

RESEARCH ARTICLE

Expression of *EMSY*, a Novel BRCA2-link Protein, is Associated with Lymph Node Metastasis and Increased Tumor Size in Breast Carcinomas

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Abstract

Background: The *EMSY* gene encodes a BRCA2-binding partner protein that represses the DNA repair function of BRCA2 in non-hereditary breast cancer. Although amplification of *EMSY* gene has been proposed to have prognostic value in breast cancer, no data have been available concerning *EMSY* tissue expression patterns and its associations with clinicopathological features. **Materials and Methods:** In the current study, we examined the expression and localization pattern of *EMSY* protein by immunohistochemistry and assessed its prognostic value in a well-characterized series of 116 unselected breast carcinomas with a mean follow up of 47 months using tissue microarray technique. **Results:** Immunohistochemical expression of *EMSY* protein was detected in 76% of primary breast tumors, localized in nuclear (18%), cytoplasmic (35%) or both cytoplasmic and nuclear sites (23%). Univariate analysis revealed a significant positive association between *EMSY* expression and lymph node metastasis (p value=0.045) and larger tumor size (p value=0.027), as well as a non-significant relation with increased risk of recurrence (p value=0.088), whereas no association with patients' survival (log rank test, p value=0.482), tumor grade or type was observed. **Conclusions:** Herein, we demonstrated for the first time the immunostaining pattern of *EMSY* protein in breast tumors. Our data imply that *EMSY* protein may have impact on clinicopathological parameters and could be considered as a potential target for breast cancer treatment.

Keywords: Breast cancer - *EMSY* - tissue microarray - immunohistochemistry - lymph node metastasis

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Introduction

Breast cancer is the most common malignancies among women worldwide, with an increase in incidence from 10.9 to approximately 20 million new cases per year by the year 2020 (Parkin et al., 2001; Lehmann et al., 2011). Breast cancer also ranks first among cancers diagnosed in Iranian women (Mousavi et al., 2009; Kolahdoozan et al., 2010; Movahedi et al., 2012).

Positive family history is a confirmed risk factor for breast cancer, as the risk to first-degree relatives of a case is almost 2 times the population risk (Collaborative group, 2001). Majority of the familial risk associated with breast cancer have a genetic origin (Ponder, 2001; Benusiglio et al., 2005).

Highly penetrate germline mutations in BRCA1 and BRCA2 account for the large majority of autosomal dominant familial breast-ovarian cancer (Bennett, 1999;

Bane et al., 2011; Mutch et al., 2013). The estimated lifetime risk of breast cancer with a BRCA1 or BRCA2 mutation can be as high as 65%-74 % (Mutch et al., 2013). The prevalence of BRCA mutations varies by population: 0.2 to 0.3 percent in general populations, 3 percent in women with breast cancer, 6 percent in women with breast cancer onset before age 40 years, 10 percent in women with ovarian cancer, and 20 percent in high-risk families (Nelson et al., 2013).

BRCA genes are implicated in RAD 51 and mediate recombinational repair of double stranded DNA breaks, chromatin remodeling and regulation of transcription (Venkitaraman, 2002; Hughes-Davies et al., 2003). Whereas, familiar breast cancer is the cause of 5% of all breast cancers, sporadic breast cancers account for more than 95% of all breast cancer types. Mutations of BRCA1/2 genes are frequently observed in hereditary breast and ovarian cancers, additionally altered expressions of

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BRCA1/2 proteins potentiate sporadic forms of breast cancer (Paul and Paul, 2014).

Nevertheless, the role of BRCA1 and BRCA2 genes in sporadic breast cancer remains unexplained, since somatic mutations in both genes are uncommon (Gayther et al., 1998). Silencing of BRCA1 allele through promoter methylation occurs in somatic breast cancer (Dobrovic and Simpfendorfer, 1997), whereas this mechanism of transcriptional suppression could not explain the absence of somatic BRCA2 mutations in sporadic cancers (Collins et al., 1997).

The discovery of *EMSY* in 2003 by Hughes-Davies et al, links the important role of BRCA2 to sporadic breast-ovarian cancer. *EMSY*, binds to exon 3 of BRCA2 and suppresses the transactivity of BRCA2. *EMSY* is over expressed in sporadic breast and ovarian cancers, suggesting that its overexpression may mimic the effects of BRCA2 inactivation, functionally equivalent to loss of BRCA genes in familial cases (Hughes-Davies et al., 2003). In addition, since *EMSY* is a BRCA-2 partner which modulates BRCA-2 function, it can be concluded that *EMSY* participates in breast cancer development (Livingston, 2004; Yao and Polyak, 2004).

EMSY localizes to the sites of DNA repair following DNA damage and maps to chromosome 11q13.5, 100 kb centromeric to the GARP gene, a locus that is known to be involved in a number of cancers including breast, ovarian and prostate cancers (Nurminen et al., 2011). Recently Nurminen et al showed that 11q13.5 contributes to prostate cancer predisposition with complex genetic structure and is associated with prostate cancer death (Nurminen et al., 2013).

Amplification of *EMSY* has been reported in 13% of sporadic breast cancer, 17% of high grade ovarian cancer and 13 % of sporadic pancreatic adenocarcinomas (Schuuring, 1995; Hughes-Davies et al., 2003; Rodriguez et al., 2004; Brown et al., 2006; van Hattem et al., 2008).

In a recent study conducted by Wilkerson et al, amplification of *EMSY* was found in 10 cancer cell lines from different anatomical origins including breast, ovary, pancreas, oesophagus, lung and the oral cavity (Wilkerson et al., 2011).

EMSY amplification has been associated with worse survival, particularly in node-negative breast cancers, suggesting that *EMSY* may be an indicative of poor outcome of the disease (Hughes-Davies et al., 2003). Therefore, *EMSY* could be a strong candidate oncogene within 11q13.5 region (Rodriguez et al., 2004).

Based on the absence of any data in the literature concerning immunohistochemical analysis of *EMSY* protein expression in tumor tissues, in this study we aimed for the first time to investigate the expression of this protein in an unselected series of breast carcinomas using tissue microarray technique. In addition, correlation of *EMSY* expression with patients and tumor characteristics was assessed.

Materials and Methods

Patients and tumor characteristics

A total of 126 breast tissues from unselected series

(either familial or sporadic) of female patients with primary breast cancer diagnosed between 1996 and 2010 were included in tissue microarrays. Of this, 10 specimens were excluded from the study due to technical problems in tissue processing or lack of possible tumor cells within the core, leaving a total of 116 cases for final evaluation.

This collection comprises female patients ranging from 30 to 79 years of age (mean=52 years) with a long-term follow-up of 2-180 months (mean=47 months). The samples were obtained from Cancer Research Centre; Shohada Hospital affiliated to Shahid Beheshti University of Medical Sciences, Tehran, Iran.

The study of prognostic markers in breast tumors was evaluated based on the REMARK criteria (McShane et al., 2005).

Patient characteristics including age, menopausal status and family history of breast cancer were procured and information on absence or presence of recurrence, and survival was also retrieved from a retrospective database. Tumor characteristics, including histological grade, tumor type (Bloom and Richardson, 1957), tumor size, lymph node involvement were collected and recorded in a database (Table 1).

The tumor grading of all breast cancers were assessed based on Bloom Richardsons system (Bloom and Richardson, 1957) by a team of pathologists including two fixed pathologists over these years.

Information on laboratory tests including Her2, Estrogen Receptor (ER), and Progesterone Receptor (PR) were also assessed routinely and included in database (Table 2). ER and PR status was assessed by immunohistochemistry (IHC) technique and a cut-off of 10% for nuclear staining was used to determine positivity. The assessment of the HER2 status was done by IHC and the results interpreted as positive (3+), negative (0-1+), or equivocal (2+). Only cases with a diffuse intense membrane staining pattern in the tumor (scored as 3+) were considered HER2 positive by IHC.

All patients received surgery (either modified radical mastectomy or breast-conserving surgery) which followed by radiotherapy (90%). Patients with lymph node metastasis even <3 had radiation therapy with at least 2 specific fields and patients with 4 or >4 involved lymph nodes received at least 3 specific fields radiation therapy plus systemic therapy.

One hundred and seven (93%) cases received chemotherapy, at least 5 cases received neoadjuvant chemotherapy (that for these cases tumor size was not a significant data due to chemotherapy efficacy), and fewer patients were given hormone therapy (either tamoxifen or aromatase inhibitors) if ER were positive (Table 2). This study was approved by Iran University of Medical Sciences (IUMS) Research Ethics Committee.

Tissue microarray (TMA) preparation

Breast cancer tissue microarrays were prepared as described previously (Kononen et al., 1998; Mehrazma et al., 2012; Sotoudeh et al., 2012; Mohsenzadegan et al., 2013). All cases were reviewed and the tumor area was marked on their H&E stained slides. Tissue arrays were then constructed by placing 0.6 mm diameter samples

from 50 different tumor samples per single block, with 1 mm spacing separating each specimen. The TMA blocks were constructed in three copies, each containing one sample from a different region of the tumor using tissue-arraying instrument (Minicore; ALPHELYS, Plaisir, France).

Immunohistochemistry

Immunohistochemical detection of *EMSY* was performed on TMA slides (Superfrost plus, Thermo Scientific, Germany) using a standard chain polymer-conjugated (Envision) technique as described previously (Madjd et al., 2011; Madjd et al., 2012; Taeb et al., 2014) applying specific Rabbit polyclonal antibody (ab 4580, abcam, Cambridge, UK). After deparaffinization in xylene and rehydration through graded alcohol, slides were immersed in methanol/hydrogen peroxide for 10 min to block endogenous peroxidase activity. Antigen was retrieved by autoclaving tissue sections for 10 minutes in sodium citrate buffer (pH 6.0). The sections were then incubated with primary antibody with optimal dilution of 1:200 for 1 hour at room temperature. After washing, the sections were incubated with anti-rabbit/anti-mouse Envision (Dako, Denmark) secondary antibody for 30 minutes. Color was developed with addition of 3, 3'-diaminobenzidine (DAB, Dako) to achieve visualization of the antigen. In the final step, sections were lightly counterstained with haematoxylin (Dako), dehydrated in alcohol, cleared in xylene and mounted for examination. The omission of primary antibody and its replacement with TBS (Tris Buffered Saline) was used as negative reagent control.

Evaluation of immunostaining

The immunostained tissue arrays were evaluated using a semi-quantitative scoring system by one observer (ZM) in a coded manner without previous knowledge of clinical and pathological parameters of patients on two separate occasions. In difficult cases, the scoring was confirmed by 2 observers and a consensus was achieved between the scorings.

Initially, the slides were scanned at 10x magnification to obtain a general impression of the overall distribution of the tumor cells and the positive cores were then assessed for localization and semi-quantitatively for expression level at higher magnifications and the final scores were given. The intensity of the staining was scored on a scale as 0 (absent), 1 (weak), 2 (moderate) or 3 (strong). The pattern of expression was categorized as nuclear, cytoplasmic, or combined nuclear and cytoplasmic staining.

Statistical analysis

Statistical analysis of data was performed using SPSS 20 statistical software (SPSS Inc, Chicago, IL). The significance of associations between *EMSY* expression and clinicopathologic variables were analyzed using Pearson's χ^2 and Pearson's R tests.

Survival rates were examined by the Kaplan-Meier method for analysis of censored data. The statistical significance of differences between the survival rates of

groups with different *EMSY* expression was analyzed using the log-rank test. A p value <0.05 was assumed statistically significant.

Results

Study population

At the time of diagnosis, patients' age ranged from 30 to 79 years (mean, 52 years). Twenty (17%) cases were younger than 40 years old, while 96 (83%) ones were over 40. Sixty five (56%) patients were in premenopausal, and 51 (44%) were in postmenopausal status. The information on family history of breast cancer in this series of patients was also recorded. Ninety four (81%) patients had no history of breast cancer in their first relatives and considered as sporadic breast carcinomas, whereas 22 cases (19%) had history of breast cancer in one of their first relatives.

Of the 116 tumors, 21 (18%) cases were grade 1, 49 (42%) cases were grade 2, and 46 (40%) were grade 3. The majority of cases were diagnosed as invasive ductal carcinoma (IDC) comprising 82% of all cases. Tumor size ranged from 1 to 12 cm (mean: 3.8 cm) and categorized in three main groups based on TNM classification of breast cancers: group 1 tumors were 2.0 cm or less in largest dimension comprising 27% (31) of cases. Group 2 tumors were 2 to 5 cm including 54% (63) cases and group 3 tumors were larger than 5.0 cm and included 19% (22) of the tumors.

At the time of the primary diagnosis, 58 (50%) cases

Table 1. Correlation of *EMSY* Expression in Invasive Breast Carcinomas with Clinicopathological Parameters (Pearson's χ^2)

Tumor and patients characteristics	No. (%)	<i>EMSY</i> expression (p-value)
Age (years, mean= 52)		
<40	20 (17)	0.26
>40	96 (83)	
Menopausal status		
Pre-menopausal	65 (56)	0.33
Post-menopausal	51 (44)	
Family history		
No	94 (81)	0.14
Yes	22 (19)	
Histological Grade		
Grade 1	21 (18)	0.48
Grade 2	49 (42)	
Grade 3	46 (40)	
Tumor size (cm)		
<2	31 (27)	0.02
2-5	63 (54)	
>5	22 (19)	
Lymph node (LN) status		0.03
LN negative	59 (51)	
LN positive	57 (49)	
Tumor type		
Invasive ductal carcinoma	95 (82)	0.39
Other tumor types*	21 (18)	
Recurrence		
Absent	84 (72)	0.08
Present	32 (28)	
Patients status		
Alive	90 (78)	0.48
Dead	26 (22)	

*Invasive lobular Carcinoma, Medullary Carcinoma, and infiltrative Carcinoma

were node negative, in 25 (22%) patients 1-3 lymph nodes were involved and in 33 (28%) cases more than 3 lymph nodes have been metastasized.

Among all patients, 90 (78%) were still alive, while 26 (22%) died from breast cancer, and recurrence occurred in 32 (28%) cases. Patients and tumor characteristics, and their correlation with *EMSY* expression are summarized in Table 1.

Expression of EMSY in breast carcinomas

Immunohistochemical expression of *EMSY* within the breast tumors was broadly heterogenous with variety of intensities. The staining pattern of expression was either nuclear, cytoplasmic, or combined pattern in tumor cells. *EMSY* stromal staining was weak and insignificant, and therefore, it was not considered in the analysis.

EMSY expression was found in 88 out of the 116 (76%) unselected invasive breast carcinomas. Nuclear, cytoplasmic and combined pattern of *EMSY* expression

were found in 21 (18%), 41 (35%) and 26 (23%) cases, respectively, whereas 28 (24%) tumors were completely negative for *EMSY* expression (Table 3, Figure 1).

EMSY expression in relation with clinicopathological features of breast carcinomas

The association between expression of *EMSY* and prognostic parameters (histological grade, lymph node involvement, tumor size, tumor type), patient characteristics (age, menopausal status, and familial history) and outcome (overall survival and recurrence) was investigated in total of 116 unselected series of patients and also separately in sporadic breast carcinomas (94/ 116). In univariate analysis of unselected (familial or sporadic) breast cancer patients, a significant positive correlation was observed between *EMSY* expression and lymph node (LN) metastasis ($p=0.045$); i.e. the higher expression of *EMSY* was more often found in LN positive breast tumors particularly those tumors with more than 3 metastatic LN. The expression of *EMSY* protein was also significantly correlated with increased tumor size ($p=0.027$), indicating higher level of expression of *EMSY* in tumors with larger than 5 cm diameter. Furthermore, a relative positive association ($p=0.088$) was evident between *EMSY* expression and recurrence of breast cancer.

To evaluate the potential role of *EMSY* in sporadic breast cancers, the proportion of *EMSY* expression in both sporadic and familial subpopulations was compared.

Table 2. Different Types of Treatment and Laboratory Tests in Breast Carcinomas

Treatment and laboratory tests	Number (%)
Surgery	116 (100)
Yes	116 (100)
No	0
Chemotherapy	107 (93)
Neoadjuvant Chemotherapy	5 (4)
No Adjuvant/Neoadjuvant chemotherapy	4 (3)
Radiotherapy	
Yes	105 (90)
No	11 (10)
Hormone therapy	
Yes	93 (80)
No	23 (20)
Her2	
Positive	49 (42)
Negative	67 (58)
ER	
Positive	71 (61)
Negative	45 (39)
PR	
Positive	71 (61)
Negative	45 (39)

Table 3. The Pattern of Expression of EMSY Protein in Breast Carcinomas

Immunostaining	Number (%)
No staining	28 (24%)
Cytoplasmic staining	41 (35%)
Nuclear staining	21 (18%)
Combined pattern (Cytoplasmic& Nuclear)	26 (23%)

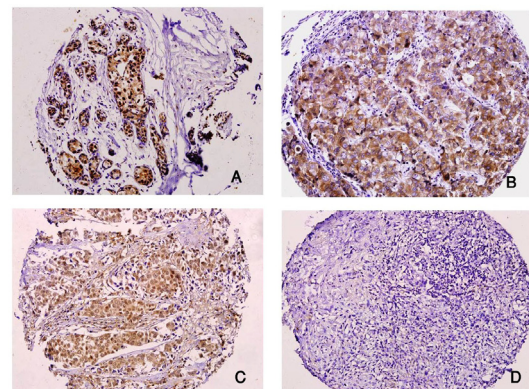


Figure 1. Expression of EMSY Protein in Breast tumor. (A) Nuclear, (B) cytoplasmic (C) combined pattern (cytoplasmic and nuclear) and (D) No staining of *EMSY* was observed in invasive breast carcinomas (magnification $\times 100$)

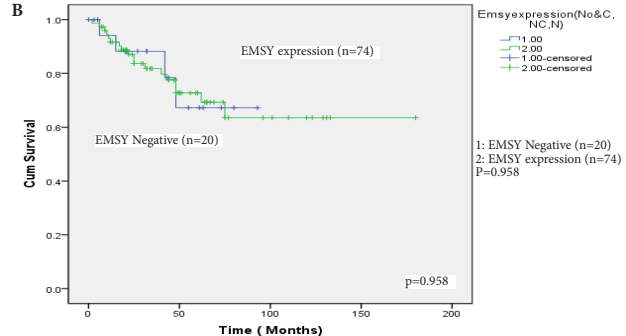
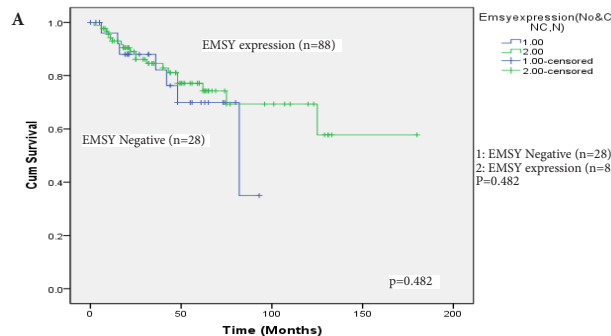


Figure 2. EMSY Expression and Overall Survival. Kaplan-Meier curves comparing breast cancer mortality between women with *EMSY* expression (cytoplasmic, nuclear and combined pattern) and *EMSY* negative breast cancers. No significant correlation was seen between expression of *EMSY* and breast cancer mortality A) 116 unselected series of breast cancer (log rank, $p=0.482$). B) 94 sporadic breast cancer patients, (log rank, $p=0.958$).

Table 4. Correlation of *EMSY* Expression with Lymph node Involvement and Tumor Size of Breast Carcinomas (Pearson's χ^2)

Tumor Features	Total Number	<i>EMSY</i> expression		p-value
		No staining n (%)	Cytoplasmic /Nuclear/ combined pattern n (%)	
Tumor size (cm)				
<2	31	12 (39)	19 (61)	0.02
2-5	63	13 (21)	50 (79)	
>5	22	3 (14)	19 (86)	
Lymph node (LN) involvement				
LN negative	58	19 (33)	39 (67)	0.04
1-3 LN	25	4 (16)	21 (84)	
>3 LN	33	5 (15)	28 (85)	

Seventy nine percent (74/94) of sporadic cases expressed *EMSY*, whereas *EMSY* expression was detected in 64% (14/22) of familial breast cancers. Although the proportion of *EMSY* expression in familial cases was relatively lower compared to sporadic ones, the difference did not reach to statistically significant level.

Univariate analysis was also performed on 94 sporadic cases to evaluate the association between *EMSY* expression and clinicopathological features. As with unselected series of breast cancer patients, in subpopulation of sporadic breast carcinomas, *EMSY* expression was significantly correlated with LN metastasis (p value =0.038) and larger tumor size (p value =0.027).

In contrast, no correlation was demonstrated between expression of *EMSY* protein and histological grade, tumor type, history of familial breast cancer, menopausal status or patient age at time of diagnosis either in total series or fraction of sporadic breast carcinomas (Table 1). Similarly no significant difference was evident between *EMSY* expression and ER status among total series (p value=0.56) and sporadic breast carcinomas (p value=0.31).

Survival analysis in relation to EMSY expression

Correlation between the expression of *EMSY*, and overall survival for all breast cancer patients, with a mean of 47 months (4 years) follow up period, was assessed. Based on Kaplan-Meier analysis, no significant association was found between the *EMSY* expression and patient survival (p value=0.482, Figure 2 A).

Similarly, no significant correlation was seen between the expression of *EMSY* and overall survival of the 94 sporadic breast cancer patients, (p value=0.958, Figure 2 B).

In addition, in spite of various patterns of *EMSY* expression in breast tumors, there was no significant difference in the localization of *EMSY* protein regarding survival data.

Discussion

Breast cancer is the most common cancer diagnosed in women worldwide (Lehmann et al., 2011). It is also reported as the most common malignancy among Iranian females (Sadjadi et al., 2005; Mousavi et al., 2009;

Kolahdoozan et al., 2010; Harirchi et al., 2011). Breast cancer, like other tumor types, is developed as a result of cumulative genetic and epigenetic changes. Although the majority of inherited breast carcinomas caused by germline mutations in the BRCA1 and BRCA2 tumor suppressor genes (Miki et al., 1994; Wooster and Stratton, 1995; Hofmann and Schlag, 2000), the involvement of these genes in sporadic cancers is still uncertain (Yao and Polyak, 2004). Sporadic breast cancers result from a serial multi step accumulation of acquired mutations in somatic genes, without any germ line mutations (Kenemans et al., 2004).

EMSY, a novel BRCA2 binding protein was identified as putative oncogene which involves the BRCA2 pathway in sporadic tumors (Hughes-Davies, 2003). *EMSY* can interact with BRCA2 and inhibit its function, therefore *EMSY* amplification provides a possible explanation for the lack of BRCA2 mutation in sporadic breast cancers and emphasizes on the contribution of this protein in breast tumorigenesis (Hughes-Davies, 2003).

This study aimed to investigate the expression of *EMSY* protein and its correlation with prognostic value and patient outcome, in a well-characterized series of breast carcinomas comprising 94 sporadic and 22 familial cases.

Our immunostaining analysis demonstrated that the majority (76%) of breast carcinomas expressed *EMSY* protein either nuclear or cytoplasmic, whereas in some cases *EMSY* localized in both cytoplasm and nuclear site. In an elegant work by Hughes-Davies et al. using immunofluorescent staining, it was demonstrated that *EMSY*, as with BRCA2, is re-localized to the nucleus in response to DNA damage (Hughes-Davies et al., 2003). In contrast to this finding, the results of our study clearly showed that in addition to nuclear localization, a large proportion of breast cancer cells express *EMSY* in their cytoplasm. It is notable that in this research work, wild type embryonic fibroblastic cell line was used as a model to assess the function of *EMSY* protein (Hughes-Davies et al., 2003). We believe that in cancer cells ectopically expressed proteins may have different localization and serve distinct function.

The proportion of nuclear staining of *EMSY* protein presented here is accordance with previous fluorescence in situ hybridization study reporting that *EMSY* was amplified in 18% (5/28) of breast cancer cell lines and in only 13% (70/551) of primary sporadic breast tumors in a TMA setting (Hughes-Davies et al., 2003).

In this study, it was observed that cell lines with *EMSY* genomic amplification had the highest levels of *EMSY* gene expression, as judged by Quantitative RT-PCR, indicating that amplification of *EMSY* gene leads to increased expression of *EMSY* (Hughes-Davies et al., 2003). Despite of comprehensive survey on *EMSY* gene expression in a complete set of breast cancer cell lines, the expression of its protein has been reported neither in breast cancer cell lines nor in primary breast tumors.

In this study, we report for the first time the expression of *EMSY* protein in primary breast cancer tissues using immunohistochemistry. Expression of *EMSY* was detected in 76% of invasive breast carcinomas investigated, whereas

only 24% of cases were completely negative. Expression of *EMSY* protein was slightly higher in subpopulation of sporadic breast carcinomas compared to unselected or familial cases, but no significant difference was found. This may be due to the limited number of familial cases in our series or involvement of other mechanisms in suppression of *BCRA2* in sporadic breast cancer.

Univariate analysis revealed a positive association between *EMSY* expression and larger tumor size, also an increased incidence of lymph node metastasis; however, there was no association between *EMSY* expression and tumor grade or type. These findings are in agreement with previous study conducted in 364 primary breast tumors using FISH showing that *EMSY* amplification was associated with larger tumor size and lymph node metastasis but no association with tumor type or grade was indicated (Bane et al., 2011). Although various patterns of *EMSY* expression (nuclear, cytoplasmic or combined) was observed in this series of breast tumors, we did not find any significant difference in the localization of *EMSY* protein regarding survival data in spite of this belief that "different localization of *EMSY*" may "serve distinct function".

A recent report on comparing *EMSY* copy number within breast ductal carcinoma in situ (DCIS) by multiplex ligation-dependent probe amplification (MLPA), showed that *EMSY* amplification was more frequent in high-grade DCIS than in low/intermediate-grade DCIS, suggesting that in high grade DCIS, *EMSY* may be potential target for treatment and/or a predictor of progression (Moelans et al., 2010; Moelans et al., 2011). We were unable to investigate *EMSY* expression separately in DCIS due to small number of cases.

In a more recent study by Kornegoor et al, the copy number changes of 21 breast cancer related genes in 110 male breast cancers were evaluated using MLPA, indicating that in male breast cancer *EGFR* and *CCND1* were more often gained than in the female breast cancer group, whereas *EMSY* and *CPD* copy number gain was less frequent (Kornegoor et al., 2012).

Our collection included only female breast cancer; therefore we were unable to show the differences in the level of expression of *EMSY* protein between female and male breast cancers.

Our results also demonstrated that expression of *EMSY* protein was relatively associated with recurrence of breast cancers. Similarly, amplification of *EMSY* was correlated with increased risk of relapse particularly in grade 1 breast tumors in a study performed by southern blotting (Rodriguez et al., 2004).

Furthermore, amplifications of *EMSY* was observed in 7.2% and 9.6% of consecutive and ER+ tamoxifen-treated patients (Kirkegaard et al., 2008) and *EMSY* amplification was correlated with positive ER status in a subset of sporadic breast cancer patients (Rodriguez et al., 2004). Nevertheless, no significant association was evident between *EMSY* expression and ER or PR status in our series of patients.

EMSY amplification has been reported to be associated with a poor patient outcome in some previous studies, using stratified patient groups including lymph node-negative (Hughes-Davies et al., 2003), lymph node-

positive (Rodriguez et al., 2004), or ER-positive breast cancers (Kirkegaard et al., 2008), whereas in other studies such association have not been demonstrated (Bane et al., 2011).

Hughes-Davies et al. showed that in sporadic breast cancers, *EMSY* amplification was associated with worse survival, particularly in node-negative breast cancer, suggesting that it may be of prognostic value, whereas among node-positive patients, no such association was found proposing that *EMSY* amplification alone is not a distinctive risk factor in all breast cancer patients (Hughes-Davies et al., 2003).

In our survival analysis using Kaplan-Meier method, there was no association between overall survival and *EMSY* expression (log-rank test $p=0.482$), even when we restricted the analysis to lymph node positive or lymph node negative tumors. The clinical significance of these findings needs further investigation in larger patient groups.

In conclusion, in the present study, we demonstrated for the first time the immunostaining pattern of *EMSY* protein in an unselected series of breast tumors using tissue microarray technique.

Collectively, our data imply that *EMSY* protein expression is correlated with increased tumor size, lymph node metastasis and relatively higher rate of recurrence. Therefore, *EMSY* may be potential target for breast cancer treatment, but further studies with larger size of patients are required before such conclusion could be consolidated.

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