Original Article
The expressions of autotaxin-lysophosphatidate signaling-related proteins in metastatic breast cancer

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Abstract: Purpose: We evaluated the expression of autotaxin-lysophosphatidate signaling-related proteins and the clinical implications for metastatic breast cancer. Methods: We constructed tissue microarrays (TMA) with 126 cases of metastatic breast cancer [31 (24.6%) bone metastases, 36 (28.6%) brain metastases, 11 (8.7%) liver metastases, and 48 (38.1%) lung metastasis], and we conducted immunohistochemical staining for the autotoxin-lysophosphatidate signaling-related proteins ATX, LPA1, LPA2, and LPA3. Results: Stromal ATX (P = 0.006) and LPA1 (P < 0.001) were differently expressed according to their metastatic organ; stromal ATX showed high expression in bone metastasis, and LPA1 showed high expression in liver and lung metastases. Stromal ATX positivity was higher than others in luminal A type tumors (P = 0.035), and stromal LPA3 positivity was correlated with a high Ki-67 labeling index (LI) (P = 0.005). In univariate analysis, tumoral LPA3 negativity was correlated with shorter overall survival (OS) (P = 0.015) in metastatic breast cancer. When analyzed according to the metastatic sites, tumoral LPA3 negativity was correlated with shorter OS (P = 0.010) in lung metastasis, whereas stromal LPA3 negativity was correlated with shorter OS (P = 0.026) in brain metastasis. In multivariate Cox analysis, tumoral LPA3 negativity was an independent poor prognostic factor (HR = 2.311, 95% CI: 1.029-5.191, P = 0.043). Conclusion: Among autotoxin-lysophosphatidate signaling-related proteins, stromal ATX was highly expressed in bone metastases, and LPA1 was highly expressed in liver and lung metastases. Tumoral LPA3 might be a prognostic factor in metastatic breast cancer.

Keywords: Autotaxin, breast cancer, lysophosphatidate receptor, metastasis

Introduction

Autotaxin (ATX) is a glycoprotein transcribed by the ENPP2 gene on chromosome 8 [1]. ATX is the same molecule as lysophospholipase D, so it converts lysophosphatidylcholine (LPC) to the bioactive lipid mediator lysophosphatidate (LPA). LPA, after binding to the appropriate receptor, activates phospholipase C, the MAPK pathway, the PI3K pathway, and the PhoA pathway, and it is involved in various cellular processes [2, 3]. The LPA receptor is a G-protein coupled receptor. There are at least 6 LPA receptors, LPA1-LPA6. LPA1-LPA3 belong to the EGD family (LPA1-EDG2, LPA2-EDG4, LPA3-EDG7), and LPA4-LPA6 are similar to the P2Y nucleotide receptor [4]. ATX-LPA signaling is involved in tumor formation, progression, and metastasis [5, 6]. The expression of ATX and LPA 1-3 has been reported to be especially high in breast cancer, and ATX, LPA, and LAP receptors in breast cancer are involved in angiogenesis, tumor cell invasion, and migration [7].

Breast cancer has both high morbidity and mortality, which is mainly attributed to distant metastasis. The main metastatic sites of breast cancer are the lung, brain, liver, and bones [8, 9]; however, most current studies have involved brain and bone metastases [10-15]. The general pathogenesis of tumor metastasis involves a reciprocal interaction between tumor cells and host tissue, consisting of adhesion, proteolysis, invasion, and angiogenesis [9, 16]. As has been reported previously, metastatic breast cancer shows specific tumor characteristics according to the metastatic site, young age, ER negativity, prior lung metastasis, HER-2 overexpression, EGFR overexpression, and basal subtype [12-14]. Lower histologic grade, ER

Table 1. Source, clone, and dilution of antibodies

Antibody	Company	Clone	Dilution
Autotaxin-related proteins			
ATX	Abcam, Cambridge, UK	Polyclonal	1:1000
LPA_1	Abcam, Cambridge, UK	EPR9710	1:100
LPA_2	Abcam, Cambridge, UK	Polyclonal	1:100
LPA_3	Abcam, Cambridge, UK	Polyclonal	1:250
Molecular subtype-related proteins			
Estrogen receptor	Thermo Scientific, San Diego, CA, USA	SP1	1:100
Progesterone receptor	DAKO, Glostrup, Denmark	PgR	1:50
Human epidermal growth factor receptor-2	DAKO, Glostrup, Denmark	Polyclonal	1:1500
Ki-67	Abcam, Cambridge, UK	SP6	1:100

Autotaxin (ATX), also known as ectonucleotide pyrophosphatase/phosphhodesterase 2 (ENPP2), LPA_1-EDG2, LPA_2-EDG4, and LPA_3-EDG7.

positivity, ER positivity/PR negativity, strand growth pattern, and the presence of fibrotic foci in invasive ductal carcinoma are associated with bone metastasis [11, 17, 18]. Consequently, it is easily presumed that metastatic breast cancer can have different tumor characteristics according to the metastatic site. ATX-LPA signaling in breast cancer has been reported to be involved in metastasis, and the expression of ATX and LPAR in breast cancer is especially associated with advanced metastatic disease [19]. The overexpression of ATX or LPA_{1-3} in transgenic mice results in lung metastasis [20]. However, studies on autotaxin-lysophosphatidate signaling-related proteins in metastatic breast cancer according to the metastatic sites have not yet been reported, so we aimed to assess the expression of autotaxin-lysophosphatidate signaling-related proteins and related clinical implications in metastatic breast cancer according to the metastatic site.

Materials and methods

Patient selection and histologic evaluation

Cases of metastatic breast cancer to the liver, lung, brain, and bone were selected from the data files of the Department of Pathology, Severance Hospital. Only patients who were diagnosed with invasive ductal carcinoma were included. This study was approved by the Institutional Review Board (IRB) of Severance Hospital. A total of 126 cases were retrieved and all the slides that were prepared per case were reviewed. Pathologic diagnoses were confirmed by 2 pathologists (JSK and WJ), and the histological grade was assessed using the Nottingham grading system [21].

Tissue microarray (TMA)

A representative area showing tumor and tumor stroma was selected on an H&E-stained slide, and a corresponding spot was marked on the surface of the paraffin block. Using a biopsy needle, the selected area was punched out, and a 3-mm tissue core was transferred to a 6 x 5 recipient block. Two tissue cores from each invasive tumor were extracted to minimize extraction bias. Each tissue core was assigned a unique tissue microarray location number that was linked to a database containing other clinicopathologic data.

Immunohistochemistry

The antibodies used for immunohistochemistry are shown in **Table 1**. Formalin-fixed, paraffin-embedded tissue sections were used for immunohistochemistry, and 3-μm-thick tissue sections were deparaffinized and rehydrated in xylene and graded alcohol. We used a Ventana Discovery XT automated stainer (Ventana Medical System, Tucson, AZ, USA). CC1 buffer (Cell Conditioning 1; citrate buffer Ph 6.0, Ventana Medical System) was used for antigen retrieval. Appropriate positive and negative controls were used for each antibody. The positive controls were used according to the manufacturer's instructions (ATX; human tonsil tissue, LPA1; human thyroid tissue, LPA2; Breast cancer tissue, LPA3; Human prostate carcinoma). A negative control was used with a sec-

ondary antibody alone and the primary antibody omitted.

Interpretation of immunohistochemical staining

All immunohistochemical markers were assessed by light microscopy. A cut-off value of 1% or more positively stained nuclei was used to define ER and PR positivity [22]. HER-2 staining was analyzed according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines using the following categories: 0 = no immunostaining; 1+ = weak incomplete membranous staining, less than 10% of tumor cells; 2+ = complete membranous staining, either uniform or weak in at least 10% of tumor cells; and 3+ = uniform intense membranous staining in at least 30% of tumor cells [23]. HER-2 immunostaining was considered positive when strong (3+) membranous staining was observed, but cases with 0 to 1+ were regarded as negative. Cases showing 2+ HER-2 expression were evaluated for HER-2 amplification by fluorescent *in situ* hybridization (FISH). The Ki-67 labeling index (LI) was defined as the percentage of tumor cells showing positive nuclear staining.

All stained slides were semi-quantitatively evaluated [24]. Tumor and stromal cell staining were assessed as 0: negative or weak immunostaining in < 1% of the tumor/stroma, 1: focal expression in 1-10% of tumor/stroma, 2: positive in 11-50% of tumor/stroma, and 3: positive in 51-100% of tumor/stroma. Entire tumor areas included in the TMA were evaluated in all cases, and a score of 0 was considered negative, while a score of 1 to 3 was considered as positive.

Western blotting

Deparaffinization and protein extraction of the FFPE tissue was carried out using a Qproteome FFPE Tissue Kit (Qiagen, 1042481) following the manufacturer's instructions. Extracted proteins were determined using a Bradford assay (BIO-RAD, 5000205). Quantified proteins were mixed with 5X SDS-PAGE loading buffer (Biosesang, S2002) at a concentration of 2 μg/μl and boiled at 95°C for 5 min. Equal quantities of protein were separated to SDS-PAGE gel and transferred to nitrocellulose membranes (Bio-Rad 1704158). Membranes were blocked by incubation in 5% skim milk in Tris-buffered

saline (TBS) with 0.1% Tween-20 and probed with antibody against ATX (1:1000, Abcam, ab140915), LPA1 (1:2000, Abcam, ab166903), and LPA2 (1:1000, Abcam ab38322) diluted in 1% BSA in TBS, 0.1% Tween 20, and 0.02% NaN$_3$. The membranes were washed and then incubated with secondary antibodies (HRP conjugated anti-mouse IgG, or anti-rabbit IgG) (1:20000, Santa Cruz) for 1 h at room temperature. The bands were visualized using WesternBright ECL (advansata, K-12045-D50) after washing the membrane and exposed to x-ray film.

Tumor phenotype classification

In this study, we classified breast cancer phenotypes according to the immunohistochemistry results for ER, PR, HER-2, Ki-67, and FISH results for HER-2 as follows [25]: *luminal A type*, ER or/and PR positive, HER-2 negative and Ki-67 LI < 14%; *Luminal B type*, (HER-2 negative) ER or/and PR positive, HER-2 negative and Ki-67 LI ≥ 14%; (HER-2 positive) ER or/and PR positive and HER-2 overexpressed or/and amplified; *HER-2 overexpression type*, ER and PR negative and HER-2 overexpressed or/and amplified; *TNBC type*: ER, PR, and HER-2 negative.

Statistical analysis

Data were analyzed using SPSS for Windows, Version 23.0 (SPSS Inc., Chicago, IL, USA). For the determination of statistical significance, Student's *t* and Fisher's exact tests were used for continuous and categorical variables, respectively. On multiple comparisons, a corrected *p*-value from Bonferroni multiple comparisons was used. Statistical significance was set to P < 0.05. Kaplan-Meier survival curves and log-rank statistics were employed to evaluate time to tumor recurrence and overall survival. Multivariate regression analysis was performed using the Cox proportional hazards model.

Results

Basal characteristics of metastatic breast cancer

A total of 126 cases of metastatic breast cancer were assessed and categorized as 31 (24.6%) bone metastases, 36 (28.6%) brain metastases, 11 (8.7%) liver metastases, and

Table 2. Basal characteristics of patients with metastatic breast cancer

Parameter	Total N = 126 (%)	Metastatic site				P-value
		Bone N = 31 (%)	Brain N = 36 (%)	Liver N = 11 (%)	Lung N = 48 (%)	
Age (years)						0.605
≤ 50	65 (51.6)	17 (54.8)	17 (47.2)	4 (36.4)	27 (56.3)	
> 50	61 (48.4)	14 (45.2)	19 (52.8)	7 (63.6)	21 (43.8)	
ER						< 0.001
Negative	59 (46.8)	6 (19.4)	25 (69.4)	2 (18.2)	26 (54.2)	
Positive	67 (53.2)	25 (80.6)	11 (30.6)	9 (81.8)	22 (45.8)	
PR						< 0.001
Negative	86 (68.3)	16 (51.6)	35 (97.2)	3 (27.3)	32 (66.7)	
Positive	40 (31.7)	15 (48.4)	1 (2.8)	8 (72.7)	16 (33.3)	
HER-2						0.032
Negative	86 (68.3)	25 (80.6)	18 (50.0)	9 (81.8)	34 (70.8)	
Positive	40 (31.7)	6 (19.4)	18 (50.0)	2 (18.2)	14 (29.2)	
Ki-67 LI						0.008
< 14	84 (66.7)	27 (87.1)	18 (50.0)	9 (81.8)	30 (62.5)	
≥ 14	42 (33.3)	4 (12.9)	18 (50.0)	2 (18.2)	18 (37.5)	
Molecular subtype						< 0.001
Luminal A	44 (34.9)	21 (67.7)	3 (8.3)	6 (54.5)	14 (29.2)	
Luminal B	24 (19.0)	5 (16.1)	8 (22.2)	3 (27.3)	8 (16.7)	
HER-2	25 (19.8)	3 (9.7)	12 (33.3)	1 (9.1)	9 (18.8)	
TNBC	33 (26.2)	2 (6.5)	13 (36.1)	1 (9.1)	17 (35.4)	
Patient death	41 (32.5)	16 (51.6)	11 (30.6)	4 (36.4)	10 (20.8)	0.041

ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2; LI, labeling index; TNBC, triple-negative breast cancer.

48 (38.1%) lung metastases. ER (P < 0.001), PR (P < 0.001), HER-2 (P = 0.032), Ki-67 LI (P = 0.008), and molecular subtype (P < 0.001) showed significantly different results according to the metastatic site. Brain metastases, for example, showed higher rates of ER negativity, PR negativity, and HER-2 positivity as well as higher Ki-67 LI when compared with tumors metastatic to other sites. Luminal A type tumors were more frequently observed in bone and liver metastases, HER-2 and TNBC in brain metastases, and TNBC in lung metastases (**Table 2**).

The expressions of autotaxin-lysophosphatidate signaling-related proteins in metastatic breast cancer according to the metastatic site

Results on the differential expressions of autotaxin-lysophosphatidate signaling-related proteins in metastatic breast cancer according to metastatic site showed that the expressions of stromal ATX (P = 0.006) and LPA1 (P < 0.001) were statistically different (**Table 3** and **Figure**

1). The expression rate of stromal ATX was the highest in bone metastases, whereas that of LPA1 in tumor cells was the highest in liver and lung metastases (**Figure 2**). The interpretation of positive and negative expressions was relatively clear-cut because none showed score 1 (1-10% positive) in ATX, LPA1, LPA2, or LPA3 staining. Also, none of the cases showed LPA1 or LPA2 expressions in the stromal cells. Those that stained positively on ATX and LPA3 staining in stromal compartment were identified as immune cells and fibroblasts. In Western blot, just as in IHC, the expression rate of stromal ATX was the highest in the bone metastases and the expression rate of LPA1 in tumor cells was the highest in the liver and lung metastases (**Figure 3**).
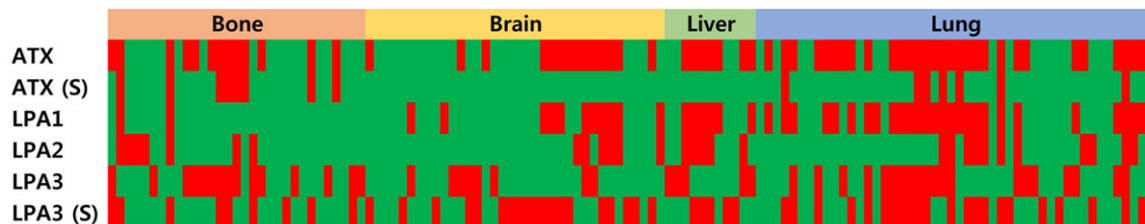
The correlation between clinicopathologic factors and the expression of autotaxin-lysophosphatidate signaling-related proteins

The correlation analysis on the expression of autotaxin-lysophosphatidate signaling-related

Table 3. Expression of autotaxin-lysophosphatidate signaling-related proteins according to the meta-static site in breast cancer metastases

Parameter	Total N = 126 (%)	Metastatic site				P-value
		Bone N = 31 (%)	Brain N = 36 (%)	Liver N = 11 (%)	Lung N = 48 (%)	
ATX (T)						0.061
Negative	61 (48.4)	18 (58.1)	22 (61.1)	4 (36.4)	17 (35.4)	
Positive	65 (51.6)	13 (41.9)	14 (38.9)	7 (63.6)	31 (64.6)	
ATX (S)						0.006
Negative	110 (87.3)	23 (74.2)	36 (100.0)	11 (100.0)	40 (83.3)	
Positive	16 (12.7)	8 (25.8)	0 (0.0)	0 (0.0)	8 (16.7)	
LPA$_1$						< 0.001
Negative	79 (62.7)	29 (93.5)	25 (69.4)	5 (45.5)	20 (41.7)	
Positive	47 (37.3)	2 (6.5)	11 (30.6)	6 (54.5)	28 (58.3)	
LPA$_2$						0.271
Negative	96 (76.2)	24 (77.4)	30 (83.3)	6 (54.5)	36 (75.0)	
Positive	30 (23.8)	7 (22.6)	6 (16.7)	5 (45.5)	12 (25.0)	
LPA$_3$ (T)						0.124
Negative	78 (61.9)	16 (51.6)	28 (77.8)	6 (54.5)	28 (58.3)	
Positive	48 (38.1)	15 (48.4)	8 (22.2)	5 (45.5)	20 (41.7)	
LPA$_3$ (S)						0.237
Negative	70 (55.6)	22 (71.0)	17 (47.2)	6 (54.5)	25 (52.1)	
Positive	56 (44.4)	9 (29.0)	19 (52.8)	5 (45.5)	23 (47.9)	

The *p*-value was calculated using Student's *t*-test.

Figure 1. Heat map of the expression of autotaxin-lysophosphatidate signaling-related proteins according to the metastatic site in metastatic breast cancer. S, stroma.

proteins and clinicopathologic factors revealed that stromal ATX positivity was higher in luminal A tumors than in other tumor subtypes (P = 0.035), and stromal LPA3 positivity was correlated with a high Ki-67 LI (P = 0.005, **Figure 4**).

The impact of autotaxin-lysophosphatidate signaling-related protein expression on the prognosis of metastatic breast cancer

Univariate analysis of the impact of autotaxin-lysophosphatidate signaling-related protein expression on the prognosis of metastatic breast cancer showed that tumoral LPA3 negativity was correlated with shorter OS (P = 0.015,

Figure 5). According to the metastatic site, tumoral LPA3 negativity was correlated with shorter OS in lung metastasis (P = 0.010) and stromal LPA3 negativity with shorter OS in brain metastasis (P = 0.026, **Table 4**). Multivariate Cox analysis showed that tumoral LPA3 negativity is a poor independent prognostic factor (HR = 2.311, 95% CI: 1.029-5.191, P = 0.043, **Table 5**).

Discussion

We assessed the expression of ATX-LPA signaling-related proteins in metastatic breast cancer according to the metastatic site. First of all,
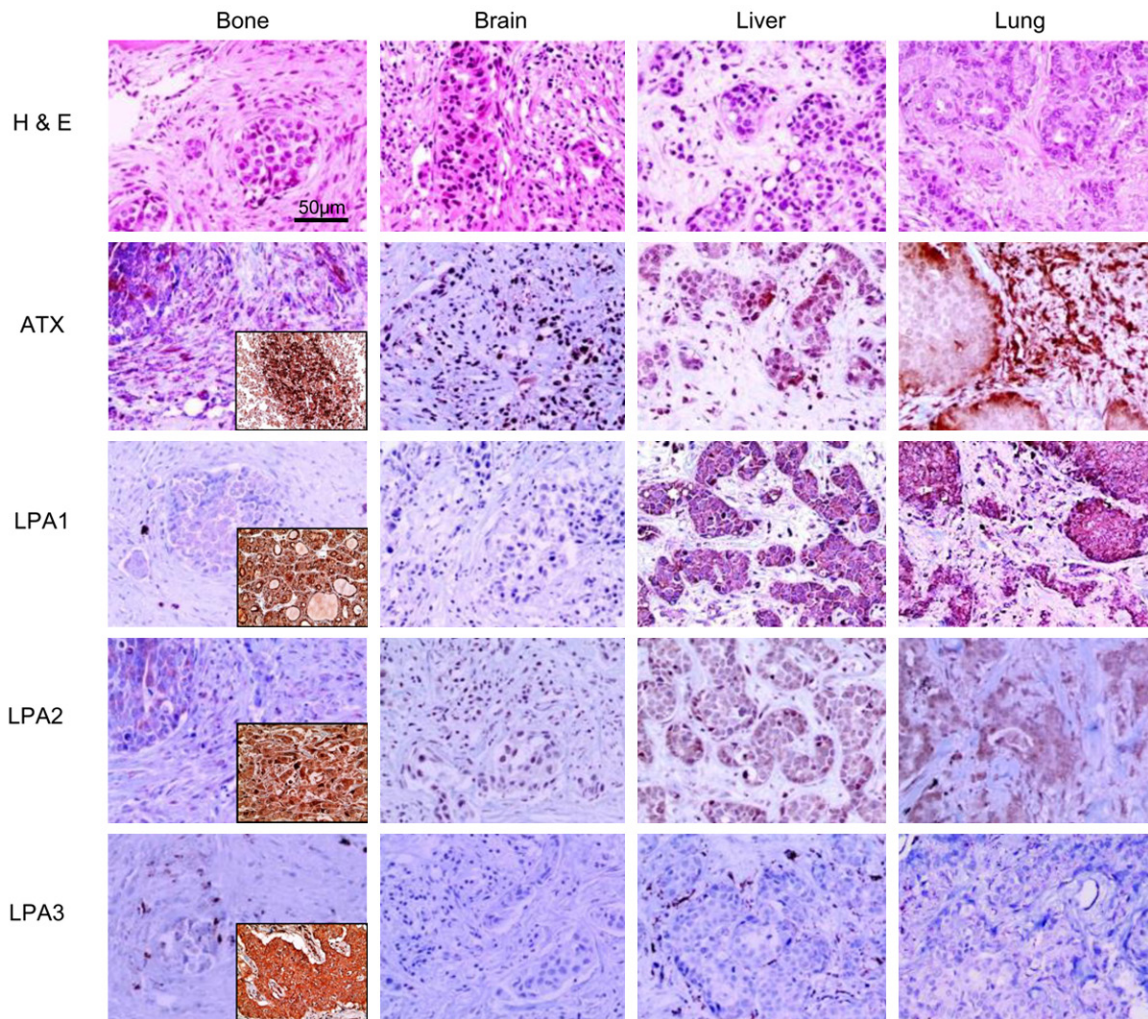
Figure 2. Expressions of autotaxin-lysophosphatidate signaling-related proteins according to the metastatic site in metastatic breast cancer. Stromal ATX is highly expressed in bone and lung metastases, and tumoral LPA1 is highly expressed in liver and lung metastases. The insets represent the positive controls.
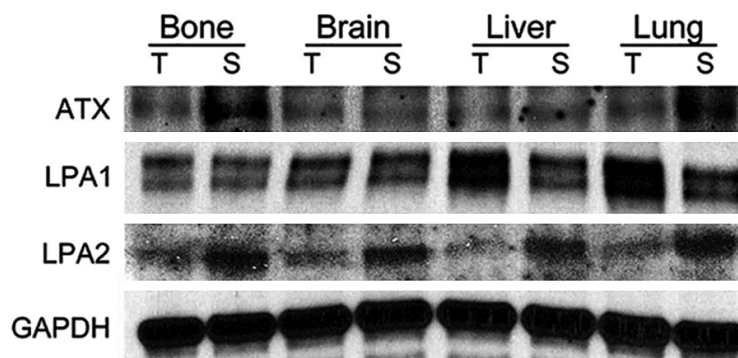

Figure 3. Western blot for autotaxin-lysophosphatidate signaling-related proteins according to the metastatic site in metastatic breast cancer. Stromal ATX is highly expressed in bone and lung metastases, and tumoral LPA1 is highly expressed in liver and lung metastases.

ATX and LPA3 were expressed not only in the tumor cells but also in the tumor stroma. ATX is often expressed in cells other than tumor cells [19, 26], such as platelets, endothelial cells, fibroblasts, and adipocytes [27-30]. Therefore, it is plausible that ATX is found in breast cancer stromal cells [19]. In addition, LPA3 is expressed in breast cancer stromal cells [19]. Our results demonstrated ATX expression in the stroma of metastatic breast cancer to the lung and bone, and the stromal expression of ATX was especially high in the latter. A previous study reported that inflammatory mediators secreted by breast cancer cells activate ATX secretions from adipocytes adjacent to the breast cancer tu-
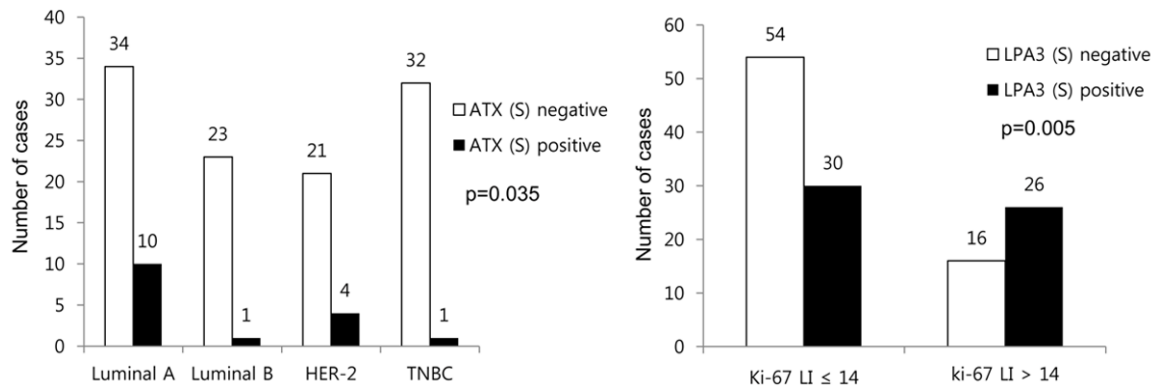
Figure 4. Correlation between clinicopathologic factors and the expression of autotaxin-lysophosphatidate signaling-related proteins.
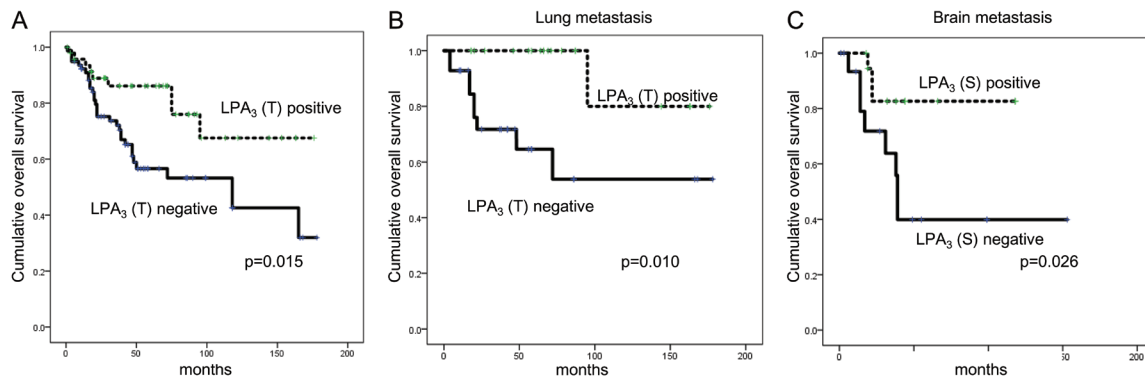
Figure 5. The impact of tumoral LPA3 on prognosis in overall metastatic breast cancer (A), lung metastasis (B), and stromal LPA3 in brain metastasis (C).

mor cells, which activates LPA signaling. Activated LPA signaling then increases the secretion of inflammatory mediators from the breast cancer cells [31]. This suggests a possibility that ATX secreted from breast cancer stromal cells enables tumor-stroma interaction, which leads to the hypothesis that ATX expressed in the stroma of metastatic breast cancer in the lung and bones may influence tumor progression. Wannecq et al. reported that ATX is involved in the formation of bone metastasis through the activation of osteoclasts by LPA signaling [32], thereby explaining the high expression of stromal ATX in bone metastasis.

In this study, LPA1 was highly expressed in liver and lung metastases. An overexpression of LPA_1 in transgenic mice results in lung metastasis [20], and lung metastasis of basal breast cancer result from the LPA1/ZEB1/miR-21-activation pathway. These results are in concordance with our study results [33].

The clinical implication of our study is that the ATX-LPA signaling axis may be a possible therapeutic target in metastatic breast cancer. Ki16425, a non-lipid competitive inhibitor of LPA1 and LAP3, actually suppresses bone metastases of breast cancer in a mouse model [30], and Debio0719, an R-stereoisomer of Ki16425, inhibits lung and bone metastases [34] and liver and lung metastasis [35] of breast cancer in a mouse model. BrP-LPA, which is a dual ATX and pan-LPAR inhibitor, inhibited cell migration and invasion in a breast cancer cell line, and also suppressed primary tumor formation and angiogenesis in a mouse xenograft study [36]. In addition, the new ATX inhibitor ONO-843050 decreases tumor growth in a breast cancer mouse model and also decreases lung metastatic nodules up to 60% [37]. Therefore, the ATX-LPA signaling axis may be an effective therapeutic target for metastatic breast cancer, and further study is necessary.

Table 4. Univariate analysis of the association between expressions of autotaxin-lysophosphatidate signaling-related proteins in metastatic breast cancers and overall survival on a log-rank test

Parameters	Total, N = 149 (%)		Metastatic site							
			Bone, *N* = 31 (%)		Brain, *N* = 36 (%)		Liver, *N* = 11 (%)		Lung, *N* = 48 (%)	
	Mean survival (95% CI) months	*P*-value	Mean survival (95% CI) months	*P*-value	Mean survival (95% CI) months	*P*-value	Mean survival (95% CI) months	*P*-value	Mean survival (95% CI) months	*P*-value
ATX (T)		0.055		0.522		0.129		0.460		0.787
Negative	100 (79-120)		79 (47-111)		71 (49-93)		47 (47-47)		140 (103-177)	
Positive	127 (104-149)		68 (49-87)		131 (103-158)		85 (52-117)		127 (95-158)	
ATX (S)		0.807		0.514		N/A		N/A		0.420
Negative	113 (97-129)		89 (58-119)		N/A		N/A		126 (96-155)	
Positive	113 (76-149)		52 (29-74)		N/A		N/A		144 (106-183)	
LPA$_1$		0.159		0.211		0.307		0.843		0.917
Negative	105 (86-124)		86 (60-112)		75 (54-96)		61 (38-85)		139 (106-172)	
Positive	122 (100-145)		16 (16-16)		125 (91-159)		80 (44-117)		119 (87-151)	
LPA$_2$		0.896		0.969		0.416		0.761		0.208
Negative	114 (97-131)		85 (55-115)		99 (73-125)		80 (47-112)		137 (109-166)	
Positive	109 (81-137)		65 (43-86)		84 (59-110)		61 (28-93)		114 (73-155)	
LPA$_3$ (T)		0.015		0.191		0.132		N/A		0.010
Negative	100 (81-120)		72 (39-105)		87 (69-104)		N/A		111 (76-146)	
Positive	136 (113-159)		86 (62-110)		82 (26-139)		N/A		159 (131-188)	
LPA$_3$ (S)		0.609		0.089		0.026		N/A		0.629
Negative	111 (92-131)		94 (64-124)		76 (42-110)		N/A		133 (103-164)	
Positive	107 (83-130)		41 (16-65)		101 (83-118)		N/A		111 (68-153)	

CI, confidence interval; n/a, not applicable.

Table 5. Multivariate Cox analysis of the association between expressions of autotaxin-lysophosphatidate signaling-related proteins in metastatic breast cancers and overall survival

Included parameters	Metastatic breast cancer		
	Overall survival		
	HR	95% CI	P-value
ER status			0.522
Negative vs. positive	1.634	0.363-7.354	
PR status			0.904
Negative vs. positive	0.943	0.360-2.466	
HER-2 status			0.147
Negative vs. positive	0.379	0.102-1.407	
Molecular subtype			0.735
TNBC vs. non-TNBC	1.337	0.249-7.173	
ATX (T)			0.459
Negative vs. positive	1.308	0.642-2.663	
LPA_3 (T)			0.043
Negative vs. positive	2.311	1.029-5.191	

HR, hazard ratio; CI, confidential interval; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor-2; TNBC, triple-negative breast cancer.

In conclusion, among autotoxin-lysophosphatidate signaling-related proteins, stromal ATX is highly expressed in bone metastases, and LPA1 is highly expressed in liver and lung metastases. Furthermore, tumor LPA3 is an independent prognostic factor in metastatic breast cancer.

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Disclosure of conflict of interest

None.

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