**Scutellaria barbata** polysaccharides inhibit tumor growth and affect the serum proteomic profiling of hepatoma H22-bearing mice

LI LI1, XIAOYI XU1, LEILEI WU1, HAICHENG ZHU2, ZHIPENG HE1, BO ZHANG3, YANJUN CHI4 and GAOCHEN SONG1

1Department of Basic Medicine, Mudanjiang Medical University, Mudanjiang, Heilongjiang 157011; 2Department of Digestive Surgery, Mudanjiang Anorectal Hospital, Mudanjiang, Heilongjiang 157000; 3Department of Clinical Laboratory, Tumor Hospital of Mudanjiang City, Mudanjiang, Heilongjiang 157009; 4Department of Brain Surgery, Mudanjiang First People's Hospital, Mudanjiang, Heilongjiang 157011, P.R. China

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**Abstract.** The present study aimed to evaluate the antitumor effect of *Scutellaria barbata* polysaccharides (SBPS) in a hepatoma mouse model and examine the serum proteins involved in the tumorigenesis and SBPS treatment. A hepatoma model was established by the subcutaneous inoculation of murine hepatocellular carcinoma into Kunming mice. The treatment (once a day) lasted until the tumor weight in the model group was ~1 g (~7-10 days after inoculation). The sera proteins from each group were then collected and subjected to two-dimensional gel electrophoresis. Differentially expressed proteins were screened out and representatives were identified using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. SBPS treatment at different doses significantly inhibited hepatoma growth (all P<0.01 vs. model group). The comparative serum proteomics showed that pseudouridine synthase 1 and chain A of the signal recognition particle Alu RNA-binding heterodimer (Srp9/14) were increased in the serum of the H22 hepatoma-bearing mice, and both were reduced by SBPS treatment. Mitochondrial ribosomal protein L24 was absent from the serum of H22 hepatoma-bearing mice, and was restored by SBPS treatment to approximately the normal level. Taken together, SBPS inhibited the growth of hepatic carcinoma in mice and affected serum proteomic profiling.

**Introduction**

*Scutellaria barbata* D. Don (*S. barbata*; Ban zhi lian in Chinese) is used as an immunomodulatory and antitumor agent in traditional Chinese medicine (1). Extracts of *S. barbata* have exhibited growth-inhibitory effects in a number of types of cancer *in vitro* and/or *in vivo*, including liver cancer (2,3). A recent study revealed that the immunomodulatory function of *S. barbata* extracts on Th1 and Th17 immune responses was involved in its antitumor effect when treating hepatoma H22-bearing mice (4). The active ingredients in *S. barbata* extracts were investigated regarding their different inhibitory effects on hepatocarcinoma and underlying mechanisms, and it was found that total flavonoids from *S. barbata* impaired the viability (5) and metastatic capability (6) of MHCC97H human hepatocarcinoma cells *in vitro*, which were associated with regulation of the mitochondrial apoptotic pathway (5) and matrix metalloproteinases (6), respectively.

*S. barbata* polysaccharides (SBPSs) are another category of main active ingredients extracted from *S. barbata* (7). SBPS have been reported to not only inhibit the proliferation of lung carcinoma cells *in vitro* and in a subcutaneous xenograft model in a dose-dependent manner, but also downregulate phosphorylated (p)-c-Met and its downstream signaling molecules, including p-extracellular signal-regulated kinase (p-ERK) and p–protein kinase B (p-AKT) (8). Yang et al showed that SBPS potently inhibited cell proliferation and human epidermal growth factor receptor (HER)2 phosphorylation in HER2-mutated non-small cell lung cancer *in vitro* and in xenograft models (9). In addition, a previous study revealed that SBPS inhibited the proliferation and elevated the apoptosis of human colon cancer HT29 cells. SBPS also inhibited the epithelial-mesenchymal transition of HT29 cells by upregulating the mRNA expression of E-cadherin and
downregulating that of N-cadherin and vimentin (10). However, there has been limited investigation of the effects of SBPS on liver cancer so far. In addition, comprehensive analyses of the molecular mechanisms underlying the antitumor effect of SBPS using high-throughput omics technologies are required.

Serum contains an abundance of proteins that are important indicators of physiological or pathological states. Therefore, comprehensive determination of the proteome of human serum with high accuracy and availability has the potential to identify disease biomarkers, monitor disease development, and identify the mechanisms underlying disease development (11). Matrix-assisted laser (induced) desorption ionization (MALDI)-based profiling of serum proteomics has been approved for routine diagnostics (12). Examining serum proteomics with the assistance of MALDI time-of-flight mass spectrometry (MALDI-TOF MS) analysis has identified several potential targets for treating hepatocellular carcinoma (13,14).

In the present study, an experimental model of mice bearing H22 hepatic carcinoma was established and treated with different dosages of SBPS. The antitumor effect of SBPS on hepatic carcinoma was investigated. In addition, two-dimensional gel electrophoresis (2-DE) was applied to establish the serum protein patterns of normal mice, tumor-bearing mice and SBPS-treated tumor-bearing mice. The differential protein spots were analyzed using MALDI-TOF MS and the resulting protein sequences were used to search the National Center for Biotechnology Information (NCBI) database for validation. Six serum proteins were identified between the normal and SBPS-treated tumor-bearing mice. This study may provide a novel perspective for treating hepatocellular carcinoma with S. barbata extracts.

Materials and methods

Establishment of the hepatic carcinoma mouse model, treatment, and sample collection. Kunming mice (23±1°C, 12 h light/dark cycle, with free access to food and water), weighing 20±2.0 g (males and females in equal numbers), were purchased from the Cancer Institute and Hospital (Chinese Academy of Sciences, Little Chalfont, UK). The mice were housed in a standard animal room maintained at 20±2°C, 60% humidity, and 12 h light/dark cycle, with free access to food and water. The mice were randomly assigned to different groups. The antitumor effect of SBPS on hepatic carcinoma was investigated. In addition, two-dimensional gel electrophoresis (2-DE) was applied to establish the serum protein patterns of normal mice, tumor-bearing mice and SBPS-treated tumor-bearing mice. The differential protein spots were analyzed using MALDI-TOF MS and the resulting protein sequences were used to search the National Center for Biotechnology Information (NCBI) database for validation. Serum was collected from the blood of normal mice, tumor-bearing mice and SBPS-treated tumor-bearing mice. The serum samples were preprocessed using the Calbiochem® ProteoExtract™ Albumin/IgG Removal kit (EMD Millipore; Merck KGaA, Darmstadt, Germany), and then precipitated with cold acetone. The precipitated samples were resolved in protein extraction buffer (200 µl per 10 mg samples) containing 1 mM PMSF, 2 mM EDTA and 10 mM DTT.

MALDI-TOF MS. The protein spots were excised from the gel manually, digested with trypsin and desalinated. The peptide mixtures were identified via Ultraflex TOF/TOF MS (Bruker Daltonics, Bremen, Germany) in the reflection mode with a 337 nm, with an ion extraction delay of 0 ns. Subsequently, 100 shots were accumulated per spectrum, and trypsin autolysis peaks were used as internal calibrant and adrenocorticotropic hormone as external calibrant to obtain peptide mass fingerprints (PMFs).

Protein identification. The signal peaks of single isotopes were acquired from the PMF images using Flexanalysis 3.0 software, and PMFs were searched against the NCBI database (https://www.ncbi.nlm.nih.gov/protein) using the Mascot program (Matrix Science, Ltd., London, UK, http://www.matrixscience.com/search_form Select.html). The search parameters were: Mus musculus; trypsin digestion; ion species: Monoisotopic and MH+; carbamidomethyl modification of cysteine as a fixed modification; oxidation of methionine as a variable modification; and mass error of peptide fragments: ±0.1%.
Statistical analysis. The data were analyzed using SPSS 13.0 statistical analysis software (SPSS, Inc., Chicago, IL, USA). The experimental results are expressed as the mean ± standard deviation. The differences among groups were compared using one-way analysis of variance and Tukey’s post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Antitumor effect of SBPS on H22 hepatoma. To evaluate the effect of SBPS on hepatoma growth, the tumor-bearing mice were injected with SBPS, CTX, or saline every day for 7 days (~7-10 days after H22 cell inoculation). The maximum tumor

Table I. Antitumor effect of SBPS on H22 hepatoma.

| Group            | Dose [mg/(kg · day)] | Tumor weight (g) | Inhibition rate (%) |
|------------------|----------------------|-------------------|---------------------|
| Model            | -                    | 1.083±0.236       | -                   |
| CTX              | 30                   | 0.390±0.119*      | 63.99               |
| High-dose SBPS   | 200                  | 0.627±0.163*a,b   | 43.03               |
| Moderate-dose SBPS| 100                 | 0.505±0.185*      | 49.68               |
| Low-dose SBPS    | 50                   | 0.680±0.157*a,b   | 37.21               |

Mice bearing H22 hepatic carcinoma were randomly divided into five groups: Model, CTX, and SBPS high-dose/middle-dose/low-dose groups. From 6 h post-inoculation, saline, CTX and SBPS were intraperitoneally injected once a day for 7 days. The mice were sacrificed 24 h after the final drug administration. The results are expressed as the mean ± standard deviation and were analyzed using analysis of variance and Tukey’s post hoc test. *P<0.01, compared with the model group; a,b P<0.05, compared with the CTX group. N=10/group. CTX, cyclophosphamide; SBPS, Scutellaria barbata polysaccharides.
Table II. Differentially expressed proteins between the normal and tumor-bearing mice with a fold-change of >3 or <0.3.

| ID  | Protein isoelectric point | MW (Da) | Fold-change (tumor vs. normal) |
|-----|--------------------------|---------|-------------------------------|
| 33a | 5.73                     | 15,799  | -                             |
| 42a | 5.09                     | 17,393  | -                             |
| 43a | 5.86                     | 17,211  | -                             |
| 45a | 5.55                     | 18,195  | -                             |
| 53a | 5.13                     | 19,925  | -                             |
| 58a | 5.12                     | 21,334  | -                             |
| 69a | 4.85                     | 24,353  | -                             |
| 176a| 5.25                     | 37,256  | -                             |
| 232a| 5.58                     | 45,714  | -                             |
| 157 | 6.18                     | 35,678  | 0.140                         |
| 79  | 6.64                     | 28,093  | 0.144                         |
| 218 | 5.10                     | 41,945  | 0.159                         |
| 97  | 6.02                     | 30,039  | 0.198                         |
| 228 | 5.24                     | 43,818  | 0.202                         |
| 262 | 5.40                     | 51,344  | 0.206                         |
| 287 | 5.49                     | 55,973  | 0.209                         |
| 336 | 6.06                     | 77,358  | 0.217                         |
| 172 | 5.99                     | 36,731  | 0.219                         |
| 159 | 5.60                     | 35,150  | 0.235                         |
| 104 | 5.39                     | 30,112  | 0.239                         |
| 167 | 5.46                     | 35,627  | 0.242                         |
| 150 | 4.85                     | 35,300  | 0.244                         |
| 186 | 5.31                     | 38,657  | 0.256                         |
| 109 | 6.23                     | 31,088  | 0.270                         |
| 122 | 6.27                     | 32,303  | 0.273                         |
| 121 | 5.05                     | 32,280  | 0.279                         |
| 215 | 5.22                     | 42,005  | 0.280                         |
| 292 | 5.68                     | 57,306  | 0.282                         |
| 274 | 5.20                     | 52,815  | 0.292                         |
| 185 | 5.60                     | 37,389  | 0.294                         |
| 148 | 5.84                     | 34,827  | 0.304                         |
| 191 | 5.49                     | 38,767  | 0.306                         |
| 115 | 5.80                     | 31,780  | 0.311                         |
| 96  | 6.66                     | 29,846  | 0.329                         |
| 348 | 4.63                     | 93,782  | 3.16                          |
| 340 | 5.37                     | 85,259  | 3.32                          |
| 296 | 6.83                     | 57,848  | 3.33                          |
| 349 | 4.54                     | 93,040  | 3.48                          |
| 281 | 6.43                     | 54,928  | 3.61                          |
| 295 | 6.68                     | 58,487  | 3.61                          |
| 226 | 6.12                     | 44,861  | 3.72                          |
| 222 | 4.54                     | 42,726  | 4.11                          |
| 343 | 4.93                     | 89,953  | 4.28                          |
| 345 | 5.07                     | 87,836  | 4.28                          |
| 246 | 5.93                     | 48,676  | 4.30                          |
| 347 | 4.71                     | 92,488  | 4.34                          |
| 341 | 5.22                     | 86,281  | 4.61                          |
| 346 | 4.82                     | 90,850  | 4.62                          |
| 225 | 4.39                     | 43,407  | 5.15                          |
| 210 | 6.20                     | 41,975  | 5.54                          |
| 229 | 4.26                     | 44,024  | 7.16                          |
| 233 | 4.15                     | 45,002  | 8.41                          |
Compared with the model group, the tumor weights of the CTX and SBPS high-, moderate-, and low-dose groups were significantly decreased (all P<0.01; Table I). Compared with the CTX group, the low- and high-dose SBPS groups were significantly different (both P<0.05; Table I); the moderate-dose SBPS group exhibited comparable antitumor effects (P>0.05; Table I). Therefore, SBPS efficiently inhibited the growth of hepatoma in mice and the inhibitory rate of the moderate-dose SBPS was the highest (49.68%; Table I).

### Table II. Continued.

| ID  | Protein isoelectric point | MW (Da) | Fold-change (tumor vs. normal) |
|-----|---------------------------|---------|-------------------------------|
| 20b | 6.19                      | 21,225  | -                             |
| 22b | 6.68                      | 21,243  | -                             |
| 192b| 4.21                      | 42,905  | -                             |
| 195b| 4.37                      | 42,243  | -                             |
| 197b| 4.28                      | 42,557  | -                             |
| 205b| 4.85                      | 44,630  | -                             |
| 238b| 6.43                      | 49,488  | -                             |
| 262b| 4.45                      | 53,826  | -                             |
| 266b| 4.41                      | 53,975  | -                             |
| 269b| 4.50                      | 53,310  | -                             |

*Proteins found in normal mice but not in tumor-bearing mice, sorted according to ID number in ascending order. Protein found in tumor-bearing mice but not in normal mice, sorted according to ID number in ascending order. All other proteins are sorted according to the fold-change of protein levels (tumor/normal) in ascending order. MW, molecular weight.

### Table III. Differentially expressed proteins between the SBPS-treated and tumor-bearing mice with a fold-change of >3 or <0.3.

| ID  | Protein isoelectric point | MW (Da) | Fold-change (SPBS vs. tumor) |
|-----|---------------------------|---------|-------------------------------|
| 12a | 4.66                      | 15,103  | -                             |
| 16a | 4.64                      | 19,473  | -                             |
| 20a | 6.19                      | 21,225  | -                             |
| 22a | 6.68                      | 21,243  | -                             |
| 100a| 4.56                      | 34,106  | -                             |
| 106a| 4.49                      | 34,385  | -                             |
| 138a| 5.92                      | 37,999  | -                             |
| 205a| 4.85                      | 44,630  | -                             |
| 238a| 6.43                      | 49,488  | -                             |
| 260a| 6.10                      | 53,604  | -                             |
| 213 | 6.69                      | 46,770  | 0.271                         |
| 266 | 4.41                      | 53,975  | 3.490                         |
| 268 | 5.20                      | 54,874  | 3.690                         |
| 93  | 5.12                      | 33,111  | 3.740                         |
| 305 | 5.07                      | 61,268  | 3.760                         |
| 144 | 6.52                      | 38,538  | 4.190                         |
| 239 | 5.76                      | 50,451  | 4.510                         |
| 18b | 6.64                      | 49,338  | -                             |

*Proteins found in tumor-bearing mice but not in SBPS-treated mice, sorted according to the ID no. in ascending order. Protein found in SBPS-treated mice but not in tumor-bearing mice. All other proteins are sorted according to the fold-change of protein levels (tumor/normal) in ascending order. SBPS, *Scutellaria barbata* polysaccharides; MW, molecular weight.

volume was ~10-15 mm³. Compared with the model group, the tumor weights of the CTX and SBPS high-, moderate-, and low-dose groups were significantly decreased (all P<0.01; Table I). Compared with the CTX group, the low- and high-dose SBPS groups were significantly different (both P<0.05; Table I); the moderate-dose SBPS group exhibited comparable antitumor effects (P>0.05; Table I). Therefore, SBPS efficiently inhibited the growth of hepatoma in mice and

2-DE mapping of serum proteins in the normal, tumor and SBPS groups. The total numbers of serum protein spots in the normal, tumor, and SBPS groups were 347±5, 335±3 and 252±7, respectively. Software analysis showed 62 protein spots with a change of >3-fold between the normal and tumor
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Analysis of the differences in the 2-DE map. The clear, large and heavily stained protein spots were selected as differentially expressed proteins from the three independent 2-DE images. The normal group vs. model group and the SBPS group vs. model group were compared to identify the differentially expressed serum proteins. The spots with fold-changes of $>3$ or $<0.3$ (tumor/normal or SBPS/tumor) were selected (Tables II and III). Table IV shows the serum proteins which were differentially expressed between the normal and tumor-bearing mice but rescued by SBPS treatment. Among them, spots 33, 176, 232 and 257 were expressed in the normal group, absent in the tumor group, and to a certain extent, restored in the SBPS group; whereas spots 20, 22, 205 and 238 exclusively appeared in the tumor group and disappeared upon SBPS treatment. In addition, spots 150, 196, 283 and 310 in the tumor group were lower than those in the normal group; however, following SBPS treatment, the expression levels of these proteins were either restored to an extent (spots 150 and 196) or even exceeded the normal level (spots 198, 283 and 310). By contrast, spots 234, 296 and 349 in the tumor group were higher than those in the normal group; following SBPS

Table IV. Serum proteins differentially expressed between the normal and tumor-bearing mice but rescued by SBPS treatment.

| ID | PI  | MW (Da) | Normal | Tumor | SBPS | Fold-change (tumor vs. normal) | Fold-change (SBPS vs. normal) |
|----|-----|---------|--------|-------|------|-------------------------------|-------------------------------|
| 257a | 6.42 | 50,071 | 1,354.16 | - | 318.46 | - | 0.235 |
| 232a | 5.58 | 45,714 | 675.51 | - | 559.23 | - | 0.828 |
| 33a  | 5.73 | 15,799 | 623.64 | - | 137.48 | - | 0.220 |
| 176a | 5.25 | 37,256 | 417.18 | - | 567.86 | - | 1.360 |
| 310  | 4.25 | 61,307 | 1,109.01 | 85.52 | 2,060.76 | 0.0771 | 1.860 |
| 218  | 5.10 | 41,945 | 847.48 | 145.76 | 444.41 | 0.172 | 0.524 |
| 150  | 4.85 | 35,300 | 435.68 | 81.82 | 307.92 | 0.188 | 0.707 |
| 283  | 6.13 | 55,710 | 668.29 | 126.31 | 1,830.14 | 0.189 | 2.740 |
| 196  | 6.38 | 39,969 | 1,319.16 | 299.31 | 1,261.29 | 0.227 | 0.956 |
| 198  | 6.70 | 40,396 | 306.08 | 116.53 | 1,047.45 | 0.381 | 3.420 |
| 325  | 4.90 | 71,029 | 187.60 | 94.61 | 357.99 | 0.504 | 1.910 |
| 296  | 6.83 | 57,848 | 761.96 | 1,954.87 | 583.35 | 2.57 | 0.766 |
| 349  | 4.54 | 93,040 | 539.96 | 1,444.62 | 187.35 | 2.68 | 0.347 |
| 234  | 6.65 | 46,292 | 263.39 | 1,485.30 | 404.69 | 5.64 | 1.540 |
| 205b | 4.85 | 44,630 | - | 138.75 | - | - | - |
| 20b  | 6.19 | 21,225 | - | 696.90 | - | - | - |
| 22b  | 6.68 | 21,243 | - | 1,382.00 | - | - | - |
| 238b | 6.43 | 49,488 | - | 2,139.15 | - | - | - |

Proteins found in normal mice but not in tumor-bearing mice, sorted in descending order of protein levels in the serum of normal mice.
Proteins found in tumor-bearing mice but not in normal mice, sorted in ascending order of protein levels in the serum of tumor-bearing mice.
All the other proteins are sorted according to the fold-change of protein levels (tumor/normal) in ascending order. SBPS, Scutellaria barbata polysaccharides; MW, molecular weight.

Figure 2. Peptide mass fingerprint of protein spot 150.
treatment, the expression of these proteins were either restored to an extent (spot 234) or were below the normal level (spots 296 and 349).

**MALDI-TOF MS analysis.** Accordingly, six representative protein spots (150, 196, 234, 296, 232 and 238) were selected for further analysis. Through MALDI-TOF MS analysis, the respective PMFs of spots 150 (Fig. 2), 196 (Fig. 3), 234 (Fig. 4), 296 (Fig. 5), 232 (Fig. 6) and 238 (Fig. 7) were obtained. The six protein spots were identified in the NCBI database (Table V). Taken together, the results showed that pseudouridine synthase 1

| ID  | Protein name | gi    | MS score | Peptides matched (n) | Predicted MW (Da) | Protein isoelectric point | Sequence coverage (%) |
|-----|--------------|-------|----------|----------------------|-------------------|---------------------------|-----------------------|
| 150 | Unnamed      | gi26352025 | 85        | 24                   | 40,885            | 9.22                      | 52                    |
| 196 | LOC10106021  | gi407256771 | 87        | 8                    | 10,789            | 11.87                     | 89                    |
| 234 | Pseudouridine synthase 1, isoform CRA_b | gi148680837 | 82        | 21                   | 51,127            | 9.10                      | 44                    |
| 296 | Chain A, signal recognition particle Alu | gi157829621 | 73        | 21                   | 26,560            | 9.64                      | 70                    |
| 232 | Mitochondrial ribosomal protein L24, isoform CRA_b | gi148623388 | 66        | 11                   | 27,036            | 9.49                      | 49                    |
| 238 | mCG16643, isoform CRA_b | gi148703223 | 77        | 27                   | 51,801            | 5.47                      | 43                    |
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Figure 7. Peptide mass fingerprint of protein spot 238.

(Pus1p; spot 234) and chain A of the signal recognition particle Alu RNA-binding heterodimer (Srpr9/14; spot 296) were increased in the serum of H22 hepatoma-bearing mice, and both were reduced by SBPS treatment. In addition, mitochondrial ribosomal protein L24 (MRPL24; spot 232) was absent from the serum of H22 hepatoma-bearing mice, and this was restored approximately to the normal level by SBPS treatment.

Discussion

In the present study, a hepatoma mouse model was established to evaluate whether SBPS had an antitumor effect on H22 hepatoma in vivo and to determine how SBPS treatment affected the serum proteome of tumor-bearing mice. The study found that SBPS at different dosages inhibited hepatoma growth. Differentially expressed proteins were identified on comparing the serum proteome of the healthy control mice, tumor-bearing mice, and SBPS-treated tumor-bearing mice. Accordingly, several abnormally expressed serum proteins in the tumor-bearing mice were rescued by SBPS administration, indicating the antitumor effect of SBPS. In the present study, the results showed that the inhibitory effects of SBPS on the tumors were not dose-dependent. The tumor inhibition rate was lower in the high-dose group than that in the moderate-dose group. It may be that excessive doses of drugs increase the burden on the liver and kidney, and may have a negative impact on the anticancer mechanisms in the body, such as immune function. Even if the dose-limiting toxicity has been examined in a previous study, additional toxicity testing of SBPS is required in order to determine the exact mechanisms of action.

Other than the direct inhibition of cancer cell proliferation, SBPS also showed antioxidant effects in vitro and anti-angiogenic and immune-regulation effects in vivo, which may systematically regulate the metabolism in patients with cancer or tumor-bearing animals. Therefore, proteomic analysis is suitable for the comprehensive evaluation of serum proteins, which can reflect the multifaceted effects of SBPS on hepatoma mice. SBPS inhibits tumor growth via different molecular pathways in various types of cancers, indicating the cancer type-dependent effects of SBPS. Seven differentially expressed proteins were identified between healthy control mice, tumor-bearing mice, and SBPS-treated tumor-bearing mice in the literature, including three functional proteins relevant to solid tumors, three proteins with unknown functions, and an unnamed protein.

PUS enzymes catalyze the site-specific isomerization of uridine to generate pseudouridine (PU) (20). Elevated PU excretion has been detected in different diseases, particularly in the urine of patients with advanced cancer (21,22). Previously, the positivity of urinary and serum PU in patients with hepatoma was reported to be 71.3% and 70.0%, respectively, and PU levels were reduced to normal levels following tumor resection (23). The present study showed that the serum level of Pus1p was low in normal mice, increased in tumor-bearing mice, but restored to a low level in SBPS-treated tumor-bearing mice. This may partially explain why PU is abnormally increased in patients with hepatoma, as Pus1p catalyzes the generation of PU. Serum Pus1p may be of clinical significance in the diagnosis and monitoring of primary liver cancer. However, the way in which SBPS inhibits the expression or secretion of Pus1p requires investigation in the future.

The heterodimeric protein complex SRP9/SRP14, as a component of the SRP, binds to 7SL RNA or cytoplasmic Alu RNA to form a complex known as Alu RNP (24). In these two forms, the SRP9/14 dimer mainly delays peptide elongation and possibly inhibits the initiation of protein synthesis (25). Reports have indicated the involvement of SRP9/14 in cancer development. For example, Rho et al combined 2-D PAGE and tandem mass spectrometry to identify five differentially expressed proteins between normal colon and colon cancer tissues. SRP9 was one of the upregulated proteins in cancer tissues, which was also confirmed by western blot analysis and immunohistochemistry, suggesting the upregulation of SRP9 is a candidate biomarker of colon cancer (26). An early study revealed that SRP9 was one of the upregulated genes in Chinese patients with hepatocellular carcinoma (27). Consistently, the expression level of SRP9 was found to be increased in tumor tissues. Regulation of the expression of SRP9/14 in liver cancer remains to be elucidated; however, alterations in the expression levels of SRP9/14 indicate that SBPS regulates specific protein synthesis within the tumor or in the whole body of tumor-bearing mice.

MRPL24 is one of the proteins encoded by nuclear genes (28). It facilitates the specific requirements of protein synthesis in mitochondria (29). In the present study, MRPL24 was expressed in healthy control mice but absent in tumor-bearing mice, suggesting that this protein was important in the process of tumorigenesis. This suggests that MRPL24 may be a novel target for the diagnosis of liver cancer.

In conclusion, the antitumor effect of SBPS was confirmed in the hepatoma mouse model and seven differentially expressed serum proteins were identified using proteomic analysis in mice with/without SBPS treatment. These differentially expressed proteins supported the antitumor mechanism of SBPS. They may provide valuable clues for the pathogenesis, diagnosis and treatment of liver cancer.

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Availability of data and materials

The dataset supporting the results of this study are included within the article.

Authors' contributions

GS and LL conceived and supervised the study; GS and LL designed experiments; LL, XX and LW performed experiments; HZ completed the animal experiments and blood sample collection; ZH developed new software and performed simulation experiments; BZ and YC analyzed data; LL, XX and LW wrote the manuscript; GS and LL made manuscript revisions. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Mudanjiang Medical University (Mudanjiang, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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