Original Article

Proteomic identification of alpha-2-HS-glycoprotein as a plasma biomarker of hypopharyngeal squamous cell carcinoma

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Abstract: Hypopharyngeal squamous cell carcinoma (HSCC) has very poor prognosis compared with other head and neck squamous cell carcinomas. Late-stage diagnosis of HSCC increases mortality. Therefore, more effective biomarkers for early diagnosis of HSCC are necessary. Unfortunately, appropriate biomarkers for clinical diagnosis and prognosis have not been identified yet. However, recent progresses in quantitative proteomics have offered opportunities to identify plasma proteins as biomarkers for HSCC. In the present study, plasma samples were analyzed by two-dimensional differential gel electrophoresis (2D-DIGE), and differentially expressed proteins were identified by matrix assisted laser desorption ionization-time of flight/time of flight mass spectrometry (MALDI-TOF/TOF MS). A total of 26 proteins representing 12 unique gene products were identified. The up-regulation proteins were alpha-2-HS-glycoprotein (AHSG), complement C4-B, haptoglobin, C-reactive protein, and ceruloplasmin, whereas the down-regulation proteins were serum albumin, angiotensinogen, alpha-1-antichymotrypsin, Ig gamma-3 chain C region, fibrinogen gamma chain, apolipoprotein A-I, and Ig kappa chain C region. Among all the differentially expressed proteins, AHSG was validated by western blot and ELISA. The results were consistent with the data from 2D-DIGE, further suggesting that AHSG may be employed as a potential biomarker for the early diagnosis of HSCC. In summary, this study was the first to use 2D-DIGE and MALDI-TOF/TOF platform to identify the potential plasma biomarkers for HSCC. The plasma AHSG showed great potential for HSCC screening.

Keywords: Hypopharyngeal squamous cell carcinoma, plasma, biomarker, proteomics, differential in-gel electrophoresis, AHSG

Introduction

Hypopharyngeal squamous cell carcinoma (HSCC) accounts for approximately 5-15% of all head and neck cancers [1]. Over the past decade, the 5-year survival rate of HSCC is estimated to be at 25-40% [2], and it has not been markedly improved despite recent advances in various treatment modalities, including surgery, radiotherapy, and chemotherapy [3]. The poor prognosis of HSCC might be because of the lack of early detection and high rate of metastasis [4]. Detection of HSCC at an earlier stage would be beneficial to patients. Many molecules, such as SIRT1, DBC1 [5], Beclin-1, LC3 [6], and Caveolin-1 [7], have been evaluated as candidate biomarkers for HSCC, but none has been widely used in practice because of the lack of understanding of the molecular mechanisms involved in HSCC development, progression, and treatment response [8]. Therefore, studies of novel and more effective molecular biomarkers for early diagnosis of HSCC are necessary.

Recent progresses in quantitative proteomics have offered promising opportunities to discover biomarkers for HSCC. To date, most studies...
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Materials and methods

Chemicals and reagents

Generic chemicals and albumin and IgG depletion kit were purchased from Sigma-Aldrich (USA). Reagents for 2D-DIGE were purchased from GE Healthcare (Sweden). All the chemicals and biochemicals used in this study were of analytical grade.

Plasma sample collection and purification

A total of 96 donors from one hospital (Nanfang Hospital, Southern Medical University, Guangdong, China) were enrolled in this study from Jan 2013 to Dec 2013. The subjects were divided into HSCC patients (n=48) and healthy donor group (n=48). Eight cases of early stage HSCC patients and eight cases of healthy donors were enrolled in proteomic and western blot analyses, whereas the other 40 cases of early stage HSCC patients and 40 cases healthy donors were enrolled in ELISA analyses. The criteria for assessing the presence of HSCC were based on the pathological diagnosis and guidelines proposed by the World Health Organization. Healthy individuals were selected with similar age and with no clinical HSCC diagnosis. This study was approved by the Institutional Research Board of Southern Medical University and carried out according to the Helsinki Declaration Principles. Written informed consent was collected from all participating subjects.

To improve the performance of proteomic analysis of the plasma samples, the albumin and immunoglobulin G in the collected plasma samples were depleted using an albumin and IgG removal kit (Sigma, USA) according to the instructions of the manufacturer. The depleted plasma samples were then precipitated with a 2D Clean-up Kit (GE Healthcare, Sweden) according to the protocol of the manufacturer, and then resuspended in Lysis Buffer (7 M urea, 2 M thiourea, 4% CHAPS, 30 mM Tris-HCl, at pH 8.5). Protein concentrations were determined by 2D Quant Kit (GE Healthcare, Sweden) according to the instructions of the manufacturer. The purified samples were then preserved at -80°C before DIGE.

2D-DIGE and gel image analysis

Before performing 2D-DIGE, plasma samples from 8 early stage HSCC patients and 8 healthy donors were pooled respectively. Approximately 50 μg of protein samples from patients and healthy donors were labeled with 400 pmol of either Cy3 or Cy5 for triplicate comparison on three 2DE gels. To facilitate image matching and cross-gel statistical comparison, 50 μg of pooled proteins (a pool of all samples) was also prepared and labeled with 400 pmol Cy2 as an internal standard for every gel. Thus, the triplicate samples and the internal standard can be run and quantified on multiple 2DE gels. At the same time, a preparative gel using 800 μg of pooled protein sample without labeling was prepared. The plasma sample arrangement for a triplicate 2D-DIGE experiment is shown in
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Table 1. Plasma samples arrangement for a triplicate 2D-DIGE experiment

<table>
<thead>
<tr>
<th>Gel No.</th>
<th>Cy2 (50 μg)</th>
<th>Cy3 (50 μg)</th>
<th>Cy5 (50 μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel 1</td>
<td>Pool</td>
<td>Healthy Donors</td>
<td>HSCC Patients</td>
</tr>
<tr>
<td>Gel 2</td>
<td>Pool</td>
<td>HSCC Patients</td>
<td>Healthy Donors</td>
</tr>
<tr>
<td>Gel 3</td>
<td>Pool</td>
<td>Healthy Donors</td>
<td>HSCC Patients</td>
</tr>
</tbody>
</table>

Table 1. The labeling reactions were performed in the dark on ice for 30 min, and then quenched with a 20-fold molar ratio excess of free L-lysine to dye for 10 min. The differently Cy3- and Cy5-labeled samples were mixed with the Cy2-labeled internal standard and reduced with dithiothreitol for 10 min. The IPG buffer, pH 3-10 nonlinear [2% (v/v), GE Healthcare], was added, and the final volume was adjusted to 450 μL with 2D-lysis buffer for rehydration. The rehydration process was performed with immobilized non-linear pH gradient (IPG) strips (pH 3-10 NL, 24 cm), which were later rehydrated by CyDye-labeled samples in the dark at room temperature overnight (at least 12 h). Isoelectric focusing was then performed using an IPGphor III apparatus (GE Healthcare) for a total of 60 kVh at 20°C. Strips were equilibrated in 6 M urea, 30% (v/v) glycerol, 1% (w/v) SDS, 100 mM Tris-HCl (pH 8.8), and 65 mM dithiothreitol for 15 min, and then in the same buffer containing 240 mM iodoacetamide for another 15 min. The equilibrated IPG strips were transferred onto 26×20 cm 12.5% polyacrylamide gels casted between low fluorescent glass plates. The strips were overlaid with 0.5% (w/v) low melting point agarose in a running buffer containing bromophenol blue. The gels were run in an Ettan™ DALT Six gel tank (GE Healthcare) at 3 Watt per gel at 10°C until the dye front had completely run off the bottom of the gels.

Afterward, the fluorescence 2DE gels were scanned directly between the low fluorescent glass plates using an Ettan™ DIGE Imager (GE Healthcare). This imager is a charge-coupled device-based instrument that enables scanning at different wavelengths for Cy2-, Cy3-, and Cy5-labeled samples. Gel analysis was performed using DeCyder 2D Differential Analysis Software v7.0 (GE Healthcare) to co-detect, normalize, and quantify the protein features in the images. Features detected from non-protein sources (e.g., dust particles and dirty backgrounds) were filtered out. Spots displaying 1.5 average-fold increase or decrease in abundance with a P<0.05 were selected for protein identification.

Protein staining and spot picking

The preparative gel was stained with colloidal Coomassie brilliant blue G-250. Bonded gels were fixed in 40% (v/v) ethanol, 10% (v/v) acetic acid for 1.5 h, washed three times (10 min each time) with ddH₂O, and then incubated in 20% (v/v) methanol, 10% (w/v) ammonium sulfate, 10% (v/v) phosphoric acid, and 0.12% w/v Coomassie brilliant blue G-250 for 5 to 7 days. No destaining step was required. The stained gels were then imaged on an Image Scanner III densitometer (GE Healthcare), which processed the gel images as TIF files. Matched spots of interest were picked automatically from the preparative gel by Ettan™ Spot Picker (GE Healthcare).

In-gel digestion and protein identification

The picked spots were destained with 50% acetonitrile (ACN)/100 mM NH₄HCO₃ for 10 min, dehydrated with 100% ACN for 10 min, and then dried using a centrifugal concentrator (TOMY SEIKO, Tokyo). Afterward, 2 μL of 25 ng/ml trypsin (Promega) diluted in 50 mM NH₄HCO₃ was added to each gel piece and incubated for 30 min at 4°C, and then 30 μL of 50 mM NH₄HCO₃ was added and the spots were incubated overnight at 37°C. Supernatants were collected, peptides were further extracted twice with 0.1% trifluoroacetic acid (TFA) in 50% acetonitrile for 15 min, and the supernatants were then pooled. Peptide extracts were vacuum-dried and dissolved with 2 μL 50% acetonitrile/0.1% TFA, and aliquots of 0.5 μL were applied on the target and air-dried. Subsequently, 0.5 μL of matrix solution (CHCA saturated in 50% acetonitrile/0.1% TFA) was added to the dried samples and allowed to dry again.

Samples on the MALDI target plates were then analyzed by ABI 4800 Proteomics Analyzer MALDI-TOF/TOF mass spectrometer (Applied Biosystems). A total of 800 shots were accumulated for MS analyses. MS/MS analyses were performed using air as collision energy of 2 KV. MASCOT search engine (version 2.1, Matrix Science) was used to search all of the tandem mass spectra. GPS Explorer™ software (version 3.6.2, Applied Biosystems) was used to create and search files with the MASCOT search for peptide and protein identification. Protein identities were obtained using Mascot searching engine against Swiss-Prot non-redundant sequence databases selected for human taxonomy.
Western blot

Western blot was used to validate the differential abundance of identified proteins by mass spectrometry. Aliquots of 20 μg of plasma proteins were diluted in Laemmli sample buffer (50 mM Tris, pH 6.8, 10% glycerol, 2% SDS, 0.01% bromophenol blue) and separated by SDS-PAGE following standard procedures. After electroblotting, the proteins were transferred onto 0.45 μm PVDF membranes (Millipore), and the membranes were blocked with 5% (w/v) skimmed milk in TBST (50 mM Tris, pH 8.0, 150 mM NaCl, and 0.1% Tween-20 (v/v)) for 1 h. Membranes were then incubated in primary antibody solution in TBST containing 0.02% (w/v) sodium azide for 2 h. Membranes were washed 3 times in TBST (5 min each time), and then incubated with the appropriate horseradish peroxidase-coupled secondary antibody for 1 h (Santa Cruz, USA). After washing 3 times with TBST, the reaction was detected by chemiluminescence with ECL reagents (Pierce Biotechnology, USA). All membranes were exposed and scanned by Carestream Image Station 4000R (Carestream Health, USA). A semi quantitative analysis based on OD was performed using Quantity One software (Bio-Rad, USA).

ELISA analysis

Polystyrene microtitration wells were coated with 50 μg of protein samples and incubated at 37°C for 2 h. The plate was washed for three times with phosphate buffered saline-Tween 20 (PBST) and three times with PBS. After the uncoated space was blocked with 100 μL of 5%
skimmed milk in PBS at 37°C for 2 h, the plate was washed three times with PBST. Antibody solution was added and incubated at 37°C for 2 h. After washing with PBST and PBS for a total of 10 times, 100 µL of peroxidase-conjugated secondary antibody in PBS was added for incubation at 37°C for 2 h. After 10 washings, 100 µL of 3,3',5,5'-tetramethyl Benzodrine (Pierce) was added. After incubation at room temperature for 30 min, 100 µL of 1 M H₂SO₄ was added to stop the reaction, followed by measurement of absorbance at 450 nm using a Stat Fax 2100 Microtiter Plate Reader (Awareness Technology Inc., FL, USA).

Statistical analysis

Statistical features in DeCyder were used for the evaluation of the DIGE gels. For all protein spots, comparison between HSCC/Control groups was calculated, as change in volume ratios and with Student t-test as selection criteria. Protein spots differentially expressed between groups (at least 50% change of ratios between groups and t-test P<0.05) were extracted. For western blot bands and ELISA analyses, two-tailed non-paired Student’s t-test was used to determine the mean differences between the two groups, and a P<0.05 was used to assess the significance of the differences using SPSS 13.0 software.

Results

Differentially expressed plasma proteins between HSCC patients and healthy donors

According to the DeCyder software analysis, about 1500 protein spots were constantly detected in each gel, which localized in the ranges of pl 3.10 and MW 10-200 kDa. On the basis of the average intensity ratios of protein spots, the spots were quantified, normalized, and inter-gel-matched. A total of 26 protein spots exhibited significant differences occurring between the HSCC patients and the healthy donors.
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honors, in which 13 protein spots were up-regulated and 13 down-regulated in HSCC patients compared to healthy controls. The differentially expressed protein figures that showed greater than 1.5-fold changes in the expression level were shown in Figure 1 (P<0.05).

Identification of differentially expressed proteins by MALDI-TOF/TOF MS

To identify the differentially expressed proteins of HSCC plasma, all 26 differentially-expressed protein spots were excised from these 2-DE gels and subjected to in-gel trypsin digestion and subsequent analyzed by MALDI-TOF/TOF MS. A total of 26 proteins representing 12 unique gene products were successfully identified by MALDI-TOF/TOF MS and database search. Figure 2 shows an example of a MALDI-TOF/TOF peptide mass fingerprint of protein spot No. 1316. The up-regulation proteins included alpha-2-HS-glycoprotein (AHSG), complement C4-B, haptoglobin, C-reactive protein, and ceruloplasmin, whereas the down-regulation proteins are serum albumin, angiotensinogen, alpha-1-antichymotrypsin, Ig gamma-3 chain C region, Ig kappa chain C region, fibrinogen gamma chain, and apolipoprotein A-I. Details of these proteins are listed in Table 2.

Validation of AHSG by western blotting

To validate the DIGE/MS-obtained results and further evaluate the nature and importance of some of the identified proteins, western blot analyses were performed. Among the varied proteins, AHSG was validated by western blot. As shown in Figure 3, AHSG was over-expressed.

Table 2. Differentially expressed plasma proteins in HSCC patients identified by MALDI-TOF/TOF compared with healthy donors

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein name</th>
<th>Gene name</th>
<th>Accession No.</th>
<th>MW/pI</th>
<th>Ion score</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>960</td>
<td>Serum albumin</td>
<td>ALB</td>
<td>P02768</td>
<td>71.3/5.92</td>
<td>85</td>
<td>-2.01</td>
</tr>
<tr>
<td>1259</td>
<td>Alpha-1-antichymotrypsin</td>
<td>SERPINA3</td>
<td>P01011</td>
<td>47.8/5.33</td>
<td>73</td>
<td>-1.70</td>
</tr>
<tr>
<td>1311</td>
<td>Alpha-2-HS-glycoprotein</td>
<td>AHSG</td>
<td>P02765</td>
<td>40.1/5.43</td>
<td>121</td>
<td>2.11</td>
</tr>
<tr>
<td>1316</td>
<td>Alpha-2-HS-glycoprotein</td>
<td>AHSG</td>
<td>P02765</td>
<td>40.1/5.43</td>
<td>145</td>
<td>4.23</td>
</tr>
<tr>
<td>1338</td>
<td>Angiotensinogen</td>
<td>SERPINA8</td>
<td>P01019</td>
<td>53.4/5.87</td>
<td>81</td>
<td>-1.73</td>
</tr>
<tr>
<td>1351</td>
<td>Ig gamma-3 chain C region</td>
<td>IGHG3</td>
<td>P01860</td>
<td>42.3/8.23</td>
<td>164</td>
<td>-1.97</td>
</tr>
<tr>
<td>1363</td>
<td>Fibrinogen gamma chain</td>
<td>FGG</td>
<td>P02679</td>
<td>52.1/5.37</td>
<td>103</td>
<td>-2.04</td>
</tr>
<tr>
<td>1374</td>
<td>Fibrinogen gamma chain</td>
<td>FGG</td>
<td>P02679</td>
<td>52.1/5.37</td>
<td>98</td>
<td>-2.74</td>
</tr>
<tr>
<td>1382</td>
<td>Fibrinogen gamma chain</td>
<td>FGG</td>
<td>P02679</td>
<td>52.1/5.37</td>
<td>215</td>
<td>-2.12</td>
</tr>
<tr>
<td>1402</td>
<td>Fibrinogen gamma chain</td>
<td>FGG</td>
<td>P02679</td>
<td>52.1/5.37</td>
<td>176</td>
<td>-1.94</td>
</tr>
<tr>
<td>1632</td>
<td>Complement C4-B</td>
<td>C4B</td>
<td>P00015</td>
<td>194.2/6.73</td>
<td>139</td>
<td>1.57</td>
</tr>
<tr>
<td>1702</td>
<td>Haptoglobin</td>
<td>HP</td>
<td>P00738</td>
<td>45.9/6.13</td>
<td>157</td>
<td>1.76</td>
</tr>
<tr>
<td>1706</td>
<td>Haptoglobin</td>
<td>HP</td>
<td>P00738</td>
<td>45.9/6.13</td>
<td>83</td>
<td>1.88</td>
</tr>
<tr>
<td>1715</td>
<td>Haptoglobin</td>
<td>HP</td>
<td>P00738</td>
<td>45.9/6.13</td>
<td>124</td>
<td>1.88</td>
</tr>
<tr>
<td>1718</td>
<td>Haptoglobin</td>
<td>HP</td>
<td>P00738</td>
<td>45.9/6.13</td>
<td>115</td>
<td>1.99</td>
</tr>
<tr>
<td>1729</td>
<td>Haptoglobin</td>
<td>HP</td>
<td>P00738</td>
<td>45.9/6.13</td>
<td>146</td>
<td>2.16</td>
</tr>
<tr>
<td>1916</td>
<td>Apolipoprotein A-I</td>
<td>APOA1</td>
<td>P02647</td>
<td>30.8/5.56</td>
<td>256</td>
<td>-2.02</td>
</tr>
<tr>
<td>1928</td>
<td>Ig kappa chain C region</td>
<td>IGKC</td>
<td>P01834</td>
<td>11.8/5.58</td>
<td>88</td>
<td>-1.62</td>
</tr>
<tr>
<td>1929</td>
<td>Ig kappa chain C region</td>
<td>IGKC</td>
<td>P01834</td>
<td>11.8/5.58</td>
<td>132</td>
<td>-1.67</td>
</tr>
<tr>
<td>1931</td>
<td>Ig kappa chain C region</td>
<td>IGKC</td>
<td>P01834</td>
<td>11.8/5.58</td>
<td>117</td>
<td>-1.54</td>
</tr>
<tr>
<td>1982</td>
<td>Ig kappa chain C region</td>
<td>IGKC</td>
<td>P01834</td>
<td>11.8/5.58</td>
<td>122</td>
<td>-1.53</td>
</tr>
<tr>
<td>1986</td>
<td>C-reactive protein</td>
<td>CRP</td>
<td>P02741</td>
<td>25.2/5.45</td>
<td>94</td>
<td>1.83</td>
</tr>
<tr>
<td>2145</td>
<td>Ceruloplasmin</td>
<td>CP</td>
<td>P00050</td>
<td>122.9/5.44</td>
<td>82</td>
<td>3.08</td>
</tr>
<tr>
<td>2227</td>
<td>Haptoglobin</td>
<td>HP</td>
<td>P00738</td>
<td>45.9/6.13</td>
<td>103</td>
<td>1.62</td>
</tr>
<tr>
<td>2232</td>
<td>Haptoglobin</td>
<td>HP</td>
<td>P00738</td>
<td>45.9/6.13</td>
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<td>2243</td>
<td>Haptoglobin</td>
<td>HP</td>
<td>P00738</td>
<td>45.9/6.13</td>
<td>172</td>
<td>1.68</td>
</tr>
</tbody>
</table>

*Spot No. is the unique sample spot protein number that refers to the labels in Figure 1; *Swiss-Prot database accession number; *Theoretical molecular weight (kDa) and theoretical pI; *Ion score of more than 66 is significant (P<0.05); *Fold change of the differentially expressed plasma proteins in HSCC patients compared with healthy donors.
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Discussion

Although early diagnosis is established as a mainstay of successful HSCC treatment, no accepted screening test exists for this cancer. Thus, new biomarkers with high sensitivity and specificity to detect HSCC at an early stage are urgently needed. Differential proteomics have been widely used to identify all types of biomarkers in cancer research [15]. Liang Zhou et al. reported that a diagnostic pattern consisting of four protein peaks with m/z values of 7796, 4216, 5927, and 5361 Da was established with a sensitivity of 94% and a specificity of 89% by SELDI-TOF-MS [13].

To the best of our knowledge, no research using 2D-DIGE/MALDI-TOF/TOF to identify plasma biomarkers of HSCC has been reported yet. Therefore, this study was the first to utilize this platform to look for potential plasma biomarkers for HSCC. Our proteomics analysis revealed 26 differentially expressed protein spots (13
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Up-regulated plasma proteins of HSCC

AHSG, also known as human fetuin-A, is a major serum glycoprotein synthesized and secreted by the liver. A number of studies have suggested that AHSG is a multifunctional protein involved in calcium homeostasis, bone development, and insulin sensitivity [16]. In addition, AHSG has the characteristics of a negative acute-phase protein, that is, the serum concentrations decrease significantly after major surgical procedures, trauma, burns, and severe inflammation [17]. Interestingly, AHSG can inhibit intestinal tumor progression by blocking TGF-β signal transduction [18]. In our results, AHSG was up-regulated in HSCC plasma, and validated by western blot and ELISA. Therefore, the up-regulation of plasma AHSG showed great potential diagnosis value in the identification HSCC from controls. The results suggested that AHSG may be a useful biomarker for HSCC.

Complement C4-B (C4B) is an isotype of complement C4. C4B functions more in the propagation of the activation pathways that lead to the formation of the membrane attack complex in attacking foreign antigens [19, 20]. Several studies, however, have reported that serum complement proteins may include efficient biomarkers related to cancers, such as ovarian cancer, breast cancer, and bladder cancer [21]. C4B was increased in our results, which might play an important role in the elimination of damaged tumor cells.

Haptoglobin (HP) is an acute phase protein synthesized by liver parenchymal cells, which is widely existing among serums and other body fluids in human and many other mammals, its main function is to combine the free hemoglobin so as to prevent the kidneys and vasculature from oxidative damage induced by free hemoglobin, and promote the cycle of heme iron [22]. Moreover, HP has important roles in the regulation of epidermal cell transformation, immune suppression in cancer, and angiogenesis. Studies have pointed out that HP has higher expressions in lung cancer, leukemia, breast cancer, prostate cancer, ovarian cancer, and gastric cancer [23-25], indicating that HP has potential clinical application value in tumor diagnosis. In this study, we found an increased change of HP between HSCC and controls. However, whether plasma HP can be a potential biomarker for screening HSCC requires further validations.

C-reactive protein (CRP), generally produced by hepatocytes, is an acute-phase protein, which will be increased in various inflammatory conditions [26]. CRP has been reported as a predictor of survival in different malignancies, including pancreatic, ovarian, pulmonary, and colorectal cancers [27]. The plasma level of CRP increased in the HSCC group, which suggested that CRP can be a predictor of HSCC.

Ceruloplasmin (CP) is the major copper-carrying protein in the blood and has a role in iron metabolism, exerting the copper-dependent activity of ferroxidase [28]. The Elevated levels of CP were identified in the serum and tissue of several malignancies and found associated with poor prognosis of cancers [29]. In our study, we found that CP expression was up-regulated in the plasma of HSCC, which was consistent to the previous reports.

Down-regulated plasma proteins of HSCC

Serum albumin (ALB), the main protein of plasma, is synthesized and secreted by the liver. ALB is generally used to assess the nutritional status, severity of disease, and disease progression and prognosis. In the hospital setting, many reports have related ALB level to in-hospital mortality, length of stay, and nosocomial infection. ALB has also been described as an independent prognosticator of survival in various cancers, such as lung, pancreatic, gastric, colorectal, and breast cancers. Low serum albumin has also been shown to be an independent indicator for prognosis in cancer patients with unknown primaries [30]. Our study revealed that ALB was down-regulated in HSCC plasma, indicating that malnutrition and inflammation of HSCC may suppress ALB synthesis.

Angiotensinogen (AGT), mainly synthesized in the liver, is the only known precursor of angio-
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tensin I. Recombinant human AGT inhibits human endothelial cell proliferation, migration, and angiogenesis in vitro [31]. A study demonstrated that an adenovirus encoding human AGT could inhibit tumor progression through an antiangiogenic effect in different mice models and represent a promising new strategy to block primary tumor growth and prevent metastasis [32, 33]. We interpreted that the down-regulated AGT in HSCC patients may play an important role in tumor growth.

Alpha-1 antichymotrypsin (ACT), is a protease inhibitor thought to limit tissue damage produced by excessive inflammation-associated proteolysis [34]. In addition, ACT has been recognized as a biomarker for certain cancers including colorectal adenocarcinoma [35], breast cancer [36], and cervical carcinoma [37]. In our experiment, ACT was down-regulated in the HSCC plasma. Thus, we speculated that ACT may be as a specific biomarker for HSCC.

Ig gamma-3 chain C region (IGHG3) and Ig kappa chain C region (IGKC) belong to the major immunoglobulin class in body secretions. High blood Igs and circulating immune complex values in patients with cancer have been repeatedly reported and suggested as tumor markers with varying regulation patterns in numerous cancers [38]. In our study, however, the decreased plasma levels of IGHG3 and IGKC were contrary to the previous reports about other cancers, revealing their potential as biomarkers for HSCC.

Fibrinogen gamma chain (FGG) is one of the fibrinogen degradation products generated during fibrinolysis. FGG has a COOH-terminal globular domain to which several integrin cell adhesion receptors bind. In addition, fibrinogen and its gamma chain contain many specific binding sites and can regulate cellular adhesion and invasion. Recently, the correlation of fibrinogen and its degradation products with carcinogenesis was reported in oral squamous carcinoma [39]. From our current DIGE results, the down-regulated FGG plasma level might be related to tumor thrombosis, which was suggested to be a useful predictor for HSCC.

Apolipoprotein A-I is a component of the high density lipoprotein responsible for the cholesterol transport into the liver. In numerous cancers, such as bladder cancer, oesophageal cancer, cholangiocarcinoma, ovarian cancer, and colon cancer, the apolipoprotein A-I level is up-regulated [40]; however, the reduced plasma apolipoprotein A-I level is associated with gastric tumor [41]. Our 2D-DIGE analysis revealed that the apolipoprotein A-I level was down-regulated in HSCC plasma. These previous reports and our results revealed that the regulation of the apolipoprotein A-I level during tumorigenesis was complicated and needed further clarification.

In conclusion, this study adopted the quantitative proteomics approach to screen the plasma biomarkers for HSCC, and 12 potential biomarkers were identified, in which AHSG might be a putative biomarker that has potential to serve as a useful tool for HSCC diagnosing and monitoring. However, the potential of utilizing these markers for screening and treating of HSCC still needs much more further investigations.

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Disclosure of conflict of interest

None.

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References


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