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Enhanced SLP-2 promotes invasion and metastasis by regulating Wnt/β-catenin signal pathway in colorectal cancer and predicts poor prognosis

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ABSTRACT

Stomatin-like protein-2 (SLP-2) gene belongs to the stomatin supergene family, and previous studies have revealed up-regulated SLP-2 expression in gallbladder cancer, lung cancer, and esophageal cancer, while the role of SLP-2 in colorectal cancer (CRC) remains unclear and needs further investigation. Therefore, the expression levels of SLP-2 in CRC tissue and cell lines were tested in this study. Besides, we further explored the role of SLP-2 in CRC invasion and metastasis at molecular level via gene intervention technique. Our results demonstrated that the positive rate of SLP-2 expression in CRC tissues was higher than that in the adjacent non-cancerous tissues ($P < 0.05$); positive SLP-2 expression predicted poorer prognosis of CRC patients as an independent risk factor ($P < 0.05$). Cell activities and the capacity of migration and invasion significantly decreased after the suppression of SLP-2 in SW620 cells ($P < 0.05$). Furthermore, the suppression of SLP-2 in SW620 cells resulted in varieties of invasion and metastasis-related genes and Wnt/β-catenin signal pathway ($P < 0.05$). The present study identified that SLP-2 may predict a poor prognosis in CRC patients as a novel marker, and SLP-2 may facilitate the migration and invasion of CRC via regulating Wnt/β-catenin pathway activities.

1. Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality and morbidity worldwide [1]. As CRC is characterized by rapid metastasis, the majority of patients has progressed to the advanced stage of CRC at diagnosis [2,3]. Although there are comprehensive treatments including surgery, radiotherapy, chemotherapy and targeted therapy, the overall outcome for CRC is not satisfactory [4–7]. Despite having known that many genes are involved in the process of CRC invasion and metastasis, the key genes have not been identified [8]. Therefore, identifying a novel gene with clinical significance is necessary, as it may assist in analyzing CRC process and confirming novel tumor markers and molecular targets.

Stomatin-like protein-2 (SLP-2), one member of the stomatin superfamily, has been reported to show enhanced expression in gallbladder cancer, lung cancer, esophageal cancer, gastric cancer and cervical cancer and participate in cancer progress [9–13]. The high expression of SLP-2 was also revealed in CRC tissues by previous studies implicating that SLP-2 may promote CRC cancer cell proliferation and inhibit cancer cell apoptosis by regulating collagen and calcium binding EGF domains 1 (CCBE1) [14]. However, the role of SLP-2 in CRC invasion and metastasis has rarely been reported. Thus, in the present study, we examined SLP-2 expression in 95 CRC tissue samples and further analyzed the prognostic value, also the molecular mechanism of SLP-2 was investigated via experiments in vitro. In the following study, we inhibited the SLP-2 expression specifically in SW620 cells via RNA intervention technology and subsequently examined the cell behaviors and the variations of SLP-2 related genes and the Wnt/β-catenin signaling pathway. In this way, we explored the role of SLP-2 in CRC invasion and metastasis and its clinical significance for prognosis.

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2. Materials and methods

2.1. Patients' data

95 patients aged between 38 and 78 years with confirmed CRC who underwent surgical removal of the primary tumors between January 2012 and December 2012 at the Fourth Hospital of Hebei Medical University were recruited. The mean age was (55.66 ± 8.64) years with females and males constituting 30 and 65 cases respectively; 16 patients with cancers in ascending colon, 13 in transverse colon, 13 in descending colon; 24 in sigmoid, 29 in rectum; 58 patients with well differentiated cancers, 37 with poorly differentiated; 28 patients in I-II TNM stages, 67 patients in III-IV TNM stages. All participants were diagnosed with CRC for the first time without any other cancers and solely received surgical removal of the cancer. Prior to undergoing operative resection, the participants had not received any chemotherapy, radiology and/or targeted drug treatments. All of them have complete medical records along with follow-up data collected until 31st of December 2017. From each of the CRC patient, a pair of paraffin-embedded tumor tissues and adjacent non-cancerous tissue samples were collected for SLP-2 expression using immunohistochemistry (IHC).

Additional 16 CRC patients having undergone operative resection between March and October 2017 were also recruited in this study. Likewise, SLP-2 expression was determined in 16 pairs of fresh tumor tissues and matched adjacent non-cancerous tissue specimens (more than 5 cm from the tumor edge with no carcinoma tissue and atypical hyperplasia detected under microscope) via real-time fluorescent quantitative PCR (qPCR) and Western blot. These results were then used to confirm the outcome from IHC. This research was approved by Medical Ethics Committee of the Fourth Hospital of Hebei Medical University, and the consents were obtained from all the participants.

2.2. Cell lines and reagents

Human CRC cell lines HT29, SW480, SW620, LOVO, Caco-2,HCT116 and normal colon epithelial cells NCM460 were purchased from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. All cell lines were preserved and cultured at Research Center of the Fourth Hospital of Hebei Medical University. The reagents were obtained from commercial sources as below: TurboFect Transfection Reagents from (Thermo, USA). Dulbecco's Modification of Eagle's Medium (DMEM) culture solution and fetal bovine serum(FBS) supplied by Gibco, USA; Cell counting KIT-8 provided by DOJINDO, Japan; Trizol, real-time CPA kit and Protein Extraction Kit purchased from Sigma, USA. qPCR primers were synthesized at Sangon Biotech, China; Jikai Gene, China supplied SLP-2-siRNA and chronic viral vector pLVX-EF1α- mCherry-N1; SLP-2, Matrix metalloproteinases-2 (MMP-2), MMP-9, MMP-7, Tissue inhibitor of metalloproteinases-1 (TIMP-1), TIMP-2, Intercellular cell adhesion molecule-1(ICAM-1), Proliferating cell nuclear antigen (PCNA), and GSK3β, p-GSK3β, β-catenin, Survivin, cyclin D1, c-myc, CD44, and internal reference β-actin antibody were obtained from Sigma, USA.

2.3. IHC analysis for SLP-2 protein detection

The paraffin embedded tissue slices were dewaxed and tested by IHC strictly following the protocols. All the slices, in which 5 fields of 400 × with 100 cells each were randomly calculated, and the SLP-2 expression was evaluated by 2 independent pathologists, In the present study, the SLP-2 protein was marked as positive by yellow or brown particles observed in cytoplasm/ cytomembrane. The positive rates were determined by a combination of two scoring methods. One scored samples according to the staining gradient or intensity on a 0–3 scale: 0, no stain; 1, pale yellow stain; 2, brown yellow strain; 3, brown strain. The other one in which scores ranging from 0 to 5 based on the percentage of cell stained positively: 0 as no cells showing positive staining; 1 as ≤10% of cells showing positive staining; 2 as cells with 11%–50% positive strained; 3 as cells showing 51%–75% of positive staining; 4 as > 75% of cells showing positive staining. We calculated the overall scores by adding up the two sub scores and defined results as negative if the overall score is no more than 2, or as positive otherwise.

2.4. qPCR examination

The total cellular RNA was extracted from cells using Trizol one-step approach and the integrity of RNA were then evaluated. 1 μg of total RNA was reverse-transcribed and examined by real-time PCR. A 20 μl PCR reaction system was established using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as internal reference, in which, 1 μl cDNA, 10 μl 2 × UltraSYBR Mixture, 1 μl forward/downward primers (10 μmol/l) and 8 μl non-DNase-RNase water were included. The cycling parameters of PCR were as the following: an initial pre-denaturation at 95 °C for 5 min. followed by a 30-second denaturation at 95 °C and then at 60 °C for 30 s and finally a 30-second extension at 72 °C, which was repeated 35 cycles and fluorescent signals were collected at each extension stage. Eventually, RQ of targeted genes (qPCR) were determined with GAPDH as internal reference and the data were further analyzed by statistics software. (see Table 1 for primer sequences)

Table 1
Primer sequences of target genes for qPCR.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
SLP-2	GATGGAGTCCTTTACCTGCG	CCAGAGAGAGTTTGCCGAG
MMP-2	CCAACTACAACTTCTTCCCTCG	TCACATCGCTCCAGACTTG
MMP-9	ACGCAGACATCGTCATCCA	AGGGACCACAACTCGTCATC
MMP-7	CAGGCTCAGGACTATCTCAAGAG	TTGGCTTCTAAACTGTTGGC
TIMP-1	TTCTGGCATCCTGTTGTTG	GTGGTCTGGTTGACTTCTGG
TIMP-2	CGACATTTATGGCAACCCT	ATTCCTTCTTTCCTCCAACG
ICAM-1	GTCATCATCACTGTGGTAGCAG	GGCTTGTGTGTTCGGTTTC
PCNA	GTGGAGAACTTGGAAATGGAAAC	TTGAAGAGAGTGGAGTGGCT
GSK3β	GACGCTCCCTGTGATTTATG	TCCAACAAGAGGTTCTGCG
β-catenin	TTACACCCACCATCCCACTG	GCACGAACAAGCAACTGAACTA
Survivin	TTTCTCAAGGACCACCGCA	AGTCTGGCTCGTTCTCAGTG
CyclinD1	CTCGGTGTCCTACTTCAAATGT	TCCTCGCACTTCTGTTCCT
c-Myc	CCACGAAACTTTGCCCATAG	CCTTGCTCGGGTGTTGTAAG
CD44	ATTCCCCGGATCCACCCCAACT	GCCGCTGCTCACGTCATCATCA
β-actin	GGTCATCACCATTGGCAA	GAGTTGAAGGTAGTTTCGTGGA

2.5. Western blot assay

Cell lysate was prepared by 10-min centrifugation at 8000 rpm at 4 °C and each protein concentration was determined using a bicinchoninic acid (BCA) assay. Following this, even quantity of proteins was isolated by 10% polyacrylamide gel electrophoresis and transferred to Polyvinylidene fluoride (PVDF) membrane, where the proteins were blocked in 5% skim milk powder for 2 h under room temperature. Subsequently, the diluted primary antibodies were utilized for incubation at 4°C overnight. The proteins were further incubated for 2 h under room temperature after rinsing with TBST for 3 times and added with secondary antibodies. Consequently, the specimens were washed with TBST for triple times and with TBS for one time. After that, chemiluminescent detection method was employed to detect the light and the image was obtained. Each protein level therefore was evaluated through densitometry and defined by the quantification of absorbance (OD value).

2.6. Cell culture

Cells were cultivated in an incubator at 37°C, 5% CO_2 in the DMEM culture medium comprised of 10% of fetal calf serum (FCS), 100 U/ml

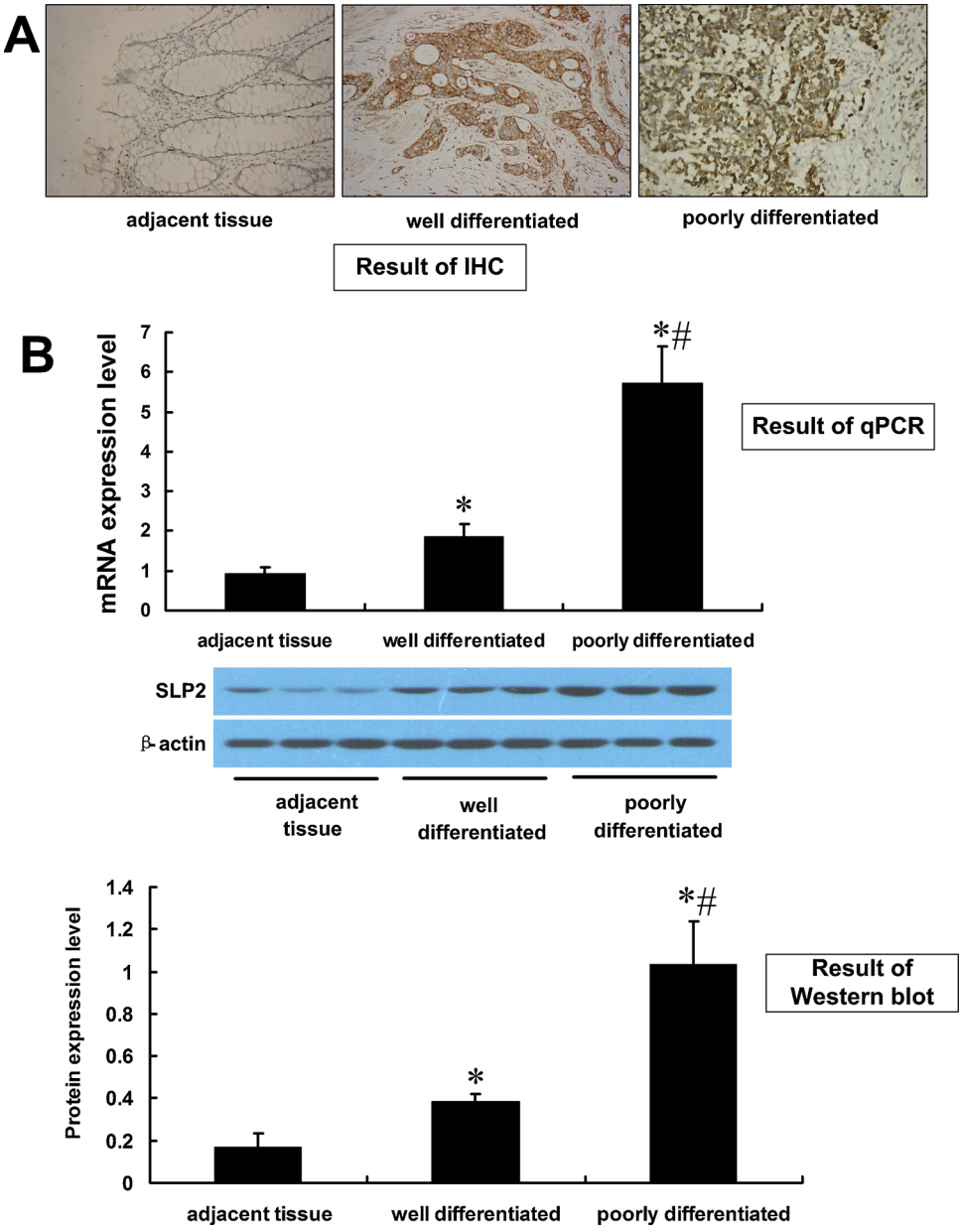


Fig. 1. Expression of SLP-2 mRNA and protein in CRC tissues and adjacent non-cancerous tissues.
Paraffin samples of 95 CRC and adjacent non-cancerous tissues (adjacent tissue)were collected and subjected to IHC to detect expression of SLP-2 protein, and the results were showed as Fig.1A. The Fresh samples from another 16 patients were selected to detect expression of SLP-2 mRNA and protein with qPCR and Western blot, and the results was shown as Fig.1 B.
*$P <$ 0.01 versus adjacent tissue; # $P <$ 0.01 versus high differentiated tissue

of penicillin and 100 mg/ml of streptomycin. Also, 0.25% of trysin(including 0.02% EDTA)was used for cell passage. Finally, cells with logarithmic growth were selected for the present study.

Sequences of SLP-2-siRNA were synthesized as following: (SLP-2-siRNA-1) 5'- GCAGAGUCUCAAGGAAAUUtt-3', (SLP-2-siRNA-2) 5'-CGACAAUGUAACUCUGCAA tt-3', (SLP-2-siRNA-3) 5'- GGCCAAGGCU AAAGCUGAA tt-3'; while the non-specific control siRNA(NS-siRNA) as 5'-GGUCUCACUCCCCAUAGAGtt-3'. Then, the each targeted fragment were inserted into pLVX-EF1α-mCherry-N1, and grouped into blank control, negative control and LV-SLP-2-siRNA group. 24 h before transfection, SW620 cells were seeded in 6-well plates with at density of 1×10^6/ml and rinsed with DMEM containing no antibiotics and FBS. 24 h post transfection, the SLP-2 expression and transfection rate were investigated.

2.7. CCK-8 assay

After transfection, cells from each group were located in 6-well plates and cultivated at an incubator for 24, 48 and 72 h, and then the medium was discarded and added with another 100 μl of medium containing CCK-8 (CCK-8 : medium = 1:9). The cells were further cultivated for 2 h. Optical density (OD) was measured at 450 nm in a microplate reader. The whole procedure was repeated for 3 times.

2.8. Wound assay

Wound assay was employed to examine the migration capacity of cells and the procedure was repeated for three times, in which SW620 was processed into single-cell suspension (1×10^6 cells/ml) after digestion and seeded in inoculated to 6-well plates. SLP-2-siRNA or NS-siRNA were transfected where cells grew at 60%–70%. The medium was then discarded at the point where cells grew to 100%. Following that, PBS was used to rinse the cells and the specimens were scratched using the sterile tips. After being rinsed with PBS, the wound healing of scratched cells was observed under optical microscope.

2.9. Transwell assay

The Transwell upper chambers were coated with 100 μl Matrigel and then exposed to ultraviolet rays. The prepared SW620 cells were seeded in 6-well plates at a density of 1×10^6 cells /ml. The transfection then was performed when the cells grew up to 60%–70%. Following a 24- hour incubation, 200 μl of specimen from for each cell group was inoculated into the upper chambers with DMEM being added to the lower champers. The Matrigel and cells in the upper chambers were removed after another 24 h of incubation and the polycarbonate membrane was fixed with methanol for 10 min. After crystal violet staining, the cells with membrane penetration were calculated. This experiment was repeated for three times.

2.10. Statistical analysis

Kaplan-Meier (*K-M*) analysis and *COX* regression were performed using statistical product and service solutions (SPSS 21.0) to analyze the clinic value of SLP-2 to predict the prognosis of patients with CRC. *t* test and ANOVA were utilized to analyze the differences between each group. The differences between Enumeration data including percentage and ratio were analyzed by X^2. A P value < 0.05 was considered as statistical significance. All statistical analysis was performed utilizing SPSS21.0.

Table 2
Relationship between SLP-2 protein and clinicopathological parameters of patients with CRC ($n = 95$).

	n	SLP-2 protein		X^2 value	*P* value
		Negative(24)	Positive(71)		
Gender					
Male	65	18	47	0.643	0.423
Female	30	6	24		
Age					
≥60	29	8	21	0.119	0.730
< 60	66	16	50		
Location					
Ascending colon	16	5	11	1.222	0.875
Transverse colon	13	3	10		
Descending colon	13	2	11		
Sigmoid	24	7	17		
Rectum	29	7	22		
Invasion depth					
Serosa invasion(-)	27	4	23	2.181	0.140
Serosa invasion (+)	68	20	48		
Differentiation					
Well	58	15	43	0.028	0.866
Poor	37	9	28		
Lymphatic metastasis					
Positive	64	11	53	6.774	0.009
Negative	31	13	18		
Vascular invasion					
Positive	34	11	23	1.410	0.235
Negative	61	13	48		
Nerve invasion					
Positive	28	7	21	0.001	0.970
Negative	67	17	50		
Distant metastasis					
M1	11	2	9	0.330	0.565
M0	84	22	62		
TNM staging					
I-II	28	13	15	9.420	0.002
III-IV	67	11	56		

3. Results

3.1. Expression of SLP-2 mRNA and protein in CRC tissues

The results of IHC showed in Fig. 1A demonstrated a significantly higher percentage of SLP-2 positive rate at 74.74% (71/95) in CRC tissue specimens than that in adjacent non-cancerous tissue specimens at 25.26% (24/95)($X^2 = 58.855$, $P < 0.001$). The results were also confirmed in both qPCR and Western blot (shown in Fig. 1B) from fresh tissues, which verified that expressions of SLP-2 mRNA and protein in cancer tissues were higher than those in adjacent non-cancerous tissues (both $P < 0.001$).

3.2. Relation between SLP-2 expression in CRC tissue specimens and the medical data of the patients

As shown in Table 2, Chi-square analysis detected a higher expression of SLP-2 in CRC tissue specimens with lymphatic metastasis and at advanced stage than that without lymphatic metastasis and at early stage ($X^2 = 6.774$, $P = 0.009$; $X^2 = 9.420$, $P = 0.002$). No other parameters (gender, age, tumor location, invasion depth, differentiation, vascular invasion, nerve invasion, distant metastasis) were found to be associated with these results (all $P > 0.05$).

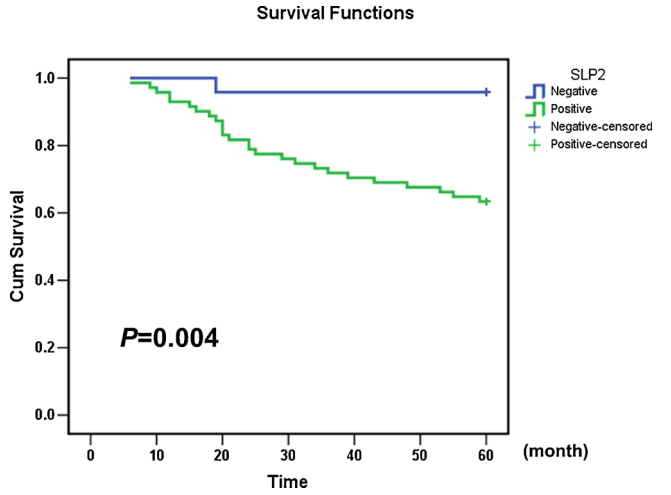


Fig. 2. Relationship between expression of SLP-2 protein and prognosis for CRC patients.
The data about expression of SLP-2 protein and prognosis of 95 CRC patients were collected, and Kaplan-Meier analysis was applied. The significance of SLP-2 for prognosis prediction was demonstrated in Fig.2.

3.3. The relation between SLP-2 expression and patient's prognosis

Median survival time of all CRC patients was (50.495 ± 1.738) months, which in patients with negative SLP-2 was (58.292 ± 1.672) months, and it was (47.859 ± 2.169) months for patients with positive SLP-2. A Survival Curve shown in Fig. 2 was produced based on the *K-M* analysis for the relation between SLP-2 expression and patients' prognosis. We found that the patients with a positive expression of SLP-2 had lower survival rates than those with a SLP-2 negative expression ($X^2 = 8.281$, $P = 0.004$). We also determined that the positive expression of SLP-2 is an independent risk factor in terms of CRC patient prognosis ($X^2 = 4.884$, $P = 0.027$), which were seen in Table.3.

3.4. The expression of SLP-2 in cell lines

Both qPCR and Western blot results revealed that the mRNA and protein expression of SLP-2 performed differently in each cell line, and

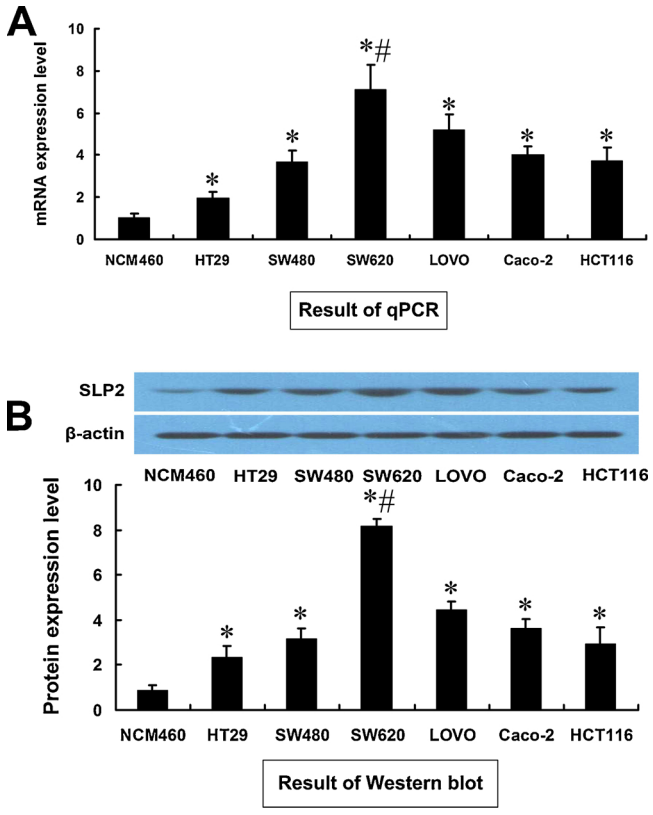


Fig. 3. Expression of SLP-2 mRNA and protein in colorectal cell lines.
Human CRC cell lines HT29, SW480, SW620, LOVO, Caco-2, HCT116 and normal colon epithelial cells NCM460 were cultured, and SLP-2 mRNA and protein were detected with qPCR and Western blot. The results of SLP-2 mRNA were showed as Fig.3A, and SLP-2 protein were showed as Fig.3B.
*$P <$ 0.01 versus NCM460 cells; # $P < 0.01$ versus HT29, SW480, LOVO, Caco-2, HCT116

it was confirmed that all CRC cell lines with higher SLP-2 expression than in NCM460 cell line ($P < 0.01$). Also it was confirmed that SW620 cell line with the highest expression in CRC cell lines ($P < 0.01$). Thus, we selected SW620 cells as targeted cell for following study (shown in Fig. 3).

Table 3
Independent risk factors patients for prognosis of patients with CRC.

	B	*SE*	*Wald*	*df*	*Sig.*	*Exp(B)*	*95.0% CI for Exp(B)*	
							Lower	Upper
Gender	0.182	0.435	0.175	1	0.675	1.200	0.512	2.813
Lymphatic metastasis	9.231	102.533	0.008	1	0.928	10205.106	0.000	1.929E+091
T	0.121	0.482	0.063	1	0.802	1.128	0.439	2.902
TNM	−8.529	102.535	0.007	1	0.934	0.000	0.000	3.750E+083
SLP-2	2.282	1.032	4.884	1	0.027	9.792	1.295	74.072
Age	0.078	0.448	0.030	1	0.862	1.081	0.449	2.603
Differentiation	−0.495	0.482	1.056	1	0.304	0.609	0.237	1.567
Vascular invasion	0.139	0.492	0.079	1	0.778	1.149	0.438	3.015
M	0.011	0.671	0.000	1	0.987	1.011	0.272	3.766
Location	0.267	0.176	2.301	1	0.129	1.305	0.925	1.842
Nerve invasion	−0.622	0.536	1.347	1	0.246	0.537	0.188	1.536

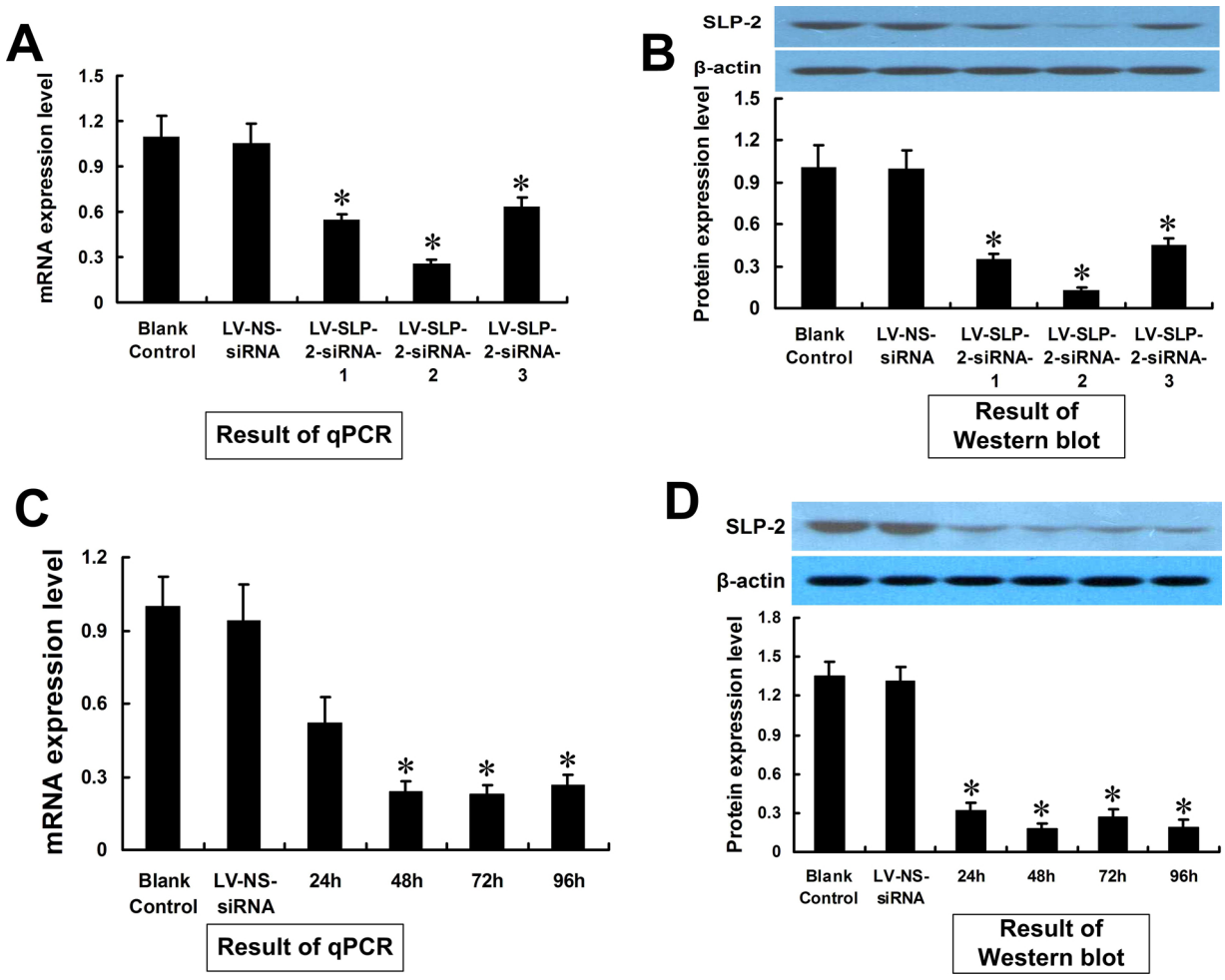


Fig. 4. Effect of LV-SLP-2-siRNA transfection on CRC cell line SW620.
SW620 cells were transfected with LV-SLP-2-siRNA or diffrenet LV-NS-siRNAs, and the effects of diffrenet LV-NS-siRNAs to expression of SLP-2 mRNA and protein was tested with qPCR(Fig.4A) and Western blot (Fig.4B), which revealed that LV-SLP-2-siRNA-2 was most effective for SLP-2 inhibition. Result of Fig.4C and 4D demonstrated that LV-SLP-2-siRNA (LV-SLP-2-siRNA-2) could inhibit SLP-2 with time-dependent manner. The results showed that LV-SLP-2-siRNA could inhibit SLP-2 expression significantly in SW620 cells.
*$P < 0.01$ versus LV-NS-siRNA group, Blank control group

3.5. The transfection of SLP-2-siRNA suppressed the SLP-2 expression in SW620 cells

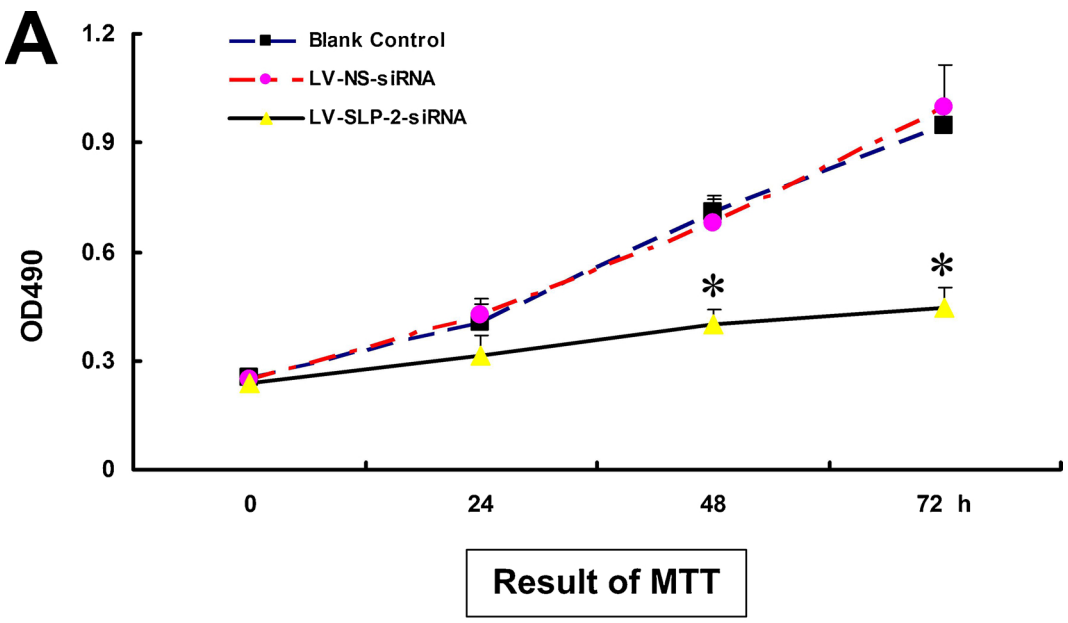
The Fig. 4 based on qPCR and Western-blot analysis shows that the expression of SLP-2 in SW620 was notably lower than the control and blank control 48 h after transfection ($P < 0.01$), which confirmed LV-SLP-2-siRNA-2 could inhibit SLP-2 expression in SW620 cells most effectively. (shown in Fig. 4). In the following experiments, LV-SLP-2-siRNA -2 was recored as LV-SLP-2-siRNA for convenience.

3.6. The transfection of SLP-2-siRNA affected the cell activity and cell cycles of SW620 cells

As shown in Fig. 5, the cell activities in LV-SLP-2-siRNA group were remarkably lower than the negative control and the blank control at 48 h, 72 h($P < 0.01$) (Fig. 5A). Results of FCM demonstrated that ratio of LV-SLP-2-siRNA group cells in G0/G1 phase was higher than that in negative control and the blank control, whereas cells of LV-SLP-2-siRNA group cells in G2/M phase was lower than that in negative control and the blank control ($P < 0.01$) (Fig. 5B), which verified that cell activity and proliferation could be inhibited after SLP-2-siRNA transfection.

3.7. The effect of SLP-2-siRNA transfection on SW620 cell capacity of invasion, migration

Results of wound assay indicated that the SW620 cell capacity of migration reduced after LV-SLP-2-siRNA transfection, which was much slower than that in the control and the blank control ($P < 0.05$), whereas no difference was detected between the two controls (shown in Fig. 6A, B). Similar results were found in Transwell chamber assay (Fig. 6C, D), which showed that SW620 cell capacity of invasion reduced significantly after SLP-2-siRNA transfection.



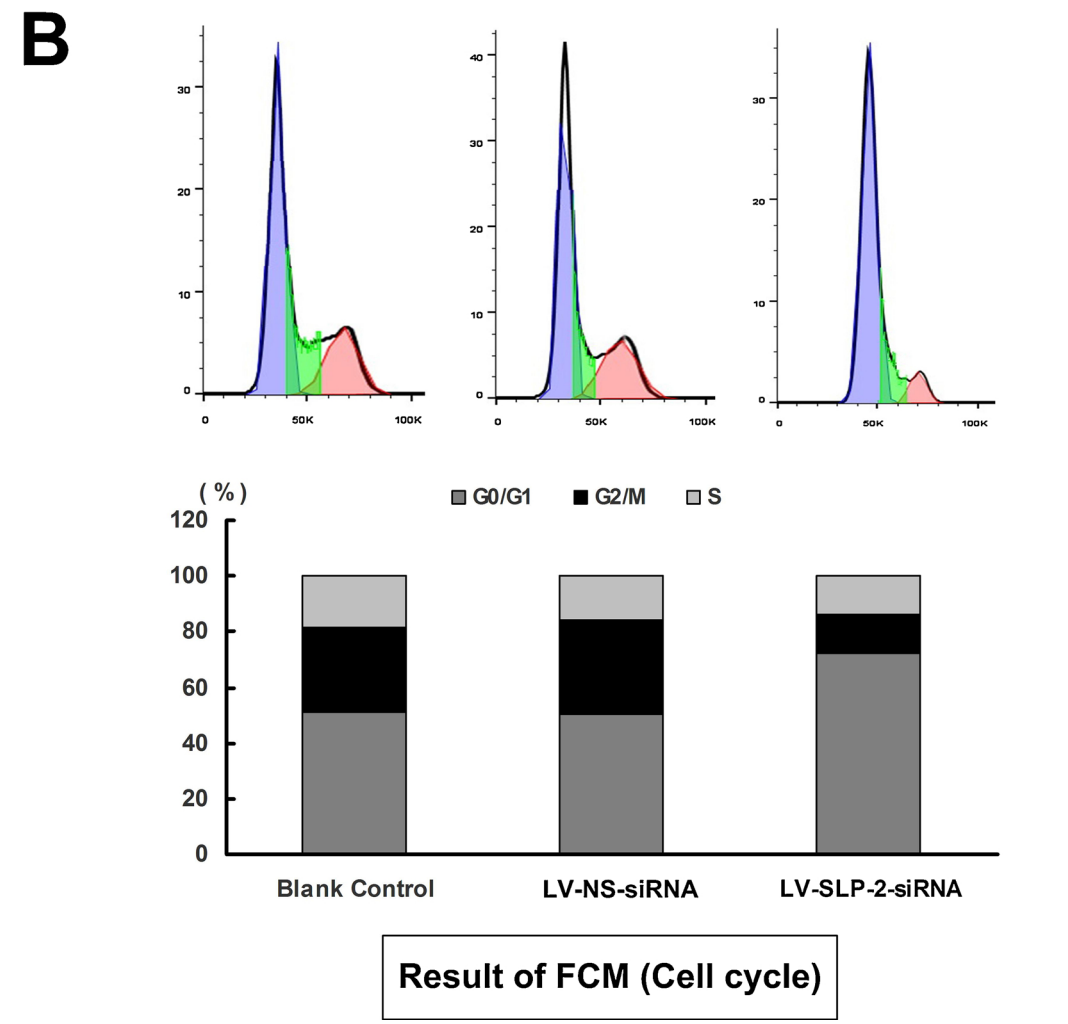


Fig. 5. Effect of LV-SLP-2-siRNA on the activity and cell cycles of SW620 cells.
After transfected with LV-SLP-2-siRNA, activity of SW620 cells decreased remarkably compared with NS-siRNA group and blank control group ($P < 0.01$). The results was shown as Fig.5A, which revealed that LV-SLP-2-siRNA transfection could inhibit activity of SW620 cells significantly. LV-SLP-2-siRNA transfection could prohibit cells from G0/G1 phase into G2/M phase (Fig. 5B).
*$P < 0.01$ versus LV-NS-siRNA group, Blank control group

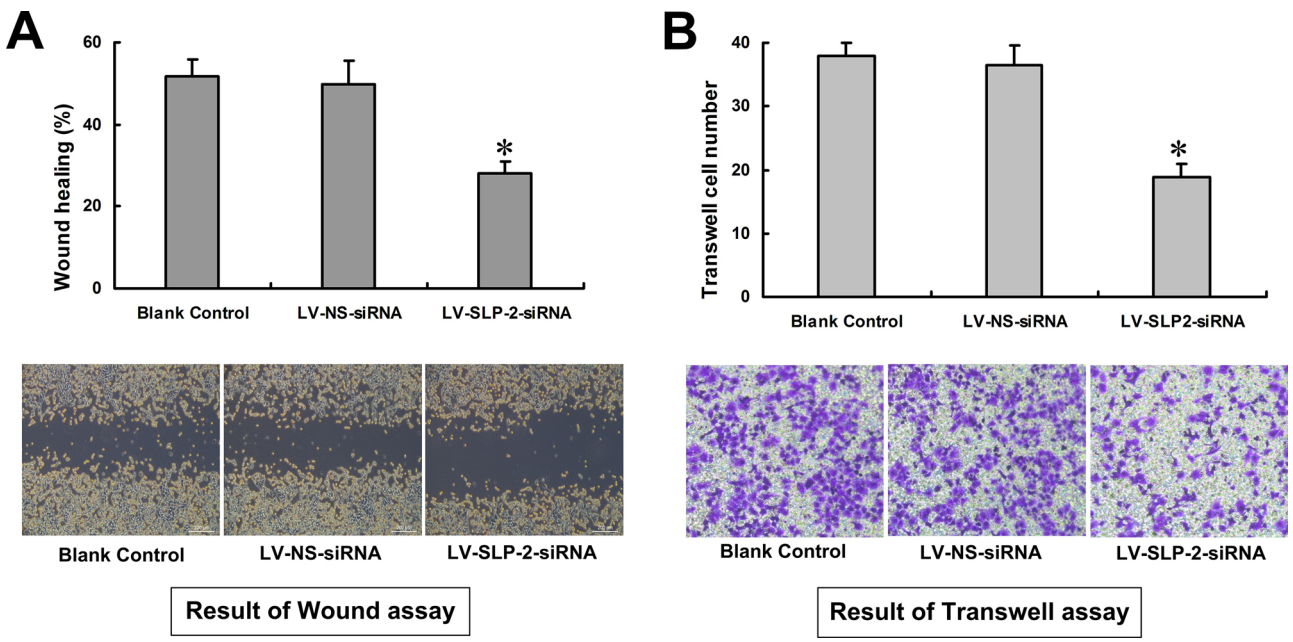


Fig. 6. Effect of LV-SLP-2-siRNA on invasion and migration of SW620 cells.
After transfected with LV-SLP-2-siRNA or NS-siRNA, SW620 cells were subjected to Wound assay (Fig6A) and Transwell assay (Fig6B) to determine the invasion, migration capacity of SW620 cells. Results demonstrated that invasion and migration of SW620 cells decreased significantly after LV-SLP-2-siRNA transfection.
*$P <$ 0.01 versus LV-NS-siRNA group, Blank control group

3.8. The impact of LV-SLP-2-siRNA transfection on each targeted gene of SW620 cells

To investigate the variation of genes after SLP-2 was inhibited, qPCR assay was utilized. Result demonstrated that the expression of mRNA and protein were downregulated in MMP-2, MMP-9, MMP-7, ICAM-1, PCNA genes at 48 h after LV-SLP-2-siRNA transfection in SW620 cells (all $P < 0.001$)while that in TIMP-1 and TIMP-2 showed no changes ($P > 0.05$). There was also no difference between negative control group and blank control group ($P > 0.05$) (shown as Fig. 7). This result revealed that SLP-2 was invovled in proliferation, migration and inhibition of CRC cells by regulating some related genes.

3.9. LV-SLP-2-siRNA transfection suppressed Wnt/β-catenin signaling pathway expression and activities

To further explore the effect of SLP-2 on Wnt/β-catenin signaling pathway, members of Wnt/β-catenin signaling pathway were detected after SLP-2 was inhibited in SW620 cells. Result showed that the variations were seen in Wnt/β-catenin signaling pathway and the related genes expression. Results of Fig. 8 demonstrates that the protein expression of GSK3β, p-GSK3β, β-catenin, Survivin, cyclin D1, c-myc and CD44 were lower than the two controls ($P < 0.05$), while there was no difference between negative control group and blank control group ($P > 0.05$).

4. Discussion

Incidence and mortality of colorectal cancer (CRC) have been increasing worldwide in recent years [15]. The rapid CRC cells invasion and migration results in poor prognosis for CRC patients [16,17]. Thus, it is crucial to explore new measures for inhibiting CRC cell migration and invasion in order to improve the outcome of CRC.

The recent studies have reported that multiple genes and signaling pathways participate in CRC invasion and migration [18,19], but the genes underlying most CRC remains unclear. In the present study, we examined SLP-2, a member from the stomatin family, which is located in 9q34.1 in the human chromosome and encode proteins of 357 acid residues [20]. SLP-2 is found to be involved in many malignant tumors [9–13,21–23]. In CRC tissue, the SLP-2 expression become abnormal, which may implicate that SLP-2 promotes CRC cell proliferation and inhibits cell apoptosis [14]. However, reports about the association between SLP-2 and CRC are limited, and therefore our present study explored the clinical significance of SLP-2 and examined this gene's molecular mechanism in CRC cells. IHC was employed to test the expression of SLP-2 in the tumors and adjacent non-cancerous tissue specimens of 95 patients and the results indicated that SLP-2 expression was higher in tumor tissue specimens compared with that in adjacent non-cancerous tissue specimens. This finding was further confirmed in both qPCR and Western blot-tests. Furthermore, we analyzed the relation between SLP-2 expression and cancer pathological parameters and found that SLP-2 expression also correlated closely to lymph nodes metastasis and clinical TNM stage. The above results suggest SLP-2 participates in CRC cells invasion and migration. Additionally, prognosis analysis discovered that SLP-2 positive expression can be regarded as an independent risk factor for CRC due to the low survival rates in patients with SLP-2 positive expression. Thus, SLP-2 may be a potential biomarker to predict the poor prognosis for CRC patients.

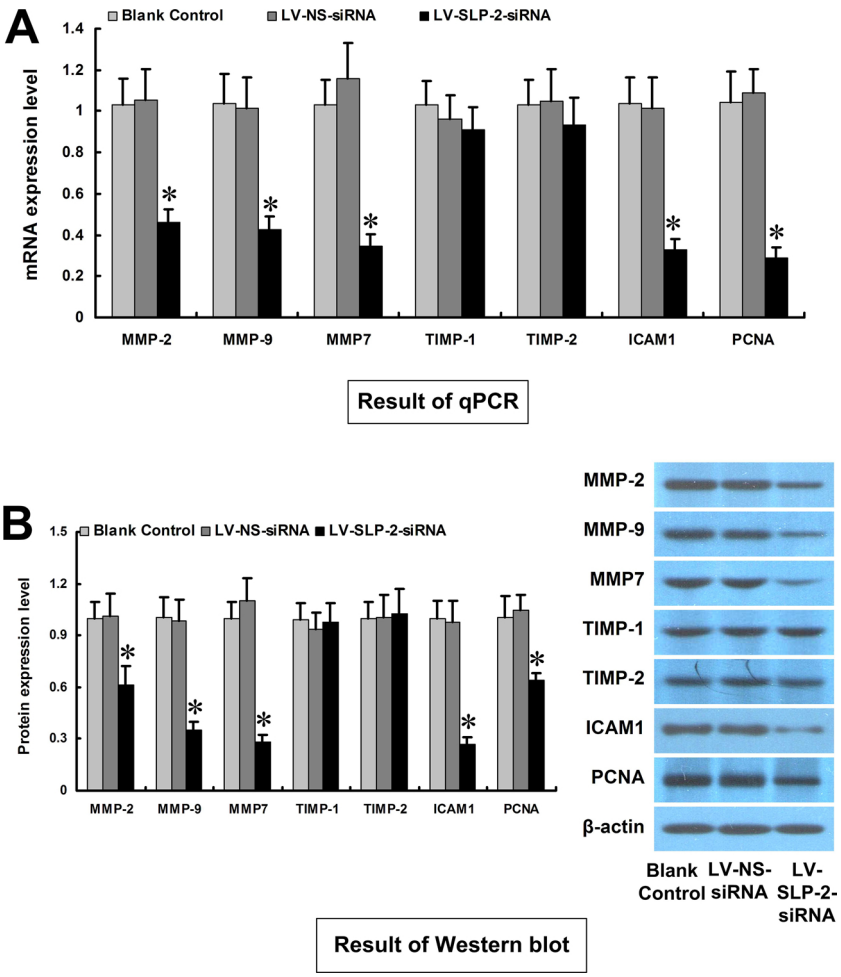


Fig. 7. Effect of LV-SLP-2-siRNA to MMP-2, MMP-9, MMP7, TIMP-1, TIMP-2, ICAM-1, PCNA on SW620 cells.
LV-SLP-2-siRNA transfection group or control groups were all subjected to qPCR (Fig.7A) and Western-blot (Fig.7B) assays to test the expression of MMP-2, MMP-9, MMP7, TIMP-1, TIMP-2, ICAM-1, PCNA. The results demonstrated that expression of MMP-2, MMP-9, MMP-7, ICAM-1, PCNA decreased significantly compared with NS-siRNA group and Blank control group.
*$P < 0.01$ versus LV-NS-siRNA, Blank control group

In order to understand the role of SLP-2 at molecular level in CRC invasion and migration, we inhibited SLP-2 expression in SW620 cells via RNA interference and the results showed the activities of SW620 reduced and the invasion and migration capacity of SW620 cells also decreased. These results indicated that SLP-2 may enhance CRC cells proliferation, invasion and migration. Meanwhile, we also examined the expression of MMP-2, MMP-9, MMP-7, TIMP-1, TIMP-2, ICAM-1, PCNA genes after inhibiting the expression of SLP-2 in SW620 cells as these genes play an important role in CRC development [24–26]. Among these genes, anti-oncogenes TIMP-1, TIMP-2 can inhibit MMPs resulting in promoting tumor cells invasion and migration [27,28]; ICAM-1 could promote cancer cell migration by regulating the adherence function of cancer cells [29], whereas PCNA is an indicator of cells proliferation capacity [30]. After investigation, we revealed that the expression of mRNA and protein in MMP-2, MMP-9, MMP-7, ICAM-1 and PCNA was downregulated, however those expression in TIMP-1 and TIMP-2 genes did not vary. It has been reported that SLP-2 inhibition could result in reduction of Survivin in non-small cell lung cancer (NSCLC) cells, and author verified that SLP-2 promote expression of Survivin by regulating β-catenin, a member of Wnt/β-catenin signaling pathway [10], so we suppose that SLP-2 might also regulate members of Wnt/β-catenin signaling pathway in CRC cells. Then in this study, we detceted the variations of Wnt/β-catenin signaling pathway after SLP-2 inhibition in CRC cells. The results of this study suggested SLP-2 may play a role in CRC cell growth through regulating Wnt/β-catenin signaling pathway, which enhances CRC cell growth remarkably [31].

Overall, the present study confirmed that the positive expression of SLP-2 could be a biomarker for poor CRC prognosis and inhibiting SLP-2 expression could be used as a method to inhibit CRC development. The data in this study implicated that SLP-2 plays a notable part in CRC cell invasion and migration so SLP-2 could be a novel biomarker in CRC and targeted gene in CRC treatments.

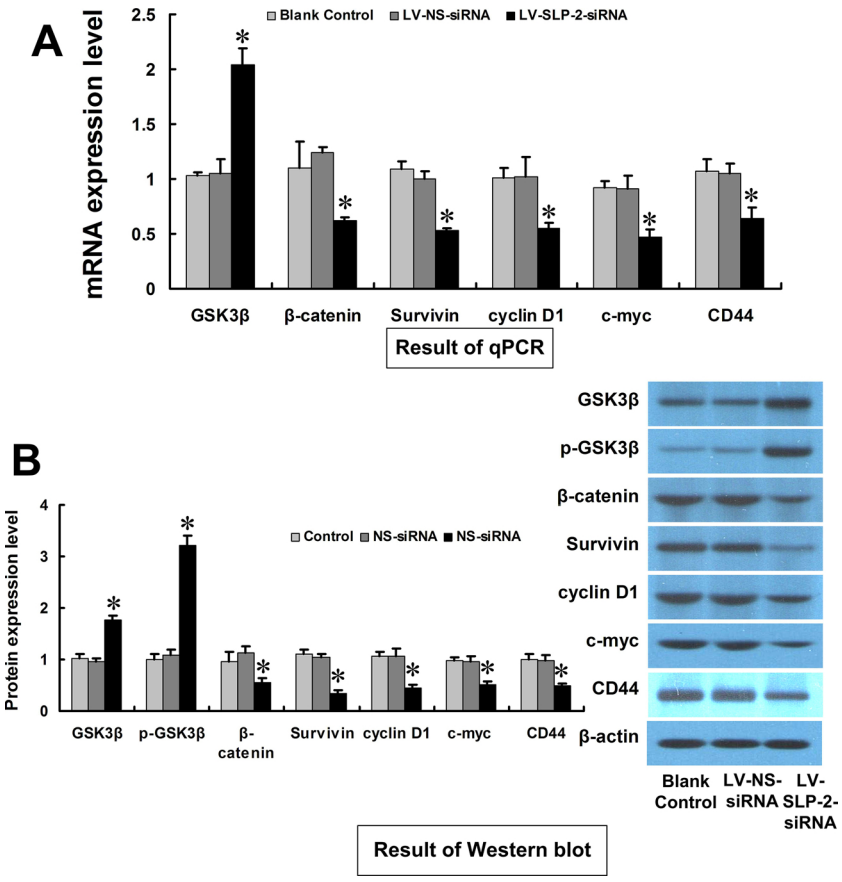


Fig. 8. Effect of LV-SLP-2-siRNA transfection on expression and activities of Wnt/β-catenin signaling pathway on SW620 cells.

LV-SLP-2-siRNA transfection group or control groups were all subjected to qPCR and Western blot assays to test expression and activities of Wnt/β-catenin signaling pathway in SW620 cells. The results revealed that expression of GSK3β, p-GSK3β, β-catenin, Survivin, cyclin D1 and c-myc were lower than the two control groups, which was shown in Fig.8A (qPCR) and 8B (Western blot).

*$P <$ 0.01 versus LV-NS-siRNA group, Blank control group

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