European Review for Medical and Pharmacological Sciences 2018; 22: 87-94

The regulatory role of SLP-2 and mechanism on CCBE1 gene expression in rectal carcinoma and adjacent lymphatic tube tissues

R.-L. GUO[1,2], X.-R. WANG[3], Q.-G. WANG[4], Z. LI[5], X. LU[6], R.-Z. MIAO[7], H. CHANG[1]

[1]Department of Hepatobiliary Surgery, Shandong Provincial Hospital affiliated to Shandong University, Jinan, Shandong, China
[2]Department of vascular surgery, Taian Central Hospital, Taian, China
[3]Department of Anus and Intestine Surgery, Second Hospital of Shandong University, Shandong, China
[4]Department of Hepatobiliary Surgery, Taian Central Hospital, Taian, China
[5]Department of Neurosurgery, Taian Central Hospital, Taian, China
[6]Department of Cardiac Surgery, Taian Central Hospital, Taian, China
[7]Department of Gastrointestinal Surgery, Shandong Provincial Hospital affiliated to Shandong University, Shandong, China

Renle Guo and Xianren Wang contributed equally to this work

Abstract. – OBJECTIVE: The incidence of rectal carcinoma (RC) has been increasing recently, and becomes the second most common digestive tumors besides gastric cancer, with a rise in the incidence of RC in younger populations. The early diagnosis and treatment are thus critical for the improvement of survival rate and life quality of patients. Stomatin-like protein 2 (SLP-2) is a type of membrane factor, which is generally found highly expressed in various tumors. Collagen and calcium-binding EGF domain (CCBE1) belongs to lymphatic tube genesis factor. The regulatory role of SLP-2 gene on CCBE1 expression in RC tumor and adjacent lymphatic tube tissues, however, has not been studied.

PATIENTS AND METHODS: 52 RC patients were recruited, and tumor and adjacent lymphatic tube tissues were collected. Real-time PCR, western blotting and immunohistochemistry (IHC) staining were used to analyze SLP-2 and CCBE1 expressions. Human lymphatic endothelial cells (LECs) were cultured in vitro and were assigned to control, scramble, and SLP-2 siRNA group. MTT assay was used to detect cell proliferation, while caspase 3 activity was detected.

RESULTS: SLP-2 and CCBE1 levels were significantly elevated in tumor lymphatic tissues, compared to that in adjacent tissues. Statistically positive correlation between SLP-1 and CCBE2 was found ($p<0.05$). The downregulation of SLP-2 by siRNA inhibited cell proliferation, elevated caspase3 activity, and decreased CCBE1 expression ($p<0.05$ compared to control group).

CONCLUSIONS: SLP-2 is up-regulated in RC lymphatic tissues, and is positively correlated with the level of CCBE1, which provides the academic the basis for the development of medicine targeting SLP-2 in the anti-rectal carcinoma therapy.

Key Words:
Stomatin-like protein 2, Collagen- and calcium-binding EGF domain 1, Rectal carcinoma, Lymphatic tube.

Introduction

Rectal carcinoma (RC) is a sort of malignant tumor invading in the gastrointestinal system and belongs to colorectal cancer[1]. RC has now become the second most common tumor in digestive tract[2,3]. The mortality of RC remains high due to the frequent metastasis and recurrence, though the diagnosis of tumor has been remarkably improved[4]. In the early phase of tumor metastasis, blood-borne or lymphatic tube transmission plays a significant role and tumor cells of RC mainly translocate to peripheral or distal organs via lymphatic tubes. Therefore, the lymphatic metastasis is closely associated with tumor invasion, and directly affects disease prognosis[5,6].

Stomatin-like protein 2 (SLP-2) gene belongs to stomatin gene superfamily and locates in short arm of human chromosome 9, encoding the protein of 357 amino acids[7]. SLP-2 coding protein is also the major component among membrane-related proteins and can participate in cell signal transduction, the regulation of membrane ion channel and the formation of cytoskeleton[8]. Studies[9,10]

Corresponding Author: Hong Chang, MD; e-mail: hongchangtt@126.com

hav found that the expression of SLP-2 elevated in ovarian cancer and cervical carcinoma, and it can facilitate tumor proliferation, metastasis, and invasion. The functional role and related mechanism of SLP-2 in tumor pathogenesis, however, has not been fully illustrated. The recent finding showed that Collagen- and calcium-binding EGF domain 1 contributed to regulatory function in lymphatic tube genesis[11,12]. This study thus investigated the effect of SLP-2 gene on CCBE1 in RC tumor and adjacent tissues, as well as its functional implications.

Patients and Methods

Patients

A total of 52 RC patients who were diagnosed and received surgical resections in Shandong Provincial Hospital affiliated to Shandong University from January 2014 to December 2014 were recruited in this study. There were 27 males and 25 females in the cohort, which had an average age of 56±7.2 years (from 43 to 78 years). There were 23, 23, and 7 cases of mammillary adenoma, mucinous adenoma, and signet ring cell carcinoma, respectively. **Inclusive criteria:** (1) Primary treatment for tumors; (2) No chemo-, radio- or biological therapy before surgery. **Exclusive criteria:** (1) History of heart or cerebral vascular disease; (2) Severe liver or kidney failure; (3) Hematological or other malignant tumors; (4) Systemic auto-immune, infectious diseases; (5) Unfinished post-op follow-ups; (6) Accident death during follow-up period; (7) Incomplete medical history. This study has been pre-approved by the Ethical Committee of Shandong Provincial Hospital affiliated to Shandong University and has obtained written consents from patients. Lymphatic tube tissues within RC tissues and adjacent tissues were collected during the surgery and were kept in liquid nitrogen. Partial tissues were fixed in 4% neutral buffered formalin solution for immunohistochemistry (IHC) staining.

Instrument and Reagent

Human lymphatic endothelial cells (LECs) were purchased from ATCC (Manassas, VA, USA). Dulbecco's Modified Eagle Medium (DMEM) medium, fetal bovine serum (FBS) and streptomycin-penicillin were bought from Hyclone (GE Healthcare Life Sciences, South Logan, UT, USA). Dimethyl sulfoxide (DMSO) and methyl thiazolyl tetrazolium (MTT) powders were from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Trypsin-EDTA lysis buffer was provided from Sigma (Merck, Temecula, CA, USA). polyvinylidene difluoride (PVDF) membrane was from Pall Life Sciences (Port Washington, NY, USA). EDTA was collected from Hyclone (GE Healthcare Life Sciences, South Logan, UT, USA). The Western blotting reagent was purchased from Beyotime (Beijing, China). ECL reagent was from Amersham Biosciences (Piscataway, NJ, USA). Rabbit anti-human SLP-2 monoclonal antibody, anti-human CCBE1 monoclonal antibody, and mouse anti-rabbit horseradish peroxidase (HRP)-conjugated IgG secondary antibody were all from Cell Signaling (Beverly, MA, USA). RNA extraction kit, and reverse transcription kit were purchased from Axygen (Thermo Fisher Scientific, Waltham, MA, USA). Labsystem Version 1.3.1 microplate reader was from Bio-Rad (Hercules, CA, USA). IHC SP kit was provided from BosterBio (Pleasanton, CA, USA). Caspase 3 activity assay kit was from BD (US). Other common reagents were from Sangon (Shanghai, China).

LECs Culture and Grouping

LECs cells stored in liquid nitrogen were resuscitated in 37°C water-bath until fully thawing. Cells were centrifuged at 1000 rpm for 3 min, and were re-suspended in 1 ml fresh medium and were removed to 50 ml culture flask, which contained 4ml fresh culture medium. Cells were kept in a humidified chamber with 5% CO_2 at 37°C for 24-48 h. LECs were seeded in 6-well plate at concentration of 1×10^5/ml with high-glucose DMEM medium containing 10% FBS (100 U/ml penicillin and 100 μg streptomycin). Cells were kept in a humidified chamber with 5% CO_2 at 37°C. Cells at log-phase of 2nd to 8th generation were randomly divided into control group; siRNA negative control (scramble) group, and SLP-2 siRNA group.

Liposome Transfection of SLP-2 siRNA into LECs

SLP-2 siRNA (Sense: 5'-GAT AAA TGG ACT TGA CAG-3'; Anti-sense: 5'-TAA ATG GAG ACT TGA CAG ACT-3') or siRNA negative control oligonucleotides (Sense: 5'-ATT CCC CTA CGA TGT AT-3'; Anti-sense: 5'-GAT TGA CAA ACT TGA CAG-3') were transfected into LECs cells. In brief, cells were cultured in 6-well plate until 70%-80% confluence. SLP-2 siRNA or negative controlled sequences were mixed with liposome in 200 μl serum-free medium for 15 min incubation at room temperature.

Lipo2000 reagent was then mixed with SLP-2 siRNA or controlled dilutions. Serum was removed from cells, followed by PBS washing and the addition of 1.6 ml serum-free culture medium. Cells were then kept in a humidified chamber with 5% CO_2 at 37°C for 6 h, followed by the change of serum-containing medium in 48 h continuous incubation for further experiments.

Real-time PCR for SLP-2 and CCBE1 mRNA Expressions

Real-time PCR was used to test the mRNA expressions of SLP-2 and CCBE1 gene in lymphatic tube tissues. Trizol reagent was used to extract RNA from all groups of Neuro-2a cells. Reverse transcription was performed according to the manual instruction, using primers designed by Primer6.0 and synthesized by Invitrogen (China) as shown in Table I. Real-time PCR was performed on target genes under the following conditions: 35 cycles each containing 92°C for 30 sec, 58°C for 45 sec, and 72°C for 35 sec (Thermo Fisher Scientific, Waltham, MA, USA). Data were collected and calculated for CT values of all samples and standards based on fluorescent quantification using GAPDH as the internal reference. A standard curve was firstly plotted, followed by semi-quantitative analysis by $2^{-\Delta Ct}$ method.

MTT Assay for Cell Proliferation in All Groups

LECs cells at log-phase were digested, counted and seeded into 96-well plate at 3000 cells per well. Cells were then randomly divided into control, siRNA negative control (scramble) group and SLP-2 siRNA group (N=5 each), and were treated as abovementioned. After 48-hour incubation, 20 µl sterile MTT solution (5 g/L) was then added into each well in triplicates. With 4 h continuous culture, the supernatant was completely removed and added with 150 µl DMSO for 10 min vortex until the crystal violet was completely resolved. Absorbance (A) values were measured at 570 nm in the microplate reader (Promega, Madison, WI, USA). The proliferation rate was calculated in each group. Each experiment was repeated in triplicates for statistical analysis using SPSS19.0 software (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA).

Caspase3 Activity Assay

Caspase 3 activity was evaluated using test kit. In brief, cells were digested with trypsin and were centrifuged at 4°C, 600 g for 5 min. The supernatant was discarded, followed by cell lysis and iced incubation for 15 min. The mixture was then centrifuged at 4°C, 20 000 g for 5 min, followed by the addition of 2 mM Ac-DECD-pNA. OD values at 450 nm wavelength were measured to evaluate Caspase 3 activity.

Western Blotting for Measuring SLP-2 and CCBE1 Protein Expression Level

LECs cell proteins were firstly extracted. In brief, RIPA lysis buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 2 µg/ml Aprotinin, 2 µg/ml Leupeptin, 1 mM PMSF, 1.5 mM EDTA and 1 mM NaVandade) was used to lyse cells on ice for 15-30 min, followed by ultrasound rupture (5s×4) and centrifugation (4°C, 10 000 g, 15 min). Supernatants were kept and quantified for protein contents, and were stored at -20°C for further Western blotting. Proteins were then separated using 10% SDS-PAGE gel, and were transferred to PVDF membrane using semi-dry method (150 mA for 1.5 h). The non-specific background was removed by 5% defatted milk powder at room temperature for 2 h, followed by incubation of anti-SLP-2 monoclonal antibody (1:1000 dilution), anti-CCBE1 monoclonal antibody (1:2000) in 4°C overnight. On the next day, the membrane was rinsed in phosphate buffered saline with Tween (PBST) and incubated with goat anti-rabbit secondary antibody (1:2000) for 30 min dark incubation. After PBST washing, ECL reagent was used to develop the membrane for 1 min, followed by exposure under X-ray. Protein imaging analysis system and Quantity One software were used to scan X-ray films for determining band density. Each experiment was

Table I. Primer sequences.

Gene	Forward primer 5′-3′	Reverse primer 5′-3′
GAPDH	ACCAGGTATCTTGGTTG	TAACCATGTCAGCGTGGT
SLP-2	TCTCGACTCCACACAGT	GCCGGGTCATTAGCTATATT
CCBE1	AGGCGACACTCCACAGT	GATTAGTGGTCGCTATATT

Table II. SLP-2 and CCBE1 expression in RC and adjacent lymphatic tubes.

	SLP-2			CCBE1		
	(-)	(+--++)	%	(-)	(+--++)	%
Adjacent	46	6	11.54	30	22	42.30
Tumor	3	49	94.23*	2	50	96.15*

Note: *, p<0.05 compared to adjacent tissues.

repeated for four times for further analysis in SPSS19.0 software (IBM, Armonk, NY, USA).

IHC Staining for SLP-2 and CCBE1 Expressions in Lymphatic Tubes from RC Tumor and Adjacent Tissues

Lymphatic tubes from tumor and adjacent tissues were prepared for tissue sections. Paraffin slices were used in IHC staining according to the instruction of SP test kit. 10% normal goat serum was used to block endogenous peroxidase, with antibodies of SLP-2 (1:1000) and CCBE1 (1:500) for 37°C incubation for 1 h. After PBS rinsing (3 times), biotin-labeled goat anti-mouse IgG was added for 20 min room temperature incubation. SABC complex was then added for 20 min incubation. After PBS washing, DAB-H_2O_2 was added, followed by hematoxylin counter-staining, ethanol dehydration, and mounting. Slices were observed under the microscope (Olympus, CX41, Shinjuku, Tokyo, Japan). Positive expression of target genes was judged by the number of yellow-brown granules: negative (-): no staining cells; positive (+): <50% positive cells; and strong positive (++): >=50% positive cells.

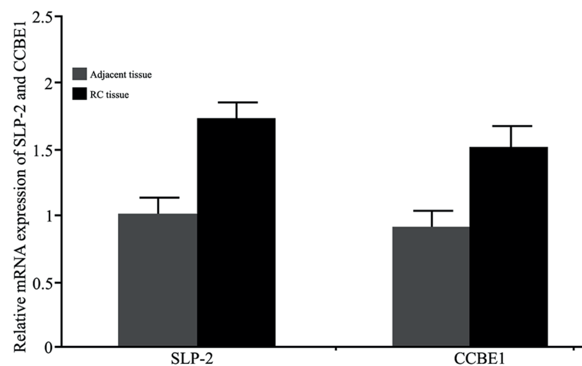

Figure 1. mRNA expression of SLP-2 and CCBE1 in lymphatic tubes of RC and adjacent tissues. *, $p<0.05$ compared to adjacent tissues.

Statistical Analysis

SPSS19.0 software was used to analyze data, in which measurement data were presented as mean±standard deviation (SD) and were compared by LSD test between groups. Enumeration data were presented by percentage (%) and were compared in chi-square manner. Spearman approach was used in correlation analysis. Statistical significance was identified when $p<0.05$.

Results

SLP-2 and CCBE1 mRNA Expression in Lymphatic Tubes of RC and Adjacent Tissues

Real-time PCR was used to test expressions of SLP-2 and CCBE1 mRNA in RC and adjacent lymphatic tubes tissues. Results showed the level of SLP-2 mRNA in RC lymphatic tubes was significantly elevated compared to that in adjacent tissues ($p<0.05$). CCBE1 mRNA expression was also significantly upregulated in RC tissues compared to that in adjacent tissues ($p<0.05$) (Figure I).

IHC staining for SLP-2 and CCBE1 Expression

To further determine the expression of SLP-2 and CCBE1 proteins in RC and adjacent lymphatic tubes, IHC staining was used. The result showed that the levels of SLP-2 and CCBE1 proteins in RC lymphatic tubes were remarkably increased, presenting as positive (+) or strongly positive (++) expressions, whereas those proteins in adjacent tissues were detected as negative (-) expression (Figure 2 and Table II).

Correlation Analysis of SLP-2 and CCBE1

We further analyzed the correlation of mRNA or protein levels between of SLP-2 and CCBE1. Results revealed the positive correlation between SLP-2 and CCBE1 expressions at both mRNA and protein level ($p<0.05$, Table III).

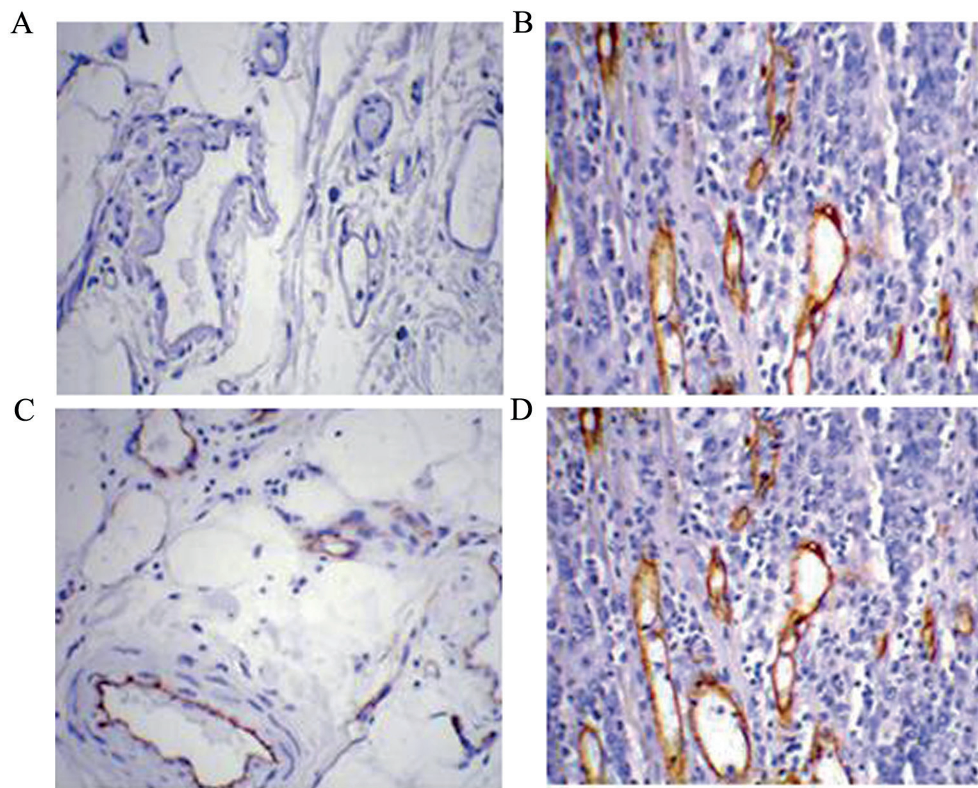

Figure 2. SLP-2 and CCBE1 expression in RC and adjacent lymphatic tubes (×400). ***A,*** SLP-2 expression in adjacent tissues; ***B,*** SLP-2 expression in RC tissues; ***C,*** CCBE1 expression in adjacent tissues; ***D,*** CCBE1 expression in RC tissues.

Effect of siRNA on SLP-2 mRNA and Protein level in LECs

We established an *in vitro* model of SLP-2 down-regulation by transfecting with siRNA to illustrate the effect of SLP-2 on LECs. Real-time PCR data showed SLP-2 mRNA expression was significantly reduced after the transfection with SLP-2 siRNA, compared with that in control group ($p<0.05$, Figure 3). Western blotting data further indicated that the level of SLP-2 protein also significantly decreased compared to that in control group, suggesting that SLP-2 siRNA effectively inhibited the expression of SLP-2 (Figure 4, Figure 5).

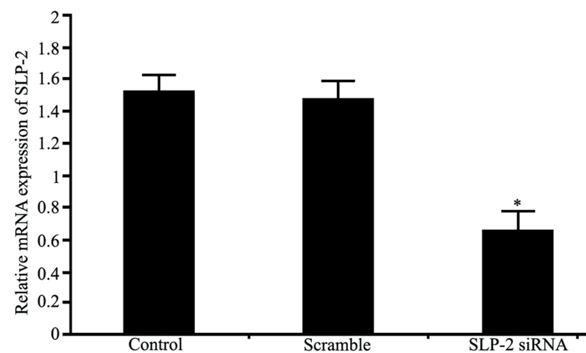

Figure 3. The effect of siRNA on SLP-2 mRNA expression of LECs. *, $p<0.05$ compared to control group.

Table III. Correlation analysis between SLP-2 and CCBE1.

	Adjacent tissue		RC tissue	
Factor	**r**	***p***	**r**	***p***
SLP-2 mRNA CCBE1 mRNA	0.874	0.032	0.751	0.041
SLP-2 protein CCBE1 protein	0.892	0.003	0.718	0.045

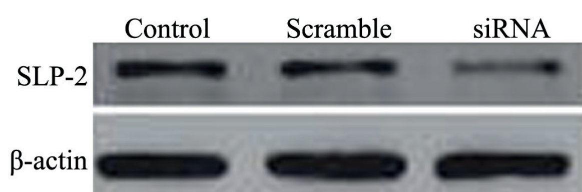

Figure 4. The effect of siRNA on SLP-2 protein level in LECs.

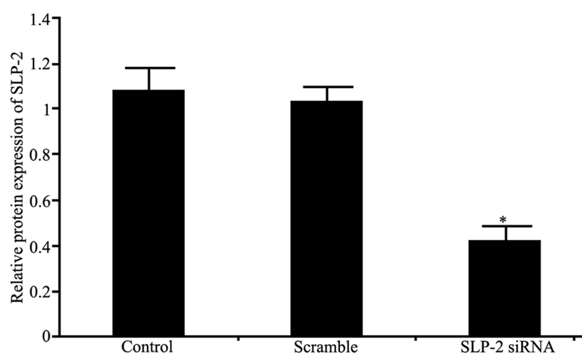

Figure 5. The effect of siRNA on SLP-2 protein level in LECs. *, $p<0.05$ compared to control group.

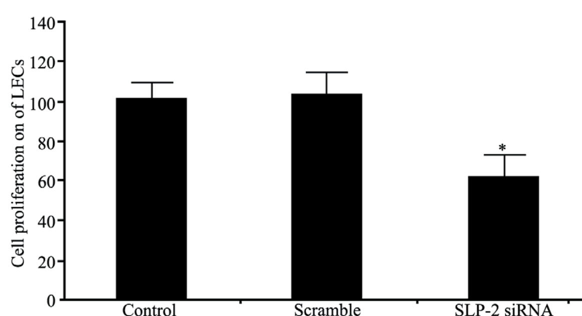

Figure 6. Effect of SLP-2 on LECs cell proliferation. *, $p<0.05$ compared to control group.

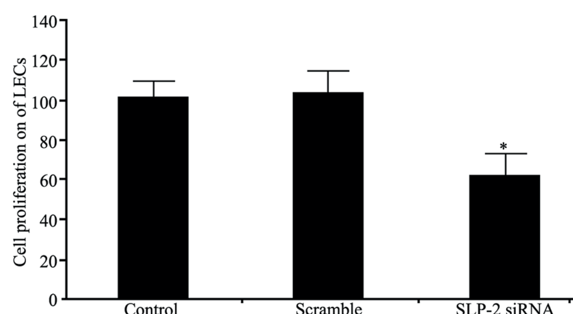

Figure 7. Effect of SLP-2 on Caspase3 activity of LECs. *, $p<0.05$ compared to control group.

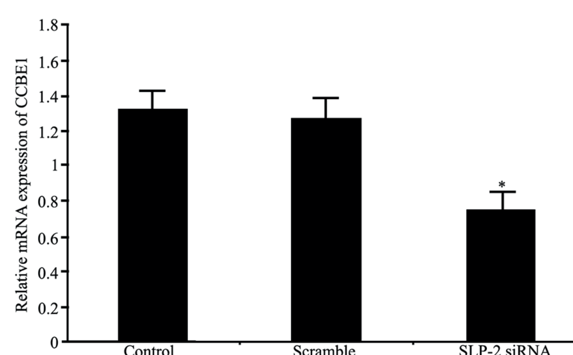

Figure 8. Regulatory effect of SLP-2 on CCBE1 mRNA in lymphatic tissues. *, $p<0.05$ compared to control group.

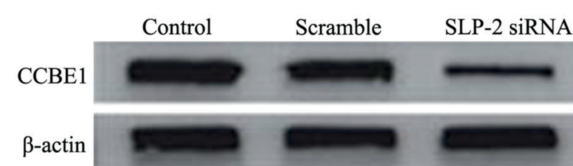

Figure 9. Regulatory effect of SLP-2 on CCBE1 protein in lymphatic tissues.

Effect of SLP-2 on LECs Cell Proliferation

MTT assay was used to test the effect of SLP-2 on the proliferation of LECs. Results showed that after the expression of SLP-2 was inhibited, LECs proliferation was significantly decreased compared to that in control group ($p<0.05$, Figure 6).

Effect of SLP-2 on Caspase3 Activity of LECs

Then, we detected and compared the Caspase3 activity of LECs with or without the treatment of SLP-2 siRNA. Results showed that the downregulation of SLP-2 significantly elevated Caspase3 activity of LECs compared to control group ($p<0.05$, Figure 7).

Regulatory Effect of SLP-2 on Level of CCBE1 mRNA and Protein in LECs

The level of CCBE1 mRNA and protein in LECs was also measured after the expression of SLP-2 was reduced. Both real-time PCR and western blotting results showed the expression of CCBE1 mRNA or protein was apparently suppressed, respectively, compared to that of control group ($p<0.05$, Figures 8, 9, and 10).

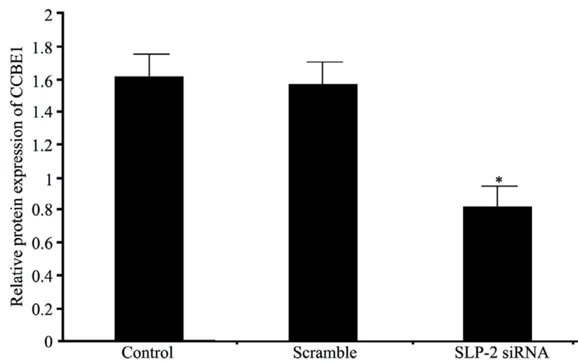

Figure 10. Analysis of regulatory effect of SLP-2 on CCBE1 protein in lymphatic tissues. *, $p<0.05$ compared to control group.

Discussion

SLP-2 protein acts as the anchor point of cytoskeleton. It binds with membrane factors for stabilizing mitochondrial structure and regulating ATP synthesis in in mitochondria[13,14], and plays an essential role in the modulation of cellular signal transduction pathway, synthesis, stabilization and metabolism of mitochondria in plants, nematode and fruit fly[13-15]. SLP-2 exists in membrane of lymphatic tube and vascular endothelial cells, where it forms the pit-like structure for genesis and development of mitochondria and endoplasmic reticulum[16]. Among various malignant tumors, SLP-2 is aberrantly high-expressed, which draws extensive interest as a novel cancer-related gene[17]. The incidence and mortality of RC remain high, mainly due to its metastasis of lymphatic tubes, which severely impedes the successful treatment[18]. Of note, our study demonstrated that SLP-2 mRNA/protein expression was significantly elevated in lymphatic tubes of RC tissues, as consistent with the phase, recurrence, and metastasis of RC[19].

Further study regarding the regulatory mechanism of SLP-2 on RC was investigated. Lymphatic tube genesis related factor CCBE1 could regulate the lymphatic tube genesis factor VEGF-C, and the VEGF-C/VEGFR3 signal pathway is closely correlated with embryonic development, tissue regeneration and genesis of tumor lymphatic tubes[20, 21]. Lymphatic tube plays an important role in maintaining body homeostasis, transporting of large molecules, stabilizing immune function, and absorption of intestinal lipids, and also participates in tumor metastasis such as RC[22,23]. This study detected the expression of CCBE1 in lymphatic tube of RC and adjacent tissues. Results showed significantly higher level of CCBE1 in RC tissues, indicating the critical role of CCBE1 in the genesis of lymphatic tube of RC. Further analysis revealed positive correlation between SLP-2 and CCBE1 in RC tissues, which was consistent with the previous study[24]. We then established *in vitro* cultured lymphatic endothelial cells line LEC, on which siRNA was used to interfere SLP-2 expression. Our data unraveled that the downregulation of SLP-2 significantly suppressed CCBE1 expression in LECs, further inhibited cell proliferation, potentiates Caspase3 activity and induced endothelial cell apoptosis. These results collectively suggested that SLP-2 in RC participates in the genesis of lymphatic tubes via regulation CCBE1 expression.

Conclusions

SLP-2 is up-regulated in lymphatic tube tissues of RC and is positively correlated with CCBE1 expression. The decrease of SLP-2 suppresses the CCBE1 expression, further participating in the genesis of lymphatic tubes. This report provides evidence for the lymphatic tube metastasis of RC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Guedj N, Maggiori L, Pote N, Norkowski E, Cros J, Bedossa P, Panis Y. Distal intramural and tumor spread in the mesorectum after neoadjuvant radiochemotherapy in rectal cancer: about 124 consecutive patients. Hum Pathol 2016; 52: 164-172.
2) Pedziwiatr M, Pisarska M, Kisielewski M, Major P, Mydlowska A, Rubinkiewicz M, Winiarski M, Budzynski A. ERAS protocol in laparoscopic surgery for colonic versus rectal carcinoma: are there differences in short-term outcomes? Med Oncol 2016; 33: 56.
3) Guenaga KF, Otoch JP, Artifon EL. Local staging of rectal carcinoma: endorectal ultrasound versus magnetic resonance imaging. Systematic review of literature and meta-analysis. Rev Gastroenterol Peru 2016; 36: 43-48.
4) Hernandez J, Molins L, Fibla JJ, Heras F, Embun R, Rivas JJ. Role of major resection in pulmonary metastasectomy for colorectal cancer in the Spanish prospective multicenter study (GECMP-CCR). Ann Oncol 2016; 27: 850-855.
5) Simkens GA, van Oudheusden TR, Braam HJ, Wiezer MJ, Nienhuijs SW, Rutten HJ, van Ramshorst B, de Hingh

IH. Cytoreductive surgery and HIPEC offers similar outcomes in patients with rectal peritoneal metastases compared to colon cancer patients: a matched case control study. J Surg Oncol 2016; 113: 548-553.

6) Kong I, Vorunganti S, Patel M, Farrell T, Timotin E, Quinlan-Davidson S, Pond G, Sur R, Hunter R. Prospective comparison of rectal dose reduction during intracavitary brachytherapy for cervical cancer using three rectal retraction techniques. Brachytherapy 2016; 15: 450-455.

7) Kucuk I, Tanoglu A, Oncu K, Yilmaz I, Kara M, Beyazit Y, Akyol T, Kaplan M, Ozari HO, Yazgan Y. Immunohistochemical activity of Prohibitin-2 and Stomatin-Like Protein-2 in patients with ulcerative colitis. Turk J Gastroenterol 2016; 27: 233-238.

8) Mitsopoulos P, Chang YH, Wai T, Konig T, Dunn SD, Langer T, Madrenas J. Stomatin-like protein 2 is required for in vivo mitochondrial respiratory chain supercomplex formation and optimal cell function. Mol Cell Biol 2015; 35: 1838-1847.

9) Sun F, Ding W, He JH, Wang XJ, Ma ZB, Li YF. Stomatin-like protein 2 is overexpressed in epithelial ovarian cancer and predicts poor patient survival. BMC Cancer 2015; 15: 746.

10) Xiao B, Xie Z, Guo L, Wu J, Zhang H. Stomatin-like protein 2 expression is associated with clinical survival in patients with cervical cancer. Int J Clin Exp Pathol 2015; 8: 1804-1809.

11) Yang X, Zang W, Xuan X, Wang Z, Liu Z, Wang J, Cui J, Zhao G. miRNA-1207-5p is associated with cancer progression by targeting stomatin-like protein 2 in esophageal carcinoma. Int J Oncol 2015; 46: 2163-2171.

12) Jackson CC, Best L, Lorenzo L, Casanova JL, Wacker J, Bertz S, Agaimy A, Harrer T. A multiplex kindred with hennekam syndrome due to homozygosity for a CCBE1 mutation that does not prevent protein expression. J Clin Immunol 2016; 36: 19-27.

13) Kedracka-Krok S, Swiderska B, Jankowska U, Skupien-Rabian B, Solich J, Buczak K, Dziedzicka-Wasylewska M. Clozapine influences cytoskeleton structure and calcium homeostasis in rat cerebral cortex and has a different proteomic profile than risperidone. J Neurochem 2015; 132: 657-676.

14) Ghose P, Park EC, Tabakin A, Salazar-Vasquez N, Rongo C. Anoxia-reoxygenation regulates mitochondrial dynamics through the hypoxia response pathway, SKN-1/Nrf, and stomatin-like protein STL-1/SLP-2. PLoS Genet 2013; 9: e1004063.

15) Liu Z, Yang Y, Zhang Y, Ye X, Wang L, Xu G. Stomatin-like protein 2 is associated with the clinicopathological features of human papillary thyroid cancer and is regulated by TGF-beta in thyroid cancer cells. Oncol Rep 2014; 31: 153-160.

16) Wang WX, Lin QF, Shen D, Liu SP, Mao WD, Ma G, Qi WD. Clinicopathological significance of SLP-2 overexpression in human gallbladder cancer. Tumour Biol 2014; 35: 419-423.

17) Inomata M, Akagi T, Nakajima K, Etoh T, Tahara K, Matsumoto T, Ogawa T, Fujii K, Shiromizu A, Kitano S. A prospective feasibility study to evaluate neoadjuvant-synchronous S-1 with radiotherapy for locally advanced rectal cancer: A multicentre phase II trial. Mol Clin Oncol 2016; 4: 510-514.

18) Cao W, Zhang B, Ding F, Zhang W, Sun B, Liu Z. Expression of SLP-2 was associated with invasion of esophageal squamous cell carcinoma. PLoS One 2013; 8: e63890.

19) Liu D, Zhang L, Shen Z, Tan F, Hu Y, Yu J, Li G. Increased levels of SLP-2 correlate with poor prognosis in gastric cancer. Gastric Cancer 2013; 16: 498-504.

20) Bui HM, Enis D, Robciuc MR, Nurmi HJ, Cohen J, Chen M, Yang Y, Dhillon V, Johnson K, Zhang H, Kirkpatrick R, Traxler E, Anisimov A, Alitalo K, Kahn ML. Proteolytic activation defines distinct lymphangiogenic mechanisms for VEGFC and VEGFD. J Clin Invest 2016; 126: 2167-2180.

21) Roukens MG, Peterson-Maduro J, Padberg Y, Jeltsch M, Leppanen VM, Bos FL, Alitalo K, Schulte-Merker S, Schulte D. Functional dissection of the CCBE1 protein: a crucial requirement for the collagen repeat domain. Circ Res 2015; 116: 1660-1669.

22) Furtado J, Bento M, Correia E, Inacio JM, Belo JA. Expression and function of Ccbe1 in the chick early cardiogenic regions are required for correct heart development. PLoS One 2014; 9: e115481.

23) Jeltsch M, Jha SK, Tvorogov D, Anisimov A, Leppanen VM, Holopainen T, Kivela R, Ortega S, Karpanen T, Alitalo K. CCBE1 enhances lymphangiogenesis via A disintegrin and metalloprotease with thrombospondin motifs-3-mediated vascular endothelial growth factor-C activation. Circulation 2014; 129: 1962-1971.

24) Zhang L, Liu FJ. Expression of SLP-2 gene and CCBE1 are associated with prognosis of rectal cancer. Eur Rev Med Pharmacol Sci 2017; 21: 1214-1218.