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Original Article

Kif2a and HER2 expression in breast cancer tissue chip and their prognostic significance

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Abstract: Objective: To investigate and evaluate the expression of Kif2a and HER2 in breast cancer tissue chip and their prognostic significance. Methods: The Kif2a and HER2 expression were detected in the cancer and adjacent tissues of 102 patients with breast cancer by tissue chip and immunohistochemistry. The 62 patients were recieved follow-up, and results were analyzed by single factor analysis or Cox regression analysis. Results: There was positive correlation between the expression of Kif2a and HER 2 in breast cancer with different TNM grade and lymph node metastasis ($P < 0.05$). Kif2a was positively correlated to HER2 ($P < 0.01$). In the following up of the 62 patients, the results of single factor analysis showed that the survival rate was correlated to the expression of Kif2a and HER2, TNM grade and lymph node metastasis, which was with independently prognostic significance by Cox regression analysis. Conclusion: Kif2a and HER2 expressions in breast cancer tissue were positively correlated to TNM grade and lymph node metastasis ($P < 0.05$). The expressions of Kif2a and HER2 were with important significance in the detection of potential metastasis of breast cancer and the evaluation of prognosis.

Keywords: Kif2a, HER2, tissue chip, breast cancer, immunohistochemistry, prognosis

Introduction

Breast cancer was one of the malignant tumors threatening female worldly. Recently, the incidence of breast cancer increases and has a tendency of younger. Tissue chip is the special biological chip technique, which contains many tissue samples in a slide with regular array and is helpful for the research of primary tissue. In the beginning, tissue chip is widely applied for the large scale, high throughput, standardization and so on.

In 1995, Vale et al. [1] found the microtubule kinesin, and previous researches proved it was up-regulation in tumors [2, 3]. Kif2a was one member of the Kinesin-13 family. Human epidermal growth factor receptor 2 (HER2), also named as c-erbB-2 gene, was an important factor in the prognosis of breast cancer.

In our research, we used immunohistochemistry (IHC) to detect the expression of Kif2a and HER2 in human breast cancer tissue chip to investigate the correlation between the expression and clinical pathological feathers, which might provide new targets and methods for treatment of breast cancer.

Material and method

Clinical pathological data

The tumor samples from 102 patients with invasive breast cancer were collected in our department of pathology from January 2000 to December 2006. All patients were female aged from 34 to 81 (average age 54). They were all without radiotherapy, chemotherapy or endocrine therapy. Besides, 75 patients received follow-up, and the data was collected from 62 cases with the follow-up rate of 82.67% (62/75). The follow-up month was between 102 and 185, with the average 145 months.

According to the WHO classification standard of invasive breast cancer, there were 81 cases with invasive ductal carcinoma, 11 cases with invasive lobular carcinoma, and 2 cases with mucinous adenocarcinoma. According to the

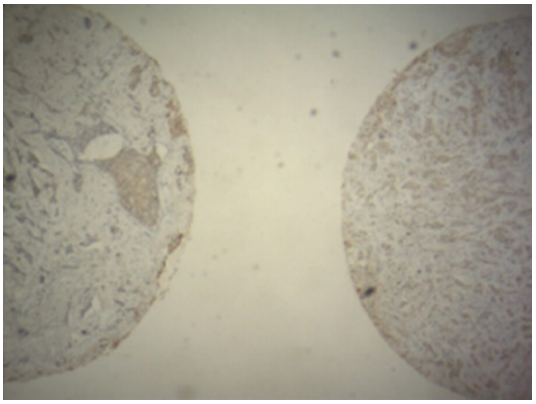

Figure 1. (SP×40). Kif2a expression in breast invasive ductal carcinoma.

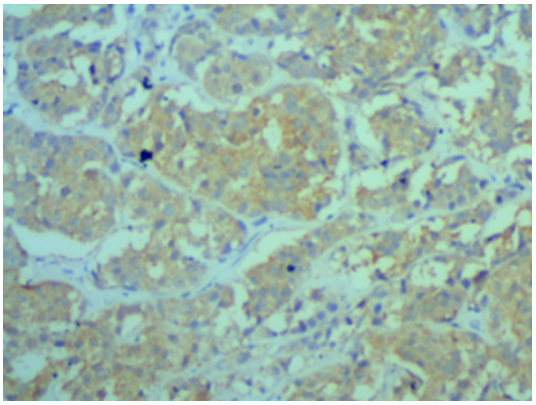

Figure 2. (SP×200). Kif2a expression in breast invasive ductal carcinoma.

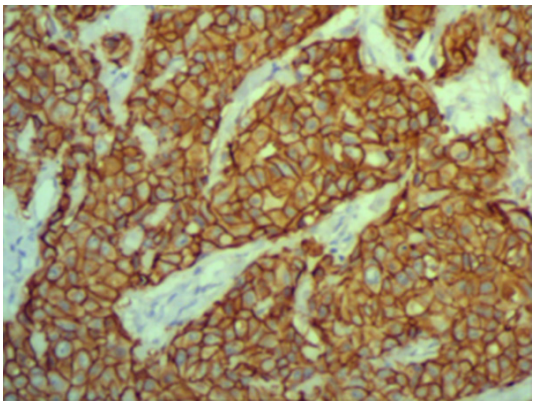

Figure 3. HER2 expression in breast invasive ductal carcinoma (SP×200).

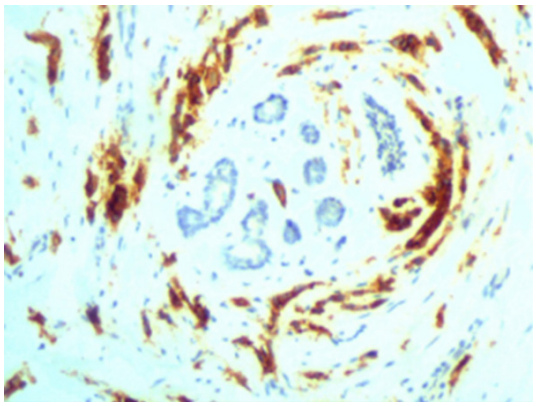

Figure 4. HER2 expression in breast invasive lobular carcinoma (SP×200).

TNM clinical stage, there were 78 cases in stage I+II, 34 cases in stage III+IV. According to the lymph node metastasis, there were 53 cases without lymph node metastasis, and 49 cases with.

Method

All fresh samples were fixed in 10% neutral formalin and embedded in paraffin after resection immediately. 102 tumor samples and adjacent tissues (within 3 cm of distance) were collected. The sections were labeled with representativeness according to HE staining. With the design of tissue chip, the tumor and adjacent tissues were made into paraffin cubes by perforate with fine needle and the machine, and then transferred on the glass slide for tissue chip. SP method was used for staining the tissue chip. The anti-human Kif2a mouse monoclonal antibody (ab55383) was obtained from Abcam plc. (Shanghai, China) according to standard working concentration (1:80). The anti-human HER2 mouse antibody was obtained from Beijing ZSGB-BIO technology Co., Ltd. (China). The positive Kif2a was mainly located in cytoplasm and nucleus of tumor cell and stained as brown-yellow. According to the scoring standard [4], the positive cells were scored as: 0 (without positive cell); 1 (positive cell < 1%); 2 (1% < positive cell < 10%); 3 (10% < positive cell < 33%); 4 (33% < positive cell < 67%); 5 (67% < positive cell). The staining intensity was scored as: 1 (light brown); 2 (moderate brown); 3 (dark brown). Combined the scores of positive cell and staining intensity, and the results were showed as: negative (score 0-1 or positive cell < 1%); weak expression (score 2-3); moderate expression (score 4-5); strong expression (score 6-8). Positive HER2 was mainly located in cytoplasm of tumor cell according to the judgment [5]: the tissue was considered as

Table 1. The expression of Kif2a, HER2 in invasive breast carcinoma and clinicopathologic feathers

	Kif2a				HER2			
Variables	n	($\bar{X}$±s)	t	P	+	-	X^2	P′
Age			-0.391	0.697			0.011	0.914
≤ 54	63	7.02±0.275			33	30		
> 54	39	7.06±0.736			20	19		
Histological type			-0.215	0.865			0.243	0.886
Invasive ductal carcinoma	81	7.03±1.423			43	38		
Invasive lobular carcinoma	11	7.05±0.921			5	6		
Others	10	7.02±1.971			5	5		
TNM stage			-2.026	0.045			15.880	0.000
I+II	78	6.85±0.843			32	46		
III+IV	24	7.22±0.531			21	3		
Lymph nodal involvement			-4.074	0.000			8.944	0.003
0	53	6.77±0.736			20	33		
1	49	7.26±0.425			33	16		

Table 2. Relation between the expression of Kif2a and HER2 in invasive breast carcinoma

		Kif2a				
HER2	n	L-Kif2a (%)	H-Kif2a (%)	P	Cramer's V	Gamma
-	49	36 (73.47)	13 (26.53)	0.000	0.414	0.709
+	53	17 (32.08)	36 (67.92)			

negative (-) when the number of cells was ≤ 10% that without staining or with incomplete cytoplasm and weak staining; positive (1+) was that the number of the cells was > 10%; positive (2+) was that the number of the cells was > 10%, or ≤ 10%; positive (3+) was that the number of the cell > 10%.

Statistical analysis

All the data was analyzed using Stata 7.0 software. The score of Kif2a was showed as $\bar{X}$±S. t-test and chi-square was used for comparing groups. The results of follow-up were analyzed with Log rank. Cox regression analysis was used in multivariate survival analysis, and the survival curve was obtained by Kaplan-Meier method. P < 0.05 was considered as statistically significant.

Results

Kif2a expression in tumor and adjacent tissues

Positive Kif2a expression was mainly located in cytoplasm and nucleus (**Figures 1**, **2**). Kif2a was positive in all invasive breast cancer tissues. The staining score had significant differences between tumor (7.03±0.825) and adjacent (6.36± 0.375) tissues (t=7.467, *P*=0.000).

Positive HER2 expression was mainly located in cytoplasm (**Figures 3**, **4**). In our results, 51.96% (53/102) of the tumor cells expressed positively. The positive rate was dramatically different between tumor (51.96%) and adjacent (9.8%) tissues (X^2= 45.46, *P*=0.000).

Correlation of Kif2a and HER2 expression and clinical pathological feathers in invasive breast carcinoma

Shown in **Table 1**, there was positive correlation between Kif2a and TNM stage, lymph node metastasis in invasive breast carcinoma (P < 0.05), while Kif2a was not related to age, histological type and so on. HER2 expression was significantly different among different TNM stages and groups with lymph node metastasis or not (P < 0.01).

Correlation between Kif2a and HER2 expressions

There were significant differences in the score of negative HER2 (6.72±0.825) and positive Kif2a (7.28±0.473) in the invasive breast cancer (t=-4.245, P=0.000).

The average value 7 of staining score was as the critical value. There were 53 cases with

Table 3. Univariant analysis and Cox stepwise proportional hazards analysis in 62 cases of invasive breast carcinoma

	Log rank		Cox's Proportional Hazard Model					
Variables	x^2	P	Hazard ratio	Standard Error	Z statistic	P′	95% confidence interval	
Age	0.02	0.881	-0.079	0.528	-0.15	0.881	-1.113	0.955
Histological type	0.37	0.829	-0.014	0.424	-0.03	0.973	-0.845	0.817
TNM stage	12.75	0.000	1.691	0.531	3.19	0.001	0.652	2.729
Lymph nodal involvement	7.74	0.005	1.845	0.760	2.43	0.015	0.354	3.335
Kif2a	4.98	0.026	1.540	0.760	2.03	0.043	0.050	3.030
HER2	5.08	0.024	1.554	0.766	2.04	0.041	0.064	3.044

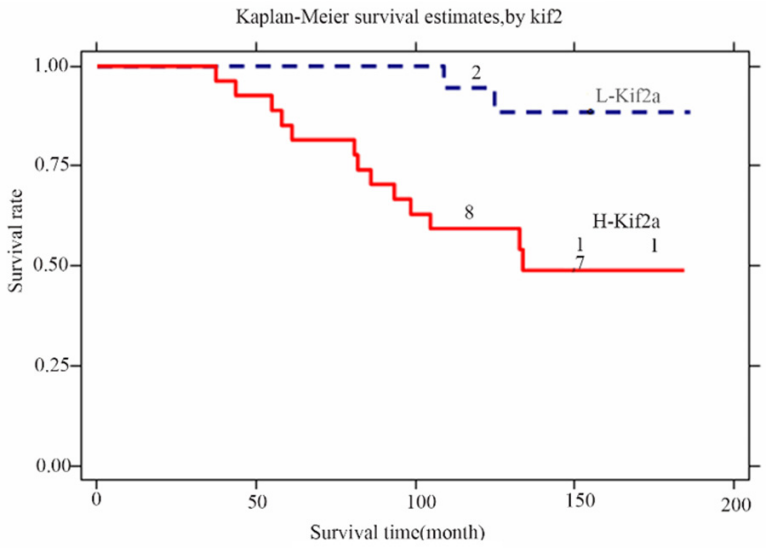

Figure 5. Kaplan-Meier survival estimates, by Kif2a.

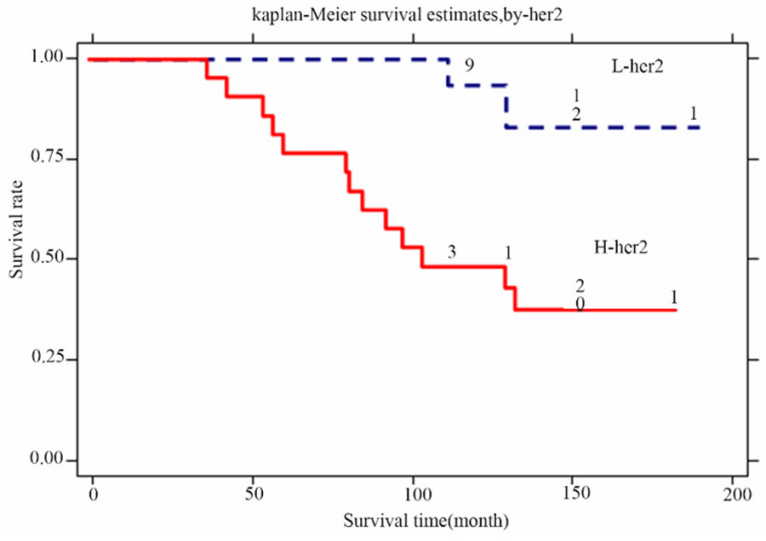

Figure 6. Kaplan-Meier survival estimates, by HER2.

L-Kif2a (≤ 7) and 49 cases with H-Kif2a (> 7) (**Table 2**).

As shown in the **Table 2**, there was positive correlation between Kif2a and HER2 expressions in breast cancer, with the gamma values of Cramer's V and Goodman-Kruskal were both > 0.

Analysis of clinicopathological feathers influencing prognosis of breast cancer

The 75 patients recieved follow-up, in which the follow-up rate was 82.67% (62/75), including 47 living cases and 15 dead. The Log rank and Cox regression analysis were shown in **Table 3**.

As shown in **Table 3**, there was correlation between lymph node metastasis, TNM stage, Kif2a, HER2 and the prognosis of breast cancer ($P < 0.05$), while there was no correlation between the age, histological type and the prognosis. The independently prognostic significances were existed among lymph node metastasis, TNM stage, Kif2a and HER2 expression in invasive breast cancer ($P < 0.05$). The risk ratios of lymph node metastasis, TNM stage, Kif2a and HER2 expression were all > 0, which indicated that the increase of these feathers would increase the mortality risk of breast cancer.

According to **Figure 5**, the survival rate of H-Kif2a was significantly lower than that of

L-Kif2a. In **Figure 6**, L-HER2 was with the higher survival rate.

Discussion

Since the promulgation of tissue chip by Kononen in 1998, it was efficiently used for research at the level of genome, transcriptome and proteome to analyze thousands of hundreds of tissue samples. The advantages of tissue chip included large scale, high throughput, standardization and so on, which promoted its wide using. The tissue chip could combine with IHC, hybridization in situ, fluorescence in situ hybridization (FISH), PCR in site and so on in many basic and clinical researches. In our study, we combined tissue chip with IHC to detect the Kif2a and HER2 expression in the breast cancer tumor and adjacent tissues of 102 patients to investigate the correlation of the two and the clinic pathological feathers.

Breast cancer usually occurred in female. The recurrence and metastasis of the tumor were the critical factors influencing the poor prognosis. Therefore, it is important to study the mechanism of tumor metastasis and improve the prognosis and search for the new therapy. Previous research [6] showed that Kinesin-13 family could regulate the changes of cytoskeleton, promote the cell proliferation and mediated the activation and migration of the cells by the depolymerization of microtubule. Kinesin-13 family contained three subfamily: Kif2a, Kif2b and Kif2c/MCAK [6]. Over-expression of Kif2c was related to tumor metastasis and prognosis [7]. However, there was few reports about the relationship between Kif2a and tumor metastasis, invasion and prognosis.

Kif2a has the function promoting the depolymerization of microtubule, participating in the assembly of spindle and promoting the cell division [8]. Tumor cell had stronger abilities of proliferation and division than normal cell. Therefore, we presumed that the abnormal expression of Kif2a was related to the invasion and metastasis of tumor cell. Human epidermal growth factor receptor 2 (HER2), also named as c-erbB-2 gene, was located at 17q12-21.32, which encoded the receptors-like transmembrane proteins with the relative molecular mass of 185000. HER2, with the activity of tyrosine kinase, could participate in the signal pathway, enhance the cell mitosis, regulate the cell growing, and promote tumor proliferation and differentiation.

In our study, Kif2a expression was strongly positive in the invasive breast cancer tissue, with significantly higher staining score in tumor tissue (7.03±0.825) than adjacent tissue (6.36±0.375) (t=7.467, P=0.000). This result indicated that Kif2a was closely related to the pathogenesis of breast cancer. Additionally, Kif2a expression was positively correlated to TNM stage and lymph node metastasis ($P < 0.05$), while not related to age or histological type. This result indicated that high expression of Kif2a promoted the malignant transformation of the cell, unstable cytoskeleton, and the invasive and metastatic tumor. These were accorded to the conclusions on Kinesin-13 family in malignant tumor drawn by Ishikawa et al. [2-4, 7, 9].

There was 51.96% positive HER2 in invasive breast cancer tissue, which was significantly different from the adjacent tissue (9.8%) (X^2=45.46, P=0.000). The HER2 expression in different TNM stages and those with lymph node metastasis or not were also significantly different ($P < 0.05$), which was according with previous studies [10, 11]. Otherwise, our results showed that there was closely relationship between Kif2a and HER2 expressions. This might due to the expressions of Kif2a and HER2 that were related to the promotion of cell division and proliferation, which was further identified that the pathogenesis and development were the complicated process with many interaction of multiple factors.

The survival rate of the patients, with high expression of Kif2a and HER2, was significantly lower than with low expression of Kif2a and HER2 ($P < 0.05$), which indicated invasive breast cancer, the lymph node metastasis, TNM stage, Kif2a and HER2 expressions were also related to the prognosis ($P < 0.05$), while the age and histological type were not. The lymph node metastasis, TNM stage, Kif2a and HER2 expressions were with the independently prognostic significance ($P < 0.05$), and the risk ratio were all > 0, indicating that these factors promoting the death risk of breast cancer.

In 2007, the drug double-targeting HER2 and EGFR was approved by Food and Drug Ad-

ministration (FDA) and got effectively therapeutic effect. It still needed further researches to study the coordinate expression between Kif2a and HER2 in the invasive breast cancer, and the association of the two with tumor pathogenesis, invasion and metastasis, which could provide new targets of anti-tumor drug and therapy, and support new thinking of the prognosis on tumor therapy.

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Disclosure of conflict of interest

None.

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