International Journal of Medical Science and Clinical Invention 5(04): 3729-3733, 2018
DOI:10.18535/ijmsci/v5i4.03 **ICV 2016: 77.2**
e-ISSN:2348-991X, p-ISSN: 2454-9576

Research Article

Utility of Different Immunohistochemical Stain for Diagnosis of Invasive Breast Cancer

Dujuan Wang[1*], Li Chen[1], Lihua Zeng[2], Shuzhen Han[3]

[1, 2, 3] Department of Clinical Pathology, Affiliated Dongguan Houjie Hospital, Guangdong Medical College, Dongguan, Guangdong523945, China

Abstract:

Breast cancer is a malignant tumor that seriously affects females' physical health, which is the leading cause of cancer death among Chinese female. Estimating early diagnostic and prognostic markers are helpful to conduct treatment for patients with breast cancer. Accumulating investigations focused on the role of Jab1 and S100A8 proteins in the development and metastasis. In our study, we performed the immunohistochemical stain for Jab1 and S100A8 in breast carcinoma and para-carcinoma samples. We have found that the positive rate of Jab1 and S100A8 in breast cancer was higher than that in para-carcinoma tissues. The expression level of Jab1 and S100A8 in breast cancer might have a close relationship with the histologic grade and lymphatic metastasis. The two proteins might be promising supplementary targets for the treatment and prognosis of breast cancers in clinical pathology.

Keywords: Breast cancer ; Jab1 ; S100A8; Immunohistochemical stain

Introduction

Breast cancer (BC) is one of the most common cancers in women and is a major cause of death wordwide with nearly 1.7 million new cases diagnosed each year. WHO (World Health Organization) estimated that worldwide over 521,000 women died in 2012 due to breast cancer [1]. Breast cancer in China has characteristics of advance morbidity peak compared with western countries, and the average occurrence age is 10 years earlier than that in foreign countries[2]. There are many risk factors that might cause breast cancer, including genetic factors, environmental factors and mental factors[3]. Recent therapeutic advances have improved survival for many patients with breast cancer. These advances have been most impressive for targeted therapies, such as those targeting the estrogen receptor (tamoxifen) and the human epidermal growth factor receptor (EGFR) 2 (Her2) [4,5]. However the clinical benefit is limited because of instrinsic and acquired drug resistance[6,7]. Identification of key signaling molecules relevant to those patients who have no specific target medicine is therefore an important step toward the goal of improving breast cancer therapy [8-10]. Based on the limited medical resources in China, we must take positive researches to explore more diagnostic and prognostic markers, which can use widely in clinical pathology.

c-Jun activation domain-binding protein-1 (Jab1) is a multifunctional signaling protein that can mediate many of its biological effects, including tumor apoptosis, cell cycle regulation and promotion of cell survival [10]. Jab1 has found to be a master regulator of the 'wound response' and it is also interacts with many components of known cell signaling pathways such as NF-κB, Smad4 and P27 in breast cancer [11,12]. Additional evidence showed that Jab1 is a key gene in breast cancer progression comes from the recent finding that it is a downstream target for Her2 [13]. Recent studies have showed that abnormal expression of S100 protein is often related to tumor, such as thyroid cancer, colorectal cancer, prostatic cancer, bladder cancer, lung cancer, breast cancer and so on[14-20]. It is known that abnormal expression of multiple S100 proteins is associated with breast cancer, including S100A2, S100A4, S100A6, S100A7, S100A8, S100A9, and S100A11[21]. Increasing studies have reported S100A8 is overexpression in lung cancer and breast cancer[22,23], and it is associated with the progress, matastasis and chemo-resistance in breast cancer[24-26]. In this study, we will use immunohistochemical stain to detect the different expression of Jab1 and S100A8 in invasive breast carcinoma and para-carcinoma tissues and show the correlationship of these two markers and clinical characteristics.

Materials and methods

Tissue specimens

The breast cancer tissues were collected between 2014 and 2016 at Affiliated Dongguan Houjie Hospital of Guangdong Medical College. The diagnostic criterion was followed Breast tumours, Pathology and Genetics (WHO2012) . The study samples consisted of 30 invasive breast cancers with non-specific patients and 30 para-carcinoma tissues. All the samples were selected for this study based on the availability of archived paraffin-embedded BC and tissue blocks for immunohistochemical analysis. Each case were reviewed for tumor histologic grade, tumor size, lymph node status.

Immunohistochemical Analysis

A total of 60 formalin-fixed, paraffin-embedded human speciments (30 primary BC specimens and 30 para-carcinoma specimens) were analyzed. Immunohistochemical (IHC) analyses of Jab1 and S100A8 were performed using the Bond-Max system (Leica Biosystems, Wetzlar, Germany). Antigens were retrieved according to the Bond Max ER1 antigen retrieval protocol. Antibodies used in this study : Jab1 (mouse anti-human, 1/1000, Santa Cruz Biotechnology, Santa Cruz, CA) and S100A8 (mouse anti-human antibody, 1/800, Lifespan Bioscience, Seattle, USA).

The percentage of tumor cells exhibiting intense staining for Jab1 and S100A8 were evaluated in 10 high-power microscopy fields. Cases were considered positive when more than 10% of tumor cells were stained or negative when 10% or less were stained[27]. For each specimen, protein staining in the nucleus were scored separately.

Statistical analysis

Statistical analyses were perfored using the GraphPad Prism 6 for Windows. Pearson chisquare test (or Fisher exact test when appropriate) was used to compare the Jab1 and S100A8 expression between groups. Paired t-tests were performed to determine whether there were significant differences between the mean percentage of them in different groups. Data were considered statistically significant when the *p*-value was less than 0.050.

Result and discussion

Patient Characteristics and Demographics

Samples from 30 patients with non-specific invasive breast cancer (median age, 48 years old; rang, 29-74 years old) were used in the present study. The cancer occured in left breast is 16cases and right breast is 14cases. All the characteristics have show in Table 1. As reported, in China the average occurrence age is 47 years old, and the most women patients are from 40-49 years old[2]. Among our patients there is 14 cases (about 47% of total patients) occured among 40-50 years old. The tumor size almost is 2~5cm. Our data is consistent with this report.

Table 1 The characteristics of Breast cancer patients

Characteristics	Age(years)			Tumor size (cm)			Histologic grade			Lymphnode status(number)			
	20~40	40~60	>60	≤2	2~5	⩾5	I	II	III	0	1~3	4~9	>9
Breast cancer	2	22	6	5	24	1	1	17	12	20	4	2	4

Jab1 Expression in breast cancer

Jab1 was initially identified as c-Jun activation domain-binding protein-1 (Jab1) and subsequently discovered to be the fifth component of the constitutive photomorphogenic-9 signalosome (COPS5) [28]. This protein regulates a variety of cellular and developmental processes, including signal transduction, cells proliferation, cell cycle, apoptosis, DNA damage response (DDR) and tumorigenesis [29]. Previous studies have reported Jab1 overexpressed in breast cancers [30]. In our study, we used immunohistochemical analysis to reveal that 96% of the BC samples has positive staining for Jab1 (Figture 1). We found that the positive cells in para-carcinomas were almost in normal ductal epithelium cells, especially in gland epithelium cells. The positive rate was higher (96%) than that in the para-carcinomas tissues, which was 63% (*P=0.0025*<0.01; Table 2). The positive rate of Jab1 between different positive percentage groups has significant difference, but there is no difference between 10~50% group and 50~75% group. Above 75% group the positive rate is up to 83%. However the different positive rate between breast cancer and para-carcinoma is not related to tumor size, lymph node status and histologic grade. This finding suggested that Jab1 might be potential biomarker in BC, and it was consistent with other researches.

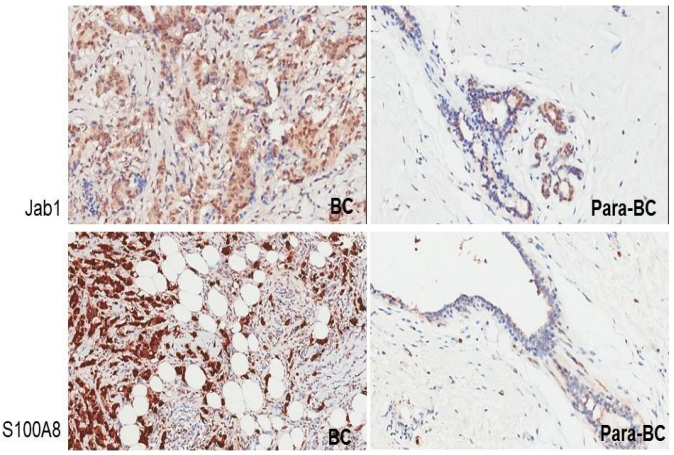

Figure 1 Expression of Jab1 and S100A8 proteins in breast cancer and para-carcinoma (×20)

S100A8 Expression in breast cancer

In breast cancer, glandular epithelial cell gets gene mutation under the action of multiple carcinogenic factors, and the immunophenotype of these disorder cells will be changed. They can interact with the surrounding microenvironment, moreover they will be invasive because the microenvironment factors will squeeze and destroy the surrounding normal tissues and damages the normal tissue structure of the breast[31,32]. S100 protein family is a kind of calcium binding protein with cell and tissue specificity[33], and increasing investigations have showed the overexpression in breast cancer. S100A8 is expressed by breast cancer cells as well as by infiltrating immune and myeloid cells. High breast cancer cell S100A8 protein expression was a significant prognostic factor for OS in 417 patients cohort[34]. Strong

expression and secretion of S100A8 may be associated with the loss of ER in BC, and may be involved in the poor prognosis of Her2+/basal-like subtypes of BC [24]. In our study, the positive rate of S100A8 in invasive breast cancer is 67%, which is higher than that in para-carcinoma(10%, *P<0.0001*; Figture1, Table 2). The percentage positive cells of 50~75% group is significantly differernt from above 75% group. As expression of Jab1 positive rate, there have no obvious ralationship with tumor size, histologic grade and lyphm node status because of the limited cancer sample number (Table 3).

The machanism about S100A8 overexpression in progression, invasion, matastasis and chemo-resistance of tumor involves the tumor microenvirenment. The S100A8 promotes the migration and invasion of human breast cancer cells through actin polymerization and epithelial-mesenchymal transition. Ye Y have demostrated that during premetastatic phase, an inflammatory response and inflammation-induced vascular hyperpermeability leading to an abnormal pulmonary microenvironment in a TGFβ-dependent manner, resulting an increasing expression of S100A8[22]. It will promote the circulating breast tumor cells to seed the lung. Becker A also reported that S100A8 has been implicated in the induction of tumor associated macrophaged (TAM) or myeloid-derived suppressor cells (MDSC), which support tumor development and spread[35]. Also there are increasing studies showed the signaling pathways of S100A8 in breast cancer, such as p38-MAPK, IL6 –JAK2-STAT3[36] and so on.

Table 2 The positive expression of Jab1 and S100A8 in different groups

Groups	n	Jab1 (%)				S100A8 (%)			
		⩽10	10~50	50~75	>75	⩽10	10~50	50~75	>75
BC	30	1	1	4	24	10	7	2	11
Para-BC	30	11	5	4	12	27	3	0	0

Table 3 The positive expression of Jab1 and S100A8 with different clinical characteristics

Characteristics		Jab1		S100A8	
		BC(n=30)	Para-BC(n=30)	BC(n=30)	Para-BC(n=30)
Tumor size (cm)					
	⩽2	5	4	2	0
	2~5	22	14	17	2
	⩾5	2	1	1	1
Tissue classified					
	Ⅰ	1	0	0	0
	Ⅱ	16	12	11	1
	Ⅲ	12	7	9	2
LN Status					
	0	20	11	14	2
	1~3	3	4	1	1
	4~9	2	1	2	0
	>9	4	3	3	0

Conclusion

Overexpression of Jab1 and S100A8 in breast cancer was testified in our study, which has significant difference between breast cancer and para-carcinoma. Moreover, the positive rate has no difference between different groups with tumor size, age, hitologic grade and lyphm node metastasis. Limited studies have not clarified the machanism of Jab1 and S100A8 in breast cancer clearly, however evaluation of Jab1 and S100A8 protein expression may provide additional prognostic information beyond traditional breast cancer prognostic biomarkers in clinical pathology.

References

[1] Lu R, Hu X, Zhou J, Sun J, Zhu AZ, Xu X, et al. COPS5 amplification and overexpression confers tamoxifen-resistance in ERα-positive breast cancer by degradation of NCoR. Nat Commun. 2016; 7:12044.

[2] Zheng Y, Wu CX, Wu F. Status and trends of breast cancer mortality in Chinese females. Zhonghua Yu Fang Yi Xue Za Zhi 2011; 45(2):150-154.

[3] Shah R, Rosso K, Nathanson SD. Pathogenesis, prevention, diagnosis and treatment of breast cancer. World J Clin Oncol 2014; 5(3):283-298.

[4] Abba MC, Hu Y, Sun H, Drake JA, Gaddis S, Baggerly K, et al. Gene expression signature of estrogen receptor alpha status in breast cancer. BMC Genomics. 2005; 6:37.

[5] Gruvberger-Saal SK, Eden P, Ringner M, Baldetorp B, Chebil G, Borg A, et al. Predicting continuous values of

prognostic markers in breast cancer from microarray gene expression profiles. Mol Cancer Ther. 2004; 3(2):161-168.

[6] Nahta R, Esteva FJ. Herceptin: mechanisms of action and resistance. Cancer Lett. 2006; 232(2):123-138.

[7] Selli C, Dixon JM, Sims AH. Accurate prediction of response to endocrine therapy in breast cancer patients: current and future biomarkers. Breast Cancer Res. 2016; 18(1): 118.

[8] Biswas DK, Iglehart JD. Linkage between EGFR family receptors and nuclear factor kappaB (NF-kappaB) signaling in breast cancer. J Cell Physiol. 2006; 209(3):645-652.

[9] Biswas DK, Shi Q, Baily S, Strickland I, Ghosh S, Pardee AB, et al. NF-kappa B activation in human breast cancer specimens and its role in cell proliferation and apoptosis. Proc Natl Acad Sci USA. 2004; 101(27):10137-10142.

[10] Emberley ED, Niu Y, Leygue E, Tomes L, Gietz RD, Murphy LC, et al. Psoriasin interacts with Jab1 and influences breast cancer progression. Cancer Res. 2003; 63(8):1954-1961.

[11] Adler AS, Lin M, Horlings H, Nuyten DS, Vijver MJ van de, Chang HY. Genetic regulators of large-scale transcriptional signatures in cancer. Nat Genet. 2006; 38(4):421-430.

[12] Adler AS, Littlepage LE, Lin M, Kawahara TL, Wong DJ, Werb Z, et al. CSN5 isopeptidase activity links COP9 signalosome activation to breast cancer progression. Cancer Res. 2008; 68(2):506-515.

[13] Hsu MC, Chai CY, Hou MF, Chang HC, Chen WT, Hung WC. Jab1 is overexpressed in human breast cancer and is a downstream target for HER-2/neu. Mod Pathol. 2008; 21(5):609-616.

[14] Puskas LG, Juhasz F, Zarva A, Hackler L Jr, Farid NR. Gene profiling identifies genes specific for well-differentiated epithelial thyroid tumors. Cell Mol Biol (Noisy-le-grand). 2005;51(2):177-186.

[15] Dahlmann M, Okhrimenko A, Marcinkowski P, Osterland M, Herrmann P, Smith J, et al. RAGE mediates S100A4-induced cell motility via MAPK/ERK and hypoxia signaling and is a prognostic biomarker for human colorectal cancer metastasis. Oncotarget 2014;5(10):3220-3233.

[16] Ye L, Sun PH, Martin TA, Sanders AJ, Mason MD, Jiang WG. Psoriasin (S100A7) is a positive regulator of survival and invasion of prostate cancer cells. Urol Oncol 2013;31(8):1576-1583.

[17] Yao R, Davidson DD, Lopez-Beltran A, MacLennan GT, Montironi R, Cheng L. The S100 proteins for screening and prognostic grading of bladder cancer. Histol Histopathol 2007;22(9):1025-1032.

[18] Hountis P, Matthaios D, Froudarakis M, Bouros D, Kakolyris S. S100A2 protein and non-small cell lung cancer. The dual role concept. Tumour Biol 2014;35(8):7327-7333.

[19] McKiernan E, McDermott EW, Evoy D, Crown J, Duffy MJ. The role of S100 genes in breast cancer progression. Tumour Biol 2011;32(3):441-450.

[20] Cormier K, Harquail J, Ouellette RJ, Tessier PA, Guerrette R, Robichaud GA. Intracellular expression of inflammatory proteins S100A8 and S100A9 leads to epithelial-mesenchymal transition and attenuated aggressivity of breast cancer cells. Anticancer Agents Med Chem 2014;14(1):35-45.

[21] Li F, Men X1, Zhang W. S100 protein in breast tumor. Indian J Cancer. 2014;51 Suppl 3:e67-71.

[22] Ye Y, Liu S, Wu C, Sun Z. TGFβ modulates inflammatory cytokines and growth factors to create premetastatic microenvironment and stimulate lung metastasis. J Mol Histol. 2015;46(4-5):365-375.

[23] Fidalgo F, Rodrigues TC, Pinilla M, Silva AG, Maciel Mdo S, Rosenberg C,et al.Lymphovascular invasion and histologic grade are associated with specific genomic profiles in invasive carcinomas of the breast. Tumour Biol. 2015;36(3):1835-1848.

[24] Bao YI, Wang A, Mo J.S100A8/A9 is associated with estrogen receptor loss in breast cancer. Oncol Lett.2016;11(3):1936-1942.

[25] Yin C, Li H, Zhang B, Liu Y, Lu G, Lu S,et al. Erratum to: RAGE-binding S100A8/A9 promotes the migration and invasion of human breast cancer cells through actin polymerization and epithelial-mesenchymal transition. Breast Cancer Res Treat. 2016; 156(2):407-408.

[26] Hsu YL, Hung JY, Tsai EM, Wu CY, Ho YW, Jian SF,et al.Benzyl butyl phthalate increases the chemoresistance to doxorubicin/cyclophosphamide by increasing breast cancer-associated dendritic cell-derived CXCL1/GROα and S100A8/A9. Oncol Rep. 2015;34(6):2889-2900.

[27] Lee HJ, Kim DI, Kwak C, Ku JH, Moon KC. Expression of CD24 in clear cell renal cell carcinoma and its prognostic significance. Urology. 2008; 72(3):603–607.

[28] Claret FX, Hibi M, Dhut S, Toda T, Karin M. A new group of conserved coactivators that increase the specificity of AP-1 transcription factors. Nature. 1996; 383: 453-457.

[29] Pan Y, Yang H, Claret FX. Emerging roles of Jab1/CSN5 in DNA damage response, DNA repair, and cancer. Cancer Biol Ther. 2014; 15(3): 256-262.

[30] Pan Y, Liu G, Yuan Y, Zhao J, Yang Y, Li Y. Analysis of differential gene expression profile identifies novel biomarkers for breast cancer. Oncotarget. 2017; 8(70):114613-114625.

[31] Fasching PA, Ekici AB, Wachter DL, Hein A, Bayer CM, Häberle L, et al. Breast cancer risk - From genetics to molecular understanding of pathogenesis. Geburtshilfe Frauenheilkd 2013;73(12):1228-1235.

[32] Evans MK, Longo DL. PALB2 mutations and breast-cancer risk. N Engl J Med 2014;371(6):566-568.

[33] Santamaria-Kisiel L, Rintala-Dempsey AC, Shaw GS. Calcium-dependent and - Independent interactions of the S100 protein family. Biochem J. 2006; 396(2):201-214.

[34] Miller P, Kidwell KM, Thomas D, Sabel M, Rae JM, Hayes DF, Hudson BI, et al.

Elevated S100A8 protein expression in breast cancer cell s and breast tumor stroma is prognostic of poor disease outcome. Breast Cancer Res Treat. 2017; 166(1):85-94.

[35] Becker A, Große Hokamp N, Zenker S, Flores-Borja F, Barzcyk K,Varga G, et al.Optical in vivo imaging of the alarmin S100A9 in tumor lesions allows for estimation of the individual malignant potential by evaluation of tumor-host cell interaction. J Nucl Med. 2015;56(3):450-456.

[36] Rodriguez-Barrueco R, Yu J, Saucedo-Cuevas LP, Olivan M, Llobet-Navas D, Putcha P, et al. Inhibition of the autocrine IL-6-JAK2-STAT3-calprotectin axis as targeted therapy for HR-/HER2+ breast cancers. Genes Dev. 2015; 29(15):1631-1648.