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The Relationship Between the Expression of Tumor Associated Fibroblasts Cav-1 and MCT4 and the Prognosis of Papillary Carcinoma of Breast

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ABSTRACT

Aim: To investigate the potential role of MCT4 in the progress of IMPC in CAFs. Methods: MCT4 and Cav-1 expression in 86 IMPC patients were detected by immunohistochemical LSAB methods and the 105 cases of non specific invasive ductal carcinoma (IDC-NOS) selected as the control. Compared the differences of the expressions of them in different issues and analysed the relationship of the main pathological features of the expression of Cav-1 and MCT4 in different ages, tumor stages and hisological grades. Results: The study conformed that the miss expression of Cav-1 on CAFs and the expression of MCT4 up-regulated were related on the poor prognosis of IMPC patients. Conclusion: It suggested that CAFs may be the target of the tumor and can supply the new basis for the treatment of breast cancer.

Keywords: *breast cancer; CAFs; Cav-1; MCT4.*

INTRODUCTION

Breast cancer is a kind of highly heterogeneous malignant tumor, which has different biological characteristics and clinical prognosis. Therefore, it is very important to understand the characteristics of breast cancer and its clinical diagnosis, treatment and prognosis, and so as improve the overall level of breast cancer treatment in a certain extent. Since 1999, we have been working on the relationship between the morphology and biological behavior of Invasive micropapillary carcinoma (IMPC). In our previous studies [1,2,3], we found that the IMPC tumor cells in the primary foci, invasion of lymphatic vessels and metastasis is the same form of small nipple or micro tube structure, and existed a form of "tumor cells of the group growth, invasion and metastasis". Well, it is not clear that the presence of these large number of mesenchymal cells in the tumor microenvironment can affect the occurrence, development and biological behavior of IMPC. It were not clear that the expression of Cav-1 and IMPC in carcinoma-associated fibroblasts (CAFs), and the correlation with the clinical pathological parameters and the relationship with the histological types of breast cancer. Through the different expression of Cav-1 and MCT4 in IMPC andnon specific invasive ductal carcinoma. Then analyzing the relationship between the expression of these two proteins and the prognosis of IMPC. The molecular basis of the growth, invasion and metastasis of breast cancer was discussed and to provide the new valuable subservience index.

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METHODS

1.1 The agents

Mouse anti human Cav-1 monoclonal IgG antibody (Abeam, Englishi, 1:200). Rabbit anti human MCT4 polyclonal IgG antibody (Santa cruz Biotechnology, America, 1:100). Mouse anti human a-SMA monoclonal IgG antibody (mouse IgQZymed, USA, 1:50). Rabbit anti human Estrogen receptor monoclonal IgG antibody (clone SP1, Zymed, America). Rabbit anti human progesterone receptor monoclonal IgG antibody (clone SP2, ZYmed, America). Mouse anti human HER-2/neu monoclonal antibody (DAKO Hercep Test TM, Denmark). Mouse anti human EMA (epithelial membrane antigen) monoclonal IgG antibody (DAKO, Denmark, 1:100). PBS phosphate buffer salt (0.01 M pH 7.27-7.4 XZLI-9062, Beijing ZhongshanJinqiao Biological Technology Co., Ltd). Citrate repair solution (PH 6.0, 0.01M) (ZLI-0965, Beijing ZhongshanJinqiao Biological Technology Co., Ltd). EDTA repair solution (1 mM PH 9.0) (ZLI-9067, Beijing Zhongshan Jinqiao Biological Technology Co., Ltd). Blocked with normal goat serum working solution (SP-9000, Beijing Zhongshan Jinqiao Biological Technology Co., Ltd). Antibody dilution (ZLI-9030, Beijing Zhongshan Jinqiao Biological Technology Co., Ltd). Biotin labeled second antibody (SP-9000, Beijing ZhongshanJinqiao Biological Technology Co., Ltd). Chain avidin of working fluid labeled with horseradish peroxidase (SP-9000, Beijing Zhongshan Jinqiao Biological Technology Co., Ltd). Endogenous peroxidase blocker (SP-9000, Beijing Zhongshan Jinqiao Biological Technology Co., Ltd). DAB HRP-OPD (ZLI-9032, Beijing ZhongshanJinqiao Biological Technology Co., Ltd). Netrual balsam (ZLI-9055, Beijing Zhongshan Jinqiao Biological Technology Co., Ltd). APES (ZLI-9001, Beijing ZhongshanJinqiao Biological Technology Co., Ltd). Xylene (Tianjin Fangde Technology Co., Ltd). Absolute ethyl alcohol (Tianjin Fangde Technology Co, Ltd)

Main instrument

Paraffin section machine (RM2235, LEICA, German). Electro-heating standing-temperature cultivator (HHB1M20, Tianjin experimental instrument factory). Stainless steel pressure cooker. Medical centrifuge (Shanghai Medical Instrument Co., Ltd). Optical microscope (BX60, Olympus, Japan). TK-C1381 image acquisition system (JVC, Japan)

Histopathological diagnosis analysis

The diagnosis was made according to the following contents by three senior breast pathological diagnoses and the results were statistically analyzed.

Diagnosis of MFC: According to morphological criteria for the classification of WHO breast cancer in 2003 and 2012 [4,5] are the morphological features of the small papillary of papillary or polar inverted tubular structures on the morphology of several tumor cell adhesion (Fig. 1). There was no significant correlation between IMPC components in tumor and the degree of lymph node metastasis in the early stage of the study [6], therefore, as long as the cancer nest contains a typical IMPC components and then diagnosed the IMPC. All cases were confirmed by IMPC membrane antigen for positive expression in the micropapillay parts or false glandular outside that cancer cells in the interstitial side, E-cadherin (ECD), in connection face with cancer cells expressed strongly. While the on the outside surface of cancer cells with stromal connection surface expression (Fig. 2, 3) theimmunohistochemical staining confirmed.

Tumor cells arranged in form of a small nipple without fiber bundle or a polarity reversal of a fiber bundle

The positive expression of the tumor cells in the stroma of the tumor cells in papillary or sham tube

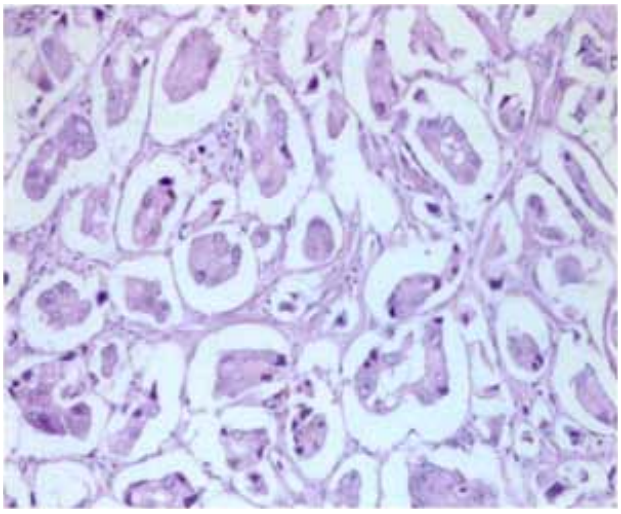

Fig.1 Morphology of IMPC (dyed with HE)

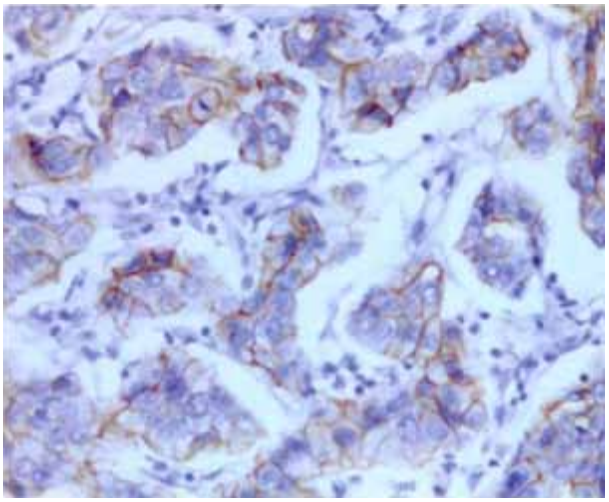

Fig. 3 IMPC immuohistochemical staining for ECD

The positive expression of the tumor cells was strongly expressed in the connection face

Tumor size in IMPC and IDC-NOS cases: The maximum diameter of IMPC and IDC-NOS was recorded. Tumor size staging: T1 staging: the maximum diameter ≤2cm; T2 staging: the maximum diameter > 2cm, <5cm;T3 staging: the maximum diameter >5 cm.

Histopathological grading of IMPC and IDC-NOS cases: ☐:The size and shape of the tumor cells were consistent with that of the chromatin, or with mild profile, no or accidentally see a nuclear fission/10 HPF; ☐ : The size and shape

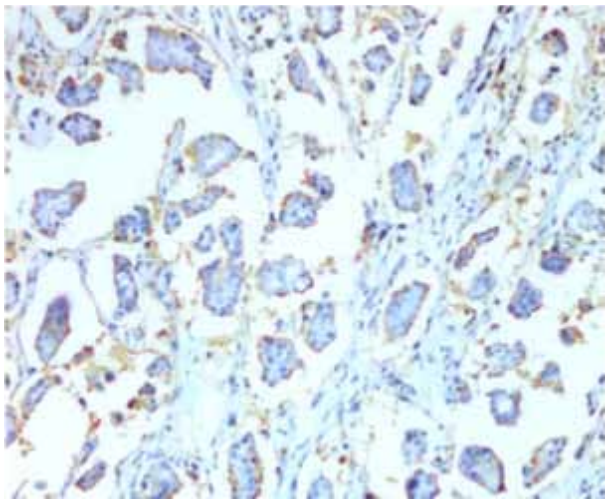

Fig. 2 IMPC immunohistochemical staining for EMA

of tumor cells were moderately shaped, and 2-3 of nuclear fissions could be seen/10HPF; ☐ : The size, shape and color of the tumor cells were stained with severe shape, and more than 3 of nuclear fission could be seen/10HPF.

Lymph node metastasis in patients with IMPC and IDC-NOS: The lymph node metastasis of aaIMPC and IDC-NOS were divided into 4 grades according to the number of metastasis: (NO: 0, N1: 1-3, N2: 4-9, N3: >9).

The research object

86 cases of IMPC were selected from the Tumor Hospital Affiliated to Tianjin Medical University from January 2003 to August 2005 in the diagnosis of breast cancer specimens. The patients were female and the median age was 52 years (28-82). At the same time, we randomly selected 105 cases of IDC-NOS in the same period as the control group. All the specimens were fixed by 10% neutral formalin and embedded in paraffin. All patients received no asjuvant therapy before surgery.

The selected IMPC patients were followed up for 1-115 months, and the median follow-up time was 64 months. The survival status of patients included death and recurrence, which was obtained by telephone, letters, and access to medical records. The recurrence of the

tumor was mainly local recurrence (ipsilateral locking, chest wall recurrence and internal mammary lymph node metastasis) and the new occurrence of contralateral and the distant metastasis (on the side of the lock, neck lymph nodes, liver, bone, lung, brain and other distant organ metastasis). The data of death of breast cancer was complete, while the survival or death of other causes was defined as the loss of information. The patients' progression-free survival (PFS) was defined as the time to first recurrence or the last follow-up from the date of surgery. The overall survival (OS) was defined as the death or the last follow-up from the date of surgery. During the follow-up, of 35 patients (41.8%) had a recurrence of the tumor and of 8 (9.3%) death of breast cancer.

Immunohistochemical staining

Using labeled streptavidin biotin (LSAB/SP) to detect the expression of Cav-1 and MCT4 in IMPC and IDC-NOS of breast cancer, using the PBS buffer instead of primary antibody, using the known positive staining as a positive control and the result dyed with DAB.

The basic principle

In this paper, the adopted method of immunohistochemistry was a highly sensitive affinity histochemistry technique, that is, the enzyme standard chain affinity biotin technology. Biotin is also known asvitamin H, is a small molecule vitamin and its molecular weight is 244; the stretavidin (SA) is a protein isolated from Streptomyces, the structure is similar to albumen of biotin with a molecular of 60 KD and 4 biotin binding sites, but it could exclude nonspecific endogenous substances and tissues with the latter, therefore the background color was light and could obtain higher signal-to noise ratio (Fig. 4).

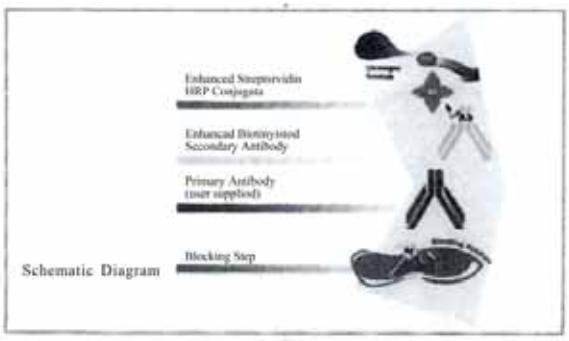

Fig. 4 Schematic diagram of SP method

EXPERIMENTAL

Slide processing: The slides soaked in soapy water overnight and washed with water, then with 95% alcohol soaked overnight and dried. Put the clean slides into the APES acetone working fluid (1:50) for 20-60s, took out for a moment, put them into the acetone solution or rinse water to wash the unbond APES, then dried and saved in box.

Paraffin embedded tissue into pieces, the thickness was about 4μm and toasted for 2 hours on the slice warmer, then placed in 60°C of constant temperature overnight to reduce a skin flick.

Paraffin section routine procedure dewaxing to water. Washed with distilled water and soaked into PBS for 5min. The paraffin section processed with antigen hot repair (citrate, PH 6.0, high pressure repair for 2 min) and cooled to room temperature. Washed 3 times with PBS for 5 min each time. Incubation in 3% H_2O_2 for 10 min at room temperature to eliminate endogenous peroxidase activity. Washed 3 times with PBS for 5 min each time. Closed with the normal serum of rabbit/rat common type and incubated for 10 min at room temperature, and poured without washing. Dropped primary antibody at 4°C overnight. Using the PBS as a negative control instead of primary antibody. Washed with PBS for 3 times and each for 5 min. Plused second antibody working fluid labeled with biotin and incubated for 20 min at 37°C.

Washed with PBS 3 times and each for 5 min. Plused the streptavidin third antibody work fluid labeled with horseradish peroxidase and incubated for 20 min at 37°C. Washed with PBS 3 times and each for 5 min. Dyed with new DAB agent for 3-10 min at temperature, observed in microscope and wash with water to end the reaction. Counterstained with hematoxylin, dehydrated and sealed with neutral gum.

Table 1 The dilution concentration of primary antibody and antigen repair conditions

anti-body	producer	dilution	antigen repair conditions
Cav-1	Abcam	1:200	PH6.0 citrate high-pressure repair
MCT4	Abcam	1:100	PH6.0 citrate high-pressure repair
EMA	DAKO	1:100	PH6.0 citrate high-pressure repair
α-SMA	Zymed	1:150	PH6.0 citrate high-pressure repair
ER	Zymed	1:100	PH6.0 citrate high-pressure repair
PR	Zmyed	1:150	PH6.0 citrate high-pressure repair
HER-2	DAKO	1:400	PH6.0 citrate high-pressure repair

Determination of immunohistochemical staining

For immunohistochemical staining, double blind method was used by the two senior doctors.

Cav-1 protein: The expression of Cav-1 in tumor cells and tumor stroma. Cav-1 was mainly located in the cytoplasm and cytoplasm with brownish yellow granular in tumor cells. The expression of Cav-1 was mainly concentrated in CAFs and the identification of CAFs was performed by α -SMA staining. Cav-1 is mainly located in the cells in the CAFs. The expression of Cav-1 in the tumor cells and tumor matrix of CAFs determined respectively. According to the determination of the percentage of positive cell scored, that is, ≤10% of positive cells was negative, 10-30% of positive cells was (+) and more than 30% positive cells was (++).

MCT4 protein: There were expression of MCT4 in tumor cells and tumor matrix. MCT4 was mainly in cell membrane and cytoplasm with brownish yellow granular. The expression of MCT4 was mainly concentrated in CAFs and the identification of CAFs was performed by α -SMA staining. MCT4 is mainly located in the cells in the CAFs. The expression of MCT4 in the tumor cells and tumor matrix of CAFs determined respectively. The expression of MCT4 in tumor cells was determined by considering the percentage and the staining intensity of positive cells, which was divided into 0 with no staining, 1 point with light staining, 2 points with light staining and 3 points with strong staining. The percentage of positive cells divided into 0 point with 0% percent cells staining, 1 point with 5% percent cells staining, 2 points with 5-50% cells staining and 3 points with more than 50% percent cells staining. Positive intensity and positive percentage scores for the final score: 0-2 was negative, 3-4 was (+) and 5-6 was (++). The expression of MCT4 in tumor matrix CAFs was determined by the percent of positive, that is, the percent cells positive less than 10% was negative, the percent of positive cells between 10%-30% was (+) and the percent positive cells more than 30% was (++).

The ER and PR staining results determined [7]. The expression of PR and ER was mainly localized in the nucleus with brownish yellow granular and defined the positive cells occupied

more than 1% in tumor cells as positive or as negative.

The HER-2 result determined [8]. The staining of positive cells was mainly localized in the cell membrane and the membrane of the infiltrating tumor cells without staining defined as (-); any proportion of infiltrating tumor cells showed weak and incomplete cell membrane staining or less than 10% of tumor cells was weak and incomplete cell membrane staining defined as (+); more than or equal to 10% percent of infiltrating tumor cells showed weak or inconsistent and complete membrane staining and less than or equal to 30% percent of infiltrating tumor cells showed strong and complete membrane staining was (++); more than 30 percent infiltrating tumor cells showed strong and complete membrane staining defined as (+++).

According to the actual situation of clinical application, this experiment would defined 0 and (+) as the HER-2 negative; (++) was defined as HER-2 positive while the cases of (++) group were analyzed by fluorescence in situ hybridization detection.

Statistical analysis of experimental data

All the research data was statistics by SPSS15.0 statistical software, and the $P<0.05$ as the statistical significant test standard. The Mann-Whitney U test was adopted to compare the difference among the clinical pathological data of the groups. It was also used to compare the expression of MCT4 and Cav-1 in IMPC group and control group of IDC-NOS. Mann-Whitney U test and Spearman rank correlation test were used to compare the expression of Cav-1 and MCT4 intra-class of IMPC group and IDC-NOS group. Spearman rank correlation test was used to analyze the correlation between the Cav-1 and MCT4 in IMPC group and the clinical pathology. Kmskal-Wallis test was used to analyze the expression of Cav-1 and MCT4 in IMPC group. Kaplan-Meier was used to draw a survival curve and log-rank test was used to survival test. Cox proportional hazard model was used to analyze the single factor and multiple factors survival.

RESULTS

The clinical pathology of breast cancer patients

Tumor size: Among the 86 breast IMPC patients, there were 14 in T1 stage, 50 in T2 stage and 20 in T3-T4 stages; there were 26 in T1 stage, 63 in T2 stage and 16 in T3 stage among the 105 IDC-NOS patients. There was no significant statistic of the tumor size difference betweenthe IMPC and IDC-NOS ($Z=-1.709$, $P=0.03$, see Table 2).

Histological grade: there were 7 in I grade, 54 in II stage, 25 in III stage among the 86 IMPC patients; there were 6 in I stage, 79 in II stage and 20 in III stage among the 105 IDC-NOS patients. There was no significant statistic of the histological grade between the IMPC group and IDC-NOS group ($Z=-1.104$, $P=0.270$, see Table 2).

Lymph node staging: 12 had no lymph node metastasis, 17 in N1 with lymph node metastasis, 20 in N2 stage and 36 in N3 among the 85 IMPC breast patients; in 85 cases, 73 cases (85.9%) had positive lymph nodes, the number of metastasis was 1-53 and the average was 11.71 ±10.14; in 104 IDC-NOS patients, 38 had no lymph node metastasis, 31 in N1 stage had lymph node metastasis, 15 in N2 stage had lymph node metastasis, 20 in N3 stage had lymph node metastasis, in 104 cases of IDC-NOS, 66 of it had positive (63.4%) lymph nodes, the number of metastasis was 1-48 and the average was 8.79 ±10.31. Compared with the group of IDC-NOS, IMPC has higher lymph node staging ($Z=-4.515$, $P<0.001$, see Table 2).

The expression of ER, PR and HER-2: The positive cases of ER and PR were 64 (75.3) and 56(65.9%) in 85 cases of IMPC patients, respectively. The positive cases of HER-2 was 15 (17.9%) in 84 cases of IMPC patients; The

positive cases of ER and PR were 69 (66.3%) and 63 (60.6%) in 104 IDC-NOS patients respectively; the positive cases of HER-2 was 24 (23.8%) in 101 IDC-NOS patients (see Table 2).

Table 2: Comparison of clinical pathological features of IMPC and IDC-NOS in the breast

clinical pathological features	IMPC	IDC-NOS	Z value	*P
age				
≤52	44(51.2)	55(52.4)	-0.167	0.867
>52	41(48.8)	50(47.6)		
size tumor (T-stage)				
T1	14(16.7)	26(24.8)	-1.709	0.073
T2	50(59.5)	63(60.0)		
T3-T4	20(23.8)	16(15.2)		
Histological grade				
	7(8.1)	6(5.7)	-1.104	0.270
	54(62.8)	79(75.2)		
	25(29.1)	20(19.1)		
Lymph node staging				
N0	12(14.1)	38(36.6)	-4.515	<0.001
N1	17(20.0)	31(29.8)		
N2	20(23.5)	15(14.4)		
N3	36(42.438)	20(19.2)		
ER stage				
negative	21(24.7)	35(33.7)	-1.337	0.918
positive	64(75.3)	69(66.3)		
PR stage				
negative	29(34.1)	41(39.4)	-0.749	0.804
positive	56(65.9)	63(60.6)		
HER-2 stage				
negative	69(82.1)	77(76.2)	-0.978	0.328
positive	15(17.9)	24(23.8)		

*Mann-Whitney U test.

The expression of Cav-1 in tumor cells and stroma of breast cancer

Cav-1 was expressed in IMPC tumor cells and tumor stroma CAFs (Fig.5), and the expression in IMPC and IDC-NOS was shown in Table 3.

In 86 cases of IMPC breast cancer patients, 49 were negative expressed of Cav-1 stroma and 37 were positive; in 86 IMPC cases, 74 cases showed negative expression of tumor epithelium and 12 showed positive expression. In IMPC group, the expression of Cav-1 in tumor stroma of CAFs was higher than the expression in tumor epithelium (43%, 37/86 vs. 14%, 12/86; P<0.001) (Table 3).

Cav-1 expressed in tumor epithelium cells and tumor stroma CAFs of breast IDC-NOS (Fig.5), the expression of it as follows: in 105 cases of breast IDC-NOS, 38 cases showed negative expression of stroma Cav-1 and 67 cases were positive; in 105 cases of breast IDC-NOS cases, 91 cases showed negative expression of tumor epithelium Cav-1 and 14 cases were positive. In IDC-NOS cases, the positive expression of tumor stroma CAFs were higher than the

tumor epithelium (63.8%, 67/105 vs. 13.3%, 14/105; P<0.001) (Table 4). The expression of stroma Cav-1 in IMPC were higher than that in IDC-NOS group (57%, 49/86 vs. 36.2%, 38/105; P=0.004). While compared the expression of tumor epithelium Cav-1 between the IMPC group and IDC-NOS group, there was no statistical significant (P=0.901) (Table 5).

In addition, the expression of Cav-1 was in normal breast tissue of the ductal and lobular myoepithelial cells generally, but it didn't express in the luminal epithelial cells, and in lobular interstitial fibroblasts and its surrounding lobular expressed little and in fat cells and vascular endothelial cells also showed positive expression (Fig. 5A).

Table 3: The comparison of expression of Cav-1 in tumor stroma and epithelium in IMPC cases

	stroma	epithelium	Z	*P
Cav-1stage				
-	49(57)	74(86)	-4.211	<0.001
+/++	37(43)	12(14)		

*Mann-Whitney U test.

Table 4: The comparison of expression of Cav-1 in tumor stroma and epithelium in IDC-NOS cases

	stroma	epithelium	Z	*P
Cav-1stage				
-	38(36.2)	91(86.7)	-7.496	<0.001
+/++	67(63.8)	14(13.3)		

*Mann-Whitney U test.

Table 5: The comparison of the expression of Cav-1 and MCT4 in IMPC and IDC-NOS cases

	IMPC	IDC-NOS	Z	*P
tumor epithelium Cav-1				
-	74(86)	91(86.7)	-0.124	0.901
+/++	12(14)	14(13.3)		
stroma CAFsCav-1				
-	49(57)	38(36.2)	-2.862	0.004
+/++	37(43)	67(63.8)		
tumor epithelium MCT4				
T(-/+)	38(44.2)	55(52.4)	-1.124	0.261
T(++)	48(55.8)	50(47.6)		
stroma CAFsMCT4				
S(-/+)	27(31.4)	54(51.4)	-2.708	0.005
S(+/++)	59(68.6)	51(48.6)		

*Mann-Whitney U test.

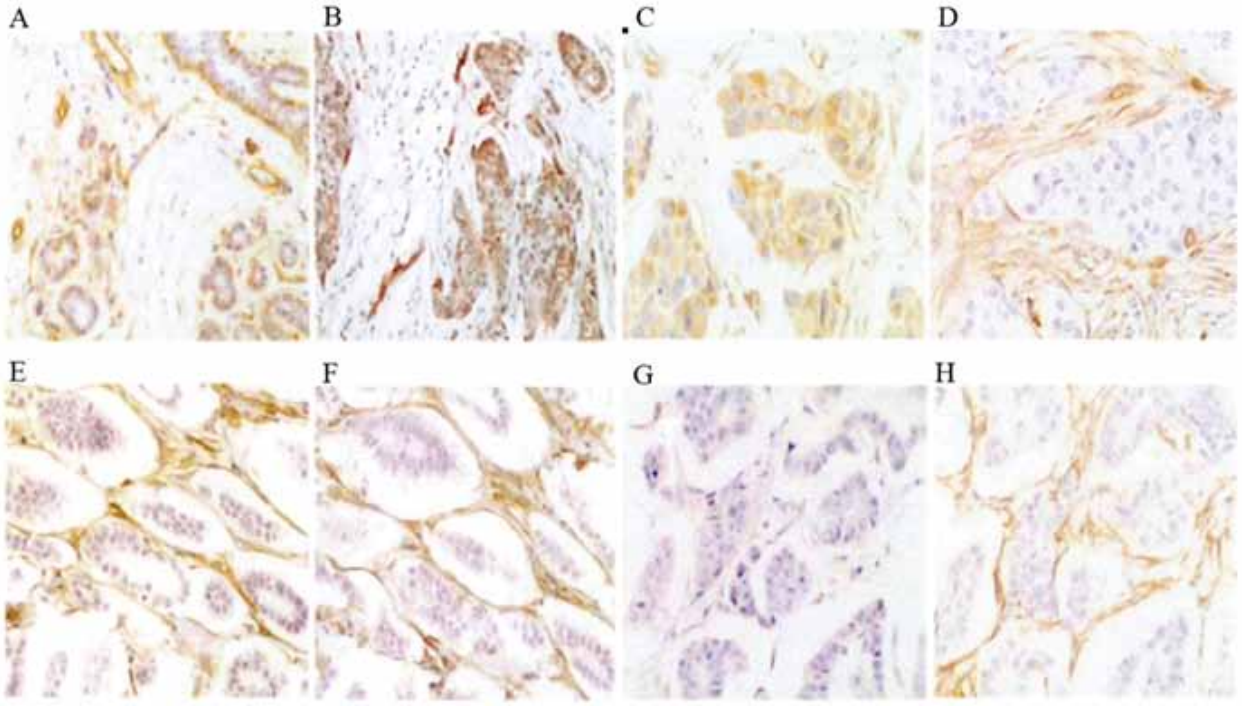

Fig. 5: Immunohistochemical staining of Cav-1 and α-SMA. Cav-1 staining was observed in lobular epithelial cells of normal breast tissue, myoepithelial cells around the lobular acini and vascular endothelial cells (A); the positive expression of Cav-1in tumor epithelium of IDC-NOS (B); the positive expression of Cav-1 in tumor epithelium of IMPC(C); the positive expression of Cav-1 of tumor stroma of IDC-NOS (D); the positive of Cav-1 in tumor stroma CAFs of IMPC (E); through the serial section of α-SMA positive staining(F); the negative expression of Cav-1 in tumor stroma CAFs (G); the determined of CAFs through the serial section of α-SMA positive staning (H).

The expression of MCT4 in breast tumor cells and stroma

MCT4 expressed in tumor epithelium cells and tumor stroma CAFs in IMPC breast (Fig.6), and the expression in IMPC and IDC-NOS was shown in Table 6.

In 86 IMPC cases, 27 cases were negative or low expression in tumor epithelium of MCT4 and 59 were high expression; in 86 cases, 38 cases were negative or low expression in tumor epithelium cells of MCT4 and 48 cases were high expression, and there was no correlation of the expression of MCT4 in tumor epithelium and stroma (rs=0.205, P=0.058) (Table 6).

In 105 IDC-NOS cases, 54 cases were negative or low expression of MCT4 in tumor stroma and 51 were high expression; in 105 IDC-NOS cases, 55 cases were negative or low expression of MCT4 in tumor epithelium and 50 were high expression, while there was no obvious correlation of the expression of MCT4 in tumor epithelium and stroma (rs=-0.125, P=0.203) (Table 7).

The expression of MCT4 in stroma of IMPC was higher than IDC-NOS significantly (68.6%, 59/86 vs. 48.6%, 51/105; P=0.005). The expression of Cav-1 in tumor epithelium there was no statistical significant between the IMPC group and IDC-NOS group (Table 5).

Table 6: The relationship of the expression of MCT4 in tumor epithelium and stroma of IMPC cases

	the expression of epithelium			
	-/+	++	rs	*P
the expression of stroma				
-/+	16(59.4)	11(22.9)	0.205	0.058
++	22(57.9)	37(7.1)		

*Spearman's Rank-Correlation test.

Table 7: The relationship of expression of MCT4 in tumor epithelium and stroma of IDC-NOS cases

	the expression of epithelium			
	-/+	++	rs	*P
the expression of stroma				
-/+	25(45.5)	29(58)	-0.125	0.203
++	30(54.5)	21(42)		

*Spearman's Rank-Correlation test.

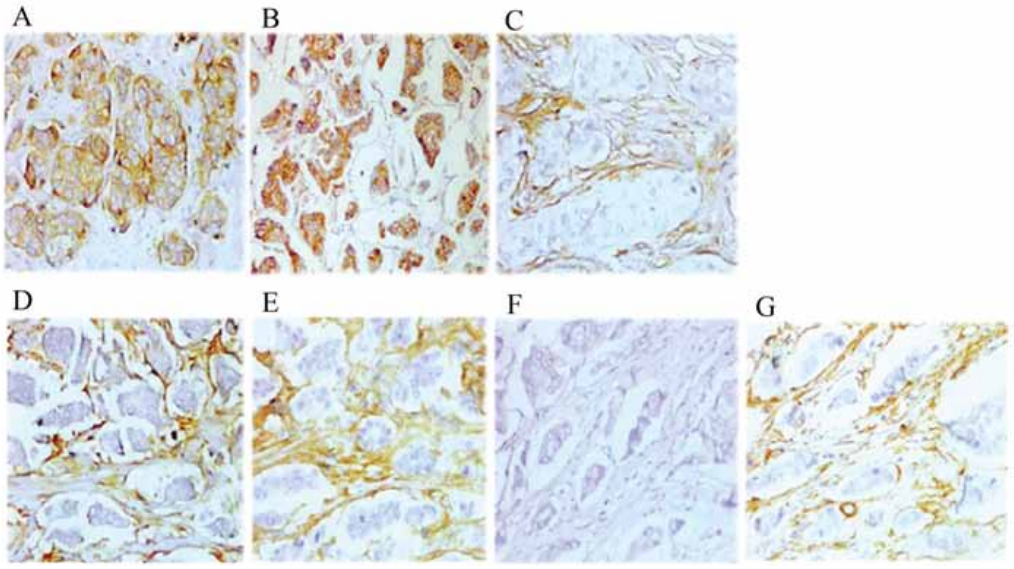

Fig. 6: Immunohistochemical staining of MCT4 and α-SMA. The negative expression of MCT4 in tumor epithelium cells (A); the positive expression of MCT4 in tumor epithelium of IMPC (B); the positive expression of MCT4 in tumor stroma of IDC-NOS (C); the positive expression of MCT4 of tumor stroma CAFs of IMPC (D); through the serial section of α-SMA positive staining(E); the negative expression of MCT4 in tumor stroma CAFs of IMPC (F); the determined of CAFs through the serial section of α-SMA positive staning (G).

The correlation of the stroma of Cav-1 and MCT4

The correlation of stroma of Cav-1 and MCT4b was shown in Table 8. The expression missing of stroma of Cav-1 was related to the high expression of MCT4 (P<0.001).

The correlation between the expression of Cav-1 and clinical pathological parameters

The correlation between the expression of

Table 8: The relationship of expression of stroma of Cav-1 and stroma of MCT4 in IMPC cases

	stroma MCT4			
	-	+	++	*P
stroma Cav-1				
-	8(16.3)	10(20.4)	31(63.3)	<0.001
+	0	0	21(100)	
++	8(50)	1(6.3)	7(43.7)	

*Kruskal-Wallis Test.

Table 9: The relationship of the expression of Cav-1 and MCT4 in tumor stroma and with the clinical pathological features of IMPC

clinical pathological features	stroma Cav-1			*P	stroma MCT4			
	-	+/++	rs		-/+	++	rs	*P
age								
≤52	27(61.4)	17(38.6)	0.091	0.406	16(59.3)	28(47.5)	0.110	0.315
>52	22(52.4)	20(47.6)			11(40.7)	31(52.5)		
tumor size (T-stage)								
T1	5(35.7)	9(64.3)	-0.252	0.021	3(11.5)	11(19)	-0.044	0.694
T2	27(54.0)	23(46.0)			17(65.4)	33(56.9)		
T3-T4	15(75.0)	5(25.0)			6(23.1)	14(24.1)		
histological grade								
□	1(14.3)	6(85.7)	-0.178	0.101	5(18.5)	2(3.4)	0..094	0.389
□	32(59.3)	22(40.7)			14(51.9)	40(67.8)		
□	16(64.0)	9(36.0)			8(29.6)	17(28.8)		
lymph nodes staging								
N0	5(41.7)	7(58.3)	-0.223	0.040	4(15.4)	8(13.6)	0.289	0.019
N1	5(29.4)	12(70.6)			8(30.8)	9(15.3)		
N2	15(75.0)	5(25.0)			9(34.6)	11(18.6)		
N3	23(63.9)	13(36.1)			5(19.2)	31(52.5)		
ER staging								
negative	14(66.7)	7(33.3)	0.105	0.341	4(14.8)	17(29.3)	-0.156	0.153
positive	35(56.1)	29(43.9)			23(85.2)	41(70.7)		
PR staging								
negative	20(69.0)	9(31.0)	0.165	0.132	11(40.7)	18(31.0)	0.095	0.386
positive	29(51.8)	27(49.2)			16(59.3)	40(69.0)		
HER-2 staging								
negative	39(56.5)	30(43.5)	-0.079	0.476	21(80.8)	48(82.8)	-0.024	0.828
positive	10(66.7)	5(33.3)			5(19.2)	10(17.2)		

*Spearman's Rank-Correlation test.

Cav-1 and clinical pathological parameters was shown in Table 9. The expression of Cav-1 was negatively correlated with tumor size (rs =-0.252, P=0.021) and lymph node staging (rs =-0.223 P=0.040), that is, the bigger the size, the higher the staging of lymph nodes, the higher the expression missing of Cav-1 in stroma. There was no obvious correlation to the age , histological grade, ER, PR and HER-2 (P>0.05).

The correlation between the expression of MCT4 and clinical pathological parameters

The correlation between the expression of MCT4 and clinical pathological parameters was shown in Table 9. The expression of MCT4 was positively correlated with lymph nodes staging (rs =0.298, P=0.019),while there was no obvious correlation to the age , tumor size, histological grade, ER, PR and HER-2 (P>0.05).

CONCLUSION

Our study confirmed that the expression missing of Cav-1 in tumor stroma CAFs and the up-regulated expression of MCT4, which was related to the poor prognosis of IMPC patients. In further study the mechanism of interaction between tumor micro-environment and tumor cells may provide a new method for the treatment of breast cancer patients.

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