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Study on the correlation of MLCK and FAP expression with uterine fibroid cell proliferation and invasion

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ABSTRACT

Objective: To study the correlation of myosin light chain kinase (MLCK) and fibroblast activation protein (FAP) expression with uterine fibroid cell proliferation and invasion. **Methods:** Uterine fibroids samples and normal uterine muscle samples next to fibroids that were surgically removed in Wuhan Red Cross Hospital between May 2014 and January 2017 were chosen, fluorescence quantitative PCR kits were used to deterct MLCK and FAP mRNA expression, and enzyme-linked immunosorbent assay kits were used to determine proliferation and invasion gene protein expression. **Results:** MLCK and FAP mRNA expression in uterine fibroids samples were significantly higher than those in normal uterine muscle samples, and Survivin, Livin, Bcl-2, Snail, N-cadherin and MMP2 protein expression were significantly higher than those in normal uterine muscle samples; Survivin, Livin, Bcl-2, Snail, N-cadherin and MMP2 protein expression in uterine fibroids samples with high MLCK and FAP expression were significantly higher than those in uterine fibroids samples with low MLCK and FAP expression. **Conclusion:** Highly expressed MLCK and FAP in uterine fibroids can promote the proliferation and invasion of uterine fibroids.

1. Introduction

Uterine fibroids is a common gynecological benign tumor, which can cause clinical symptoms such as irregular menstruation, dysmenorrhea, and infertility. At present, the pathogenesis of uterine fibroids is not entirely clear, the abnormal signal transduction in stromal cells around tumor cells is thought to be closely associated with the occurrence of tumor, and the cell function change caused by abnormal signaling pathways within stromal cells can provide favorable microenvironment for the occurrence and development of tumor[1,2]. Fibroblasts are the important stromal cells around the tumor during the development and change of uterine fibroids, and more than 80% of fibroblasts in lesions are in the activated state and regarded as the tumor-associated fibroblasts[3]. Activated fibroblasts can secrete a large amount of extracellular matrix, which are the important part of micro environment for uterine fibroids cell growth, and can promote the growth of uterine fibroids lesions. Myosin light chain kinase (MLCK) and fibroblast activation protein (FAP) are the molecules closely related to fibroblast activation, the correlation of MLCK and FAP expression with uterine fibroid cell proliferation and invasion was analyzed in detail in the following research.

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2. Clinical sample information and experiment methods

2.1 Clinical sample information

Uterine fibroids samples and normal uterine muscle samples next to fibroids that were surgically removed were selected. All the clinical samples were taken from the patients who received hysteromyomectomy in Wuhan Red Cross Hospital between May 2014 and January 2017, there were a total of 52 patients, and they were 44-58 years old. The uterine fibroids samples were collected from the central part of the uterine fibroids lesions, and the normal uterine muscle samples were collected from the area 1cm from the uterine fibroids lesions. All clinical samples were collected, then washed with saline, quickly frozen in liquid nitrogen, then taken out and stored at -80 ℃ for test.

2.2 Gene expression detection methods

2.2.1 mRNA expression detection methods

Uterine fibroids samples and normal uterine muscle samples were taken and added in Trizol lysis buffer to extract RNA, reverse transcription kit was used to synthesize RNA into cDNA, then fluorescence quantitative PCR kit was used to amplify MLCK, FAP and β-actin, β-actin was the reference, and the amplification curve was referred to calculate MLCK and FAP mRNA expression.

2.2.2 Protein expression detection methods

Uterine fibroids samples and normal uterine muscle samples were taken and added in RIPA lysis buffer to extract total protein, and enzyme-linked immunosorbent assay kits were used to detect Survivin, Livin, Bcl-2, Snail, N-cadherin and MMP2 protein expression.

2.3 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 MLCK and FAP mRNA expression

MLCK mRNA expression in uterine fibroids samples and normal uterine muscle samples were (2.41±0.35) and (1.05±0.18) respectively, and FAP mRNA expression were (2.77±0.51) and (1.02±0.14) respectively. After t test, MLCK and FAP mRNA expression in uterine fibroids samples were significantly higher than those in normal uterine muscle samples. Differences in were statistically significant in MLCK and FAP mRNA expression in uterine fibroids samples and normal uterine muscle samples ($P<0.05$).

3.2 Proliferation gene and invasion gene expression

Analysis of proliferation genes Survivin (ng/mL), Livin (ng/mL) and Bcl-2 (ng/mL) as well as invasion genes Snail (pg/mL), N-cadherin (pg/mL) and MMP2 (ng/mL) expression in uterine fibroids samples and normal uterine muscle samples was as follows: Survivin, Livin, Bcl-2, Snail, N-cadherin and MMP2 protein expression in uterine fibroids samples were significantly higher than those in normal uterine muscle samples. Differences were statistically significant in Survivin, Livin, Bcl-2, Snail, N-cadherin and MMP2 protein expression in uterine fibroids samples and normal uterine muscle samples ($P<0.05$).

3.3 Correlation of MLCK with proliferation genes and invasion genes

Analysis of proliferation genes Survivin (ng/mL), Livin (ng/mL) and Bcl-2 (ng/mL) as well as invasion genes Snail (pg/mL), N-cadherin (pg/mL) and MMP2 (ng/mL) expression in uterine fibroids samples with different MLCK mRNA expression was as follows: Survivin, Livin, Bcl-2, Snail, N-cadherin and MMP2 protein expression in uterine fibroids samples with high MLCK expression were significantly higher than those in uterine fibroids samples with low MLCK expression. Differences were statistically significant in Survivin, Livin, Bcl-2, Snail, N-cadherin and MMP2 protein expression in uterine fibroids samples with different MLCK mRNA expression ($P<0.05$).

3.4 Correlation of FAP with proliferation genes and invasion genes

Analysis of proliferation genes Survivin (ng/mL), Livin (ng/mL) and Bcl-2 (ng/mL) as well as invasion genes Snail (pg/mL), N-cadherin (pg/mL) and MMP2 (ng/mL) expression in uterine fibroids samples with different FAP mRNA expression was as follows: Survivin, Livin, Bcl-2, Snail, N-cadherin and MMP2 protein

Table 1.

Proliferation gene and invasion gene expression in uterine fibroids samples and normal uterine muscle samples.

Sample origin	*n*	Survivin	Livin	Bcl-2	Snail	N-cadherin	MMP2
Uterine fibroids	52	1.94±0.22	1.25±0.17	2.77±0.42	252.32±33.25	174.52±22.56	3.49±0.52
Normal uterine muscle	52	0.78±0.09	0.48±0.07	1.25±0.15	114.28±14.27	75.42±9.32	1.32±0.18
T		12.985	14.249	13.215	15.028	13.218	17.598
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 2.

Proliferation gene and invasion gene expression in uterine fibroids samples with different MLCK expression.

MLCK expression	*n*	Survivin	Livin	Bcl-2	Snail	N-cadherin	MMP2
High expression	26	2.77±0.31	1.62±0.22	3.88±0.51	327.49±41.62	244.21±30.93	5.01±0.77
Low expression	26	1.19±0.16	0.71±0.10	1.65±0.22	168.45±21.38	110.37±15.82	1.82±0.23
T		14.298	11.329	13.239	9.392	14.293	22.194
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 3.

Proliferation gene and invasion gene expression in uterine fibroids samples with different FAP expression.

FAP expression	*n*	Survivin	Livin	Bcl-2	Snail	N-cadherin	MMP2
High expression	26	2.62±0.28	1.69±0.26	3.62±0.47	341.33±46.25	238.61±32.14	4.48±0.82
Low expression	26	1.28±0.18	0.67±0.09	1.74±0.25	159.68±17.62	117.25±14.27	2.15±0.20
T		11.398	13.429	10.582	12.482	10.497	13.583
P		＜0.05	＜0.05	＜0.05	＜0.05	＜0.05	＜0.05

expression in uterine fibroids samples with high FAP expression were significantly higher than those in uterine fibroids samples with low FAP expression. Differences were statistically significant in Survivin, Livin, Bcl-2, Snail, N-cadherin and MMP2 protein expression in uterine fibroids samples with different FAP mRNA expression (P<0.05).

4. Discussion

Uterine fibroids lesions are made up of tumor cells, activated fibroblasts, and a large amount of extracellular matrix, and fibroblast activation can secrete a large amount of extracellular matrix and make up of tye microenvironment necessary for tumor cell proliferation and invasion, which is beneficial to the growth of uterine fibroids. MLCK and FAP are two molecules that are closely related to fibroblast activation. MLCK is the key regulatory protein of myosin light chain phosphorylation status, which could catalyze the dephosphorylation of myosin light chain process, enhance the activity of the myosin, and thus promote fibroblast activity through the myosin activity[4,5]; FAP is a type of serine protease, which regulates various growth factors such as vEGF, bFGF, TGF-β and IGF-1, and is a symbol after fibroblast activation[6,7]. In the study, analysis of MLCK and FAP expression in uterine fibroids lesions and normal uterine muscle next to fibroids showed that MLCK and FAP mRNA expression in uterine fibroids samples were significantly higher than those in normal uterine muscle samples. This shows that the high expression of MLCK and FAP is closely related to the occurrence of uterine fibroids, and promoting the activation of fibroblasts may be the pathway for MLCK and FAP to participate in uterine fibroids.

The growth of uterine fibroids is closely related to the abnormal proliferation of cells, and Survivin, Livin and Bcl-2 are the anti-apoptotic genes that promote cell proliferation. Survivin is a molecule with extensive anti-apoptotic effect, which can not only antagonize the apoptotic cascade mediated by multiple caspase molecules, but can also promote the process of the cell cycle[8,9]; Livin is a new anti-apoptotic molecule discovered in recent years, which has inhibiting effect on the pro-apoptotic effects of caspase-3, caspase-7 and other molecules; both Bcl-2 gene and pro-apoptotic gene Bax belong to the Bcl-2 family, the product encoded by Bcl-2 gene is located in the mitochondrial membrane, and can form heterodimer with Bax, prevent cytochrome C release from mitochondria into cytoplasm, and thereby inhibit the apoptosis mediated by cytochrome C[10,11]. In the study, analysis of the proliferation gene expression in uterine fibroids lesions and normal uterine muscle next to fibroids showed that Survivin, Livin and Bcl-2 protein expression in uterine fibroids samples were significantly higher than those in normal uterine muscle samples. This suggests that the highly expressed anti-apoptotic genes Survivin, Livin and Bcl-2 as well as the cell proliferation mediated by them are closely related to the occurrence of uterine fibroids.

Uterine fibroid cells can infiltrate towards surrounding tissue on the basis of abnormal proliferation, and they have the feature of invasive growth. MMP2 in the MMPs family can degrade various types of gelatine, collagen and elastin, and is a key molecule that promotes tumor cell invasion[12,13]. Snail is an important transcription factor that regulates epithelial-mesenchymal transition, and it can be combined with the promoter region of epithelial marker gene E-cadherin to inhibit its expression, and then make the epithelial phenotype of cells transit to mesenchymal phenotype, which is accompanied by the decreased expression of epithelial marker gene E-cadherin and the increased expression of mesenchymal marker gene N-cadherin[14,15]. Cells of mesenchymal phenotype have strong motion and invasion properties. In the study, the analysis of above invasion gene expression in uterine fibroids lesions and normal uterine muscle next to fibroids showed that Snail, N-cadherin and MMP2 protein expression in uterine fibroids samples were significantly higher than those in normal uterine muscle samples. This indicates that the highly expressed invasion genes Snail, N-cadherin and MMP2 as well as the cell invasion mediated by them are closely related to the occurrence of uterine fibroids.

Fibroblast activation can create favorable micro-environment for the growth of uterine fibroids, and the excessively activated fibroblasts in local lesions can promote uterine fibroids cell proliferation and invasion. As mentioned earlier, fibroblast activation-related molecules MLCK and FAP showed a trend of high expression in uterine fibroids lesions, and in order to define whether the highly expressed MLCK and FAP participated in the growth of uterine fibroids, the correlation of MLCK and FAP expression with above uterine fibroid cell proliferation and invasion gene expression was further analyzed in the study. Comparison of proliferation gene and invasion gene expression in uterine fibroids samples with different MLCK and FAP expression was as follows: Survivin,

Livin, Bcl-2, Snail, N-cadherin and MMP2 protein expression in uterine fibroids samples with high MLCK and FAP expression were significantly higher than those in uterine fibroids samples with low MLCK and FAP expression. It means that highly expressed MLCK and FAP in uterine fibroids lesions can increase the expression of proliferation genes and invasion genes to promote uterine fibroids cell proliferation and invasion, which is conducive to the growth of uterine fibroids lesions.

To sum up, it is believed that MLCK and FAP are highly expressive in uterine fibroids; highly expressed MLCK and FAP can promote proliferation gene and invasion gene expression to promote the proliferation and invasion of uterine fibroid cells.

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