Journal of Hainan Medical University

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Relationship of JAK2V617F gene mutation with cell proliferation and coagulation function in myeloproliferative neoplasms

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ARTICLE INFO

Article history:
Received 7 Jul 2016
Received in revised form 17 Jul 2016
Accepted 12 Jul 2016
Available online 24 Jul 2016

Keywords:
Myeloproliferative neoplasms
Jauns kinase
Gene mutation
Proliferation

ABSTRACT

Objective: To study the relationship of JAK2V617F gene mutation with cell proliferation and coagulation function in myeloproliferative neoplasms. **Methods:** Patients who were diagnosed with BCR-ABL-negative myeloproliferative neoplasms in Anyang District Hospital between June 2014 and August 2016 were selected, JAK2V617F gene mutation was detected, and according to the test results, the patients were divided into mutation-positive group and mutation-negative group. The expression of JAK2/STATs signaling pathway molecules and cell proliferation genes in bone marrow fluid as well as the coagulation function indexes in peripheral blood were detected. **Results:** p-JAK2, p-STAT3, p-STAT5, Survivin, C-myc, CyclinD1 and ASXL1 protein expression in myeloproliferative neoplasms of mutation-positive group were significantly higher than those of mutation-negative group, and peripheral blood PT and APTT levels were significantly lower than those of mutation-negative group while TT and FIB levels were not significantly different from those of mutation-negative group. **Conclusion:** JAK2V617F gene mutation in myeloproliferative neoplasms can promote the cell proliferation and cause the hypercoagulable state.

1. Introduction

Myeloproliferative neoplasms (MPNs) is a common hematologic disease, which is characterized by the clonal proliferation of one-line or multiple-line bone marrow cells, and includes polycythemia vera, thrombocythemia, myelofibrosis and so on[1,2]. The abnormal activation of JAK2-STAT3 and JAK2-STAT5 in Jauns kinases (JAKs)/signal transducer and activators of transcriptions (STATs) signaling pathways has played an important role in the abnormal proliferation of bone marrow cells[3]. During the abnormal activation of the signaling pathway, JAK2's autophosphorylation is closely associated with the mutation of JAK2V617F[4]. However, the downstream regulatory molecules of JAK2-STAT3 and JAK2-STAT5 pathways during abnormal proliferation of bone marrow cells are not yet clear. In the following study, the relationship of JAK2V617F gene mutation with cell proliferation and coagulation function in myeloproliferative neoplasms was analyzed.

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Fund Project: Correlation Study Between Myeloproliferative Neoplasms Coagulation Disorder and JAK2V617F Gene Mutation No: Puyang Science and Technology Key Project 130638.

2. Subjects and methods

2.1 Research subjects

Patients who were diagnosed with BCR-ABL-negative myeloproliferative neoplasms in Anyang District Hospital between June 2014 and August 2016 were selected as the research subjects, all patients were diagnosed with myeloproliferative neoplasms by bone marrow biopsy and they had not been treated. A total of 64 cases were enrolled and divided into mutation-positive group and mutation-negative group according to JAK2V617F gene mutation. Mutation-positive group (n=30) included 18 male cases and 12 female cases that were 42-56 years old; mutation-negative group (n=34) included 20 male cases and 14 female cases that were 41-58 years old. There was no significant difference in general information between two groups of patients (P>0.05).

2.2 Research methods

2.2.1 JAK2V617F gene mutation detection

2 mL bone marrow liquid was collected, JAK2 (V617F) mutation detection kits from Shanghai Huaguan Biochip Co., Ltd., were used for experiments, the method was TaqMan probe method, and the amplification permissions were used to determine whether there were JAK2V617F gene mutations.

2.2.2 Gene expression detection in lesions

5 mL bone marrow liquid was collected, and enzyme-linked immunosorbent assay kits were used to determine the protein expression of p-JAK2, p-STAT3, p-STAT5, Survivin, C-myc, CYCLIND1 and ASXL1.

2.2.3 Coagulation funciton index detection

4ml fasting venous blood was collected from all of the patients before treatment in the morning, anti-coagulated with 0.38% sodium citrate anticoagulant and then centrifuged at 3 000 r/min for 10 min to separate plasma, and then Rayto RT-2204C coagulation analyzer was used to determine plasma prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT) and plasma fibrinogen (FIB-C).

2.4 Statistical methods

SPSS 20.0 software was used to input and analyze data, measurement data analysis between two groups was by t test and $P<0.05$ meant statistical significance in differences.

3. Results

3.1 JAK2/STATs signaling pathway expression in lesions

Analysis of JAK2/STATs signaling pathway molecules p-JAK2 (ng/mL), p-STAT3 (pg/mL) and p-STAT5 (pg/mL) expression in myeloproliferative neoplasms between mutation-positive group and mutation-negative group was as follows: p-JAK2, p-STAT3 and p-STAT5 protein expression in myeloproliferative neoplasms of mutation-positive group were significantly higher than those of mutation-negative group. Differences in p-JAK2, p-STAT3 and p-STAT5 protein expression in myeloproliferative neoplasms were statistically significant between mutation-positive group and mutation-negative group ($P<0.05$).

Table 1.

JAK2/STATs signaling pathway expression in lesions of two groups of patients.

Groups	n	p-JAK2	p-STAT3	p-STAT5
Mutation-positive group	30	4.68±0.76	178.65±22.52	242.15±37.84
Mutation-negative group	34	2.03±0.32	76.84±9.35	115.27±15.72
T		13.298	14.282	11.395
P		<0.05	<0.05	<0.05

3.2 Cell proliferation gene expression in lesions

Analysis of cell proliferation genes Survivin (ng/mL), C-myc (ng/mL), CyclinD1 (pg/mL) and ASXL1 (pg/mL) expression in myeloproliferative neoplasms between mutation-positive group and mutation-negative group was as follows: Survivin, C-myc, CyclinD1 and ASXL1 protein expression in myeloproliferative neoplasms of mutation-positive group were significantly higher than those of mutation-negative group. Differences in Survivin, C-myc, CyclinD1 and ASXL1 protein expression in myeloproliferative neoplasms were statistically significant between mutation-positive group and mutation-negative group ($P<0.05$).

Table 2.

Cell proliferation gene expression in lesions of two groups of patients.

Groups	*n*	Survivin	C-myc	CyclinD1	ASXL1
Mutation-positive group	30	3.51±0.52	5.03±0.84	168.54±22.31	232.15±33.63
Mutation-negative group	34	1.35±0.18	2.42±0.36	73.58±8.93	103.65±16.68
T		14.297	10.397	12.557	13.407
P		<0.05	<0.05	<0.05	<0.05

3.3 Peripheral blood coagulation function indexes

Analysis of peripheral blood coagulation function indexes PT (s), APTT (s), TT (s) and FIB (mg/dL) levels between mutation-positive group and mutation-negative group was as follows: peripheral blood PT and APTT levels of mutation-positive group were significantly lower than those of mutation-negative group while TT and FIB levels were not significantly different from those of mutation-negative group. Differences in peripheral blood coagulation function indexes PT and APTT levels were statistically significant between mutation-positive group and mutation-negative group ($P<0.05$).

Table 3.

Peripheral blood coagulation function indexes of two groups of patients.

Groups	*n*	PT	APTT	TT	FIB
Mutation-positive group	30	14.35±1.85	36.52±3.25	18.44±3.04	218.27±78.93
Mutation-negative group	34	18.21±2.75	52.35±4.41	17.53±1.21	244.89±79.04
T		7.938	8.574	0.752	0.993
P		<0.05	<0.05	>0.05	>0.05

4. Discussion

JAKs/STATs signaling pathways are the important pathways regulating cell proliferation, differentiation and apoptosis, the JAK2 phosphorylation can induce downstream STAT3 and STAT5 phosphorylation, and the phosphorylated STAT3 and STAT5 are able to form dimers and enter the nucleus to regulate the expression of multiple genes[5]. In recent years, the relationship of abnormal activation of JAK2-STAT3 and JAK2-STAT5 signaling pathways with myeloproliferative neoplasms has received more and more attention[6,7], but the mechanism of abnormal signal pathway activation mediated by JAK2 is not yet clear. JAK2V617F mutation is an important reason affecting JAK2 function, the mutation is

in the 1849th site of JAK2 gene, and it can lead to the weakened effect of JH2 structure domain on inhibiting autophosphorylation, and thus result in JAK2 phosphorylation increase[8]. To clarify the relationship between JAK2V617F mutation and myeloproliferative neoplasms, the effect of gene mutations on JAK2 itself and the activation of downstream signaling molecules was first analyzed in the study, and the result showed that p-JAK2, p-STAT3 and p-STAT5 protein expression in myeloproliferative neoplasms of mutation-positive group were significantly higher than those of mutation-negative group. This suggests that the JAK2V617F gene mutation in the myeloproliferative neoplasms will cause JAK2's autophosphorylation, which in turn starts JAK2-STAT3 and JAK2-STAT5 signaling pathways.

The abnormal cell proliferation is an important feature of the myeloproliferative neoplasms[9], and the expression of multiple genes in cell proliferation is mediated by JAK2-STAT3 and JAK2-STAT5 signaling pathways. Survivin is a highly conservative anti-apoptotic gene, and the product encoded by it can on the one hand, antagonizing caspase molecule function to inhibit apoptosis, and on the other hand, accelerate the cell cycle process to promote cell proliferation; C-myc is a kind of proto-oncogene closely related to a variety of malignant tumors, and the product encoded by it can coordinate with cyclinD1 to promote the DNA replication in cell cycle, and thus promote cell proliferation[10]; ASXL1 is a type of Asx homologous gene that can coordinate with SRC-1 and PPAR-ɣ to regulate gene expression and promote cell proliferation[11]. In order to define the relationship of JAK2V617F mutation-induced JAK2-STAT3 and JAK2-STAT5 signaling pathway activation with cell proliferation gene expression, the effect of gene mutation on above cell proliferation gene expression was analyzed in the study, and the results showed that Survivin, C-myc, CyclinD1 and ASXL1 protein expression in myeloproliferative neoplasms of mutation-positive group were significantly higher than those of mutation-negative group. This suggests that the JAK2V617F mutation in the myeloproliferative neoplasms will cause the increased expression of multiple cell proliferation genes.

In the outcome process of myeloproliferative neoplasms, thrombosis is the leading cause of death, and the blood coagulation pathway activation and hypercoagulable state are closely related to thrombosis[12-14]. PT and APTT reflect the functions of extrinsic coagulation pathway and the intrinsic coagulation pathway respectively, and the shortening of the time can reflect that the corresponding coagulation pathways are activated, and the blood is in hypercoagulable state[15]; TT and FIB can reflect the quality and quantity of fibrinogen respectively, and the delay of TT and the increase in FIB content reflect the state of hyperfibrous hyperlysis. In the study, analysis of the effect of JAK2V617F mutation on blood coagulation function indexes showed that the peripheral blood PT and APTT levels of mutation-positive group were significantly lower than those of mutation-negative group while TT and FIB levels were not significantly different from those of mutation-negative group. It means that JAK2V617F mutation in myeloproliferative neoplasms will significantly activate the extrinsic coagulation pathway and intrinsic coagulation pathway and cause the hypercoagulable state without significantly affecting the fibrinolytic system.

To sum up, it is believed that JAK2V617F mutations in myeloproliferative neoplasms can promote the JAK2 autophosphorylation, significantly activate the JAK2-STAT3 and JAK2-STAT5 signaling pathways, and then promote cell proliferation and cause hypercoagulable state through the activation of signaling pathways.

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