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Research Article

Cancer Epidemiology, Biomarkers & Prevention

ST14 Gene Variant and Decreased Matriptase Protein Expression Predict Poor Breast Cancer Survival

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Abstract

Background: Matriptase plays a role in carcinogenesis, but the role of its genetic variation or that of the hepatocyte growth factor activator inhibitor-1 (HAI-1) has not been evaluated. This study aimed to examine the genetic variation of matriptase (*ST14* gene) and HAI-1 (*SPINT1* gene) in breast cancer risk and prognosis, to assess matriptase and HAI-1 gene and protein expression in breast tumors, and to identify their clinicopathologic correlations and prognostic significance.

Methods: Five single nucleotide polymorphisms in *ST14* and three in *SPINT1* were genotyped in 470 invasive breast cancer cases and 446 healthy controls. Gene expression analysis was done for 40 breast cancer samples. Protein expression was assessed by immunohistochemical analyses in 377 invasive breast tumors. The statistical significance of the associations among genotypes, clinicopathologic variables, and prognosis was assessed.

Results: The *ST14* single nucleotide polymorphism rs704624 independently predicted breast cancer survival, a poor outcome associated with the minor allele ($P = 0.001$; risk ratio, 2.221; 95% confidence interval, 1.382-3.568). Moreover, *ST14* gene expression levels were lower among the minor allele carriers ($P = 0.009$), and negative/low matriptase protein expression was independently predictive of poorer survival ($P = 0.046$; risk ratio, 1.554; 95% confidence interval, 1.008-2.396).

Conclusions: The *ST14* variant rs704624 and protein expression of matriptase have prognostic significance in breast cancer. This study adds to the evidence for the role of matriptase in breast cancer and has found new evidence for the genotypes having an impact in breast cancer.

Impact: This is the first study showing that genetic variation in matriptase has clinical importance. The results encourage further study on the genetic variation affecting protein levels and function in type II transmembrane serine proteases. *Cancer Epidemiol Biomarkers Prev; 19(9); 2133–42. ©2010 AACR.*

Introduction

Matriptase, also known as membrane-type serine protease-1 (MT-SP1), suppressor of tumorigenicity-14 (ST14), tumor-associated differentially expressed gene-15 (TADG-15), and SNC19, is a type II transmembrane serine protease (TTSP) encoded by the *ST14* gene on chromosome 11 (1-5). TTSPs are transmembrane proteins with a COOH-terminal serine protease domain and a cytoplasmic NH_2-terminal domain enabling functions in intracellular signal transduction (6, 7). In addition, the location of these proteases on the cell surface is ideal for interaction with other cell surface proteins, soluble proteins, and components of the extracellular matrix (6, 7). TTSPs have the ability to degrade extracellular matrix components, suggesting a possible role in cancer, as impaired proteolysis is a hallmark of cancer (7). Indeed, the substrates of matriptase include urokinase-type plasminogen activator (uPA), hepatocyte growth factor (HGF), macrophage-stimulating protein 1, matrix metalloproteinase 3, and insulin-like growth factor binding protein-related protein-1 (8-12). Matriptase is also critical in maintaining epithelial integrity (13-15), and the mouse homologue of matriptase, epithin, is involved in mammary epithelial growth and morphogenesis (16). Experiments with a mouse model showed that small perturbations of matriptase expression can initiate malignant transformation and critically potentiate the effect of genotoxic exposure, suggesting a causal role of the transmembrane serine protease in human carcinogenesis (17).

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Hepatocyte growth factor activator inhibitor-1 (HAI-1), encoded by the serine peptidase inhibitor, Kunitz type 1 gene (*SPINT1*) on chromosome 15 (18, 19) inhibits matriptase activity (17, 20-22). Interaction between HAI-1 and matriptase is also needed for the activation of matriptase, together with endoproteolytic cleavages (20, 22-24). The cleavage of matriptase at the canonical activation motif of its zymogen may be carried out by another matriptase zymogen molecule as a process of transactivation (24). The newly produced, active matriptase may also activate latent matriptase (24). In the absence of HAI-1, the biosynthesis of matriptase can only reach a low level, due to autoproteolytic activation, leading to a harmful effect on the trafficking of the protease and the cessation of further matriptase translation (24, 25). In the presence of HAI-1 the uncontrolled spontaneous autoproteolytic activation does not occur, or is instead immediately quenched to the levels that allow proper trafficking of matriptase (24, 25). Maintenance of a high HAI-1 to matriptase ratio is necessary to prevent uncontrolled spontaneous matriptase activation, and activated matriptase is bound with HAI-1 immediately after the generation of the former (24, 25). All these considered, HAI-1 is essential for preventing spontaneous activation of matriptase, which is important for the extracellular trafficking and proper expression as well as for preventing uncontrolled activation of matriptase (25).

Matriptase and HAI-1 are expressed in many types of epithelial cells in most epithelium-containing tissues (26-28) and are also shed from epithelial cells (1, 18, 29). Matriptase expression has been reported in a variety of epithelia-derived human cancers, e.g., breast cancer and ovarian cancer (21, 26, 30, 31). The cellular location of matriptase activation and inhibition and the secretory route for the matriptase-HAI-1 complex may vary along with the functional divergence of different epithelial cells (29). Aberrant subcellular localization of the protease may also contribute to its role in breast cancer malignancy (21). Matriptase protein overexpression has been reported to associate with poorer breast cancer survival among node-negative breast cancer cases (31). In another study no association was observed between *ST14* mRNA levels and survival (32). Instead, *SPINT1* mRNA levels were decreased in grade 3 breast tumors compared with grade 1 (32). Recently, a coordinate overexpression of the *ST14, MSP* (macrophage stimulating protein), and *MST1R* (macrophage stimulating protein receptor) genes was observed to associate with metastasis and poor breast cancer prognosis (33). Among Chinese breast cancer cases, matriptase protein expression was increased in more advanced cancers (34).

The mutation G827R in the catalytic domain of matriptase causes autosomal recessive ichthyosis (35, 36), but so far, despite the obvious role in cancer progression, the influence of the genetic variation of matriptase (e.g., polymorphisms) on the risk of breast (or other) cancer or prognosis has not been reported. Previously we carried out an autosome-wide screening using microsatellites to identify new chromosomal regions associated with breast cancer risk in an isolated Eastern Finnish population (37). One of the breast cancer risk-associated regions is the *TMPRSS6* gene encoding TTSP matriptase-2 (38). Due to the sequence similarity between matriptase and matriptase-2 and the various roles that TTSPs may have in cancer progression (39) we determined *ST14* to be a suitable candidate for further investigation.

This is the first study evaluating the role of *ST14* and *SPINT1* gene variants in breast cancer. We genotyped single nucleotide polymorphisms (SNP) on these genes to investigate their association with clinical parameters and with breast cancer survival, and to estimate the risk of breast cancer associated with the variants. We also determined the effect of the genetic variation on gene expression levels and protein expression in breast tumor tissue, and its association with patient outcome (breast cancer survival).

Materials and Methods

DNA samples

DNA from 470 patients with invasive breast cancer and 446 control subjects from the Kuopio Breast Cancer Project (KBCP) sample set were available for genotyping (Supplementary Table S1). The KBCP sample set consisted of 497 prospective breast cancer cases and 458 controls from the province of Northern Savo in Eastern Finland. The cases represent 96% of the total 516 breast cancers diagnosed at Kuopio University Hospital from April 1990 to December 1995. The 458 age- and long-term area-of-residence-matched controls were selected from the National Population Register during the same time period. The KBCP sample material is characterized in more detail by Hartikainen et al. (37) and Pellikainen et al. (40). Genomic DNA was extracted from peripheral blood lymphocytes of both cases and controls using standard procedures (41). The KBCP was approved by the joint ethics committee of Kuopio University and Kuopio University Hospital.

Tumor tissue samples for RNA extraction and cDNA preparation

From 40 breast cancer cases in the KBCP fresh frozen tissue samples of the primary breast tumor were included in the RNA expression analysis (Supplementary Table S1). The tumor samples were obtained during the initial cancer surgery and immediately after resection were covered with optimum cutting temperature compound (Sakura Finetek Europe B.V.) cooled in liquid isopentane and liquid nitrogen and stored in −70°C. RNA extraction and cDNA preparation were done as described by Nykopp et al. (42).

Breast cancer tumor tissue samples in microarray

Paraffin-embedded tumor tissue from the primary tumor was obtained in breast cancer surgery from all breast cancer patients participating in the KBCP. For the present study tissue samples from 377 invasive breast carcinomas

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were available in a tissue microarray format (Supplementary Table S1). All the materials had been fixed in 10% buffered formalin and embedded in paraffin. The array blocks were constructed with a MTA-1 Manual Tissue Arrayer instrument (Beecher Instruments, Inc.). The sample diameter of the tissue core in the array block was 1,000 μm. Each block was produced in triplicate (three biopsies from each sample) for the immunohistochemical staining of matriptase and HAI-1.

SNP selecting

The SNP markers on the *ST14* and *SPINT1* genes were selected utilizing the HapMap (43) and dbSNP (44) databases so that they would cover all linkage disequilibrium blocks on the gene's region according to the HapMap, and the minor allele and rare homozygous genotype frequency would be as high as possible (minimum 0.05). The primary criterion was at covering the linkage disequilibrium blocks, and if the suggested SNPs had a low minor allele frequency a neighboring SNP with a higher minor allele frequency was selected.

SNP genotyping

Genotyping was done by 5′ nuclease assay (TaqMan) using the Mx3000P Real-Time PCR System (Stratagene) according to the manufacturer's instructions. Primers and probes for SNPs rs689589, rs4924508, and rs8028552 in *SPINT1* and rs704624, rs610005, rs530351, rs478795, and rs7123119 in *ST14* were supplied by Applied Biosystems as Assays-on-Demand TaqMan Genotyping Assays. Assays were carried out in 10-μL reactions in 96-well format, with each plate containing 80 to 92 test samples and 4 negative controls (with no DNA). TaqMan Universal PCR Master Mix No AmpErase UNG (Applied Biosystems) and 20 ng of genomic DNA together with the genotyping assay mix were used in the reactions. The thermal profile for the TaqMan reactions was 2 minutes at 50°C, 10 minutes at 95°C, following 50 to 60 cycles of 15 seconds at 92°C and 1 minute at 60°C. Genotypes were determined using the MxPro-Mx3000P v3.01 or MxPro-Mx3000P v4.00 software (Stratagene). Duplicate genotypes were done for 3% to 5% of samples per SNP for quality control. Greater than 98% overall concordance was required. If the duplicate and its pair were discordant the genotypes for this sample would be discarded. If the overall concordance among the duplicates was <98% then the whole SNP would be discarded.

Gene expression analysis

Real-time gene expression analysis was done using TaqMan Gene Expression Assays (Applied Biosystems) according to the manufacturer's instructions and as described by Nykopp et al. (42). The assay numbers for the genes studied were Hs00173678_m1 for *SPINT1*, Hs00222707_m1 for *ST14*, and Hs99999909_m1 for *HPRT1* (hypoxanthine phophoribosyltransferase 1), which was used as the endogenous control (45). The maximum deviation between the expression values of each triplicate sample was allowed to be 0.2. The mean value of the triplicates was used as the raw expression value. Relative gene expression values were calculated as the ratio between the target gene and *HPRT1* and were used in the analyses.

Matriptase and HAI-1 immunohistochemistry

Matriptase and HAI-1 immunohistochemical staining was done in microarray format containing tissue samples of 377 invasive breast carcinomas and using the staining procedure described by Voutilainen et al. (46). The primary antibodies, rabbit polyclonal anti-human matriptase (A300-221A, Bethyl Laboratories Inc.; refs. 34, 47) and rabbit polyclonal anti-human HAI-1 (RP1-HAI-1, Triple Point Biologies Inc.), were diluted with 1% bovine serum albumin in PBS to 1:500 and 1:1,500 working solutions, respectively. Breast cancer tissue sample used as a positive control was selected during the optimization of the antibody dilution. Tissue from the same tumor was also used as the negative staining control. Matriptase and HAI-1 immunostainings were done in triplicate and assessed by three experienced pathologists (V-MK, YS, and RS). Matriptase and HAI-1 immunostainings in the cytoplasm of epithelial cells of the breast tumors were evaluated separately for intensity (0, negative; 1, low intensity; 2, moderate intensity; 3, strong intensity) and quantity (0, 0-5% of cells positive; 1, >5% and ≤50% of cells positive; 2, >50% and ≤75% of cells positive; 3, >75% of cells positive). The score obtained from evaluating the intensity and quantity in each case was added and thus a sum score was obtained with a minimum of 0 and a maximum of 6. A sum score of ≤3 was assessed as negative/low and ≥4 as moderate/strong. Discordant samples were re-evaluated to reach a consensus. For estimating the statistical significance of the combined matriptase and HAI-1 protein expression the matriptase and HAI-1 immunohistochemical staining was combined in the following four groups: 0, negative/low matriptase and negative/low HAI-1; 1, negative/low matriptase and moderate/high HAI-1; 2, moderate/high matriptase and negative/low HAI-1; 3, moderate/high matriptase and moderate/high HAI-1 staining.

Statistical analyses

The significance levels for comparisons of the SNP genotype frequencies between cases and controls, and for the overall association of the genotypes and protein expression with the clinical variables were computed using Fisher's exact test and Monte Carlo approximation implemented in SPSS v 14.0 (SPSS Inc.). The logistic regression analysis implemented in SPSS v 14.0 (SPSS Inc.) was used for computing the significance levels for the risks [odds ratios (OR)] of the associated variables. Consistency of the genotypes with Hardy-Weinberg equilibrium was calculated using the standard χ^2 test. The association between *ST14* and *SPINT1* gene expression with SNP genotypes and protein expression was computed using the independent samples *T*-test in SPSS v 14.0. Univariate survival

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analyses were done using the Kaplan-Meier method and log-rank test in SPSS v 14.0. Multivariate survival analyses providing the risk ratios (RR) for breast cancer death were carried out using Cox's proportional hazards model in a forward stepwise manner with the log-likelihood ratio significance test in SPSS. Breast cancer survival was defined as the time between the date of diagnosis and the date of death due to breast cancer. Metastatic breast cancer cases (n = 16) and one case with stage 0 were omitted from survival analyses and other analyses in which clinical variables were used. Linkage disequilibrium between two SNPs was estimated by calculating the pairwise D' values for the three possible pairs of the *SPINT1* SNPs and the ten pairs of the *ST14* SNPs using cases and controls combined (altogether 861-909 samples for each SNP; Supplementary Tables S2 and S3; ref. 48). $P \leq 0.05$ was considered significant in all analyses and all statistical tests were two sided. P values were not corrected for multiple testing so as not to eliminate potentially important findings. Some of the results may need to be interpreted with caution and could be replicated in independent data sets.

Results

ST14 genetic variant rs704624 predicts poor breast cancer survival

ST14 SNP rs530351, rs7123119, rs704624, and rs610005 genotypes were significantly associated with (invasive) breast cancer survival in the univariate analysis (Supplementary Table S4, Supplementary Fig. S1). The minor allele associated with poorer survival of the patients compared with the major allele. In the multivariate analysis rs704624, rs7123119, and rs610005 remained significant when studied separately (Supplementary Table S5). However, the results concerning rs610005 should be taken cautiously due to a low frequency of the minor allele. In the multivariate analysis including all three SNPs only rs704624 remained significant and thus is considered as an independent factor influencing breast cancer survival [P = 0.001; RR, 2.221; 95% confidence interval (95% CI), 1.382-3.568; Table 1, Fig. 1]. In addition, nodal status, tumor grade, tumor histology, and human epidermal growth factor receptor 2 (HER2) status remained significant prognostic factors in the multivariate analysis. Of these, only nodal status proved to be a more important prognostic factor than rs704624 genotypes (Table 1). The prognostic association of the rs704624 rare allele G was further supported by the association with advanced clinical stage III (P = 0.023; OR, 2.206; 95% CI, 1.140-5.938) and larger tumor size (T_2, T_3, T_4; P = 0.043; OR, 1.495; 95% CI, 1.012-2.209). Moreover, the heterozygous genotype AG associated with a positive nodal status (P = 0.025; OR, 1.639; 95% CI, 1.064-2.524). The *SPINT1* SNP genotypes were not associated with breast cancer survival (data not shown). Association of the rare alleles of *SPINT1* rs4924508 and rs8028552 with a negative progesterone receptor status, with P values of 0.042 and 0.049, and OR of 1.601 (95% CI, 1.017-2.519) and 1.573 (95% CI, 1.001-2.472), respectively, were the only significant associations between *SPINT1* genotypes and clinical parameters. None of the *ST14* or *SPINT1*

Table 1. Parameters significantly associated with breast cancer survival in the multivariate analysis

Variable	B (SE)	Wald	RR (95% CI)	*P*
Nodal status				
Negative		Ref.		
Positive	1.079 (0.228)	22.472	2.943 (1.883-4.598)	0.0000021
rs704624 genotype				
AA		Ref.		
AG&GG	0.798 (0.242)	10.874	2.221 (1.382-3.568)	0.001
Grade				
I		Ref.		
II	1.022 (0.330)	9.557	2.778 (1.453-5.309)	0.002
III	0.807 (0.371)	4.724	2.242 (1.083-4.642)	0.030
HER2 status				
Negative		Ref.		
Positive	0.592 (0.280)	4.478	1.807 (1.045-3.126)	0.034
Histology				
Medullary and other		Ref.		
Ductal	0.073 (0.315)	0.054	1.076 (0.580-1.995)	0.816
Lobular	0.763 (0.355)	4.615	2.145 (1.069-4.303)	0.032

NOTE: Tumor grade, histologic type, tumor size, nodal status, estrogen receptor status, HER2 status, rs704624 genotype, rs7123119 genotype, and rs610005 genotype were included in the analyses.
Abbreviation: B (SE), B coefficient with standard error.

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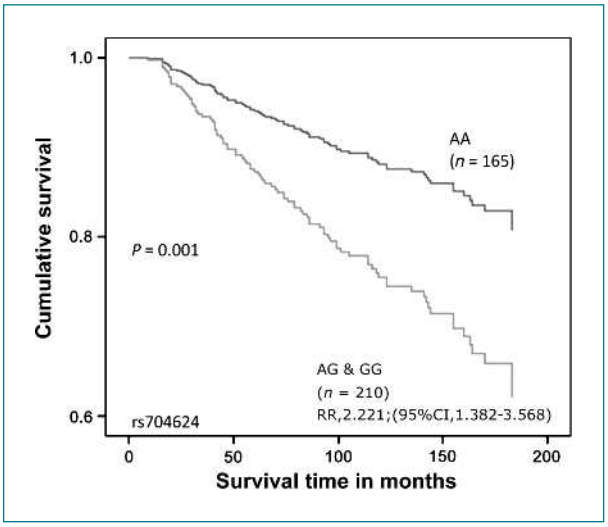

Figure 1. Breast cancer survival in multivariate analysis according to rs704624 genotypes. Analysis stratified by tumor grade, histologic type, tumor size, nodal status, estrogen receptor status, HER status, rs7123119 genotype, and rs610005 genotype.

SNPs associated with breast cancer risk (Supplementary Table S6).

rs704624 rare allele carriers express less *ST14* mRNA

ST14 gene expression levels were significantly lower among the rare allele carriers of the markers rs704624, rs7123119, and rs610005 compared with the common homozygotes (Table 2). Moreover, the *ST14* to *SPINT1* gene expression level ratio was lower among *ST14* rs704624 rare allele carriers (Table 2). *SPINT1* genotypes had no association with *ST14* gene expression, *SPINT1* gene expression, or *ST14* and *SPINT1* gene expression ratio (data not shown). A significant positive correlation was observed between *ST14* and *SPINT1* gene expression (Pearson's correlation $P = 0.000005$).

Decreased matriptase protein expression associates with poorer breast cancer survival

Results of the immunohistochemical staining were obtained from 366 invasive breast cancer samples, and clinical data were available for 348 cases (Fig. 2). In the univariate survival analysis negative/low matriptase protein expression associated with poorer survival than moderate/high protein expression (Fig. 3A). In the multivariate analysis (Cox regression) matriptase protein expression remained an independent factor affecting survival, in addition to nodal status and HER2 status (Fig. 3B). This was further supported by the association of decreased (negative/low) matriptase expression with larger tumor size and more advanced stages, and by association of moderate/high matriptase expression with an early stage and a small tumor size (Table 3). Negative/low matriptase immunohistochemical staining associated also with a lobular histology and positive estrogen receptor status (Table 3). The association of negative/low matriptase

Table 2. Association of *ST14* SNP genotypes with *ST14* mRNA expression and *ST14/SPINT1* mRNA expression level ratio

SNP genotypes	*n*	Mean	Mean difference (95% CI)	*P**
rs478745			0.074 (–4.369 to 4.517)	0.973
TT	23	12.119		
TC+CC	16	12.046		
rs610005			5.965 (0.319-11.611)	**0.039**
GG	34	12.904		
GA+AA	6	6.938		
rs530351			–1.192 (–5.478 to 3.094)	0.574
GG	21	11.443		
GA+AA	19	12.635		
rs7123119			4.095 (0.381-7.808)	**0.032**
TT	28	13.298		
TC+CC	12	9.203		
rs704624			5.332 (1.404-9.260)	**0.009**
AA	20	14.675		
AG+GG	20	9.343		
ST14/SPINT1 ratio				
rs704624			0.342 (0.125-0.672)	**0.043**
AA	20	1.0329		
AG+GG	20	0.695		

**P* value from independent samples *t*-test for equality of means.

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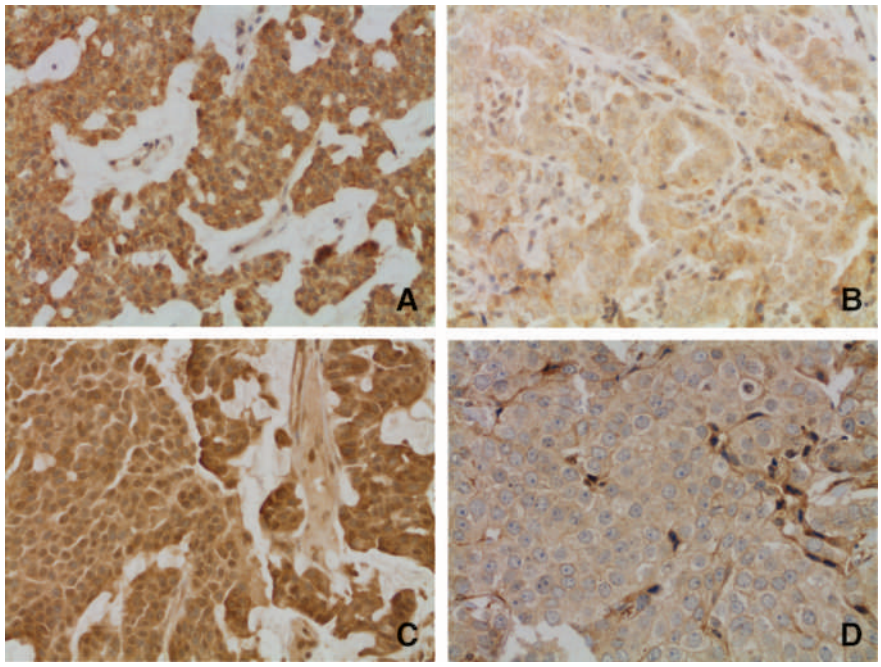

Figure 2. Expression of matriptase and HAI-1 in invasive breast cancer cells. The figures illustrate high (A) and low (B) matriptase and high (C) and low (D) HAI-1 protein expression. Original magnification, ×400.

protein expression with poorer breast cancer survival remained significant also among the node-negative cases only (P = 0.034 in the univariate analysis; P = 0.035; RR, 2.230; 95% CI, 1.059-4.694 in the multivariate analysis; Supplementary Fig. S2).

Matriptase protein expression did not directly associate with *ST14* SNP genotypes or gene expression levels (data not shown). HAI-1 protein expression had no association with breast cancer survival, but negative/low HAI-1 expression associated with more advanced stages, larger tumor sizes, high grade tumors, and a lobular histology (Table 3). Accordingly, moderate/high HAI-1 protein expression associated with early stage, small tumor size, and grade I and II tumors (Table 3). HAI-1 protein expression had no association with *SPINT1* genotypes or gene expression (data not shown).

The combined matriptase and HAI-1 protein expression was also tested for association with breast cancer survival, SNP genotypes, and clinical parameters. The common homozygotes of *SPINT1* SNPs associated with combined moderate/strong matriptase and HAI-1 expression (Supplementary Table S7). There was a positive association between matriptase and HAI-1 protein expression (logistic regression, $P < 10^{-16}$; OR, 9.148; 95% CI, 5.520-15.163; data not shown) and hence the significant associations of the combined matriptase and HAI-1 protein expression with clinical variables were similar to matriptase or HAI-1 alone (Supplementary Table S8). The combined matriptase and HAI-1 protein expression also associated with breast cancer survival similar to matriptase alone (P = 0.046; RR, 1.565; 95% CI, 1.009-2.428 in multivariate analysis; Supplementary Table S9, Supplementary Fig. S3).

Discussion

Although matriptase plays a role in carcinogenesis, the role of its genetic variation and that of HAI-1 in cancer progression or prognosis have not been evaluated. Our results show that the genetic variation of *ST14* influences breast cancer survival. Compared with the homozygous common allele carriers, having the minor allele (*G*) of *ST14* SNP rs704624 predicts significantly worse breast cancer survival. The minor allele of rs704624 is also associated with clinical parameters indicative of advanced tumors and poorer prognosis, such as a positive nodal status, and thus supports the connection with survival. Of the studied SNPs rs704624 had the strongest association with prognosis; it locates in the 3′ untranslated region of the *ST14* gene and it affects the *ST14* gene expression level, which makes it an attractive candidate for the functional variant. Also according to *in silico* analysis the single nucleotide change from A to G at SNP rs704624 (NT_033899.8: g. 33642433) affects putative binding site for transcription factors. However, rs704624 may also be in linkage disequilibrium with the exact functional variant, and the mechanism of its function needs to be investigated. In addition to mutating the possible binding sites of transcription factors and therefore affecting gene expression, sequence variations such as SNPs can disrupt the microRNA-binding site and the microRNA-mRNA interaction and thus affect the expression of microRNA targets.

We also observed that decreased matriptase protein expression associated with poorer survival compared with moderate/high matriptase expression. Because the minor

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alleles of *ST14* SNPs associated with a lower *ST14* gene expression level it could be hypothesized that the *ST14* SNP genotypes with the minor allele would lead to decreased matriptase protein expression. However, the low matriptase protein expression did not associate directly with the rare allele genotypes. It is likely, however, that the protein expression is affected by several factors, including rs704624 genotype, and therefore a final conclusion cannot be made about the relation of *ST14* genetic variation and protein expression. This implies that the genetic variation defined by the SNPs analyzed here would not itself be a factor increasing the risk of breast cancer but instead influences the prognosis. However, future studies using larger sample sets could clarify the issue of *ST14* as a breast cancer risk factor.

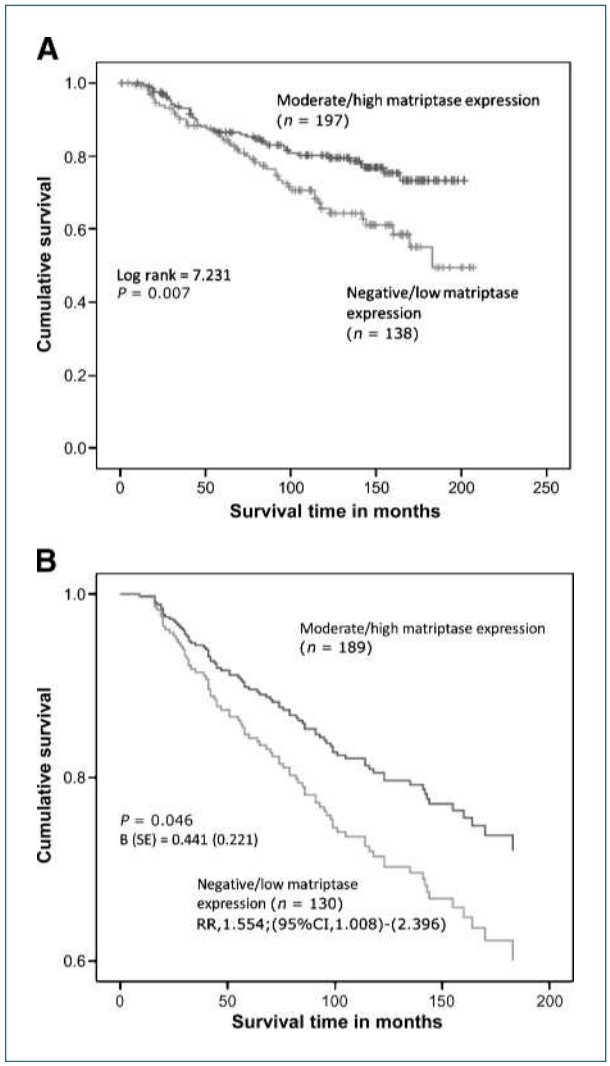

Figure 3. Breast cancer survival according to matriptase immunohistochemical staining. A, univariate analysis (Kaplan-Meier). The percentages of cases alive in each group at the end point time were 73.3% among moderate/high matriptase expression and 49.5% among negative/low matriptase expression. B, multivariate analysis, stratified by tumor grade, histologic type, tumor size, nodal status, estrogen receptor status, and HER2 status. Other parameters significantly associated with breast cancer survival in the multivariate analysis were positive nodal status [$P = 0.00003$; B (SE), 0.974 (0.233); RR, 2.649; 95% CI, 1.677-4.185] and positive HER2 status [$P = 0.039$; B(SE), 0.544 (0.263); RR, 1.722; 95% CI, 1.028-2.885]. B(SE), B coefficient with standard error.

Matriptase has several functions in tumor initiation, cancer progression, metastasis (i.e., activation of uPA and HGF), and cell adhesion and development. Some of these functions require an increased matriptase activity or protein level; thus, one would assume that in progressed cancer a high matriptase level would be expected. In our material a more intense matriptase staining associated with better survival. Previously, Kang et al. (31) reported a worse survival among node-negative breast cancer cases with increased matriptase protein level. We replicated this by selecting the node-negative cases only for the survival analysis. In our material the negative/low matriptase protein expression associated with poorer survival in this subgroup as well. This discrepancy could be explained by the different antibodies used in the studies.

We also observed a significant positive association between matriptase and HAI-1 protein expression. Indeed, it has been reported that in the absence of HAI-1 the biosynthesis of matriptase can only reach a low level (25). The antibodies that we used most likely recognize the total matriptase and HAI-1, not just the activated forms of the proteins. Thus, it is possible that in the samples in which we observed an increased matriptase and HAI-1 protein expression the level of HAI-1 was sufficient to inhibit the activated matriptase, and cancer progression–promoting abnormal activation of matriptase did not occur (most of the total matriptase being latent). Likewise, in the samples in which a decreased matriptase level associated with a poor prognosis the level of HAI-1 might not have been sufficient for matriptase inhibition. Taken together, the functional connection between breast cancer prognosis/survival and matriptase or HAI-1 protein remains to be resolved. It has been suggested that the increased activity of the protease (matriptase) in breast cancer is due to mechanisms other than a simple increase in matriptase protein or mRNA (26). Furthermore, it has been proposed that matriptase may be at the pinnacle of an as yet poorly characterized protease cascade, and activation of matriptase and the putative protease cascade would occur only at the right time, when the exogenous inducers are present, and only at the right place, such as at cell-cell junctions where its substrates might be colocalized or cotranslocated (24). Thus, there might be other yet unknown substrates for matriptase that play a role in breast cancer.

Previously, it was observed in epithelial ovarian cancer that increased matriptase expression was associated with early stage of the disease (30, 49). Moreover, patients with matriptase-positive tumors had longer survival compared with matriptase-negative (49). It was concluded that increased expression of matriptase is mainly associated with early events in the development of malignancy and is downregulated during cancer progression, which

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Table 3. Significant association of matriptase and HAI-1 protein expression with clinical variables

Clinical variable	Protein expression		*P**	OR (95% CI)†
	No. of negative/low	No. of Moderate/high (Ref.)		
Matriptase				
Tumor size			**0.002***	
T_1	49	108		Ref.
T_2, T_3, T_4	84	88	**0.001**	**2.104 (1.340-3.303)**
Tumor stage			**0.019***	
I	37	80		Ref.
II, III	96	116	**0.016**	**1.789 (1.114-2.875)**
Estrogen receptor status			**0.001***	
Negative	19	59		Ref.
Positive	114	134	**0.001**	**2.642 (1.488-4.691)**
Histology			**0.027***	
Medullary and other	15	41		Ref.
Ductal	87	125	0.053	0.526 (0.274-1.009)
Lobular	31	30	**0.009**	**2.824 (1.300-6.135)**
HAI-1				
Tumor stage			**0.008***	
I	40	77		Ref.
II, III	106	106	**0.006**	**1.925 (1.206-3.072)**
Tumor size			**0.00002***	
T_1	51	107		Ref.
T_2, T_3, T_4	95	76	**0.000026**	**2.623 (1.673-4.112)**
Tumor grade			**0.022***	
I	28	52		Ref.
II	62	86	0.310	NS
III	55	45	**0.008**	**2.270 (1.239-4.158)**
Histology			**0.046***	
Medullary and other	18	37		Ref.
Ductal	94	119	0.128	NS
Lobular	34	27	**0.014**	**2.588 (1.215-5.516)**

NOTE: Ref., reference category in the logistic regression analysis.
Abbreviation: NS, not significant.
*P value for overall comparison from χ^2 test for difference in frequencies. Other P values from logistic regression analysis.
†OR and 95% CI values for association with negative or low matriptase and HAI-1 expression from logistic regression analysis.

suggests that matriptase may be important in establishing primary ovarian tumors and it later may function directly or indirectly as a progression inhibitor (49). A recent study also showed that negative matriptase immunohistochemical staining in ovarian cancer specimens associated with poorer survival and that tumors at an early stage of the disease were more often matriptase positive (47). Interestingly, the study used tissue microarray and the same antibody against matriptase as we did (47). Based on these data, and considering our own results, it can be drawn together that, similar to ovarian cancer, matriptase may be important in the early phases of breast cancer development and may later be decreased.

In conclusion, our results suggest that the matriptase genetic variation rs704624 influences the progression and prognosis of breast cancer. In addition, decreased matriptase protein expression, either alone or together with HAI-1, associates with a poorer breast cancer prognosis. This study provides further support for the role of TTSPs in breast cancer progression and encourages further study on the genetic variation affecting protein levels and function in TTSPs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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