse transcription-PCR. For cDNA synthesis, 1 μg of total RNA was reverse-transcribed in a 20 μL volume of reaction mixture using a cDNA cycle kit (Invitrogen). For quantitative PCR, the primers used were as follows:

LAMP3 (cDNA)
5′-GCTCTGTTCACTCACGCACTTG-3′ (forward)
5′-CCGTCTCAGATCCAGAGACAGTT-3′ (reverse)
5′-FAM-CCGACTGCTGTCTGGAACATCACCAC-TAMRA-3′ (probe).

The *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene expression was quantified using the TaqMan GAPDH Control Regents for normalization of the *LAMP3* gene expression. Templates were loaded into a MicroAmp Optical 96-well Reaction Plate (Applied Biosystems, Foster City, CA). In brief, 5 μL of cDNA template was placed in microwells for each tissue sample, followed by the addition of 25 μL of the TaqMan Universal PCR Master Mix (Applied Biosystems), 0.5 μmol/L of each primer (forward and reverse), 0.2 μmol/L of each probe, 5 μL of H_2O to a total volume of 50 μL. The GeneAmp 7700 Sequence Detection System (Perkin-Elmer Life Analytical Sciences, Inc., Boston, MA) was used for amplification using a

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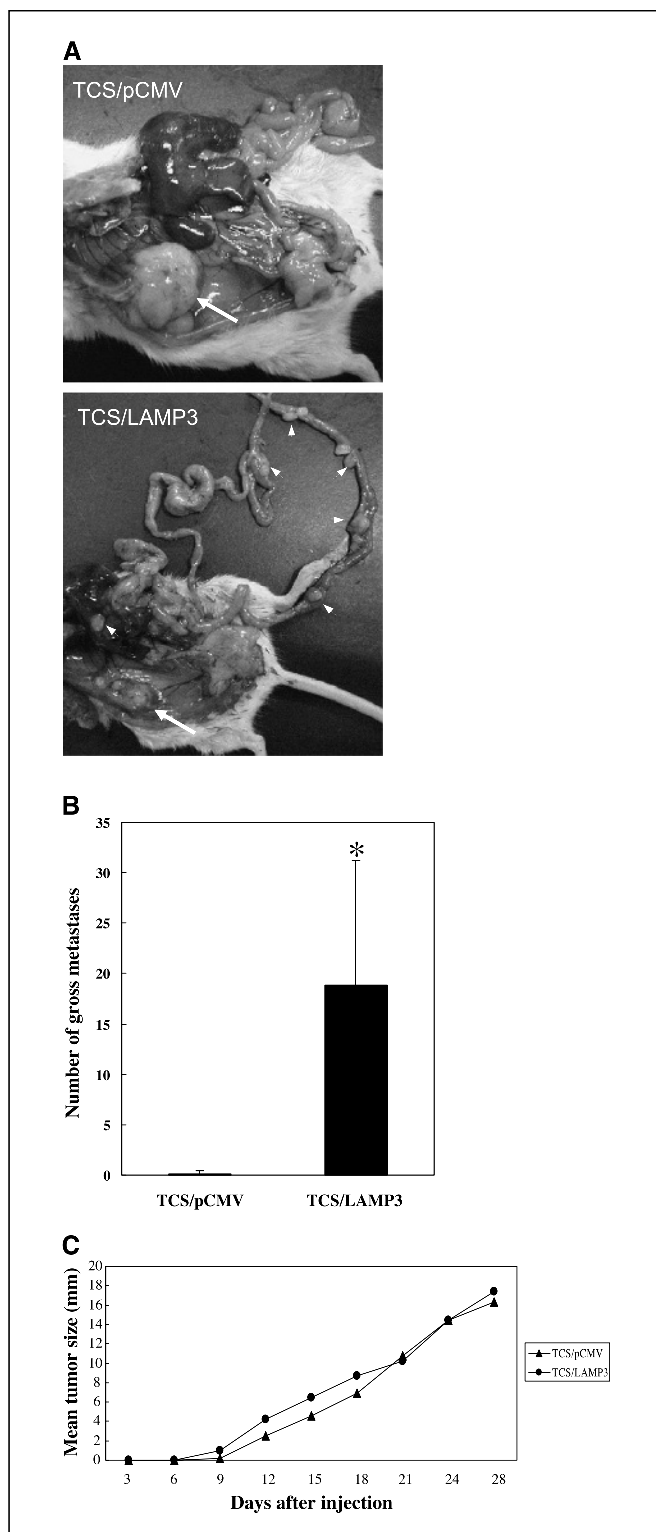

Figure 2. Increased metastasis of TCS/*LAMP3* transfectants. Eleven 7-week-old female SCID mice injected s.c. with 10^7 TCS/*LAMP3* cells developed multiple metastatic tumors in the liver, lungs, and para-aortic and mesenteric lymph nodes (*A, bottom, arrowheads*). S.c. primary tumors in the back showed similar growth in both control and TCS/*LAMP3*-injected mice (*A, arrows*). *B,* the number of gross metastases was significantly higher in the TCS/*LAMP3*-injected mice than in control mice. *C,* the growth curves (*points,* mean) of the original s.c. tumors were similar between the control mice and the TCS/*LAMP3*-injected mice. *$P < 0.05$, Student's *t* test.

two-temperature cycling program (denaturation at 95°C for 15 seconds followed by an annealing and extension cycle at 60°C for 1 minute) for a total of 40 cycles. The C_t value (minimum threshold cycle where the amount of product is significantly separated from the background baseline) was generated by ABI PRISM 7700 SDS (version 1.7) software. Samples with a high copy number of the target gene show an earlier increase of fluorescence, resulting in a lower C_t value. The expression of *LAMP3* was presented as ΔC_t ($GAPDHC_t - LAMP3C_t$). Therefore, a higher ΔC_t indicates a higher copy number of *LAMP3* mRNA. Results were calibrated against normal brain tissue cDNA.

Western blot analysis. TCS/*LAMP3* and TCS cells were harvested and boiled in SDS buffer for 5 minutes. Equal aliquots (20 μg) of total protein from the whole cell extracts were fractionated on a 10% denaturing SDS-polyacrylamide gel and transferred to polyvinylidene difluoride membranes. Nonspecific interactions were blocked with 5% nonfat milk/0.05% Tween 20. Proteins were identified using mouse monoclonal antibodies against DC-LAMP/LAMP3 (1:50; Immunotech, Marseilles, France), with a 1-hour incubation at room temperature followed by incubation with peroxidase-conjugated secondary antibodies (goat anti-mouse) for 30 minutes. Membrane-bound antibodies were detected using ECL Western Blotting Detection System (Amersham Biosciences Corp., Piscataway, NJ). Immunoblots were exposed on X-OMAT-AR film (Eastman Kodak, Rochester, NY) under standard conditions. Equivalent loading of proteins was checked using β-actin antibody (1:1,000).

Immunohistochemistry. Sectioned tissues were dewaxed, blocked with methanol, and incubated with a 1:10 dilution of the anti–DC-LAMP/LAMP3 antibody (Immunotech). The mouse anti-human recombinant E02B02 monoclonal antibody was originally produced by de Saint-Vis et al. (2). Signals were detected by use of a VECTASTAIN Elite ABC kit (Vector Laboratories, Burlingame, CA).

Statistical analyses. Univariate analyses were done by the Mann-Whitney *U* test and the Student's *t* test. Survival rates were calculated by the Kaplan-Meier method. For all tests, the level of significance was set at $P < 0.05$.

Results

Overexpression of LAMP3 in TCS cells. To establish a cell line overexpressing LAMP3, an expression vector for *LAMP3* was stably transfected into the human uterine cervical cancer cell line TCS. Quantitative real-time reverse transcription-PCR (RT-PCR) showed that the TCS/*LAMP3* cells expressed ~250-fold more *LAMP3* mRNA than the TCS cells (Fig. 1*A*). ΔC_t of TCS/pCMV and TCS/*LAMP3* were 3.5, and 11.5, respectively. The RT-PCR analysis was done in triplicate and showed similar results. Western blotting analysis showed a single band of 70 kDa in size. An increase of LAMP3 protein in the TCS/*LAMP3* cells is apparent and is consistent with mRNA (Fig. 1*B*).

***TCS/LAMP3* cells display increased cell motility.** Using an *in vitro* invasion assay, we observed higher motility in TCS/*LAMP3* cells than in the parental TCS cells. Figure 1*C* shows the cells migrating to the lower side of the filter, indicating invasive phenotype. The average number ± SD of invaded TCS cells in 10 high power fields of each filter was 4.0 ± 1.5; for TCS/pCMV, it was 5.8 ± 2.1; for TCS/*LAMP3* (clone 1), it was 89.4 ± 6.1; for TCS/*LAMP3* (clone 2), it was 71.2 ± 10.0; for TCS/*LAMP3* (clone 3), it was 73.2 ± 12.9; for TCS/*LAMP3* (clone 4), it was 73.0 ± 9.0; and for TCS/pCMV without Matrigel, the number was 16.6 ± 4.5 (Fig. 1*D*). Cell counts for all of four isolated TCS/*LAMP3* clones were significantly higher than for vector control (TCS/pCMV). The number of invading cells of TCS/pCMV and TCS were not different, indicating that transfection with the empty vector did not affect cell migration. The cell counts of TCS/pCMV without Matrigel were higher than TCS/pCMV, showing that 25 μL of Matrigel was

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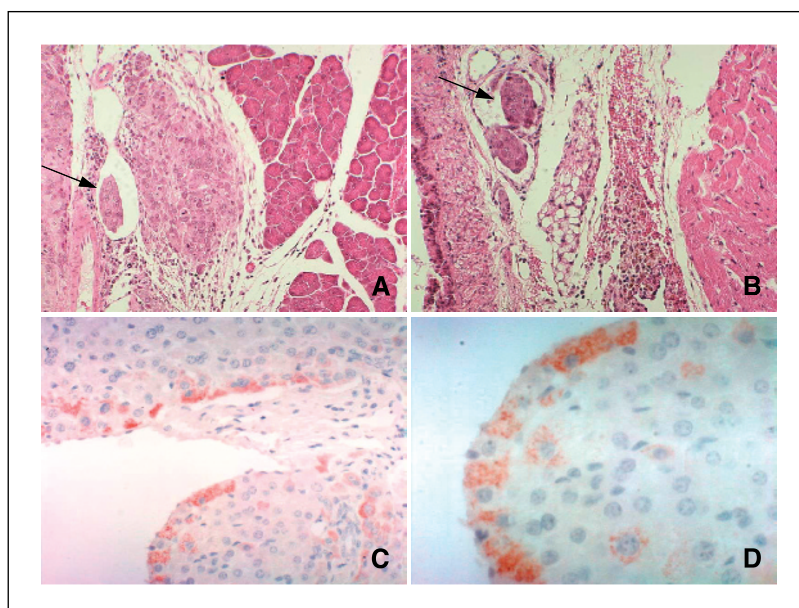

Figure 3. Invasion into lymph-vascular space in TCS/*LAMP3*-injected mice. H&E-stained sections of metastatic tumors [lymph-vascular invasion at intestine (*A*) and hilum of lung (*B*)] observed in TCS/*LAMP3*-injected mouse were shown. Invasion into lymph-vascular space (*arrows*) was obvious in each section. Immunohistochemistry for LAMP3 showed positive cytoplasmic staining in the tumor cells at expanding and invading areas (*C* and *D*). Magnification, ×40 (*A* and *B*), ×100 (*C*), and ×200 (*D*).

sufficient for the invasion assay. These findings indicate that overexpression of *LAMP3* enhances cell invasion.

TCS/*LAMP3* transfectants possess increased metastatic potential *in vivo*. S.c. injection of TCS/*LAMP3* or TCS/pCMV (vector control) cells into SCID mice produced xenografts in the back of the mice and subsequent metastases. Metastatic tumors were detected more frequently in TCS/*LAMP3*-injected mice than in control mice (Fig. 2*A*); 9 of 11 (83%) TCS/*LAMP3*-injected mice developed metastases and only 1 of 11 (9%) of the control mice did. The metastatic target sites were the liver, lung, and para-aortic and mesenteric lymph nodes. The number of gross metastases was significantly higher in TCS/*LAMP3*-injected mice (18.8 ± 12.2 versus 0.009 ± 0.3) as shown in Fig. 2*B*. On the other hand, the growth curves of the primary s.c. tumors were similar between the control mice and the TCS/*LAMP3*-injected mice (Fig. 2*C*), suggesting that overexpression of *LAMP3* do not affect primary tumor growth. H&E-stained sections of the metastatic tumors of a TCS/*LAMP3*-injected mouse are shown in Fig. 3. Aggressive invasion into the lymph-vascular space was observed in the TCS/*LAMP3*-injected mice, but not in the control mice. In the immunohistochemistry for LAMP3, tumor cells that were invading into the surrounding tissues were more strongly positive than other areas. The cytoplasm of the tumor cells was stained, suggesting that LAMP3 was located in or near the lysosomal membrane as are other LAMP family members, LAMP1 and LAMP2. These findings indicate that overexpression of LAMP3 is actively involved in tumor invasion through increased migration into lymph-vascular spaces.

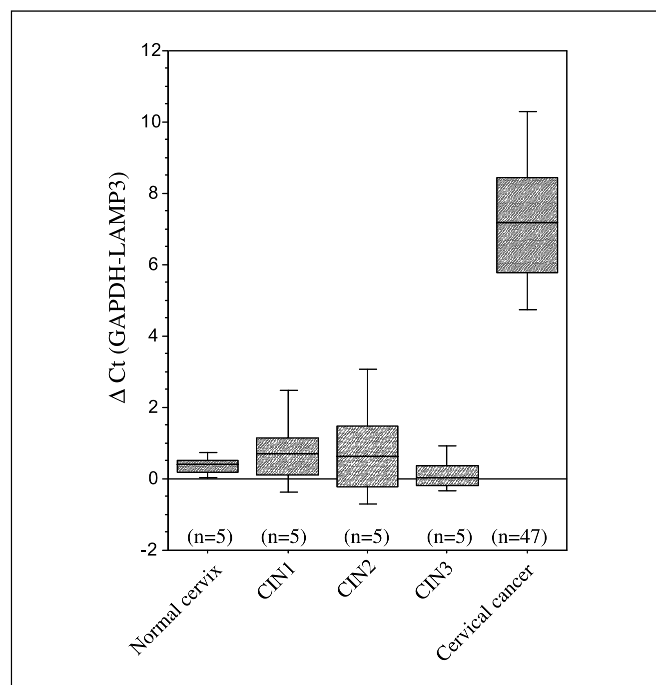

Figure 4. Expression of the *LAMP3* in primary uterine cervical cancers. In clinical samples, quantitative real-time RT-PCR showed that cervical cancers expressed significantly higher levels of *LAMP3* mRNA than others. The expression of *LAMP3* mRNA was presented as ΔC_t (*GAPDH* C_t − *LAMP3* C_t). Therefore, higher ΔC_t indicates higher copy number of *LAMP3* mRNA. In 47 cervical cancers tested, 47 samples (100%) revealed overexpression of *LAMP3* mRNA, whereas normal cervixes and CINs showed very low levels of expression. Average ± SD of ΔC_t in normal cervix, CIN1, CIN2, CIN3, and cervical cancer were 0.37 ± 0.26, 0.13 ± 0.48, 0.78 ± 1.42, 0.74 ± 1.05, and 7.34 ± 2.1, respectively.

Expression of the *LAMP3* gene in primary uterine cervical cancers. In clinical samples, quantitative real-time RT-PCR showed that cervical cancers expressed ~100-fold more *LAMP3* mRNA than normal uterine cervixes (Fig. 4). In 47 cervical cancers tested, 47 samples (100%) revealed overexpression of *LAMP3* mRNA. In 15 CINs, two samples did (a single case of CIN2 and a single case of CIN3). Overexpression was not detected in five normal cervixes. The averages ± SD of ΔC_t in normal cervix, CIN1, CIN2, CIN3, and cervical cancer were 0.37 ± 0.26, 0.13 ± 0.48, 0.78 ± 1.42, 0.74 ± 1.05, and 7.34 ± 2.1, respectively.

Immunohistochemistry. In an immunohistochemical study using clinical samples, we found that LAMP3 protein was highly expressed in primary cervical cancers (Fig. 5*A*). Metastatic tumors in lymph nodes and tumor cells in lymph-vascular spaces also

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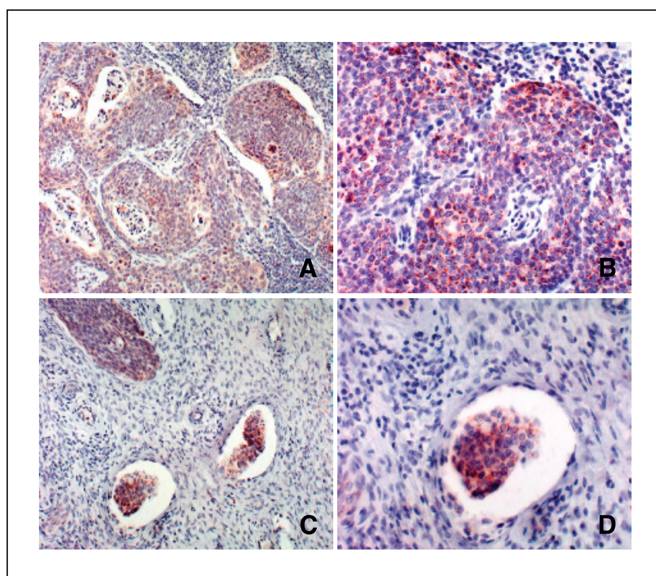

Figure 5. Immunohistochemistry. *A,* positive staining for LAMP3 was detected at the primary site of uterine cervical cancer. *B,* metastatic tumor in a lymph node showed strong expression of LAMP3. *C* and *D,* tumor cells in lymph vessels are also strongly stained. Magnification, ×40 (*A* and *C*) and ×200 (*B* and *D*).

showed a high expression of LAMP3 as well (Fig. 5*B-D*). Squamous epithelium of the normal uterine cervix had negligible LAMP3 staining (data not shown).

High *LAMP3* expression was significantly correlated with poor prognosis in patients with stage I or II cervical cancers. For the 47 cervical carcinomas we examined, 24 patients were evaluated for their prognosis. All of them were Fesddration Internationale des Gynaecologistes et Obstetristes stage I or II (24). Radical hysterectomy, with or without postoperative whole pelvic radiation, was applied to all the patients. To clarify the significance of the *LAMP3* gene expression in prognosis, we divided these 24 patients into groups A and B by the degree of expression of *LAMP3* mRNA of the tumor. Group A included nine cases with a ΔC_t of >7; group B included 15 cases with a ΔC_t of <7. The correlation between expression of *LAMP3* mRNA and overall patient survival was analyzed by the Kaplan-Meier method (Fig. 6). The prognosis for group A was significantly worse than that of group B by the log-rank test ($P < 0.05$). This suggests that high levels of *LAMP3* are associated with uncontrollable distant metastasis and a higher mortality.

Discussion

Surgery and radiation therapy are relatively effective for controlling uterine cervical cancer at its primary site. Mortality from this tumor is usually caused by its metastasis to lymph nodes and distant organs. Our data indicate that the presence of overexpressed *LAMP3* is significantly associated with the promotion of a metastatic potential both *in vitro* and *in vivo*. Furthermore, in clinical samples, we found, by quantitative real-time RT-PCR, overexpression of *LAMP3* mRNA in cervical cancer compared with normal uterine cervix and CINs. Interestingly, we also observed that higher *LAMP3* expression was significantly correlated with poorer prognosis of patients with stage I/II cervical cancer.

Metastasis consists of several sequential steps (i.e., growth at the primary site, invasion into the stroma, migration into blood vessels or lymphatics, arrest at a distant site, extravasation, and proliferation). Recent studies have shown that vascular endothelial growth factors (VEGF)-C and VEGF-D play pivotal roles in lymphangiogenesis and in lymph node metastasis in human cancers. Skobe et al. (25) have shown that overexpression of VEGF-C in human breast cancer cell line increases both lymphangiogenesis and lymphatic metastases in mice. VEGF-D also promotes the metastatic spread of tumor cells via the lymph systems (26). In their study, VEGF-D promotes not only lymphatic metastasis but also tumor growth and angiogenesis. These effects were inhibited by anti–VEGF-D antibodies. Association between overexpression of VEGF-C and/or VEGF-D and lymph node metastasis has been reported in various human cancers as reviewed by Pepper et al. (27). Thus, the molecular mechanisms of lymphangiogenesis in tumor have begun to be elucidated.

Certain mechanisms of metastasis have been found to be similar to those of inflammation in that many of the mediators involved are similar in both processes. For example, the chemokine receptors CXCR4 and CCR7, which were originally identified in leukocytes, are highly expressed in human breast cancer cells and metastases. Their respective ligands, CXCL12/SDF-1α and CCL21/6Ckine, exhibit the highest levels of expression in organs representing the first destinations of breast cancer metastasis (28). Correlations between overexpression of CCR7 in tumor cell and lymph node metastasis have been reported in gastric cancers (29), murine melanoma (30), non–small cell lung cancer (31), and head and neck cancer (32). These similarities between metastasis and inflammation have suggested that further study of the

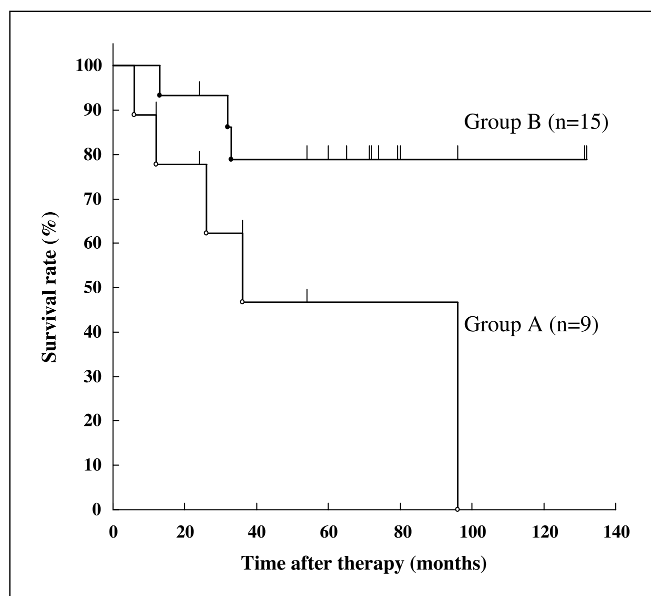

Figure 6. High *LAMP3* expression was significantly correlated with poor prognosis in patients with stage I or II cervical cancer. To clarify the significance of *LAMP3* expression in prognosis, 24 patients with stage I or stage II uterine cervical cancers were divided into two groups, group A and group B, by degrees of expression of *LAMP3* mRNA. Group A includes nine cases with a ΔC_t of >7, indicating higher expression levels of *LAMP3* mRNA. Group B includes 15 cases with a ΔC_t of <7, indicating relatively low levels of *LAMP3* mRNA. A correlation between expression of *LAMP3* and overall survival was shown by Kaplan-Meier analysis. The prognosis of group A was significantly worse than that of group B by the log-rank test ($P < 0.05$).

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crosstalk between inflammation and the metastatic process may be worthy of consideration.

Migration of cancer cells into lymph vessels is of keen interest, but its mechanism is largely unknown. On the other hand, the mechanisms of cellular traffic of peripheral dendritic cell in lymphatics have been studied extensively, as reviewed by von Andrian et al. (33). After exposure to inflammatory stimuli, peripheral dendritic cell undergoes maturation that allows them to enter lymph vessels and to access the T-cell area in draining lymph nodes. In this process, CCR7 participates in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes (34), during which the dendritic cell marker LAMP3 (DC-LAMP) is up-regulated transiently (2). Thus, LAMP3 is transiently expressed in dendritic cell upon maturation and may be a key molecule for their migration from the periphery into lymph vessels, although such a definitive function for LAMP3 has not yet been proven. Taken together, by mimicking dendritic cell, tumor cells expressing LAMP3 may migrate into surrounding afferent lymph vessels and reach the draining lymph nodes.

Additionally, *LAMP3* is on chromosome 3q, where many previous studies have implicated the existence of an oncogene(s). Therefore, we propose that *LAMP3*, which promotes lymph node metastasis, should be given serious consideration as being one of the candidates for this implied oncogene.

In a support of our idea, using a comparative genomic hybridization technique, Allen et al. (35) have reported that a high occurrence of a 3q gain was more frequent at the primary site of uterine cervical cancers with lymph nodes metastases than in those without metastases (64% versus 28%), although not statistically significant. In their 37 cases of stage Ib uterine cervical cancers, the patient mortality was linked to a higher presence of 3q amplification compared with survivors (80% versus 37%), indicating the importance of 3q amplification in lymph node metastasis and prognosis.

Our data give additional new evidence for similarities between cancer metastasis and inflammation at the level of LAMP3 expression. Because this similarity is common in human cancers, overexpression of LAMP3 may also occur in other types of cancer as well.

In conclusion, our data suggest that *LAMP3* promotes metastasis and can thus be a prognostic factor and serve as a novel target antigen for the therapy of patients with cervical cancers that are refractory to standard treatment modalities.

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