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BRIEF REPORT

EMSY copy number variation in male breast cancers characterized for *BRCA1* and *BRCA2* mutations

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Abstract
Purpose Male breast cancer (MBC) is a rare disease that shares some similarities with female breast cancer (FBC). Like FBC, genetic susceptibility to MBC can be referred to mutations in *BRCA1* and, particularly, *BRCA2* genes. However, only about 10 % of MBCs are caused by *BRCA1/2* germ-line mutations, while the largest part are sporadic cancers and may derive from somatic alterations. *EMSY*, a *BRCA2* inactivating gene, emerged as a candidate gene involved in the pathogenesis of sporadic FBC, and its amplification was suggested to be the somatic counterpart of *BRCA2* mutations. Considering the relevant role of *BRCA2* in MBC, we aimed at investigating the role of *EMSY* gene copy number variations in male breast tumors.
Methods *EMSY* copy number variations were analyzed by quantitative real-time PCR with TaqMan probes in a selected series of 75 MBCs, characterized for *BRCA1/2* mutations.
Results We reported *EMSY* amplification in 34.7 % of MBCs. A significant association emerged between *EMSY* amplification and *BRCA1/2* mutations ($p = 0.03$). We identified two amplification subgroups characterized by low and high amplification levels, with *BRCA2*-related tumors mostly showing low *EMSY* amplification.
Conclusions Our results show a high frequency of *EMSY* amplification in MBC, thus pointing to a role of *EMSY* in the pathogenesis of this disease. *EMSY* amplification may be a new feature that might uncover underlying molecular pathways of MBCs and may allow for the identification of MBC subgroups with potential clinical implication for targeted therapeutic approaches.

Keywords Male breast cancer · *EMSY* · Copy number variation · *BRCA1/2* germ-line mutations

Introduction

Male breast cancer (MBC) is a rare disease representing less than 1 % of all breast cancers (BCs) and less than 1 % of cancers in men [1–3]. Due to its rarity, information about this disease is still fragmented and clinical approach is mostly based upon data derived from its largely known female counterpart. Indeed, MBC shares some similarities with female breast cancer (FBC) [4, 5].

On the genetic level, MBC susceptibility can be particularly referred to mutations in the two major BC genes, *BRCA1* and, mainly, *BRCA2* [3]. Besides the two well-known BC susceptibility genes, several genes functionally related to *BRCA1* and *BRCA2*, such as *CHEK2* and *PALB2*, have been associated with MBC susceptibility [6–9]. Overall, only about 10 % of MBCs are hereditary diseases. The largest part of MBCs are so-called sporadic cancers and may derive from genetic alterations at somatic level [10–12].

EMSY (c11orf30) gene maps on chromosome 11q13.5, a locus harboring several potential oncogenic drivers frequently amplified in BC. *EMSY* encodes for a protein that

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can bind and inactivate BRCA2 and was shown to localize to sites of repair after DNA damage [13]. Since its first description, EMSY inactivating function over BRCA2 pointed to a major role for *EMSY* gene into DNA repair mechanism and in cancer pathogenesis. As expected, *EMSY* involvement in tumorigenesis has been primarily reported in *BRCA2*-related tumors, mostly breast and ovarian cancers [13, 14].

Studies focusing on sporadic FBC have shown that these tumors may carry amplification of *EMSY*, suggesting that *EMSY* amplification could be the somatic counterpart of *BRCA2* mutations and may thus explain sporadic BC development [13, 15]. In fact, FBCs with *EMSY* amplification and *BRCA2* deletion seem to display the same pathology, suggesting that overexpression of *EMSY* and deletion of *BRCA2* may insist on a specific pathway [13].

Given the role of *EMSY* in the DNA repair process, it has been hypothesized that *EMSY* amplification could represent a putative biomarker to evaluate the sensitivity to treatment with drugs targeting DNA repair mechanisms, such as PARP inhibitors. PARP inhibitors are generally used for treatment of several cancer types characterized by *BRCA1/2* mutations [16]. Recent clinical data reported a possible use for these drugs also in patients with sporadic cancers, mostly ovarian cancers (OC) [17]. The contribution of *EMSY* amplification in affecting PARP inhibitors sensitivity in sporadic BCs and OCs has recently been investigated with contrasting results [18, 19].

Over the recent years, *EMSY* amplification in FBC has been studied and described with a frequency ranging from 7 to 25 % [13, 15, 20–23]. The parallel view between MBC and FBC and the growing data about an implication of gene copy number variations (CNVs) as a relevant pathogenic mechanism in sporadic MBC [24–28] lead to the attempt to characterize *EMSY* CNV in MBC. In 2012, Kornegoor et al. reported *EMSY* amplification in MBC as a less frequent event compared to FBC [27], but information about the real impact of this gene on MBC development, particularly in relation to *BRCA1/2* mutations, are still lacking.

In order to deeply characterize *EMSY* involvement in male breast tumor pathogenesis, we analyzed *EMSY* CNV in a selected series of MBC cases, characterized for *BRCA1* and *BRCA2* mutations.

Materials and methods

A total of 75 MBC cases were included in the study. The series included 10 *BRCA1/2*-positive (2 *BRCA1* and 8 *BRCA2*) and 65 *BRCA1/2*-negative tumors. Cases were also characterized for the main clinical-pathologic features, including estrogen and progesterone receptor (ER and PR) status, HER2 and MIB1 expression, and tumor grade (G). As shown in Table 1, 86.3 % of the tumors were ER-positive, 78.1 % PR-positive, 71 % HER2-negative, 57.1 % MIB1-negative and 47.1 % were G2.

Tumor DNA was extracted from FFPE and fresh frozen tissue sections following standard protocols. After extraction, DNA quantity was assessed through NanoDrop 1000 spectrophotometer (Thermo Scientific).

EMSY CNVs were analyzed by quantitative real-time PCR (qRT-PCR) using TaqMan probes (Life Technologies, Carlsbad, California, USA). TaqMan Copy Number assay for *EMSY* (Hs02953503_cn) and TaqMan Copy Number RNaseP Reference Assay containing primer and probe mix for target and housekeeping gene, respectively, were used (Life Technologies, Carlsbad, California, USA). Real-time PCR reaction was carried out in quadruplicate using a PRISM 7500 Fast platform (Life Technologies, Carlsbad, California, USA). *EMSY* copy number status, relative to the housekeeping gene *RNaseP*, was assessed using the $2^{-\Delta\Delta C_t}$ method and considering a normal male breast tissue sample as a calibrator. For each sample, a RQ value (where $RQ = 2^{-\Delta\Delta C_t}$) was obtained. Samples with RQ value <1.5, between 1.5 and 2, and ≥2 were defined as having no amplification, gain and amplification, respectively. Based on RQ values, low and high amplification levels were defined as follows: low amplification with $2 \leq RQ < 3$, high amplification with $RQ \geq 3$. Associations between

Table 1 Clinical-pathologic features of the male breast cancer series analyzed

Clinical-pathologic features[a]	*N* (%)
BRCA1/2 mutation status	
BRCA1 mutation	2 (2.6)
BRCA2 mutation	8 (10.7)
Negative	65 (86.7)
ER status	
Negative	10 (13.7)
Positive	63 (86.3)
PR status	
Negative	16 (21.9)
Positive	57 (78.1)
HER2 status	
Negative	49 (71.0)
Positive	20 (29.0)
MIB1 status	
Negative	40 (57.1)
Positive	30 (42.9)
Grade	
G1	13 (18.6)
G2	33 (47.1)
G3	24 (34.3)

[a] Some data are missing

EMSY CNV and clinical-pathologic features were evaluated using 2 × 3 Fisher's Exact test. A p value <0.05 was considered statistically significant.

Informed consent was obtained from all individual participants included in the study. The study was approved by the local ethical committee (Sapienza University of Rome, Protocol 264/12).

Results

Analysis of *EMSY* CNV was performed in a selected series of 75 MBC cases. As reported in Table 2, among the 75 tumors examined, 30 cases (40 %) showed no *EMSY* amplification, 19 cases (25.3 %) were characterized by *EMSY* gain, and 26 cases (34.7 %) showed *EMSY* amplification. Based on RQ values, two different subgroups were observed among the 26 cases with *EMSY* amplification: a first subgroup with RQ values between 2 and 3, defined as low amplification, included 17 cases (65.4 %), and a second subgroup with RQ values ≥3, defined as high amplification, included nine cases (34.6 %). Of the eight *BRCA2*-related tumors, five (62.5 %) showed low *EMSY* amplification and three (37.5 %) showed no *EMSY* amplification. Of the two *BRCA1*-related cases, one showed low *EMSY* amplification and the other showed high *EMSY* amplification (Fig. 1).

Possible associations between *EMSY* CNV and *BRCA1/2* mutation status, and between *EMSY* CNV and main pathologic features were evaluated. Considering both *BRCA1* and *BRCA2* mutations, a significant association between *EMSY* amplification and *BRCA1/2* mutations emerged ($p = 0.03$). However when considering only *BRCA2* mutations, no statistically significant association between *EMSY* amplification and *BRCA2* mutations was observed ($p = 0.10$). Associations between *EMSY* CNV and pathologic features, including ER and PR status, HER2 and MIB1 expression and tumor grade were also evaluated but no statistically significant associations emerged (Table 2).

Discussion

Aimed at investigating whether *EMSY* CNV could be a candidate pathogenic mechanism for MBC, we analyzed *EMSY* CNV in a selected series of MBC cases characterized for *BRCA1/2* mutations.

Table 2 Results from *EMSY* copy number variation analysis on 75 male breast cancer cases and associations with clinical-pathologic features

	No amplification (RQ < 1.5)	Gain (1.5 ≤ RQ < 2)	Amplification (RQ ≥ 2)	p[a]
Number of cases (%)	30 (40.0)	19 (25.3)	26 (34.7)	
BRCA1/2 mutation				
Pos	3	0	7	**0.03**
Neg	27	19	19	
BRCA2 mutation				
Pos	3	0	5	0.10
Neg	27	19	21	
ER				
Pos	24	19	20	0.10
Neg	5	0	5	
PR				
Pos	20	18	19	0.09
Neg	9	1	6	
HER2				
Pos	11	3	6	0.22
Neg	17	16	16	
MIB1				
Pos	14	9	7	0.38
Neg	14	10	16	
Grade				
G1/G2	17	15	14	0.18
G3	12	3	9	

Pos positive, *neg* negative

[a] 2 × 3 Fisher's Exact test; statistically significant associations ($p < 0.05$) are reported in bold

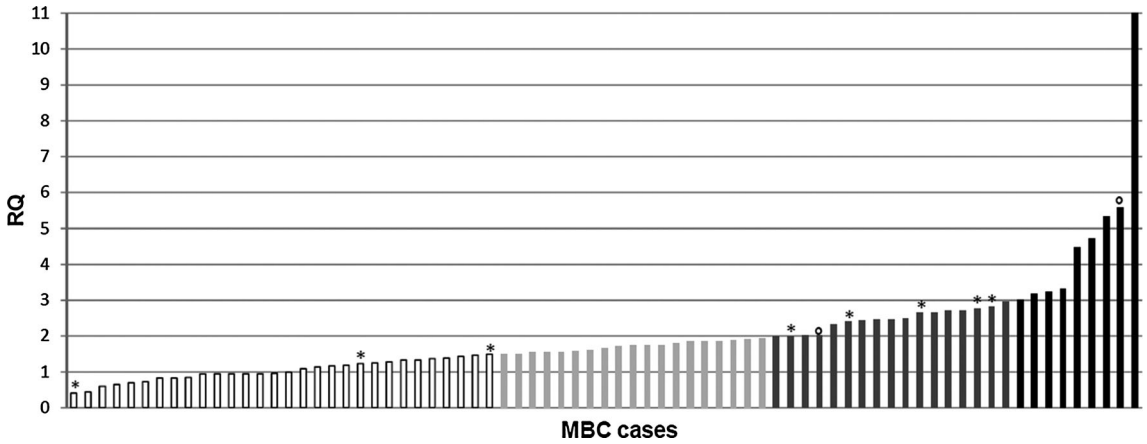

Fig. 1 Histogram showing *EMSY* copy number variation analysis in 75 male breast cancer cases characterized for *BRCA1/2* mutations. Distribution of RQ values is shown. Four different subgroups were identified by RQ values: No amplification (RQ < 1.5; *white columns*); Gain ($1.5 \leq$ RQ < 2; *light gray columns*); Low amplification ($2 \leq$ RQ < 3; *gray columns*); High amplification (RQ ≥ 3; *black columns*). **BRCA2*-related cases, °*BRCA1*-related cases

To date, only one study has investigated the involvement of *EMSY* alterations in MBC [27]. In this study, by Kornegoor et al. *EMSY* CNV was evaluated in a series of MBCs, not characterized for *BRCA1/2* mutations, by MLPA using a gene panel. Results from this study showed *EMSY* amplification in 2 % and *EMSY* gain in 10 % of MBCs. In our study, we performed a targeted analysis of *EMSY* copy number variations by qRT-PCR with TaqMan probes, a sensitive and specific quantitation method. We found a frequency of 34.7 and 25.3 % for *EMSY* amplification and *EMSY* gain, respectively. Compared with the study of Kornegoor et al. we reported a higher frequency of *EMSY* CNVs in MBC. Our data are in line with the frequency of *EMSY* amplification in FBC and indicate a similarity among the somatic alterations involved in tumor development in female and male breast tumors.

We also investigated possible differences between *BRCA*-related and not related MBCs and observed a statistically significant association between *EMSY* amplification and the presence of *BRCA1/2* mutations ($p = 0.03$). This result may suggest that *EMSY* amplification and *BRCA1/2* mutations may be involved in a specific pathogenic pathway in MBC.

However, the association was not confirmed when considering only *BRCA2* mutations. Brown et al. reported that in FBC *EMSY* gene amplification was a frequent event in *BRCA1*-related tumors, while was less common in *BRCA2*-related tumors [21]. Although it is limited by the low number of *BRCA1*-related tumors analyzed, there was evidence of similar trend in our MBC series. Furthermore, we defined two subgroups within cases with *EMSY* amplification characterized by low and high amplification levels. We observed that *BRCA2*-related tumors do not show high *EMSY* amplification but rather they were characterized by low or no *EMSY* amplification. Intriguingly, both *BRCA1*-related tumors showed *EMSY* amplification, one at high and the other at low level. Thus, our findings would suggest a possible relationship between *BRCA1* mutations and a high increase in *EMSY* gene copy number and between *BRCA2* mutations and low or no increase in *EMSY* gene copy number.

Previous studies on FBCs described an association between *EMSY* amplification and ER-positive status and poor prognosis [15, 20, 21]. In our study, no significant association between *EMSY* CNVs and ER status emerged, but this could be affected by the high proportion of ER-positive tumors in our series, as MBCs are more likely to be ER-positive than FBCs [3]. In addition, no significant associations between *EMSY* amplification status and the other main pathologic features (including PR status, HER2 and MIB1 expression and tumor grade) emerged. These observations deserve further investigation on a larger sample series.

Due to its role in DNA repair mechanism, *EMSY* has been investigated for its possible role as predictor of response to PARP inhibitors [19]. Indeed, it has been proposed that, as for *BRCA* mutations, amplification of *EMSY* may also lead to enhanced sensitivity to PARP inhibitors [13, 19]. Thus, our data might be eventually relevant for identification of MBC cases that would possibly benefit from targeted therapeutic approaches.

Recently, a work by Viré et al. reported a new intriguing *EMSY* function [29]. In particular, they identified a group of miRNAs whose expression seems to be deregulated by *EMSY* amplification in BC cells. One of these miRNAs, miR-31, is a known regulator of metastatic mechanism in BC, thus suggesting a role for *EMSY* also in the regulation of the metastatic process in BC. Based on these findings, it

could be hypothesized that the different MBC subgroups identified in our work may be characterized by different miRNA profiles related to different *EMSY* amplification levels. Further studies are needed to prove this hypothesis.

In conclusion, our study may contribute to define molecular mechanisms underlying MBC pathogenesis. The characterization of MBC cases by *EMSY* amplification could help in unraveling the heterogeneity of male breast tumors allowing the definition of MBC subgroups with features possibly relevant for clinical management and therapeutic approach.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The experiments comply with the current laws of Italy. Informed consent was obtained from all individual participants included in the study. The study was approved by the local ethical committee (Sapienza University of Rome, Protocol 264/12).

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