

Relationship between Expression of Cell Adhesion Molecules and the Metastatic Mechanism in Invasive Micropapillary Carcinoma of the Breast

Yu Fan

Li Fu

Ronggang Lang

Ying Wang

Xiangcheng Zhi

Baocun Sun

Department of Breast Pathology and Surgery, Tianjin Medical University Cancer Hospital, Tianjin 300060, China.

Correspondence to: Li Fu
E-mail: fulijyb@hotmail.com

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E-mail: cocr@eyou.com Tel(Fax): 0086-022-23522919

OBJECTIVE To explore the expression and the function of cell adhesion molecules in invasive micropapillary carcinoma (IMPC) of the breast, and to investigate the metastatic mechanism of IMPC.

METHODS The expression of E-cadherin, α -catenin and β -catenin was detected by immunohistochemical staining in 64 cases of IMPC, and compared with that of invasive ductal carcinoma (IDC).

RESULTS E-cadherin and β -catenin were mainly expressed on the cell membrane of tumors, and α -catenin was expressed in the cytoplasm and/or on the cell membrane. The expression of E-cadherin in IMPC was significantly higher than that in IDC. Furthermore, the expression of E-cadherin was mainly on the intercellular contact surface of the tumor cell clusters in IMPC, while that on the outer surface of the tumor cell clusters decreased or could not be detected. The degree of lymph nodes metastases in IMPC was significantly higher than that in IDC. The co-expressions of α -catenin and β -catenin in cases of lymph nodes metastases along with the expression of E-cadherin in IMPC were significantly higher than that in IDC.

CONCLUSIONS These findings indicated that the adhesiveness of the intercellular contact surfaces of tumor clusters in IMPC was strong, while that of the outer surface of tumor clusters was decreased or lost. It is suggested that the adhesive characteristic of the cells in IMPC might play an important role in its higher metastatic potential.

KEYWORDS: invasive micropapillary carcinoma, breast, E-cadherin, α -catenin, β -catenin, metastases.

Invasive micropapillary carcinoma (IMPC) of the breast is one subtype of IDC, and has been studied widely in recent years^[1-4] because of its uniqueness in morphology and biology. IMPC is also called lymphophile carcinoma with a high risk of metastases^[3,4] because of its high tendency for lymphatic invasion, high potential of lymph nodes metastases and poor prognosis. We have investigated the morphology, the ultrastructure and the molecular biology of IMPC in our previous studies^[5,6], which indicated that there is a relationship between the morphology and biological behavior of IMPC, and found that the tumor cells in the primary tumor focus, lymphatics and metastatic focus presented as clusters. We call it "conglomerate infiltration and metastasis of tumor cells".

Epithelial cadherin (E-CD), with the molecular weight of

124,000, is one of the key adhesive molecules in the epithelium. In the presence of Ca^{2+} , the "slide fastener" structure is formed between the $-\text{N}^+\text{H}_3$ ends of E-CD in the outside of cells, which makes the tight junction among cells. The $-\text{COOH}$ ends inside cells adhere to the actin in the cytoplasm by the mediation of catenin (Cat), which makes E-CD attach to the cell skeleton. E-CD-mediated cell-cell adhesiveness is the molecular basis of the tight junction among cells. α -Cat is essential to the adhesive function of E-CD, and β -cat takes part in the adhesiveness by mediating the interaction between α -cat and E-CD^[7].

Until now, most of the data indicated that the loss of E-cadherin function, besides causing loss of cell-cell adhesion, might also convey signals that actively induce tumor-cell invasion and metastasis. We have observed before that there were integrated tight junctions and desmosomes among tumor cells in IMPC^[5,6]. The result suggested that E-CD was highly expressed in the tumor clusters and played an important role in the metastases of the clusters.

In order to confirm the function of E-CD in the invasion and metastases of the tumor clusters, to identify whether the presence or loss of E-CD function might convey signals that actively induce tumor-cell invasion and metastasis, and to explain the resistance of tumors to the treatment of recovery of E-CD induced adhesion, the expression of E-CD, α -cat and β -cat were detected by immunohistochemical staining in 64 cases of IMPC and compared with 57 cases of IDC.

MATERIALS AND METHODS

Materials

The mammary tumor tissues were obtained from female patients who had undergone mastectomy in Tianjin Medical University Cancer Hospital from January 2001 to May 2003. Their average age was 51.4 (39~66) years.

Methods

Pathological diagnosis and classification: all of the sections were reviewed by three pathologists, and classified into IMPC, IDC and the special types according to the WHO Histological Classification of Tumors of the Breast^[8] on the basis of previously published criteria^[1-7]. For controls 57 cases were selected randomly from IDC.

Immunohistochemical staining: the expression of E-cadherin, α -catenin and β -catenin were detected by immunohistochemical staining of the IMPC and IDC. Mouse monoclonal antibody anti-E-CD, anti-

α -cat, anti- β -cat and a LSAB kit (ZMED) were purchased from the Beijing Zhongshan Biotechnology Co. Ltd. The normal breast tissue was used as a positive control and PBS replaced the primary antibody for negative control. The criteria of scoring were based on Jwahari's method^[9,10]: the positive expression of E-CD and β -cat was brown-yellow on the cell membrane. The scoring was as follows: 0: no positive cell; 1: the expression was in the cytoplasm and the number of positive cells was $\leq 25\%$; 2: the expression was in the cytoplasm and the expression on the cell membrane became weaker, the number of positive cells was 26%~75%; 3: the expression was normal and the expression on the cell membrane was intact, the number of positive cells was $\geq 75\%$, and recorded as (+); a score of 0, 1 and 2 was recorded as (-). The positive expression of α -cat was brown-yellow in the cytoplasm and/or on the cell membrane. The criteria were: the normal expression was recorded as (+), the percentage of positive cell was $\geq 90\%$; the abnormal expression: no positive cells were in the section or the positive cells were dispersed in the section, the percentage of positive cells was $< 90\%$, and recorded as (-)^[11].

Statistical Analysis: Statistical evaluation was performed with χ^2 test, exact test and *t* test.

RESULTS

The incidence of IMPC

There were 64 cases of IMPC in this study and the frequency of IMPC was 4.2% among the 1524 cases of mammary carcinomas during the same period. The proportion of IMPC was as follows: $< 25\%$ 5 cases (7.8%), 26%~50% 8 cases (12.5%), 51%~75% 12 cases (18.8%) and more than 75% 39 cases (60.9%).

The metastasis rate in lymph nodes between the two groups

The metastasis rate in lymph nodes in IMPC (85.9%) was significantly higher than that in IDC (52.6%) ($P < 0.0001$). The average number of metastatic lymph nodes in IMPC (13.3) was significantly higher than that in IDC (8.8) ($P < 0.001$). Even if the tumors smaller than 2cm or the component in the IMPC was lower than 25%, the metastasis rate in lymph nodes was significantly higher than that in IDC as well ($P < 0.05$) (Table 1, Fig.1).

The results of immunohistochemistry

E-CD expression: the expression of E-CD in IMPC (85.9%) was significantly higher than that in IDC (43.9%) ($P < 0.0001$). It was strongly positive on the

intercellular contact surface of the tumor clusters but weakly positive/no expression on the outer surface of the tumor clusters in IMPC (Fig.2). But there were no such characteristics in IDC(Fig.3, Table 2).

Table 1. The state of metastasis in lymph nodes

| | Number | lymph nodes | |
|------------------|--------|---------------------------|-------------------------|
| | | Positive | Average positive number |
| IMPC | 64 | 55 (85.9%) ^a | 13.25 ^c |
| ≤25% | 5 | 5 (100.0%) ^b | 8.8 |
| IDC | 57 | 30 (52.6%) ^{a b} | 5.72 ^c |
| tumor size<2.0cm | | | |
| IMPC | 15 | 10 (66.7%) ^d | 10.4 ^c |
| IDC | 14 | 4(28.6%) ^d | 4.8 ^c |

a:χ²=16.0 P<0.0001 b:χ²=4.20 P<0.05
c:t=12.44 P<0.001 d:χ²=4.06 P<0.05 e:t=2.06 P<0.05

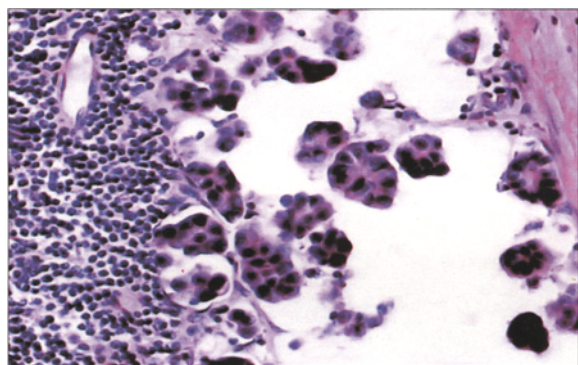


Fig. 1. Lymph nodes metastasis showed the same arrangement found in the primary tumor (Hematoxylin and eosin; original magnification ×200).

Table 2. The expression of E-CD in IMPC and IDC (cases, percent)

| | Number | E-CD(+) | E-CD(-) |
|------|--------|----------|----------|
| IMPC | 64 | 55(86.0) | 9(14.1) |
| IDC | 57 | 25(43.9) | 32(56.1) |

χ²=23.83 P<0.0001

The expression of α-cat and β-cat: the expression rate of α-cat was 65.6% in IMPC and 40.4% in IDC. The expression rate of β-cat in IMPC and IDC was 53.13% and 50.9% respectively. There were no significant differences between the two groups (Table 3).

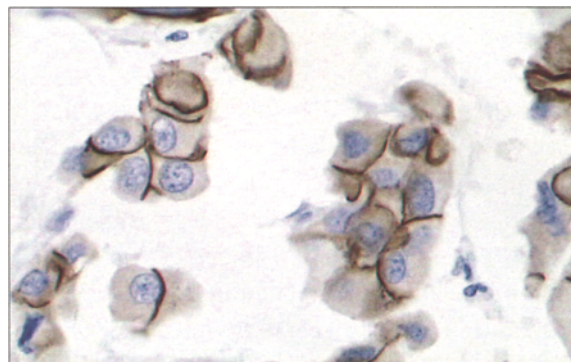


Fig. 2. There is strong, linear, thick membranous staining along the intercellular contact but no expression on the external surface of the tumor cell clusters (E-cadherin with hematoxylin counterstain; original magnification ×400).

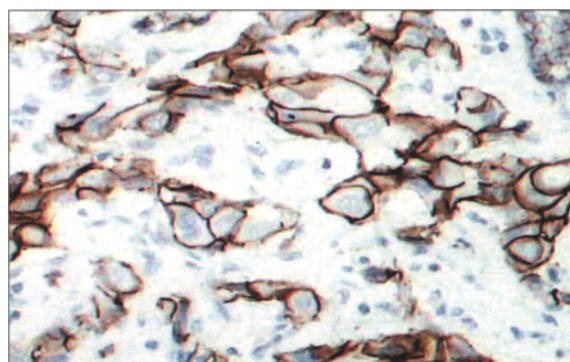


Fig. 3. The membranous staining along the intercellular contact and external surface of the tumor cell clusters were observed (E-cadherin with hematoxylin counterstaining; original magnification ×400).

Table 3. The expression of α-cat and β-cat in IMPC and IDC

| | Number | α-cat(%) | β-cat(%) |
|------|--------|----------|----------|
| IMPC | 64 | 42(65.6) | 34(53.1) |
| IDC | 57 | 23(40.4) | 29(50.9) |

The relationship between the expression of E-CD, α-cat and β-cat and metastasis in lymph nodes

The relationship between the expression of E-CD and metastases in lymph nodes: The metastasis rate in lymph nodes in IMPC (92.7%)with the expression of E -CD was significantly higher than that in IDC (52.0%)(P<0.001)(Table 4).

Table 4. The relationship between E-CD expression and lymph nodes metastasis (cases, percent)

| | ECD(+) | lymph nodes(+) | lymph nodes(-) |
|------|--------|----------------|----------------|
| IMPC | 55 | 51(92.7) | 4(7.3) |
| IDC | 25 | 13(52.0) | 12(48.0) |

$\chi^2=16.84$ $P<0.001$

The expression rate of α -cat, β -cat in cases with the metastasis in lymph nodes and expression of E-CD. The co-expression rate of α -cat and β -cat in IMPC (45.1%) in cases of metastasis in lymph nodes with expression of E-CD was significantly higher than that in the control group (15.4%) ($P<0.05$) (Table 5).

Table 5. The expression of α and β -cat in cases of lymph nodes metastasis with the expression of E-CD

| | lymph nodes(+) E-CD(+) | α -cat(+) | β -cat(%) | α,β -cat(%) |
|------|---------------------------|-----------------------|-----------------|------------------------|
| IMPC | 51 | 36(70.6) ^a | 29(56.9) | 26(45.1) ^a |
| IDC | 13 | 4(30.8) ^a | 8(61.5) | 2(15.4) ^b |

a: $\chi^2=7.01$ $P<0.01$ b: $\chi^2=5.33$ $P<0.05$

DISCUSSION

Many studies have reported that the mechanism of the invasion and metastases of tumor cells is very complicated, involving many adhesive molecules, stromatinase, cell factors, changes in pathway of signal transduction and changes of some tumor-related genes. Though researchers have focused on IMPC in recent years, there have been no reports concerning the invasion and metastasis of tumor clusters, and the mechanism of metastasis of single tumor cells is not suitable to explain the invasion and metastasis of all the tumors.

Until now, most of the data indicated that the loss of E-cadherin function, besides causing loss of cell-cell adhesion, might also convey signals that actively induce tumor-cell invasion and metastasis [12].

This study on IMPC sheds light on the difficulty with this hypothesis. The results regarding the IMPC indicated that E-CD was highly expressed in the cells of IMPC clusters (Fig.1), which were in contrast to results previously reported. Furthermore, it was found that the co-expression rate of α -cat and β -cat of IMPC clusters was significantly higher than that of IDC (Table 5). The decreased or loss of expression was also seen on the outside surface of the cells. The result suggested that the high expression of E-CD might play an important role in the cluster metastases of IMPC. Possible mechanisms were as follows. 1) Tumor cells ag-

gregated together, forming micro-papillary cell clusters through E-CD adhesion. Therefore, the stability of tumor cells in the circulation may have increased and their metastatic ability increased. Single tumor cells in the circulation and lymphatic fluid might be subjected to mechanical injury or be easily killed by the immune system. This was one possible reason as to why no metastatic focus cancer cells could be detected in the blood. 2) The transformation and movement ability of tumor cells increased through their micropapillary clustering adhesion, which could increase points of intercellular stress and change intercellular protein structure and function through adhesion. 3) The decreased or lost expression on the outer surface of the tumor clusters in IMPC (Fig.1), besides causing loss of cell-stroma adhesion, might also convey signals that actively induce tumor-cell invasion and metastasis.

Totally 157 archival primary mammary carcinomas were studied by Schiesche *et al* [13] revealing the relationship between loss of E-cadherin expression and invasive tumor growth and/or metastasis. They found that there were no metastases in lymph nodes in the primary mammary carcinomas with normal expression of E-CD, and they speculated that the abnormal expression of E-CD might induce the metastases in tumors. In our study, the metastatic rate in lymph nodes in IMPC was significantly higher than that in IDC (Table 1), and that it was positively correlated with the high expression of E-CD (Table 4). The result revealed that the co-expression degree of α -cat and β -cat in MPC in cases of metastasis in lymph nodes along with expression of E-CD was significantly higher than that in IDC. This indicated that the adhesive system in IMPC was special. It showed IMPC had the capacity for greater invasion, metastatic potential and poorer prognosis due to its micropapillary arrangement in morphology, which could increase the capacity for cell adhesion. Also, the expression of E-CD in lymph node-positive cases in IDC was mostly negative, while α -cat expression decreased significantly in few cases of high expression of E-CD. In two cases of E-CD, α -cat, β -cat co-expression, only cytoplasm expression was observed while cell membrane expression was lost. Lymph node metastasis in these cases was a single locus or big flake-like in morphology. This might cause instability of the E-CD-cat-cat complex leading to loose intracellular adhesion and cell exfoliation.

In conclusion, E-cadherin-mediated cell-cell adhesion might have a different function in the metastasis of IDC and IMPC. The high expression and special location of E-CD in the tumor cells of IMPC

might be useful in evaluation of the metastatic potential of mammary carcinomas. Further studies are necessary.

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