Int J Clin Exp Pathol 2016;9(7):7040-7049
www.ijcep.com /ISSN:1936-2625/IJCEP0022520

*Original Article*

# Expression of autophagy-related proteins in metastatic breast cancer of different site

Woo-Young Sun[1], Hye Min Kim[2], Ja Seung Koo[2]

*[1]Department of Surgery, Daejeon St. Mary's Hospital, The Catholololic University of Korea College of Medicine, Daejeon, South Korea; [2]Department of Pathology, Yonsei University College of Medicine, Severance Hospital, Seoul, South Korea*

Received December 22, 2015; Accepted March 7, 2016; Epub July 1, 2016; Published July 15, 2016

**Abstract:** The aim of this study was to evaluate the expression of autophagy-related proteins and their clinical implications in primary and metastatic breast cancer. Immunohistochemical staining of autophagy-related proteins (beclin-1, LC3A, LC3B) in 162 metastatic breast cancers (bone metastasis = 47, brain metastasis = 39, liver metastasis = 24, and lung metastasis = 52) was performed using tissue microarray (TMA). The expression of autophagy-related proteins in tumor cells varied according to metastatic site. Tumoral LC3A expression was high in brain and lung metastasis (P<0.001), stromal LC3A in bone metastasis (P<0.001), and stromal LC3B in liver metastasis (P = 0.017), respectively. In univariate analysis, beclin-1 positivity (P = 0.002) was associated with shorter overall survival (OS). In analysis by metastatic site, beclin-1 positivity (P = 0.002) and activated autophagy status (P = 0.009) in bone metastasis as well as beclin-1 positivity (P = 0.016) and tumoral LC3A positivity (P = 0.038) in lung metastasis were related to shorter OS. In conclusion, the expression of autophagy-related proteins varied according to the site of metastasis and was correlated with prognosis.

**Keywords:** Autophagy, breast cancer, metastasis

## Introduction

Breast cancer is associated with high morbidity and mortality rates due to frequent metastasis to sites such as the lung, brain, liver, and bone [1, 2], with brain and bone being the most well-characterized [3-8]. The general mechanism of tumor metastasis is interaction between tumor cells and host tissue, and it includes adhesion, proteolysis, invasion, and angiogenesis [2, 9]. Because not all tumors show the same metastatic pattern, the seed and soil hypothesis proposes that a specific tumor (seed) can survive in a specific organ (soil) [10]. Metastatic breast cancer characteristics differ according to metastatic site; previous reports show that young age, ER negativity, HER-2 overexpression, EGFR overexpression, and basal subtype are specific for brain metastasis [5-7], and factors suggesting bone metastasis include lower histologic grade, ER positivity/PR negativity, strand growth pattern, and the presence of fibrotic foci [4, 11, 12].

Cancer cells can survive difficult conditions such as hypoxia, lack of nutrients, or chemotherapy. However, highly aggressive malignant tumors often have high metabolic demand and, in some cases, require an alternative metabolic pathway. These cells can use energy supplied by recycling cytoplasmic components through autophagy [13, 14]. Hence, we hypothesize that autophagy plays an important role in tumor metabolism. Several proteins can be used to evaluate the activity of autophagy including beclin-1, which participates in the nucleation process [13, 14], and LD3, which participates in the elongation process and helps form autophagosomes [15-17]. These autophagy pathways are under current investigation as new possible targets for tumor therapy [18-21]. Since target therapy is used in both primary and metastatic tumors, evaluation of autophagy status at primary and metastatic sites is necessary. However, tumor characteristics vary according to metastatic site and there are few studies focused on autophagy at these sites. Therefore, the aim of this study is to investigate the expression of autophagy-related proteins at different metastatic sites and the resulting clinical implications.

**Table 1.** Clone, dilution, and source of antibodies used

| Antibody | Clone | Dilution | Source |
|---|---|---|---|
| Beclin-1 | Polyclonal | 1:100 | Abcam, Cambridge, UK |
| LC3A | EP1528Y | 1:100 | Abcam, Cambridge, UK |
| LC3B | SQSTM1 | 1:100 | Abcam, Cambridge, UK |

## Materials and methods

*Patient selection*

Data files were selected for invasive primary breast cancer and metastasis to distant organs (liver, lung, brain, and bone) from the Department of Pathology of Severance Hospital. Only patients with diagnosed invasive ductal carcinoma were included. This study was approved by the institutional review board. A total of 162 cases were included, with 49 cases paired between the primary tumor site and metastatic site. All slides were reviewed and pathologic diagnoses were approved by 2 pathologists (JSK and WJ). Histological grade was assessed using the Nottingham grading system [22].

*Immunohistochemistry*

Antibodies used for immunohistochemistry are shown in **Table 1**. Formalin-fixed, paraffin-embedded tissue sections were used for IHC staining. Tissue was sectioned to 3-μm thickness, deparaffinized in xylene, and rehydrated with alcohol solution. Detection was performed using a Ventana Discovery XT automated stainer (Ventana Medical System, Tucson, AZ, USA). Antigen retrieval was performed using CC1 buffer (Cell Conditioning 1; citrate buffer Ph 6.0, Ventan Medical System). IHC staining included adequate positive and negative controls.

*Interpretation of immunohistochemical results*

A cut-off value of ≥1% positively stained nuclei was used to define ER and PR positivity [23]. 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 [24]. HER-2 immunostaining was considered positive when strong (3+) membranous staining was observed, whereas cases with 0 to 1+ were regarded as negative.

IHC staining was evaluated by calculating the proportion of stained cells multiplied by immunostaining intensity. The proportion of stained cells was scored as 0 = negative, 1 = positive below than 30%, and 2 = positive when 30% or more. Immunostaining intensity was defined as 0 = negative, 1 = weak, 2 = moderate, and 3 = strong. The multiplication score of the proportion of stained cells by immunostaining intensity was defined as negative if 0 or 1, and positive if 2-6 [25]. Cases with two or more positive autophagy-related markers were considered to be autophagy-activated.

*Statistical analysis*

Statistical analyses were performed using SPSS for Windows, version 12.0 (SPSS Inc., Chicago, IL, USA). Correlation analysis of immunostaining results between primary breast cancer and metastatic breast cancer were calculated by the McNemar test. Comparative statistics were performed using chi-squared analysis. Statistical significance was assumed when $P<0.05$. Kaplan-Meier survival curves and log-rank statistics were employed to evaluate time to tumor metastasis and time to survival.

## Results

*Basal characteristics of patients (**Table 2**)*

Among 162 patients, lung metastasis was observed in 52 cases (32.1%), bone metastasis in 47 cases (29%), brain metastasis in 39 cases (24.1%), and liver metastasis in 24 cases (14.8%). ER positivity and PR positivity were high in bone and liver metastases ($P<0.001$), and HER-2 positivity was high in brain metastasis ($P = 0.017$). There were more luminal A types in bone and liver metastases and more TNBC types in brain and lung metastases ($P<0.001$).

*Expression of autophagy-related proteins in breast cancer metastasis according to metastatic site (**Table 3**; **Figure 1**)*

Analysis of the expression of autophagy-related proteins showed that the expression of tumoral

**Table 2.** Basal clinicopathologic characteristics of breast cancer metastasis according to the metastatic sites

| Parameters | Total N = 162 (%) | Bone metastasis N = 47 (%) | Brain metastasis n = 39 (%) | Liver metastasis N = 24 (%) | Lung metastasis n = 52 (%) | *P*-value |
|---|---|---|---|---|---|---|
| Age (years) | | | | | | 0.022 |
| ≤50 | 81 (50.0) | 27 (57.4) | 17 (43.6) | 6 (25.0) | 31 (59.6) | |
| >50 | 81 (50.0) | 20 (42.6) | 22 (56.4) | 18 (75.0) | 21 (40.4) | |
| ER | | | | | | <0.001 |
| Negative | 69 (42.6) | 8 (17.0) | 26 (66.7) | 6 (25.0) | 29 (55.8) | |
| Positive | 93 (57.4) | 39 (83.0) | 13 (33.3) | 18 (75.0) | 23 (44.2) | |
| PR | | | | | | <0.001 |
| Negative | 109 (67.3) | 23 (48.9) | 38 (97.4) | 12 (50.0) | 36 (69.2) | |
| Positive | 53 (32.7) | 24 (51.1) | 1 (2.6) | 12 (50.0) | 16 (30.8) | |
| HER-2 | | | | | | 0.017 |
| Negative | 114 (70.4) | 38 (80.9) | 20 (51.3) | 19 (79.2) | 37 (71.2) | |
| Positive | 48 (29.6) | 9 (19.1) | 19 (48.7) | 5 (20.8) | 15 (28.8) | |
| Molecular subtypes | | | | | | <0.001 |
| Luminal A | 67 (41.4) | 33 (70.2) | 4 (10.3) | 15 (62.5) | 15 (28.8) | |
| Luminal B | 27 (16.7) | 7 (14.9) | 9 (23.1) | 3 (12.5) | 8 (15.4) | |
| HER-2 | 30 (18.5) | 5 (10.6) | 12 (30.8) | 3 (12.5) | 10 (19.2) | |
| TNBC | 38 (23.5) | 2 (4.3) | 14 (35.9) | 3 (12.5) | 19 (36.5) | |
| Patients death | 53 (32.7) | 23 (48.9) | 11 (28.2) | 7 (29.2) | 12 (23.1) | 0.040 |

**Table 3.** Expression of autophagy related proteins in tumor cell compartment of breast cancer metastasis according to the metastatic sites

| Parameters | Total N = 162 (%) | Bone metastasis N = 47 (%) | Brain metastasis n = 39 (%) | Liver metastasis N = 24 (%) | Lung metastasis n = 52 (%) | *P*-value |
|---|---|---|---|---|---|---|
| Beclin-1 | | | | | | 0.739 |
| Negative | 97 (59.9) | 26 (55.3) | 25 (64.1) | 16 (66.7) | 30 (57.7) | |
| Positive | 65 (40.1) | 21 (44.7) | 14 (35.9) | 8 (33.3) | 22 (42.3) | |
| LC3A (T) | | | | | | 0.001 |
| Negative | 137 (84.6) | 45 (95.7) | 26 (66.7) | 23 (95.8) | 43 (82.7) | |
| Positive | 25 (15.4) | 2 (4.3) | 13 (33.3) | 1 (4.2) | 9 (17.3) | |
| LC3A (S) | | | | | | 0.001 |
| Negative | 138 (85.2) | 34 (72.3) | 30 (76.9) | 24 (100.0) | 50 (96.2) | |
| Positive | 24 (14.8) | 13 (27.7) | 9 (23.1) | 0 (0.0) | 2 (3.8) | |
| LC3B (T) | | | | | | 0.281 |
| Negative | 112 (69.1) | 32 (68.1) | 28 (71.8) | 20 (83.3) | 32 (61.5) | |
| Positive | 50 (30.9) | 15 (31.9) | 11 (28.2) | 4 (16.7) | 20 (38.5) | |
| LC3B (S) | | | | | | 0.017 |
| Negative | 157 (96.9) | 45 (95.7) | 39 (100.0) | 21 (87.5) | 52 (100.0) | |
| Positive | 5 (3.1) | 2 (4.3) | 0 (0.0) | 3 (12.5) | 0 (0.0) | |
| Autophagy status | | | | | | 0.363 |
| Non-activated | 119 (73.5) | 33 (70.2) | 31 (79.5) | 20 (83.3) | 35 (67.3) | |
| Activated | 43 (26.5) | 14 (29.8) | 8 (20.5) | 4 (16.7) | 17 (32.7) | |

LC3A (P = 0.001), stromal LC3A (P = 0.001), and stromal LC3B (P = 0.017) were different among metastatic sites. Tumoral LC3A expression was high in brain and lung metastases,

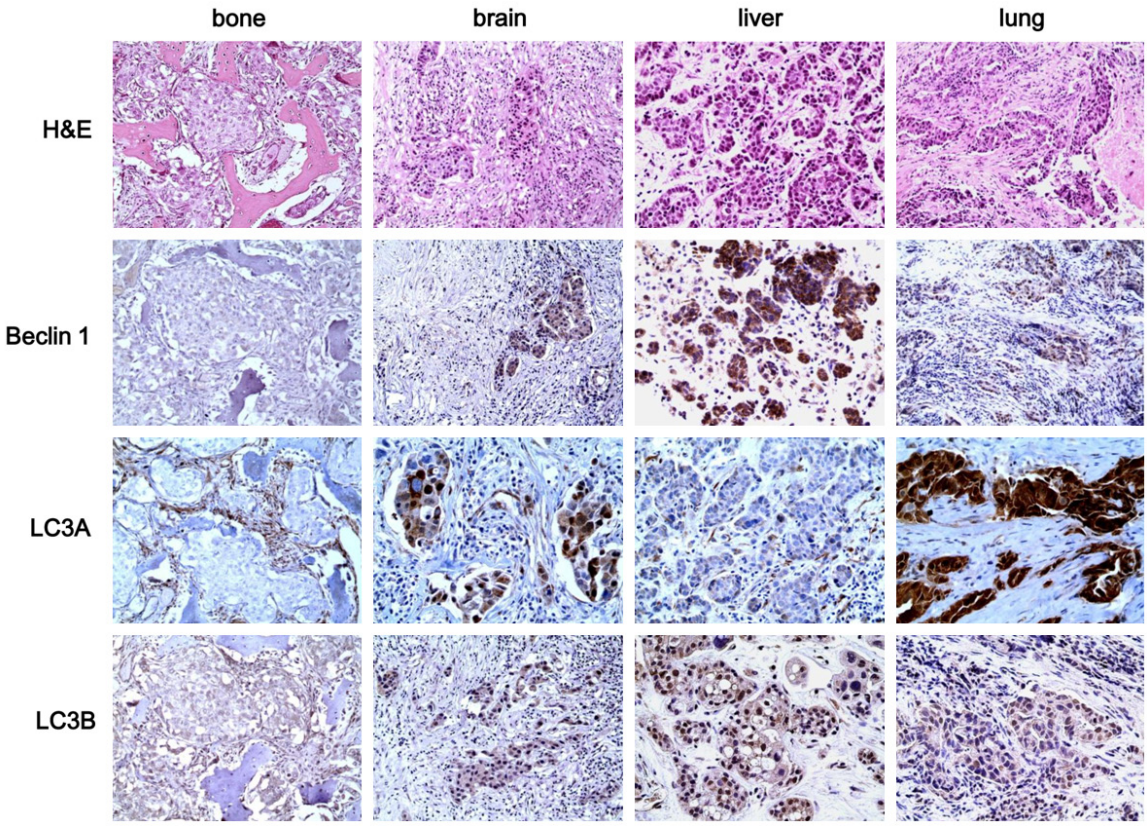


**Figure 1.** Expression of autophagy-related proteins in metastatic breast cancer according to metastatic site. Tumoral LC3A expression was high in brain and lung metastases, while stromal LC3A and stromal LC3B were high in brain and liver metastases, respectively.

**Table 4.** Correlation of expression of autophagy related proteins between primary and metastatic breast cancer according to the metastatic sites

| Parameters | Total | | Bone metastasis | | Brain metastasis | | Liver metastasis | | Lung metastasis | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N = 49(%) | *P*-value | N = 13 (%) | *P*-value | N = 9 (%) | *P*-value | N = 4 (%) | *P*-value | N = 23 (%) | *P*-value |
| Beclin-1 | | 0.424 | | 0.500 | | 0.375 | | 1.000 | | 0.688 |
| (+) → (+) | 5 (10.2) | | 1 (7.7) | | 1 (11.1) | | 0 (0.0) | | 3 (13.0) | |
| (+) → (-) | 9 (18.4) | | 0 (0.0) | | 4 (44.4) | | 1 (25.0) | | 4 (17.4) | |
| (-) → (+) | 5 (10.2) | | 2 (15.4) | | 1 (11.1) | | 0 (0.0) | | 2 (8.7) | |
| (-) → (-) | 30 (61.2) | | 10 (76.9) | | 3 (33.3) | | 3 (75.0) | | 14 (60.9) | |
| LC3A | | 0.688 | | n/a | | 1.000 | | 1.000 | | 0.625 |
| (+) → (+) | 4 (8.2) | | 0 (0.0) | | 2 (22.2) | | 0 (0.0) | | 2 (8.7) | |
| (+) → (-) | 2 (4.1) | | 0 (0.0) | | 0 (0.0) | | 1 (25.0) | | 1 (4.3) | |
| (-) → (+) | 4 (8.2) | | 0 (0.0) | | 1 (11.1) | | 0 (0.0) | | 3 (13.0) | |
| (-) → (-) | 39 (79.6) | | 13 (100.0) | | 6 (66.7) | | 3 (75.0) | | 17 (73.9) | |
| LC3B | | 0.815 | | 1.000 | | 1.000 | | 1.000 | | 1.000 |
| (+) → (+) | 7 (14.3) | | 2 (15.4) | | 1 (11.1) | | 0 (0.0) | | 4 (17.4) | |
| (+) → (-) | 10 (20.4) | | 2 (15.4) | | 3 (33.3) | | 0 (0.0) | | 5 (21.7) | |
| (-) → (+) | 8 (16.3) | | 1 (7.7) | | 2 (22.2) | | 1 (25.0) | | 4 (17.4) | |
| (-) → (-) | 24 (49.0) | | 8 (61.5) | | 3 (33.3) | | 3 (75.0) | | 10 (43.5) | |

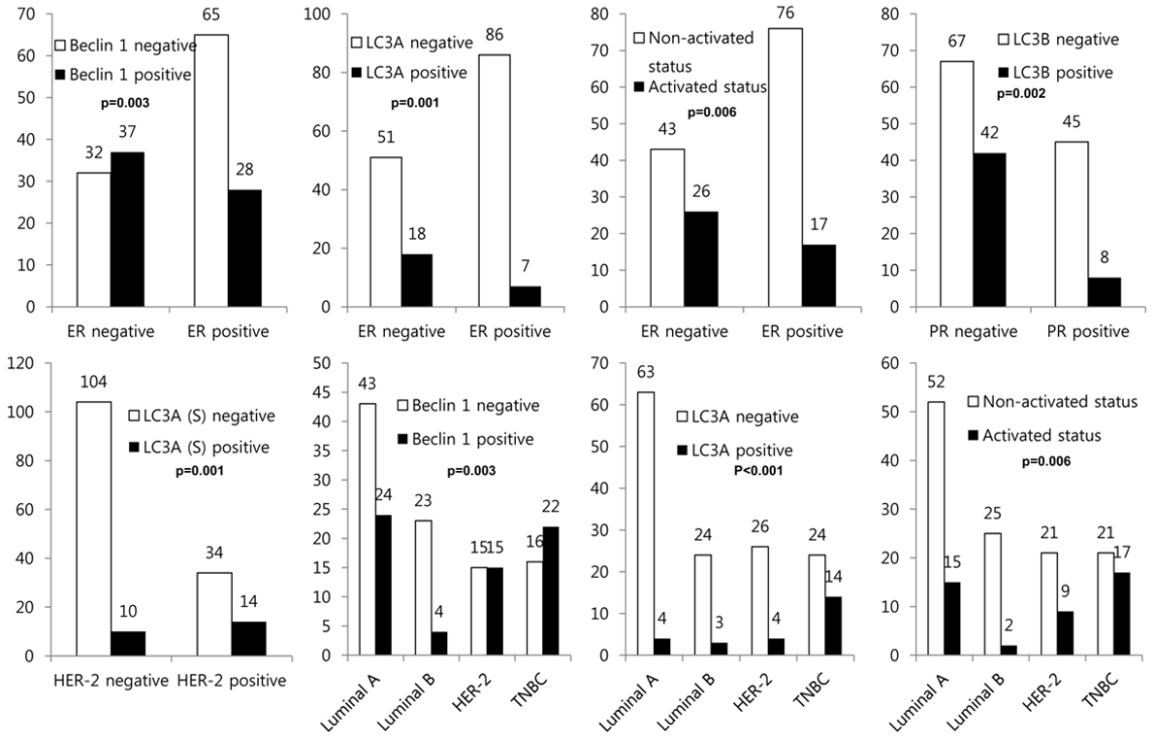


**Figure 2.** Correlation between pathologic factors and expression of autophagy-related proteins in metastatic breast cancer.

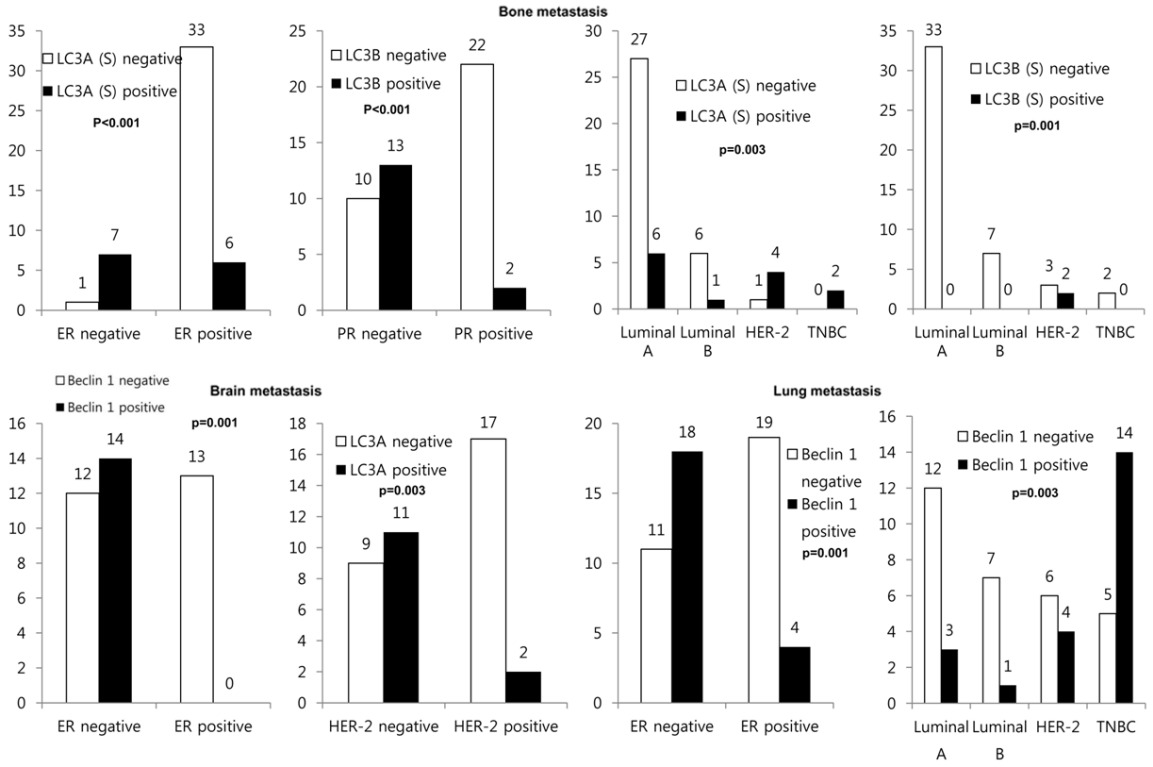


**Figure 3.** Correlation between pathologic factors and expression of autophagy-related proteins according to metastatic site.

**Table 5.** Univariate analysis of the impact of expression of autophagy related proteins in metastatic breast cancers on overall survival by the log-rank test

| Parameters | Total N = 162 (%) | | Bone metastasis N = 47 (%) | | Brain metastasis N = 39 (%) | | Liver metastasis N = 24 (%) | | Lung metastasis N = 52 (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 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 |
| Beclin-1 | | 0.002 | | 0.004 | | 0.788 | | 0.850 | | 0.016 |
| Negative | 127 (111-143) | | 113 (84-143) | | 140 (77-131) | | 80 (58-103) | | 150 (125-175) | |
| Positive | 61 (52-71) | | 51 (39-63) | | 77 (55-98) | | 66 (44-88) | | 59 (45-73) | |
| LC3A (T) | | 0.249 | | 0.441 | | 0.870 | | n/a | | 0.038 |
| Negative | 113 (99-128) | | 85 (64-107) | | 85 (67-103) | | n/a | | 139 (115-163) | |
| Positive | 80 (48-112) | | 58 (26-90) | | 118 (84-152) | | n/a | | 51 (29-73) | |
| LC3A (S) | | 0.520 | | 0.187 | | 0.678 | | n/a | | n/a |
| Negative | 110 (94-125) | | 86 (65-106) | | 108 (83-134) | | n/a | | n/a | |
| Positive | 78 (58-99) | | 63 (34-93) | | 90 (65-115) | | n/a | | n/a | |
| LC3B (T) | | 0.588 | | 0.123 | | 0.147 | | n/a | | 0.732 |
| Negative | 113 (97-130) | | 93 (67-118) | | 116 (92-141) | | n/a | | 126 (96-156) | |
| Positive | 104 (83-126) | | 57 (40-74) | | 63 (38-87) | | n/a | | 128 (96-161) | |
| LC3B (S) | | 0.597 | | n/a | | n/a | | 0.232 | | n/a |
| Negative | 111 (97-125) | | n/a | | n/a | | 86 (66-106) | | n/a | |
| Positive | 45 (36-54) | | n/a | | n/a | | 40 (28-51) | | n/a | |
| Autophagy status | | 0.079 | | 0.009 | | 0.186 | | n/a | | 0.737 |
| Non-activated | 119 (104-134) | | 101 (75-128) | | 113 (89-136) | | n/a | | 132 (106-159) | |
| Activated | 65 (54-76) | | 50 (35-65) | | 63 (32-93) | | n/a | | 69 (54-83) | |

while stromal LC3A expression was high in bone metastases and stromal LC3B expression was high in liver metastases.

*Correlation of expression of autophagy-related proteins with primary and metastatic breast cancer according to metastatic site (**Table 4**)*

Of 49 paired cases of primary tumor and its metastatic site, there were no differences in the expression of autophagy-related proteins between primary and metastatic breast cancer.

*Correlation between pathologic factors and expression of autophagy-related proteins (**Figure 2**)*

ER negativity was related to beclin-1 positivity (P = 0.003), LC3A positivity (P = 0.001), and activated autophagy status (P = 0.006). PR negativity was related to LC3B positivity (P = 0.002), and HER-2 positivity was related to stromal LC3A positivity (P = 0.001). Beclin-1 positivity (P = 0.003), LC3A positivity (P<0.001), and autophagy status (P = 0.006) varied according to molecular subtype, the expressions of beclin-1 and LC3A were high and activated autophagy status showed in TNBC.

*Correlation between pathologic factors and expression of autophagy-related proteins according to metastatic site (**Figure 3**)*

In bone metastasis, ER negativity was related to stromal LC3A (P<0.001), PR negativity was related to LC3B positivity (P<0.001), and HER-2 type was related to stromal LC3A and LC3B expression (P = 0.003, and 0.001, respectively). ER negativity was associated with beclin-1 positivity (P = 0.001) and HER-2 negativity was associated with LC3A positivity (P = 0.003) in brain metastasis. In lung metastasis, beclin-1 positivity was related to ER negativity (P = 0.001) and TNBC (P = 0.003).

*The impact of autophagy-related proteins on patient prognosis (**Table 5; Figure 4**)*

Univariate analysis revealed that beclin-1 positivity (P = 0.002) was related to shorter overall survival (OS). Analysis by metastatic site showed that beclin-1 positivity (P = 0.002) and activated autophagy status (P = 0.009) in bone metastasis, and beclin-1 positivity (P = 0.016) and tumoral LC3A positivity (P = 0.038) in lung metastasis, correlated with shorter OS.

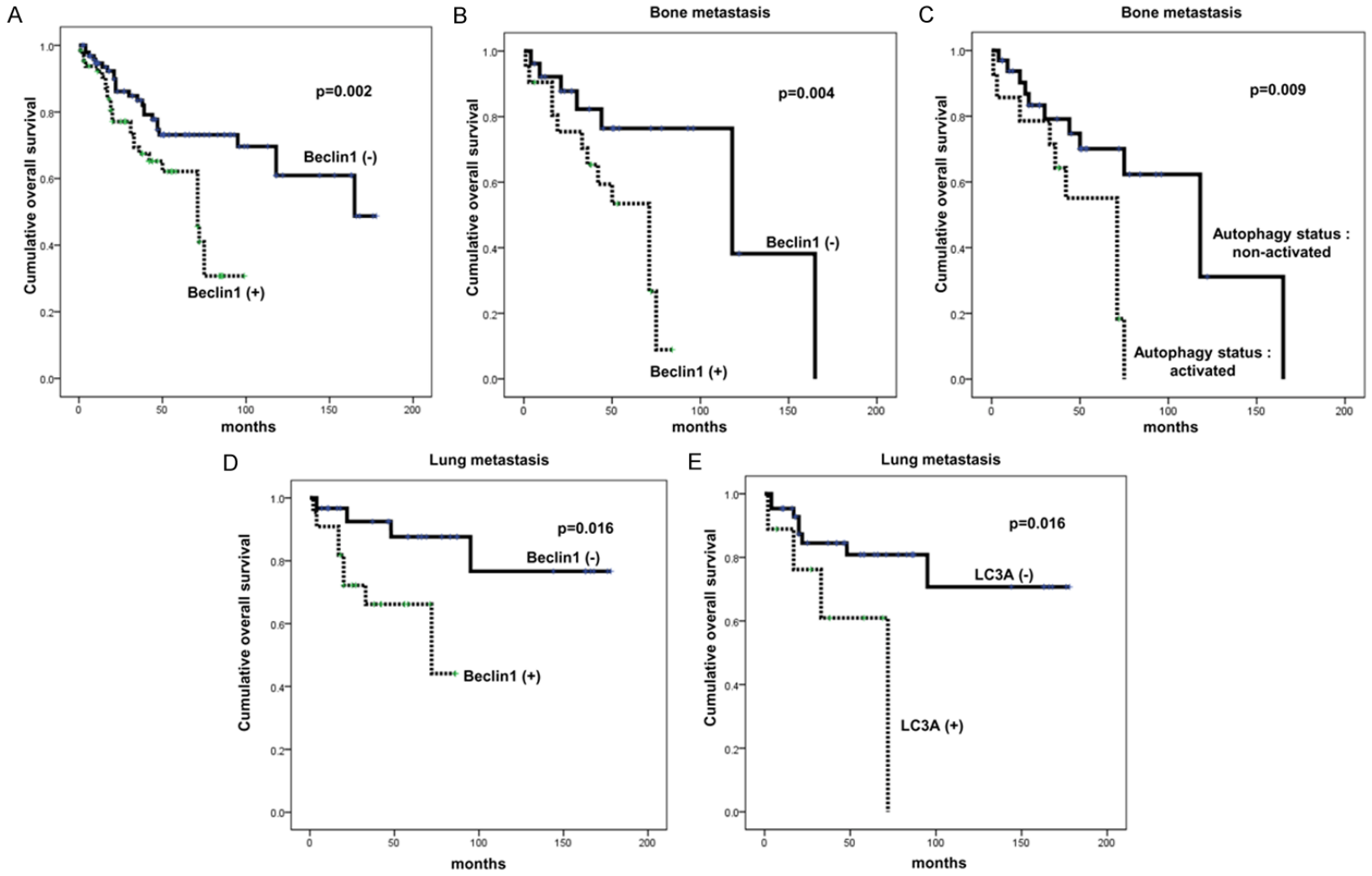


**Figure 4.** The impact of autophagy-related proteins on patient prognosis in metastatic breast cancer (A), bone metastasis (B, C), and lung metastasis (D, E).

## Discussion

In this study, we analyzed the expression of autophagy-related proteins in primary and metastatic tumors. Results showed a difference in the expression of autophagy-related proteins according to metastatic site. Tumoral LC3A expression was high in brain and lung metastases ($P<0.001$), stromal LC3A expression was high in bone and brain metastases ($P<0.001$), and stromal LC3B expression was high in liver metastases ($P = 0.017$). There have been few studies on autophagy in metastatic breast cancer, although one report suggested that expression of autophagy-related proteins was high in TNBC [26]. Our study indicated that tumoral LC3A expression is high in brain and lung metastases (frequent metastatic sites in TNBC) in concordance with a previous report.

High expression of autophagy-related proteins in TNBC is associated with tumor hypoxia status, the representative signal of autophagy induction, and pathologic characteristics include central necrosis and high proliferative activity [31]. We can presume the expression of autophagy-related proteins is higher in TNBC than in other subtypes due to the increased autophagy status by hypoxia.

This study showed that expression of autophagy-related proteins in the stromal tissue of metastatic breast cancer differs according to metastatic site. Autophagy in the stroma of breast cancer can be explained by the reverse Warburg effect, where there is interaction between breast cancer cells and cancer stromal cells. According to this theory, glycolysis, mitochondrial dysfunction and increased autophagic activity occur in stromal cells due to reactive oxygen species released from breast cancer cells. Ketone bodies and lactate produced by stromal glycolysis enter the tumor cell, which produces ATP via oxidative phosphorylation [27-29].

Previous research has shown that autophagy phenotypes differ among molecular subtypes, and luminal type shows reverse Warburg effect type and TNBC shows Warburg effect type [30]. Our study showed high expression of stromal LC3A and LC3B in bone and liver metastases, respectively, and these are frequent metastatic sites for the luminal type. Therefore, the high expression of stromal autophagy-related proteins can be attributed to the reverse Warburg effect.

The expression of beclin-1 was related to poor prognosis, consistent with previous studies showing that expression of beclin-1 corresponded with poor prognosis in colon [31], ovarian [32] and hypopharyngeal cancer [33]. However, beclin-1 expression correlated with good prognosis in other cancers, including hepatocellular carcinoma [34] and non-small cell lung cancer [35]. Further studies are needed to clarify these incompatible results.

One limitation of this study is the use of IHC staining of autophagy-related proteins like beclin-1, LC3A, and LC3B as indicators of autophagic activity. Evaluation of autophagic activity using a static method like IHC may be less accurate because autophagy is a multistep, dynamic process. While we expect that autophagic activity is increased when LC3A and LC3B expression increases, these markers can also be increased by autophagosome degradation.

The results of this study have a clinical implication as regulation of autophagy may be a therapeutic target for cancer. Recent studies have shown that autophagy inhibitors can suppress growth of several types of tumors [18-21].

In conclusion, the expression of autophagy-related proteins differed according to the site of metastasis. Tumoral LC3A expression was high in cases with brain and lung metastases and frequent metastatic sites in TNBC, while stromal LC3A and LC3B expression were high in cases with bone and liver metastases and frequent metastatic sites in more luminal cancer types.

## Acknowledgements

This study was supported by a grant from the National R&D Program for Cancer Control, Ministry of Health & Welfare, and Republic of Korea (1420080). This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2015R1A1A1A050012-09).

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Ja Seung Koo, Department of Pathology, Yonsei University College of Medicine, Severance Hospital, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, South Korea. Tel: 82-2-2228-1772; Fax: 82-2-362-0860; E-mail: kjs-1976@yuhs.ac

## References

[1] Weil RJ, Palmieri DC, Bronder JL, Stark AM and Steeg PS. Breast cancer metastasis to the central nervous system. Am J Pathol 2005; 167: 913-920.

[2] Woodhouse EC, Chuaqui RF and Liotta LA. General mechanisms of metastasis. Cancer 1997; 80: 1529-1537.

[3] Abali H and Celik I. High incidence of central nervous system involvement in patients with breast cancer treated with epirubicin and docetaxel. Am J Clin Oncol 2002; 25: 632-633.

[4] Colleoni M, O'Neill A, Goldhirsch A, Gelber RD, Bonetti M, Thurlimann B, Price KN, Castiglione-Gertsch M, Coates AS, Lindtner J, Collins J, Senn HJ, Cavalli F, Forbes J, Gudgeon A, Simoncini E, Cortes-Funes H, Veronesi A, Fey M and Rudenstam CM. Identifying breast cancer patients at high risk for bone metastases. J Clin Oncol 2000; 18: 3925-3935.

[5] Evans AJ, James JJ, Cornford EJ, Chan SY, Burrell HC, Pinder SE, Gutteridge E, Robertson JF, Hornbuckle J and Cheung KL. Brain metastases from breast cancer: identification of a high-risk group. Clin Oncol (R Coll Radiol) 2004; 16: 345-349.

[6] Gaedcke J, Traub F, Milde S, Wilkens L, Stan A, Ostertag H, Christgen M, von Wasielewski R and Kreipe HH. Predominance of the basal type and HER-2/neu type in brain metastasis from breast cancer. Mod Pathol 2007; 20: 864-870.

[7] Hicks DG, Short SM, Prescott NL, Tarr SM, Coleman KA, Yoder BJ, Crowe JP, Choueiri TK, Dawson AE, Budd GT, Tubbs RR, Casey G and Weil RJ. Breast cancers with brain metastases are more likely to be estrogen receptor negative, express the basal cytokeratin CK5/6, and overexpress HER2 or EGFR. Am J Surg Pathol 2006; 30: 1097-1104.

[8] Lorincz T, Toth J, Badalian G, Timar J and Szendroi M. HER-2/neu genotype of breast cancer may change in bone metastasis. Pathol Oncol Res 2006; 12: 149-152.

[9] Nicolson GL. Organ specificity of tumor metastasis: role of preferential adhesion, invasion and growth of malignant cells at specific secondary sites. Cancer Metastasis Rev 1988; 7: 143-188.

[10] Paget S. The distribution of secondary growths in cancer of the breast. Lancet 1889; 1: 571-572.

[11] Hasebe T, Imoto S, Yokose T, Ishii G, Iwasaki M and Wada N. Histopathologic factors significantly associated with initial organ-specific metastasis by invasive ductal carcinoma of the breast: a prospective study. Hum Pathol 2008; 39: 681-693.

[12] Wei B, Wang J, Bourne P, Yang Q, Hicks D, Bu H and Tang P. Bone metastasis is strongly associated with estrogen receptor-positive/progesterone receptor-negative breast carcinomas. Hum Pathol 2008; 39: 1809-1815.

[13] Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, Mukherjee C, Shi Y, Gelinas C, Fan Y, Nelson DA, Jin S and White E. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 2006; 10: 51-64.

[14] Roy S and Debnath J. Autophagy and tumorigenesis. Semin Immunopathol 2010; 32: 383-396.

[15] Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y and Yoshimori T. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J 2000; 19: 5720-5728.

[16] Sivridis E, Koukourakis MI, Zois CE, Ledaki I, Ferguson DJ, Harris AL, Gatter KC and Giatromanolaki A. LC3A-positive light microscopy detected patterns of autophagy and prognosis in operable breast carcinomas. Am J Pathol 2010; 176: 2477-2489.

[17] Yoshioka A, Miyata H, Doki Y, Yamasaki M, Sohma I, Gotoh K, Takiguchi S, Fujiwara Y, Uchiyama Y and Monden M. LC3, an autophagosome marker, is highly expressed in gastrointestinal cancers. Int J Oncol 2008; 33: 461-468.

[18] Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, Thomas-Tikhonenko A and Thompson CB. Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. J Clin Invest 2007; 117: 326-336.

[19] Carew JS, Medina EC, Esquivel JA 2nd, Mahalingam D, Swords R, Kelly K, Zhang H, Huang P, Mita AC, Mita MM, Giles FJ and Nawrocki ST. Autophagy inhibition enhances vorinostat-induced apoptosis via ubiquitinated protein accumulation. J Cell Mol Med 2010; 14: 2448-2459.

[20] Carew JS, Nawrocki ST, Kahue CN, Zhang H, Yang C, Chung L, Houghton JA, Huang P, Giles FJ and Cleveland JL. Targeting autophagy augments the anticancer activity of the histone deacetylase inhibitor SAHA to overcome Bcr-

Abl-mediated drug resistance. Blood 2007; 110: 313-322.
[21] Gupta A, Roy S, Lazar AJ, Wang WL, McAuliffe JC, Reynoso D, McMahon J, Taguchi T, Floris G, Debiec-Rychter M, Schoffski P, Trent JA, Debnath J and Rubin BP. Autophagy inhibition and antimalarials promote cell death in gastrointestinal stromal tumor (GIST). Proc Natl Acad Sci U S A 2010; 107: 14333-14338.
[22] Elston CW and Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991; 19: 403-410.
[23] Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, Hicks DG, Lester S, Love R, Mangu PB, McShane L, Miller K, Osborne CK, Paik S, Perlmutter J, Rhodes A, Sasano H, Schwartz JN, Sweep FC, Taube S, Torlakovic EE, Valenstein P, Viale G, Visscher D, Wheeler T, Williams RB, Wittliff JL and Wolff AC. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010; 28: 2784-2795.
[24] Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM and Hayes DF. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007; 25: 118-145.
[25] Won KY, Kim GY, Kim YW, Song JY and Lim SJ. Clinicopathologic correlation of beclin-1 and bcl-2 expression in human breast cancer. Hum Pathol 2010; 41: 107-112.
[26] Choi J, Jung W and Koo JS. Expression of autophagy-related markers beclin-1, light chain 3A, light chain 3B and p62 according to the molecular subtype of breast cancer. Histopathology 2013; 62: 275-286.
[27] Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG, Casimiro MC, Wang C, Fortina P, Addya S, Pestell RG, Martinez-Outschoorn UE, Sotgia F and Lisanti MP. The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. Cell Cycle 2009; 8: 3984-4001.
[28] Bonuccelli G, Tsirigos A, Whitaker-Menezes D, Pavlides S, Pestell RG, Chiavarina B, Frank PG, Flomenberg N, Howell A, Martinez-Outschoorn UE, Sotgia F and Lisanti MP. Ketones and lactate "fuel" tumor growth and metastasis: Evidence that epithelial cancer cells use oxidative mitochondrial metabolism. Cell Cycle 2010; 9: 3506-3514.
[29] Martinez-Outschoorn UE, Balliet RM, Rivadeneira DB, Chiavarina B, Pavlides S, Wang C, Whitaker-Menezes D, Daumer KM, Lin Z, Witkiewicz AK, Flomenberg N, Howell A, Pestell RG, Knudsen ES, Sotgia F and Lisanti MP. Oxidative stress in cancer associated fibroblasts drives tumor-stroma co-evolution: A new paradigm for understanding tumor metabolism, the field effect and genomic instability in cancer cells. Cell Cycle 2010; 9: 3256-3276.
[30] Choi J, Kim do H, Jung WH and Koo JS. Metabolic interaction between cancer cells and stromal cells according to breast cancer molecular subtype. Breast Cancer Res 2013; 15: R78.
[31] Han Y, Xue XF, Shen HG, Guo XB, Wang X, Yuan B, Guo XP, Kuang YT, Zhi QM and Zhao H. Prognostic significance of Beclin-1 expression in colorectal cancer: a meta-analysis. Asian Pac J Cancer Prev 2014; 15: 4583-4587.
[32] Cai M, Hu Z, Liu J, Gao J, Liu C, Liu D, Tan M, Zhang D and Lin B. Beclin 1 expression in ovarian tissues and its effects on ovarian cancer prognosis. Int J Mol Sci 2014; 15: 5292-5303.
[33] Wang J, Pan XL, Ding LJ, Liu DY, Lei DP and Jin T. Aberrant expression of Beclin-1 and LC3 correlates with poor prognosis of human hypopharyngeal squamous cell carcinoma. PLoS One 2013; 8: e69038.
[34] Qiu DM, Wang GL, Chen L, Xu YY, He S, Cao XL, Qin J, Zhou JM, Zhang YX and E Q. The expression of beclin-1, an autophagic gene, in hepatocellular carcinoma associated with clinical pathological and prognostic significance. BMC Cancer 2014; 14: 327.
[35] Zhou W, Yue C, Deng J, Hu R, Xu J, Feng L, Lan Q, Zhang W, Ji D, Wu J, Liu Q and Liu A. Autophagic protein Beclin 1 serves as an independent positive prognostic biomarker for non-small cell lung cancer. PLoS One 2013; 8: e80338.