Correlations of Cancer Stem Cell-Related Markers $CD44^{+}/CD24^{-/low}$ and ALDH1 Expression in Breast Cancer and Its Significance

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***Abstract*—In this study, we tried to explore the characteristics of cancer stem cell-related markers CD44 and CD24 expression in breast cancer, and to investigate the relationship between $CD44^{+}/CD24^{-/low}$ cells, ALDH1 and the expression of HER-2, ER, PR, CK5/6, and its correlation with clinical pathological factors. The single and double streptavidin-biotin-peroxidase immunohistochemistry of CD44 and CD24 were performed on 10 cases of breast lobular hyperplasia, 10 cases of simple ductal hyperplasia, 42 cases of ductal carcinoma in situ and 463 cases of breast invasive ductal carcinoma. And Immunohistochemistry of ALDH1, HER-2, ER, PR, CK5/6 was used on 463 cases of invasive ductal carcinoma, which the expression of the conditions as immunophenotyping. Results showed that CD44 positive cells located in the membrane. The positive rate was statistically different between the invasive carcinoma and the ductal carcinoma in situ. The expression of CD24 was found in luminal border of a tubule of non-cancerous breast tissues, but it was expressed both lumincal border and cytomembrane of cancerous tissues. The positive rate of CD24 in invasive carcinoma was higher than that in ductal carcinoma in situ. The $CD44^{+}/CD24^{-/low}$ phenotype is enriched in basal-like breast tumors. $CD44^{+}/CD24^{-/low}$ cells have a high expression rate of breast carcinoma in situ and poorly differentiated cancer than invasive cancer and well differentiated cancer. The change of CD24 expression from the luminal border in non-cancerous tissues to the cytomembrane in cancerous tissues suggested CD24 may involve in carcinogenesis of breast cancer.**

Keywords-breast cancer; CD44; CD24; $CD44^{+}/CD24^{-/low}$; ALDH1; IHC

I. INTRODUCTION

Recent studies have found that a small group has the characteristics of cancer stem cells in tumors induced in tumor cells and promote tumor growth and resistance characteristics, became the source of tumor recurrence. The study showed that $CD44^{+}/CD24^{-/low}$ breast cancer cells had characteristics of stem cell-like and tumor infiltrating [1-5]. These cells not only have the tumorigenicity and high invasion characteristics, but also can resistance chemotherapy and radiotherapy. The higher proportion of $CD44^{+}/CD24^{-/low}$ cell was more prone to distant metastases in patients [2-4]. But research results were from animal experiments and cell-culture, detection method most applications flow cytometry instrument, there were inconsistent from detected using single and double streptavidin-biotin-peroxidase immunohistochemistry (IHC) of $CD44^{+}/CD24^{-/low}$ stained positioning, distribution characteristics, the positive rate and clinicalpathological factors [6-9].

In this study, we used single and double streptavidin-biotin-peroxidase IHC on breast lobular hyperplasia, simple ductal hyperplasia, ductal carcinoma in situ and invasive ductal breast cancer to explore the characteristics of cancer stem cell-related markers CD44 and CD24 expression in breast cancer, and to investigate the relationship between $CD44^{+}/CD24^{-/low}$ cells and the expression of ALDH1, HER-2, ER, PR, CK5/6 and its correlation with clinical pathological factors.

II. MATERIAL AND METHODS

A. Source of Specimen

We selected 463 cases of invasive ductal breast cancer that had purposes of simplify radical surgery, preoperative treatment, clinical data complete among 1028 cases of surgical removal of breast cancer which from the department of Pathology of Jiaxing Maternal and Child Health Hospital, Jan. 2005 to Dec. 2012. All cases were women, aged 26 to 79 years old, with a median of 46 years old. There were 102 cases lymph node metastases, and 361 cases without lymph node metastasis. Histological grade reference Bloom-Richardson semiquantitative grading which 115 cases of well-differentiated, 264 cases of moderately differentiation, 84 cases of the low differentiated, and selected the same period 10 cases of breast lobular hyperplasia, 10 cases of simple ductal hyperplasia and 42 cases of ductal carcinoma in situ as control. All specimens were fixed in 10% formalin, embedded in paraffin, 4 slices which thick was 4 μm by serial sections, for stain HE and IHC of ALDH1, CD44, CD24, ER, PR, HER-2 and CK5/6.

B. Main Reagents and Experimental Methods

The monoclonal antibody mouse anti-human CD24 (SN3b), monoclonal antibody mouse anti-human CD44 (156-3C11), monoclonal antibody mouse anti-human HER-2 (CB11), monoclonal antibody mouse anti-human CK5/6 (D5/16B4), monoclonal antibody mouse anti-human ER (SP1), monoclonal antibody mouse anti-human PR (SP2) were purchased from

NeoMarkers (USA), and ALDH1 were purchased from BD Biosciences (USA), and single standard and double standard SP IHC kit were purchased from ZSGB-Bio (China). The IHC single stain was performed by kit operating instructions, the IHC double stain of CD44 by BCIP/NBT coloring, CD24 by AEC coloring.

C. Result Judgment

Results determined in accordance with the method of the literature [5], CD24 and CD44 positive criteria was that the positive cells was 1% to 10% (+), 11% to 50% (++), 51% to 75% (+++), more than 75% (++++). HER-2, ER, PR and CK5/6 positive standard referenced literature [5]. Instead of primary antibody with PBS as negative control, known positive breast slices as a positive control.

D. Statistical analysis

All the experimental data were analyzed by one-way or two-way ANOVA and Chi-square test from Excel 2003 database and the SPSS 11.5 statistical package. The probability value of 0.05 was accepted as significant for differences between groups of data.

III. RESULTS

A. The expression of CD44 in breast cancer

The CD44 positive confined to the basal cell and (or) myoepithelial cell membrane / cytoplasm, and scattered distribution in epithelial cells, positive cells were scattered in interstitial in non-cancerous breast tissue. In cancer tissues, CD44 expression was the strongly positive in cell membrane, the positive rate was statistically difference between the invasive carcinoma (59.9%) and the ductal carcinoma in situ (85.7%) (P<0.05). In the highly, moderately and poorly differentiation tissue of invasive breast cancer, CD44 positive rates were 70.4%, 62.5% and 36.9% respectively, which differences were significant (P<0.05), as shown in Table 1.

TABLE 1. THE EXPRESSION OF CD44 IN GROUPS

Histological type	n	Staining intensity					Positive rate
		-	+	2+	3+	4+	(%)
Lobular hyperplasia	10	8	1	1	0	0	20.0
Simple ductal hyperplasia	10	6	3	1	0	0	40.0
Ductal carcinoma in situ	42	6	10	11	10	5	85.7*
Invasive carcinoma							
Well-differentiated	115	34	18	52	7	4	70.4#
Moder-differentiated	264	99	69	31	30	35	62.5
Poorly-differentiated	84	53	6	4	5	16	36.9

*P<0.05 *vs* invasive carcinoma, #P<0.05 *vs* among groups in invasive carcinoma.

B. The expression of CD24 in breast cancer tissue

The CD24 positive occasionally existed in small tube cavity edge in lobular hyperplasia and simple ductal hyperplasia. CD24 positive cell exist in cavity edge or membranous in cancer tissue. In 328 of 463 cases with invasive carcinoma showed cavity edge-positive and the remaining 135 cases were membranous-positive, the positive rate was 41.5% and that was 64.3% in carcinoma in situ in ductal. Positive rates were 20% in lobular hyperplasia and ductal hyperplasia, and the difference was significant compare with breast cancer (P<0.05). In the breast tissue of various degrees of differentiation, CD24 positive rate were 37.4%, 40.9% and 39.3%, the difference wasn't significant (P>0.05), as shown in Table 2.

TABLE 2. THE EXPRESSION OF CD24 IN GROUPS

Histological type	n	Staining intensity					Positive rate
		-	+	2+	3+	4+	(%)
Lobular hyperplasia	10	8	2	0	0	0	20.0
Simple ductal hyperplasia	10	8	2	0	0	0	20.0
Ductal carcinoma in situ	42	15	10	15	1	1	64.3*
Invasive carcinoma							
Well-differentiated	115	68	12	28	4	3	40.9#
Moderately differentiated	264	152	45	26	19	22	42.4
Poorly differentiated	84	51	5	9	7	12	39.3

*P<0.05 *vs* invasive carcinoma, #P>0.05 *vs* among groups in invasive carcinoma.

C. The expression of $CD44^+/CD24^{-/low}$ in breast cancer

According to the expression $CD44^+/CD24^{-/low}$ positive cells in single and double streptavidin-biotin-peroxidase IHC, 463 cases of invasive ductal carcinoma were divide into $CD44^+/CD24^{-/low}$ group (165, 35.6%), $CD44^-/CD24^-$ group (120, 25.9%), $CD44^-/CD24^+$ and $CD44^+/CD24^+$ group (178, 38.4%). According to the expression ER, PR, HER-2 and CK5/6 positive cells in single and double streptavidin-biotin-peroxidase IHC, 463 cases of invasive ductal carcinoma were divide into Luminal A type (ER^+, PR^+, HER-2^-) (140, 30.2%), Luminal B type (ER^+, PR^+,HER-2^+) (121, 26.1%), Over expression of HER-2 type (ER^-, PR^-, HER-2^{+++}) (123, 26.6), and basal cell type (ER^-, PR^-, HER-2^-, CK5/6^+) (79, 17.1%).

The $CD44^+/CD24^{-/low}$ positive rate was 55.7% in Luminal A type, was 55.4% in Luminal B type, was 17.6% in over-expression of HER-2 type, and was 83.5% in basal cell type. The differences of express was significance between the various types (P<0.05). The difference of express wasn't significant between Luminal A type and Luminal B type (P>0.05). The expression of $CD44^+/CD24^{-/low}$ was 71.4% in intraductal carcinoma and 40.4% in invasive carcinoma, the difference was significant (P<0.05). $CD44^+/CD24^{-/low}$ expression were 38.3%, 40.2% and 44.0% in breast cancer tissue of various degrees of differentiation, the difference was statistically significant (P<0.05), as shown in Table 3.

TABLE 3. THE EXPRESSION OF $CD44^+/CD24^{-/low}$ IN TYPE OF IMMUNE AND PATHOLOGY*

Type of IHC and Pathology	n	Staining intensity					Positive rate
		-	+	2+	3+	4+	(%)
Type of Immune							
Luminal A	140	62	17	23	25	13	55.7*
Luminal B	121	54	14	20	22	11	55.4
O-expression her2	123	89	7	11	15	1	17.6
Basal cell	79	13	12	19	17	18	83.5
Histological type							
Ductal carcinoma in situ	42	12	7	13	5	5	71.4#
Invasive carcinoma							
Well-differentiated	115	71	10	13	12	5	38.3**
Moder-differentiated	264	158	21	28	30	27	40.2
Poorl-differentiated	84	47	7	14	14	2	44.0

*P<0.05 *vs* among groups of immune, #P<0.05 *vs* invasive carcinoma, **P>0.05 *vs* among groups in invasive carcinoma.

D. The CD44$^+$/CD24$^{-/low}$ and ALDH1 expression in patient cohort

There wasn't statistically significant between CD44$^+$/CD24$^{-/low}$ cell number with patient age, menopausal status, tumor size, lymph node metastasis and distant metastasis (P>0.05), as shown in Table 4.

Immunohistochemical data of ALDH1 expression were available for 426 of the 463 patients (92.0%). Of these patients, 297 (69.7%) were < 48 years at diagnosis and 129 (30.3%) were > 48 years at diagnosis. Complete lack of expression of ALDH1 of any tumor cell was found in 8.0% of the tumors. ALDH1 expression was inversely correlated with age and was significantly higher in patients aged <48 years (79.8%) than in patients aged >48 years (33.3%; P<0.05). The association of ALDH1 expression with classic patient, tumor and treatment characteristics was shown in Table 4. ALDH1 expression was significantly correlated with ages, estrogen-receptor expression and progesterone-receptor expression in patients.

TABLE 4. ASSOCIATION OF CD44$^+$/CD24$^{-/low}$ AND ALDH1 WITH CLINICOPATHOLOGICAL CHARACTERISTICS

Characteristic	CD44$^+$/CD24$^{-/low}$ (n=463)		ALDH1 (n=426)	
	Negative (%)	*Positive (%)*	*Negative (%)*	*Positive (%)*
Ages*				
<48	114(48.5)	121(51.5)	60(20.2)	237(79.8)
≥48	109(47.8)	119(52.2)	86(66.7)	43(33.3)
Menopausal status				
Premenopausal	161(52.6)	145(47.4)	150(48.4)	160(51.6)
Postmenopausal	72(45.9)	85(54.1)	(52.6)	55(47.4)
Tumor size (mm)				
<20	189(55.3)	153(44.7)	166(54.2)	140(45.8)
≥20	71(58.7)	50(41.3)	55(45.8)	65(54.2)
Lymph node metastasis				
Negative	195(54.2)	166(45.8)	169(46.8)	192(53.2)
Positive	48(47.1)	54(52.9)	27(41.5)	38(58.5)
Distant metastasis				
Negative	211(53.0)	187(47.0)	227(57.1)	171(42.9)
Positive	35(53.8)	30(46.2)	15(53.6)	13(46.4)
ER status*				
Negative	103(47.9)	112(52.1)	39(30.5)	89(69.5)
Positive	132(53.2)	116(46.8)	95(31.9)	203(68.1)
PR status*				
Negative	109(50.2)	108(49.8)	32(45.6)	82(71.1)
Positive	130(52.8)	116(47.2)	105(33.7)	207(66.3)
HER-2 status				
Negative	134(55.8)	106(44.2)	114(49.1)	118(50.9)
Positive	118(52.9)	105(47.1)	102(52.6)	92(47.4)
CK5/6 status				
Negative	99(46.9)	112(53.1)	124(54.6)	103(45.4)
Positive	138(54.8)	114(45.2)	88(44.2)	111(55.8)

*P<0.05 in ALDH1 expression.

IV. DISCUSSION

CD24 membrane glycoprotein mediated adhesion between cells, cells and extracellular matrixwas that initially considered being the B-cell differentiation markers which played a role in lymphocyte maturation process [10-16]. In recent years, studies have shown that the gene played a key role in tumor occurrence, development process, and high expression in a variety of tumors [17]. CD24 associated with the occurrence of pancreatic carcinoma, bile duct carcinoma and colorectal cancer, and its high expression correlated with prognosis in patients, but the mechanism remains unclear.

CD24 expression was less in normal breast tissue, and it mainly exists in the cell membrane of ductal atrophy epithelial. CD24 expression patterns in invasive breast cancer is mainly cytoplasmic, the membrane simultaneously stained, its expression was related to lymph node metastasis of breast cancer that can be used as prognostic indicators of breast cancer [14, 15]. The study found that the CD24 positive occasionally in the tubular cavity margin in the lobular hyperplasia, ductal hyperplasia. In cancer tissue, CD24 positive performed as the cavity edge or film slurry. In 328 of 463 cases of invasive ductal carcinoma with positive margin for the cavity, the rest were membranous, the positive rate was 41.5%. The positive rate was 64.3% in ductal carcinoma in situ.

In normal physiological conditions, CD24 can mediate monocytes or neutrophils adhesion to P-selection activated endothelial cells or platelets. This study found that the CD24 positive can be found in the vascular endothelial cells and interstitial inflammatory cells may be associated with the expression of the above functions.

A small group tumor cells with stem cell function were isolated from patients with breast tumor tissue of, which was defined as breast cancer stem cells, and it been generally accepted that CD44$^+$/CD24$^{-/low}$ is the most specific surface markers of these cells.

Research shown the CD44$^+$/CD24$^{-/low}$ phenotype of breast cancer stem cells was closely related with basal cell-like breast cancer. It has not been reported that the relationship between type of luminal A and B and breast cancer stem cells, but studies have shown that breast cancer stem cells with the phenotype of side population cells can be isolated from the cell lines of MCF-7 luminal A type. This study demonstrates that the CD44$^+$/CD24$^{-/low}$ positive rate was 55.7% in luminal A type, 55.4% in luminal B type, 17.6% in HER-2 over-expression type and 83.5% in basal cell type. The results suggest that the CD44$^+$/CD24$^{-/low}$ have higher expression rate in basal cell type of breast cancer.

According to recently study, we had known that a biological explanation of the qualitative age-interaction of the prognostic effect of ALDH1 expression might be that of a changing micro-environment in elderly patients, which may result in hampered signal transduction between tumor stem cells and the micro-environment [17-23]. Moreover, changes in metabolic processes might limit the role of tumor stem cells in elderly patients [23]. In this study, we demonstrated that the presence of ALDH1 expression was significantly higher in young breast cancer patients than in elderly patients. We demonstrated that ALDH1 expression is an independent risk factor for decreased survival in young breast cancer patients, but not in elderly patients. We found that expression of the putative breast cancer stem cell marker ALDH1 and its prognostic effect are age-ER-PR-dependent in breast cancer patients. This study support the hypothesis that breast cancer biology of elderly patients and their younger counterparts is distinct and emphasizes the importance of analyzing and reporting age-specific effects in breast cancer research.

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