RESEARCH COMMUNICATION

Clinical Significance of Basal-like Breast Cancer in Chinese Women in Heilongjiang Province

Ying Liu[1]*, Qiu-Ying Jiang[1], Tao Xin[1], Li Cai[2], Chang-Hong Zhao[2]

Abstract

Background: Our objective was to clarify the clinical and biological characteristics of basal-like breast cancer (BLBC) and non-basal-like breast cancer (TN3BKE) in Heilongjiang. Methods: We examined, by immunohistochemistry, expression of biological markers cytokeratin (CK) 5/6 and epidermal growth factor receptor (EGFR) and B cell specific moloney murine leukemia virus integration site 1(Bmi-1) in triple-negative breast cancer (TNBC). We studied the correlation between BLBC and several factors related to tumor progression, along with its prognostic value. Results: In the 229 cases of operable TNBC, BLBC was detected in 178 (77.7%) and TN3BKE- in 51 (22.2%). There was no significant difference in clinicopathological factors between them, However, BLBC was significantly associated with Bmi-1 expression (P=0.000) and shorter disease-free survival (DFS) (P = 0.045) and overall survival (OS) (P = 0.041). Conclusions: Compared with the non-basal group, patients with BLBC have a high expression of Bmi-1 and a poor prognosis.

Keywords: Basal like - breast cancer - prognosis

Asian Pacific J Cancer Prev, **13**, 2735-2738

Introduction

Breast cancer comprises an extraordinarily diverse group of diseases in terms of presentation, morphology, molecular profile, and response to therapy, Gene expression analysis has identified molecular classes of breast cancer that are biologically and clinically distinct, and by that breast cancer was classified into five subtypes: luminal A, luminal B, HER2-overexpressing, normal-breast-like, and BLBC. Among the five intrinsic subtypes, BLBC have drawn particular attention (Perou et al., 2000; Sorlie et al., 2001; Sorlie et al., 2003; Sotiriou et at., 2003; Shipitsin et al., 2007; Chuthapisith et al., 2012),because they express neither ER, PR, nor HER-2, and therefore would not be expected to benefit from anti-estrogen hormonal therapies nor from trastuzumab. So BLBC are associated with poor prognosis (Perou et al., 2000; Sorlie et al., 2001; Sorlie et al., 2003; Sotiriou et at., 2003; Shipitsin et al., 2007). Although gene expression profiling is considered the ''gold standard'' method for identification of BLBC, this approach is not currently feasible for large-scale clinical applications or retrospective studies using formalin fixed, paraffin-embedded samples. Therefore, as an alternative, expression of basal/myoepithelial cell proteins identified by immunohistochemical staining has been used as a surrogate of gene expression. Nielsen et al. immunohistochemically characterized this subtype by analyzing a panel of 21 basal-like tumors that had been previously classified using gene expression profiles. The investigators found that four antibodies (ER, EGFR, HER2, and CK5/6) can be used to identify BLBC immunohistochemically. The BLBC was defined as lacking both ER and HER2 expression, but it was positive for the expression of CK5/6 and EGFR. The panel using these four antibodies showed a specificity of 100% and a sensitivity of 76% for the identification of BLBC (Nielsen et al., 2004). This definition is currently considered one of the most pragmatic and widely accepted definitions of BLBC (Matos et al., 2005; Carey et al., 2006; Livasy et al 2007; Cheang et al., 2008). Furthermore, several studies have shown that Clinical significance of BLBC in TNBC (Foulkes et al., 2003; Lakhani et al., 2005; Kobayashi et al., 2008; Yutaka et al., 2009; Brady-West et al., 2011). However, the relative contribution of ER, PR, HER-2, and basal markers, in predicting BLBC in Chinese patients especially Heilongjiang province, has not been fully studied. To assess this, we analysis an cohort of breast cancer cases between January 2003 and December 2006.

Materials and Methods

We studied a cohort of 1259 patients with invasive breast cancer who were treated at Second Affiliated Hospital of Harbin Medical University and Affiliated Oncology Hospital of Harbin Medical University between January 2003 and December 2006. and for whom clinical information and a paraffin-embedded tumor specimen was available. The pathology and clinical characteristics (age at initial diagnosis; menopausal status (pre- or post-menopause); family history of breast cancer; information

*[1]Department of Oncology, Second Affiliated Hospital of Harbin Medical University [2]Department of Oncology, Affiliated Oncology Hospital of Harbin Medical University, Harbin, Heilongjiang, China *For correspondence: yingliu1983@126.com*

Table 1. Clinicopathological Factors According to BLBC in TNBC

Variables	TNBC		P
	BLBC	TN3BKE-	
Age (years)			
≤35	13	4	0.897
>35	165	47	
Menopause			
Pre	100	32	0.403
Post	78	19	
Tumor size (cm)			
≤2	36	9	0.683
>2	142	42	
Nodal status			
Negative	79	27	0.280
Positive	99	24	
Type of surgery			
Radical mastectomy	9	5	0.212
Modified	164	46	
clinical stages			
III	56	13	0.493
II	81	28	
I	41	10	
Histology			
Invasive ductal Carcinoma	159	45	0.503
Others	18	7	
Family history of breast cancer			
Yes	39	5	0.160
No	139	46	
Lactation			
Yes	163	41	0.307
No	14	9	
Bmi-1			
Positive	140	19	0.000
Negative	38	32	

on therapy; clinical follow-up; relapses; histological type and grade of the primary tumor; tumor size; lymph node status; and pathologic stage at diagnosis) were retrieved from the medical records. Follow-up has been maintained by the database coordinator by periodic review of the clinical charts and by contacting the patient or her physician by telephone. For deceased patients, dates and causes of death were obtained from the medical records.

Immunohistochemistry

Pathology specimens were retrieved from storage and reviewed. A representative section of tumor tissue was selected. A tissue microarray was constructed, which rep-resented the 1259 breast cancer cases in triplicate, in 1 mm cores. A panel of three antibodies was used ER, PR, HER2. A positive and negative control for each antibody was included in every analysis. ER and PR were categorized as negative (0%), low-positive (1–10%) and positive (>10%). HER2 positivity was based on the CAP (Canadian Association of Pathologists) guidelines (2007); only tumors with complete strong membranous staining of at least 30% of cells were considered to be positive. Each breast cancer sample was represented in triplicate; in cases where the staining results were discordant, we coded the highest value observed among the three. We categorized the patients as triple-negative if they were negative for

Table 2. Relationship Between BLBC and TN3BKE- and Pattern of Distant Metastasis After 5 Years of Follow-up

	Tumor groups		X^2	P
	BLBC n	TN3BKE- n		
Distant metastasis	27	6	0.407	0.523
Distant metastasis site				
Brain	4	1	0.017	0.898
Lung	9	3	0.051	0.822
Bone	9	1	0.921	0.337
Liver	3	0	0.876	0.349
Others	2	1	0.210	0.646
Local recurrence	25	3	2.497	0.114

ER, PR, Her-2. Among the triple-negative subgroup, we detected anther three antibodies–CK5/6 EGFR and Bmi-1. CK5/6, EGFR expression was ≥ 10% of the cytoplasmic membrane staining were positive for these two markers. and Bmi-1 was categorized as positive ≥ 10% of tumor nuclei. continuous variables, based on the proportion of tumor cells which stained positive (1–100%), regardless of staining intensity. tumors were classified as basal-like if they stained positive for either CK5/6 or EGFR and as normal like if they were negative for both EGFR and CK5/6. The remaining cancers were divided into ER-positive tumors and HER2-neu positive (cancers that were positive both for ER and for HER2-neu were assigned to the HER2-neu group). Whereas this article we just discuss the Clinicopathological features and survival of BLBC in TNBC.

Statistical analysis

For statistical analysis, the chi-squared test and unpaired t test were used for analysis of two unpaired samples. Disease-free survival and overall survival rates and time periods after surgical resection were calculated by the Kaplan–Meier method, and differences in survival curves were assessed by the log-rank test. The Cox proportional hazards model was used for multivariate analysis. All analyses were performed with the package SPSS 17.0 for Windows. A P value of less than 0.05 was regarded as statistically significant. All statistical tests were two-sided.

Results

Basal-like and non-basal-like breast cancer

TNBC was classified into two sub-types (BLBC and TN3BKE-) according to the expression of CK5/6 and EGFR. Immunohistochemical staining of CK5/6 and/or EGFR was identified in the cytoplasmic membrane of tumor cells. The expression of CK5/6 or EGFR was found in 81 (35.3%) and 153 (66.8%) of the 229 cases of TNBC, respectively. BLBC or TN3BKE- was detected in 178 (77.7%) and 51 (22.2%) cases of TNBC, respectively.

Correlations between basal-like subtype and clinicopathological factors

Correlations between the BLBC versus TN3BKE- and clinico- pathological factors are shown in Table 1.

There was no significant difference between BLBC

Table 3. Univariate and Multivariate Analyses of Basal-like Subtype in Triple-negative Cancer: Overall-survival

	Univariate analysis			Multivariate analysis		
	Exp(B)	95% CI	P value	Exp(B)	95% CI	P value
Nodal status	6.660	2.799-15.846	0.000	1.047	0.177-6.186	0.960
Bmi-1 index	1.96	1.272-3.193	0.000	1.481	0.89-2.58	0.132
clinical stages	0.103	0.045-0.234	0.000	0.075	0.019-0.296	0.000

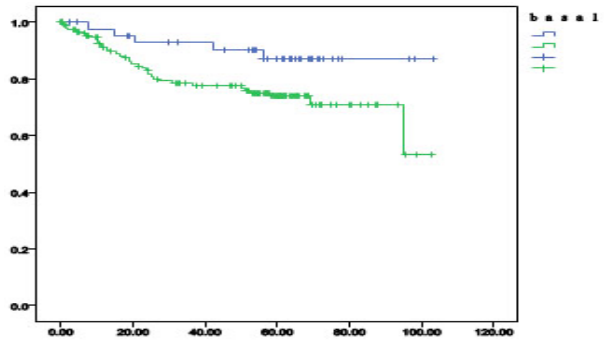

Figure 1. Disease-free Survival (P=0.045)

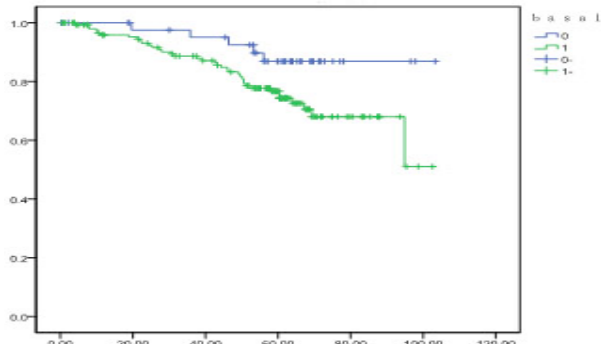

Figure 2. Overall Survival (P = 0.041)

and TN3BKE- regarding patients age, menopausal status, primary tumor size, histology, Family history of breast cancer, Lactation. When compared with TN3BKE-tumors, BLBC showed an association with higher prevalence of Bmi-1 expression (P = 0.000).

Patients' outcome

Patterns of metastatic dissemination .Distant metastases developed in 27 cases of BLBC compared with 6 cases in TN3BKE- (Table2).

Survival analysis

In univariate survival analysis, patients with BLBC had shorter DFS (P = 0.045) and OS (P = 0.041) than patients with TN3BKE- (Figure 1, 2). In multivariate analysis of DFS by Cox regression analysis, no independent prognostic factor was identified among the prognostic factors that had been obtained from univariate analysis. However, In multivariate analysis of OS, BLBC was correlate with clinical stages.

Discussion

We demonstrated that TNBC can be classified into at least two subtypes of cancer according to the expression of basal markers CK5/6 and EGFR. In addition, we showed the clinicopathological significance of these subtypes, including their prognostic impact on survival in patients with TNBC. In our study, the BLBC was detected in 77.7% of TNBC. This proportion is among of previously published data, which included BLBC rates ranging from 53% to 84% in cases of TNBC (Bidar et al., 2007; Rakha et al., 2007; Tischkowitz et al., 2007; Cheang et al., 2008; Sasa et al., 2008; Tan et al., 2008). The definition of TNBC in this study was ER expression in <10% of tumor nuclei, PR expression in <10% of tumor nuclei, and negative expression of HER2 expression in <30% of tumor membrane . In addition, the cutoff value for CK5/6 and EGFR expression was ≥10% of the cytoplasmic membrane staining positive for these two markers. Our study's cutoff values for these markers differed from previously reported definitions. For example, Nielsen et al. reported that ER positivity was identified by any staining of tumor nuclei, HER2 positivity was defined as strong membranous staining, and CK5/6 and EGFR positivity was defined as any cytoplasmic and/or nuclei staining in tumor specimens (Nielsen et al., 2004). Our definition of TNBC, such as those from the St. Gallen International Consensus Conference of 2007 (Goldhirsch et al., 2003), and this definition is widely accepted in the adjuvant setting. BLBC and TN3BKE- did not have distinct difference with Clinicopathological factors in our study, Except Bmi-1 high expression. Whereas, Cheang et al. demonstrated that the BLBC was significantly associated with high grade (87% of cases were grade 3) and young age (18.8% were <40 years old) when the BLBC was compared with cancer that was negative for all five markers (ER, PR, HER2, CK5/6, and EGFR). The investigators showed that the five-marker classification of the BLBC identified a subset of particularly high-risk patients(Cheang et al., 2008).

Sasa et al. also showed that BLBC was associated with tumor size and nuclear grade (Sasa et al., 2008) .We also showed that the BLBC was associated with a high Bmi-1 expression, The high Bmi-1 expression is related to the mitotic index and high levels of cell proliferation (Xu et al., 2011). Bmi-1 status is an independent prognostic factor, which also is associated with tumor histological grade and basal-like phenotype. The high proportions of positive Bmi-1 expression in BLBC may be related to the high aggressiveness behavior of this subtype of breast cancer (Wang et al., 2012). Apart from this study, Arnes et al. have evaluated the relationship between Bmi-1 expression and basal-like phenotype. They found that Bmi-1 was inversely related to basal-like factors (Arnes et al., 2008). The same as our study, Further studies are needed to verify these results.

In this study, BLBC did not have a special transfer pattern. But Rakha, et al. reported that Metastatic BLBC tumors showed a unique pattern of distant metastasis with frequent metastasis to two or more sites, BLBC showed more frequent metastasis to brain and lung but less

frequently metastasize to other lymph node groups than TN3BKE-. However, no significant difference was noted in case of bone or liver metastases in BLBC compared with TN3BKE- (Rakha et al. 2009).

In univariate analysis, three factors: nodal status (P = 0.000), Bmi-1 (P = 0.000), and clinical stages(P = 0.000), were identified as prognostic factors affecting OS. However, in multivariate analysis, clinical stages was identified as a independent prognostic factor. One of the reasons is that only a small number of patients were examined in this study, making it difficult to reach statistical significance. In a previous large study, basal markers (CK5/6 and/or EGFR) were identified as independent prognostic factors (Rakha et al., 2007; Cheang et al., 2008). Taken together, the available data suggest that TNBC can be classified into at least two subgroups based on basal markers, which are predictive of different prognosis.

In conclusion, our study showed TNBC to be a heterogeneous disease that consists of at least two phenotypes based on the expression of basal markers (CK5/6 and/or EGFR). BLBC has a poor prognosis, although all the study patients were treated with standard therapy according to conventional practice guidelines. Based on the poor prognosis associated with the basal-like subtype, a novel treatment strategy for this type of breast cancer is urgently needed.

Acknowledgements

Informed consent had been obtained, and the Ethics Committee of Harbin Medical University approved this study. The author(s) declare that they have no competing interests.

References

Arnes JB, Collett K, Akslen LA, et al (2008). Independent prognostic value of the basal-like phenotype of breast cancer and associations with EGFR and candidate stem cell marker BMI-1. *Histopathology*, **52**, 370-80.

Bidard FC, Conforti R, Boulet T, et al (2007).negative phenotype accurately identify basal-like tumour? An immunohistochemical analysis based on 143 'triple-negative' breast cancers. *Ann Oncol*, **18**, 1285-6.

Brady-West DC, McGrowder DA (2011). Triple negative breast cancer: therapeutic and prognostic implications. *Asian Pac J Cancer Prev*, **12**, 2139-43.

Carey LA, Perou CM, Livasy CA, et al (2006). Race, breast cancer subtypes, and survival in the Carolina Breast CancerStudy. *JAMA*, **295**, 2492-502.

Cheang MC, Voduc D, Bajdik C, et al (2008). Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res*, **14**, 1368-76.

Chuthapisith S, Permsapaya W, Warnnissorn M, et al (2012). Breast cancer subtypes identified by the ER, PR and HER-2 status in Thai women. *Asian Pac J Cancer Prev*, **13**, 459-62.

EmadA Rakha, SomaiaE Elsheikh, MuhammedA Aleskandarany, et al (2009). Triple-Negative Breast Cancer: Distinguishing between Basal and Non-basal Subtypes. *Clin Cancer Res*, **15**, April1.

Foulkes WD, Stefansson IM, Chappuis PO, et al (2003). Germ line BRCA1 mutations and a basal epithelialphe-no type in breast cancer. *J Natl Cancer Inst*, **95**, 1482-5.

Goldhirsch A, Wood WC, Gelber RD, et al (2003). Meeting highlights: updated international expert consensus on the primary therapy of early breast cancer. *J Clin Oncol*, **21**, 3357-65.

Kobayashi S (2008). Basal-like subtype of breast cancer: a review of its unique characteristics and their clinical significance. *Breast Cancer*, **15**, 153-8.

Lakhani SR, Reis-Filho JS, Fulford L, et al (2005). Prediction of BRCA1 status inpatients with breast cancer using estrogen receptor and basal phenotype. *Clin Cancer Res*, **11**, 5175-80.

Livasy CA, Perou CM, Karaca G, et al (2007). Identification of a basal-like subtype of breast ductal carcinoma insitu. *HumPathol*, **38**, 197-204.

Matos I, Dufloth R, Alvarenga M, er al (2005). p63, cytokeratin 5, and P-cadherin: three molecular markers to distinguish basal phenotype in breast carcinomas. *Virchows Arch*, **447**, 688-94.

Nielsen TO, Hsu FD, Jensen K, et al (2004). Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res*, **10**, 5367-74.

Perou CM, Sorlie T, Eisen MB, et al (2000). Molecular portraits of human breast tumors. *Nature,* **406**, 747-52.

Rakha EA, El-Sayed ME, Green AR, et al (2007). Prognostic markers in triple-negative breast cancer. *Cancer*, **109**, 25-32.

Sasa M, Bando Y, Takahashi M, et al (2008). Screening for basal marker expression is necessary or decision of therapeutic strategy for triple-negative breast cancer. *J Surg Oncol*, **97**, 30-4.

Shipitsin M, Campbell LL, Argani P, et al (2007). Molecular definition of breast tumor heterogeneity. *Cancer Cell*, **11**, 259-73.

Sorlie T, Perou CM, Tibshirani R, et al (2001). Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA,* **98**, 10869-74.

Sorlie T, Tibshirani R, Parker J, et al (2003). Repeated observation of breast tumor subtypes in independent gene expression datasets. *Proc Natl Acad Sci USA*, **100**, 8418-23.

Sotiriou C, Neo SY, McShane LM, et al (2003). Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Natl Acad Sci USA*, **100**, 10393-8.

Tan DS, Marchio C, Jones RL, et al (2008). Triple negative breast cancer: molecular profiling and prognostic impact in adjuvant anthracycline-treated patients. *Breast Cancer Res Treat*, **111**, 27-44.

Tischkowitz M, Brunet JS, Begin LR, et al (2007). Use of immunohistochemical markers can refine prognosis in triple negative breast cancer. *BMC Cancer*, **7**, 134.

Wang Y, Zhe H, Ding Z, Gao P, Zhang N, Li G (2012). Cancer stem cell marker Bmi-1 expression is associated with basal-like phenotype and poor survival in breast cancer. *World J Surg*, **36**, 1189-94.

Xu Z, Liu H, Lv X, et al (2011). Knockdown of the Bmi-1 oncogene inhibits cell proliferation and induces cell apoptosis and is involved in the decrease of Akt phosphorylation in the human breast carcinoma cell line MCF-7. *Oncol Rep,* **25**, 409-18.

Yamamoto Y, Ibusuki M, Nakano M, et al (2009). Clinical significance of basal-like subtype in triple-negative breast cancer. *Breast Cancer*, **16**, 260-7.