European Review for Medical and Pharmacological Sciences 2017; 21: 2167-2171

Mechanism of the high coagulation state of breast cancer tissue factor

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Abstract. – OBJECTIVE: We conducted this study to analyze the mechanism behind the high coagulation state induced by circulating plasma microparticle tissue factor (MP-TF) in patients with breast cancer.

PATIENTS AND METHODS: 87 cases of breast cancer patients (10 cases of TNM stage I, 16 cases of II, 32 cases of III, 29 cases of IV; 8 cases of pathological type in situ carcinoma, 15 cases of ductal carcinoma, 64 cases of invasive cancer) were used as the observation group and 20 cases of benign breast lesions were used as the control group to compare MP-TF levels of plasma and coagulation parameters including prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (FIB) and D-dimer body (D-D) level and NF-κB signaling pathway index including P50, p65, TAK1 and IκBα levels.

RESULTS: The plasma MP-TF level in the observation group was significantly higher than that in control group, and the level of MP-TF in the observation group increased with an increase in depth of TNM stage and tumor invasion; differences were statistically significant ($p<0.05$). In the observation group, the plasma PT and APTT were shortened, and the levels of FIB and D-D were increased; the differences were statistically significant ($p<0.05$). In the observation group, the levels of P50, p65, TAK1, IκBαin circulating blood were higher than those in control group; the differences were statistically significant ($p<0.05$). After the Pearson test, the plasma levels of MP-TF in patients with breast cancer were negatively correlated with PT and APTT, and positively correlated with FIB, D-D values and the levels of p50, p65, TAK1 and IκBα (4 $p<0.05$).

CONCLUSIONS: MP-TF can lead to high blood coagulation in patients with breast cancer through the activation of NF-κB signaling pathway, which may become a new target for the intervention of the disease.

Key Words:
Breast cancer, Tissue factor particle, High coagulation state, NF-κB signaling pathway.

Introduction

The incidence of breast cancer is second only to cervical cancer in women[1]. Patients with advanced breast cancer have significant coagulation abnormalities and increased thromboembolic events, which is an important factor that impacts both quality of life and survival outcomes[2]. A study found[3] that with an increase of tumor malignancy, the high coagulation state of the body may be a response to the invasion and metastasis of tumor cells. In recent years, the role of MP-TF in thrombosis has been investigated, and tissue factor (TF) is the main promoter of coagulation *in vivo*, which is both activated by the endogenous and the exogenous coagulation pathway[4]. The formation of MP is expressed with more TF; MP with TF as the carrier forms MP-TF. Patients with shortened prothrombin time (PT) had a higher level of MP-TF, which suggests that MP-TF is involved in the occurrence of high coagulation[5]. However, it is not very clear whether it plays the same role in breast cancer and related mechanisms. Based on prior research, our study analyzed MP-TP to provide a reference for the basic research and clinical treatment of breast cancer.

Patients and Methods

Sample Selection

We successively selected 87 cases from our hospital from January 2014 to January 2016 that were diagnosed with breast cancer for the first time as the observation group. Another 20 cases of benign breast lesions were selected as the control group. Both groups were females. Inclusion criteria: (1) histopathological examination confirmed the diagnosis; (2) diagnosis of primary breast cancer, without systematic surgery, radiotherapy

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and chemotherapy and endocrine therapy, etc.; (3) informed consent was signed. Exclusion criteria: (1) metastatic tumors of the breast and other parts of the primary malignant tumor; (2) underlying diseases, such as severe weight, liver, lung, kidney, brain and other organs dysfunction, autoimmune diseases, blood system diseases, hemodialysis, taking anticoagulant or antiplatelet drugs; (3) recent bleeding or major surgical history; (4) participation in other clinical research at the same time; (5) incomplete clinical data.

The average age of the observation group was (56.8 ± 13.4) years old. The TNM stages of the tumors were 10 cases of stage I, 16 cases of II, 32 cases of III, 29 cases of IV. The maximum diameter of the tumor was 0.5-3.6 cm, with an average (2.2 ± 1.3) cm. Patients had between 1-4 tumors, with the average (1.6 ± 0.5). 8 cases of pathological type *in situ* carcinoma, 15 cases of ductal carcinoma and 64 cases of invasive cancer. The average age of the control group was (54.3 ±12.5) years old, with 10 cases of breast hyperplasia, 4 cases of papilloma and 6 cases of fibrous tumor.

Research Method and Detection Index

We compared peripheral blood MP-TF levels and coagulation function including Pt, activated partial thromboplastin time (APTT), fibrinogen (FIB), and D-Dimer (D-D) between the two groups; levels of P50, p65, TAK1 and IκBα in the signal pathway of NF-κB.

MP-TF Level Detection

We took 6 ml of fasting peripheral venous blood, of which 2 ml were used for MP-TF detection, 2 ml for blood coagulation function and 2 ml for NF-κB signal pathway molecular detection. By gradient centrifugation method (Biyuntian Technology Co., Ltd., Beijing, China), we separated circulating blood particles (MP) in an Eppendorf tube 27°C in centrifugal (1500 g, 15 min) and obtained rich plasma platelets. We centrifuged the supernatant again at 300 g for 15 min to obtain platelets poor plasma. At this time retained substances in plasma were MP and frozen stored at -80°C refrigerator for spare. FACS Caliber flow cytometry (BD Company, Franklin Lakes, NJ, USA) was used to measure MP-TF levels, FITC-CD142 monoclonal antibody (Sigma-Aldrich, St. Louis, MO, USA,) was used to mark MP-TF, at 27°C to avoid light for incubation for 30 min before testing to add the standard microspheres (Bio-Rad Company, Hercules, CA, USA). 0.8 μm, 3 μm each 5 μl and 0.8 μm standard microspheres were set as the forward detection zone, and the expression levels of MP-TF -CD142-FITC were determined. MP-TF standard is diameter 0~0.8 μm, surface marker CD142 positive.

Detection of Blood Coagulation Function Index

PT kit was purchased from Shanghai Sun Biological Technology (Co., Ltd., Shanghai, China) batch thromboplastin 301A029, buffer batch 301B029 and reference values 12-14s. APTT kit was purchased from Beijing Shidi Scientific Instruments Inc., (Beijing, China) lot number ST20201-51, CaCl2 lot ST20202-51; reference value 35-45s. FIB Kit (Clauss method) was purchased from Beijing Shidi Scientific Instruments Inc., (Beijing, China) lot STG20401-32; reference value 2-4 g/L. D-D used the immune turbidity method; reagent kit was purchased from the Beckman Coulter, Inc., (Brea, CA, USA) > 500 μg/L was positive. Detection instrument was JG-R-80B computer blood viscosity tester and LG-PABER-1 platelet aggregation coagulation factor analyzer purchased from Beijing Shidi Scientific Instruments Inc. We followed protocol strictly according to the manufacturer's directions on both the instrument and reagent box in order detect the blood coagulation function index twice and used the average.

Molecular Detection of NF-κB Signaling Pathway

We centrifuged at serum at 2000 g for 20 min (Anke LXJ-IIB centrifuge was purchased from Shanghai Anting Scientific Instrument Factory), and then used the enzyme-linked immunosorbent assay (ELISA) method to determine the NF-κB signaling pathway. The kit was purchased from R&D Company (Minneapolis, MN, USA) and the standard enzyme instrument was purchased from Invitrogen Company (Carlsbad, CA, USA). We followed protocol strictly according to the manufacturer's directions on both the instrument and reagent box in order detect the blood coagulation function index twice and used the average.

Statistical Analysis

The SPSS22.0 software (SPSS Inc., Chicago, IL, USA) was used for data analysis, measurement data was expressed by mean ±standard deviation, and the comparison was conducted by *t*-test. After the normal test correlation analysis, we used the Pearson test. $p<0.05$ indicates that differences are statistically significant, with two-sided test level.

Results

MP-TF level comparison

The plasma MP-TF level in the observation group is significantly higher than that in the control group, and the levels of MP-TF in the observation group are increased with an increase in depth of TNM stage and tumor invasion; differences were statistically significant (p<0.05) (Figure 1).

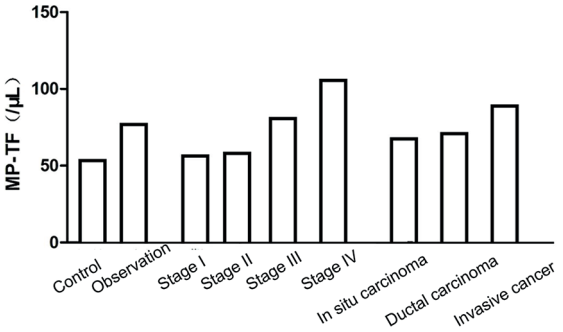

Figure 1. Comparison of plasma MP-TF levels.

Comparison of Blood Coagulation Function

In the observation group, the plasma PT and APTT were shortened, and the levels of FIB and D-D were increased; differences were statistically significant (p<0.05) (Table I).

Molecular Level Detection of NF-κB Signal Pathway

In the observation group, the levels of P50, p65, TAK1, IκBα in circulating blood were higher than those in control group; differences were statistically significant (p<0.05) (Table II).

Correlation Between MP-TF Levels and Coagulation Parameters and Signal Molecules of NF-κB in the Observation Group

After the Pearson test, the plasma levels of MP-TF in patients with breast cancer were negatively correlated to PT and APTT, and positively correlated with the value of FIB and D-D and the levels of p50, p65, TAK1 and IκBα (p<0.05) (Table III).

Discussion

Patients with malignant tumors are often in a hypercoagulable state; in fact, many patients with advanced cancer die of thromboembolic disease[6]. Therefore, finding the mechanism of blood coagulation in patients with tumors is an important method to treat the disease and improve the prognosis. MP-TF is a combination of tissue factor expression on the formation of microparticles. High levels of MP-TF, multiple myeloma progression and thrombosis are closely related[7]; MP-TF is also involved in the formation of acute myeloid leukemia[8]. Many scholars[9] believe that the abnormal MP-TF levels are involved in high blood coagulation and late thrombosis in malignant tumors.

Research confirms[10] that MP-TF is directly related to the risk of venous thromboembolism in patients with pancreatic cancer, and it can be used as a reliable method for risk stratification in patients with pancreatic cancer. Patients with bre-

Table I. Comparison of blood coagulation function.

Group	PT (s)	APTT (s)	FIB (g/L)	D-D (μg/L)
Observation group	8.4±1.9	28.3±2.7	6.2±0.7	857.6±74.8
Control group	13.5±1.6	40.8±1.9	2.9±0.4	354.5±56.7
t	5.821	6.283	5.283	6.492
p	0.018	0.012	0.022	0.006

Table II. Molecular level detection of NF-κB signal pathway (μg/L).

Group	p50	p65	TAK1	IκBα
Observation group	8.4±1.9	28.3±2.7	6.2±0.7	857.6±74.8
Control group	13.5±1.6	40.8±1.9	2.9±0.4	354.5±56.7
t	5.821	6.283	5.283	6.492
p	0.018	0.012	0.022	0.006

Table III. Correlation between MP-TF levels and coagulation parameters and signal molecules of NF-κB in the observation group.

Index	Correlation coefficient	p
PT	-0.328	0.021
APTT	-0.393	0.016
FIB	0.421	0.012
D-D	0.473	0.009
p50	0.428	0.011
p65	0.398	0.015
TAK1	0.383	0.018
IκBα	0.409	0.016

ast cancer are in a state of abnormal activation of blood coagulation. This study concluded that the plasma MP-TF levels in the observation group are significantly higher than that in the control group, and the levels of MP-TF in the observation group increase with an increase of TNM stage and tumor invasion depth; the differences were statistically significant. These results suggested that MP-TF is closely related to the occurrence and development of breast cancer, and also correlated to the severity of the disease[11]. FIB plays an important role in tumor micro-metastasis, which provides the necessary material and location to aid the micro metastasis of tumor cells, while simultaneously avoiding an immune cell attack[12]. The shortening of PT and APTT also directly promotes thrombosis in patients with malignant tumors since circulating tumor cells can survive in the lost nest, and metastasis occurs in the distance[13]. One or more of the abnormal blood coagulation parameters can be detected in 50% of the patients with primary malignant tumors[14], suggesting that the presence of high blood coagulation status in patients with breast cancer may be related to the progression of the disease.

Many studies[15] suggest that there are micro-inflammatory statuses in patients with malignant tumors, and that the inflammatory reactions are related to the abnormal function of the blood coagulation system. The excessive release of inflammatory mediators may be related to endothelial cell dysfunction, coagulation and dissolved system indirect activation, which causes an acceleration of inflammatory factors release from neutrophils and mononuclear cells, thus forming a vicious cycle of coagulation activation[16]. Studies confirm[17] that NF-κB signaling pathway affects the body's inflammatory process, and plays an important role in the blood coagulation state in patients with osteoarthritis. Recent studies[18] have found that the NF-κB signaling pathway is also involved in the progression of liver cancer and colon cancer. This study concluded that circulating blood P50, p65, TAK1 and IκBα levels in the observation group were higher than those in the control group; differences were statistically significant. In physiological conditions, NF-κB is present in the form of a P50 and p65 dimer, which does not show activity in combination with IκBα. After stimulation by inflammation, tumor and other factors, TAK1 is activated and after IκBα degradation, NF-κB signaling pathway activation regulates a series of cell factor gene over-expression[19]. Activation of NF-κB signaling pathway in patients with breast cancer may affect the levels of inflammatory mediators, and participate in the body's high coagulation state[20].

Conclusions

Pearson correlation analysis demonstrated that plasma MP-TF levels in patients with breast cancer were negatively correlated with PT and APTT, and were positively correlated with FIB and D-D values and levels of p50, p65, TAK1 and IκBα. It showed that the high expression of MP-TF in patients with breast cancer is directly related to the circulating high coagulation state, which is an important factor that causes the abnormal activation of coagulation function. High levels of MP-TF can mediate the high coagulation state of the tumor through the activation of NF-κB signaling pathway. Therefore, the intervention of MP-TF, or affecting some key molecular expression in the NF-κB signaling pathway, is expected to become the new method which effectively treats circulation hypercoagulable state of breast cancer, thereby blocking tumor cell micro-thrombus invasion and metastasis pathway.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Yung KW, Yung TT, Chung CY, Tong GT, Liu Y, Henderson J, Welbeck D, Oseni S. Principles of cancer staging. Asian Pac J Surg Oncol 2015; 1: 1-16.

2) Moon D, Lee SN, Lee BW, Lee K, Lee EK, Son SW. Transient midventricular ballooning syndrome with thrombus post pericardiocentesis in a patient

with malignant breast cancer. J Clin Ultrasound 2016; 24: 14-15.

3) Nielsen VG, Ley ML, Waer AL, Alger PW, Matika RW, Steinbrenner EB. Plasmatic hypercoagulation in patients with breast cancer: role of heme oxygenase-1. Blood Coagul Fibrinolysis 2013; 24: 809-813.
4) Mukherjee SD, Swystun LL, Mackman N, Wang JG, Pond G, Levine MN, Liaw PC. Impact of chemotherapy on thrombin generation and on the protein C pathway in breast cancer patients. Pathophysiol Haemost Thromb 2010; 37: 88-97.
5) Hisada Y, Alexander W, Kasthuri R, Voorhees P, Mobarrez F, Taylor A, McNamara C, Wallen H, Witkowski M, Key NS, Rauch U, Mackman N. Measurement of microparticle tissue factor activity in clinical samples: a summary of two tissue factor-dependent FXa generation assays. Thromb Res 2016; 139: 90-97.
6) Cho YK, Park BW, Lee MH, Hyon MS, Bang DW, Kim YJ. Fatal massive and recurring pulmonary embolism followed by thrombocytopenia developed after heparin and warfarin treatment in a patient with metastatic breast cancer with an incidental large right atrial thrombus. Blood Coagul Fibrinolysis 2016; 27: 213-215.
7) Auwerda JJ, Yuana Y, Osanto S, de Maat MP, Sonneveld P, Bertina RM, Leebeek FW. Microparticle-associated tissue factor activity and venous thrombosis in multiple myeloma. Thromb Haemost 2011; 105: 14-20.
8) Gheldof D, Chatelain C, Dogné JM, Mullier F. Microparticle-associated tissue factor activity and overt disseminated intravascular coagulation in patients with acute myelocytic leukemia. Thromb Res 2014; 134: 213-214.
9) Bharthuar A, Khorana AA, Hutson A, Wang JG, Key NS, Mackman N, Iyer RV. Circulating microparticle tissue factor, thromboembolism and survival in pancreaticobiliary cancers. Thromb Res 2013; 132: 180-184.
10) Date K, Hall J, Greenman J, Maraveyas A, Madden LA. Tumour and microparticle tissue factor expression and cancer thrombosis. Thromb Res 2013; 131: 109-115.
11) Mukherjee SD, Swystun LL, Mackman N, Wang JG, Pond G, Levine MN, Liaw PC. Impact of chemotherapy on thrombin generation and on the protein C pathway in breastcancer patients. Pathophysiol Haemost Thromb 2010; 37: 88-97.
12) Manly DA, Wang J, Glover SL, Kasthuri R, Liebman HA, Key NS, Mackman N. Increased microparticle tissue factor activity in cancer patients with Venous Thromboembolism. Thromb Res 2010; 125: 511-512.
13) Falanga A, Santoro A, Labianca R, De Braud F, Gasparini G, D'Alessio A, Barni S, Iacoviello L. HYPERCAN Study Group. Hypercoagulation screening as an innovative tool for risk assessment, early diagnosis and prognosis in cancer: the HYPERCAN study. Thromb Res 2016; 140: S55-59.
14) Tormoen GW, Cianchetti FA, Bock PE, McCarty OJ. Development of coagulation factor probes for the identification of procoagulant circulating tumor cells. Front Oncol 2012; 2: 110.
15) Rückerl R, Schneider A, Hampel R, Breitner S, Cyrys J, Kraus U, Gu J, Soentgen J, Koenig W, Peters A. Association of novel metrics of particulate matter with vascular markers of inflammation and coagulation in susceptible populations -results from a panel study. Environ Res 2016; 150: 337-347.
16) Campello E, Spiezia L, Radu CM, Simioni P. Microparticles as biomarkers of venous thromboembolic events. Biomark Med 2016; 24: 17-18.
17) de Andrés MC, Takahashi A, Oreffo RO. Demethylation of an NF-κB enhancer element orchestrates iNOS induction in osteoarthritisand is associated with altered chondrocyte cell cycle. Osteoarthritis Cartilage 2016; 13: 15-16.
18) Kong XX, Lv YR, Shao LP, Nong XY, Zhang GL, Zhang Y, Fan HX, Liu M, Li X, Tang H. HBx-induced MiR-1269b in NF-κB dependent manner upregulates cell division cycle 40 homolog (CDC40) to promote proliferation and migration in hepatoma cells. J Transl Med 2016; 14: 189.
19) Elghonaimy EA, Ibrahim SA, Youns A, Hussein Z, Nouh MA, El-Mamlouk T, El-Shinawi M, Mostafa Mohamed M. Secretome of tumor-associated leukocytes augment epithelial-mesenchymal transition in positive lymph node breast cancer patients via activation of EGFR/Tyr845 and NF-κB/p65 signaling pathway. Tumour Biol 2016; 22: 14-15.
20) Zhang P, Liu J, Tan B, Zhu F, Fang L. Hypercoagulable state is associated with NF-kappa B activation and increased inflammatory factors in patients with rheumatoid arthritis. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2016; 32: 364-368.