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Application of Quantum Dot Labeled Bimolecular Probe in the Analysis of Co-Expression of Her-2 and Ki-67 in Breast Cancer

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ABSTRACT. *Objective: To investigate the application of quantum dot labeled bimolecular probes in the analysis of synergistic expression of HER-2 and Ki-67 in breast cancer. Methods: Quantum dot labeled dual-color fluorescence imaging, image acquisition, spectral separation and quantitative separation of biomarkers. Results: HER-2 showed green fluorescence in breast cancer cell membrane and Ki-67 showed red fluorescence in cancer cell nucleus. The contrast between HER-2 and Ki-67 was obvious, which was helpful for quantitative analysis by spectral stripping. The median 8-DFS was 11.7 months (7.0-26.6 months) and 89.7 months (7.4-96.0 months) in the high and low expression group of Ki-67 (P<0.01). The median 8-DFS in the high HER-2 and high Ki-67 subgroups was shorter than that in the high HER-2 and low Ki-67 subgroups [11.7 months (95% CI: 11.0-26.6) vs 60.1 months (95% CI: 7.4-96.0 months), P < 0.05]; the low HER-2 and high Ki-67 subgroups were significantly shorter than the low HER-2 and low Ki-67 subgroups [16.4 months (95% CI: 6.97-25.67) vs 96.0 months. (95% CI: 16.0-96.0), P < 0.01); high HER-2 high Ki-67 group was significantly shorter than low HER-2 low Ki-67 group [11.7 months (95% CI: 11.0-26.6) vs 96.0 months (95% CI: 16.0-96.0), P < 0.01]; high and low HER-2 group had no significant difference compared with high Ki-67 high HER-2 group [11.7 months (95% CI: 11.0-26.6)] vs 96.0 months (95% CI: 16.0-96.0), P < 0.01]. CI: 11.0-26.6) vs 16.4 months (95% CI: 6.97-25.67), P = 0.586). Conclusion: Quantum dot labeled probe technique is helpful to quantitatively analyze the synergistic expression of HER-2 and Ki-67 in breast cancer.*

KEYWORDS: *Quantum dot labeled bimolecular probe; Breast cancer; HER-2; Ki-67; Co-expression*

1. Introduction

Human epidermal growth factor receptor-2,HER-2 and Ki-67 and Ki-67 are

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important clinicopathological indicators of breast cancer. They are used to judge the prognosis of breast cancer. However, the prognostic weights and the prognostic value of synergistic expression of Ki-67 and Ki-67 are still unclear. One of the main reasons is that conventional methods can not simultaneously quantify their expression levels in situ. Quantum dots (QDs) is a new type of semiconductor fluorescent nanoparticles. Its emission spectrum is narrow, its excitation spectrum is wide, its fluorescence intensity is high and stable, and its photobleaching resistance is strong. Therefore, nanotechnology based on quantum dots is helpful to multi-component simultaneous imaging in vitro, especially in the field of molecular pathology of multi-component in situ quantitative analysis of tumors[1]. The expressions of HER-2 and Ki-67 in breast cancer was quantitatively detected in situ by quantum dot labeled bimolecular technique, and the prognostic value of their co-expression was analyzed.

1. Materials and methods

1.1 Materials

In the clinicopathological database of breast cancer established by Zhongnan Hospital of Wuhan University, 33 breast cancer pathological specimens with complete treatment and prognosis information were selected for quantum dot staining. Follow-up data of all cases were complete. The median disease-free survival (DFS) of 8 years was 62.9 months (7.0-96.0 months).

1.2 Methods

(1) Reagents

Mouse anti-human HER-2 monoclonal antibody (CB11) was purchased from Fuzhou Maixin Company, rabbit anti-human Ki-67 monoclonal antibody (SP6) was purchased from Wuhan Jiayuan Biology Company, and quantum dot labeled sheep anti-mouse QDs 525 and sheep anti-rabbit QDs 655 were purchased from Life Technologies Company of the United States.

(2) Quantum dot labeled dual-color fluorescence imaging

According to the fluorescence imaging method of quantum dot labeling, two kinds of one-antibody from different species (mouse and rabbit) were mixed, and two different antigens on tissue slices were identified at the same time. Then the two kinds of two-antibody mixture labeled by two quantum dot probes were combined with the corresponding one-antibody respectively to achieve simultaneous coloration. Main steps: tissue section dewaxing, hydration, antigen repair (citric acid thermal repair), 2% BSA closure, incubation of first-antibody mixture (4 C, 16-18 h), PBS rinse, re-closure, incubation of second-antibody mixture labeled by quantum dots (37 C, 2 h), PBS rinse, fluorescence microscopy observation[2].

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(3) Image acquisition

The sections were observed by fluorescence microscopy equipped with Olympus DP72 camera and Cri Nuance multispectral imaging system. Ultraviolet light (330-385 nm) was used to excite both QDs 525 and QDs 655. The emission of QDs 525 made HER-2 green and the emission of QDs 655 made Ki-67 red. In Nuance multispectral imaging system, all spectral cubes (i.e. cube) containing spectral information collected from 490 to 720 nm at intervals of 10 nm are captured under the same conditions[3].

(4) Spectral Separation and Quantitative Separation of Markers

The spectral information of each cube is analyzed by image analysis software package. The fluorescence signal intensity and distribution area of each cube are calculated. The quantum dot/biomarker expression in the distribution area is quantified. The average value of cube in five different parts of each slice is used as the final experimental data[4].

1.3 Statistical analysis

SPSS 22.0 software was used for statistical analysis. Xtile software judged the best cut-off point of HER-2 and Ki-67 expression signal value; Kaplan-Meier method analyzed the difference of 8-DFS; Cox regression analysis quantified the prognostic weight of HER-2 and Ki-67, $P < 0.05$ was the difference.

2. Results

2. 1 Quantum dot immunofluorescence imaging

HER-2 showed green fluorescence on breast cancer cell membrane and Ki-67 showed red fluorescence on cancer cell nucleus. The contrast between HER-2 and Ki-67 was obvious, which was conducive to quantitative analysis by spectral stripping.

2.2 Correlations between expression of HER-2, Ki-67 and 8-DFS

According to X-tile software, the best cut-off point of HER-2 signal value was 214 651.0, and patients were divided into two groups: high expression (n = 14) and low expression (n = 19); the best cut-off point of Ki-67 was 3 684 227.0, and patients were divided into two groups: high expression (n = 5) and low expression (n = 28). The median 8-DFS was 36.3 months (95% CI: 7.4-96.0 months) and 93.0 months (95% CI: 7.0-96.0 months) in the high and low expression group of HER-2 ($P = 0.09$), and 11.7 months (7.0-26.6 months) and 89.7 months (7.4-96.0 months) in the high and low expression group of Ki-67 ($P < 0.01$).

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2.3 Synergistic expression of HER-2 and Ki-67 and prognostic analysis of 8-DFS

Table 1 ients were divided into four subgroups: high HER-2 high Ki-67, high HER-2 low Ki-67, low HER-2 high Ki-67, low HER-2 high Ki-67, low HER-2 high Ki-67 and low HER-2 low Ki-67. The median 8-DFS in the high HER-2 and high Ki-67 subgroups was shorter than that in the high HER-2 and low Ki-67 subgroups [11.7 months (95% CI: 11.0-26.6) vs 60.1 months (95% CI: 7.4-96.0 months), $P < 0.05$]; the low HER-2 and high Ki-67 subgroups were significantly shorter than the low HER-2 and low Ki-67 subgroups [16.4 months (95% CI: 6.97-25.67) vs 96.0 months. (95% CI: 16.0-96.0), $P < 0.01$); high HER-2 high Ki-67 group was significantly shorter than low HER-2 low Ki-67 group [11.7 months (95% CI: 11.0-26.6) vs 96.0 months (95% CI: 16.0-96.0), $P < 0.01$]; high and low HER-2 group had no significant difference compared with high Ki-67 high HER-2 group [11.7 months (95% CI: 11.0-26.6)] vs 96.0 months (95% CI: 16.0-96.0), $P < 0.01$]. CI: 11.0-26.6) vs 16.4 months (95% CI: 6.97-25.67), $P = 0.586$ (Table 1).

Table 1 Prognostic analysis of 8-DFS by co-expression of HER-2 and Ki-67

HER-2 vs Ki-67	Statistics	Low HER-2 Low Ki-67	High HER-2 Low Ki-67	Low HER-2 High Ki-67	High HER-2 High Ki-67
Low HER-2 Low Ki-67	χ^2	-	3.439	10.671	14.504
	P	-	0.064	0.001	0.000
High HER-2 Low Ki-67	χ^2	3.439	-	4.417	4.716
	P	0.064	-	0.036	0.030
Low HER-2 High Ki-67	χ^2	10.671	4.417	-	0.297
	P	0.001	0.036	-	0.586
High HER-2 Low Ki-67	χ^2	14.504	4.716	0.297	-
	P	0.000	0.030	0.586	-

2.4 Weight Analysis of HER-2 and Ki-67 Influencing 8-DFS

The results showed that the weight of Ki-67 on the prognosis of breast cancer was greater than that of HER-2. Cox regression was used to quantify the weight of HER-2 and Ki-67 on the prognosis. The risk ratio of Ki-67 was 4.493 (95% CI: 1.207-16.726), while that of HER-2 was 1.481 (95% CI: 0.473-4.643). The risk ratio of 8-DFS is about three times that of HER-2.

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3. Discussion

The expressions of HER-2 and Ki-67 in breast cancer was detected simultaneously in situ for the first time by quantum dot labeled bimolecular probe technique. The image was clear and the contrast was obvious[5]. HER-2 is a predictor of poor prognosis of breast cancer. Patients with over-expression of HER-2 have a higher risk of recurrence[6]. The median 8-DFS in the high-expression group of HER-2 is shorter than that in the low-expression group (36.3 months vs 93.0 months). There is no significant difference (P = 0.09). This is inconsistent with the above-mentioned reports, and may be the standard. The quantity of HER-2 was relatively small, and the immunohistochemical detection of HER-2 in all specimens was 2 + and 3 + related. Yang et al. found that high expression of Ki-67 was a poor prognostic factor for 5-DFS in breast cancer patients. Sun et al. also considered that Ki-67 score was an independent prognostic factor for HER-2 positive (non-epithelial) breast cancer patients. The difference of 8-DFS between high and low Ki-67 expression groups was significant (11.7 months vs 89.7 months, P < 0.01), suggesting that the relapse risk of high Ki-67 expression group was higher than that of low Ki-67 expression group (11.7 months vs 89.7 months, P < 0.01).

At present, there are few studies on the weight of HER-2 and Ki-67 on the prognosis of breast cancer, and the prognostic value of their synergistic expression is not clear[7-8]. In this experiment, the expression of HER-2 and Ki-67 in breast cancer was quantitatively detected in situ for the first time, and their synergistic expression was analyzed. The median 8-DFS in high HER-2 and high Ki-67 group was shorter than that in high HER-2 and low Ki-67 group (11.7 months vs 60.1 months, P < 0.05), while the low HER-2 and high Ki-67 group was significantly shorter than that in low HER-2 and low Ki-67 group (16.4 months vs 96.0 months, P < 0.01), suggesting that both high and low expression of HER-2 and Ki-67 had an effect on breast cancer 8-DFS, and had an effect on breast cancer 8-DFS in H-67 group. The low expression of ER-2 had an independent effect; the median 8-DFS had no significant difference between the high Ki-67 low HER-2 group and the high Ki-67 high HER-2 group (P = 0.586), while the low Ki-67 high HER-2 group was shorter than the low Ki-67 low HER-2 group, but the difference was only close to the edge of statistical significance (60.1 months vs 96.0 months, P = 0.064), suggesting that Ki-67 high HER-2 group was lower than the low Ki-67 low HER-2 group. - HER-2 had some effect on 8-DFS at low expression of 67, but had no effect on 8-DFS at high expression of Ki-67. The results showed that both HER-2 and Ki-67 were poor prognostic indicators of breast cancer, and the effect of Ki-67 on prognosis was greater than that of HER-2. Further Cox multivariate regression analysis showed that the risk ratio of Ki-67 was 4.493, while that of HER-2 was 1.481, suggesting that the weight of adverse prognosis of Ki-67 was about 3 times that of HER-2 (4.493/1.481).

In conclusion, quantum dot labeled probes can help quantitatively analyze the synergistic expression of HER-2 and Ki-67 in breast cancer. The weight of adverse prognosis of breast cancer affected by Ki-67 is about three times that of HER-2. Due to the limited number of experimental specimens in this group, it is necessary to

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further calculate and validate the exact relationship between HER-2 and Ki-67 in breast cancer in large sample studies.

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