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Experimental study of HSP27 differential expression in left sided colon cancer and right sided colon cancer

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Abstract: **Objective**   To provide molecular genetic basis for oncobiological difference in left sided colon cancer and right sided colon cancer. Differentially expressed proteins in left sided colon cancer and right sided colon cancer were screened by proteomic technique. **Methods**   Tissue samples including left sided colon cancer and right sided colon cancer were collected and preserved in the $-80℃$ refrigerator. In the first part of our experiment, protein was separated by 2-dimensional gel electrophoresis (2-DE) and the images of the gels were acquired by the scanner and then analyzed to find the differentially expression protein-spots in different groups. The peptide mass fingerprintings (PMF) was acquired by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and the proteins were identified by data searching in the Mascot-database. Differentially expressed proteins were assayed by RT-PCR, Western blot, and immunohistochemical method. **Results**   Altogether 55 differentially expressed protein spots were screened and 21 spots of them were identified. Compared with the right sided colon cancer, 14 proteins were up-regulated and 7 proteins down-regulated including HSP27 in the left sided colon cancer. HSP27 expressed higher in the right sided colon cancer than in the left sided colon cancer. **Conclusion**   There are differentially expressed proteins in left sided colon cancer and right sided colon cancer, especially difference in HSP27 expression at mRNA and protein level, which may be molecular genetic basis for oncobiological difference in left sided colon cancer and right sided colon cancer.

Key words: left sided colon cancer;   right sided colon cancer;   heat shock protein27;   proteomics;   immunohistochemistry

HSP27 在左侧结肠癌和右侧结肠癌差异表达的实验研究

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[摘要]　目的:应用蛋白质组学技术筛选左侧结肠癌和右侧结肠癌组织中差异表达的蛋白,为左侧结肠癌(left sided colon cancer, LSCC)和右侧结肠癌(right sided colon cancer, RSCC)在肿瘤生物学方面的差异提供分子遗传学依据。方法:收集人 LSCC 和 RSCC 组织标本,置 -80℃超低温冰箱中保存。应用双向凝胶电泳、质谱分析和生物信息学分离和鉴定 LSCC 和 RSCC 中差异表达的蛋白质。应用 RT-PCR,Western 印迹和免疫组织化学技术检测差异表达蛋白的表达状态。结果:筛选出 55 个差异蛋白质点,成功鉴定出 21 种差异蛋白质。与 RSCC 比较,14 种蛋白在 LSCC 表达上调,7 种蛋白在 LSCC 表达下调,其中 LSCC 中 HSP27 表达下调。通过 RT-PCR,Western 印迹和免疫组织化学方法证实:在 mRNA 和蛋白水平,LSCC 中 HSP27 的表达均低于 RSCC。结论:LSCC 和 RSCC 的蛋白质组存在差异表达,特别是 HSP27 在 mRNA 和蛋白水平均存在差异,这些可能是 LSCC 和 RSCC 生物学行为差异的分子遗传学基础。

[关键词]　左侧结肠癌;　右侧结肠癌;　热休克蛋白 27;　蛋白质组学;　免疫组织化学

The incidence and death rate of colorectal cancer (CRC) is in a rising trend all around the world. The colon is anatomically divided into 2 parts by spleen: the left sided colon (LSC), including the splenic flexure, the descending colon, and the sigmoid colon, and the right sided colon (RSC), including the ileocecal junction, the ascending colon, and the transverse colon. After long-term clinical observation, it is found that the left sided colon carcinoma (LSCC) and the right sided colon carcinoma (RSCC) shows different clinical manifestations. For example, the RSCC is mainly manifested as constitutional symptoms and anemia, and is commonly seen in the aged and females[1-2]. Poorly differentiated carcinoma, mucinous adenocarcinoma, and signet ring cell carcinoma are usually seen in RSCC patients[3-4]. The LSCC is mainly manifested by bowel obstruction, constipation, diarrhea and hematochezia, etc. In the research done by Nawa, et al. [5], it was found that LSCC and RSCC differed in pathomorphology in their early stage, and that the symptoms of RSCC, mainly obstruction or enclosed mass in the abdomen, appeared more late. The findings indicate that LSCC and RSCC may differ in their carcinogenic pathways and biological behaviors. But there is still no evidence showing whether these differences have molecular genetics basis. In this research, we selected the differentially expressed proteins in RSCC and LSCC using proteomic method, and further studied the differential expression of heat shock protein 27 (HSP27).

1　MATERIALS AND METHODS

1.1　Materials

1.1.1　Source of samples

We collected the issues cut from 7 RSCC and 7 LSCC patients in their surgeries in Xiangya Hospital, Central South University (Changsha, China). The 14 patients all had sporadic colon cancer, and did not have radiotherapy or chemotherapy before the surgery. The cancer was identified as moderately differentiated adenocarcinoma of stage Ⅱ - Ⅳ as defined by American Joint Committee on Cancer (AJCC), and the cutting edge was negative. The ages of the patients were 38 - 78 years old, with a median age of 59. One hundred cases of colon cancer were used in the immunohistochemical assay, including 76 males and 24 females, their age being 31 - 78, and average age (53. 2 ± 12. 2). The cancer was evaluated to be in stage Ⅱ - Ⅳ as defined by AJCC. The cases were randomly selected from the Department of gastrointestinal Surgery of Xiangya Hospital using the random number table. All the patients had the pathologic examination performed by 2 expert pathologists, and were divided into 2 groups, the RSCC group and the LSCC group, according to the position of their surgeries. The tissues samples were fixed with 10% neutral formalin, and made into paraffin embedded slices. They were identified as moderately differentiated adenocarcinoma. The ages, sexes, and clinical stages (sizes of tumor, depth of intestinal wall infiltration) of the 2 groups were not significantly different ($P > 0.05$).

1.1.2　Main reagents

The reagents used in this research include: BCA Protein Assay Kit from Pierce Company (USA); thiourea, dithiothreitol (DTT), iodoacetamide, standard protein for Two Dimensional Gel Electrophoresis, TPCK trypsin, potassium ferricyanide, trifluoroacetic acid (TFA), and the matrix α-Cyano-4-hydroxycinnamic Acid from Sigma Company (USA); IPG strip (pH4-7L, 24 cm), IPG buffer (pH4-7), ampholyte (pharmalyte, pH4-7), drystrip

cover fluid, low molecular weight protein, Silver Staining Kit, urea, acrylamide, N, N'-methylene-bis-acrylamide (MBA), glycine, Tris, 3-cholanido-propyl (CHAPS) and sodium dodecyl sulfate (SDS) from AB company (USA); DNA polymerase and RNA polymerase from Promega Company (USA); domestic Analytical reagents sodium thiosulfate and calcium chloride; domestic chromatographically pure reagent acetonitrile; TRIzol reagent (Invitrogen, Cat. 15596-026; USA); ReverTra Ace-α-TM First Strand cDNA Synthesis Kit (TOYOBO, #FSK-100; Japan); Taq DNA Polymerase (recombinant, Fermentas # EP0402; USA); dNTP Mix (2 mmol/L each Fermentas # R0241; USA); DNA marker DL2 000 (TaKaRa D501A; Japan); nitrocellulose filter membrane(PIERCE, Cat. #88018; USA); filter paper(Whatman, 3MM CHR; England); P-HSP27 (S15) antibody(Bioworld Technology Inc. ; USA). The primer of *HSP27* gene was (Homo-*HSP*27-F) 5'-GACGAGCATGGCTACATCT-3', (Homo-*HSP*27-R) 5'-ATGGTGATCTCGTTGGACT-3'. The length of the segment was 158 bp. The primer of the internal control gene *GAPDH* was (Homo-*GAPDH*-F) 5'-ACCACAGTCCATGCCATCAC-3', (Homo-*GAPDH*-R) 5'-TCCACCACCCTGTTGCTGTA-3'. The length of the segment was 450 bp.

1.1.3   Main equipments

Equipments used in this research included IPG-phor isoelectric focusing (IEF) apparatus, imageMaster 2 D Elite 4. 01 gel image analyzing software, imagescanner, labscan software for scanning control and analysis pre-processing, ProTEAN Vertical Electrophoresis cell, PowerPAC3000 electrophoresis apparatus (produced by Bio-Rad Company; USA), SaVant freeze concentration system (USA); matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometer (ABI Company, USA), Elx800 ELISA (Bio-Tek Instruments, Inc. ; USA).

1.2   Methods

1.2.1   Processing of specimens and extraction of total protein

We took fresh colon cancer tissues from surgery, washed them repeatedly with normal saline to get rid of the blood and other unnecessary matter, and cut off unnecessary tissues as far as possible. The processed specimens were immediately preserved at the temperature of −80℃. The total protein was extracted after tissue disruption using Total protein Extraction Kit (ProMab, Cat. SJ-200501; USA). 0. 5 mg cancer tissue was cut down with scissors, and placed into a tissue homogenizer. It was added with 1 mL total protein extract, and grinded for 20 min so that the tissue was fully disrupted. Then it was put on ice for 20 min, and grinded for another 20 min, and then removed into a 1. 5 mL centrifuge tube. The cancer tissue homogenate was centrifuged at the speed of 9 000 r/min for 10 min after 3 ultrasounds, 3 s once. A certain amount of the supernate was removed to a new 1. 5 mL centrifuge tube and preserved at −20℃. The concentration of the protein was assayed using the 2-D Quant Kit which was dedicated to protein extraction optimization.

1.2.2   2-D electrophoresis with immobilized pH gradients and mass spectrographic (MS) analysis

The 2-D Electrophoresis with immobilized pH gradients was performed using IPGphor isoelectric focusing system and Gorg method. The rehydration and focusing of IPG strips automatically occurred at the temperature of 20℃. Amersham IPG strips of 24 cm and pH3 − 10 were taken out and thawed, and then 400 μg sample was loaded. The sample volume was calculated based on the protein concentration. The total voltage-time product was 8 000 Vh. The hydration was done at a low voltage of 30 V for 13 h and then the isoelectric focusing was performed at 100 V for 1 h, 500 V for 1 h, 1 000 V for 1 h, 5 000 V for 1 h, and finally at 8 000 V for 8.5 h. After the focusing, the IPG strip equilibration was performed, and the strips were removed to 0. 75 mm-thick 12.5% separation gel for the SDS-PAGE. The product of SDS-PAGE 2-DE was silver-stained following the operating manual of the Silver Staining Kit. Then we did the image analysis, using imagescanner and labscan software to obtain the images, and PDQuest-V 8. 0. 1 analysis software to do analysis such as the intensity correction, spot detection, background elimination, matching, adjustment, and averaged gel establishment. MS analysis was done for differentially expressed spots. The protein spots were positioned in an EP tube, and washed with ultrapure water 3 times to get rid of foreign matter. Decoloration solution was added to the tube until the color of the protein spots faded away and the gel turned lucent. Then, the ultrapure water was discarded, and 100% acetonitrile was added for dehydration. The gel was frozen and dried thoroughly, and enzymolysis solu-

tion was added to react at 37℃ overnight. After that, the supernate was removed to a 0.5 mL EP tube, mixed with extraction solution, and frozen and condensed to $2-5$ uL. Then 0.5 uL of it was applied to a stainless steel plate, and air-dried for the future analysis. Lastly, the prepared samples were performed MALDI-TOF mass spectroscopy. The samples were loaded to the mass spectrometer, which was set on the Reflectance Mode, positive ion. The accelerating voltage of ion source was 20 KU, voltage reflection coefficient 1.12, N2 laser wave length 337 nm, pulse width 3 ns, extraction delay time 100 ns, vacuum degree 4×10^{-7} Torr, and signal was obtained by accumulation of 50 single scanning. Ion peak of trypsin autolysis was applied as the internal standard calibration, and peptide mass fingerprinting (PMF) was obtained. The peaks of each protein sample were measured 3 times. Mascot software was used to searching Uniprot database for the protein evaluation.

1.2.3　Western blot

Firstly, SDS-PAGE was carried out. The tissue lysate (100 μg) was mixed with $5 \times$ Loading buffer, and heated up to 100 ℃ for 3 min to denature the protein. The voltage was set on 200 V, and the reaction was terminated when bromophenol blue moved to 0.5 cm from the bottom of separation gel. Then Western blot was performed. The first step was to transfer the protein to the membrane. We put the gel-membrane into transfer tank, connected the electrodes, set it on 300 mA, and did the transferring for 70 min. After that, the membrane was placed into 5% non-fat milk to be blocked at 37℃ for 2 h. The next step was detection of target proteins with primary antibody. The primary antibody (1 : 2 000) was diluted with 5% non-fat milk, and incubated with blotting membrane at room temperature for 1 h. Then the membrane was washed with TBS/0.1% Tween 20 (TBS-T) for 3 times, 15 min for each time. Substrate for electrochemilu miluminescence (ECL) assay reagent was prepared on light plastics membrane to react with it for $3-5$ min. The film was exposed to light in a cassette for 3 min, and then taken out for image development and fixation. The housekeeping protein GAPDH was used as loading control. The positive bands were analyzed with the Gel pro 4.0 Potodensitometry Software, and their integrated optical density (IOD) was measured.

1.2.4　Extraction of total RNA and RT-PCR

TRIzol (1 mL) was added into each sample for digestion. The homogenate was stilled at room temperature for 10 min and then added with chloroform (0.2 times volume of the homogenate). After being shaken for 15 s, the mixture was centrifuged at 4℃, 10 000 r/min for 15 min. Then the water-like layer was transferred to a clean tube, and isopropanol (0.5 times volume of the fluid) was added. The two was fully mixed, stilled at the temperature of -20 ℃ for $10-15$ min, and then centrifuged at 4℃, 10 000 r/min for 10 min. The RNA sediment was washed with 1 mL 75% ethylalcohol after the removal of upper layer liquid, and centrifuged at 4℃, 8 000 r/min for 5 min. Then the supernate was discarded, the RNA sediment was dried moderately, and a certain amount of RNase-free water was added so that the RNA sediment was fully dissolved. Then, it was centrifuged at a speed of 2 000 r/min for 20 s.

The RT system (volume of 20 μL) included: 1 μL total RNA (1 μg/μL), 1 μL Oligo(dT) 20 (10 pmol/μL), 10 μL RNase free H_2O, 4 μL $5 \times$ RT Buffer, 2 μL dNTP mixture(10 mmol/L), 1 μL RNase Inhibitor (10 u/μL), and 1 μL ReverTra Ace. The RT procedure was as follows: 42℃, 20 min; 99℃, 5 min; 4℃, 5 min, and instant centrifugation. The PCR system (volume of 20 μL) included: 1 μL Template (RT product), 0.5 μL Primer sense (100 μmol/L), 0.5 μL Primer anti-sense (100 μmol/L), 2 μL dNTP Mixture(2 mmol/L), 1.5 μL $MgCl_2$(25 mmol/L), 2 μL $10 \times$ PCR Buffer, 0.5 μL Taq DNA Polymerase (500 U), and 12.0 μL PCR H_2O. The procedure of PCR amplification was as follows: 95℃, 5 min; 94℃, 30 s; 55℃, 30 s; 72℃, 30 s; 72℃, 5 min; preservation at 4℃, 40 circles. The detection of RT-PCR products was done using agarose gel electrophoreses (1.5% agarose gel, sample was 10 μL, and voltage 120 V). After that, the products were stained with ethidium bromide (EB), observed in ultraviolet (UV) light, and photographed. The positive bands were analyzed with the Gel pro 4.0 Potodensitometry Software, and their IOD was measured.

1.2.5　Immunohistochemical assay

Antigen retrieval was performed with the paraffin sections. We applied blocking serum and primary antibody (HSP27, 1:200) to the sections, placed them in the refrigerator of 4℃ overnight, and then washed them with PBS. The antigen retrieval was

performed following the instruction of the SP kit. HSP27 positive signal was mainly observed in the cytoplasm, and appeared to be yellow or brown grains. The results were evaluated based on the ratio of the positive cell number to the total cancer cell number, and the staining intensity of the positive cells. A: the ratio being less than 1/3 was scored 1 point, between 1/3 and 2/3 2 points, and more than 2/3 3 points; B: being not stained was scored 0, pale yellow 1 point, dark yellow 2 points, and brown 3 points. The final score = A × B. A × B = 0 was marked as (−), A × B = 1 − 2 (+), A × B = 3 − 4 (+ +), and A × B = 6 − 9 (+ + +). "−"represents negative, "+—+ + +"represents positive.

1.3　Statistical analysis

The statistical analysis was obtained by use of the statistical software SPSS13.0. The enumeration data and sample matching were tested with Chi-square test (χ^2), and the measurement data was analyzed with t test. $P < 0.05$ was considered as statistically significant.

2　RESULTS

2.1　2-D electrophoresis and MS analysis of LSCC and RSCC tissue

2-D electrophoresis analysis of 7 LSCC and 7 RSCC cases was performed, which was repeated for 3 times under the same condition. The 3 electrophoresis patterns were very alike and the protein spots were clearly seen. Analyzing the 2 electrophoresis patterns with PDQuest 8.0.1 Software, we found that there were (890 ± 64) spots in RSCC tissue, (865 ± 77) spots in RSCC tumor-adjacent tissues, (881 ± 48) in LSCC tissue, and (798 ± 72) spots in LSCC tumor-adjacent tissues. The number of differentially expressed proteins of the 2 groups was 55 (|score (d) | ⩾ 2, i.e. fold change ⩾ 2 or fold change ⩽ 0.5). MALDI-TOF-MS was done with the 55 differentially expressed protein spots, except for 1 spot which was lose during the experiment, and their PMF were examined. PeptIdent Software was employed to search the Uniprot and Trembl database. The search result was determined after a combined consideration of number of matched segments, and rate of coverage, etc. Finally 21 proteins were selected. The expressions of 7 proteins among them [elongation factor 1-delta, heat shock protein beta-1, ATP synthase subunit beta (mitochondrial), stress-70 protein (mitochondrial), transthyretin, serum albumin, heat shock protein beta-6] were found to be down-regulated in the LSCC tissues; the expressions of 14 proteins [keratin (type II cytoskeletal 8), 60 kD HSP (mitochondrial), proteindisulfide-isomerase, 78 kD glucose-regulated protein, gamma-enteric smooth muscle, transitional endoplasmic reticulum ATPase, alpha-1B-glycoprotein, apolipoprotein A-I, prohibitin, thioredoxin domain-containing protein 5, T-complex protein 1 subunit epsilon, protein disulfide-isomerase A3, isocitrate dehydrogenase[NAD] subunit alpha, mitochondrial macrophage-capping protein] were found to be up-regulated. The 12th differentially expressed protein was identified by PMF and Mascot Wizard retrieval to be HSP27 (heat shock protein beta-1, HSPβ1) (Fig. 1 − 3).
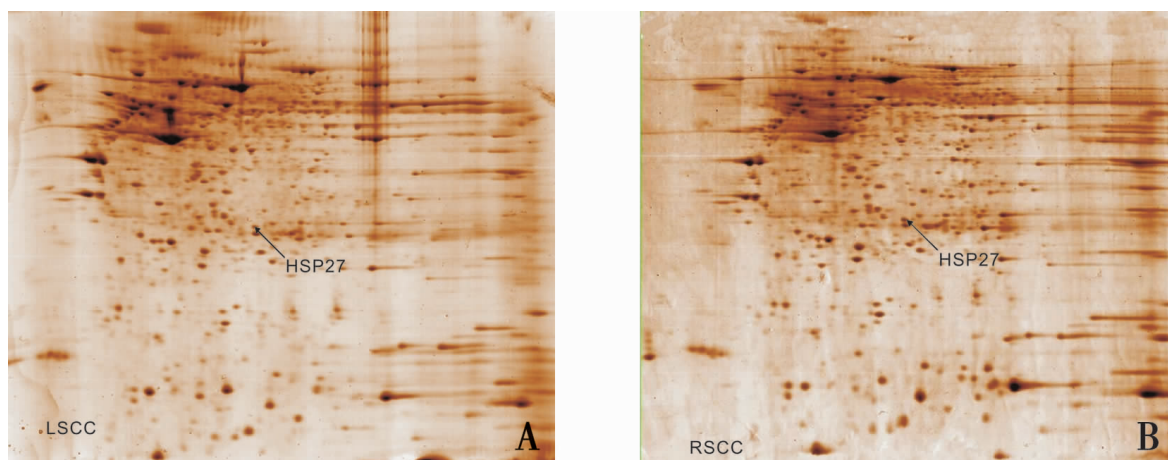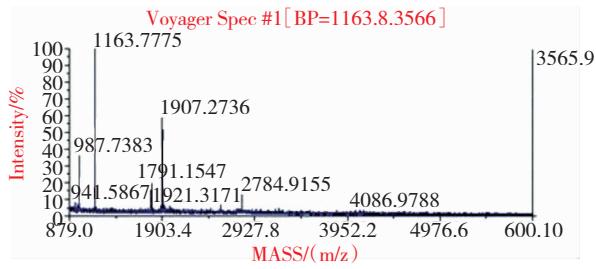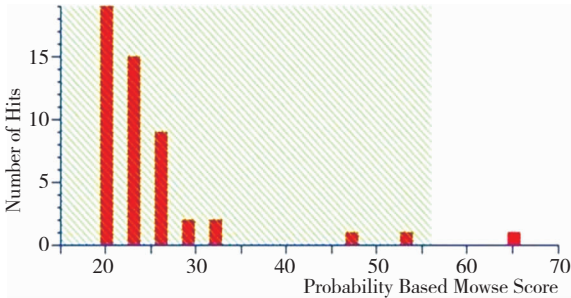

Fig. 1　Representative images of 2-dimensional gel for LSCC (A) tissues and RSCC tissues (B).

Fig. 2　Peptide mass fingerprinting of protein from spot 12(HSP27)in 2-DE map.

Fig. 3　MALDI-TOF-MS analysis of HSP27 protein spot. X axis stands for the score of Mascot, Y axis stands for the matching number, the Mascot score of protein spot 65 is considered as statistically significant.

2.2　RT-PCR showing the expression of HSP27 in RSCC and LSCC tissues

The expression level of HSP27 mRNA in the RSCC and LSCC tissues was detected with RT-PCR.

The positive bands were analyzed with Gel pro 4.0 Potodensitometry Software to measure their IOD. The IOD value of the gene of interest was compared to that of the internal control housekeeping gene *GAPDH* to obtain the corrected IOD value. To compare the expression level of *HSP27* mRNA in the RSCC tissues to that in the LSCC tissues, and found that the average corrected IOD value of LSCC was 0.4006, and that of RSCC was 0.6737. Statistical analysis showed that $t = -2.583$, $P = 0.024$. As $P < 0.05$, we could conclude that the expression level of *HSP27* mRNA in RSCC was higher than that in LSCC, and the difference between the 2 was statistically significant (Fig.4, Tab.1).

Fig. 4　RT-PCR showing the expression of HSP27 in RSCC and LSCC tissues. 1 – 7: LSCC; 8 – 14: RSCC.

Tab. 1　Semi-quantitative analysis HSP27 expressed in LSCC and RSCC tissues by RT-PCR

Genes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
HSP27	10196	8971	19591	8843	11662	11166	10421	18611	19350	21192	13702	12757	33661	30665
GAPDH	29850	27057	27304	32238	28208	27507	32551	32046	29353	30818	31558	32450	32721	32888
Ratio	0.3415	0.3315	0.7175	0.2743	0.4134	0.4059	0.3201	0.5807	0.6592	0.6876	0.4341	0.3931	1.0287	0.9324

1 – 7: LSCC; 8 – 14: RSCC.

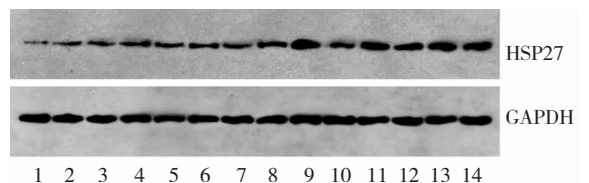## 2.3　Western blot showing the expression of HSP27 in RSCC and LSCC tissues

The expression level of HSP27 protein in the RSCC and LSCC tissues was detected with Western blot. The positive bands were analyzed with Gel pro 4.0 Potodensitometry Software to obtain the reference value of their IOD. The IOD value of the protein of interest was compared to that of the internal control housekeeping protein GAPHD to obtain the corrected IOD value. To compare the expression level of HSP27 protein in the RSCC tissues to that in the LSCC tissues, and found that the average corrected IOD value of LSCC was (0.18 ± 0.04), and that of RSCC was

(0.51 ± 0.10). Statistical analysis showed that $t = -2.966$, $P = 0.018$. So we conclude that the expression level of HSP27 protein in RSCC was higher than that in LSCC (Fig. 5, Tab. 2).

Fig. 5　Western blot showing the protein expression of HSP27 in RSCC and LSCC tissues. 1 – 7: LSCC; 8 – 14: RSCC.

Tab. 2   Semi-quantitative analysis HSP27 expressed in LSCC and RSCC tissues by Western blot

Genes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
HSP27	67.0	117.3	151.6	208.4	163.0	263.2	178.1	272.6	1 016.3	261.5	617.6	509.7	650.7	575.3
GAPDH	1 331.1	1 324.8	1 237.6	966.2	580.7	737.6	1 020.0	970.7	1 067.4	1 445.0	1 126.2	1 270.7	819.9	1 316.1
Ratio	0.05	0.08	0.12	0.21	0.28	0.35	0.17	0.28	0.95	0.18	0.55	0.40	0.79	0.43

1 – 7：LSCC；8 – 14：RSCC.

2.4   Immunohistochemistry showing the protein expression of HSP27 in RSCC and LSCC tissues

The expression level of HSP27 protein in the RSCC and LSCC tissues was detected with immuno-histochemistry and the result showed that the HSP27 positive signals located mainly in the cytoplasm, manifested as granules or pieces with different color shades ranging from pale yellow to dark brown, depending on the signal strength (Fig. 6). The ratio of expressed HSP27 in the 50 cases of LSCC was 28%, and in the 50 cases of RSCC was 60%. The difference between the 2 was statistically significant, indicating that expression of HSP27 in LSCC and in RSCC was of significant difference (Tab. 3).
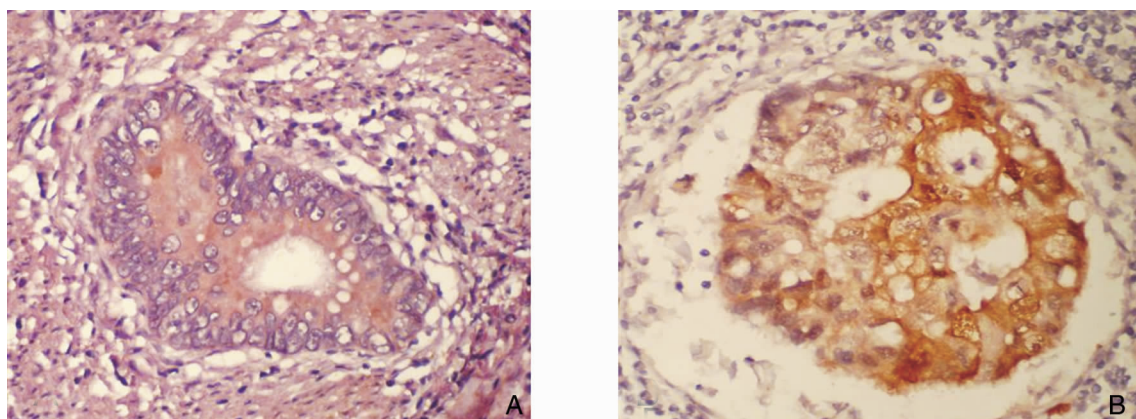
Fig. 6   Immunohistochemistry showing the expression of HSP27 in LSCC (A) and RSCC (B).

Tab. 3   Expression of HSP27 in LSCC and RSCC tissues detected by immunohistochemistry method (n = 50)

Groups	Positive case/No.	Positive rate/%
LSCC	14	28.0
RSCC	30	60.0

χ^2 = 10.390, P = 0.001.

3   DISCUSSION

It is found in clinical practice that the biological behaviors of LSCC and RSCC are different, and more and more experiments[6-12] have proved this difference in cellular and molecular level, involving chromosome, DNA, tumor and tumor suppressor gene, DNA mismatch repair gene, etc. For example, the expression level of c-myc and Ras is much higher in LSCC than in RSCC, and the mutation rate of Ras is higher in LSCC than in RSCC. Research findings showed that the allelic loss rate of APC gene at 5q, DCC gene at 18p, and p35 gene at 17q was obviously higher in LSCC than in RSCC. Also, the mutation of P53, P14, P16 in the RSCC is different from that in LSCC. The microsatellite instability (MSI) rate of RSCC is much higher than that of the LSCC and rectal cancer, indicating that the genetic mechanisms of RSCC and LSCC are different, i. e. the cause of RSCC is the mutation of cancer-related gene resulted from replication error (RER), whereas the reason of LSCC is the mutation of cancer and cancer suppressor gene resulted from loss of heterozygosity. Colon cancer is caused by continuous gene mutation, which may occur in intestinal tract stem cells. It is found that the low incidence of RSCC may be related to the short survival time of mutated crypt stem cells.

The difference between RSCC and LSCC in protein level is rarely reported. Protein is the performer of cell functions, such as regulation of gene expression and disease occurrence and serving as the tar-

gets of many drugs. The proteomics has gradually become an important and efficient method in study of proteins. At present, there are already many protein expression profiles and databases, but no report on differentially expressed protein pattern of RSCC and LSCC is published yet. In this research, based on the previous proteomic study of colorectal cancer's occurrence and metastasis, we established the expression patterns of differentially expressed proteins of RSCC and LSCC tissues using proteomic techniques, obtained 2-D electrophoreses images of high-definition background, high resolution, and good repeatability. We found 55 differentially expressed protein spots of RSCC and LSCC, identified 21 differentially expressed proteins, of which 14 were found to have up-regulated expression in LSCC, and 7 of them have up-regulated expression in RSCC. These proteins fell into categories such as metabolism-related enzymes, molecular chaperone, transference-related proteins, transcription-and-translation-related proteins, skelemin and peroxiredoxins, depending on their functions. Together with the findings in the previous study[13], we found that HSP27, the protein that was related to occurrence and metastasis of colorectal cancer, was differentially expressed in LSCC and RSCC. And the RT-PCR, Western blot, and immunohistochemistry also proved that the expression of HSP27 was less in LSCC than in RSCC in terms of both mRNA and protein.

HSP27 belongs to the low-molecular-weight heat shock proteins subfamily, having the feature of ligomerization and phosphorylation, depending on its status. The overexpression of HSP27 can inhibit cell apoptosis, and various matters involved in stress and receptor-induced apoptosis, such as caspases and cytochrome C. HSP27 can also increase the antioxidation ability of cells by reducing reactive oxygen. HSP27 is closely related to colon cancer. Firstly, the abnormal expression of HSP27 may be connected with the occurrence and malignant evolution of colon cancer. It is found that in the REG cells of colon cancer, the expression of HSP27 can reduce the apoptosis of cancer cell and increase their tumorigenicity. The overexpression of HSP27 may be involved in the metastasis and development of colorectal cancer. T lymphoma invasion and metastasis 1 (Tiam1), a new gene related to the metastasis of colorectal cancer, can up-regulate the expression of HSP27 in the colorectal cancer cells[14-16]. This re-

sult is in accordance with the result of our early research[13], indicating that RSCC and LSCC may differ in their mechanisms, especially that of metastasis. The different expression of HSP27 in RSCC and in LSCC might be one of the important mechanisms that caused the difference between RSCC and in LSCC.

Another important function of HSP27 is related to the chemotherapy sensitivity or tolerance[17-21]. HSP27 is the reactive protein of 5-FU. 5-FU can increase the expression of HSP27 in colorectal cancer tissues, and down-regulated expression of HSP27 can decrease the tolerance of colon cancer to 5-FU. 5-FU can phosphorylate the p38 mitogen-activated protein kinase (MAPK) and HSP27 in colon cancer cells, and blocking of p38 MAPK can greatly inhibit the 5-FU-induced HSP27 phosphorylation. Also, it is found in colorectal cancer that HSP27 is related to the tolerance to chemotherapy drug Irinotecan, and inhibition the expression of HSP27 can increase the sensitivity of cancer cells to the drug. The interaction of cancer cells with normal cells can affect the expression of HSP27, HSP72, and multidrug resistance protein (MRP). In addition, HSP27 is related to thermal therapy and radiotherapy[22-23]. In study on thermal therapy of colon cancer, it is found that down-regulated expression of HSP27 will increase the heat-induced cancer cell apoptosis, while up-regulated expression of HSP27 is connected with the increased sensitivity of colon cancer cells to ultraviolet. These findings indicate that HSP27 is related to various therapies for cancers, and the mutual regulation between HSP27 and cell apoptosis is one of the important mechanisms. From the fact that the expression of HSP27 in RSCC is different from that in LSCC, we may conclude that the RSCC and LSCC may differ in the treatment, especially the sensitivity to chemotherapy. The relationship between the expression of HSP27 and biological behaviors of cancer is quire complicated. And how the expression of HSP27 in RSCC and LSCC affects their biological behaviors remains a question, and requires further study.

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