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SRSF3 Promotes DNA Damage Non-Homologous End Joining Repair to Regulate Radiotherapy Resistance in Rectal Cancer

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Abstract In recent years, SRSF3 has been shown to be a proto-oncogene involved in a variety of tissue-specific splicing events and is closely related to human diseases. The splicing factor SRSF3, an important member of the serine- and arginine-rich protein family, is abnormally highly expressed in a variety of tumors and plays an important role in tumor cell proliferation, migration, and invasion. In this paper, in order to deeply analyze the role of SRSF3 in rectal cancer, RNA sequencing technology was used to determine its role in gene expression regulation. By preparing relevant reagents and materials, carrying out experimental work such as cell recovery, cell culture and passaging according to the steps, emphasizing the extraction of total intracellular proteins, we analyzed the expression of the shear factor SRSF3 in the tumor tissues of rectal cancer. The clinical case parameters of 60 rectal cancer patients were selected for correlation analysis, and the correlation coefficient between SRSF3 and clinicopathological parameters was less than 0.05, that is, the expression of SRSF3 was positively correlated with the pathological grading of colorectal tumors, while it was not correlated with other pathological features. Moreover, the expression of SRSF3 in rectal cancer tumor tissues was significantly higher than that in normal tissues, which could promote rectal cancer development by regulating the splicing of a series of cancer-related genes.

Index Terms SRSF3, Rectal cancer, RNA sequencing, Gene expression

I. Introduction

With the economic development and improvement of living standards, the dietary structure changes, and the incidence and mortality of colorectal cancer continue to increase [1]. Many patients are found to be in advanced stages, and traditional treatments are unable to eradicate advanced, especially metastatic colorectal cancer, and immunotherapy has gradually become the development direction of future tumor treatment [2], [3]. The therapeutic strategy of neoadjuvant radiotherapy combined with surgery has become the conventional treatment for rectal cancer patients, and preoperative radiotherapy can effectively reduce the tumor volume and increase the rate of anorectal preservation, thus effectively improving the prognosis of patients [4]-[6]. However, in clinical practice, there are still some rectal cancer patients who are insensitive or even resistant to radiotherapy, and radioresistance of tumor cells is the main obstacle limiting the efficacy of treatment [7], [8].

The generation of radioresistance may involve a variety of factors, such as the biological characteristics of tumor cells, DNA damage repair ability, cell cycle regulation, and tumor microenvironment [9], [10]. Because of the heterogeneity of tumors, some tumor cells have innate radioresistance [11]. Meanwhile, multiple divided irradiation induces some cells to develop acquired radiotherapy resistance, leading to radiotherapy failure in patients [12]. Currently, the splicing factor SRSF3 has been reported to play the roles of assisting immune escape, promoting angiogenesis and inhibiting apoptosis of CRC cells in colorectal cancer [13], [14]. Therefore, it is important to explore the role and molecular mechanism of SRSF3 in colorectal cancer cell metabolism and transport to discover new SRSF3 target genes to improve the sensitivity of colorectal cancer patients to radiotherapy.

This paper analyzes the composition and structure of the shear factor SRSF3 with the current stage of research development. The percentage of colorectal cancer cases and the high incidence of rectal cancer are noted. In order to further analyze the role of SRSF3 in colorectal cancer, experimental methods such as cell recovery, cell culture and passaging, cell spreading and cell transfection were used by performing reagent preparation. The experimental analysis discussed the expression level of SRSF3 in the tissues of colorectal cancer patients, the correlation between the high expression of SRSF3 and the pathological grading as well as the prognosis of colorectal cancer. The increased level of SRSF3 expression in colorectal cancer cell lines was verified. Combine the total intracellular protein extraction method to analyze the expression level after experiencing DNA damage with the expression of transcript level after receiving radiation in different rectal cancer cell lines.

II. Experimental basis

II. A. Shear factor SRSF3

Variable splicing (AS) of RNA is a prevalent regulatory mechanism in gene expression, which refers to the formation of different mRNAs and proteins from pre-mRNAs of individual genes through a variety of different splicing modes.

It has been found that the vast majority of eukaryotic genes undergo variable splicing during the generation of transcription products. Among the many proteins associated with the spliceosome, there are two highly conserved families of proteins: known as serine- and arginine-rich splicing factor proteins (SRs) and heterogeneous nuclear ribonucleoproteins (hnRNPs.) SR proteins facilitate splice site recognition and gene exon splicing by binding to regulatory sequences present in intronic and exonic splicing enhancers in precursor mRNAs, usually. While heterogeneous nuclear ribonucleoproteins inhibit SR protein binding or splice site recognition by spliceosomal snRNP through recognition of intronic splicing silencers (ISS) or exonic splicing silencers (ESS) on the pre-mRNA.SR proteins are universally expressed in cells, and so far, 12 members of the SR protein family, mainly including: SRSF1-12, have been identified.

And SRSF3 as is the smallest member of the highly conserved SR-rich splicing factor family. Its gene is located on chromosome 6p21.31, and the molecular weight of the protein is about 19 kDa. It consists of 164 amino acids, and its structure is composed of an RNA recognition motif (RRM) and an RS structural domain (RS structural domain). The former binds RNA at the N-terminus and the latter binds other proteins at the C-terminus.The RS structural domain facilitates the interaction between different SR splicing factors, which are rich in serine and arginine residues [15]-[17].

SRSF3 is mainly involved in variable splicing of genes in the human body, and in recent years it has been found to be involved in the regulation of many other cellular functions, such as: termination of transcription, translation of proteins, regulation of miRNAs, and regulation of chromatin structure and function.

In recent years, SRSF3 has been shown to be a proto-oncogene involved in a variety of tissue-specific splicing events and is closely related to human diseases. Recent studies have shown that SRSF3 is highly expressed in a variety of tumors, including oral squamous carcinoma, ovarian carcinoma, osteosarcoma, and cervical carcinoma, and that overexpression significantly promotes cancer progression.

SRSF3, as a proto-oncogene, is involved in the progression of many tumors, but its mechanism of action is different in different types of tumors. Currently, the mechanisms of SRSF3 in colon cancer, such as invasion and migration, are rarely reported, and since most of the tumors are related to the aberrant shearing of SRSF3, it is hypothesized that there may be a similar molecular mechanism for the progression of SRSF3 in colon cancer as well.

II. B. Cancer of the colon and rectum

Colorectal cancer, which includes both colon and rectal cancers, is the third most common cancer in men and the second most common in women worldwide. More than 1.9 million people are diagnosed with colorectal cancer each year, accounting for 10 percent of new cancer diagnoses globally, with nearly one-third of these cases being rectal cancer.

Although the high incidence of rectal cancer poses a serious threat to patients' lives, the survival period of rectal cancer patients has been significantly extended in recent years with the continuous advancement and improvement of anti-cancer treatment techniques [18], [19].

According to reports, the 5-year survival rate of rectal cancer patients diagnosed with stage I can reach 70%, while the 5-year survival rates of rectal cancer patients diagnosed with stage II and stage III are close to 60% and 62%, respectively.

III. Reagents and materials

III. A. Cell lines

The cell line types are shown in Table 1. The cell lines were tested to be free of mycoplasma contamination.

Table 1: Cell line

Name	Source
Human colorectal cancer cell HCT-116	ATCC The United States
Human colorectal cancer cell SW480	ATCC The United States

III. B. Plasmids and siRNAs

The plasmids and siRNAs are shown in Table 2.

Table 2: Plasmid and siRNA

Name	Source
pcDNA3.1(+)-Flag	Suzhou biotechnology co., LTD., suzhou
Flag-DNCR24	Shanghai jama gene co., LTD., Shanghai
SiDHCR24	Shanghai jama gene co., LTD., Shanghai
E3-4/minigene	Shanghai jama gene co., LTD., Shanghai
E7-9/minigene	Shanghai jama gene co., LTD., Shanghai

III. C. Primary antibodies

The major antibodies are shown in Table 3.

Table 3: Main antibody

Name	Clone number	Source
SRSF3 antibody	ab198291(monoclonal antibody)	Abcam The United States
DHCR24 antibody	#2033(monoclonal antibody)	CST The United States
DDDDK(Flag) antibody	M185-3L(monoclonal antibody)	MBL JAPAN
GAPDH antibody	AF0006(polyclonal antibody)	Biyuntian biotechnology co., LTD

III. D. Main materials and reagents

The main materials and reagents are shown in Table 4.

Table 4: Main materials and reagent

Name	Source
PCR tube	Coming, USA
Black 96 orifice plate	Coming, USA
0.25 μM Filter membrane	Merck Millipore Germany
Random primer	TaKaRa JAPAN
Oligo (dT)	TaKaRa JAPAN
100 bp DNA ladder	Thermo Scientific USA
$Premix \times Taq^{\cdots}$	Thermo Scientific USA
RNase Inhibitor	Thermo Scientific USA
RT enzyme	Thermo Scientific USA
Protein A+G	Biyuntian biotechnology co., LTD
IgG (rabbit)	Biyuntian biotechnology co., LTD
AGAR sugar	China's weihai, Beijing
dNTP Mixture Solution	Bioengineering co., LTD., Shanghai
GelRed Nucleic acid dye	Biotium USA
6 × DNA loading buffer	Thermo Scientific USA
RT Buffer	Thermo Scientific USA
Trichloromethane	National drug collectivization co., LTD., Shanghai
Isopropyl alcohol	National drug collectivization co., LTD., Shanghai
Anhydrous ethanol	National drug collectivization co., LTD., Shanghai
EGTA	National drug collectivization co., LTD., Shanghai
HEPES	National drug collectivization co., LTD., Shanghai
NaF	National drug collectivization co., LTD., Shanghai
TritonX-100	National drug collectivization co., LTD., Shanghai
DTT	Leffsi bio-testing equipment co., LTD., wuxi
RNAiso Plus	Leffsi bio-testing equipment co., LTD., wuxi
DEPC water	Zhongwang technology co., LTD., suzhou
Test kit for reactive oxygen detection	Biyuntian biotechnology co., LTD
Quick gel extraction kit	Full gold, Beijing

III. E. Main instrumentation

The main instrumentation is shown in Table 5.

Table 5: Main equipment

Name	Type	Source
PCR meter	S1000TM Thermal Cycler	Bio-Rad USA
Horizontal calibrator	HE-120	Bio-Rad USA
Vertical electrophoresis	Mini-PROTEIN	Bio-Rad USA
Tachometer	Mini-PROTEIN	Bio-Rad USA
Gel imager	CHEMIDOC	Bio-Rad USA
Full wavelength enzyme spectrometer	M1000	Tecan Switzerland
Spectrophotometer	Nanodrop 2000	Thermo Scientific USA
Microwave oven	M1-L202B	Midea China
Rotary mixer	WH-986	Its linbel China

IV. Experimental methods

IV. A. Cell Resuscitation

(1) Pre-open the thermostatic water bath and set the temperature to 37.2°C.

(2) Remove the cell cryotubes from liquid nitrogen and place them in the 37.2°C water bath until completely melted.

(3) In an ultra-clean bench, aspirate 6mL of DMEM or DMEM/F12 culture base containing 10% FBS into a 15mL sterilized centrifuge tube.

(4) Aspirate the cytosol from the frozen tube into a 15mL centrifuge tube, blow gently to mix well, and centrifuge at 800rpm for 5min.

(5) Discard the supernatant medium, add 1mL of fresh culture medium to resuspend, and blow gently to disperse the cells evenly.

(6) Take another 10mL of medium into a 100mm cell culture dish and add the resuspended cells, blow gently to make them evenly dispersed.

(7) Put the cells into a constant temperature incubator and observe the cell status regularly.

Note: Generally start the experiment from the cells with stable growth by passaging to ensure the scientific and accuracy of the experimental results. All the gun tips and centrifuge tubes used in the experiment should be autoclaved and then used.

IV. B. Cell culture and passaging

(1) HCT-116 and HCT-8 cell culture medium: DMEM high sugar medium containing 10% FBS. HUVEC cell culture medium: DMEM/F12 medium containing 10% FBS. Cell culture conditions were saturated humidity, 37°C, containing 5% CO_2.

(2) HCT-116, HCT-8, and HUVEC are all wall-adherent cells, and should be digested with trypsin containing 0.25% trypsin when passaged.

(3) Observe the cell status every day, and when the cell density growth is about 80%, the cells need to be operated for passaging. Discard the old culture solution, add 3mL of PBS buffer to wash 1-3 times to remove dead cells and other impurities, then add 2mL of 0.25% trypsin digestion and observe under the microscope. The digestion was stopped when the cells showed a single round shape, trypsin was discarded, and 5mL of fresh medium containing 10% FBS was added to terminate the digestion. Blow gently to prepare cell suspension, take 0.6mL of cell suspension into a petri dish with 10mL of fresh medium added, “8-word” shaking method, mix well, and put it into a constant temperature incubator for cultivation.

IV. C. Cell spreading plates

(1) After cell passaging, aspirate 10 μL cell suspension on a counting plate, count under a microscope and calculate the cell density.

(2) According to the experimental needs, choose 96-well plate, 24-well plate, 12-well plate or 6-well plate, as well as the appropriate amount of cells, and add the cell suspension to the corresponding wells after mixing well with fresh culture fluid.

(3) Slowly place the laid plate into the cell culture incubator and try not to shake it.

Note: When the 96-well plate is laid out, it is necessary to add 10 μL PBS to the outermost circle to prevent edge effect.

IV. D. Cell transfection (transfection of siRNA in 12-well plate as an example)

(1) One day before transfection, cells in logarithmic growth phase are passaged and spread in 12-well plates at 1.2×10^5 cells per well and incubated overnight.

(2) When the cells in the 12-well plate grow to about 80%, the transfection operation can be performed. Before transfection, suck out the culture solution in the 12-well plate and add 1mL of fresh culture solution.

(3) Take 100 μL opti-MEM solution and 3 μL siRNA in an EP tube, and mix them well (the final concentration of siRNA is 50nM, and the dosage of plasmid for a single well is 1000ng).

(4) Take another new EP tube, mix 100 μL opti-MEM solution with 2.5 μL Lipo2000, and let it stand for 5min (the above is the dosage for single well, and according to the needs of the experiment, multiple wells can be prepared in batch).

(5) Mix the above two tubes of solution, blow gently to make it homogeneous, and let it stand for 30min.

(6) According to the 200 μL per well called the mixed solution slowly added to the 12-well plate, “8” shaking method to mix well, into the constant temperature incubator to continue to cultivate.

IV. E. Total cellular RNA extraction by Trizol method (12-well plate as an example)

(1) Remove the culture plate from the incubator, discard the old culture solution, and add 1mL PBS solution per well to clean the wells of impurities and floating dead cells.

(2) Add 1mL of RNAiso Plus lysis solution to each well, and let it stand at room temperature for 20min, so that the cells can be fully lysed.

(3) Blow repeatedly with a gun to completely dislodge the cells and aspirate the cell lysate into an RNase-free EP tube.

(4) 5℃, 12000g, centrifuge for 6min, transfer the supernatant to a new 1.5mL EP tube and add 1/5 volume of trichloromethane (200 μL) to the cell lysate, cover tightly, turn up and down for 12 times to make it mixed, and let it stand for 6min at room temperature (trichloromethane has a lower boiling point, and is easy to evaporate, so be careful in the operation).

(5) 5℃, 12000g, centrifuge for 15min, RNA exists in the upper aqueous phase, aspirate 400 μL supernatant in another set of RNase-free EP tubes, add 45 μL isopropanol in the same volume as the supernatant, turn up and down for 10 times to make it mix well, and let it stand for 12min at room temperature.

(6) Centrifuge at 5℃, 12000g, for 10min, RNA precipitated at the bottom of the tube, discard the supernatant, pour it on a paper towel, aspirate the supernatant and remove the organic solvent.

(7) Add 1mL of 75% ethanol (DEPC water preparation) to each EP tube, 5℃, 12000g, centrifuge for 5min.

(8) Discard the supernatant, invert on a paper towel, absorb the water and place in a fume hood to dry for 20 min.

(9) Add 20 μL sterilized DEPC water to dissolve the RNA precipitate, the tip of the gun was gently blown to mix, Nanodrop UV determination of its concentration and purity, put in -80 ℃ storage.

IV. F. Preparation of cDNA fragments

(1) Take the RNA template and centrifuge it, take 1 μg RNA and 2 μL Oligo (dT) in an EP tube and replenish it to 10 μL with DEPC water.

(2) Close the cap of the EP tube tightly and flick the bottom of the tube to mix the reverse transcription system well, and centrifuge for a short time.

(3) Place the EP tube in a 70°C water bath for 12 min, followed by an ice bath for 3 min.

(4) Centrifuge for a short time, do not shake violently when mixing.

(5) Add RT Buffer, dNTP (10mM), RT enzyme (200 $U/\mu L$), RT inhibitor (40 $U/\mu L$), and DEPC water sequentially and mix gently.

(6) The reaction solution was allowed to stand at room temperature for 10 min before being subjected to a water bath at 40°C for 60 min.

(7) Heat at 70°C for 10min to abort the above reaction, place at 5°C for cooling and -80°C for long-term storage.

IV. G. PCR amplification

(1) Primer design: The primer design of the target gene was done by Suzhou Biotechnology Co.

(2) Preparation of amplification system: 2 μL reverse transcription products, 1 μL upstream primer (10 μM), 1 μL downstream primer (10 μM) and 12 μL $Premix\times Taq^{TM}$ were added to EP tubes in turn, and 8 μL sterilized deionized water to make a total volume of 25 μL . The above operations were done on ice.

(3) Up + Set: PCR amplification program.

Stage 1: 90℃ for 5min for pre-denaturation.

Stage 2: denaturation at 90°C for 30s, annealing at 60°C for 30s, and extension at 75°C for 1min. Total 40 cycles.

Stage 3: 70℃ for 5min extension.

Stage 4: Cool down at 5℃, store at -20℃ or electrophoresis directly after the end.

The qPCR reaction was compared with GAPDH as internal reference, and the experiment was repeated at least 5 times and the average value was taken.

IV. H. DNA agarose gel electrophoresis

(1) Prepare the electrophoresis tank: take the electrophoresis gel electrophoresis tank of Plexiglas, wash and dry it, seal the gaps between the gel tanks with adhesive tape, put it on a horizontal working table, and insert a sample comb at one end.

(2) According to the size of the isolated target DNA molecules, weigh the appropriate amount of agarose, dissolve it in 0.5× TBE electrophoresis buffer, place it in a boiling water bath or microwave oven and heat it up until it is completely dissolved, then take it out and shake it well.

(3) When the gel solution cools down to about 60℃, pour it gently onto the horizontal plate of electrophoresis tank for filling.

(4) After the agarose gel has solidified, add the prepared electrophoresis buffer into the electrophoresis tank, and then carefully pull out the comb.

(5) Mix the DNA sample to be tested with 6× spiking buffer in the ratio of 4:1, and then carefully add it to the sample tank with a micropipette, adding 10-20 μL per tank.

(6) Install the electrode wire, connect the electrophoresis instrument with the electrophoresis tank, turn on the power supply, adjust the voltage to 3V/cm, electrophoresis for 1-3h, and stop the electrophoresis when it is observed that the bromophenol blue is moved to about 1cm away from the front edge of the gel.

(7) Remove the gel and place it in the staining solution containing ethidium bromide (EB) for staining (30 min).

(8) After staining, observe the gel under a 255 nm UV lamp and record the electrophoretic profile photographically.

IV. I. qPCR

(1) Primer design: The primer design of the target gene was done by Suzhou Biotechnology Co.

(2) Preparation of qPCR reaction system (20 μL): 10 μL called SYBR Green fluorescent dye, 6 μL DEPC water, 1 μL PCR upstream primer, 1 μL PCR downstream primer and 2 μL cDNA solution.

(3) On-line + setup program.

Stage 1: 90°C for 5min (1 cycle) for pre-denaturation.

Stage 2: 90°C for 10s, 60°C for 20s, 70°C for 5s (40 cycles) for PCR reaction.

Stage 3: Dissolution curve is 70-90°C, 0.4°C/s for dissolution.

Stage 4: 40°C for 30s for cooling and -20°C for storage after the end.

Confirm the amplification curve of qPCR and the fluorescence quantification lysis curve at the end of the reaction, make a standard curve when performing PCR quantification, and count the differences in the relative expression of the target genes between the different two subgroups separately, and repeat the experiment at least 5 times for each group of specimens. GAPDH or β-actin was used as an internal reference, and data were analyzed using the $2^{-\square\square Cq}$ relative quantitative data analysis method.

IV. J. WB detection of protein expression

Total intracellular protein extraction:

(1) Remove the cell culture plate or cell culture dish, discard the supernatant, and add an appropriate amount of pre-cooled PBS solution in advance to wash the cells 1-3 times.

(2) According to the ratio of 100:1, mix RIPA lysate with protease phosphatase inhibitor mixture, mix well (ready to use).

(3) Add appropriate amount of cell lysis working solution to the plate and leave it on ice for 30 min, during which the cells are fully lysed by constant blowing with the tip of the gun.

(4) Thoroughly scrape the cells with a spoon scraper, and aspirate the cell lysate from the wells into a pre-cooled EP tube at 5℃, 12000g, and centrifuge for 10min.

(5) Put the EP tube on ice, set the sonicator 3s/time and sonicate 6 times to make the cell lysis complete.

(6) 5℃, 12000g, centrifugation for 15min, cell debris sink to the bottom of the wall, aspirate the supernatant in another set of pre-cooled EP tubes, put on ice for spare.

V. Experimental results

V. A. SRSF3 is highly expressed in colorectal cancer patient tissues

First, human SRSF3-specific detection primers were designed to detect SRSF3 mRNA expression using fluorescence quantitative PCR. Relative to normal colorectal tissues, SRSF3 mRNA expression was significantly elevated in colorectal cancer tissues, with a statistically significant difference of up to 51.67% in the proportion of cases with high SRSF1-1.5-fold or higher expression.

Subsequently, the protein expression of SRSF3 in 42 pairs of tissue samples was detected using immunoblotting experiments, and relative to normal colorectal tissues, the protein expression level of SRSF3 was significantly increased in colorectal cancer tissues with statistical differences.

The increased expression of SRSF3 mRNA and protein in human colorectal cancer samples is shown in Figure 1. The figure shows the statistics of the relative expression of SRSF3 protein in 42 human colorectal cancer tissues, and the data were normally distributed with a p-value less than 0.0001.

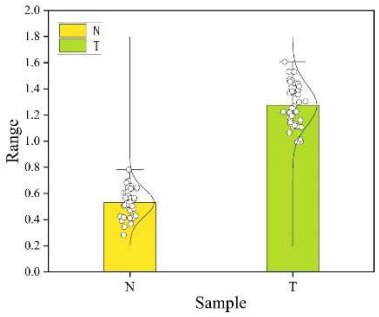

Figure 1: Increased expression of rsf3 mrna and protein in samples of colorectal cancer

Table 6: Correlation analysis of srsf3 and clinical pathological parameters

Clinical Characteristics	Non-increased SRSF3 (N=28) No (%)	Increased SRSF3(N=32) NO (%)	Test of significance
Gender			c2=1.5261 P=0.2518
Male	20(71.43)	21(65.63)	
Female	8(28.57)	11(34.38)	
Age			P=0.5336
≤50	4(14.29)	5(15.63)	
>50	24(85.71)	27(84.38)	
Tumor site			c2=1.0824 P=0.2518
Colon	12(42.86)	14(43.75)	
Rectum	16(57.14)	18(56.25)	
T stage			P=1.0137
T1	2(7.14)	3(9.38)	
T2	4(14.29)	4(12.50)	
T3	5(17.86)	5(15.63)	
T4	17(60.71)	20(62.50)	
N stage			P=0.8119
N0	12(39.29)	15(46.88)	
N1	13(42.86)	12(38.50)	
N2	5(17.86)	5(15.63)	
M stage			P=1.0000
M0	25(89.29)	26(81.25)	
M1	3(10.71)	6(18.75)	
Tumor grade			P=0.0265
Ⅰ	5(17.86)	3(9.38)	
Ⅱ	12(42.86)	8(25.00)	
Ⅲ	5(17.86)	15(46.88)	
Ⅳ	6(21.43)	6(18.75)	

V. B. High SRSF3 expression and colorectal cancer pathologic grading and prognosis

It was clarified that SRSF3 was highly expressed in colorectal cancer tissues. Next, the expression of SRSF3 was correlated with clinicopathological parameters in 60 patients.

The correlation analysis between SRSF3 and clinicopathological parameters is shown in Table 6. The results showed that the expression of SRSF3 was positively correlated with the pathological grade of colorectal tumors ($p<0.05$), while it was not correlated with other pathological features.

Kaplan-Meier survival curves were analyzed to correlate SRSF3 expression with the prognostic outcomes of colorectal cancer patients.

The correlation analysis between SRSF3 expression and the prognosis of 57 colorectal cancer patients is shown in Figure 2. Sixty patients were returned for follow-up, of which three were lost. Among the remaining 57 cases, the analysis results showed that the three-year survival rate of patients with high SRSF3 mRNA-1.5-fold or more expression was significantly decreased, which indicated that there was a correlation between high SRSF3 expression and poorer prognosis of colorectal cancer, and the results were significantly different ($P=0.0109<0.05$).

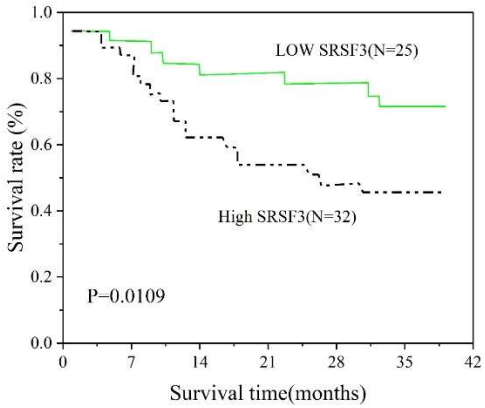

Figure 2: SRSF3 expression and the correlation analysis of the patient's prognosis

V. C. SRSF3 expression is increased in colorectal cancer cell lines

From the above experimental results, it was concluded that SRSF3 expression was increased in colorectal cancer tissues and showed some correlation with tumor grade in clinicopathological features. Meanwhile, Kaplan-Meier survival curves illustrate that SRSF3 expression presents a certain correlation with the prognosis of colorectal cancer.

Then the expression of SRSF3 in colorectal cancer cell lines was further explored. The mRNAs of normal colonic epithelial cell line CCD841, colorectal cancer cell lines SW620, HCT116, SW480, RKO and HT29 were received. The expression of SRSF3 in each cell line was detected using fluorescence quantitative PCR.

The expression of SRSF3 in colorectal cancer cell lines is shown in Figure 3, in which the data were normally distributed. The results showed that the expression of SRSF3 in colorectal cancer cell lines was significantly higher than that in normal colorectal cells.

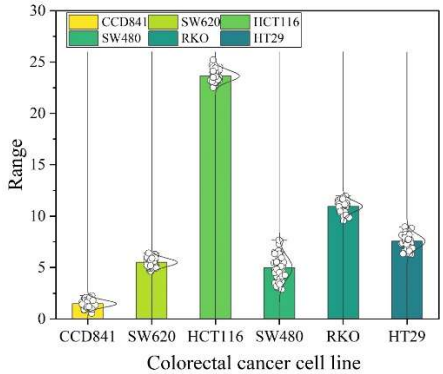

Figure 3: The expression of SRSF3 in the cell line of colorectal cancer

V. D. Expression after undergoing DNA damage in different rectal cancer cell lines

The main target of damage by ionizing radiation is DNA, and it damages DNA in the form of DNA single-strand breaks (SSB), DNA double-strand breaks (DSB), and base damage. To determine whether SRSF3 plays a role in the radiation response, the temporal relationship between SRSF3 expression and DNA damage such as radiation or cytotoxic drugs was first explored.

To confirm that SRSF3 is involved in the DNA damage response, i.e., more than just radiation-induced DNA damage, the commonly used clinical cytotoxic drugs etoposide (ETO) cephalexin (CPT), and olaparib (Olaparib) were further used to induce DNA damage.

Etoposide is a cell cycle-specific antitumor drug that targets topoisomerase II. ETO forms a stable ETO-Topo II-DNA ternary complex with the Topo II-DNA complex. Resulting in damaged DNA cannot be repaired.

CPT, which has been clinically proven to be effective against a variety of tumors, targets topoisomerase I (Topo I), which irreversibly binds to the DNA-Topo I complex, thereby blocking DNA replication and repair processes and triggering DNA damage.

Olaparib, a PARP inhibitor, is a drug that has been newly approved in recent years for clinical use in BRCA1 mutations.

It is noteworthy that PARP plays an important role in the DNA damage response, and the application of PARP inhibitors has raised the curtain on the use of DDR-derived drugs in clinical therapy. Therefore, ETO, CPT and Olaparib are commonly used as DNA damage inducers in the field of DNA damage response research.

V. E. Expression of transcript levels in different rectal cancer cell lines after radiation exposure

To further confirm that ionizing radiation may induce high SRSF3 expression, changes in SRSF3 transcription after DNA damage were explored. Colorectal cancer cells HCT116 and HT29 were similarly treated with 8 Gy of radiation using γ rays emitted from a 60Co radiation source, and the RNA of the cells was extracted at each time point at 0h (blank control), 4h, 8h, 12h, and 24h, respectively, after radiation.

RNA reverse transcription and RT-PCR were utilized to detect changes in the transcript levels of SRSF3 in HCT116 and HT29 cells after DNA damage. The mRNA expression level of SRSF3 in HCT116 and HT29 cells subjected to radiation was also confirmed to show a significant increase at 4h after the occurrence of DNA damage, followed by a gradual decrease.

VI. Conclusion

SRSF3, as a proto-oncogene, is involved in the progression of a variety of tumors, but has different mechanisms of action in different types of tumors. In this paper, based on the fact that SRSF3 is mainly involved in variable splicing of genes in human body, we found that the splicing factor SRSF3 presents higher expression around the blood vessels of rectal cancer. SRSF3 can regulate the secretion of VEGF by tumor cells, affect the ability of human umbilical vein endothelial cells HUVEC to traffic, invade and form tubes, and participate in the regulation of the angiogenic process of rectal cancer. In turn, it promoted the proliferation of colorectal cancer cells and inhibited cellular DNA damage, thus promoting the development of colorectal cancer.

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