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Original Article

Molecular subtypes of male breast cancer by immunohistochemistry

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Abstract: Male breast cancer is a rare disease, accounting for less than 1% of all breast cancer cases worldwide. Compared to female breast cancer, the incidence of male breast cancer has risen in recent years, and the relationship between molecular subtype and clinical behavior has rarely been studied. In this study, we examined the molecular subtypes of male breast cancers based on the expression profile of immunomarkers and their association with clinicopathological features. A total of 98 male breast carcinoma patients were investigated retrospectively using immunostaining for estrogen receptor (ER), progesterone receptor (PR), HER2, Ki-67, and P53. Results were interpreted based on the molecular subtype classification of the 13th St Gallen International Expert Consensus (2013). HER2 expression that was 2+ or 3+ was also evaluated by fluorescent in situ hybridization (FISH) for further validation. The luminal subtype A was the most common in all patients (83.7%, 82/98), followed by the luminal subtype B (16.3%, 16/98). HER-2 subtype and basal-like subtype were not detected. Male breast cancers were classified into luminal subtype A and luminal subtype B, and the HER-2 over-expressing and basal-like subtypes were not found in this study.

Keywords: Molecular subtype, male breast cancer, immunohistochemistry

Introduction

Male breast cancer (MBC) is an uncommon disease, accounting for approximately 1% of all breast cancer cases and less than 1% of all malignancies in men [1, 2]. MBC is a specific subgroup of breast cancer due to its rarity, different therapy strategy, and the poor prognosis if the diagnosis is delayed due to lack of awareness as compared with female breast cancer [3-6]. Different from female breast cancer (FBC), MBC occurs later in life and resembles postmenopausal breast cancer in females [7]. In cases of familial breast cancer, BRCA2 carriers are more common in men that in women. Lesions may be easier to find in men due to the smaller breast size. Furthermore, large and randomized clinical trials for individual therapy of male breast cancer are lacking.

Immunohistochemistry (IHC) is used for breast cancer classification because the routine application of microarray gene expression analysis is not feasible. Based on gene expression studies, updated IHC subtype definitions as luminal subtype A (estrogen receptor [ER]+ and/or progesterone receptor [PR]+, human epidermal growth factor receptor 2 [HER2]-), luminal subtype B (ER+ and/or PR+, HER2+), HER2+/ER- subtype (ER-, PR-, HER2+), and basal-like subtype (ER-, PR-, HER2-, CK5/6+) [8]. In 2013, the 13th St. Gallen International Expert Consensus revised the subtypes as follows: luminal subtype A (ER+/HER2-/Ki-67+, ≤ 14% and PR+, ≥ 20%), luminal subtype B (HER2-) (ER+/HER2-/Ki-67 +, > 14% or PR+, < 20%), luminal subtype B (HER2+) (ER+/HER2+/Ki-67± and PR±), HER2 over-expressing subtype (ER-/HER2+ and PR-), basal-like subtype (ER-/HER2- and PR-) [9, 10]. This change in classification allowed different breast cancer subtypes to reflect specific genetic alterations in the process of carcinogenesis and progression.

Despite great achievement in the prevention, diagnosis, and treatment of FBC, strategies for MBC are limited and the principles of management are largely derived from randomized trials performed in women. The relationship between molecular subtypes of MBC and clinical behaviors has rarely well studied [11, 12]. In current study, we evaluated the molecular subtypes of 98 cases of MBC by immunohistochemistry and examined the correlation with clinicopathological features.

Materials and methods

Patients

A total of 106 MBC patients treated at the Harbin Medical University Cancer Hospital from January 1, 1993 to July 31, 2013 were identified in the hospital database. Clinical data including age, stage of breast cancer at diagnosis according to TNM Classification of Malignant tumors defined by the Sixth Edition of the American Joint Committee on Cancer (AJCC), and vital status were available for 98 patients, and thus 98 patients were included in the analysis. The study protocol was carried out with approval by the Ethics Committee of Harbin Medical University.

Specimens

All samples were surgical specimens collected between January 1, 1993 and July 31, 2013 at the Department of Pathology of the Third Hospital of Harbin Medical University (Harbin, China). Each sample was subjected to immunohistochemistry for determination of ER, PR, Ki-67, P53, and HER2 status according to established clinical guidelines. HER2 was scored by using the current American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines [13].

Immunohistochemistry

Tissue sections were dried at 70°C for 3 h. After de-paraffinization and hydration, sections were washed in phosphate-buffered saline (PBS) three times, 3 min each time. The washed sections were treated with 3% H_2O_2 in the dark for 5-20 min. After washing in distilled water, sections were washed in PBS three times, 5 min each time. Antigen retrieval was performed in citrate buffer (pH 6.0). Each section was then treated with 300-500 ml of rabbit monoclonal antibody solution at a suitable dilution according to the manufacturer's instructions (Abcam, Hong Kong) at 4°C overnight. After washing in PBS three times, 3 min each time, each section was incubated with 300-500 ml secondary antibody at room temperature for 30 min. After washing in PBS three times, 5 min each time, each section was treated with 300-500 ml of a diaminobenzidine working solution at room temperature for 3-10 min, and then washed in distilled water.

Immunohistochemistry evaluation

The staining patterns and intensities of each of the markers were interpreted by two pathologists independently who were unaware of other tumor characteristics or staining results. For ER and PR, nuclear staining in more than 10% of tumor cells was classified as positive staining. Staining intensity was divided into four grades, with grades 0 and 1 considered as negative, grade 2 as indeterminate, and grade 3 as positive. Positive HER2 staining was defined as > 2+ membranous staining of tumor cells based on the conventional three-tier grading criteria. All cases with ≥ 2+ HER2 immunostaining were further confirmed by fluorescence in situ hybridization (FISH).

New IHC molecular subtype criteria

The immunohistochemistry-based definition of breast cancer subtypes used in this study was the molecular subtype classification scheme proposed by the 13th St. Gallen International Expert Consensus in 2013: luminal subtype A (ER+/HER2-/Ki-67+, ≤ 14% and PR+, ≥ 20%), luminal subtype B (HER2-) (ER+/HER2-/Ki-67 +, > 14% or PR+, < 20%), luminal subtype B (HER2+) (ER+/HER2+/Ki-67± and PR±), HER2 over-expressing subtype (ER-/HER2+ and PR-), basal-like subtype (ER-/HER2- and PR-) [9, 10].

Statistical analysis

The associations between categorical variables were assessed by χ^2 test or Fisher's exact test. All analyses were performed using SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA). Two-sided values of $P < 0.05$ were considered to indicate statistical significance.

Results

The clinicopathological characteristics of the 98 cases of MBC are summarized in **Table 1**.

Table 1. Clinicopathologic characteristics of 98 male breast cancer patients

Variables	No. of case (%)	Luminal A (%)	Luminal B (%)	X^2	*P* value
Age				0.09	0.768
< 60	40 (40.8)	34 (34.7)	6 (6.1)		
≥ 60	58 (59.2)	48 (49.0)	10 (10.2)		
Tumor size				4.84	0.089
pT1	22 (22.4)	20 (20.4)	2 (2.0)		
pT2	62 (63.3)	53 (54.1)	9 (9.2)		
pT3/pT4	14 (14.3)	9 (9.2)	5 (5.1)		
Histology Grade				21.22	0.000
G1	45 (45.9)	42 (42.9)	3 (3.0)		
G2	42 (42.9)	36 (36.7)	6(6.2)		
G3	11 (11.2)	4 (4.1)	7 (7.1)		
Histologic type				1.42	0.491
Invasive ductal carcinoma	85 (86.7)	72 (73.5)	13 (13.2)		
Invasive lobular carcinoma	11 (11.2)	8 (8.2)	3 (3.0)		
Mixed (ductal/lobular)	2 (2.1)	2 (2.1)	0 (0.0)		
Lymph node status				5.85	0.054
pN0	34 (34.7)	30 (30.6)	4 (4.1)		
pN1	55 (56.1)	47 (47.9)	8 (8.2)		
pN2/pN3	9 (9.2)	5 (5.1)	4 (4.1)		

The mean patient age was 63 years (range, 48 to 86 years). No statistically significant difference in age was found between patients with luminal subtype B tumors and luminal subtype A tumors (P = 0.25). Most of the patients were diagnosed with stage I to II disease (81.6%, 80/98), and they were treated surgically (94.9%, 93/98) either by lumpectomy or mastectomy. Many patients also received other treatments including hormonal therapy (87.8%, 86/98), chemotherapy (64.3%, 63/98), and radiation therapy (19.4%, 19/98).

Histologic characteristics

Of the 98 MBC cases, 85 (86.7%) were invasive ductal carcinomas, 11 (11.2%) were invasive lobular carcinomas, and 2 (2.0%) were mixed (ductal/lobular) type based on IHC analysis. Invasive ductal carcinoma was more often found in luminal subtype A breast cancer as compared to luminal subtype B (P = 0.491, **Table 1**). Invasive lobular carcinomas were seen both in luminal subtype A and subtype B, while the mixed type tumor was detected in luminal subtype A only (**Table 1**). With respect to nuclear grade, the majority part of the luminal subtype A tumors (95.1%, 78/82) had a low and intermediate nuclear grade (modified Black's nuclear grade 1 and 2) and the remaining (4.9%, 4/82) had a high nuclear grade (grade 3). In comparison, high nuclear grade (grade 3) tumors were more frequent in luminal subtype B tumors (43.8%, 7/16, **Figure 1A**) than in luminal subtype A tumors (P = 0.000, **Table 1**). Patients with luminal subtype B tumors had a trend towards pT2 and pT3/pT4 tumor size (88.5%, 14/16) as compared with those with luminal subtype A tumors (75.6%, 62/82) (P = 0.089). With respected to lymph node metastasis, luminal subtype B tumors also had a trend towards more lymph node involvement (75.0%, 12/16) as compared with patients with luminal subtype A tumors (63.4%, 52/82) (P = 0.054).

IHC expression profiles

The pathological expression profiles of the MBCs are shown in **Table 2**. The luminal A subtype was the most common subtype in this study (83.7%, 82/98), followed by the luminal B subtype (16.3%, 16/98). The IHC staining pictures of luminal A subtype tumor and luminal B subtype tumor are shown in **Figure 1**. There were no cases of the HER2 over-expressing subtype or the basal-like subtype. ER expression was noted in 95.9% (94/98) of the specimens, and PR expression in 60.2% (59/98) of the specimens. Luminal subtype A tumors had a higher frequency of PR expression (64.6%, 53/82) than luminal subtype B tumors (37.5%, 6/16) (P = 0.043). High Ki-67 expression was seen in 24 cases, while low expression was seen in 74. There was a trend towards high Ki-67 expression in luminal subtype A as compared to luminal subtype B tumors (P = 0.106). No significant difference in P53 expression was noted between luminal subtype A and B tumors (P = 0.373).

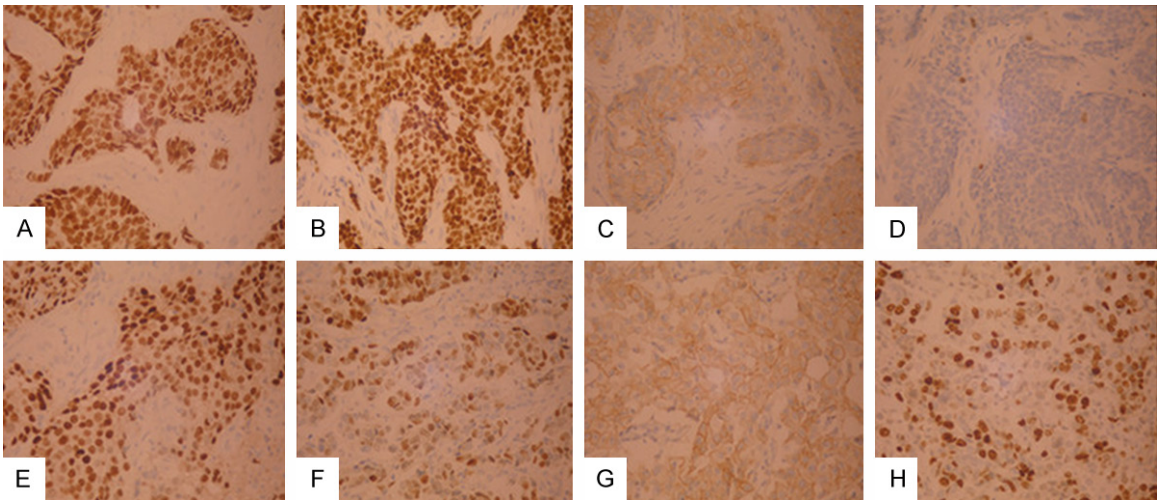

Figure 1. Representative pictures of MBC specimen with Immunohistochemistry. The upper four pictures from luminal (A) subtype tumors show the expression of ER (A), PR (B), Her-2 (C), Ki-67 (D), respectively. The lower four pictures from luminal (B) subtype tumors show the expression of ER (E), PR (F), Her-2 (G), Ki-67 (H), respectively. Top panel, magnification×200; bottom panel, magnification ×200.

Of the 98 breast cancer specimens, expression of HER2 in eight and 11 subjects was regarded as 2+ and 1+, respectively. FISH was performed on the eight 2+ cases; results showed that no specimen was the HER2 over-expressing subtype.

Discussion

Due to its rarity, MBC is a specific subgroup of breast cancer and the principles of management are largely derived from the experiences of treatment of FBC. Molecular subtypes of FBC have been well studied while few studies, especially in China, have examined molecular subtypes in MBC.

According to IHC subtype definitions, luminal subtype A (83.7%) and subtype B (16.3%) were observed in this study, while basal-like subtype and HER2 over-expressing subtype were not observed. This result is different than that of other studies of MBC which reported much higher frequencies of luminal subtype B [14, 15]. ER expression was seen in 95.9% of the samples in our study, which is consistent with previous studies that have shown MBCs exhibit a higher percentage of ER positivity (81% to 100%) than FBCs [16-18]. The variations of results between studied may be due to different races examined and different IHC definitions [19-22].

The basal-like subtype is associated with high-grade tumors, younger age, and an overall worse prognosis, and is seen in approximately 16% of FBCs [23, 24]. While the current study showed no basal-like breast cancer in men, it is in agreement with other published researches by immunohistochemistry assay [25-27]. HER2 over-expression is associated with poor survival in patients with FBC, and approximately 25-30% of invasive FBCs exhibit HER2 over-expression [28]. However, studies of HER2 over-expression are conflicting and inconsistent. HER2 over-expression was reported to be similar or higher in men than women in several studies [29-35]. A study reported that 11% of 99 MBC patients were HER2+ by FISH assay [36]. In our study of 98 cases, eight MBC patients were HER2 2+ and 11 were 1+ by IHC analysis but none were HER2+ by FISH validation. Differences in race, cancer heterogeneity, pathological scoring systems, and cut-off values for positive immunostaining and FISH analysis may be the reasons for inconsistence in these studies.

In this study, invasive lobular carcinomas were seen both in luminal subtype A and subtype B tumors, followed by the mixed invasive lobular carcinoma (ductal/lobular) type. Other pathological subtypes were not detected in the 98 MBC patients. Luminal subtype B carcinomas exhibited a larger size than luminal subtype A tumors, and high nuclear grade (grade 3) tumors were more frequent in luminal subtype B than in luminal subtype A lesions ($P = 0.000$) while the majority of luminal subtype A tumors

Table 2. Immunohistochemistry characteristics of 98 male breast cancer patients

Variables	No. of case (%)	Luminal A (%)	Luminal B (%)	X^2	*P* value
ER status				0.81	0.367
Positive	94 (95.9)	78 (79.6)	16 (16.3)		
Negative	4 (4.1)	4 (4.1)	0 (0.0)		
PR status				4.11	0.043
Positive	59 (60.2)	53 (54.0)	6 (6.2)		
Negative	39 (39.8)	29 (29.6)	10 (10.2)		
Ki67 status				3.84	0.050
≤ 14%	74 (75.5)	65 (66.3)	9 (9.2)		
> 14%	24 (24.4)	17 (17.3)	7 (7.1)		
P53 status				0.79	0.373
Positive	64 (65.3)	52 (53.1)	12 (12.2)		
Negative	34 (34.7)	30 (30.6)	4 (4.1)		
Her-2 status				-	-
Positive	0 (0.0)	0 (0.0)	0 (0.0)		
Negative	98 (100.0)	82 (83.7)	16 (16.3)		

had low and intermediate nuclear grade (modified Black's nuclear grade 1 and 2). With respected to lymph node metastasis, luminal subtype B tumors were associated with greater lymph node involvement that luminal subtype A tumors. Our results are in line with a prior study that reported luminal subtype B tumors were more frequently associated with high nuclear grade in both women and men [37]. High Ki-67 expression tumors might be more common in luminal subtype B than in luminal subtype A lesions, though we found no statistically significant difference in this study (P = 0.106). These results may explain why luminal subtype B tumors are associated with a poorer prognosis as compared with luminal subtype A tumors.

Due to high rates of lost-to-follow-up of MBC patients in this study, we cannot obtain the effective data that associated with MBC patient survival. A study indicated that AJCC stage, tumor size, lymph node state, molecular subtypes and adjuvant chemotherapy treatment were related to poor overall survival in MBC [38]. The outcome of MBC patients in terms of disease free survival or overall survival was inferior to FBC patients in previous study [39-41]. Researchers also demonstrated superior survival for FBC patients than MBC patients via population-based comparison of SEER data [42]. There are two main reasons for this phenomenon. The difference of molecular biology and the response to chemotherapy management in MBC patients may lead to the survival disparities [7, 43]. Furthermore, MBC patients were more likely to die of second primary cancers included colon, prostate, lung than the FBC patients [42]. Thus, prevention and treatment of comorbid tumor may be an effective strategy for improving survival of MBC patients.

The characteristic subtypes of FBC which have provided new insights into future study of the diagnosis, drug sensitivity and resistance, clinical management, and prognosis cannot be applied to MBC patients directly [44-46]. This current research cannot meet the needs for MBC patients due to two limitations of our study. First, as the incidence of MBC is low, the size of our study cohort is small. The number of cases collected in this study may affect the statistical power of the analysis and thus the results. Second, the current study was unable to provide a relationship between the subtype and survival due to a short period of follow-up and high lost rate of follow-up.

Luminal subtype A and luminal subtype B are the major subtypes of MBC in Chinese patients. In MBC patients, most tumors express hormone receptors. Luminal subtype B tumors tend to have a high nuclear grade and larger tumor size than luminal subtype A tumors. Despite being limited by a small number of patients, our study provides valuable information on the distribution of the molecular subtypes of MBC.

Disclosure of conflict of interest

None.

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