EFFECTS OF CYPA ON PROLIFERATION, MIGRATION, AND INVASION OF COLORECTAL CANCER CELLS THROUGH ERK/MAPK SIGNAL TRANSDUCTION PATHWAY

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ABSTRACT

Objective: *To investigate the effects of cyclophilin A (CyPA) on the proliferation, migration, and invasion of colorectal cancer cells through the ERK / MAPK signal transduction pathway.*

Methods: *Human colorectal cancer cell line HCT116 and normal colorectal mucosa cells were cultured in vitro, and were then transfected with CyPA overexpression vector (CyPA overexpression group) and interference vector (CyPA interference group), respectively. Western blot was used to detect the expression of CyPA protein in normal colorectal cells and colorectal cancer cells; CCK-8 method was used to detect the proliferation ability of each group; Transwell test was used to detect the migration and invasion ability of each group; Western blot method was also used to detect the expression of ERK / MAPK signaling pathway-related proteins.*

Results: *The expression of CyPA protein in HCT116 cells was significantly higher than in normal colorectal cells (P<0.05). Compared with the blank control group, the proliferation ability of HCT116 cells in the CyPA overexpression group was significantly increased at 24 h, 48 h, and 72 h, while the cell proliferation ability of the CyPA interference group was significantly decreased (P<0.05). Compared with the blank control group, the number of migration and invasion cells in the CyPA overexpression group was significantly increased, while the number of migration and invasion cells in the CyPA interference group was significantly decreased (P<0.05). Compared with the blank control group, the expression of CD147 and p-ERK1 / 2 protein in the CyPA overexpression group was significantly increased, while the expression of CD147 and p-ERK1/2 protein in the CyPA interference group was significantly decreased (P<0.05).*

Conclusion: *CyPA is highly expressed in colorectal cancer cells, which can promote cell proliferation, migration, and invasion. The related mechanism may be achieved by regulating the ERK/MAPK signal transduction pathway.*

Keywords: *CyPA, ERK/MAPK signaling pathway, colorectal cancer cells, proliferation, migration, invasion.*

DOI: 10.19193/0393-6384_2021_6_485

Received *March 15, 2020;* ***Accepted*** *October 20, 2020*

Introduction

Colorectal cancer ranks among the top three cancers in the world in terms of incidence, and is one of the most common malignant tumors in the digestive system. In recent years, the incidence in China specifically has been increasing annually and rising among younger patients. The early detection rate of colorectal cancer is low, and the cancer is prone to metastasis. Patients often come to see a doctor due to diarrhea, abdominal pain, and lower abdominal masses. Since these symptoms present in the middle and advanced stages, most patients have already lost the opportunity for radical surgery[1]. In recent years, the gene molecular level has become an area of interest in the clinical study of colorectal cancer pathogenesis, and the relationship between cyclophilins (Cyps) and tumors has attracted increased attention[2]. Cyclophilin A (CyPA) is one of the members of the cyclophilin family, and is a

widely distributed and highly conserved biological protein. It was initially considered the receptor of immunosuppressant CSA, with the two interacting to prevent rejection following organ transplantation[3]. CypA performs the activity of peptide proline CIS trans isomerase, participates in cholesterol metabolism and immune function regulation, and plays the role of pro-inflammatory factors.

Mitogen-activated protein kinase (MAPK) is one of many intracellular signaling pathways, and is involved in cell proliferation, apoptosis, and other physiological functions. ERK is one of the MAPK signaling pathways, and is involved in normal tissue growth and differentiation[4]. Relevant data show that the ERK/MAPK signaling pathway can stimulate cell proliferation and invasion, and that inhibition of this pathway can in turn inhibit tumor activity[5]. The purpose of this study was to analyze the effect of CyPA on the proliferation, migration, and invasion of colorectal cancer cells through the ERK / MAPK signaling pathway.

Materials and methods

Experimental reagents and instruments

Experimental reagents and instruments used included the following: human colorectal cancer cell line HCT116 (Shanghai Huzhen Industry Co., Ltd.); Trizol Extraction Reagent (Wuhan Purity Biotechnology Co., Ltd.); Kanamycin (Shanghai Baoman Biotechnology Co., Ltd.); DMEM / F-12 medium (Shunran (Shanghai) Biotechnology Co., Ltd.); Lipofectamin2000 (Invitrogen, USA); CCK8 Kit (Shanghai Jizhi Biochemical Technology Co., Ltd.); Transwell board (Shanghai Weijin Biotechnology Co., Ltd.); Matrigel (Shanghai Shanran Biotechnology Co., Ltd.); BCA protein concentration determination kit (Shanghai Jizhi Biochemical Technology Co., Ltd.); and PVDF film (Beijing Baiaoyijie Technology Co., Ltd.).

Also used were: an inverted microscope (Beijing Jiayuan Xingye Technology Co., Ltd.); constant temperature oscillator (Hangzhou Notting Scientific Equipment Co., Ltd.); microplate reader (Shanghai Yanhui Biotechnology Co., Ltd.); low temperature high speed centrifuge (Beijing Taize Jiaye Technology Development Co., Ltd.); horizontal gel electrophoresis tank (Beijing Qian Ming Gene Technology Co., Ltd.); vortex mixer (Shanghai Rongweida Industrial Co., Ltd.); autoclave (Beijing Jiayuan Xingye Technology Co., Ltd.); constant temperature magnetic stirrer (Beijing Qianming Gene Technology Co., Ltd.); and an ultra low temperature refrigerator (Beijing Taize Jiaye Technology Development Co., Ltd.).

Cell culture

The colorectal cancer cell line HCT116 was routinely cultured in RPMI-1640 medium, and was cultured in a constant temperature incubator at 37°C and 5% CO_2. The culture medium was replaced according to the cell growth situation for 2 to 3 days, and the cells on the bottle wall grew to a degree of confluence. 80–90% of the cells were passaged. CypA overexpression vector and interference vector were used to transfect HCT116 cells, and a blank control group was set up using blank lentiviral vector.

Detection methods

The CCK-8 method was used to detect cell proliferation ability. The procedure for this method was as follows: observe the cell morphology under a microscope, and inoculate the cells collected by trypsin digestion and centrifugation in a 96-well plate, counting plate technology, 2000 per well. Following transfection, add 15% FBS-DMM culture medium. Incubate overnight at 37°C. Take out 96-well plates and add CCK-8 (0.5mg/ml) at each time point of 0, 24, 48, and 72 hours. Incubate in a 37°C incubator for 1 hour. The microplate reader detects the absorbance at 450mm.

A Transwell test was used to detect cell migration and invasion. The procedure for the cell migration test was as follows: resuspend the colorectal cancer cell line HCT116 in serum-free high-sugar DMEM medium at a density of 1×10^5 cells/ml, and slowly add 200μL of cell suspension to the Transwell chamber. Add 600μL of high-sugar DMEM medium containing 10% FBS to the lower chamber, and incubate in a 37°C incubator for 24 hours. Wipe the remaining cells in the upper chamber with a cotton swab, fix with methanol for 30 minutes, stain with 0.1% crystal violet for 15 minutes, wash 3 times with PBS, and observe using a microscope. Next, randomly select 5 fields to count, and take the average value as the number of migrated cells. For the cell invasion experiment, use Matrigel gel matrix as an invasion model and add cell suspension. The remainder of the operation follows the same procedures described for the cell migration experiment. The procedure for the detection of related protein expression by Western blot was as follows: collect logarithmic growth of colorectal cancer cell line HCT116 in each group, add appropriate amount of RIPA lysate to fully lyse the

cells, and quantify protein according to the working solution prepared by the BCA kit. Prepare separation gel and layered gel, mix 30μg protein with 4× loading buffer, and centrifuge to get the supernatant. Separate by SDS-polyacrylamide gel electrophoresis, place the PVDF membrane and filter paper in the transfer solution, and soak for 5 minutes. The membrane was transferred for 1 hour; 5% skimmed milk powder was blocked at room temperature for 2 hours, while the PVDF membrane was incubated with the primary antibodies CypA, CD147, p-ERK1/2, and ERK1/2 and incubated overnight in a refrigerator at 4°C. After washing the membrane with TBST, add the corresponding secondary antibody and incubate at room temperature for 1-2h. TBST washes off the secondary antibody that is non-specifically bound to the membrane, and the color is developed by ECL chemiluminescence method.

Statistical methods

The data in this study were analyzed by the SPSS21.0 software package, and the measurement data were all expressed by ($\bar{x}\pm s$). The comparison of data between the two groups was tested by t, and the comparison of data between multiple groups was analyzed by variance analysis. P<0.05 was regarded as statistically significant.

Results

Expression of CypA protein in normal colorectal cells and colorectal cancer cells

The Western blot method showed that the expression of CypA protein in colorectal cancer cells HCT116 was significantly higher than in normal colorectal cells, and the difference was statistically significant (P<0.05). Data are presented in Table 1.

Group	Cases	CypA
Normal cell	10	0.93±0.05
HCT116 cell	10	4.34±0.06
t	137.662	
P	<0.001	

Table 1: Expression of CypA protein in normal colorectal cells and colorectal cancer cells.
Note: Compared with normal colorectal cells.

The effect of overexpression and interference with CypA on the proliferation of HCT116 cells

The CCK8 method test results showed that compared with the blank control group, the proliferation ability of HCT116 cells in the CypA overexpression group was significantly enhanced at 24h, 48h, and 72h, while the cell proliferation ability of the CypA interference group was significantly reduced (P<0.05). Data are presented in Table 2.

Group	Cases	0h	24h	48h	72h
Blank control group	10	0.20±0.09	0.34±0.08	0.37±0.08	0.78±0.13
CypA overexpression group	10	0.19±0.09	0.41±0.11[a]	0.69±0.12[a]	1.19±0.08[a]
CypA interference group	10	0.18±0.08	0.21±0.09[a]	0.25±0.11[a]	0.33±0.10[a]
F		0.13	11.62	47.17	116.7
P		0.876	<0.001	<0.001	<0.001

Table 2: The effect of overexpression and interference with CypA on the proliferation of HCT116 cells.

The effect of overexpression and interference with CypA on the migration and invasion ability of HCT116 cells

The results of the Transwell migration and invasion experiments showed that, compared with the blank control group, the number of HCT116 cells in the CypA overexpression group increased significantly, while the number of cells in the CypA interference group decreased significantly (P<0.05). Data are presented in Table 3.

Group	N	Number of migrating cells	Number of invasion cells
Blank control group	10	53.78±5.45	42.32±5.41
CypA overexpression group	10	155.23±14.78[a]	124.23±11.69[a]
CypA interference group	10	60.29±4.76[a]	51.48±6.23[a]
F		357.23	295.15
P		<0.001	<0.001

Table 3: Comparison of the number of HCT116 cell migration and invasion cells in each group ($\bar{x}\pm s$).
Note: Compared with the blank control group; [a]P<0.05.

The effect of overexpression and interference with CypA on the ERK/MAPK signaling pathway in HCT116 cells

Western blot detection of ERK/MAPK signaling pathway-related proteins showed that, compared with the blank control group, the expression of CD147 and p-ERK1/2 protein in the CypA overexpression group was significantly increased, while the protein expression of CD147 and p-ERK1/2 in the CypA interference group was significantly decreased (P<0.05). Data are presented in Table 4.

Group	N	CD147	p-ERK1/2	ERK1/2
Blank control group	10	0.45±0.08	0.55±0.07	0.92±0.13
CypA overexpression group	10	0.89±0.10[a]	0.90±0.11[a]	0.91±0.08[a]
CypA interference group	10	0.50±0.06[a]	0.60±0.08[a]	0.90±0.07[a]
F		87.05	45.94	0.11
P		<0.001	<0.001	0.900

Table 4: Comparison of CD147, p-ERK1/2, and ERK1/2 protein expression between groups ($\bar{x}\pm s$).
Note: Compared with the blank control group; [a]P<0.05.

Discussion

With the improvement of general living standards and subsequent changes in diet structure, the incidence rate of colorectal cancer is increasing annually, posing a serious threat to human health. Surgery, combined with radiotherapy and chemotherapy, is still the most effective method for the clinical treatment of colorectal cancer. The five-year survival rate of patients following early radical surgery can be as high as 90%; however, due to the invasion, metastasis, and recurrence of the tumors, the general five-year survival rate of patients is less than 10%[6, 7]. The pathogenesis of colorectal cancer is complex and diverse, and most researchers believe that it is related to genetic, environmental, lifestyle, and other factors. Its occurrence requires participation through a variety of signaling pathways, all of which affect its malignant biological behavior. Tumor cells separate from the primary focus, invade the stroma and blood vessels, and flow with the blood to distant organs and tissues, continuing to proliferate and form tumors[8]. Relevant data show that 90% of colorectal cancer deaths are caused by tumor metastasis and diffusion, and inhibition of tumor angiogenesis can significantly inhibit tumor growth and metastasis[9].

CyPA is an important member of the cyclophilin family and a target of immunosuppressant cyclosporine A in cells. CyPA is composed of 165 amino acids, and exists throughout subcellular structures such as cytoplasm, nuclear membrane, and Golgi apparatus. Due to its highly conserved structure, there is little difference in the expression of CyPA in various tissues. CypA has peptide-proamide cis-trans isomerase activity, which acts as a key rate-limiting enzyme in the process of protein folding and organization[10]. Relevant data show that the peptide-proamide cis-trans isomerase domain of CypA can bind to the dynein complex in the cytoplasm and play an important role in the process of CypA activating ERK1/2[11]. CypA also has immunoregulatory functions. CypA-CsA interaction activates the nuclease activity of CypA and promotes the degradation and apoptosis of T cell DNA; CypA-CsA complex blocks the activation of p38 and MAPK in the MAPK signaling pathway effect. It also inhibits the entry of transcription factor activator protein into the human nucleus DNA binding, inhibiting the transcription process of interleukin and other genes and thereby inhibiting the proliferation of T cells[12].

Many tumors suggest that high expression of CyPA can promote tumor cell proliferation and prevent apoptosis, and can also regulate cell malignant transformation and metabolism. Some scholars have found that CyPA gene knockout can significantly inhibit the proliferation of endometrial cancer cells induced by paclitaxel and can increase apoptosis, suggesting that CyPA gene knockout can reverse the drug resistance of tumor cells to paclitaxel. However, the full extent of the role of CyPA protein in colorectal cancer is not yet understood[13]. The results of this study showed that the expression of CyPA protein in colorectal cancer HCT116 cells was significantly higher than in normal colorectal cells ($P<0.05$). Compared with the blank control group, the proliferation, migration, and invasion of HCT116 cells were significantly increased in the CyPA overexpression group, while the proliferation, migration, and invasion of HCT116 cells were significantly decreased in the CyPA interference group ($P<0.05$). These results suggest that CyPA is highly expressed in colorectal cancer cells and can promote cell proliferation, invasion, and migration.

The MAPK signaling pathway involves a series of protein kinases. Once activated, MAPK plays a key role in regulating cell proliferation, differentiation, and angiogenesis. The ERK/MAPK pathway is the most important pathway for cell proliferation, and it can regulate cell cycle and cytoskeleton deformation[14, 15]. In this study, ERK / MAPK signaling pathway-related proteins were detected by Western blot. The results showed that, compared with the blank control group, the expression of CD147 and p-ERK1/2 protein in the CyPA overexpression group was significantly increased, while the expression of CD147 and p-ERK1/2 protein in the CyPA interference group was significantly decreased ($P<0.05$). These results suggest that the regulation effects of CyPA on malignant biological behavior of

colorectal cancer cells may be related to the ERK / MAPK signaling pathway.

In summary, CypA is highly expressed in colorectal cancer cells and can promote malignant biological behaviors such as cell proliferation, migration, and invasion. The related mechanism may be achieved by regulating the ERK/MAPK signal transduction pathway.

References

1) Patel SG, Ahnen DJ. Colorectal Cancer in the Young. Curr Gastroenterol Rep 2018; 20(4): 15.
2) Lucas C, Barnich N, Nguyen HTT. Microbiota, Inflammation and Colorectal Cancer. Int J Mol Sci 2017; 18(6): 1310.
3) Liu W, Li J, Zheng W, Shang Y, Zhao Z, et al. Cyclophilin A-regulated ubiquitination is critical for RIG-I-mediated antiviral immune responses. Elife 2017; 6: e24425.
4) Li W, Liu W, Chen C, Fan W, Zhang H, et al. Effect of extracellular cyclophilin A on inflammatory response and anti-inflammatory activity of antibody against cyclophilin A. Sheng Wu Gong Cheng Xue Bao 2018; 34(1): 90-101.
5) 5)Donahue DA, Porrot F, Couespel N, Schwartz O. SUN2 Silencing Impairs CD4 T Cell Proliferation and Alters Sensitivity to HIV-1 Infection Independently of Cyclophilin A. J Virol 2017; 91(6): e02303-16.
6) Levi F, Pasche C, Lucchini F, La Vecchia C. Macronutrients and colorectal cancer: a Swiss case-control study. Ann Oncol 2002; 13(3): 369-373.
7) Assenat, Eric. FOLFIRINOX for the treatment of colorectal cancer: latest evidence from clinical trials. Colorectal Cancer 2017; 1(3): 181-184.
8) Yang B, Gapstur SM, Newton CC, Jacobs EJ, Campbell PT. Alcohol intake and mortality among survivors of colorectal cancer: The Cancer Prevention Study II Nutrition Cohort. Cancer 2017; 123(11): 2006-2013.
9) Al Dahhan SA, Al Lami FH. Epidemiology of Colorectal Cancer in Iraq, 2002-2014. Gulf J Oncolog 2018; 1(26): 23-26.
10) Dawar FU, Wu J, Zhao L, Khattak MN, Mei J, et al. Updates in understanding the role of cyclophilin A in leukocyte chemotaxis. J Leukoc Biol 2017; 101(4): 823-826.
11) Pasetto L, Pozzi S, Castelnovo M, Basso M, Estevez AG, et al. Targeting Extracellular Cyclophilin A Reduces Neuroinflammation and Extends Survival in a Mouse Model of Amyotrophic Lateral Sclerosis. J Neurosci 2017; 37(6): 1413-1427.
12) von Ungern-Sternberg SNI, Vogel S, Walker-Allgaier B, Geue S, Maurer A, Wild AM, et al. Extracellular Cyclophilin A Augments Platelet-Dependent Thrombosis and Thromboinflammation. Thromb Haemost 2017; 117(11): 2063-2078.
13) Wang Y, Liu G, Zhang J, Zhu L, Yu J, et al. Overexpression of Cyclophilin A in Human Periapical Lesions. J Endod 2019; 45(12): 1496-1503.
14) Liao T, Wen D, Ma B, Hu JQ, Qu N, et al. Yes-associated protein 1 promotes papillary thyroid cancer cell proliferation by activating the ERK/MAPK signaling pathway. Oncotarget 2017; 8(7): 11719-11728.
15) He F, Che J, Shi G, Liu W, Yang S. Long non-coding RNA MALAT1 promotes the proliferation and metastasis in non-small cell lung cancer through the ERK/MAPK pathway. Int J Clin Exp Pathol 2017, 10(1): 417-424.

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