Down-Regulation of *LATS1* and *LATS2* mRNA Expression by Promoter Hypermethylation and Its Association with Biologically Aggressive Phenotype in Human Breast Cancers

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ABSTRACT

Purpose: **LATS1 and LATS2 are tumor suppressor genes implicated in the regulation of cell cycle. Methylation status of the promoter regions of these genes as well as its correlation with their mRNA levels were studied in human breast cancers. Correlation of *LATS1* and *LATS2* mRNA levels with clinicopathologic characteristics of breast tumors were also studied.**

Experimental Design: **Methylation status of promoter regions of *LATS1* and *LATS2* was studied by a methylation-specific PCR and mRNA expression levels of *LATS1* and *LATS2* were determined by a real-time PCR assay in 30 breast cancers. In addition, correlation of *LATS1* and *LATS2* mRNA levels with clinicopathologic characteristics was studied in 117 breast cancers.**

Results: **Methylation-specific PCR showed that of 30 tumors, *LATS1* promoter region was hypermethylated in 17 tumors (56.7%) and *LATS2* promoter region was hypermethylated in 15 (50.0%) tumors. *LATS1* mRNA levels in breast tumors with hypermethylation (2.15 ± 0.37, mean ± SE) were significantly ($P < 0.01$) lower than those without hypermethylation (6.09 ± 1.38), and *LATS2* mRNA levels in breast tumors with hypermethylation (1.42 ± 0.66) were also significantly ($P < 0.01$) lower than those without hypermethylation (3.10 ± 1.00). The decreased expression of *LATS1* or *LATS2* mRNA was significantly associated with a large tumor size, high lymph node metastasis, and estrogen receptor and progesterone receptor negativity. Furthermore, the decreased expression of *LATS1* mRNA, but not *LATS2* mRNA, was significantly ($P < 0.05$) associated with a poor prognosis.**

Conclusions: **Hypermethylation of the promoter regions of *LATS1* and *LATS2* likely plays an important role in the down-regulation of their mRNA levels in breast cancers, and breast cancers with a decreased expression of *LATS1* or *LATS2* mRNA levels have a biologically aggressive phenotype.**

INTRODUCTION

The *lats* gene, which encodes a putative serine/threonine kinase, has been identified as a tumor suppressor gene in *Drosophila* (1, 2). Deterioration of the *lats* gene function results in promotion of cell proliferation and tumor formation in *Drosophila* (2). Two mammalian homologues of the *Drosophila lats*, *LATS1* and *LATS2*, have been identified thus far. Because *LATS1*-deficient mice develop soft tissue sarcomas or ovarian stromal cell tumors, *LATS1* has been considered as a tumor suppressor gene (3). The human *LATS1* gene has been localized to chromosome 6q24-25 (4). A frequent loss of heterozygosity (LOH) at this locus has been reported in ovarian (5, 6), cervical (7) and breast cancers (8–10). Overexpression of *LATS1* causes G_2-M arrest through the inhibition of CDC2 kinase activity in breast cancer cell line *in vitro* (11). Furthermore, overexpression of *LATS1* significantly suppresses the tumorigenicity *in vivo* by inducing apoptosis (11, 12). *LATS2* (also known as KPM) was isolated as a second mammalian homologue of the *lats* tumor suppressor family (13, 14). The human *LATS2* gene was mapped to chromosome 13q11-12 (13). A frequent LOH of this locus has also been reported in various cancers including breast, ovary, and liver (15–17). Overexpression of *LATS2* causes G_1-S arrest through the inhibition of cyclin E/CDK2 *in vitro* as well as suppresses the tumorigenicity of NIH/v-*ras*–transformed cells *in vivo*, suggesting that *LATS2*, like *LATS1*, is a tumor suppressor gene (18).

Inactivation of a typical tumor suppressor gene is induced by mutation of one allele and LOH of the other allele, resulting in the complete loss of the gene function. With respect to *LATS1* and *LATS2*, LOH is frequently observed, as mentioned above, in various human tumors, but no somatic mutation of *LATS1* was reported in 25 breast cancers (19) and only one mutation was reported in 60 esophageal tumors in the *LATS2* gene (20). These results seem to indicate that loss of function of *LATS1* and *LATS2* is unlikely to be induced by the combination of somatic mutation and LOH but is more likely to be induced by other mechanisms such as hypermethylation, which has been shown to play an important role in the inactivation of several other tumor suppressor genes (21–23). This speculation seems to be supported by the recent report that showed the hypermethylation of the promoter region of the *LATS1* gene in six of seven soft tissue sarcomas with a decreased expression of *LATS1* mRNA (24). On the other hand, hypermethylation of the promoter region of the *LATS2* gene has never been studied yet in human tumors.

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Because all the data thus far obtained on *LATS1* and *LATS2* strongly indicate that both genes serve as a tumor suppressor gene and hypermethylation might play a significant role in the inactivation of these genes in various human tumors, we have analyzed, in the present study, the methylation status of these genes as well as its correlation with their mRNA levels in human breast cancers. In addition, correlation of *LATS1* and *LATS2* mRNA levels with clinicopathologic characteristics of breast tumors has also been investigated.

MATERIALS AND METHODS

Tumor Specimens and Patients. Tumor specimens were obtained at surgery from 117 female patients with breast cancer who underwent mastectomy or breast-conserving surgery at Osaka University Hospital from February 1998 to August 2001. Adjacent, normal breast tissues, which were histologically confirmed as cancer free, were also obtained from 6 patients. Histological diagnosis of breast cancers was obtained in all patients (99 invasive ductal carcinomas, 2 invasive lobular carcinomas, 1 noninvasive ductal carcinoma, and 15 others). The mean age of the patients was 53.1 years (range, 30-83 years). The specimens were snap-frozen in liquid nitrogen and kept at −80°C. Informed consent was obtained from each patient. Patients had a physical examination every 3 months for 2 years postoperatively, then every 6 months thereafter. Blood test and chest X-ray were obtained every 6 months postoperatively. Seven patients received no adjuvant therapy. Tamoxifen (20 mg/d) was given to 65 patients, goserelin to 3 patients, and both to 6 patients. Six cycles of CMF [cyclophosphamide (100 mg/d p.o., days 1-14) + methotrexate (40 mg/m^2 i.v., days 1 and 8) + 5-fluorouracil (600 mg/ m^2 i.v., days 1 and 8)] were given to 16 patients, four cycles of CE [cyclophosphamide 600 mg/ m^2 i.v. (day 1) + epirubicin 60 mg/ m^2 i.v. (day 1)] to 27 patients, four cycles of docetaxel (600 mg/ m^2 i.v., day 1) to 18 patients, and other chemotherapies to 3 patients. Twenty-eight patients were treated with hormonal therapy plus chemotherapy. Indication for adjuvant treatment was decided essentially according to St. Gallen recommendations (25, 26). The median follow-up period was 40 months, ranging from 19 to 52 months. Seventeen patients developed recurrences: 5 developed soft tissue metastases, 5 developed bone metastases, 5 developed lung metastases, 3 developed liver metastases, and 2 developed brain metastases. Ipsilateral breast recurrences after breast-conserving surgery were not counted as recurrences.

RNA Extraction and Reverse Transcription. Total RNA was extracted from the frozen tumor specimens using TRIZOL reagent according to the protocol provided by the manufacturer (Molecular Research Center, Cincinnati, OH). Three micrograms of total RNA were reverse-transcribed for single strand cDNA, using oligo$(dT)_{15}$ primer and Superscript II (Life Technologies, Inc., Gaithersburg, MD). Reverse transcription reaction was done at 42°C for 90 minutes followed by heating at 70°C for 10 minutes.

Real-time PCR Assay of *LATS1* and *LATS2* mRNA Levels. Real-time PCR reactions of *LATS1* and *LATS2* were carried out using the ABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA). The sequence of the probes for *LATS1* and *LATS2* were 5′-TACTATCAGAGTGGTGACCATCC-3′ and 5′-TGCTCC-TCCGCAAAGGGTACACTCA-3′, respectively. Both probes were labeled by 6-carboxyfluorescein as a reporter. The amplification primer pairs were 5′-TGGTCATATTAAATT-GACTGAC-3′ and 5′-CCACATCGACAGCTTGAGGG-3′ for *LATS1*, and 5′-TAGAGCAGAGGGCGCGGAAG-3′ and 5′-CCAACACTCCACCAGTCACAGA-3′ for *LATS2*. PCR conditions for *LATS1* and *LATS2* were as follows: after incubation at 50°C for 2 minutes and denaturing at 95°C for 10 minutes, 45 cycles of 95°C for 15 seconds, 58°C for 30 seconds, and 67°C for 30 seconds. To normalize transcript content in each sample, we used the *β-glucoronidase* transcripts as the quantitative control. The primer and probe mixture for *β-glucoronidase* was purchased from Perkin-Elmer Applied Biosystems and used according to the manufacturer's protocol. The standard curves for *LATS1*, *LATS2*, and *β-glucoronidase* mRNA were generated using serially diluted solutions of plasmid clones inserted with *LATS1*, *LATS2*, or *β-glucoronidase* cDNA as templates, and the amount of target gene expression was calculated from these standard curves. Finally, mRNA expression levels of *LATS1* and *LATS2* were shown as ratios to those of *β-glucoronidase*. Real-time PCR assays were conducted in duplicate for each sample, and the mean value was used for calculation of the relative expression levels.

Bisulfite Modification and Direct Sequencing. Genomic DNA extracted from tumor tissues and adjacent normal breast tissues was treated by CpGenome DNA modification kit (Serologicals Corporation, Norcross, GA), according to the manufacturer's protocol. Briefly, 1 g of genomic DNA was denatured using 0.2 mol/L NaOH and subsequently incubated with a sodium salt of bisulfite ion (HSO_4^-) at 50C for 16 hours. Bisulfite-modified DNA was amplified by two sets of primers to amplify the regions A and B including three putative CpG islands of *LATS2* promoter region (Fig. 1). The amplification primers were 5′-TTTTGAGATGGAGTTTTGTT-3′ and 5′-AATTCAAAACCAACCTAACC-3′ for region A, and 5′-TGGTTGTGGAGGAGTAGGG-3′ and 5′-CTAAAACTAC-TACTAACCCC-3′ for region B. PCR conditions were as follows: after initial denaturation at 94C for 1 minute, 35 cycles of 94C for 15 seconds, 54C for 30 seconds, and 68C for 30 seconds. Methylated cytosine residues were identified by direct sequencing using ABI PRISM 310 sequencer (Perkin-Elmer Applied Biosystems).

Methylation-Specific PCR. The methylation status in the CpG islands of *LATS1* and *LATS2* promoter regions was determined by methylation-specific PCR (27). The methylation site in the promoter region of *LATS1* was previously reported (24). Bisulfite-modified DNA was amplified with primers specific for methylated or unmethylated sequences. The methylated DNA of *LATS1* was amplified using M set primers, 5′-GGAGT-TTCGTTTTGTC-3′ and 5′-CGACGTAATAACGAACGCCTA-3′, and the unmethylated DNA of *LATS1* was amplified using U set primers, 5′-TAGGTTGGAGTGTGGTGGT-3′ and 5′-CCCAACATAATAACAAACACCT-3′. The methylated DNA of *LATS2* was amplified using M set primers, 5′-ATTT-CGGTTTATTGTAATTTTC-3′ and 5′-AACCAACATAA-TAAAACCCCG-3′, and the unmethylated DNA of *LATS2* was amplified using U set primers, 5′-TTTGTTTTTTG-GGTTTAAGT-3′ and 5′-CCAACATAATAAAACCCCA-3′.

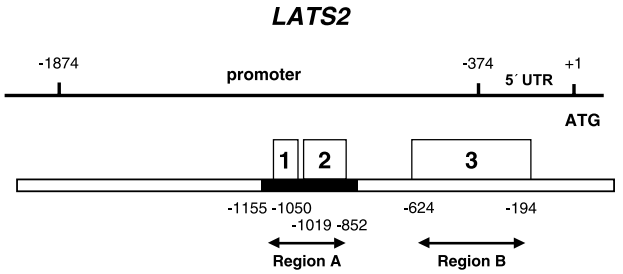

Fig. 1 Schematic representation of *LATS2* 5′ untranslated region and promoter region. Three open boxes (*1*, *2*, and *3*) correspond to the putative CpG islands. Arrows, regions (*Region A* and *Region B*) that were analyzed in their methylation status as described in MATERIALS AND METHODS. Closed box shows the highly homologous (81.4%) region between *LATS1* and *LATS2*.

Methylation-specific PCR reaction of *LATS1* was as follows: after initial denaturation at 94°C for 1 minute, 23 cycles (methylated) or 25 cycles (unmethylated) of amplification at 94°C for 15 seconds, 58°C (methylated) or 53°C (unmethylated) for 15 seconds, and 68°C for 30 seconds. Methylation-specific PCR reaction of *LATS2* was as follows: after initial denaturation at 94°C for 1 minute, 30 cycles of 94°C for 15 seconds, 58°C (methylated) or 50°C (unmethylated) for 15 seconds, and 68°C for 30 seconds.

Estrogen Receptor and Progesterone Receptor Assay. Enzyme immunoassay was conducted for the measurement of estrogen receptor and progesterone receptor protein levels in breast cancers using the kits provided by Abbott Research Laboratories (Chicago, IL) according to the manufacture's instructions. The cutoff value for estrogen receptor and progesterone receptor was 5 fmol/mg protein.

Statistical Methods. *LATS1* and *LATS2* mRNA expression levels between various groups were evaluated using Mann-Whitney test. The comparison of *LATS1* or *LATS2* mRNA levels among various tumor sizes or histologic grades was calculated using Kruskal-Wallis test. Relapse-free survival curves were calculated by the Kaplan-Meier method and the log rank test was used to evaluate the difference in relapse-free survival between the *LATS1* mRNA high and low groups and between the *LATS2* mRNA high and low groups. Multivariate analysis (Cox proportional hazards model) was conducted to estimate the independence of each prognostic factor. Statistical significance was assumed for $P < 0.05$.

RESULTS

Methylation Analysis of *LATS1* and *LATS2*. *LATS2* promoter region obtained from the University of California Santa Cruz genome browser contained three putative CpG islands that fulfilled the criteria of observed/expected CpG ratio >0.60 and percent C + percent G >50.00 using the CpG plot program (http://www.ebi.ac.uk/Tools). Then, in order to identify methylation sites, PCR reaction was done using bisulfite-modified DNA by two sets of primers that were designed to amplify the regions A and B including the three putative CpG islands of *LATS2* promoter region (Fig. 1). Methylated cytosine residues were identified in region A but not in region B, and the methylation sites in the *LATS2* corresponded to the previously reported methylation sites of *LATS1*. Thus, in the following study on the methylation status of the promoter region of *LATS2*, only region A was analyzed.

Methylation status of *LATS1* and *LATS2* were analyzed in 30 breast tumors and 6 adjacent normal breast tissues by methylation-specific PCR. Methylation-specific PCR showed that 17 (56.7%) of 30 breast tumors were hypermethylated in the *LATS1* promoter region and that 15 (50.0%) of 30 breast tumors were hypermethylated in the *LATS2* promoter region (Fig. 2). On the other hand, hypermethylation of *LATS1* and *LATS2* promoter regions was not observed in any of 6 adjacent normal breast tissues.

Relationship of Methylation Status of *LATS1* and *LATS2* with Their mRNA Levels. *LATS1* and *LATS2* mRNA levels were assayed by real-time PCR and compared between tumors with and without hypermethylation (Fig. 3). *LATS1* mRNA levels in breast tumors with hypermethylation (2.15 ± 0.37, mean ± SE) were significantly ($P < 0.01$) lower than those without hypermethylation (6.09 ± 1.38), and *LATS2* mRNA levels in breast tumors with hypermethylation (1.42 ± 0.66) were also significantly ($P < 0.01$) lower than those without hypermethylation (3.10 ± 1.00).

Correlation of *LATS1* and *LATS2* mRNA Levels with Various Clinicopathologic Parameters. *LATS1* and *LATS2* mRNA levels determined by a real-time PCR assay in 117 breast tumors are shown according to the various clinicopathologic parameters in Table 1. The decreased expression of *LATS1* and *LATS2* mRNA was significantly associated with a large tumor size ($P < 0.05$ for *LATS1* and $P < 0.01$ for *LATS2*). *LATS1* and *LATS2* mRNA levels were significantly ($P < 0.05$ for *LATS1* and $P < 0.01$ for *LATS2*) lower in tumors with lymph node metastasis than in tumors without lymph node metastasis and were also significantly ($P < 0.01$) lower in estrogen receptor- and progesterone receptor–negative tumors than estrogen receptor- and progesterone receptor–positive tumors, respectively. Other parameters such as menopausal status and histologic grade did not show a significant correlation with the *LATS1* and *LATS2* mRNA levels.

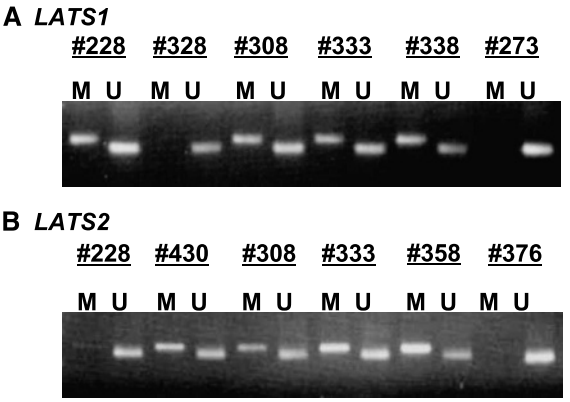

Fig. 2 Representative results of methylation-specific PCR analysis of *LATS1* (*A*) and *LATS2* (*B*) in six breast tumors. *M,* methylated; *U,* unmethylated.

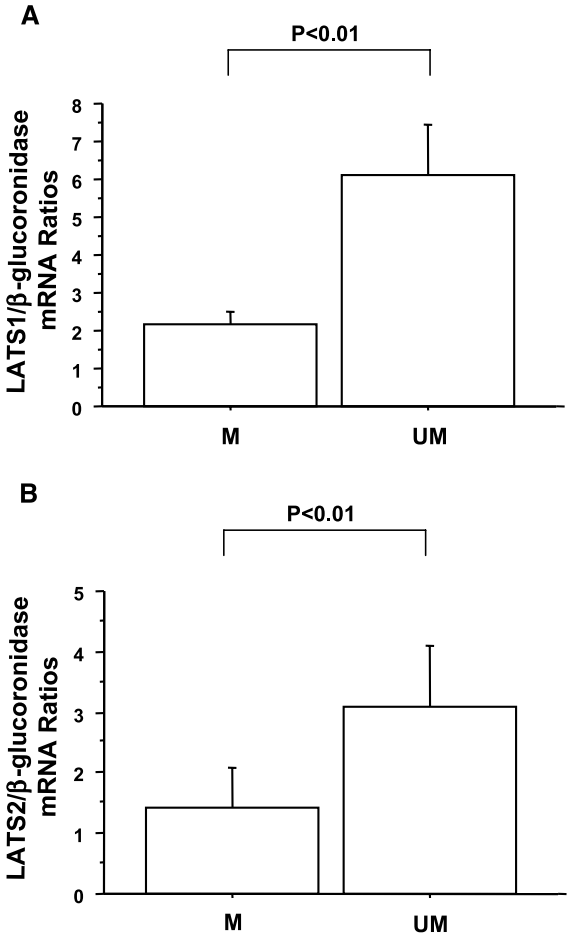

Fig. 3 Columns, mean value of *LATS1* (*A*) and *LATS2* (*B*) mRNA levels between tumors with hypermethylation (*M*) and without hypermethylation (*UM*). Bars, SE.

Patients were dichotomized into high and low *LATS1* and *LATS2* mRNA level groups using a median value of *LATS1* and *LATS2* mRNA levels, respectively, as a cutoff value. Patients with low *LATS1* mRNA levels showed a significantly poorer relapse-free survival than those with high *LATS1* mRNA levels (Fig. 4). Multivariate analysis has shown that the *LATS1* mRNA levels are a significant ($P < 0.05$) prognostic factor, being independent of lymph node metastases (Table 2). On the other hand, relapse-free survival was not significantly different between patients with the high and low *LATS2* mRNA levels (Fig. 4).

DISCUSSION

Classic tumor suppressor genes are supposed to be inactivated by a combination of LOH of one allele and somatic mutation of the other allele. Although a relatively high frequency of LOH at the locus containing *LATS1* or *LATS2* has been reported in breast cancers (10, 15), somatic mutation of *LATS1* has been shown to be not detected in breast cancers (19) and, in addition, we have found no somatic mutation of *LATS2* in 20 breast cancers (data not shown). These results show that these two tumor suppressor genes are unlikely to be inactivated by such a classic manner as a combination of LOH and somatic mutation. Another possible mechanism of inactivation of a tumor suppressor gene is hypermethylation of the promoter region, as has already been shown in several other tumor suppressor genes (21–23). In breast cancers, aberrant methylation in *p16* and *BRCA1* genes have been shown to be possibly involved in the pathogenesis of tumor progression (28–31). Thus, we have studied the methylation status of promoter regions of both *LATS1* and *LATS2* in the present study and have found that the promoter regions of *LATS1* and *LATS2* were hypermethylated in as high as 56.7% and 50.0% of breast tumors, respectively. More importantly, tumors with hypermethylated *LATS1* and *LATS2* showed a significantly lower expression of *LATS1* and *LATS2* mRNA, respectively, suggesting that hypermethylation of the promoter regions down-regulates the transcription of these genes. Our observation is consistent with the report on soft tissue sarcomas, which showed that hypermethylation of *LATS1* was associated with a decreased expression of *LATS1* mRNA (24). Taken together with the fact that hypermethylation of *LATS1* and *LATS2* was not seen in any of adjacent normal breast tissues, it is indicated that inactivation of *LATS1* and *LATS2* induced by hypermethylation may be involved in the pathogenesis of breast cancer.

Because *LATS1* and *LTAS2* play an important role in the regulation of cell cycle, tumors with a decreased expression of these genes are speculated to have a high proliferation rate and, thus, to show a biologically aggressive phenotype. In fact, we have found that a down-regulation of *LATS1* and *LATS2* mRNA levels are associated with biologically aggressive phenotypes of breast tumors such as large tumor size, high frequency of lymph node metastases, and estrogen receptor and progesterone receptor negativity. In addition,

Table 1 Relationship between *LATS1* or *LATS2* mRNA expression levels and clinicopathologic parameters

	No. of tumors	mRNA levels (mean ± SE)	
		LATS1	*LATS2*
Menopausal status			
Premenopausal	56	7.44 ± 1.17	4.24 ± 0.60
Postmenopausal	61	5.34 ± 0.61	3.35 ± 0.43
Tumor size*			
≤2 cm	33	8.68 ± 1.66†	5.70 ± 0.82‡
>2, ≤5cm	60	6.43 ± 0.79	3.56 ± 0.43
>5cm	23	2.85 ± 0.46	1.59 ± 0.67
Histological grade*			
I	22	5.33 ± 1.07	3.04 ± 0.61
II	67	6.49 ± 0.96	4.29 ± 0.58
III	27	6.88 ± 1.24	3.10 ± 0.45
Lymph node metastasis			
Negative	59	7.55 ± 0.89†	4.41 ± 0.47‡
Positive	58	5.11 ± 0.92	3.13 ± 0.56
Estrogen receptor			
Positive	71	7.89 ± 0.98‡	4.53 ± 0.53‡
Negative	46	3.95 ± 0.51	2.60 ± 0.40
Progesterone receptor			
Positive	61	7.98 ± 0.99‡	4.61 ± 0.55‡
Negative	56	4.56 ± 0.76	2.87 ± 0.45

*Excludes one noninvasive ductal carcinoma.
†$P < 0.05$.
‡$P < 0.01$.

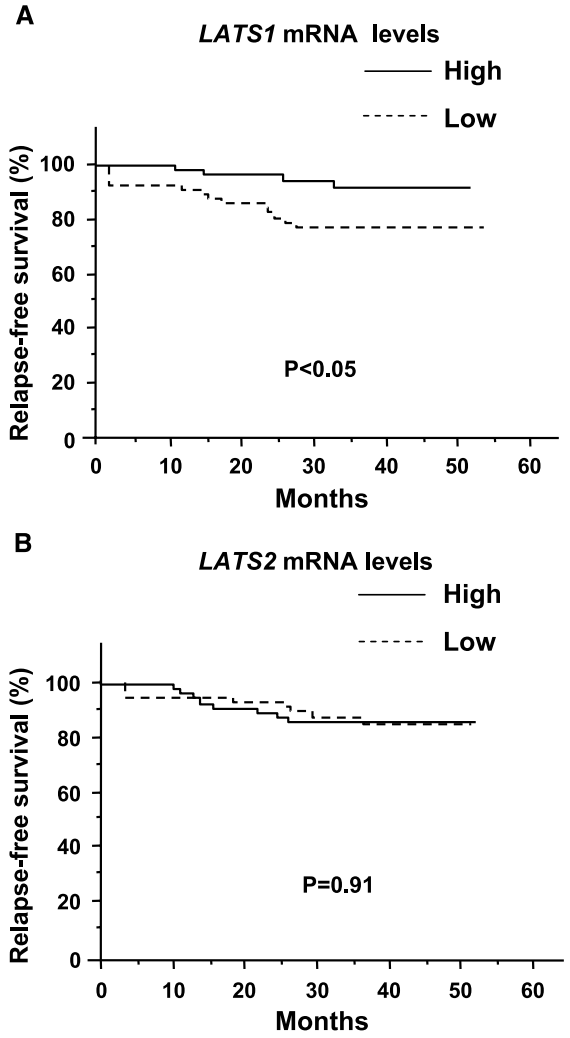

Fig. 4 Relapse-free survival of patients according to the expression levels of *LATS1* mRNA (*A*) and *LATS2* mRNA (*B*). One patient with noninvasive ductal cancer was omitted from this analysis.

tumors with a decreased expression of *LATS1* mRNA were significantly associated with a poor prognosis, and multivariate analysis has shown that a decreased *LATS1* mRNA expression is a significant prognostic factor, being independent of the other classic prognostic factors such as lymph node status. These results suggest a possibility that *LATS1* mRNA levels can be clinically useful for the prediction of patient prognosis.

The reason why *LATS2* mRNA levels were not associated with patient prognosis, although they were significantly associated with biologically aggressive phenotypes of breast tumors, is unclear. Because most of the patients recruited in the present study were treated with adjuvant hormonal therapy and/or chemotherapy, it is possible that difference in sensitivity to the adjuvant therapy between tumors with the high and the low *LATS2* mRNA levels might have masked the difference in prognosis. Ideally, the prognostic significance of *LATS1* and *LTAS2* mRNA levels needs to be tested in patients without adjuvant therapy. However, because most patients with breast cancer are recently treated with adjuvant therapy as the standard of care, it is practically almost infeasible to evaluate the real prognostic significance. Therefore, although we have shown a significant association of *LATS1* mRNA levels and prognosis in the present study, it is hard to conclude that *LAST1* mRNA levels can serve as a true prognostic factor. *LATS1* mRNA might serve as a predictive factor of response to adjuvant therapy or as a both prognostic and predictive factor. It seems to be of interest to study the relationship of *LATS1* and *LATS2* mRNA levels with sensitivity to various hormonal therapies and chemotherapies in future. Our present study is also vulnerable to the criticism that the *LATS1* and *LATS2* mRNA levels in tumors as well as the frequency of tumors with hypermethylation of *LATS1* or *LATS2* promoter region are affected by contamination with nontumor cells. The *LATS1* and *LATS2* mRNA levels in tumors might be overestimated and the frequency of tumors with hypermethylation of *LATS1* or *LATS2* promoter region might be underestimated by contamination with nontumor cells that express the *LATS1* and *LATS2* mRNA levels and lack the hypermethylation of *LATS1* or *LATS2* promoter region. To circumvent this contamination problem, we are planning to conduct a study wherein tumor cells are selectively collected using laser microdissection.

In conclusion, we have shown that hypermethylation of the promoter regions of *LATS1* and *LATS2* likely plays an important role in the down-regulation of their mRNA expression levels in breast cancers, and breast cancers with a decreased expression of *LATS1* or *LATS2* mRNA levels are significantly associated with a biologically aggressive phenotype. In addition, we have

Table 2 Univariate and multivariate analysis of various prognostic factors

	Univariate		Multivariate*	
	HR† (95% CI)	*P*	HR† (95% CI)	*P*
Tumor size	3.17 (0.72-13.89)	0.13	—	
Lymph node status	3.53 (1.15-10.87)	<0.05	4.00 (1.30-12.35)	<0.05
Histological grade	1.93 (0.70-2.70)	0.21	—	
ER status	0.83 (0.31-2.24)	0.71	—	
PR status	0.69 (0.24-1.96)	0.49	—	
LATS1 mRNA level	3.50 (1.14-10.74)	<0.05	4.04 (1.31-12.44)	<0.05
LATS2 mRNA level	0.95 (0.37-2.46)	0.91	—	

Abbreviations: HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor.

*Multivariate analysis of lymph node status and *LATS1* mRNA levels.

†Hazard ratio of large tumor size (>2.0 cm) against small tumor size (≤2.0 cm), lymph node positive against lymph node negative, histologic grade III against grade I + II, ER-positive against ER-negative, PR-positive against PR-negative, *LATS1* mRNA low against high levels, and *LATS2* mRNA low against high levels.

showed a possibility that a decreased *LATS1* mRNA expression might serve as a significant prognostic factor being independent of the other classic prognostic factors. Because the follow-up period in the present study is relatively short, the prognostic significance of *LATS1* and *LATS2* mRNA levels needs to be further investigated by additional studies including a larger number of patients with a longer follow-up period.

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