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Original Article

Correlation of LF-PRL-R expression with ER/PR and HER-2 expression in breast cancer

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Abstract: Background and objective: The activation of prolactin receptor (PRL-R) may contribute to the development and progression of breast cancer, which is mainly mediated by the long form of PRL-R (LF-PRL-R). Therefore, we analyzed the correlation of LF-PRL-R with ER, PR, and HER-2 expression in breast cancer. Methods: One hundred and thirty female patients with breast cancer (median age, 46 years; age range 26-77 years) undergone surgery without new adjuvant therapy at Sun Yat-sen University Cancer Center between Jan 2000 and Jun 2001 were included. The expression of LF-PRL-R, ER, PR, and HER-2 in the primary lesion from each patient was detected by immunohistochemistry. The correlation of LF-PRL-R expression with ER, PR, and HER-2 in breast cancer was assessed by Chi-square test. Results: Among 130 patients, 89 showed positive LF-PRL-R expression. Stratification of the statistical analysis showed that in the HER-2-positive sub-layer, LF-PRL-R expression was positively correlated with ER and PR expression ($P < 0.05$), while no correlation was noted in the HER-2-negative sub-layer ($P > 0.05$). In the ER (or PR) positive sub-layer, LF-PRL-R expression was positively correlated with HER-2 expression ($P < 0.05$), while no such correlation was noted in the ER (or PR)-negative sub-layer ($P > 0.05$). Conclusion: The positive correlation of LF-PRL-R expression with ER/PR in breast cancer relies on the positive expression of HER-2, while the positive correlation with HER-2 expression relies on the positive expression of ER/PR, which suggesting combined anticancer therapy based on the individual target site may benefit patients with breast cancer.

Keywords: Breast cancer, long form of prolactin receptor (LR-PRL-R), ER, PR, HER-2

Introduction

Approximately 70% of human breast cancer tissues express prolactin receptor (PRL-R), which may contribute to the development and progression of breast cancer. In fact, the biological effect of PRL-R is mainly induced through the long form of PRL-R (LF-PRL-R) [1-6]. The expression of ER/PR and HER-2 is an important prognostic indicator of breast cancer. Anticancer therapies based on these two targets have been considered as two important therapeutic methods for breast cancer. Laboratory studies have indicated that positive regulation exists between PRL-R and ER/PR, as well as HER-2, which suggests that there may be some complex interrelationships among PRL-R, ER/PR and HER-2 [7-10]. Studies on these interrelationships among them may help to find out some clues for optimizing anti-PRL-R, anti-ER/PR, and anti-HER-2 combination therapy. Thus, we attempted to elucidate the complex interrelationship between LF-PLR-R expression and the expression of ER/PR and HER-2 based on clinical pathological evaluation.

Materials and methods

Patients

One hundred and thirty female breast cancer patients undergone surgery without new adjuvant chemotherapy from Jan 2000 to Jun 2001 at Sun Yat-sen University Cancer Center were retrospectively collected. Paraffin-embedded primary cancer tissues were well-preserved. The patients were 26-77 years of age (median age: 46 years old). There were 80 pre- and 50 post-menopausal patients. The primary lesions were in the inner/central and outer quadrants in 48 and 82 patients, respectively. According to the pathologic classification of breast cancer

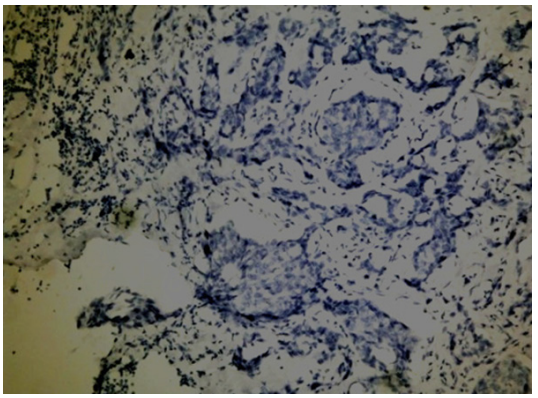

Figure 1. PRL-R negative expression (×200).

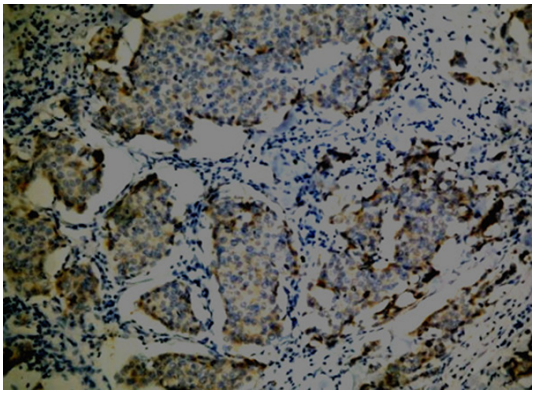

Figure 2. PRL-R positive expression (×200).

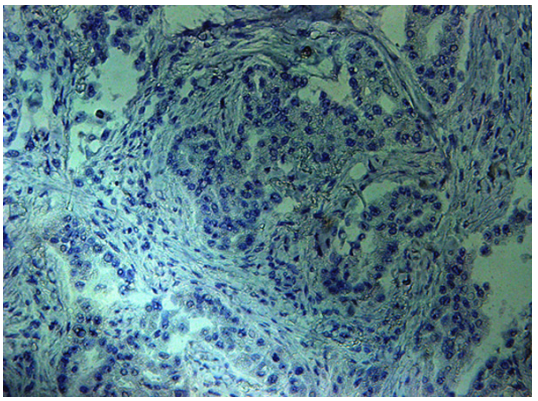

Figure 3. ER negative expression (×200).

by WHO, 119 patients had invasive ductal carcinoma, 5 had early invasive ductal carcinoma, and 6 had other types of carcinoma.

Immunohistochemistry

The expression of LF-PRL-R, ER, PR and HER-2 in post-operative samples was detected by

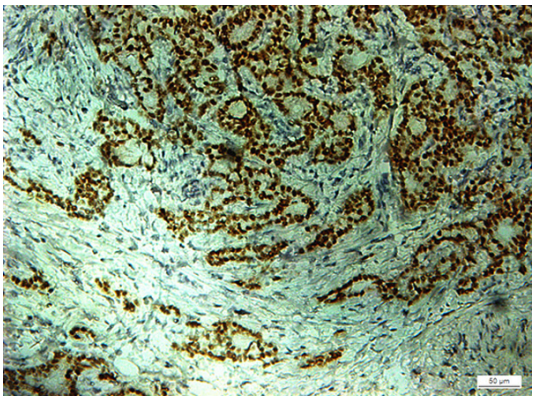

Figure 4. ER positive expression (×200).

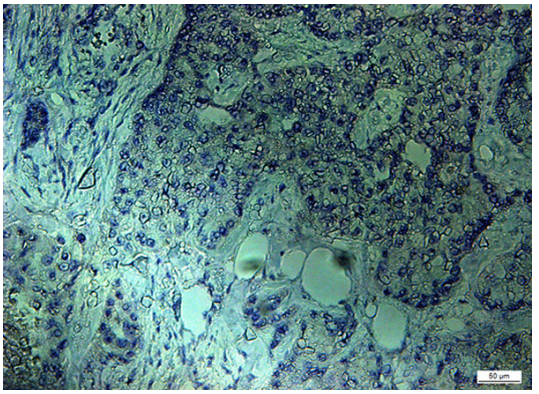

Figure 5. PR negative expression (×200).

immunohistochemistry with LSAB kit according to the manufacturers' instructions. Briefly, 6-μm slices were obtained from the biopsy specimen embedded in paraffin, heated in the thermostat at 60°C for 2 h and cooled in liquid at 37°C. Then, the tissue slices were placed into a fresh xylene tank twice (5 min each time), dipped into 95% ethanol twice (3 min each time), 70% ethanol twice (3 min each time), distilled water for at least 30 s, and pre-heated 0.01 M citric acid buffer solution (100°C, pH 8.0) for 20 min, and stood still at room temperature for 20 min. After dipped in distilled water for 3 min, the tissue slices were delineated from 2 mm away with an anti-seepage pen. Then, the slices were incubated with solution A (3% H_2O_2) and solution B (normal serum) for 10 min at room temperature, respectively. Then the tissue slices were incubated with mouse primary monoclonal antibodies (1:50) (ZYMED Laboratories, U.S.A) overnight at 4°C. The primary antibody was discarded and the tissue slices were thrice-dipped (5 min each time) in PBS (0.01 M, pH 7.4). Then the slices were incu-

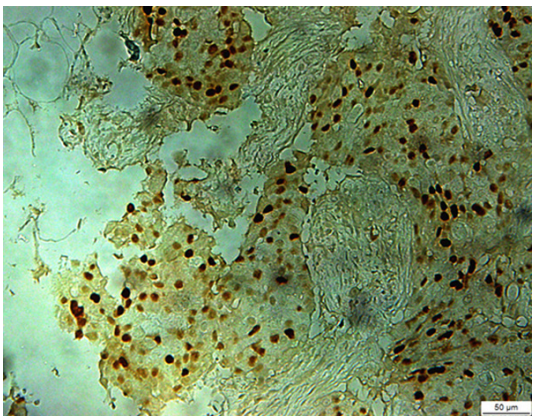

Figure 6. PR positive expression (×200).

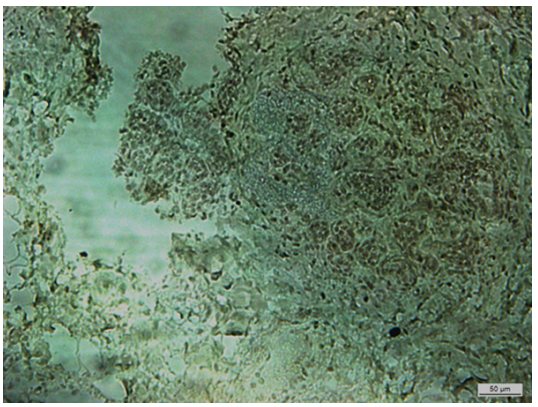

Figure 7. HER-2 negative expression (×200).

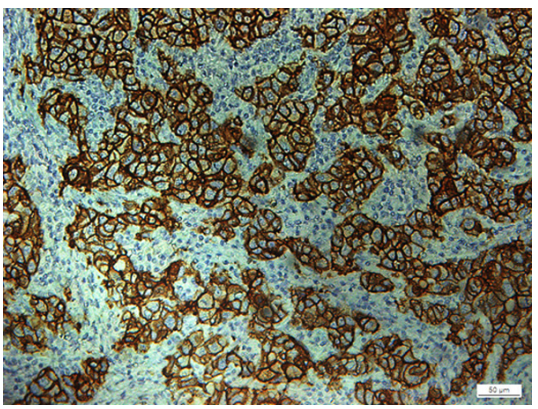

Figure 8. HER-2 positive expression (×200).

bated with solution C (biotin-labeled secondary antibody) and solution D (horseradish peroxidase-labeled streptavidin) at 37°C for 15 min respectively and developed with fresh DAB developer solution for 10 min. After rinsed with running water and stained in hematoxylin solution for approximately 30 s, the slices were dried and sealed in neutral balsam for observation.

The entire tissue slice was observed under an optical microscope by pathologists with extensive experience who were blinded to the clinical data. When the percentage of positive cells were < 10% of the total number of cells, the tissue was categorized as "negative expression" and when the percentage was > 10%, the tissue was categorized as "positive expression."

Statistical analysis

SPSS 13.0 for Windows was used for statistical analysis. The correlation of LF-PRL-R expression with ER, PR, and HER-2 expression in breast cancer was determined by Chi-square test. $P < 0.05$ was defined as statistically significant.

Results

Overall results

Among the 130 patients, 89 showed positive LF-PRL-R expression (positive rate: 68.5%), 70 showed positive ER expression (positive rate: 53.8%), 88 showed positive PR expression (positive rate: 67.7%) and 97 showed positive HER-2 expression (positive rate: 74.6%), as shown in **Figures 1** to **8**. The positive rate of LF-PRL-R expression in ER-positive patients was greater than ER-negative patients ($P < 0.05$), suggesting LF-PRL-R expression is positively correlated with ER expression. The positive rates of LF-PRL-R expression in PR-negative and -positive patents, and HER-2-negative and -positive patients were not statistically different ($P > 0.05$) (**Table 1**).

Results of stratification analysis

The positive rate of LF-PRL-R expression in ER-positive patients was greater than ER-negative patients (LF-PRL-R expression was positively correlated with ER expression). This correlation was only limited in the HER-2-positive sub-layer ($P < 0.05$), while no such correlation was noted in the HER-2-negative sub-layer ($P > 0.05$). Similarly, the positive rate of LF-PRL-R expression in PR-positive patients was greater than PR-negative patients (LF-PRL-R expression was positively correlated with PR expression), which was only shown in

Table 1. Correlation of LF-PRL-R expression with ER, PR, and HER-2 expression in all patients

Clinical factor		Total cases	PRL-R (+) cases	Positive rate (%)	X^2 value	*P* value
ER	-	60	35	58.1	5.294	0.021
	+	70	54	77.1		
PR	-	42	25	59.5	2.295	> 0.05
	+	88	64	72.7		
HER-2	-	33	19	57.6	2.472	> 0.05
	+	97	70	72.2		

Table 2. Correlation of LF-PRL-R expression with ER and PR expression after stratificated by HER-2 expression

HER-2 sub-layer			Cases	LF-PRL-R (+) cases	Positive rate (%)	X^2 value	*P* value
HER-2 (-)	ER	-	16	10	62.5	0.308	> 0.05
		+	17	9	52.9		
	PR	-	11	7	63.6	0.248	> 0.05
		+	22	12	54.5		
HER-2 (+)	ER	-	44	25	56.8	9.442	0.002
		+	53	45	84.9		
	PR	-	31	18	58.1	4.510	0.034
		+	66	52	78.8		

Table 3. Correlation of LF-PRL-R expression with HER-2 expression after stratificated by ER/PR expression

Stratification factor	Sub-layer	HER-2	Cases	LF-PRL-R (+) cases	Positive rate (%)	X^2 value	*P* value
ER	-	-	16	10	62.5	0.156	> 0.05
		+	44	25	56.8		
	+	-	17	9	52.9	7.458	0.006
		+	53	45	84.9		
PR	-	-	11	7	63.6	0.105	> 0.05
		+	31	18	58.1		
	+	-	22	12	54.5	4.889	0.027
		+	66	52	78.8		

the HER-2-positive sub-layer (P = 0.034 < 0.05), but not in the HER-2-negative sub-layer (P > 0.05) (**Table 2**).

The positive rate of LF-PRL-R expression in HER-2 positive patients was higher than HER-2-negative patients (LF-PRL-R expression was positively correlated with HER-2 expression), which was only shown in the ER (or PR)-positive sub-layer (P = 0.006 and 0.027 < 0.05, respectively), but not in the ER (or PR)-negative sub-layer (P > 0.05) (**Table 3**).

Discussion

PRL-R and PRL have been shown to contribute to the development and progression of breast cancer [1-4]. The subtypes of PRL-R expressed in breast cancer include the long form (LF), medium form (MF) and short form (SF). Studies suggest that LF and SF may have distinct biological and expression features. The enhancement of PRL-R on the growth/proliferation and invasion of breast cancer cells is mainly mediated by the LF subtype (LF-PRL-R) [6]. The mouse primary monoclonal antibodies for PRL-R detection in our study was used to detect LF-PRL-R selectively and the positive criterion was the same criterion used by Gill [11].

ER and PR are sex hormone receptors, the positive expression of which indicates the endocrine therapy is effective. Basic studies have shown that mutually-positive regulation exists in the PRL-R-PRL and ER/PR (estrogen/progestin receptor) ligands system in breast cancer cells [7, 10]. In clinical studies, Touraine and Gill showed that PRL-R expression is positively correlated with ER expression [11, 12], while other study has reported negative result [13]. Some scholars hold that the correlation between PRL-R and ER expression in breast cancer may be affected by different subtypes and other potential factors. The studies mentioned above were not subjected to a thorough analysis [14]. LF-PRL-R was specifically detected in this study. The overall results showed that LF-PRL-R expression was positively correlated with ER expression. However, according to the stratification of HER-2 expression, the aforementioned difference was only limited in the HER-2-positive sub-layer (not noted in the HER-2-negative sub-layer). Thus, HER-2 expression may be a strong impact factor for the correlation between LF-PRL-R and ER expression, and

the positive correlation relies on the positive expression of HER-2.

According to previous study, the correlation between PRL-R and PR is lower than the correlation between PRL-R and ER [11]. Most studies reported negative results [11, 14, 15]. No correlation between LF-PRL-R expression and PR expression was noted in all patients in our study. However, according to the stratification of HER-2, in the HER-2-positive sub-layer, LF-PRL-R expression was positively correlated with PR expression (no such correlation was noted in the HER-2-negative sub-layer). Thus, HER-2 expression may be also a strong impact factor for the correlation between PRLR expression and PR expression. Only when HER-2 was positive did a positive correlation exist between PRLR expression and PR expression.

It has been shown that the biological effect mediated by PRL-R may enhance the biological activity of HER-2 [8, 9]. However, up to now we have only found one study about the correlation between PRL-R expression and HER-2 expression,which didn't demonstrated any positive correlations [13]. No correlation was demonstrated between LF-PRL-R expression and HER-2 expression for all patients in our study. However, according to the stratification of ER or PR expression, LF-PRL-R expression was positively correlated with HER-2 expression in the ER- or PR-positive sub-layer, but not in the ER- or PR-negative sub-layer. The results suggest that the positive correlation between LF-PRL-R expression and HER-2 expression relies on the positive expression of ER or PR. ER/PR are thus the impact factors for the correlation between LF-PRL-P and HER-2.

This study investigated the expression of PRL-R subtype (LF-PRL-R) in breast cancer tissues, which was closest to the occurrence and development of breast cancer. We also analyzed the complicated correlation between LF-PRL-R and ER, PR, and HER-2. Based on clinical pathology, this study confirmed the complex regulation and dependence which exists between LF-PRL-R expression and ER/PR and HER-2 expression, which provides potential clues for combined therapy based on PRL-R, ER/PR, and HER-2 target sites and also put forward a new thinking for the anti-PRL-R treatment on breast cancer.

Disclosure of conflict of interest

None.

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