Original Article
Expression of reactive oxygen species-related proteins in metastatic breast cancer is dependent on the metastatic site

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Abstract: This study was performed to investigate the expression of reactive oxygen species (ROS)-related proteins and to analyze the implications for primary and metastatic breast cancer. We constructed a tissue microarray containing 143 metastatic breast cancers (52 lung metastases, 38 bone metastases, 37 brain metastases, and 16 liver metastases) and performed immunohistochemical staining for ROS-related proteins (catalase, GSTπ, TxNIP, and MnSOD). Analysis of ROS-related protein expression in metastatic breast cancers according to the metastatic sites revealed site-specific expression patterns. The expression of tumoral catalase was lower in bone metastases (P = 0.012), and stromal GSTπ expression was higher in bone and liver metastases (P < 0.001). The highest ROS activation status was observed for lung metastases, while non-activated ROS was observed for bone metastases (P = 0.001). Primary cancers were positive for stromal GSTπ, but a subset of lung metastases were negative (P = 0.021). Univariate analysis revealed that shorter overall survival (OS) was associated with negative catalase expression of the tumor (P = 0.026). Furthermore, univariate analyses according to the metastatic sites revealed that shorter OS was associated with TxNIP-positive tumors (P = 0.032) and the expression of stromal catalase (P = 0.032) in brain metastases. Tumors that were negative for MnSOD expression (P < 0.001) but positive for stromal catalase expression (P = 0.022) were associated with shorter OS in patients with liver metastases. In conclusion, cancer cells and stromal tissues showed different ROS-related protein expression patterns according to the metastatic site. In addition, the expression of ROS-related proteins is associated with patient prognosis.

Keywords: Breast cancer, metastasis, reactive oxygen species

Introduction

Among all cancers, breast cancer has one of the highest rates of morbidity and mortality due to the occurrence of distant metastases. The major organs affected in metastatic breast cancer are the lung, brain, liver, and bone [1, 2]. However, most of the existing studies are performed for metastases to the brain and bone [3-8]. Not all cancers show similar metastatic patterns, raising the seed and soil hypothesis, which states that a specific tumor (the seed) grows in a specific visceral organ (the soil) [9]. Metastatic breast cancer also displays specific characteristics depending on the metastatic site. According to previous studies, the characteristics associated with brain metastases include young age, estrogen receptor (ER) negativity, prior lung metastasis, human epidermal growth factor receptor 2 (HER-2) overexpression, epidermal growth factor receptor (EGFR) overexpression, and basal subtype [5-7]. In contrast, the characteristics associated with bone metastases are lower histologic grade, ER positivity, ER positivity/progesterone receptor (PR) negativity, strand growth pattern, and the presence of fibrotic foci in invasive ductal carcinoma [4, 10, 11]. Therefore, the fact that different metastatic cancers display different characteristics depending on the metastatic site is well supported.

Neoplastic cells differ from normal cells; the most significant difference is that they receive constant stimulation from growth promoting signals. The resulting infinite cell proliferation allows for different metabolic activities of the neoplastic cells compared to the normal cells.

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

Antibody	Clone	Dilution	Source
Catalase	Polyclonal	1:600	Abcam, Cambridge, UK
GSTπ	Polyclonal	1:500	Assay Design, Michican, USA
TxNIP	JY2	1:100	MBL International Corporation, Woburn, USA
MnSOD	Polyclonal	1:100	Abcam, Cambridge, UK

GSTπ, Glutathione S-transferase π; TxNIP, Thioredoxin interacting protein; MnSOD, Manganese superoxide dismutase.

Specifically, these changes lead to accelerated cell proliferation and impose increased oxidative stress, which results in an alteration of the normal redox balance.

A previous study showed that the redox buffering systems such as the thioredoxin, glutathione, and antioxidant systems (for example catalase and superoxide dismutase) are either deregulated or overexpressed in tumors [12-14]. The altered redox process can lead to oncogenic transformation or mutations within the tumor. Consequently, the ROS pathway was recently investigated as a new possible target for therapy [15]. Because targeted therapy can be utilized not only for primary cancer but also for metastatic cancer, it is important to understand the ROS status of both primary and metastatic cancers. It has previously been shown that the ER, PR, and HER-2 status of primary and metastatic breast cancers can be discordant. Therefore, the relationship between the ROS status of primary and metastatic breast cancers needs to be investigated. To address this discrepancy, this study investigates the expression of ROS-related proteins and its implications in primary and metastatic breast cancers.

Materials and methods

Patient selection

Patients with invasive primary breast cancer and metastases to distant organs (lung, bone, brain, and liver) were selected using their medical records at the Department of Pathology of Severance Hospital. Only patients with a diagnosis of invasive ductal carcinoma were included. In total, 143 patients were identified, and for 38 of those patients samples of primary and metastatic cancers were available. All slides were reviewed and the resulting pathologic diagnoses were approved by 2 pathologists (JSK and WHJ). The histological grade was assessed based on the Nottingham grading system [16]. This study was approved by the Institutional Review Board.

Immunohistochemistry

The antibodies used for immunohistochemistry (IHC) in this study are shown in **Table 1**. Formalin-fixed, paraffin-embedded (FFPE) tissue samples were used as follows: 3 μm-thick slices from the FFPE tissue block were deparaffinized and rehydrated in xylene and alcohol solutions and stained using the Ventana Discovery XT automated stainer (Ventana Medical System, Tucson, AZ, USA). Antigen retrieval was performed with Cell Conditioning 1 buffer (citrate buffer pH 6.0, Ventana Medical Systems). Appropriate positive and negative controls were used.

Interpretation of immunohistochemical results

A cut-off value of 1% or more was used to define ER and androgen receptor positivity in positively stained nuclei [17]. The HER-2 staining was analyzed according to the American Society of Clinical Oncology/College of American Pathologists guidelines using the following categories: (0) = no immunostaining; (1+) = weak and/or incomplete membrane staining in less than 10% of tumor cells; (2+) = complete membrane staining that is either uniform or weak in at least 10% of tumor cells; and (3+) = uniform, intense membrane staining in at least 30% of tumor cells [18]. The HER-2 immunostaining was considered positive when a strong (3+) membrane staining was observed, whereas it was considered negative when none or weak (0 to 1+) staining was seen.

IHC result interpretation was based on the product of the proportion of stained cells and the immunohistochemical staining intensity. A product between 0 and 1 was regarded as negative, a product between 2 and 4 as a low positive, and a product between 5 and 6 as a high positive [19]. The proportion of stained cells was scored as 0 for negative, 1 for positive with less than 30% of the cells stained, and 2 for positive with greater than or equal to 30% of cells stained. The staining intensity was scored

Table 2. Basal clinicopathological characteristics of breast cancer metastases according to the metastatic sites

Parameters	Total n = 143 (%)	Bone metastasis n = 38 (%)	Brain metastasis n = 37 (%)	Liver metastasis n = 16 (%)	Lung metastasis n = 52 (%)	P-value
Age (years)						0.169
≤ 50	75 (52.4)	22 (57.9)	17 (45.9)	5 (31.2)	31 (59.6)	
> 50	68 (47.6)	16 (42.1)	20 (54.1)	11 (68.8)	21 (40.4)	
ER						< 0.001
Negative	66 (46.2)	7 (18.4)	26 (70.3)	4 (25.0)	29 (55.8)	
Positive	77 (53.8)	31 (81.6)	11 (29.7)	12 (75.0)	23 (44.2)	
PR						< 0.001
Negative	100 (69.9)	21 (55.3)	36 (97.3)	7 (43.8)	36 (69.2)	
Positive	43 (30.1)	17 (44.7)	1 (2.7)	9 (56.2)	16 (30.8)	
HER-2						0.035
Negative	99 (69.2)	31 (81.6)	19 (51.4)	12 (75.0)	37 (71.2)	
Positive	44 (30.8)	7 (18.4)	18 (48.6)	4 (25.0)	15 (28.8)	
Molecular subtypes						< 0.001
Luminal A	54 (37.8)	27 (71.1)	3 (8.1)	9 (56.2)	15 (28.8)	
Luminal B	24 (16.8)	5 (13.2)	8 (21.6)	3 (18.8)	8 (15.4)	
HER-2	29 (20.3)	4 (10.5)	12 (32.4)	3 (18.8)	10 (19.2)	
NBC	36 (25.2)	2 (5.3)	14 (37.8)	1 (6.2)	19 (36.5)	
Patient death	48 (33.6)	21 (55.3)	11 (29.7)	4 (25.0)	12 (23.1)	0.010

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

as 0 for negative, 1 for weak, 2 for moderate, and 3 for strong.

Statistical analysis

Data were statistically processed using SPSS for Windows, version 12.0 (SPSS Inc., Chicago, IL, USA). Correlation analysis of the immunostaining results between primary and metastatic breast cancer was performed using the McNemar test. Comparative statistics were performed using chi-square analysis. Statistical significance was assumed for *P* < 0.05. Kaplan-Meier survival curves and log-rank statistics were used to evaluate the time until tumor metastasis and the time of survival.

Results

Basal characteristics of patients

Our study included a total of 143 patients. Of these, 52 (36.4%) had lung metastases, 38 (26.6%) had bone metastases, 37 (25.9%) had brain metastases, and 16 (11.2%) had liver metastases. The proportion of patients that were ER-positive and PR-positive was higher

among those with bone and liver metastases compared to patients with metastases to other sites (*P* < 0.001). The proportion of patients that were HER-2 positive was higher in patients with brain metastases compared to patients with metastases to other sites (*P* = 0.035). Furthermore, luminal A type breast cancer was more common among patients with bone and liver metastases, while triple negative breast cancer (TNBC) was more common among patients with brain and lung metastases (*P* = 0.010) (**Table 2**).

Expression of ROS-related proteins in breast cancer metastasis depending on the metastatic site

The analysis of ROS-related protein expression depending on the metastatic site in metastatic breast cancer revealed site-specific expression patterns (**Figure 1**). The expression of catalase in tumor cells was lower in bone metastases (*P* = 0.012), and the expression of stromal glutathione S-transferase π (GSTπ) was higher in bone and liver metastases (*P* < 0.001). The ROS status defined by the expression of ROS-related proteins differed depending on the met-
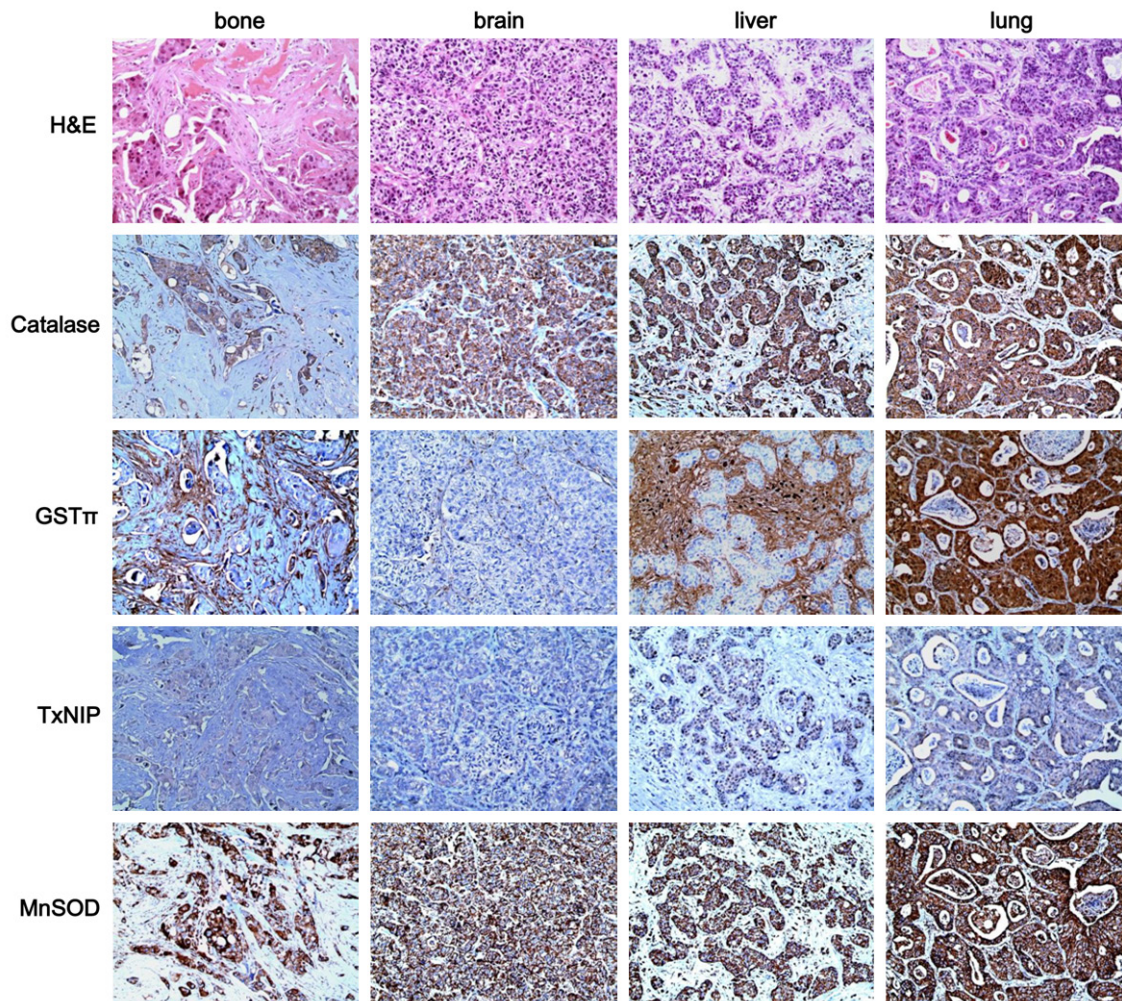
Figure 1. Expression of ROS-related proteins in metastatic breast cancers according to the metastatic sites. The expression of tumoral catalase was lower in bone metastasis, and that of stromal GSTπ was higher in bone and liver metastasis.

astatic site; the highest ROS activation status was observed for lung metastases, while non-activated ROS was observed in cancer cells derived from bone metastases (*P* = 0.001) (**Table 3**).

Correlation of expression of ROS-related proteins between primary and metastatic breast cancer depending on the metastatic site

We analyzed the expression levels of ROS-related proteins in primary and metastatic cancers in 38 patients from whom both samples were available, and found that the expression levels were not different. However, in metastatic cancer patients, primary cancers were positive for stromal GSTπ, while a subset of lung metastases were negative (*P* = 0.021) (**Table 4**).

The impact of autophagy-related protein expression on patient prognosis

Using univariate analysis, we analyzed the association of ROS-related protein expression with respect to patient prognosis (**Table 5** and **Figure 2**). We found that the factor associated with shorter overall survival (OS) was a tumor with negative catalase expression (*P* = 0.026). Using univariate analysis with respect to the metastatic site, we found that the factors associated with shorter OS in patients with brain metastases were tumors with positive Thioredoxin Interacting Protein (TxNIP) staining (*P* = 0.032) and stromal catalase expression (*P* = 0.032). Tumors with negative manganese superoxide dismutase (MnSOD) staining (*P* < 0.001) and positive stromal catalase expres-

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

Parameters	Total n = 143 (%)	Bone metastasis n = 38 (%)	Brain metastasis n = 37 (%)	Liver metastasis n = 16 (%)	Lung metastasis n = 52 (%)	P-value
Cancer cell compartment						
Catalase						0.012
Negative	75 (52.4)	27 (71.1)	21 (56.8)	8 (50.0)	19 (52.4)	
Positive	68 (47.6)	11 (28.9)	16 (43.2)	8 (50.0)	68 (47.6)	
GSTπ						0.051
Negative	102 (71.3)	31 (81.6)	28 (75.7)	13 (81.2)	30 (57.7)	
Positive	41 (28.7)	7 (18.4)	9 (24.3)	3 (18.8)	22 (42.3)	
TxNIP						0.126
Negative	126 (88.1)	34 (89.5)	36 (97.3)	14 (87.5)	42 (80.8)	
Positive	17 (11.9)	4 (10.5)	1 (2.7)	2 (12.5)	10 (19.2)	
MnSOD						0.198
Negative	5 (3.5)	1 (2.6)	3 (8.1)	1 (6.2)	0 (0.0)	
Positive	138 (96.5)	37 (97.4)	34 (91.9)	15 (93.8)	52 (100.0)	
ROS status						0.001
Non-activated	113 (79.0)	35 (92.1)	32 (86.5)	14 (87.5)	32 (61.5)	
Activated	30 (21.0)	3 (7.9)	5 (13.5)	2 (12.5)	20 (38.5)	
Stromal cell compartment						
Catalase						0.080
Negative	130 (90.9)	31 (81.6)	36 (97.3)	14 (87.5)	49 (94.2)	
Positive	13 (9.1)	7 (18.4)	1 (2.7)	2 (12.5)	3 (5.8)	
GST π						< 0.001
Negative	99 (69.2)	21 (55.3)	32 (86.5)	4 (25.0)	42 (80.8)	
Positive	44 (30.8)	17 (44.7)	5 (13.5)	12 (75.0)	10 (19.2)	
TxNIP						0.623
Negative	142 (99.3)	38 (100.0)	37 (100.0)	16 (100.0)	51 (98.1)	
Positive	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.9)	
MnSOD						0.110
Negative	118 (82.5)	30 (78.9)	34 (91.9)	15 (93.8)	39 (75.0)	
Positive	25 (17.5)	8 (21.1)	3 (8.1)	1 (6.2)	13 (25.0)	
ROS status						0.426
Non-activated	136 (95.1)	35 (92.1)	37 (100.0)	15 (93.8)	49 (94.2)	
Activated	7 (4.9)	3 (7.9)	0 (0.0)	1 (6.2)	3 (5.8)	

GSTπ, Glutathione S-transferase π; TxNIP, Thioredoxin interacting protein; MnSOD, Manganese superoxide dismutase; ROS, Reactive oxygen species.

sion (P = 0.022) were associated with shorter OS in patients with liver metastases.

Discussion

In this study, differences in the expression of ROS-related proteins were observed among patients with metastatic breast cancer depending on the metastatic site. Our analysis revealed site-specific expression patterns of catalase in tumor cells, with lower expression levels in patients with bone metastases (P = 0.012).

Furthermore, higher expression levels of stromal GSTπ were observed in patients with bone and liver metastases (P < 0.001). A comparison based on previous studies was not possible due to the lack of ROS-related protein expression data of metastatic breast cancers. ROS is widely known to play an important role in tumor metastasis [20]. ROS promotes metastasis by activating the signaling pathway that is involved in tumor metastasis. Mechanistically, ROS activates key signal transduction proteins through

Table 4. Correlation of ROS-related protein expression in cancer cells between primary and metastatic breast cancers with respect to the metastatic sites

Parameters	Total		Bone metastasis		Brain metastasis		Liver metastasis		Lung metastasis	
	n = 38 (%)	P-value	n = 7 (%)	P-value	n = 7 (%)	P-value	n = 3 (%)	P-value	n = 21 (%)	P-value
Cancer cell compartment										
Catalase		0.118		1.000		0.500		1.000		0.453
(+) → (+)	8 (21.1)		0 (0.0)		0 (0.0)		1 (33.3)		7 (33.3)	
(+) → (-)	5 (13.2)		2 (28.6)		0 (0.0)		0 (0.0)		3 (14.3)	
(-) → (+)	11 (28.9)		3 (42.9)		2 (28.6)		1 (33.3)		5 (23.8)	
(-) → (-)	14 (36.8)		2 (28.6)		5 (71.4)		1 (33.3)		6 (28.6)	
GSTπ		0.453		1.000		1.000		1.000		0.375
(+) → (+)	2 (5.1)		0 (0.0)		0 (0.0)		0 (0.0)		2 (9.1)	
(+) → (-)	2 (5.1)		0 (0.0)		1 (14.3)		0 (0.0)		1 (4.5)	
(-) → (+)	5 (12.8)		1 (14.3)		0 (0.0)		0 (0.0)		4 (18.2)	
(-) → (-)	30 (76.9)		6 (85.7)		6 (85.7)		3 (100.0)		15 (68.2)	
TxNIP		1.000		1.000		1.000		1.000		1.000
(+) → (+)	2 (5.1)		0 (0.0)		0 (0.0)		0 (0.0)		2 (9.1)	
(+) → (-)	3 (7.7)		1 (14.3)		0 (0.0)		0 (0.0)		2 (9.1)	
(-) → (+)	3 (7.7)		1 (14.3)		0 (0.0)		0 (0.0)		2 (9.1)	
(-) → (-)	31 (79.5)		5 (71.4)		7 (100.0)		3 (100.0)		16 (72.7)	
MnSOD		1.000		1.000		0.500		1.000		0.500
(+) → (+)	34 (87.2)		6 (85.7)		5 (71.4)		3 (100.0)		20 (90.9)	
(+) → (-)	2 (5.1)		0 (0.0)		2 (28.6)		0 (0.0)		0 (0.0)	
(-) → (+)	3 (7.7)		1 (14.3)		0 (0.0)		0 (0.0)		2 (9.1)	
(-) → (-)	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
Stromal cell compartment										
Catalase		1.000		1.000		1.000		1.000		1.000
(+) → (+)	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
(+) → (-)	5 (12.8)		1 (14.3)		1 (33.3)		1 (33.3)		2 (9.1)	
(-) → (+)	4 (10.3)		0 (0.0)		0 (0.0)		0 (0.0)		3 (13.6)	
(-) → (-)	30 (76.9)		6 (85.7)		2 (66.7)		2 (66.7)		17 (77.3)	
GSTπ		0.096		1.000		1.000		1.000		0.021
(+) → (+)	5 (12.8)		0 (0.0)		0 (0.0)		1 (33.3)		4 (18.2)	
(+) → (-)	13 (33.3)		2 (28.6)		2 (28.6)		0 (0.0)		9 (40.9)	
(-) → (+)	5 (12.8)		2 (28.6)		1 (14.3)		1 (33.3)		1 (4.5)	
(-) → (-)	16 (41.0)		3 (42.9)		4 (57.1)		1 (33.3)		8 (36.4)	
TxNIP		1.000		1.000		1.000		1.000		1.000
(+) → (+)	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
(+) → (-)	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
(-) → (+)	1 (2.6)		0 (0.0)		0 (0.0)		0 (0.0)		1 (4.5)	
(-) → (-)	38 (97.4)		7 (100.0)		7 (100.0)		3 (100.0)		21 (95.5)	
MnSOD		1.000		1.000		1.000		1.000		0.688
(+) → (+)	3 (7.7)		0 (0.0)		0 (0.0)		0 (0.0)		3 (13.6)	
(+) → (-)	5 (12.8)		1 (14.3)		2 (28.6)		0 (0.0)		2 (9.1)	
(-) → (+)	6 (15.4)		1 (14.3)		1 (14.3)		0 (0.0)		4 (18.2)	
(-) → (-)	25 (64.1)		5 (71.4)		4 (57.1)		3 (100.0)		13 (59.1)	

GSTπ, Glutathione S-transferase π; TxNIP, Thioredoxin interacting protein; MnSOD, Manganese superoxide dismutase.

Table 5. Univariate analysis of the impact of ROS-related protein expression in metastatic breast cancers on overall survival using the log-rank test

Parameters	Total n = 143 (%)		Bone metastasis n = 38 (%)		Brain metastasis n = 7 (%)		Liver metastasis n = 16 (%)		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
Cancer cell compartment										
Catalase		0.026		0.940		0.390		0.940		0.139
Negative	94 (75-114)		81 (57-105)		96 (64-128)		66 (47-84)		111 (68-153)	
Positive	120 (102-137)		60 (39-82)		87 (62-112)		85 (52-117)		124 (95-153)	
GSTπ		0.177		0.653		0.659		0.248		0.052
Negative	116 (100-133)		87 (62-113)		105 (79-132)		91 (70-112)		144 (117-171)	
Positive	99 (72-126)		73 (29-116)		75 (39-111)		22 (13-31)		90 (35-145)	
TxNIP		0.383		0.467		0.032		n/a		0.061
Negative	114 (99-129)		80 (58-102)		107 (84-130)		n/a		146 (124-167)	
Positive	67 (48-85)		79 (73-85)		19 (19-19)		n/a		66 (42-90)	
MnSOD		0.970		0.171		n/a		<0.001		n/a
Negative	126 (34-218)		165 (165-165)		n/a		11 (11-11)		n/a	
Positive	110 (96-125)		71 (56-86)		n/a		92 (72-112)		n/a	
ROS status		0.340		0.499		0.427		n/a		0.306
Non-activated	108 (91-124)		80 (58-102)		99 (72-125)		n/a		142 (117-167)	
Activated	88 (72-104)		79 (73-85)		98 (63-132)		n/a		72 (55-90)	
Stromal cell compartment										
Catalase		0.140		0.094		0.032		0.022		n/a
Negative	112 (97-127)		87 (65-110)		107 (84-130)		91 (70-112)		n/a	
Positive	91 (49-134)		37 (15-60)		19 (19-19)		21 (21-21)		n/a	
GSTπ		0.551		0.317		0.824		n/a		0.215
Negative	114 (97-131)		82 (65-100)		105 (81-128)		n/a		126 (100-152)	
Positive	100 (74-126)		76 (37-116)		72 (29-115)		n/a		130 (81-153)	
TxNIP		n/a		n/a		n/a		n/a		n/a
Negative	n/a		n/a		n/a		n/a		n/a	
Positive	n/a		n/a		n/a		n/a		n/a	
MnSOD		0.466		0.687		n/a		n/a		0.968
Negative	108 (92-123)		83 (60-106)		n/a		n/a		130 (103-158)	
Positive	120 (90-151)		50 (31-69)		n/a		n/a		123 (84-163)	
ROS status		0.716		0.264		n/a		n/a		n/a
Non-activated	109 (94-124)		84 (62-105)		n/a		n/a		n/a	
Activated	117 (62-173)		32 (0-65)		n/a		n/a		n/a	

GSTπ, Glutathione S-transferase π; TxNIP, Thioredoxin interacting protein; MnSOD, Manganese superoxide dismutase; ROS, Reactive oxygen species.
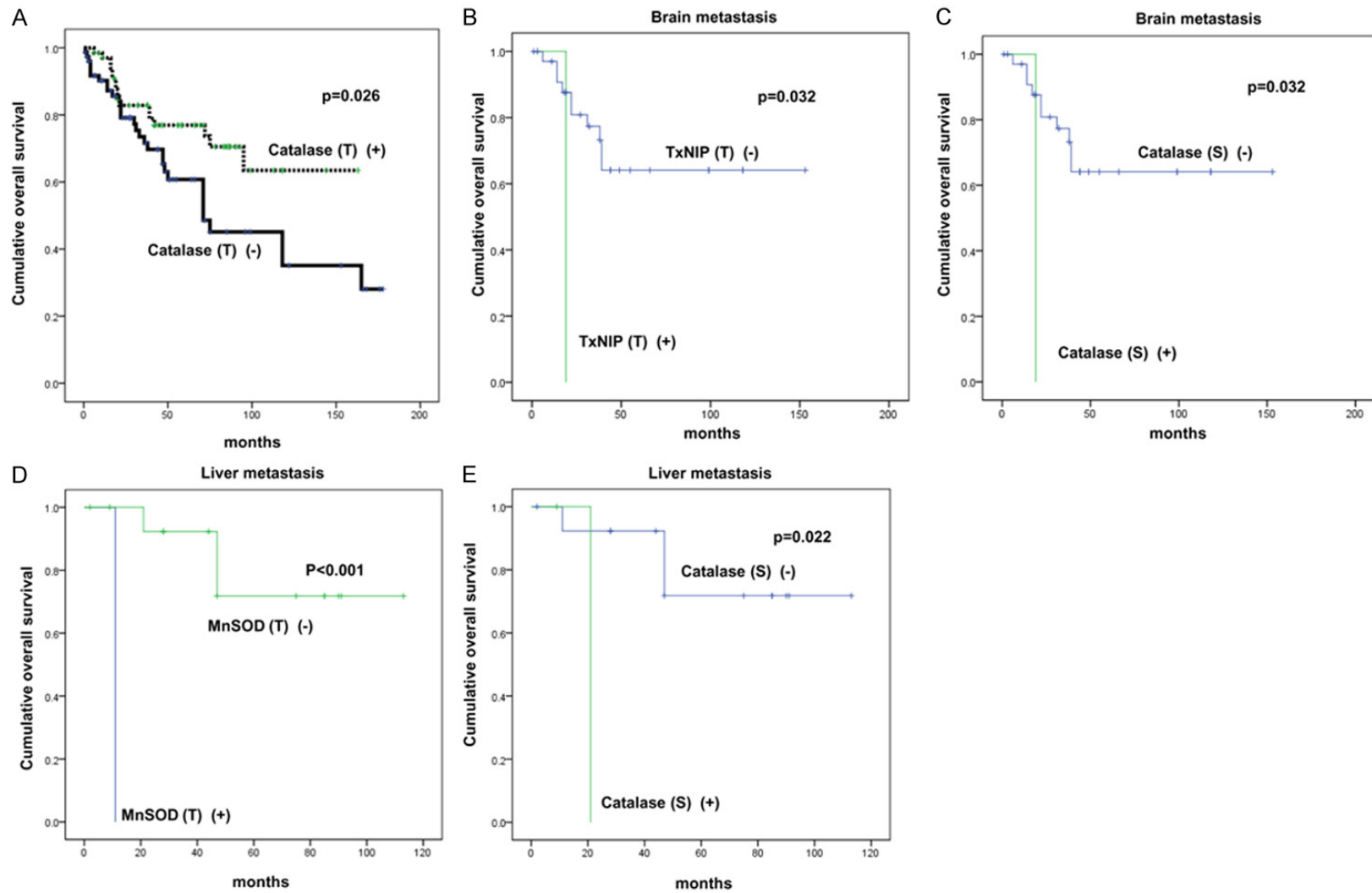
Figure 2. The impact of autophagy-related proteins on patient prognosis in metastatic breast cancer (A), brain metastasis (B, C), and liver metastasis (D, E).

oxidative posttranslational modifications of redox-sensitive proteins [21]. Therefore, metastatic cancers are expected to show an increase in the expression of ROS-related proteins. In this study, the expression of ROS-related proteins differed depending on the metastatic site. Previous studies reported different expression patterns of the most important biomarkers of breast cancer (ER, PR, and HER-2) according to the metastatic site. Most brain metastases are TNBC or HER-2 positive [6], whereas most bone metastases are hormone receptor positive [4]. These results were in agreement with our study. The proportion of hormone receptor positive cancer was high in liver metastases, and the proportion of TNBC was high in lung metastases. Our study showed different biological characteristics depending on the metastatic site. Previous studies showed a reverse correlation between ER and ROS. Specifically, the suppression of ER resulted in increased ROS production in breast cancer cells [22]. In this study, patients with bone metastases showed a high proportion of ER positivity and non-activated ROS status, while patients with lung metastases showed a low proportion of ER positivity and activated ROS status. Thus, the difference of ROS status according to the metastatic site could be the result of the hormone receptor status. Further research is required to substantiate this hypothesis.

Stromal tissues showed different expression patterns of ROS-related proteins; stromal GSTπ showed higher expression levels in bone and liver metastases. The possible theory, which can explain the expression of ROS-related proteins in stromal tissue, is the reverse-Warburg effect theory. According to this theory, cancer cells generate ROS (such as nitric oxide), which can cause oxidative stress and trigger glycolysis, autophagy (mitophagy), and mitochondrial dysfunction through NFκB and HIF-1α in stromal cells. Ketone bodies and lactate are formed through glycolysis in stromal cells that enter the cancer cells and produce ATP effectively through oxidative phosphorylation in the mitochondria of the cancer cells, which as a whole contributes to their survival and growth [23-27]. According to the reverse Warburg effect theory, to maintain the metabolic support of cancer cells, stromal cells should withstand the oxidative stress caused by ROS secreted from cancer cells. If detoxifying enzymes such as GSTπ are expressed at high levels, stromal cells could maintain the metabolic support of cancer cells under such oxidative stress environments. Previous studies reported that the reverse Warburg effect occurs in a high proportion of hormone receptor-positive luminal type breast cancers [28]. In this study, bone and liver metastases, of which a high proportion was of the luminal type, showed high stromal GSTπ expression. This can be because most bone and liver metastases display a reverse Warburg effect. However, further validation is required to generalize the findings of this study.

The clinical significance of this study is that ROS regulators may be a treatment modality. Previous studies reported that the substance increasing ROS caused ROS-induced apoptosis in cancer cells, while normal cells were not affected [29, 30]. However, the substance decreasing ROS accelerated tumor growth [31]. Therefore, ROS modulators can be considered as potential therapeutic agents that cause cancer cell death by further increasing ROS levels in targeted metastatic cancers with already increased ROS levels. However, more research is required to validate this hypothesis.

In conclusion, our study showed different expression patterns of ROS-related proteins in cancer cells and stromal tissues in metastatic breast cancers depending on the metastatic site. The expression of catalase was lower in bone metastases and that of stromal GSTπ was higher in bone and liver metastases.

Disclosure of conflict of interest

None.

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