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Clinical Usefulness of Cytokeratin 5/6 as an Immunohistochemical Marker to Predict Prognosis in Triple-negative Breast Cancer

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Abstract

Background : Traditional prognostic markers for breast cancer include estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2. Negative staining for all three markers defines the 'triple-negative' phenotype. By adding markers for cytokeratin 5/6 and epidermal growth factor receptor, triple-negative breast cancers can be divided into 'basal-like' and 'non-basal-like' subgroups. The aim of this study is to asscess the usefulness of cytokeratin 5/6 as a distinguishable marker in the basal-like subgroup of triple-negative breast cancers.

Methods : We examined, by immunohistochemistry, the expression of biological markers cytokeratin 5/6 in triple-negative breast cancers. We classified triple-negative breast cancerpatients into two groups as cytokeratin 5/6-positive and cytokeratin 5/6-negative. The clinicopathological features, such as disease free survival (DFS) and overall survival (OS) for patients with cytokeratin 5/6-positive were compared with those of the cytokeratin 5/6-negative patients.

Results : In the 131 cases of operable triple-negative breast cancer, cytokeratin 5/6-positive group was detected in 15 (11.5%) and cytokeratin 5/6-negative group was detected in 116 (88.5%). Significant correlation was observed between cytokeratin 5/6-positive groupwith tumor size, pathologic lymph-node metastasis and American Joint Committee on Cancer staging (pT, pN and stage, P = 0.001). No association was detected between cytokeratin 5/6-positive cancerand other biological markers. Patients with cytokeratin 5/6-positive showed shorter disease-free survival (P = 0.031) and overall survival (P = 0.018) than patients with cytokeratin 5/6-negative.

Conclusion : Our results show that cytokeratin 5/6 is important markers that can be used to predict prognosis in triple-negative breast cancer. But, there is a need for larger number of cases, more immunohistochemical markers and gene investigation to observe more accurate disease free survivaland overall survival rate.

Key words : CK5/6, Triple negative, breast cancer.

Introduction

Triple negative breast cancers (TNBCs) represent a heterogeneous group of diseases, characterized by significant variability in morphological and pathological features. These tumors are defined as lack ofthree most significant therapeutic markers for clinical management of breast cancer patients: human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), and progesterone receptor (PR). TNBC has been a particular focus of attention because this phenotype has no confirmed therapeutic molecular target and a poor prognosis.[1,2)]

DNA microarray profiling studies on breast cancer have identified distinct subtypes of this cancer with different clinical outcomes. In recent years, TNBC has been classified into basal-like and non-basal-like subgroups according to immunohistochemical (IHC) expressions. Nielsen et al. [3] recently proposed a definition of basal-like tumours should be negative for ER, PR and HER2 and positive for CK5/6 and/or EGFR. Also, many studies have described various molecular markers used to

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define a basal-like subtype in TNBC: expression of basal cytokeratins (CK5/6, CK14 and CK17), p63[3-6] epidermal growth factor receptor (EGFR)[3,7]and c-kit.[8] In addition to the expression of these basal markers, some investigators have required tumoral and nodalstatus, or ER-negative and HER2-negative status for defining a basal-like breast cancer (BLBC). [3-6] Kobayashi et al. reported aggressive characteristics of BLBC, which has high histological grade, p53 mutation, epidermal growth factor receptor (EGFR) overexpression, c-MYC amplification, loss of function of BRCA1, and cytogenetic abnormalities.[9-12]

Taken together, these data indicate that basal-like cancer is not identical to triple-negative cancer, according to the results of microarray-based gene expression profiling, which is considered to be the gold standard for the identification of basal-like cancer. Therefore, we sought to clarify the clinicopathological significance of CK5/6-positive as a marker of basal-like subtype and CK5/6-negative in triple-negative cases, and estimate the usefulness of CK5/6 as a prognostic marker in TNBCs.

Materials and methods

Patients and specimens

This retrospective Cohort study was approved by the Kosin Gospel Hospital. Between Jannuary of 2001 and December of 2005, a total of 1,237 patients were enrolled. Among 1,237 patients, we studied 131 womens as identified to TNBCs by IHC stain after proper surgery. The specimens of primary invasive carcinoma were obtained from resected tumor. None of these cancer patients received treatment prior to surgery. Thepatients underwent standard or partial mastectomy with fully resected axillary dissections. Institutional review board-approved informed consent was obtained from each patient prior to tissue collection.

Histology and immunochemistry

All data were collected from the pathology reports. Histopathological features such as hormone receptor status, and HER2/neu status on IHC (Dako, Copenhagen, Denmark) were all analyzed at the Department of Pathology at the Kosin Gospel Hospital. Expressions of P53, estrogen receptor alpha (ERα), Ki-67 and ErbB2 were determined immunohistochemically on paraffin sections using antibodies against ERα (Dako, Copenhagen, Denmark), Ki67 (Dako, Copenhagen, Denmark), ErbB2 (Dako, Copenhagen, Denmark), p53 (Dako, Copenhagen, Denmark). Histologic grading was performed using the criteria of Bloom and Richardson. Lymphatic or vascular invasion (LVI) was defined as the presence of tumor emboli in peritumoral lymphatic spaces, capillaries or post capillary venules. ER status and PRstatus were taken as positive if more than 10% of tumor cells showed staining. Immunohistochemical score of 3+ for HER2 was accepted as HER2 positivity. And all of 2+ for HER2 specimens were undertaken additional fluorescent in situ hybridization (FISH). We also defined FISH + as HER2 positive.

The patients are grouped into 2 categories according to CK5/6 determined in surgical specimens: Group I consist with CK5/6 negative; Group II consist with CK5/6 positive.

Statistical Analysis

Statistical tests were performed using the SPSS 12.0 statistical software package for Windows (SPSS Inc, Chicago, IL). The survival function was calculated from the time of the oneset of disease to the occurrence of death. Survival data were censored on December31, 2009, which was the date on which the survival data were correlated with the death registry for the last time or 5 years after the onset of the disease. Kaplan-Meier estimates are presented for the survival function. And differences in survival were analyzed using the log rank test. Associations between specific immunohistochemicaland

clinical survival estimates and curves were established using the Kaplan-Meier method and differences in observed survival distribution among patient subgroups were tested with two-sided log-rank test. All survival rates were presented with their standard errors. We used Pearson's correlation to determine the association of explanatory variables and differences in qualitative variables were evaluated by Chi-squared test, where necessary. All *p*-values were two-sided and a *p*-value of less than 0.05 was considered to indicate a statistically significant difference.

Results

The median age was 48.6 years old, median tumor size was 2.72cm. Median follow-up period was 30.4 months. In the 131 cases of operable TNBCs among enrolled patients, CK5/6-positive group was detected in 15 cases (11.5%) and CK5/6-negative group in 116 cases (88.5%). Among 131 TNBC patients, 19 (14.5%) cases were recurred (5 were in bone, 3 were in lung, 1 in liver, 7 were in brain, 1 in thyroid, 1 in neck node, and 1 in contralateral breast), and 13 (9.9%) patients died because of recurrent breast cancer. No correlation was seen among age, histologic grade, p53 mutation, Ki-67 staining, vascular invasion and lymphatic invasion (Table 1). But strong correlation was seen among pathologic T-staging, pathologic N-staging and AJCC staging (pT, pN and stage, p=0.001). The 5-year overall survival and disease-free survival probabilities calculated by Kaplan-Meier Estimates (p-value for log rank test) are shown in Table 2 and 3. The univariate analysis for prognostic factors associated with DFS revealed that the nodal status as N1, N2, or N3 was statistically significant (Table 2,

Table 1. Association between patient's characteristics and the basal types of immunohistochemistry

Charicteristics		CK 5/6		P value
		Negative, n=116, n(%)	Positive, n=15, n(%)	
Age	≤35	10 (8.6)	2 (13.3)	0.552
	>35	106 (91.4)	13 (86.7)	
pT	0	1 (0.9)	0 (0)	0.001
	1	46 (40.4)	3 (20.0)	
	2	54 (46.5)	12(80.0)	
	3	15(12.9)	0 (0)	
pN	0	65 (56.0)	0 (0)	0.001
	1	25 (21.5)	8 (53.3)	
	2	12 (10.7)	3 (20.0)	
	3	14 (12.5)	4 (26.7)	
Stage	0	1 (0.9)	0 (0)	0.001
	I	31 (26.7)	0 (0)	
	II	50 (43.1)	2 (13.3)	
	III	33 (28.4)	13 (86.7)	
	IV	1 (0.9)	0 (0)	
Histologic grade	Gx	0 (0)	0 (0)	0.399
	G1	11 (9.5)	0 (0)	
	G2	35 (30.2)	4 (26.7)	
	G3	70 (60.3)	11 (73.3)	
P53	Negative	56 (48.2)	8 (53.3)	0.399
	Positive	60 (51.8)	7 (46.7)	
Vascular invasion	Negative	91 (78.4)	11 (73.3)	0.653
	Positive	25 (21.6)	4 (26.7)	
Lymphatic invasion	Negative	82 (70.7)	10 (66.7)	0.748
	Positive	34 (29.3)	5 (33.3)	
Ki67	Low (≤11%)	44 (37.9)	5 (33.4)	0.934
	High (>11%)	72 (62.1)	10 (66.6)	

Table 2. 5-years disease-free survival probabilities as calculated by Kaplan-Meier estimates(P-value for log rank test).

Characteristics		*N(events)	5-year(%)	P-value
Age	≤35	2(1)	50.0	0.558
	>35	13(4)	69.2	
pT	1	3(2)	33.3	0.249
	2	12(3)	75.0	
pN	1	8(0)	100	0.0001
	2	3(1)	66.7	
	3	4(4)	0	
Stage	II	2(0)	100	0.264
	III	13(5)	61.5	
Histologic grade	G2	4(2)	50.0	0.678
	G3	11(3)	72.7	
Ki-67	low(≤11%)	5(3)	40.0	0.749
	high(>11%)	10(3)	70.0	
P53	negative	8(3)	62.5	0.779
	positive	7(2)	71.4	
Vascular invasion	negative	11(3)	72.7	0.292
	positive	4(2)	50.0	
Lymphatic invasion	negative	10(2)	80.0	0.193
	positive	5(3)	40.0	
†EIC	negative	11(3)	72.7	0.761
	positive	4(2)	50.0	

*N(events) : Total patient number of the subgroup (patient number of recurrence in the subgroup)
†EIC : extensive intraductal component.

p=0.0001). And the univariate analysis for prognostic factors associated with OS revealed the N-staging was statistically significant (Table 3, p=0.001).

Table 3. 5-years overall survival probabilities as calculated by Kaplan-Meier estimates(P-value for log rank test).

Characteristics		*N(events)	5-year(%)	P-value
Age	≤35	2(1)	50.0	0.622
	>35	13(3)	76.9	
pT	1	3(2)	33.3	0.129
	2	12(2)	83.3	
pN	1	8(0)	100	0.001
	2	3(0)	100	
	3	4(4)	0	
Stage	II	2(0)	100	0.328
	III	13(4)	69.2	
Histologic grade	G2	4(2)	50.0	0.531
	G3	11(2)	81.8	
Ki-67	low(≤11%)	5(2)	60.0	0.778
	high(>11%)	10(3)	70.0	
P53	negative	8(3)	62.5	0.458
	positive	7(1)	85.7	
Vascular Invasion	negative	11(2)	81.8	0.117
	positive	4(2)	50.0	
Lymphatic Invasion	negative	10(1)	90.0	0.113
	positive	5(3)	40.0	
†EIC	negative	11(3)	72.7	0.762
	positive	4(1)	75.0	

*N(events) : Total patient number of the subgroup (patient number of recurrence in the subgroup)
†EIC : extensive intraductal component.

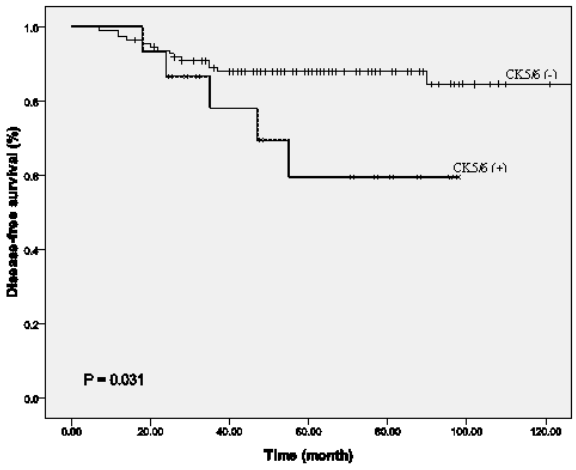

Fig. 1. Disease-free survival curves according to the immunohistochemical expression of cytokeratin 5/6 in immunohistochemistry.

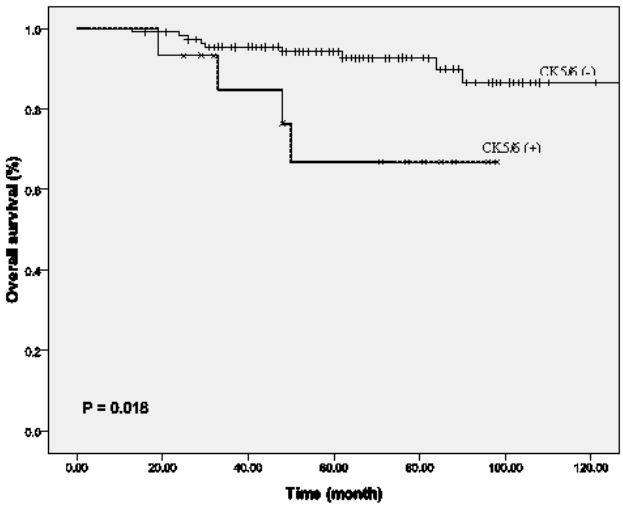

Fig. 2. Overall survival curves according to the immunohistochemical expression of cytokeratin 5/6 in immunohistochemistry.

Disease-free survival and overall survival rate between CK 5/6 positive and CK 5/6 negative in immunohistochemistry was shown in statistically significant differences (p=0.031, p=0.018, Fig. 1, and Fig. 2).

Discussion

It is well-known that breast cancer could be classified into 4 subgroups (luminal A, luminal B, HER2, and basal) according to its IHC characteristics. In 2000, Perou et al.[13] proposed that breast cancers could be classified by the characteristic gene expression profile. They defined five subgroups, including basal-like breast cancers, which expressed genes typical of basal epithelial breast cells. Gene array technology is not in routine clinical use, and for practical purposes, most studies define patient groups using IHC. In the current study, basal-like breast cancers could be defined as those that were negative for ER, PR and HER2-neu, but positive for either CK 5/6 or EGFR. This criteria was proposed by Nielsen et al in 2004, who found that defining basal-like breast cancer in this way resulted in 76% sensitivity and 100% specificity, when the gene expression signature is used as the gold standard.[3]

Most, but not all triple-negative breast cancers are

basal-like.[8] We considered whether or not the evaluation of CK5/6 would provide additional prognostic information in patients with triple-negative tumors. The addition of CK5/6 stains to the standard panel of three permitted the subdivision of the triple-negative category into basal-like and normal-like subcategories.

Basal-like cancer correlated with lymph node metastasis in this study. Sasa et al.[14] showed that basal-like cancer was associated with tumor size and nuclear grade but not nodal status. Cheang et al.[15]also demonstrated that the basal-like subtype was significantly associated with high grade (87% of cases were grade 3) and young age (18.8% were <40 years old) when the basal-like subtype 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 basal-like subtype identified.

The incidence rate of triple negative breast cancer was reported as 10~15% of total breast cancer in many studies. Mi-Jung Kim et al. reported that the frequency of TNBCs was 14%.[8] Sasa et al. described that the frequency of TNBC in Japanese women was 15% and Xin and coworkers reported that the frequency of TNBCs in Chinese women was 11%.[13,18]In the clinical feature of TNBCs, the higher incidence rate was shown in African-American women and the median age was younger than non-TNBCs. Mi-Jung Kim et al. reviewed in her study with other recent Korean journal about the frequency of TNBCs. In the report, the frequency of TNBCs is similar to those of African-American (24~26%) and raises the possibility of a similarity in the manifestation of TNBC between Korean and African-American women.[8,19] But, similar to Chinese report, we observed 10.6% of TNBCs in breast cancer cases.[18]

The proportion of basal like subtype in TNBCs reported varies (11%~87%) according to use which of immunohistochemical marker as inclusion criteria. Nielsen et al.[3] classified each tumor in a practical way based on its ER and HER2 expression. If it was both ER- and HER2-negative but positive for at least one basal marker (P-CDand/or p63 and/or CK5), it would be classified as basal (ER-/HER2-). Our study was shown 11.5% of basal like subtype in total TNBCs. This is because we investigated CK5/6 expression only in immunohistochemistry as inclusion criteria of basal-like breast cancer in TNBCs. It is necessary to use more markers to identify basal like subgroup in breast cancer cases, such as CK14, EGFR or gene investigation, such as BRCA1 mutation.

Conclusion

Our results show that CK5/6 is important immunohistochemical markers that can be used to predict prognosis in TNBCs. But, there need more larger number of cases, more IHCmarkers and gene investigation to observe more accurate DFS and OS rate.

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Reference

1) Cleator S, Heller W, Coombes RC:Triple-negative breast cancer: therapeutic options. Lancet Oncol ;8:235-244, 2007.
2) Reis-Filho JS, Tutt AN: Triple negative tumours: a critical review. Histopathology ;52:108-118, 2008
3) Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, Hernandez-Boussard T, Livasy C, Cowan D, Dressler L, Akslen LA, Ragaz J, Gown AM, Gilks CB, van de Rijn M, Perou CM: Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 10:5367-5374, 2004.
4) Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS,

Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein Lønning P, Børresen-Dale AL:Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA98:10869-10874, 2001.

5) van de Rijn M, Perou CM, Tibshirani R, Haas P, Kallioniemi O, Kononen J, Torhorst J, Sauter G, Zuber M, Köchli OR, Mross F, Dieterich H, Seitz R, Ross D, Botstein D, Brown P : Expression of cytokeratins 17 and 5 identifies a group of breast carcinomas with poor clinical outcome. Am J Pathol 161:1991-1996, 2005.

6) Matos I, Dufloth R, Alvarenga M, Zeferino LC, Schmitt F: P63, cytokeratin 5, and P-cadherin: three molecular markers to distinguish basal phenotype in breast carcinomas. Virchows Arch 447:688-694, 2005.

7) Livasy CA, Karaca G, Nanda R, Tretiakova MS, Olopade OI, Moore DT, Perou CM: Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Mod Pathol 19:264-271, 2006.

8) Kim MJ, Ro JY, Ahn SH, Kim HH, Kim SB, Gong GS: Clinicopathologic significance of the basal-like subtype of breast cancer: a comparison with hormone receptor and HER2/neu-overexpressing phenotypes. Hum Pathol 37:1217-1226, 2006.

9) Kobayashi S:Basal-like subtype of breast cancer: a review of its unique characteristics and their clinical significance. Breast Cancer 15:153-158, 2008.

10) Dawson AE, Norton JA, Weinberg DS: Comparative assessment of proliferation and DNA content in breast carcinoma by image analysis and flow cytometry. Am J Pathol 136:1115-1124, 1990.

11) Vielh P, Chevillard S, Mosseri V, Donatini B, Magdelenat H: Ki67 index and S-phase fraction in human beast carcinomas. Comparison and correlations with prognostic factors. Am J Clin Pathol 94:681-686, 1990.

12) Elledge RM, Green S, Pugh R, Allred DC, Clark GM, Hill J, Ravdin P, Martino S, Osborne CK: Estrogen receptor (ER) andprogesterone receptor (PgR) , by ligand-binding assay compared with ER, PgR, pS2, by immune-histochemistry in predicting response to tamoxifen in metastatic breast cancer: a Southwest Oncology Group Study. Int J Cancer 89(2):111-117, 2000.

13) Sasa M, Bando Y, Takahashi M, Hirose T, Nagao T:Screening for basal marker expression is necessary for decision of therapeutic strategy for triple-negative breast cancer. J Surg Oncol 97:30-34, 2008.

14) Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D: Molecular portraits of human breast tumours. Nature 406:747-752, 2000.

15) Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK: Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res 14:1368-1376, 2008.

16) Foulkes WD, Ingunn M, Stefansson S, Chappuis PO, Bégin LR, Goffin JR, Wong N, Trudel M, Asklen LA:Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst 95:1482-1485, 2003.

17) Palacios J, Honrado E, Osorio A, Cazorla A, Sarrio D, Barroso A, Rodriguez S, Cigudosa JC, Diez O, Alonso C, Lerma E, Sanchez L, Rivas C, Benitez J: Immunohistochemical characteristics defined by tissue microarray of hereditary breast cancer not attributable to BRCA1 or BRCA2 mutations: differences from breast carcinomas arising in BRCA1 and BRCA2 mutation carriers. Clin Cancer Res 9:3606-3614, 2003.

18) Xin Zhou, Qian Liu. Clinicopathological Characters of Triple Negative Breast Cancer. Chin J Cancer Res 22(1):17-20, 2010

19) Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC: Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 295:2492-2502, 2006.