LX2-CM PROMOTES PROLIFERATION, INVASION AND MIGRATION OF LIVER CANCER CELLS BY ACTIVATING C-MYC

Lejun Lin[1,#], Zhiying Xu[1,#], Bofeng Cao[2,*]
[1]Department of Nuclear Medicine, The Affiliated Yantai Yuhuangding Hospital of Qingdao University Medical College, Yantai, PR China - [2]Department of Radiology, The Affiliated Yantai Yuhuangding Hospital of Qingdao University Medical College, Yantai, PR China
[#]*These authors contributed equally to this work as co-first author*

ABSTRACT

Objective: *This study examines the mechanism of an LX2-conditioned medium (LX2-CM) in promoting the proliferation, invasion and migration of liver cancer cells.*

Methods: *Human liver cancer cells HepG2 and hepatic stellate cells LX2 were cultured to obtain LX2-CM. The experiment was divided into a control group (DMEM-CM), control and medicated group (DMEM-CM with ERK1/2 pathway blocker U0126), research group (LX2-CM), and research and medicated group (LX2-CM with U0126), in which dimethyl sulfoxide was added to both the control and research groups, U0126 was added to the control and medicated group, and the research and medicated group was protected from light for 1.5 h. The transwell experiment was used to detect the changes in cell invasion ability and migration ability. The expression levels of vimentin, p-ERK1/2 and c-Myc of each group were measured using western blotting.*

Results: *With the increase of U0126 concentration, the cell proliferation ability of both medicated groups showed a downward trend. Compared with the LX2-CM group, the cell proliferation ability of the DMEM group was significantly reduced at U0126 concentrations of 0 μmol/L, 5 μmol/L and 15 μmol/L ($P<0.01$). When the U0126 concentration was 25 μmol/L, the cells proliferative capacity in the DMEM group was significantly reduced compared with the LX2-CM group, but the difference between the two groups was not statistically significant ($P>0.05$). Compared with the control group, the cell invasion and metastasis ability of the control and medicated group were significantly reduced but were significantly improved in the research group ($P<0.05$). Compared with the research group, the cell invasion and metastasis ability of the research and medicated group were significantly reduced ($P<0.05$). The results of the western blotting showed that compared with the control group, the expression levels of vimentin, p-ERK1/2 and c-Myc in the control and medicated group were significantly reduced, and the levels in the research group significantly increased ($P<0.05$). Finally, compared with the research group, the expression levels of vimentin, p-ERK1/2 and c-Myc in the research and medicated group were significantly reduced ($P<0.05$).*

Conclusion: *LX2-CM can activate c-Myc and can significantly promote the proliferation, invasion and migration of hepatocellular carcinoma cells, which may be closely related to the ERK1/2 signalling pathway.*

Keywords: *LX2-CM, c-Myc, liver cancer cells, proliferation, invasion, migration.*

DOI: 10.19193/0393-6384_2021_2_122

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

Introduction

Hepatocellular carcinoma is one of the most common lethal malignant tumours, and its morbidity and mortality rates are rapidly increasing. According to statistics, in China, the incidence and mortality rates of liver cancer are second only to lung cancer in men. Among women, the morbidity rate ranks fifth and the death rate ranks third(1). With the development of the social economy, the treatment of liver cancer has continuously improved, and the early diagnosis and treatment effects of liver cancer have made significant progress. However, the mortality rate of patients with liver cancer is still high, which seriously affects the quality of life of patients and worsens their families' economic burden.

Studies have shown that the occurrence and development of liver cancer has an important relationship with the tumour microenvironment(2). In the liver, the hepatic stellate cell is a kind of multifunctional special liver interstitial cell that plays a major role in the process of liver inflammation and fibrosis, which

is involved in the formation of the chronic inflammation microenvironment of the liver[3]. According to reports, there are reciprocal signalling pathways between hepatic stellate cells and precancerous hepatocytes or liver cancer cells, such as extracellular signal-regulated kinase 1/2 (ERK1/2), which promotes tumour proliferation, invasion and migration and the formation of metastases (4). This study explores the mechanism by which an LX2 conditioned medium (LX2-CM) promotes the proliferation, invasion and migration of liver cancer cells.

Materials and methods

Experimental cells

Human liver cancer cell lines HepG2 (Shanghai Hengfei Biotechnology Co., Ltd.) and hepatic stellate cells LX2 (Guangzhou Focusbio Biotechnology Co., Ltd.) were obtained.

Main instruments and reagents

The following were used in the experiment: low-temperature high-speed centrifuge (Changsha Xiangrui Centrifuge Co., Ltd., model: TG16-WS); inverted biological microscope (Shanghai Putan Optical Instrument Co., Ltd., model: MM-XH500); ultra-low temperature refrigerator (Shandong Boke Biological Industry Co., model: BDF-40H100); DMEM medium (Shanghai Thermo Fisher Scientific Technology Co., Ltd.); trypsin (Shanghai Ruji Biotechnology Development Co., Ltd.); β-actin polyclonal antibody (Shanghai Yuduo Biotechnology Co., Ltd.); rabbit anti-ERK1/2 monoclonal antibody (Beijing Luyuan Bird Biotechnology Co., Ltd.); MTT solution (Shanghai Xinyu Biotechnology Co., Ltd.); c-Myc monoclonal antibody (Beijing Huaxia Ocean Technology Co., Ltd.); and foetal cattle serum (Shanghai Jianglin Biotechnology Co., Ltd.).

Grouping

Cell culture: Human hepatocellular carcinoma cells HepG2 and hepatic stellate cells LX2 were cultured in DMEM with 10% foetal bovine serum and 5% carbon dioxide at 37 °C. The culture medium was changed daily. When the cell growth density reached 85% and grew to the logarithmic phase, it was seeded in a 96-well plate, 6-well plate, or 30 cm2 medium for subsequent experiments. After the LX2 cells in the culture medium reached about 70%, the dead cells and impurities of the supernatant of the cultured cells were removed, and the LX2-CM was obtained by filtering. The DMEM medium without the foetal bovine serum was incubated for 12 h, and the culture solution was collected as a control group.

Experimental grouping: The experimental group was divided into a control group (DMEM-CM), control and mediated group (DMEM-CM with ERK1/2 pathway blocker U0126), research group (LX2-CM), and research and mediated group (U0126 was added to LX2-CM), in which dimethyl sulfoxide was added to both the control and research groups, U0126 was added to the control and mediated group, and the research and mediated group was protected from light for 1.5 h.

Observation indicators

MTT method to detect cell proliferation: HepG2 cells were made into a cell suspension and were seeded in a 96-well plate at 40,000 cells/mL. After 24 h of being cultured, they were divided into the DMEM group and the LX2-CM group. There were four compound holes in each group, 100 μL per well. Various concentrations of U0126 (0 μmol/L, 5 μmol/L, 15 μmol/L and 25 μmol/L) were added to each well. After 12 h of incubation, 100 μL of MTT solution was added to each well and cultured at room temperature for 1 h. The absorbance at 470 nm was measured. Multiple experiments were performed to reduce errors.

Transwell cell migration experiment: The cells used in each group were collected and a cell suspension was prepared. Next, 200 μL of the cell suspension was inoculated into the upper chamber of the transwell chamber, and 300 μL of each group of the mixed solution was added to the lower chamber. The transwell chamber was placed into 24 wells and routinely incubated for 24 h. The chamber was taken out, washed and fixed, the adherent cells in the chamber were wiped with a cotton swab, and crystal violet was used for staining. Four fields were randomly selected, and the number of cell migrations in each group was observed with a microscope. Multiple experiments were performed to reduce errors.

Transwell cell invasion experiment: The matrix gel was diluted with a concentration of 50 mg/L at a ratio of 1:8, and the upper chamber surface of the bottom membrane of the transwell chamber was coated with the matrix gel diluent and air-dried at room temperature to aspirate excess liquid. The procedure was the same as the transwell cell migration experiment. Multiple experiments were performed to reduce errors. The expression levels of vimentin, p-ERK1/2 and c-Myc were measured via western blotting.

Statistical methods

In this study, the measurement data were compared using an independent sample t-test between the control and research groups, and a single factor multi-sample mean comparison was used for multiple groups. The MTT method was used to detect the cell proliferation ability of each group. The transwell experiment was used to detect the changes in cell invasion ability and migration ability. The expression levels of vimentin, p-ERK1/2 and c-Myc within the groups were determined by western blotting. If the result was P<0.05, it was regarded as statistically significant. The SPSS20.0 software package was used for the statistical data analysis.

Results

Changes of cell proliferation ability in LX2-CM group and DMEM group

As shown in Table 1, the results indicated that with the increase of U0126 concentration, the cell proliferation ability of the LX2-CM and DMEM groups followed a downward trend.

Compared with the LX2-CM group, the cell proliferation ability of the DMEM group was significantly reduced at U0126 concentrations of 0 μmol/L, 5 μmol/L and 15 μmol/L (P<0.01). When the U0126 concentration was 25 μmol/L, the cells in the DMEM group were significantly reduced compared with the LX2-CM group, but the difference between the two groups was not statistically significant (P>0.05).

Groups	Cases (n)	0 μmol/L U0126	5 μmol/L U0126	15 μmol/L U0126	25 μmol/L U0126
LX2-CM group	4	1.75±0.16	1.33±0.16	0.93±0.18	0.44±0.10
DMEM group	4	1.14±0.11	0.87±0.09	0.51±0.07	0.39±0.06
t		6.283	5.012	4.349	0.858
P		0.001	0.002	0.005	0.424

Table 1: Changes in cell proliferation capacity between the control and research groups ($\bar{x}$±s).

Changes of cell invasion ability in each group

As shown in Table 2, compared with the control group, the cell invasion ability of the control and mediated group was significantly reduced, and the cell invasion ability of the research group was significantly enhanced (P<0.05). Compared with the research group, the cell invasion ability of the research and mediated group was significantly reduced (P<0.05).

Groups	Changes of cell invasion ability (cases)
Control group	51.26±6.33
Control and mediated group	21.53±7.49[a]
Research group	81.74±18.66[a]
Research and mediated group	37.84±10.37[b]

Table 2: Changes of cell invasion ability in each group ($\bar{x}$±s).

Note: [a]P<0.05 compared with the control group; [b]P<0.05 compared with the research group.

Changes in cell migration capacity in each group

As shown in Table 3, compared with the control group, the cell migration ability of the control and mediated group was significantly decreased, and the cell migration ability of the research group was significantly enhanced (P<0.05). Compared with the research group, the cell migration ability of the research and mediated group was significantly reduced (P<0.05).

Groups	Changes of cell invasion ability (cases)
Control group	96.47±11.15
Control and mediated group	47.23±14.71[a]
Research group	205.67±36.28[a]
Research and mediated group	62.25±19.87[b]

Table 3: Changes in cell migration capacity of each group ($\bar{x}$±s).

Note: [a]P<0.05 compared with the control group; [b]P<0.05 compared with the research group.

Changes in expression levels of vimentin, p-ERK1/2 and c-Myc in each group

As shown in Figure 1, the results of the western blotting indicated that compared with the control group, the expression levels of vimentin, p-ERK1/2 and c-Myc in the control and mediated group were significantly reduced, whereas the levels in the research group significantly increased (P<0.05).

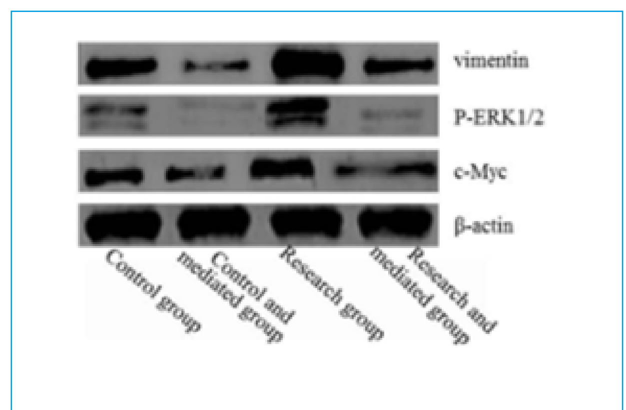

Figure 1: Changes in expression levels of vimentin, p-ERK1/2 and c-Myc in each group.

Compared with the research group, the expression levels of vimentin, p-ERK1 / 2 and c-Myc in the research and mediated group were significantly reduced (P<0.05).

Discussion

Liver cancer is a malignant tumour with high lethality. Although treatment methods for liver cancer have increased, it is prone to metastasis inside and outside the liver, and, therefore, the overall prognosis of patients with liver cancer is still poor[5]. According to reports, the most important processes in liver cancer metastasis include the proliferation, invasion and migration of cancer cells[6]. Therefore, inhibiting the growth of liver cancer cells and blocking their invasion and migration has become a key focus in the treatment of this disease. With the continuous improvement of isolation methods and deepening knowledge, an increasing amount of studies have shown that hepatic stellate cells are not only involved in liver development, regeneration, immunoregulation and the proliferation and differentiation of liver progenitor cells but also related to hepatitis, cirrhosis and the occurrence and development of liver cancer[7-8]. In this study, LX2-CM was used as an experimental reagent to observe its effect on liver cancer cells.

The MTT method is commonly used to detect changes in cell proliferation[9]. In this study, MTT experiments were used to detect changes in cell proliferation, and it was found that the cell proliferation of the LX2-CM group was significantly higher than that of the DMEM group. As the concentration increased, the cell's proliferation ability was significantly weakened. It is thus suggested that LX2-CM can significantly promote the proliferation of liver cancer cells.

The migration of tumour cells is a nonnegligible part of tumour cell metastasis. Hepatoma cells can pass through the basement membrane and extracellular matrix into the vasculature during metastasis. This enhances the ability of liver cancer cells to migrate[10]. Jin et al.[11] found that hepatic stellate cells can promote the invasion and migration of liver cancer cells with over-secreted laminin 5. In this study, compared with the control group, the cell invasion and metastasis ability of the control and mediated group were significantly reduced but were significantly enhanced in the research group (P<0.05). Compared with the research group, the cell invasion of the research and mediated group was significantly reduced (P<0.05). This indicates that LX2-CM has an important role in promoting the invasion and migration ability of liver cancer cells.

Numerous studies point out that the close interaction between tumour cells and their adjacent tumour stroma is an important regulator in the occurrence and development of liver cancer. Many factors and complex signal pathways in the tumour microenvironment participate in tumour cells. An epithelial-mesenchymal transition occurs, and the tumour microenvironment is closely related to the maintenance of tumour stem cells[12-13].

C-Myc is a member of the Myc family that consists of 439 amino acid residues, and it plays an important role in cell proliferation and differentiation. Studies have found that the activation of c-Myc is closely related to the occurrence and development of various tumours. It not only promotes the malignant proliferation and transformation of cells but also inhibits cell differentiation[14]. Vimentin is a characteristic phenotypic protein of mesenchymal cells that can maintain the normal morphology of cells and promote cell adhesion and migration. Some scholars have found that overexpression of vimentin is an important marker of epithelial-mesenchymal transition and has a central relationship with the malignant development of tumour cells[15]. An ERK1/2 signalling pathway is an effective way to regulate cell growth and cell cycle progression and can also regulate the transcription of multiple proteins[16].

In this study, compared with the control group, the expression levels of vimentin, p-ERK1/2 and c-Myc in the control and mediated group were significantly reduced, but their levels in the research group significantly increased (P<0.05). Compared with the research group, the expression levels of vimentin, p-ERK1/2 and c-Myc in the research medication group were significantly reduced (P<0.05). It is suggested that LX2-CM can activate the expression of c-Myc and ERK1/2 and induce the occurrence of epithelial mesenchyme.

In summary, LX2-CM can activate c-Myc, which has a significant promotion effect on the proliferation, invasion and migration of liver cancer cells and may be closely related to the ERK1/2 signalling pathway.

References

1) Stvilia K, Spradling PR, Asatiani A, Gogia M, Kutateladze K, et al. Progress in Testing for and Treatment of Hepatitis C Virus Infection Among Persons Who Inject Drugs - Georgia, 2018. MMWR. MMWR Morb Mortal Wkly Rep 2019; 68: 637-641.
2) Qin J, Yang L, Sheng X, Sa Z, Huang T, et al. Antitumor effects of brucine immuno-nanoparticles on hepatocellular carcinoma in vivo. Oncol Lett 2018; 15: 6137-6146.
3) Li ZQ, Wu WR, Zhao C, Zhao C, Zhang XL, et al. CCN1/Cyr61 enhances the function of hepatic stellate cells in promoting the progression of hepatocellular carcinoma. Int J Mol Med 2018; 41: 1518-1528.
4) Hu WH, Hu Z, Shen X, Dong LY, Zhou WZ, et al. C5a receptor enhances hepatocellular carcinoma cell invasiveness via activating ERK1/2-mediated epithelial-mesenchymal transition. Exp Mol Pathol 2016; 100: 101-108.
5) VoPham T, Bertrand KA, Tamimi RM, Laden F, Hart JE. Ambient PM2.5 air pollution exposure and hepatocellular carcinoma incidence in the United States. Cancer Causes Control 2018; 29: 563-572.
6) Wang ZY, Song D, Huang P. MicroRNA 340 inhibits tumor cell proliferation, migration and invasion, and induces apoptosis in hepatocellular carcinoma. Mol Med Rep 2017; 16: 7649.
7) Moscat J. Abstract SY22-02: Tumor suppression by p62 through the metabolic reprogramming of the stroma. Cancer Res 2017; 77: 22.
8) Murray B, Barbier-Torres L, Fan W, Mato JM, Lu SC. Methionine adenosyltransferases in liver cancer. World J Gastroenterol 2019; 25: 4300-4319.
9) Huang HL, Zhang YL, Chen WF, Wu CL. Effects of aqueous extracts of gecko on stemness of hepatocellular carcinoma. Zhongguo Zhong Yao Za Zhi 2017; 42: 3990-3995.
10) Xu T, Ma M, Chi Z, Si L, Sheng X, et al. High G2 and S-phase expressed 1 expression promotes acral melanoma progression and correlates with poor clinical prognosis. Cancer Sci 2018; 109: 1787-1798.
11) Jin H, Cui M. Gene silencing of heparanase results in suppression of invasion and migration of gallbladder carcinoma cells. Biosci Biotechnol Biochem 2018; 82: 1116-1122.
12) Atanasov G, Dino K, Schierle K, Dietel C, Aust G, et al. Immunologic cellular characteristics of the tumour microenvironment of hepatocellular carcinoma drive patient outcomes. World J Surg Oncol 2019; 17: 97.
13) Wu Q, Zhou L, Lv D, Zhu X, Tang H. Exosome-mediated communication in the tumor microenvironment contributes to hepatocellular carcinoma development and progression. J Hematol Oncol 2019; 12: 53.
14) Ji F, Zhang ZH, Zhang Y, Shen SL, Cao QH, et al. Low expression of c-Myc protein predicts poor outcomes in patients with hepatocellular carcinoma after resection. BMC Cancer 2018; 18: 460.
15) Dong Q, Zhu X, Dai C, Zhang X, Gao X, et al. Osteopontin promotes epithelial-mesenchymal transition of hepatocellular carcinoma through regulating vimentin. Oncotarget 2016; 7: 12997-13012.
16) Chen P, Lei L, Wang J, Zou X, Zhang D, et al. Down-regulation of Talin1 promotes hepatocellular carcinoma progression via activation of the ERK1/2 pathway. Cancer Sci 2017; 108: 1157-1168.

Corresponding Author:
Bofeng Cao
Email: gx45fz@163.com
(China)