Pathological features and prognosis of different molecular subtypes of breast cancer

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Abstract. To examine the pathological features and prognosis of different molecular subtypes of breast cancer, the clinical data of 892 breast cancer patients were retrospectively analyzed and divided into four subtypes according to hormone receptor expression in breast cancer tissue: Her-2 overexpression, luminal A, luminal B and basal-like subtypes. The pathological data and prognosis of these subtypes were compared. Of the 892 breast cancer patients, there were 46 cases (5.2%) with Her-2 overexpression-type, 698 cases (78.3%) with luminal A-type, 38 cases (4.3%) with luminal B-type and 110 patients (12.2%) with basal-like-type. Immunohistochemistry was used to identify the progesterone and estrogen receptors in the tumor tissues. The χ^2 test was used to verify the measurement data. The Cox proportional hazard regression model was used for the univariate and multivariate analyses. Results showed there was no statistical difference for lymphatic metastasis among the various molecular subtypes of breast cancer (P>0.05). The distant metastatic rate of patients with Her-2-type breast cancer was significantly higher compared to patients with the other three subtypes (P<0.05). The difference in local recurrence among molecular subtypes was not significantly significant (P>0.05). Lymph node metastasis, age and different molecular subtypes were found to have an impact on patient overall survival (OS) and disease-free survival (DFS). Her-2 overexpression-type breast cancer patients had the lowest 9-year DFS and 7-year OS compared to the other subtypes (P<0.05). Thus, Her-2-type was associated with the worst prognosis. In conlusion, the molecular typing of breast cancer has important clinical value in prognosis estimation and is expected to affect breast cancer treatment approaches.

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Introduction

Treatment for breast cancer is mainly guided by clinical staging and TNM staging, which are based on the size of the primary tumor and the degree of metastasis to regional lymph nodes and blood circulation (1). Prognosis of breast cancer may differ even with common characteristics in their pathological type or clinical stage (2). Therefore, individualized treatment may depend on the molecular characteristics of breast cancer (3). Previous theories in 'breast cancer genotyping' (1) suggested that different molecular subtypes are associated with different prognoses (4). In this study, a retrospective analysis was conducted of the pathological data and prognosis of breast cancer patients (n=892) admitted to our hospital between January 2000 and December 2009.

Materials and methods

General information. Patients (n=892) who had invasive breast cancer and received surgical treatment in our hospital (from January 2009 to December 2011) were selected for this study. The study was approved by the Ethics Committee of the First Affiliated Hospital of Harbin Medical University. The study included 882 females and 10 males with a maximum age of 86 years, minimum age of 21 years and mean age of 56.2±21.3 years. In total, 712 patients received modified radical mastectomy, 89 patients received radical mastectomy, 55 patients received modified and extended radical mastectomy, and 36 patients received breast-conserving surgery. Postoperative pathological examination confirmed mucinous adenocarcinoma in 17 cases, invasive lobular carcinoma in 19 cases, infiltrating ductal carcinoma in 72 cases, invasive ductal carcinoma in 732 cases and other types of cancer in 52 cases. Regarding lymph node involvement, 496 cases had lymph node metastasis and 396 cases had no lymph node metastasis; the percentage of patients with metastasis was 55.6%. The surgical stages in patients with lymph node metastasis were as follows: 289 cases in stage N1 (metastasis in ≤3 lymph nodes); 136 cases in stage N2 (metastasis in 4-9 lymph nodes); and 71 cases in stage N3 (metastasis in ≥10 lymph nodes). Using the cancer staging guidelines established in 2002 by AJCC, the results were as follows: 2 cases in stage T0, 212 cases in stage T1, 536 cases in stage T2, 64 cases in stage T3 and 42 cases in unspecified stages. The clinical stages were:

Table I. Comparison of pathology data among the different molecular subtypes of breast cancer [n (%)].

Pathological features	No.	Her-2 overexpression	Basal-like	Luminal A	Luminal B	χ^2 test	P-value
Age (years)						51.888	0.001
≤35	28	2 (4.2)	6 (5.5)	12 (1.8)	8 (21.1)		
36-50	468	24 (50.0)	54 (49.1)	380 (54.4)	10 (26.3)		
≥51	396	22 (45.8)	50 (45.5)	306 (43.8)	20 (52.6)		
Lymphonodus						0.970	0.809
N (-)	396	20 (30.6)	50 (32.9)	312 (45.6)	14 (47.8)		
N (+)	496	26 (69.4)	60 (67.1)	386 (54.4)	24 (52.2)		
N1	289	12 (46.2)	32 (53.3)	235 (60.9)	10 (41.7)	7.369	0.288
N2	136	10 (38.4)	20 (33.3)	98 (25.4)	8 (33.3)		
N3	71	4 (15.4)	8 (13.3)	53 (13.7)	6 (25.0)		
Tumor size[a]						44.181	0.000
T1	212	12 (26.1)	30 (27.3)	155 (23.6)	15 (44.1)		
T2	536	24 (52.2)	62 (56.4)	442 (67.2)	8 (23.5)		
T3	64	6 (13.0)	10 (9.1)	39 (5.9)	9 (26.5)		
T4	36	4 (8.7)	8 (7.3)	22 (3.3)	2 (5.9)		
Clinical stages[b]						23.720	0.001
I	162	10 (21.7)	30 (25.0)	112 (16.6)	10 (27.8)		
II	468	23 (50.0)	58 (48.3)	380 (56.4)	7 (19.4)		
III	246	13 (28.3)	32 (26.7)	182 (27.0)	19 (52.8)		

[a]Does not include patients in unknown and T0 stages; [b]does not include patients that could not be divided into specific clinical stages.

162 cases in stage I, 468 cases in stage II, 246 cases in stage III and 16 cases which could not be clinically staged. Patients received adjuvant therapy following surgery: 656 cases with chemotherapy, 212 cases with radiotherapy and 417 cases with endocrine therapy.

Immunohistochemical methods. The ready-to-use non-biotin EliVision™ two-step method was used to reveal the progesterone receptor (PR) and estrogen receptor (ER) subtypes in tumor tissues. Immunohistochemistry was performed using the DAB developing process. The paraffin-embedded tissue was cut into serial sections of 4-μm, mounted on glass slides that were treated with 3-aminopropyltriethoxy-silane (APES) acetone solution, and baked in an oven at 60˚C for 1 h. Slides were dewaxed and incubated at room temperature with 3% H_2O_2 for 10 min. A microwave (100˚C) was used to repair the antigens for 15 min after the inactivation of endogenous peroxidase. Ready-to-use monoclonal antibodies were used as the primary antibodies. The ready-to-use broad-spectrum EliVision plus kits, DAB color-developing agents and antibodies were purchased from Fuzhou Maixin Biotechnology Development Co., Ltd. (Fujian, China). A positive expression of PR and ER was manifested as ≥10% of tumor cells with the expression of labeled hormone receptors. According to the expression of Her-2, PR and ER in tumor tissues, breast cancer was divided into four molecular subtypes: Her-2 overexpression, luminal A, luminal B and basal-like.

Follow-up. Until December 2011, patients in the study were followed up by letter, telephone or clinic visit to confirm their prognosis. Definite diagnosis of recurrence was made by image diagnosis or clinical manifestation. In this study, 872 patients had follow-up results, with a follow-up rate of 97.8% and mean follow-up period of 46.2±13.7 months.

Statistical analysis. The SPSS16.0 statistical software was used for statistical analysis. The χ^2 test was used to verify the measurement data. The Cox proportional hazard regression model was used to perform multivariate analysis to determine the survival analysis: inclusion, α=0.05 and exclusion, α=0.1. P<0.05 indicates a statistically significant difference.

Results

Molecular typing results of breast cancer patients. Breast cancer patients were divided into four subtypes: 46 patients (5.2%) with Her-2 overexpression, 698 patients (78.3%) with luminal A, 38 patients (4.3%) with luminal B and 110 patients (12.2%) with basal-like subtypes of breast cancer.

Clinicopathological characteristics of breast cancer patients. Lymph node metastasis was not significantly different among the various molecular subtypes of breast cancer (P>0.05). However, patient age, tumor size and clinical stage among the molecular subtypes were statistically significant (P<0.05) (Table I).

Distant metastasis and local recurrence of different molecular subtypes of breast cancer. Sixty-two of the 872 patients who were followed up had distant metastasis, including

Table II. Metastasis and recurrence in patients with different molecular subtypes of breast cancer [n (%)].

	No.	Basal-like	Her-2	Luminal A	Luminal B	χ^2 test	P-value
Distant metastasis							
No	810	91 (11.2)	30 (3.7)	652 (80.5)	37 (4.6)	58.702	0.001
Yes	62	9 (14.5)	16 (25.8)	36 (58.1)	1 (1.6)		
Local recurrence							
No	860	99 (11.5)	44 (5.1)	680 (79.1)	37 (4.3)	4.800	0.028
Yes	12	1 (8.3)	2 (33.3)	6 (50.0)	1 (8.3)		

Table III. Univariate analysis of factors that affect breast cancer prognosis.

Influencing factors	No.	7-year DFS			9-year OS		
		No. (%)	χ^2 test	P-value	No. (%)	χ^2 test	P-value
Age (years)			0.436	0.791		3.009	0.112
≤35	26	91.2			97.9		
36-50	442	90.8			93.8		
≥51	404	93.7			94.2		
Lymphonodus			13.249	0.000		23.293	0.000
Positive	492	85.2			86.8		
Negative	380	97.9			97.9		
Molecular typing			7.629	0.039		12.357	0.012
Her-2 overexpression	100	81.9			84.3		
Luminal A	46	92.9			96.3		
Luminal B	686	94.2			96.2		
Basal-like	38	88.7			91.8		

DFS, disease-free survival; OS, overall survival.

19 cases of basal-like-type, 7 cases of Her-2-type, 35 cases of luminal A-type and 1 case of luminal B-type. The distant metastatic rate in patients with Her-2-type breast cancer was significantly higher compared to the other subtypes ($P<0.05$); 12 patients had local recurrence, including 2 cases of basal-like-type, 1 case of luminal A-type and 9 cases of luminal B-type. The local recurrence rate was not significantly different ($P>0.05$) among the molecular subtypes of breast cancer (Table II).

Univariate analysis of overall survival (OS) and disease-free survival (DFS) in patients with different molecular subtypes of breast cancer. Univariate analysis results showed that the 7-year DFS and 9-year OS of lymph node-positive patients were both shorter compared to node-negative patients ($P<0.05$). Her-2 overexpression-type patients had the lowest 9-year DFS and OS among the four molecular subtypes ($P<0.05$) (Table III).

Multivariate Cox regression analysis of DFS and OS in patients with different molecular subtypes of breast cancer. Lymph node metastasis, patient age and different molecular subtypes have an impact on patient DFS and OS. Compared to the Her-2 overexpression-type, the other three molecular subtypes had a better DFS and OS prognosis (Tables IV and V).

Discussion

Histomorphological characteristics of tumors are the gold standard for pathological diagnosis, and therefore the basis for clinical treatment. Traditional clinical staging has been significant for determining patient prognosis. However, with new advances in molecular medicine, traditional pathological staging cannot meet the needs of modern cancer diagnosis and treatment (5,6). Currently, results of genetic testing methods for breast cancer are inconclusive. Breast cancer is usually divided into four subtypes according to immunohistochemical indicators commonly used in clinical practice: Her-2 overexpression, luminal A, luminal B and basal-like subtypes (7,8).

This study showed that luminal A-type had the highest constituent ratio in 892 cases of invasive breast cancer patients, while luminal B-type had the lowest constituent ratio; findings that are consistent with the results of previous studies (9-12). Age, tumor size and clinical stages among patients with different molecular subtypes were statistically significant ($P<0.05$). Nevertheless, clinicopathological parameters of

Table IV. Results of multivariate Cox regression analysis of disease-free survival.

Variables	β	SE	Wald χ^2 test	V	P-value	OR	95% CI
Lymph node metastasis	1.237	0.381	23.458	1	0.000	3.989	2.139-6.892
Age	-0.017	0.203	0.029	1	0.892	0.895	0.586-1.329
Luminal A	-1.306	0.412	11.239	1	0.006	0.217	0.118-0.783
Luminal B	-1.312	0.712	3.683	1	0.059	0.234	0.069-1.238
Her-2 overexpression			12.019	3	0.003	1	
Basal-like	-0.769	0.397	4.789	1	0.036	0.379	0.128-0.869

Table V. Results of multivariate Cox regression analysis of overall survival.

Variables	β	SE	Wald χ^2 test	V	P-value	OR	95% CI
Lymph node metastasis	0.239	0.395	23.497	1	0.000	5.987	2.397-11.897
Age	0.179	0.318	0.739	1	0.421	1.397	0.696-2.038
Luminal A	-1.589	0.397	16.236	1	0.000	0.128	0.057-0.659
Luminal B	-1.493	0.912	2.982	1	0.071	0.312	0.051-1.198
Her-2 overexpression			15.672	3	0.001	1	
Basal-like	-1.213	0.478	7.536	1	0.009	0.209	0.176-0.864

breast cancer patients have multiple influencing factors, thus more studies are required to confirm these conclusions.

The key to the therapeutic success of breast cancer treatment lies in limiting distant metastasis, which is correlated with molecular typing (13,14). The results of this study revealed that the distant metastatic rate of Her-2-type breast cancer patients was significantly higher compared to other subtypes ($P<0.05$). The local recurrence rate was not significantly different among the molecular subtypes ($P>0.05$). These results indicate that the molecular typing of breast cancer is of great value in the prognosis and treatment options available for breast cancer patients, particularly gene-targeted therapy for the Her-2 overexpressing-type. The prognosis of patients with breast cancer is also closely correlated with the molecular subtype (15). This study found that patients with Her-2 overexpression had the lowest 9-year OS and 7-year DFS among all the molecular subtypes. Adjuvant therapy may therefore be necessary for patients overexpressing the Her-2 marker.

Molecular typing of breast cancer may benefit the clinical prediction of tumor response to therapy and patient prognosis (14). Improvements should be made to existing personalized treatment plans based upon the molecular typing of breast cancer. Additionally, gaining a better understanding and knowledge in this field may prolong survival and improve the quality of life for breast cancer patients.

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