American Journal of Cancer Science
American Journals of Cancer Science
http://ivyunion.org/index.php/ajcs/
Vol 3 Article ID 201400424, 9 pages

Research Article

Differential Expression of Survivin in Mammary Gland Diseases

Aung Thiha[1], Yoshiyuki Osamura[2] Yoko Oishi[3], Hitoshi Ohashi[4], Yoshiko Yashiro[4], Hisaki Fukushima[4], Hajime Kuroda[1*]

[1]Department of Pathology, International University of Health and Welfare Hospital, Japan
[2]Department of Pathology, International University of Health and Welfare Mita Hospital, Japan
[3]Department of Breast Surgery, Nissan Tamagawa Hospital, Japan
[4]Department of Breast Surgery, International University of Health and Welfare Hospital, Japan

Abstract

Objective: Unlike other inhibitors of apoptosis proteins (IAP), survivin is expressed during fetal development, cannot be detected in normal adult tissues, and is re-expressed in most of the common human cancers. However, the clinicopathological significance of the expression of survivin in human breast carcinoma has not been fully elucidated. Therefore, we examined the accumulation of survivin in pathological specimens of mammary gland diseases to identify new protein markers that may lead to improvements in patient management.

Methods: We investigated the expression of survivin using immunohistochemistry in 180 cases of breast disease accessioned in the Department of Pathology, International University of Health and Welfare Hospital and Nissan Tamagawa Hospital between 2003 and 2013.

Results: Ninety-eight out of 141 malignant tumor cases (69.5%) stained positive for survivin, with no significant staining being detected in the remaining cases. Staining for survivin was completely absent in the epithelial cells of 34 of 39 cases of benign disease. No correlation was observed between the expression of survivin and major prognostic factors in breast carcinomas including patient age, tumor size, histological grade, axillary lymph node metastasis, local recurrence, and visceral metastasis status. However, a significant difference was observed in the expression of survivin between malignant and benign tumors and other benign diseases.

Conclusion: The results of the present study suggest that the expression of survivin is strongly selective for cancer cells and may be useful for identifying and quantifying human breast cancer.

Keywords: surviving; histological typing; mammary gland
Academic Editor: Xiaoning Peng, Hunan Normal University School of Medicine, China
Received: June 9, 2014; **Accepted**: July 26, 2014; **Published:** September 9, 2014
Competing Interests: The authors have declared that no competing interests exist.

***Correspondence to:** Hajime Kuroda, Department of Pathology, International University of Health and Welfare Hospital, 537-3 Iguchi, Nasushiobara, Tochigi 329-2763, Japan. E-mail: hajimek@iuhw.ac.jp

Introduction

Survivin is a member of the inhibitor of apoptosis protein (IAP) gene family, which has been implicated in both the inhabitation of apoptosis and regulation of mitosis [1]. Survivin is associated with the microtubules of mitotic spindles and is expressed in the G2M phase of the cell cycle [2]. Disruption of the survivin-microtubule interaction leads to the loss of survivin function and increases the activities of proapoptotic caspases 3 and 7. Unlike other IAPs, survivin is expressed during fetal development, cannot be detected in normal adult tissues, and is re-expressed in most of the common human cancers [3, 4]. Several immunohistochemical markers, such as P-53, Ki-67, HER2/neu, EGFR, estrogen receptor, CK5/6, and E-cadherin, have been evaluated for the discrimination of breast proliferative lesions. The development of a novel biomarker to prevent subjective errors may be necessary to assist pathologists in making accurate diagnose. To confirm these possibilities, we examined malignant, premalignant, and benign diseases and determine the use of survivin expression in mammary gland diseases. We investigated the accumulation of survivin in pathological specimens of mammary gland diseases to identify new protein markers that may lead to improvements in patient management.

Materials and methods

Patients and tissue samples

We identified 180 cases accessioned in the Department of Pathology, International University of Health and Welfare Hospital and Nissan Tamagawa Hospital between 2001 and 2013 lumpectomy specimens. Case histories were reviewed for age, tumor size, histological grading, lymph node metastasis, local recurrence, and visceral metastasis for the cancer patients. Patients were followed-up until 2014 and 74 for the patients with 141 breast carcinomas were available with 10-year follow-up data. The diagnosis of recurrent disease was based on clinical and radiological examinations, and was confirmed by the pathological findings. Survival time was defined as the period between the primary treatment and death in each case. Pathological diagnoses were assigned retrospectively according to the criteria adopted by the WHO classification [6].

Immunohistochemistry analysis

Sufficient lumpectomy specimens of breast diseases remained in blocks to permit the cutting of additional sections for immunohistochemical staining. Representative blocks were then selected for immunostaining. Survivin was evaluated by immunohistochemical analysis using a specific antibody. We examined survivin (Monoclonal mouse, clone 12C4, dilution of 1:50, DAKO Japan) protein expression using a fully automated system(NICHIREI BIOSCIENCES INC. Histostainer 36A). Briefly, five μm-thick unstained sections were placed onto an electrostatically charged glass slide and baked to allow for tissue adherence. Each immunostained slide was evaluated for the overexpression of survivin in the cancer cells. Cases were considered to be survivin-positive if more than 1% of the cell showed cytoplasmic and/or nuclear staining. Negative controls were obtained by omitting positive controls. All histological findings and immunostains were initially reviewed and scored by 2 pathologists (A.T. and H.K.).

Statistical analysis

The Social Survey Research Information Co., Ltd. Toukei 2012 software for Windows was used for statistical analysis. A $\chi 2$ test was used to assess statistical associations among variables, and the Mann-Whitney test was used to compare means. The Kaplan-Meier method was used for survival analysis, and differences in survival were estimated using the log-rank test. Differences were considered significant when p was less than 0.01.

Results

Clinicopathological characteristics

The clinicopathological characteristics of the 180 breast disease cases identified in this study are summarized in Tables 1, 2, and 3. There were 141 cases of malignant tumors. Among these, there were 110 cases of invasive carcinoma, no special type (IC), 4 of invasive lobular carcinoma, 22 of ductal carcinoma *in situ* (DCIS), and 5 of tumors of special type. There were 39 cases of benign diseases (24 fibroadenoma, 6 mastitis, 4 papilloma, 3 fibrocystic disease, and 2 phyllodes). We also selected 8 cases of usual ductal hyperplasia (UDH) and 11 of atypical ductal hyperplasia (ADH) accompanied by malignant mammary gland tumors in the same specimen. The respective lesion was used in the investigation. All patients were women with breast carcinoma, and their ages ranged from 32 to 88 years (mean 59.7). The mean tumor size was 2.1 cm at the maximum diameter. The 115 cases of invasive carcinoma were Elston and Ellis modified Bloom–Richardson grade 1 in 51 case, grade 2 in 25 cases, and grade 3 in 39 cases.

Table 1 Relationships between the characteristics of carcinoma and the survivin marker

	Survivin		P value
	Positive	Negative	
No.(141 carcinoma cases)	98	43	
Age	59.8	59.6	0.8436
Size(cm)			
Tumor size 2cm≧	51	27	0.2372
Tumor size 2cm<	47	16	
Histological grading (115 cases of invasive carcinoma*)	78	37	
Ⅰ	32	19	
Ⅱ	17	8	0.5050
Ⅲ	29	10	
Lymph node metastasis (%)	21 (21.4%)	12 (27.9%)	0.4029
Local recurrence (%)	6 (6.1%)	6 (14.0%)	0.2276
Visceral metastasis (%)	18 (18.4%)	6 (14.0%)	0.5208

*

Excluding invasive lobular carcinoma and ductal carcinoma in situ from malignant tumor.

Table 2 Relationships between survivin expression in benign diseases vs malignant tumors

	Histological type	No	Survivin		
			Positive	Negative	P value (malignant vs benign)
Malignant	Invasive carcinoma, no special type (IC)	110	74	36	
	Invasive lobular carcinoma	4	2	2	
	Neuroendocrine carcinoma	1	1	0	
	Mucinous carcinoma	1	1	0	
	Invasive micropapillary carcinoma	2	2	0	
	Spindle cell carcinoma	1	0	1	
	Ductal carcinoma *in situ* (DCIS)	22	18	4	
	Total	141	98	43	<0.0001
Benign	Fibrocystic disease	3	1	2	
	Fibroadenoma	24	3	21	
	Phyllodes	2	0	2	
	Papilloma	4	1	3	
	Mastitis	6	0	6	
	Total	39	5	34	

Table 3 Relationships between survivin expression in benign diseases vs UDH, ADH, DCIS, and IC.

Histological type	No	Survivin		
		Positive	Negative	P value
Benign disease	39	5	34	
Usual ductal hyperplasia (UDH)	8	2	6	0.7366
Atypical ductal hyperplasia (ADH)	11	7	4	0.0020
Ductal carcinoma *in situ* (DCIS)	24	19	5	<0.0001
Invasive carcinoma, no special type (IC)	110	74	36	<0.0001

Relationship between the expression of survivin with the clinicopathological characteristics of human breast diseases

The expression of survivin was observed in 98 of 141 cases of breast cancer (69.5%) and 5 of 39 (12.8%) cases of benign tumors and other benign diseases (Table 1,2). The staining pattern of breast carcinoma was predominantly characterized by the nuclear staining of epithelial cells (Figure 1). Very weak cytoplasmic staining was observed in only 4 of the 9 other cases of benign diseases. The expression of survivin was significantly higher in IC, DCIS, and ADH than in the benign disease group. A correlation was not observed between the expression of survivin and UDH (Table 3).

Correlations between the expression of survivin and major factors in breast carcinoma including

patient age, tumor size, histological grade, axillary lymph node, metastasis, local recurrence, and visceral metastasis status were examined. However, based on our results, survivin expression did not correlate with these prognostic factors.

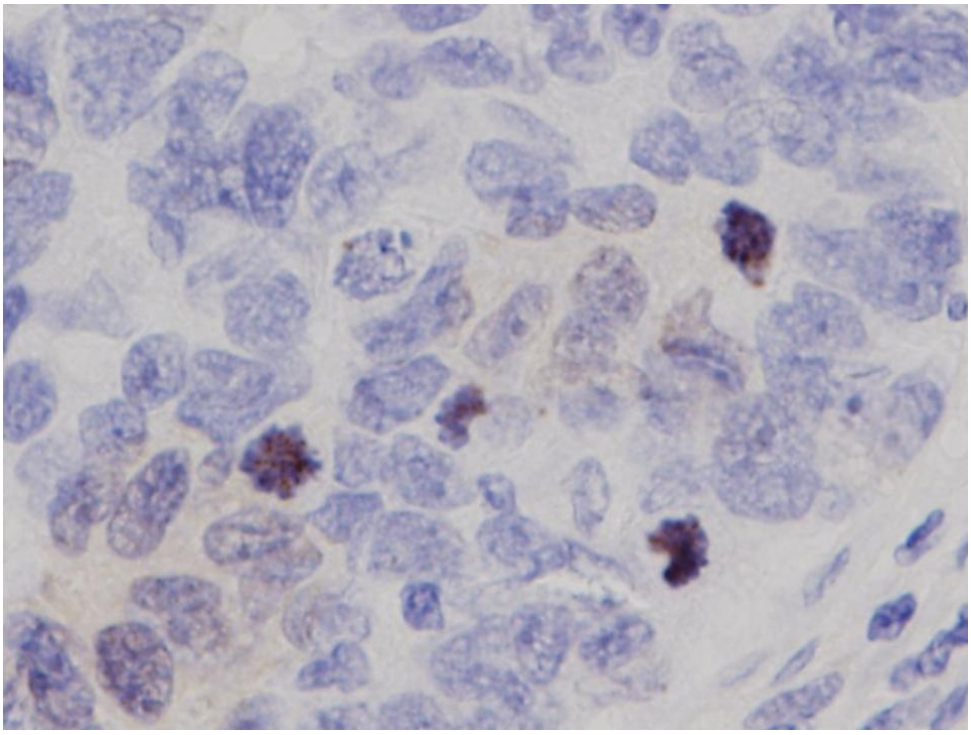

Figure 1 Carcinoma with strong nuclear reactivity in tumor cells to the survivin antibody scored as positive (400x).

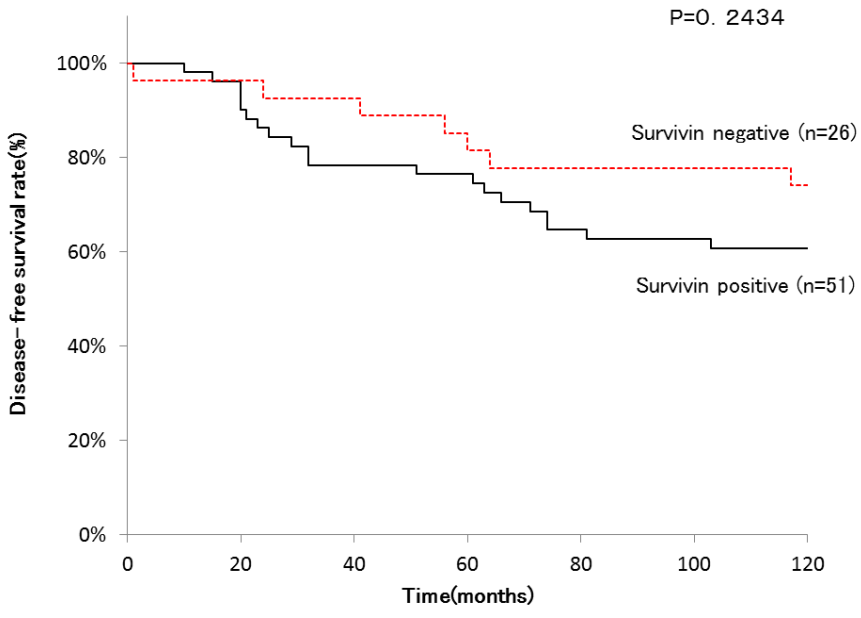

Figure 2

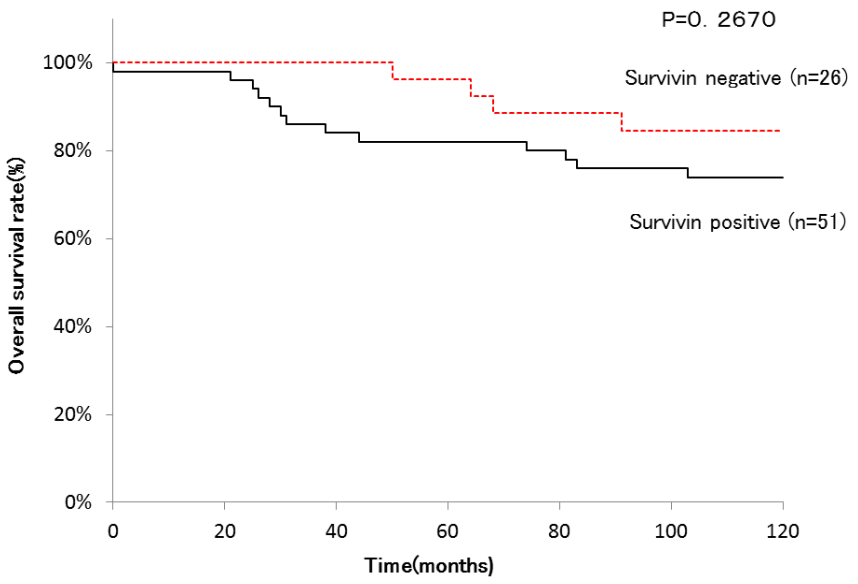

Figure 3

Figure 2, 3 These graphs illustrate the findings of disease-free and overall survival rates among breast cancer patients staining positive for survivin. Kaplan-Meier survival curves are shown for disease-free survival (Figure 2) and overall survival (Figure 3) rates.

Follow-up data were available in 77 of the 141 patients diagnosed with breast carcinomas. Of these, 17 died of the disease following the initial diagnosis of the primary lesion. Sixty patients were alive 10 years after the initial diagnosis of the primary lesion. The 10-year survival rate, when corrected for intercurrent deaths, was 13 (25.4%) for survivin positive patients and 4 (15.3%) for patients with survivin-negative tumors. A higher frequency associated with disease recurrence and death in survivin-positive patients (Figure 2, 3).

Discussion

A preliminary limited survey of previous studies indicated the differential expression of survivin in several types of cancers [3,4,7-9]. Similar to previous findings, in spite of histological variations in cancers, the expression of survivin was observed in the malignant cell components of most of our cases [4,5,7-21]. This result was in contrast to our benign disease control group, including fibroadenoma, mastitis, and fibrocytic disease, in which the expression of survivin was reduced or absent in the epithelial components. Al-Joudi et al recently described 382 women with invasive ductal carcinoma of the breast, and survivin was positive in 260 (68.1%) of these cases [17]. They emphasized the importance of detecting of survivin in breast carcinoma to aid in its diagnosis and confirm malignancy. However, they only examined the expression of survivin in cases of invasive ductal carcinoma of the breast and did not compare it with premalignant lesions, DCIS, or benign diseases. Based on our results compared with benign and malignant diseases, immunohistochemical staining for survivin, we agree with these conclusions. Therefore, using a protocol to detect the strong

expression of survivin may contribute to a histological diagnosis in mammary gland tumors.

The expression of survivin has also been detected in DCIS as well as in invasive lesions in breast tumors. Okumura et al reported the expression of survivin in DCIS and microinvasive carcinoma of the breast [22]. In contrast to our results, the frequency of a positive expression ratio of survivin was significantly higher in microinvasive carcinoma than in DCIS. However, DCIS is considered to be distinct from IC because of the lack of stromal invasion and a good prognosis. However, once stromal invasion occurs in DCIS, the biological behavior of these tumors becomes as aggressive as that of IC. The significantly lower expression of survivin in UDH than in ADH, DCIS, and IC may be due to its non-neoplastic potential. The expression of survivin was even observed in ADH lesions, but not in non-neoplastic regions, which is consistent with its selective expression in transformed cells. In addition, the number of survivin-expressing cells increased when ADH progressed to DCIS and IC. Similar to our results, the expression of survivin in precancerous lesions has been reported in several organs, which suggests that its expression occurs in the early stage of malignant transformation [7-9,20]. Thus, the expression of survivin may be induced during the transformation from benign to malignant tumors in the breast.

We demonstrated that the expression of survivin was not associated with prognostic factors (age, tumor size, histological grade, axillary lymph node metastasis, local recurrence, and visceral metastasis) or survival. The prognostic value of survivin has been reported in several studies on breast carcinoma [10-16,18-21]. Using immunohistochemistry staining, recent studies reported a correlation between the expression of survivin and clinicopathological parameters. However, the findings of previous studies on the association between survivin and the prognostic parameters of breast carcinoma patients were inconclusive or contradictory. Chu et al reported that the expression of survivin correlated with clinicopathological parameters [12]. However, it does not have significance as a marker for predicting overall or disease-free survival. Similar to their findings, Nassar et al reported that the expression of survivin correlated with histological parameters, but not with overall survival [19]. Kayaselcuk et al also reported that the expression of survivin did not correlate with any of the pathological parameters examined [13]. Furthermore, surviving did not correlate with local recurrence or distant metastasis.

However, the prognoses of patients in our study were based on an analysis of small groups; therefore, the meaning of these results is inconclusive.

This is the first study to compare the expression of survivin immunohistochemically between breast carcinoma and premalignant and benign mammary diseases with the aim that such information will provide a basis for studying the expression of survivin in the diseased breast. The results obtained with the survivin antibody suggest that survivin may be useful for identifying and quantifying human breast cancer. However, these results need to be replicated and expanded to more cases before any recommendations can be made for the use of this antibody in the routine identification of breast diseases.

Acknowledgments

The authors thank Kazuaki Hachimura, Masumi Seki, Satoko Souma, Toshihide Kikuchi and Shinichi Kamikura (Department of Pathology, International University of Health and Welfare Hospital) for their technical assistance with immunohistochemical staining.

References

1. Altieri DC, Marchisio PC. Survivin apoptosis: an interloper between cell death and cell proliferation in cancer. ***Lab Invest***. 1999, 79:1327-1333
2. Li F, Ambrosini G, Chu EY, Plescia J, Tognin S, Marchisio PC, Altieri DC. Control of apoptosis and mitotic spindle checkpoint by survivin. ***Nature***. 1998, 396:580-584
3. Adida C, Crotty PL, McGrath J, Berrebi D, Diebold J, Altieri DC. Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. ***Am J Pathol.*** 1998, 152:43-49
4. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. ***Nat Med***. 1997, 3:917-921
5. Simpson JF and Page DL. Pathology of preinvasive and excellent-prognosis breast cancer. ***Curr Opin Oncol***. 2001, 13:426-430
6. Lakhani SR, Ellis IO, Schnitt SJ Tan PH, van de Vijver MJ. WHO Classification of tumours of the breast, 4th ed. ***IARC Press***., *Lyon* 2012
7. Grossman D, McNiff JM, Li F, Altieri DC. Expression of the apoptosis inhibitor, survivin, in nonmelanoma skin cancer and gene targeting in a keratinocyte cell line. ***Lab Invest***. 1999, 79:1121-1126
8. Kawasaki H, Toyoda M, Shinohara H, Okuda J, Watanabe I, Yamamoto T, Tanaka K, Tenjo T, Tanigawa N. Expression of survivin correlates with apoptosis, proliferation, and angiogenesis during human colorectal tumorigenesis. ***Cancer***. 2001, 91:2026 -2032
9. Satoh K, Kaneko K, Hirota M, Masamune A, Satoh A, Shimosegawa T. Expression of survivin is correlated with cancer cell apoptosis and is involved in the development of human pancreatic duct cell tumors. ***Cancer***. 2001, 92:271-278
10. Tanaka K, Iwamoto S, Gon G, Nohara T, Iwamoto M, Tanigawa N. Expression of survivin and its relationship to loss of apoptosis in breast carcinomas. ***Clin Cancer Res***. 2000, 6:127-134
11. Kennedy SM, O'Driscoll L, Purcell R, Fitz-Simons N, McDermott EW, Hill AD, O'Higgins NJ, Parkinson M, Linehan R, Clynes M. Prognostic importance of survivin in breast cancer. ***Br J Cancer***. 2003, 88:1077-1083
12. Chu JS, Shew JY, Huang CS. Immunohistochemical analysis of survivin expression in primary breast cancers. ***J Formos Med Assoc***. 2004, 103:925-931
13. Kayaselcuk F, Nursal TZ, Polat A, Noyan T, Yildirim S, Tarim A, Seydioglu G. Expression of survivin, bcl-2, P53 and bax in breast carcinoma and ductal intraepithelial neoplasia (DIN 1a). ***J Exp Clin Cancer Res***. 2004, 23:105-112
14. Barnes N, Haywood P, Flint P, Knox WF, Bundred NJ. Survivin expression in *in situ* and invasive breast cancer relates to COX-2 expression and DCIS recurrence. ***Br J Cancer***. 2006, 94:253-258
15. Sohn DM, Kim SY, Baek MJ, Lim CW, Lee MH, Cho MS, Kim TY. Expression of survivin and clinical correlation in patients with breast cancer. ***Biomed Pharmacother.*** 2006, 60:289-292
16. Hinnis AR, Luckett JC, Walker RA. Survivin is an independent predictor of short-term survival in poor prognostic breast cancer patients. ***Br J Cancer***. 2007, 96:639-645
17. Al-Joudi FS, Iskandar ZA, Hasnan J, Rusli J, Kamal Y, Imran AK, Ahmed M, Zakaria J. Expression of survivin and its clinicopathological correlations in invasive ductal carcinoma of the breast. ***Singapore Med J***. 2007, 48:607-614
18. Al-Joudi FS, Iskandar ZA, Imran AK. Correlations in survivin expression with the expression of p53 and bcl-2 in invasive ductal carcinoma of the breast. ***Southeast Asian J Trop Med Public Health***. 2007, 38:904-910

19. Nassar A, Lawson D, Cotsonis G, Cohen C. Survivin and caspase-3 expression in breast cancer: correlation with prognostic parameters, proliferation, angiogenesis, and outcome. ***Appl Immunohistochem Mol Morphol***. 2008, 16:113-120

20. Tsai WC, Chu CH, Yu CP, Sheu LF, Chen A, Chiang H, Jin JS. Matriptase and survivin expression associated with tumor progression and malignant potential in breast cancer of Chinese women: tissue microarray analysis of immunostaining scores with clinicopathological parameters. ***Dis Markers***. 2008, 24:89-99

21. Athanassiadou AM, Patsouris E, Tsipis A, Gonidi M, Athanassiadou P. The significance of Survivin and Nectin-4 expression in the prognosis of breast carcinoma. ***Folia Histochem Cytobiol***. 2011, 49:26-33

22. Okumura Y, Yamamoto Y, Zhang Z, Toyama T, Kawasoe T, Ibusuki M, Honda Y, Iyama K, Yamashita H, Iwase H. Identification of biomarkers in ductal carcinoma in situ of the breast with microinvasion. ***BMC Cancer***. 2008, 8:287