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Homeodomain-interacting Protein Kinase 1 (HIPK1) Expression in Breast Cancer Tissues

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Objective: This study investigated the incidence and clinical significance of homeodomain-interacting protein kinase 1 expression in breast cancer patients.
Methods: We investigated immunohistochemical homeodomain-interacting protein kinase 1 expression from tissue microarrays of 1032 patients. The association of homeodomain-interacting protein kinase 1 expression pattern, clinicopathologic factors and survival outcome was evaluated. Tumors with $\geq$10% stained cells were considered positive for homeodomain-interacting protein kinase 1.
Results: Non-cancerous breast tissue, pTis and pT1mic lesions did not show homeodomain-interacting protein kinase 1 expression at any sites. Of the 859 invasive tumors, 124 (14.4%) showed homeodomain-interacting protein kinase 1 expression with three different expression patterns: cytoplasmic (2.4%), nuclear (6.3%), and both cytoplasmic and nuclear (5.7%). Cytoplasmic homeodomain-interacting protein kinase 1-positive tumors showed distinctive features such as fewer nodal metastases, but were frequently Grade III, estrogen receptor-negative, progesterone receptor-negative, HER2-positive, highly proliferative and molecular apocrine tumors. No significant difference in clinicopathologic features was identified between negative and nuclear homeodomain-interacting protein kinase 1-positive tumors. Both cytoplasmic and nuclear HIPK1-positive tumors represent frequent small size, node negativity and moderately differentiated features. Survival was not significantly different by homeodomain-interacting protein kinase 1 expression patterns.
Conclusions: Homeodomain-interacting protein kinase 1 expression was identified only in invasive breast cancer cells with three different patterns: cytoplasmic, nuclear, and both cytoplasmic and nuclear. Although the mechanism is not certain, the subcellular localization of HIPK1 expression is associated with tumor histopathologic characteristics and different functions.

Key words: breast cancer – homeodomain-interacting protein kinase 1 – molecular apocrine tumor

INTRODUCTION

The homeodomain-interacting protein kinases (HIPKs) are members of a small family of nuclear serine/threonine kinases that includes HIPK1, HIPK2 and HIPK3 (1). HIPK1 is primarily localized in the nucleus where it is sumoylated (2,3). HIPKs may recognize multiple cellular inputs and

regulate cell proliferation and apoptosis by regulating the activity of interacting proteins and subsequently the transcription of various target genes (4). HIPKs might be involved in the integration of various extracellular stimuli and the mediation of appropriate cellular responses during embryogenesis.

HIPK2 is relatively well studied and is involved in DNA-damage response, signaling to p53 by phosphorylating Ser-46, which triggers an apoptotic response through the upregulation of pro-apoptotic p53 target genes (5–7). Nodale et al. (8) reported that HIPK2 overexpression can contribute to the inhibition of breast cancer cell migration and invasion by vimentin downregulation.

On the contrary, HIPK1 is less well studied than HIPK2 and its function appears to be complex. The HIPK1 gene is highly expressed from human chromosome band 1p13, a site frequently altered in cancers. Mouse HIPK1 has been suggested to act as a transcriptional co-repressor interacting with the homeoproteins NKx-1,2 and NK-1 (2). Kondo et al. (9) isolated the p53-binding kinase HIPK1 and showed the upregulation of HIPK1 in many tumor cell lines. They also showed that carcinogen-treated HIPK1 −/− mice developed fewer and smaller skin tumors than HIPK1 +/+ mice and that the loss of HIPK1 had a protective effect on malignant progression by demonstrating that HIPK1 −/− transformed mouse embryonic fibroblast colonies grew more slowly than equivalent HIPK1 +/+ cells (9). Lee et al. (10) also reported that the cell growth rate of HIPK1 knockdown cell lines was markedly reduced compared with parental and control A549 cells and the tumorigenic activity of A549 lung cancer cells was reversed on suppression of HIPK1 expression in a soft agar assay and xenograft nude mice model. Taken together, these data suggest antiapoptotic and oncogenic function for HIPK1. However, HIPK1 is desumoylated in response to tumor necrosis factor (TNF)-α, and desumoylated HIPK1 is exported to cytoplasm where it binds to the AIP1-ASK1 signaling complex leading to the activation of ASK1-JNK/P38 signaling and endothelial cell apoptosis (11). Therefore, HIPK1 may play contradictory roles of antiapoptotic and apoptotic by desumoylation and relocalization.

Since sumoylation is associated with the modification of protein function and subcellular localization, desumoylation and relocalization of HIPK1 may affect the tumor growth or differentiation. Few studies have explored HIPK1, and its function has not been investigated or identified. Therefore, we designed this study to investigate the incidence of HIPK1 expression, patterns of expression and its association with clinicopathologic parameters in breast cancer.

PATIENTS AND METHODS

Patient Selection

Tumor samples were collected between November 1999 and August 2005, and formalin-fixed and paraffin-embedded. Archival hematoxylin and eosin (H&E)-stained slides for each case were reviewed by breast pathologists. Immunohistochemistry (IHC) was interpreted in a blind fashion, without information regarding clinical parameters or outcomes. Among the initial study population of 1200 cases, 157 cases (13.1%) with unreadable or duplicated HIPK1 expression and 11 cases (0.9%) with metastatic or recurrent disease at diagnosis were excluded. A total of 1032 cases were enrolled for analyses. Of these, 106 cases (10.3%) with invasive carcinoma that did not have invasive foci upon the review of archival H&E-stained slides were included and represented only extensive intraductal components (EIC). This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System, Seoul, Korea (4-2011-0354).

Patient characteristics, recurrence patterns and survival were regularly updated with regular follow-up information obtained from the breast cancer registry of the Yonsei University Severance Hospital. Patients received standard surgical procedures, either total mastectomy or breast-conservation surgery and sentinel lymph node biopsy or axillary lymph node dissection. After surgery, local radiotherapy or adjuvant treatments were decided by the results of risk evaluation. Clinical follow-up included history-taking, physical examinations, laboratory tests and radiologic imaging every 6–12 months for detection of relapse. Tumor stage was based on the Sixth American Joint Committee on Cancer criteria (12). Histological grade was assessed by the modified Bloom–Richardson classification (13). Locoregional recurrence was defined as tumor recurrence in the ipsilateral breast, chest wall and regional lymph node. Any recurrence at a distant site including contralateral axillary or supraclavicular lymph nodes was considered as a distant metastasis. Disease-free survival (DFS) was measured from the date of the first curative surgery to the date of the first locoregional or systemic recurrence, or death before any type of relapse. Overall survival (OS) was calculated from the date of the first surgery to the date of the last follow-up or death from any cause.

Tissue Microarray and IHC

Formalin-fixed, paraffin-embedded tissue blocks were arrayed using a tissue-arraying instrument (AccuMax Array, Petagen, Inc., Seoul, Korea). On each H&E slide of tumor blocks, invasive and/or intraductal components were selected and corresponding areas were marked on the surface of tumor blocks. The designated zone of each donor block was punched with a tissue cylinder and a core sample of 3 mm in diameter was transferred to a 6 × 5 recipient block in a grid pattern. Each core was assigned a unique tissue microarray (TMA) location number linked to a database containing clinicopathologic parameters.

TMA blocks were subjected to IHC. Briefly, 5 μm sections were obtained, deparaffinized and rehydrated. After treatment with 3% hydrogen peroxide solution for 10 min to block endogenous peroxidase, the sections were pretreated in

10 mM citrate buffer for antigen retrieval in a microwave oven for 20 min. After incubation with primary antibodies against HIPK1 (monoclonal, 1:30; catalog No. ab58136, Abcam, Cambridge, MA), estrogen receptor (ER) (SP1, 1:100; Thermo Scientific, Fremont, CA), progesterone receptor (PR) (PgR 636, 1:50; DAKO, Glostrup, Denmark), human epidermal growth factor receptor 2 (HER2) (polyclonal, 1:1,500; DAKO), Ki-67 (MIB-1, 1:100; DAKO), or androgen receptor (AR) (AR 441, 1:100; Thermo Scientific), immunodetection was performed with biotinylated antimouse/rabbit immunoglobulin, followed by peroxidase-labeled streptavidin using a labeled streptavidin biotin kit with 3,3′-diaminobenzidine chromogen as a substrate. Slides were counterstained with hematoxylin. Appropriate control tissues including skeletal muscle for HIPK1 were used as positive controls and non-cancerous breast tissue samples were also included in the TMA blocks.

Tumors with 10% or more cells expressing HIPK1 in the cytoplasm, nucleus, or at both sites were considered to be positive. Therefore, HIPK1 expression was categorized as either negative or positive for the cytoplasm, nucleus, or both. Tumors with $\geq$1% nuclear-stained cells were considered positive for ER and PR according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines (14). HER2 IHC and fluorescence *in situ* hybridization (FISH) were performed in all cases. HER2 IHC was interpreted as 0, 1+, 2+ or 3+ and FISH was defined as positive in cases with an absolute HER2 gene copy number >6 or HER2 gene/chromosome 17 copy number ratio >2.2 according to the ASCO/CAP guidelines (15). HER2 was considered to be positive in cases with IHC 3+ score or positive FISH regardless of IHC results. Tumors with 10% or more positively nuclear-stained cells were considered positive for AR (16). Based on IHC or FISH results of ER, PR, HER2 and Ki-67, molecular subtype was categorized as follows: luminal A (ER+ and/or PR+, HER2− and Ki-67 <14%); luminal B (ER+ and/or PR+, HER2− and Ki-67 $\geq$14% or ER+ and/or PR+ and HER2+ irrespective of Ki-67 expression); HER2-enriched (ER−, PR− and HER2+); and triple negative breast cancer (TNBC) (ER−, PR− and HER2−). The molecular apocrine subtype was immunohistochemically defined as ER-negative and AR-positive tumors (17).

Statistical Analysis

Differences between the groups were evaluated by a chi-square test. Fisher's exact test was used when appropriate. Survival curves were plotted using the Kaplan-Meier method and group differences in survival time were investigated by a log-rank test. All statistical tests were two-sided and $P < 0.05$ was considered statistically significant. SPSS for Windows version 18.0 (SPSS, Inc., Chicago, IL) was used for all statistical analysis.

RESULTS

Whole-population HIPK1 Expression

The study population of 1032 tumors was composed of 38 *in situ* carcinomas (pTis, 3.7%), 29 microinvasive cancer (pT1mic, 2.8%), 106 EIC of invasive tumors (10.3%), 385 pathologic tumor stage 1 (pT1, 37.3%), 448 pT2 (43.4%) and 26 pT3-4 (2.5%) carcinomas. Of a total 1032 cases, 907 (87.9%) tumors were negative for HIPK1 expression, 21 (2.0%) were cytoplasmic, 55 (5.3%) were nuclear and 49 (4.7%) showed both (Fig. 1A–D.). Non-cancerous breast tissue did not show HIPK1 expression at any sites (Fig. 1E). The frequency of HIPK1 expression according to tumor stage is in Table 1. All core samples from the pT1mic and EIC represented intraductal tumor cells by review of archival H&E slides. No lesions with pTis and pT1mic showed HIPK1 expression at any sites. Of 106 EIC lesions, only one (0.9%) showed HIPK1 expression. The other 105 samples (99.1%) did not show HIPK1 expression. Among invasive cancers, expression patterns of HIPK1 were not significantly different between pT1 and pT2-4 carcinomas (chi-square test, $P = 0.127$). Among 859 invasive cancers, 124 cases (14.4%) showed HIPK1 expression in the cytoplasm (21 cases, 2.4%), nucleus (54 cases, 6.3%) or both (49 cases, 5.7%) (Table 1).

Clinicopathologic Parameters of Invasive Cancer Patients

Clinicopathologic characteristics and survival outcomes were investigated for 859 patients with invasive cancer. The mean with standard deviation (SD) of age at diagnosis was 49.0 $\pm$ 10.5 years (range, 20–87). Among the 859 patients, 385 (44.8%) patients had pT1 carcinoma and 435 (50.6%) presented axillary node-negative tumors. Histologic grade was I in 157 (18.3%), II in 463 (53.9%) and III in 239 (27.8%) cases. Expression was positive for ER in 618 (71.9%), for PR in 536 (62.4%) and AR in 472 cases (54.9%). HER2 overexpression or amplification was identified in 211 tumors (24.6%). Of the 858 patients who were available for Ki-67 proliferative index, 477 (55.6%) showed Ki-67 <10%, 229 (26.7) had a Ki-67 index of 10–19% and 152 (17.7%) had a Ki-67 index of 20% or higher. Breast-conservation surgery was performed in 246 (28.6%) patients and local radiotherapy was administered to 411 (47.8%). Adjuvant chemotherapy and endocrine therapy was administered to 745 (86.7%) and 565 (65.8) patients, respectively.

Characteristics and Survival According to HIPK1 Expression among Invasive Cancers

Of the 859 invasive cancers, HIPK1 expression was negative in 735 cancers (85.6%), cytoplasmic in 21 (2.4%), nuclear in 54 (6.3%), and both cytoplasmic and nuclear in 49 (5.7%) tumors. Positive HIPK1 expression was seen in 124 (14.4%)

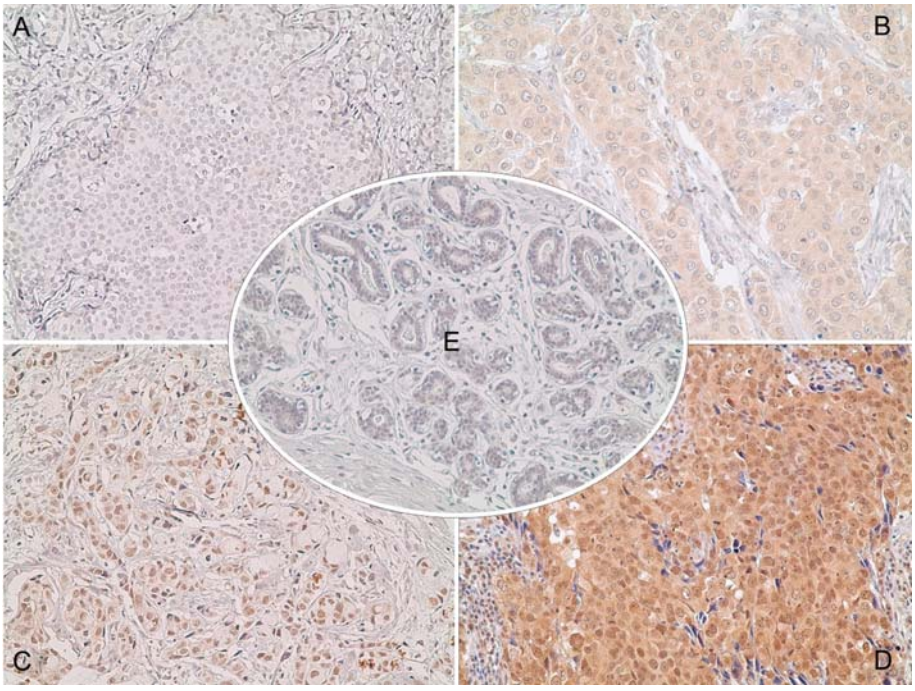

Figure 1. Expression pattern of HIPK1 in cancerous and non-cancerous breast tissues. Microscopic findings of negative (A), cytoplasmic (B), nuclear (C) and both cytoplasmic and nuclear (D) HIPK1-expressing tumors and non-cancerous breast tissue (E) (×200).

Table 1. HIPK1 expression according to pathologic tumor stage (pT)

	pTis ($n = 38$, %)	pT1mic ($n = 29$, %)	EIC ($n = 106$, %)	pT1 ($n = 385$, %)	pT2–4 ($n = 474$, %)
HIPK1					
Negative	38 (100)	29 (100)	105 (99.1)	323 (83.9)	412 (86.9)
Cytoplasmic	0 (0.0)	0 (0.0)	0 (0.0)	9 (2.3)	12 (2.5)
Nuclear	0 (0.0)	0 (0.0)	1 (0.9)	23 (6.0)	31 (6.5)
Both	0 (0.0)	0 (0.0)	0 (0.0)	30 (7.8)	19 (4.0)

Is, *in situ* carcinoma; mic, microinvasive carcinoma; EIC, extensive intraductal components associated with invasive cancer.

patients, in three patterns. Patient characteristics by HIPK1 expression are in Table 2.

No significant difference was seen in clinicopathologic features between negative and nuclear HIPK1-expressing tumors (chi-square tests between negative and nuclear HIPK1 subgroups, $P > 0.05$ for each clinicopathologic characteristic). The most prominent features were for cytoplasmic HIPK1-expressing tumors. Compared with tumors with negative HIPK1 expression, those with cytoplasmic HIPK1 expression showed fewer regional node metastases, but were frequently poorly differentiated Grade III, ER-negative, PR-negative, HER2-positive and highly proliferative Ki-67-positive cancers. They were also associated with HER-enriched subtype and molecular apocrine tumors. Tumors with HIPK1 expression in both the cytoplasm and nucleus showed the characteristics between nuclear and cytoplasmic expression. They were frequently small, node-negative cancers, but were associated with moderately differentiated tumor. A moderate frequency of HER2 overexpression and Ki-67 positivity were also noted. For molecular subtype defined by IHC, tumors expressing cytoplasmic HIPK1 were associated with HER2-enriched subtype. Tumors expressing both cytoplasmic and nuclear HIPK1 were associated with luminal B subtype, but the proportion of TNBC did not differ by HIPK1 expression patterns.

The median follow-up duration was 86.3 months (range, 5–137) for all patients. The sample sizes of the expression categories were too small to determine significant survival difference according to HIPK1 expression patterns. DFS and OS according to HIPK1 expression are shown in Fig. 2. Five-year DFS rates for patients with tumors that were HIPK1 negative, cytoplasmic, nuclear or both were 83.2, 85.7, 83.3 and 83.7%, respectively; 5-year OS rates were 89.9, 95.2, 83.3 and 91.8%. Survival outcomes were not

Table 2. Clinicopathologic characteristics according to HIPK1 expression in 859 patients with invasive carcinoma

	HIPK1 expression patterns				*P* value
	Negative ($n = 735$, %)	Cytoplasmic ($n = 21$, %)	Nuclear ($n = 54$, %)	Both cytoplasmic and nuclear ($n = 49$, %)	
Age at diagnosis (years)					
≤35	54 (7.3)	0 (0.0)	5 (9.3)	5 (10.2)	0.484*
>35	681 (92.7)	21 (100)	49 (90.7)	44 (89.8)	
Age at diagnosis (years)					
≤50	465 (63.3)	10 (47.6)	41 (75.9)	29 (59.2)	0.098
>50	270 (36.7)	11 (52.4)	13 (24.1)	20 (40.8)	
Pathologic tumor stage					
T1	323 (43.9)	9 (42.9)	23 (42.6)	30 (61.2)	0.085*
T2	392 (53.3)	12 (57.1)	27 (50.0)	17 (34.7)	
T3-4	20 (2.7)	0 (0.0)	4 (7.4)	2 (4.1)	
Pathologic node stage					
N0	359 (48.8)	16 (76.2)	25 (46.3)	35 (71.4)	0.005*
N1	226 (30.7)	3 (14.3)	14 (25.9)	3 (6.1)	
N2	93 (12.7)	2 (9.5)	9 (16.7)	5 (10.2)	
N3	57 (7.8)	0 (0.0)	6 (11.1)	6 (12.2)	
Histologic grade					
I	139 (18.9)	2 (9.5)	12 (22.2)	4 (8.2)	<0.001
II	400 (54.4)	4 (19.0)	25 (46.3)	34 (69.4)	
III	196 (26.7)	15 (71.4)	17 (31.5)	11 (22.4)	
Estrogen receptor					
Negative	205 (27.9)	10 (47.6)	15 (27.8)	11 (22.4)	0.190
Positive	530 (72.1)	11 (52.4)	39 (72.2)	38 (77.6)	
Progesterone receptor					
Negative	265 (36.1)	12 (57.1)	22 (40.7)	24 (49.0)	0.069
Positive	470 (63.9)	9 (42.9)	32 (59.3)	25 (51.0)	
HER2					
Negative	568 (77.3)	10 (47.6)	38 (70.4)	32 (65.3)	0.004
Positive	167 (22.7)	11 (52.4)	16 (29.6)	17 (34.7)	
Androgen receptor					
Negative	338 (46.0)	10 (47.6)	21 (38.9)	18 (36.7)	0.473
Positive	397 (54.0)	11 (52.4)	33 (62.1)	31 (63.3)	
Ki-67 (%, $n = 858$)					
<10	415 (56.5)	3 (14.3)	35 (64.8)	24 (49.0)	0.001
≥10	319 (43.5)	18 (85.7)	19 (35.2)	25 (51.0)	
Subtype ($n = 858$)					
Luminal A	398 (54.2)	4 (19.0)	28 (51.9)	22 (44.9)	0.015*
Luminal B	152 (20.7)	7 (33.3)	11 (20.4)	16 (32.7)	
HER2-enriched	58 (7.9)	6 (28.6)	5 (9.3)	4 (8.2)	
TNBC	126 (17.2)	4 (19.0)	10 (18.5)	7 (14.3)	
Molecular apocrine tumor					

Continued

Table 2. *Continued*

	HIPK1 expression patterns				*P* value
	Negative ($n = 735$, %)	Cytoplasmic ($n = 21$, %)	Nuclear ($n = 54$, %)	Both cytoplasmic and nuclear ($n = 49$, %)	
Apocrine subtype	46 (6.3)	5 (23.8)	4 (7.4)	4 (8.2)	0.035*
Other types	689 (93.7)	16 (76.2)	50 (92.6)	45 (91.8)	

HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.
*Fisher's exact test.

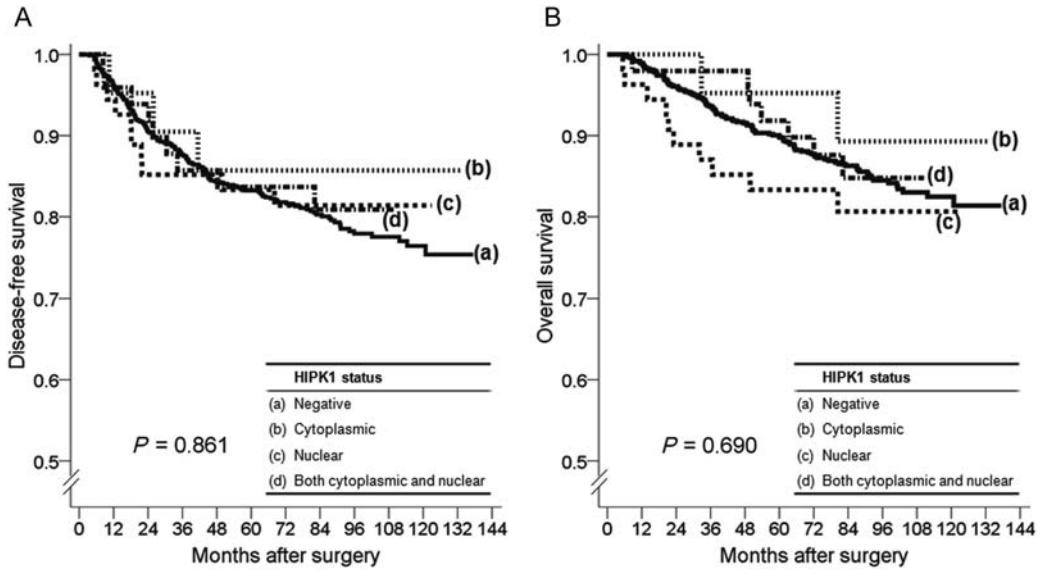

Figure 2. Disease-free (A) and overall (B) survival according to HIPK1 status. The solid line (a) represents negative HIPK1; dotted line (b), cytoplasmic HIPK1; dashed line (c), nuclear HIPK1 and dash-dotted line (d), both cytoplasmic and nuclear HIPK1 expression.

significantly different according to the HIPK1 expression status (DFS, $P = 0.861$; OS, $P = 0.690$). DFS was not significantly different when stratified by the lymph node, ER, HER2 or Ki-67 status or according to the HIPK1 status when stratified by TNM stage or molecular subtype, though cytoplasmic HIPK1-postive tumors showed a relatively better survival trend in node-positive subgroup, HER2-postive and ER-negative subgroups (data not shown). DFS and OS according to HIPK1 expression for 59 molecular apocrine subtype tumors are shown in Fig. 3. No recurrence or death was seen in five patients with molecular apocrine tumors expressing cytoplasmic HIPK1, although this was not statistically significant.

DISCUSSION

In our study, HIPK1 expression was detected in only 1 of the 173 intraductal tumor samples (0.58%) but increased expression of HIPK1 was detected in 124 of 859 (14.4%) invasive breast cancer tissues. We observed three HIPK1 expression patterns: cytoplasmic (2.4%), nuclear (6.3%) and both cytoplasmic and nuclear (5.7%) expression. HIPK1 is expressed mainly in invasive breast cancer cells but not in intraductal cancer cells, which suggests that HIPK1 expression might not be an early event in cancer development. HIPK1 is sumoylated and primarily localized in the nucleus with a novel dot-like subnuclear distribution (2,3), they are also found in the cytoplasm (3,18). Since sumoylation is a post-translational modification by a small ubiquitin-like modifier and can modify the protein function and desumoylation is associated with cytoplasmic translocation of HIPK1. Therefore, HIPK1 may play different roles depending on the sites of expression such as localization in the nucleus, in the cytoplasm or in both sites.

As shown in Table 2, significantly different clinicopathologic features were noted by HIPK1 expression patterns. Significant differences were seen in cytoplasmic HIPK1-expressing tumors, which showed fewer regional node metastases but frequently had poorly differentiated

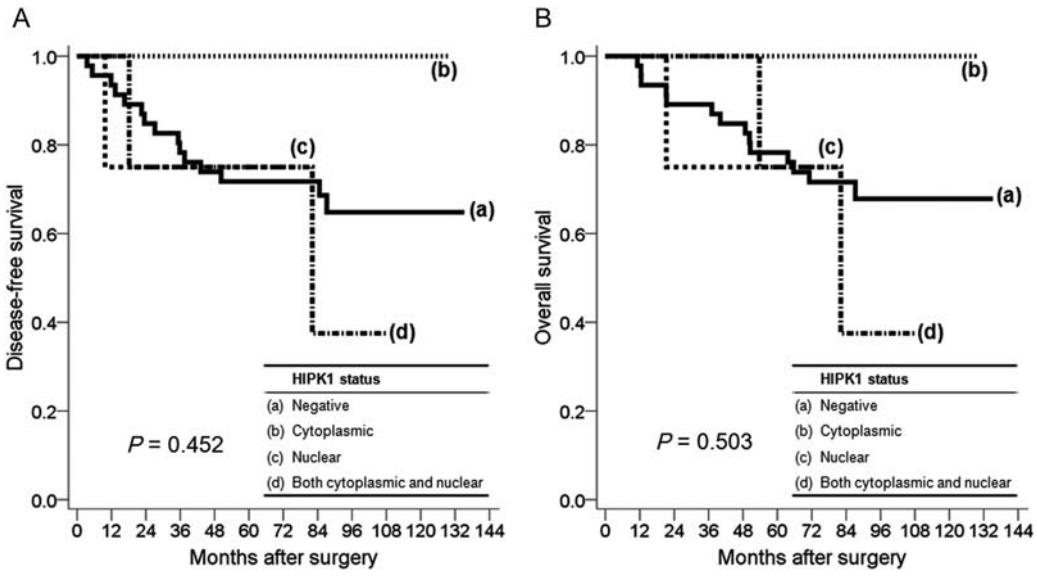

Figure 3. Disease-free (A) and overall (B) survival of 59 molecular apocrine tumors according to HIPK1 status. The solid line (a) represents negative HIPK1; dotted line (b), cytoplasmic HIPK1; dashed line (c), nuclear HIPK1 and dash-dotted line (d), both cytoplasmic and nuclear HIPK1 expression.

Grade III, ER-negative, PR-negative, HER2-positive and highly proliferative Ki-67-positive features. They were also associated with HER-enriched subtype and molecular apocrine tumors. However, no significant difference was seen between HIPK1-negative and nuclear HIPK1-positive tumors. Tumors with HIPK1 expression in both the cytoplasm and nucleus showed frequent small size, node-negativity and moderately differentiated features. The mechanisms or reasons why cytoplasmic HIPK1-expressing tumors showed different clinicopathologic features have not been investigated, but sumoylation and other post-translational modification might be involved.

In terms of function, the subcellular localization of HIPK represents an important mechanism in defining its functional specificity. HIPK2 can promote apoptosis by down-regulation of the transcriptional co-repressor CtBP inside the nucleus (19), while cytoplasmic HIPK1 appears to transduce signals by death receptors through interaction with TNF receptor type 1 associated death domain protein and Fas-associated death domain protein (20,21). HIPK1 has been reported to be oncogenic and might enhance cell growth and migration (9,10). However, HIPK1 is desumoylated in response to TNF-α, and desumoylated HIPK1 is exported to cytoplasm where it binds to the AIP1-ASK1 signaling complex leading to the activation of ASK1-JNK signaling and endothelial cell apoptosis (11). Based on the previous *in vitro* models, therefore, cytoplasmic HIPK1 expression might be associated with enhancing apoptosis and less aggressive biologic behavior by activation of ASK1-JNK signaling in breast cancer. However, contradictory findings were noted by our clinical analyses and cytoplasmic HIPK1 expression was associated with aggressive clinicopathologic features such as high grade, negative hormone receptors status, HER2-overexpression and high proliferation. Further study should be necessary.

No significant difference in survival was seen by HIPK1 expression. Since HIPK1 expression pattern varied, the number of tumors in each group was too small to investigate the statistical significance. However, survival showed different trends by HIPK1 expression patterns in subgroup analyses. Molecular apocrine tumors constitute 8–12% of breast cancers (17,22), and are ER negative but AR positive. Even though the number of cases was small, a cytoplasmic HIPK1 pattern was significantly associated with molecular apocrine type and none of the five cases of cytoplasmic HIPK1-expressing molecular apocrine type breast cancers showed recurrence or death for up to 10.8 years (71.3–130.6 months; mean: 102 months; median: 101 months) after surgery (Fig. 3). A large number of cases should be investigated for the confirmation of prognostic implications of HIPK1 expression patterns in molecular apocrine tumors. Cytoplasmic HIPK1-postive tumors showed a relatively better survival trend in the node-positive subgroup, HER2-postive and ER-negative subgroups (data not shown), which suggests that the cytoplasmic HIPK1 might play a protective role in a certain subgroup of breast cancers but details of molecular mechanism should be explored and independent validation using a large data set should be necessary.

Although there are some limitations including the necessity of independent verification of immunoreactions using commercially available antibody for the detection of novel molecule HIPK1, this study showed that ~15% of invasive breast cancers express HIPK1 with various patterns and cytoplasmic expression is associated with distinctive histopathologic characteristics. Sumoylation seems to be associated

with subcellular localization and different functions of HIPK1; further experimental and clinical investigations would be necessary.

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Conflict of interest statement

None declared.

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