



Correlation of PI3K/Akt signaling pathway with cell apoptosis and invasion in mantle cell lymphoma

Ying-Juan Lu, Xiao-Shen Xia[✉]

¹ Traditional Chinese Medicine Hospital of Shijingshan District, Beijing 100043, China

² The Ninth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 201999, China

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ABSTRACT

Objective: To study the correlation of PI3K/Akt signaling pathway with cell apoptosis and invasion in mantle cell lymphoma. **Methods:** A total of 38 patients who were diagnosed with mantle cell lymphoma in Xijing Hospital Affiliated to the Fourth Military Medical University between June 2014 and March 2017 were selected as the MCL group of the research, 55 patients who were diagnosed with reactive lymphoid hyperplasia in Xijing Hospital Affiliated to the Fourth Military Medical University during the same period were selected as the control group of the research, and lymph node tissue was collected to detect the protein expression of p-PI3K and p-AKT as well as the mRNA expression of apoptosis genes and invasion genes. **Results:** p-PI3K and p-AKT protein expression as well as SOX11, cyclinD1, TNFAIP3, XIAP, PCNA, MMP2, MMP7, MMP9 and VEGF mRNA expression in lymph node of MCL group were significantly higher than those of control group while TNFAIP3 mRNA expression was significantly lower than that of control group; SOX11, cyclinD1, XIAP, PCNA, MMP2, MMP7, MMP9 and VEGF mRNA expression in MCL lymph node with high p-PI3K expression were significantly higher than those in MCL lymph node with low p-PI3K expression while TNFAIP3 mRNA expression was significantly lower than that in MCL lymph node with low p-PI3K expression. **Conclusion:** The activation of PI3K/Akt signaling pathway in mantle cell lymphoma is closely related to the tumor cell apoptosis disorder and invasion enhancement.

1. Introduction

Mantle cell lymphoma (MCL) is a special type of B-cell non-Hodgkin lymphoma. It has strong invasiveness, the clinical treatment is very difficult and the survival rate is lower[1]. The main pathological features of MCL are that the tumor cells within lymph nodes massively express B cell antigen and T cell antigen and show mantle zone-like invasion[2,3], but it is still not clear about the mechanism of abnormal tumor cell invasion in MCL lesions. There is t(11;14)(q13;q32) chromosome translocation within the MCL lesions, it is accompanied by the abnormal expression of a variety of apoptosis and invasion genes, and exploring the regulation mechanism of tumor cell apoptosis and invasion gene

expression contributes to exploring the pathogenesis of MCL. Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB, also known as AKT) is the important signaling pathway that regulates cell apoptosis and invasion in the body, which, after activated in the form of phosphorylation, can regulate the expression of a variety of downstream apoptosis and invasion genes[4]. In the following studies, we specifically analyzed the correlation of PI3K/Akt signaling pathway with cell apoptosis and invasion in mantle cell lymphoma.

2. Research subjects and methods

2.1 Research subjects

A total of 38 patients who were diagnosed with mantle cell lymphoma in Xijing Hospital Affiliated to the Fourth Military Medical University between June 2014 and March 2017 were selected as the MCL group of the research, they were

[✉]Corresponding author: Xiao-Sheng Xia, Hematology Department, Xijing Hospital Affiliated to the Fourth Military Medical University in Shaanxi Province, Xi'an City, Shaanxi Province, 710032.

Tel: 15691783235

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diagnosed with mantle cell lymphoma by lymph node biopsy and immunohistochemical staining, and they included 29 men and 9 women that were 42-59 years old; 55 patients who were diagnosed with reactive lymphoid hyperplasia in Xijing Hospital Affiliated to the Fourth Military Medical University during the same period were selected as the control group of the research, they were diagnosed with reactive lymphoid hyperplasia by lymph node biopsy and immunohistochemical staining, and they included 39 men and 16 women that were 40-58 years old. There was no statistically significant difference in general information between the two groups ($P>0.05$).

2.2 Gene expression detection

2.2.1 Gene protein expression detection

Right amount of lymph node tissue for biopsy was taken, added in RIPA lysate and fully grinded, the obtained suspension was centrifuged for 20 min at a speed of 12 000 r/min to separate supernatant, and then enzyme-linked immunosorbent assay kit was used to determine the contents of p-PI3K and p-AKT.

2.2.2 Gene mRNA expression detection

Right amount of lymph node tissue for biopsy was taken, added in Trizol lysate and fully grinded, the RNA in tissue was separated and synthesized into cDNA by reverse transcription, then fluorescence quantitative PCR amplification was conducted, and the amplification curve was referred to calculate the SOX11, cyclinD1, TNFAIP3, XIAP, PCNA, MMP2, MMP7, MMP9 and VEGF mRNA expression.

2.3 Statistical methods

SPSS 20.0 software was used to input data, the median of p-PI3K protein expression of MCL group was calculated, patients with p-PI3K expression $>$ the median were judged as those with higher expression, and patients with p-PI3K expression $<$ the median were judged as those with lower expression. Measurement data analysis between two groups was by t test and correlation analysis was by Pearson test. $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 p-PI3K and p-AKT expression in lymph node

p-PI3K and p-AKT protein expression in lymph node of MCL group were (5.28 ± 0.72) ng/mL and (2.91 ± 0.38) ng/mL respectively; p-PI3K and p-AKT protein expression in lymph node of control group were (2.38 ± 0.37) ng/mL and (1.28 ± 0.17) ng/mL respectively. After t test, p-PI3K and p-AKT protein expression in lymph node of MCL group were significantly higher than those of control group, and differences in p-PI3K and p-AKT protein expression in lymph node were statistically significant between two groups of patients ($P<0.05$).

3.2 Apoptosis gene expression in lymph node

Analysis of apoptosis genes SOX11, cyclinD1, TNFAIP3, XIAP and PCNA mRNA expression in lymph node between MCL group and control group was as follows: SOX11, cyclinD1, XIAP and PCNA mRNA expression in lymph node of MCL group were significantly higher than those of control group while TNFAIP3 mRNA expression was significantly lower than that of control group. Differences in SOX11, cyclinD1, TNFAIP3, XIAP and PCNA mRNA expression in lymph node were statistically significant between two groups of patients ($P<0.05$).

Analysis of apoptosis genes SOX11, cyclinD1, TNFAIP3, XIAP and PCNA mRNA expression in MCL lymph node with different p-PI3K expression was as follows: SOX11, cyclinD1, XIAP and PCNA mRNA expression in MCL lymph node with high p-PI3K expression were significantly higher than those in MCL lymph node with low p-PI3K expression while TNFAIP3 mRNA expression was significantly lower than that in MCL lymph node with low p-PI3K expression. Differences were statistically significant in SOX11, cyclinD1, TNFAIP3, XIAP and PCNA mRNA expression in MCL lymph node with different p-PI3K expression ($P<0.05$).

Table 1.

Apoptosis genes SOX11, cyclinD1, TNFAIP3, XIAP and PCNA expression in lymph node.

Groups	n	SOX11	CyclinD1	TNFAIP3	XIAP	PCNA
MCL group	38	2.65±0.36	2.77±0.38	0.36±0.06	3.18±0.45	2.92±0.37
Control group	55	0.97±0.11	1.05±0.14	0.98±0.12	1.02±0.14	1.01±0.14
T		14.592	15.637	12.392	22.138	19.2984
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 2.

Apoptosis genes SOX11, cyclinD1, TNFAIP3, XIAP and PCNA expression MCL lymph node with different p-PI3K expression.

p-PI3K	n	SOX11	CyclinD1	TNFAIP3	XIAP	PCNA
Low expression	19	1.60±0.21	1.63±0.20	0.59±0.09	1.89±0.24	2.03±0.29
High expression	19	3.71±0.46	3.81±0.49	0.19±0.03	4.52±0.68	3.90±0.48
T		13.294	14.521	27.582	15.029	9.283
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 3.

Invasion genes MMP2, MMP7, MMP9 and VEGF expression in lymph node.

Groups	n	MMP2	MMP7	MMP9	VEGF
MCL group	38	2.38±0.35	2.74±0.39	2.19±0.32	3.37±0.47
Control group	55	1.03±0.17	1.01±0.14	1.05±0.12	0.98±0.12
T		13.298	16.239	10.498	23.485
P		<0.05	<0.05	<0.05	<0.05

Table 4.

Invasion genes MMP2, MMP7, MMP9 and VEGF expression in MCL lymph node with different p-PI3K expression.

p-PI3K	n	MMP2	MMP7	MMP9	VEGF
Low expression	19	1.61±0.22	1.71±0.24	1.52±0.19	2.03±0.31
High expression	19	3.11±0.46	3.86±0.51	2.84±0.39	4.52±0.58
T		9.298	11.349	8.947	12.395
P		<0.05	<0.05	<0.05	<0.05

3.3 Invasion gene expression in lymph node

Analysis of invasion genes MMP2, MMP7, MMP9 and VEGF mRNA expression in lymph node between MCL group and control group was as follows: MMP2, MMP7, MMP9 and VEGF mRNA expression in lymph node of MCL group were significantly higher than those of control group. Differences in MMP2, MMP7, MMP9 and VEGF mRNA expression in lymph node were statistically significant between two groups of patients ($P<0.05$).

Analysis of invasion genes MMP2, MMP7, MMP9 and VEGF mRNA expression in MCL lymph node with different p-PI3K expression was as follows: MMP2, MMP7, MMP9 and VEGF mRNA expression in MCL lymph node with high p-PI3K expression were significantly higher than those in MCL lymph node with low p-PI3K expression. Differences were statistically significant in MMP2, MMP7, MMP9 and VEGF mRNA expression in MCL lymph node with different p-PI3K expression ($P<0.05$).

4. Discussion

t(11;14)(q13;q32) chromosome translocation within mantle cell lymphoma (MCL) lesions can cause abnormal expression of multiple apoptosis and invasion genes, and then result in the abnormal proliferation of tumor cells and the mantle zone-like invasion[5-7]. At present, the mechanism regulating apoptosis and invasion gene expression in MCL lesions is still unclear. PI3K/AKT is the signaling pathway that regulates cell proliferation, invasion, differentiation, metabolism and other biological process in the body, the p-PI3K from the phosphorylation of PI3K can make downstream molecule AKT phosphorylated, p-AKT has the effects of activating eIF-4E-binding protein and ribosomal protein p70S6K, and it can make the corresponding transcription factors transfer into the nucleus and regulate gene expression[8,9]. It has been reported that the specific inhibitor of PI3K can inhibit the proliferation and invasion of mantle cell lymphoma cell lines and induce apoptosis [10]. In the study, in order to further clarify the role of PI3K/AKT

signaling pathway in the development of mantle cell lymphoma, the PI3K/AKT pathway reactive molecule expression in mantle cell lymphoma tissue were analyzed, and the results showed that p-PI3K and p-AKT protein expression in lymph node of MCL group were significantly higher than those of control group and the expression of p-PI3K and p-AKT are positively correlated. This indicates that the activation of PI3K can synchronously trigger the activation of AKT, and the overactivation of PI3K/AKT signaling pathway is closely related to the occurrence of mantle cell lymphoma.

The activation of the PI3K/AKT signaling pathway in the mantle cell lymphoma lesions can affect cell proliferation and invasion by regulating the expression of downstream genes. SOX11, cyclinD1, TNFAIP3, XIAP and PCNA are the genes closely associated with the proliferation and apoptosis of mantle cell lymphoma cells. SOX11 is a transcription factor that plays a regulatory role in the differentiation and maturation of a variety of cells[11], it can increase the expression of cyclinD1 through the Rb-E2F pathway, and high-protein cyclinD1 can form complexes with CDK4 and CDK6 to promote cell cycle progression and inhibit apoptosis[12,13]; TNFAIP3 is the expression product of A20 gene, which has zinc finger structure domain and can inhibit the activation of NF- κ B and thus promote cell apoptosis[14]; XIAP is a molecule with anti-apoptotic activity, which can inhibit the apoptosis cascade activation mediated by various caspase molecules; PCNA is the proliferating cell nuclear antigen that participates in DNA replication, which has promoting effect on cell proliferation and has inhibitory effect on apoptosis. In the study, analysis of these apoptosis gene expression in mantle cell lymphoma lesions showed that SOX11, cyclinD1, XIAP and PCNA mRNA expression in lymph node of MCL group were significantly higher than those of control group while TNFAIP3 mRNA expression was significantly lower than that of control group. This indicates that the high protein of anti-apoptosis genes and the high expression of pro-apoptosis genes are closely related to the occurrence of mantle cell lymphoma. Further analysis of the correlation between PI3K/AKT signaling pathway and apoptosis gene expression showed that SOX11, cyclinD1, XIAP and PCNA mRNA expression in MCL lymph node with high p-PI3K expression were significantly higher than those in MCL lymph node with low p-PI3K expression while TNFAIP3 mRNA expression was

significantly lower than that in MCL lymph node with low p-PI3K expression. This indicates that the activation of PI3K/AKT signaling pathway in the mantle cell lymphoma lesions can cause changes in the expression of downstream apoptosis genes, which can lead to the excessive cell proliferation and apoptosis disorder.

On the basis of abnormal proliferation, the cells in mantle cell lymphoma lesions will show the characteristic of invasive growth, cell migration and invasion are the important pathological links for mantle cell lymphoma to complete invasive growth, and MMP2, MMP7, MMP9, VEGF and various molecules are closely related to the cell migration and invasion[15]. A variety of molecules in MMPs family are involved in the migration and invasion process of a variety of malignant tumor cells, and they can hydrolyze the collagen, elastin, laminin and other components in intercellular matrix and basement membrane, and then cause the cells to leave the primary lesion and infiltrate to the adjacent tissue; MMP2, MMP7 and MMP9 are the members of the MMPs family that are associated with cell invasion in mantle cell lymphoma[16,17]. VEGF is the active cytokine that regulates endothelial cell proliferation and migration as well as angiogenesis, and it can increase the number of new blood vessels inside the lesions and promote the tumor to break away from primary lesion and infiltrate to the adjacent tissue[18,19]. In the study, analysis of above invasion gene expression in mantle cell lymphoma lesions showed that MMP2, MMP7, MMP9 and VEGF mRNA expression in lymph node of MCL group were significantly higher than those of control group. This indicates that the high expression of the invasion genes is closely related to the occurrence of the lymphoma. Further analysis of the correlation between PI3K/AKT signal pathway and invasion gene expression indicated that MMP2, MMP7, MMP9 and VEGF mRNA expression in MCL lymph node with high p-PI3K expression were significantly higher than those in MCL lymph node with low p-PI3K expression. This indicates that the activation of PI3K/AKT signaling pathway in the mantle cell lymphoma lesions can cause changes in the expression of downstream invasion genes, which can lead to excessive cell invasion.

In conclusion, it is believed that the PI3K/Akt signaling pathway is overactive in mantle cell lymphoma; the activation of PI3K/Akt pathway can inhibit cell apoptosis and promote cell invasion so as to participate in the invasive growth of the mantle cell lymphoma.

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