Proteomic Analysis of Primary Colon Cancer and Synchronous Solitary Liver Metastasis

EUN-KYUNG KIM1, MIN-JEONG SONG1, YUNJAE JUNG2, WON-SUK LEE3 and HO HEE JANG1

1Department of Biochemistry, College of Medicine, Lee Gil Ya Cancer and Diabetes Institute, Gachon University, Incheon, Republic of Korea; 2Department of Microbiology, College of Medicine, Gachon University, Incheon, Republic of Korea; 3Department of Surgery, Gil Medical Center, Gachon University, Incheon, Republic of Korea

Abstract. Background/Aim: Colon cancer is prone to distant metastases to other sites and the risk of recurrence is relatively high. Therefore, the identification of liver metastasis-related factors is important for the diagnosis or treatment of colon cancer. The aim of this study was to identify the metastasis-related factors that are differentially expressed in synchronous solitary liver metastasis compared to primary colon cancer. Materials and Methods: Tissues of primary colon cancer and associated with liver metastases of five patients were used for mass spectrometry. Identified proteins were validated by western blotting. The in silico analysis was performed using the STRING database and GeneMANIA. Results: We identified 58 differentially expressed proteins (DEPs), including 51 under-expressed and 7 over-expressed proteins among a total of 164 identified proteins. Major hubs of protein-protein networks were ACTC1, PRDX6, TPI1, and ALDH1A1. DEPs were located in the extracellular region and cytoplasm and were involved in the regulation of enzymatic activity. The metabolic process was significantly enriched in biological processes and an involvement in the KEGG pathway. Conclusion: These DEPs can potentially be used as biomarkers for the diagnosis of liver metastasis and they may provide a new strategy for developing anti-metastatic liver drugs in colon cancer patients.

This article is freely accessible online.

Correspondence to: Ho Hee Jang, Department of Biochemistry, College of Medicine, Gachon University, Incheon 21999, Republic of Korea. Tel: +82 328996317, Fax: +82 328996318, e-mail: hhjang@gachon.ac.kr and Won-Suk Lee, Department of Surgery, Gil Medical Center, Gachon University, Incheon 21565, Republic of Korea. Tel: +82 324603216, Fax: +82 324603009, e-mail: lws@gilhospital.com

Key Words: Colon cancer, differentially expressed proteins, gene ontology, liver metastatic cancer, mass spectrometry analysis, protein-protein interactions.
DEPs provides a set of potential diagnostic biomarkers and possible targets for anti-liver metastatic drugs.

Materials and Methods

Materials. All chemicals (iodoacetamide, 4-Sulfophenyl isothiocyanate, acetonitrile, a-cyano-4-hydroxycinnamicacid (CHCA), sodium bicarbonate, urea, bis-acrylamide, trifluoroacetic acid, thiourea, ammonium bicarbonate, Bradford solution, acrylamide, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate (CHAPS), SDS, DTT, benzamidine, and α-cyano-4-hydroxycinnamic acid) were in grade of electrophoresis or analytical and were purchased from Sigma (St. Louis, MO, USA). Pharmalyte (pH 3.5-10) was purchased from Amersham Biosciences (Piscataway, NJ, USA). Modified porcine trypsin of sequencing grade was purchased from Promega (Madison, WI, USA). Antibodies against carbonic anhydrase I (CA1, sc-39340), Serpin A1 (sc-59438), N-acetylneuraminate synthase (NANS, sc-374133), transferrin (sc-365871), and GAPDH (sc-47724) were all purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Biopsy samples. The study proposal was finalized and approved by the institutional review board of Gil Hospital, Gachon University (IBR No. GIRBA2216), prior to the investigation. Written informed consent was obtained from all participants. The samples were retrieved from the Gil Hospital, Gachon University (Incheon, Republic of Korea) between June 2015 and June 2017. The eligibility criteria for hepatic metastasis samples are as follows: i) synchronous colon cancer and single hepatic metastasis confirmed by spiral abdomino-pelvic computed tomography and liver MRI, ii) no evidence of tomography or other metastasis following positron emission tomography iii) liver metastasis as the first manifestation of M1 disease without contagious disease due to preoperative expression, protein spots were selected variations that deviated more than twice the expression level compared to normal samples. The spectra were taken at 300 shots per spectrum over the m/z range 600-3,000 and corrected with a two-point internal calibration by trypsin auto-digestion peaks (m/z 842.5099, 2211.1046). A list of peaks was created using Flex Analysis 3.0 (Bruker Daltonics). The thresholds used for peak-picking were as follows: the minimum resolution of monoisotopic mass was 500 and the S/N was 5. MASCOT, a search program developed by Matrixscience (http://www.matrixscience.com/), was used to identify proteins using peptide mass fingerprinting. The

---

**Table I. Clinicopathological features of colon cancer liver metastasis patients used for proteomic analysis.**

| Sample ID | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 |
|-----------|--------|--------|--------|--------|--------|
| Gender    | M      | M      | M      | M      | F      |
| Age       | 55     | 63     | 62     | 54     | 77     |
| Dx        | Sigmoid colon | Sigmoid colon | Sigmoid colon | Sigmoid colon | Sigmoid colon |
| MSI status| Stable  | Stable  | Stable  | Stable  | Stable  |
| KRAS status| Wild Type | Mutant | Wild Type | Wild Type | Mutant |
| Tumor size (mm) | 37.0 | 52.0 | 45.0 | 58.0 | 75.0 |
following parameters were used in the database search: i) trypsin digestion, ii) a maximum of one lost cleavage, iii) a complete modification of 2-iodoacetamide (Cys), iv) oxidation (Met) as partial modification, v) monoisotopic masses, and vi) a mass tolerance of ±0.1 Da. PMF acceptance criteria were used in the probability score calculation. The heat map of 58 DEPs was generated by average linkage clustering using online Heatmapper (http://www.heatmapper.ca/) (12, 13).

**Western blotting.** Sample extracts were lysed in RIPA lysis buffer consisting of 50 mM Tris (pH 8.0), 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, and 1X protease inhibitor mixture (GenDEPOT, Barker, TX, USA). Tissue samples were incubated in lysis buffer for 30 minutes at 4˚C. We centrifuged lysates at 12,000 × g for 15 minutes at 4˚C. To measure protein concentrations in the collected supernatants the method of Bradford protein assay was used (Bio-Rad). Equal concentrations of protein were loaded in 12% SDS-PAGE gel and, were then transferred onto nitrocellulose membranes (Pall Gelman Laboratory, MI, USA). Prior to probing with primary the antibody at 4˚C overnight, the membranes were blocked with 8% skimmed milk or 5% bovine serum albumin. Following washing, the membranes were incubated with a peroxidase-conjugated 2nd antibody. The membranes were developed using the Amersham ECL western blotting detection reagent.

**Analysis of protein-protein interaction (PPI) and gene ontology by STRING.** The 58 identified DEPs were analyzed and visualized to predict PPI using the STRING software [v10.5, http://string-db.org, (14)], which employs search tools for multiple proteins. Network data from the STRING database displayed a combination of data, including text mining, neighborhood, co-expression, co-occurrence, gene fusion, and experiments. The score of minimum required interaction was medium confidence (0.400). The pathways of 58 DEPs were analyzed using GeneMANIA (15, 16).

Gene Ontology (GO) by STRING was used to classify DEPs according to functional enrichments. The network of 58 DEPs was analyzed with regard to: i) cellular component, ii) molecular function, iii) biological process, and iv) KEGG pathways.

### Results

**Identification of differentially expressed proteins between primary colon cancer and liver metastasis cancer.** To identify distinct biomarkers for liver metastatic colon cancer, tissue lysates of primary colon cancer and liver metastasis cancer were fractionated on a 2D-PAGE gel (Figure 1). Analysis of the extracted peptides was performed using mass spectrometry. In total, 164 individual spots showed DEPs between primary colon cancer and synchronous solitary liver metastasis. Seven DEPs had higher synchronous solitary liver metastasis compared to primary colon cancer (Table II). Fifty-one DEPs had lower expression for liver metastasis compared to primary colon cancer. Figure 2 shows the heatmap of protein expression differences between primary colon cancer and liver metastatic colon cancer.

**Western blotting analysis of liver metastasis-related factors.** To validate the results of mass spectrometry analysis, we performed western blotting for detection of liver metastasis-related proteins (Figure 3). We randomly selected two factors of increased and decreased proteins. Serpin A1 and CA1 are as representative of up-regulated markers in liver metastasis. Serpin A1 is a serine protease inhibitor related to tumor migration. (17). Expression of Serpin A1 by mass spectrometry analysis showed a 2.68-fold increase in metastatic liver cancer over the level in primary colon cancer (Table II). Western blot analysis of Serpin A1 showed also 1.7- or 3.6-fold increase in metastatic liver cancer samples (M of cases 4 and 5) compared to that of primary colon cancer samples (P of cases 4 and 5). CA1 is a zinc metalloenzyme that catalyzes of the reversible hydration of carbon dioxide (18). Mass spectrometry analysis of CA1 showed a 1.52-fold increase in metastatic liver cancer samples (Table II). Immunoblotting of CA1 showed 1.1-fold
Table II. Identified different expression of proteins in colon cancer liver metastasis samples.

| Spot No. | Protein name | Gene | Intensity | Fold |
|----------|--------------|------|-----------|------|
| 103      | Calpain small subunit 1 | CAPNS1 | 421.4 | 328.1 | 0.78 |
| 503      | Serpin family A member 1 | SERPINA1 | 1281.6 | 3435.9 | 2.68 |
| 1002     | Apolipoprotein A1 | APOA1 | 2682.5 | 4088.6 | 1.52 |
| 1006     | Coactosin like F-actin binding protein 1 | COTL1 | 1576.3 | 493.6 | 0.31 |
| 1202     | Actin gamma 1 | ACTG1 | 9021.5 | 4673.6 | 0.52 |
| 1305     | Actin, alpha, cardiac muscle 1 | ACTC1 | 14998.8 | 9968.9 | 0.66 |
| 1315     | Intelectin 1 | ITLN1 | 146.7 | 592.7 | 4.04 |
| 1410     | ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide | ATP5B | 2865.2 | 1581.4 | 0.55 |
| 1420     | Protein disulfide isomerase family A member 5 | PDI5 | 2472.6 | 1109.2 | 0.45 |
| 1508     | Mutant desmin | DES | 610.6 | 733.6 | 1.20 |
| 2001     | S100 Calcium binding protein A9 | S100A9 | 1599.7 | 1539.4 | 0.96 |
| 2101     | Proteasome subunit beta 3 | PSMB3 | 949.4 | 481.3 | 0.51 |
| 2102     | Glyceraldehyde 3-phosphate dehydrogenase | GAPDH | 1129.7 | 613.9 | 0.54 |
| 2203     | Pyruvate dehydrogenase E1 beta subunit | PDHB | 2017.7 | 361.3 | 0.18 |
| 2204     | Dimethylarginine dimethylaminohydrolase 1 | DDAH1 | 1057.7 | 542.0 | 0.51 |
| 2302     | Creatine kinase B | CKB | 4180.1 | 1932.8 | 0.46 |
| 2405     | Ubiquinol-cytochrome c reductase core protein 1 | UQRC1 | 2204.1 | 1409.4 | 0.64 |
| 2605     | Annexin A6 | ANXA6 | 1232.3 | 781.7 | 0.64 |
| 3002     | Superoxide dismutase 1 | SOD1 | 4362.8 | 1765.2 | 0.40 |
| 3009     | Actin-related protein 2/3 complex subunit 5 isoform 1 | ARP5C | 623.6 | 72.2 | 0.12 |
| 3105     | Capping actin protein of muscle Z-line beta subunit | CAPZB | 2520.1 | 10400.0 | 0.41 |
| 3109     | Dimethylarginine dimethylaminohydrolase 2 | DDAH2 | 678.7 | 350.1 | 0.52 |
| 3407     | ARP3 Actin related protein 3 homolog | ACTR3 | 888.3 | 353.9 | 0.40 |
| 3704     | Gelsolin | GSIN | 1373.1 | 610.7 | 0.44 |
| 4404     | Parkinsonism associated deglycase | PARK7 | 2440.2 | 907.4 | 0.37 |
| 4509     | Diazepam binding inhibitor, acyl-CoA binding protein | DBH | 1448.7 | 1773.2 | 1.22 |
| 4101     | Cytochrome c oxidase subunit 6B1 | COX6B1 | 852.6 | 254.6 | 0.30 |
| 4104     | Endoplasmic reticulum protein 29 | ERP29 | 1293.5 | 862.5 | 0.67 |
| 4105     | Thiopurine S-methyltransferase | TPMT | 722.6 | 249.2 | 0.34 |
| 4108     | Pyrophosphatase (inorganic) 2 | PPA2 | 746.8 | 353.0 | 0.47 |
| 4204     | Crystallin lambda 1 | CRYLL1 | 634.8 | 292.2 | 0.46 |
| 5001     | Peroxiredoxin 3 | PRDX3 | 1690.7 | 576.6 | 0.34 |
| 5101     | Heat shock protein family B (small) member 1 | HSPB1 | 1390.7 | 911.4 | 0.66 |
| 5103     | Persulfide dioxygenase | ETHE1 | 1292.5 | 835.0 | 0.65 |
| 5106     | Enoyl-CoA hydratase, short chain 1 | ECHS1 | 1159.4 | 772.2 | 0.67 |
| 5401     | Selenium binding protein 1 | SELENBP1 | 3948.4 | 1806.5 | 0.46 |
| 6003     | Peroxiredoxin 6 | PRDX6 | 2193.0 | 1156.2 | 0.53 |
| 6104     | Triosephosphate isomerase 1 | TPI1 | 1488.8 | 984.1 | 0.66 |
| 6106     | Proteasome subunit alpha 6 | PSMA6 | 1081.2 | 601.9 | 0.58 |
| 6204     | Mercaptopyruvate sulfurtransferase | MPST | 593.4 | 361.8 | 0.61 |
| 6210     | N-Acetylenzyme synthetase | NAMS | 386.2 | 120.7 | 0.31 |
| 6211     | Acyl-CoA dehydrogenase short chain | ACADS | 552.1 | 170.6 | 0.31 |
| 6507     | Coronin 1A | CORO1A | 892.4 | 523.9 | 0.59 |
| 6511     | WD repeat domain 1 | WDR1 | 1251.3 | 595.3 | 0.48 |
| 6512     | Aldehyde dehydrogenase 1 family member A1 | ALDH1A1 | 828.2 | 413.1 | 0.50 |
| 6516     | Phosphoglucomutase 1 | PGM1 | 608.1 | 253.7 | 0.42 |
| 6609     | Succinate dehydrogenase complex flavoprotein subunit A | SDHA | 500.1 | 437.5 | 0.87 |
| 6710     | Transferrin | TF | 729.4 | 212.1 | 0.29 |
| 6712     | Aconitase 1 | ACO1 | 669.5 | 310.5 | 0.46 |
| 6719     | Programmed cell death 6 interacting protein | PDCD6IP | 852.1 | 367.1 | 0.43 |
| 7101     | Carbonic anhydrase 1 | CA1 | 2030.0 | 3039.2 | 1.52 |
| 7102     | Phosphoglycerate mutase 1 | PGAM1 | 1395.5 | 768.3 | 0.55 |
| 7301     | Tu Translation elongation factor, mitochondrial | TUFM | 1353.8 | 737.7 | 0.54 |
| 7302     | Isocitrate dehydrogenase (NADP(+) 1, cytosolic | IDH1 | 1153.7 | 598.4 | 0.52 |
| 7406     | 3-Hydroxy-3-methylglutaryl-CoA synthase 2 | HMGC2 | 796.6 | 406.9 | 0.51 |
| 7612     | Lamin A/C | LMNA | 819.4 | 542.9 | 0.66 |
| 8014     | S100 Calcium binding protein A8 | S100A8 | 1882.2 | 1923.5 | 1.02 |
| 8102     | Carbonic anhydrase 2 | CA2 | 2472.5 | 1317.7 | 0.53 |

Identified different expression of proteins in colon cancer liver metastasis samples.
and 7.5-fold increases in metastatic liver cancer cases 4 and 5, respectively, compared to their levels in primary colon cancer in these cases.

The selected decreased factors in liver metastasis were NANS and transferrin. NANS works as an enzyme in the biosynthetic pathways of sialic acids (19). In the results of mass spectrometry analysis, NANS was 0.31-fold lower in liver metastatic cancer compared to primary colon cancer (Table II). Western blot analysis demonstrated almost absence of expression (0.1-fold or 0) in liver metastatic cancer. Transferrin has the function of transporting iron and manganese (20), and mass spectrometry analysis showed that it was 0.29-fold lower in liver metastatic cancer compared to primary colon cancer (Table II). Western blot analysis showed that the level of transferrin also decreased by 0.7-fold and 0.3-fold in metastatic liver cancer cases 4 and 5, respectively, compared to cases of primary colon cancer in these cases.

Protein-protein interaction analysis. To explore PPI networks among the identified proteins, the 58 identified DEPs were analyzed using the STRING software (Figure 4A). Eight of the 58 DEPs did not connect to any type of network (STRING interaction score=0.4). Forty-two of the DEPs were connected to networks by complex relationships. ACTC1, WDR1, HSPB1, SDHA, PRDX6, TPI1, and ALDH1A1 showed network hubs.

Figure 2. Heat map analysis of 58 differentially expressed proteins (DEPs) from mass spectrometry. The relative levels of the 58 proteins found to exhibit differential expression between primary colon cancer and the isolated liver metastases were expressed as expression-based heat maps. The Y-axis is a list of 58 DEPs.

Figure 3. Validation of liver metastasis-related factors. Western blot analysis showed that CA1 and Serpin A1 were up-regulated and that NANS and transferrin were down-regulated in liver metastases (M) compared to primary colon cancer (P).
Figure 4. Analysis of protein–protein interaction (PPI) by online bioinformatics. (A) PPI of DEPs analyzed against the STRING Database for association networks. Known interactions are edges of pink (experimentally determined) and deep sky blue (database obtained). Predicted interactions are edges of green (gene neighborhood), blue (gene co-occurrence), and red (gene fusions). Edges of yellowish green are text-mining. Edges of black color mean co-expression. Edges of light purple mean protein homology. (B–C) Physical interaction and involvement of DEPs in the KRAS pathway (B) or EGF and EGFR (C), as analyzed by GeneMANIA.
highly associated with other factors in PPI. In addition, single networks were formed between PSMA6 and PSMB3, SELENBP1 and CA1, S100A9 and S100A8, and DDAH2 and DDAH1.

Progression of colon cancer related with KRAS and epidermal growth factor receptor (EGFR) (21-23). The GeneMANIA database was used to evaluate the relationship of KRAS or EGFR signaling among 58 DEPs. The network analysis of DEPs showed that KRAS associated with the Fc receptor signaling pathway and physically interacted with UQCRC1 and GLOD4 (Figure 4B). The network between DEPs and epidermal growth factor (EGF) showed that EGF physically related with ACO1, COX6B1, GSN, HSPB1, and LMNA (Figure 4C). The network analysis of DEPs with EGFR showed that EGFR physically interacted with NANS, TP11, S100A9, GCN, ARPC5, HSPB1, and PDCD6IP (Figure 4C). Common functions of TP11, NANS, and EGF were involved in the carbohydrate biosynthetic process.

Gene ontology analysis of identified proteins in liver metastasis. To investigate gene ontology of the 58 DEPs, we used bioinformatics to profile the cellular component, biological process, molecular function, and involvement in the KEGG pathway. The GO analysis of 58 DEPs is shown in Figure 5. Most of the identified proteins were located in the cytoplasm, extracellular region including membrane-bound vesicles, and exosome (Figure 5A). Molecular function was primarily associated with binding or multiple-enzyme activity

Figure 5. Analysis of GO annotation of 58 identified DEPs. A total of 58 proteins analyzed with regard to their cellular component (A) and molecular function (B). 58 DEPs were involved in a biological process (C) and in the KEGG pathway (D). These data were analyzed by the gene ontology from the STRING database.
(Figure 5B). The biological process of DEPs belonged to: i) metabolic processes, ii) response to stress, and iii) homeostatic process (Figure 5C). According to the analysis of the biological process, the identified DEPs that were increased are involved in various cellular processes, including processes without actin filaments, phagocytosis, pyruvate metabolic processes, and gluconeogenesis (Figure 5C). This result matches the analysis of the cellular position and suggests that liver metastasis from colon cancer induces down-regulation of glucose-related metabolism; however, other cellular metabolisms were up-regulated. Most of the 58 DEPs had a role in the KEGG metabolic pathway (Figure 5D).

**Discussion**

Patients with metastatic colorectal cancer with liver or lung metastases have a 5-year overall survival rate of 30-50% (24). Conventional chemotherapy for metastatic colorectal cancer with leucovorin and fluorouracil may extend the progression-free survival and overall survival, regardless of the use of oxaliplatin or irinotecan (25); however, the long-term results are not satisfactory. Therapeutic chemotherapy treatment for colon cancer with liver metastases is not currently possible. Stage IV colon cancer is a heterogeneous disease in terms of genomic and transcriptomic alterations with respect to patient survival and chemotherapy responses. Currently, the Colorectal Cancer Subtyping Consortium suggests four consensus molecular subtypes (CMS) that combines six independent classification systems based on gene expression analysis: i) CMS1 (MSI immune subtype; strong immune activation, microsatellite unstable, and hypermutated), ii) CMS2 (canonical subtype; MYC and WNT signal pathway activation), iii) CMS3 (metabolic subtype; metabolic dysregulation), and iv) CMS4 (mesenchymal subtype; TGF-β activation, stromal infiltration and angiogenesis) (26).

Despite this classification, CRCSC do not include an evaluation of the concordance and shifts of CMS calls in primary and matched metastatic samples, as well as the predictive value of the CRC subtypes. It is absolutely critical to understand that their marked biological differences are likely to develop new targeted therapies in CRC.

In this study, we investigated tumor metastatic contributors from primary colon cancer to liver metastasis using a proteomics approach. Samples of patients were chosen from primary colon cancer tissue to liver metastasis tissue. The 164 identified proteins included factor expression levels that were higher, lower, and unchanged. Proteins considerably increased the expression are: i) SERPINA1, ii) APOA1, iii) ITLN1, iv) DES, v) DBI, vi) SDHA, and vii) CA1.

Protein-protein interaction data show that central proteins in the networks are: i) ACTC1, ii) WDR1, iii) HSPB1, iv) SDHA, v) PRDX6, vi) TP11, and vii) ALDH1A1 (Figure 4). Unexpectedly, one of the hub proteins, SDHA, only belongs to increased DEPs. Others are involved in decreased DEPs. This result suggests that progression of metastasis induces imbalance in protein networks changing protein expression levels. These DEPs in liver metastasis tissue may be inducers of metastasis from the colon to liver or residue products of the liver-micro environment following the establishment of metastasis at a distant site. Alternative expression of proteins in primary colon cancer induces the possibility of quickly transitioning to a new progression status that may be involved in metastasis, drug resistance, cancer stem cell, supporting cell, and adapting cell.

The metastatic feature of cancer cells is their increasing energy metabolism and decreasing immune cell-related migration (27-29). Serpin A1 and ITLN1 are involved with the regulation of the innate immune system. Parts of decreased DEPs are related to energy metabolism. These data suggest that metastatic cancers have individual different conditions with regard to energy metabolism and immune system during tumor progression.

According to the analysis of cellular components, increased DEPs (SERPINA1, APOA1, ITLN1, DES, DBI, SDHA, and CA1) and decreased DEPs were located in various regions. The main location of increased DEPs is the extracellular region and exosome or in membrane-bounded vesicles. This characteristic means it such DEPs can be used as biomarkers for the detection of liver metastatic colon cancer using serum or colon samples. Especially, the level of Serpin A1 in serum is exceptionally high in patients with prostate, colorectal, lung, breast cancers, and insulinomas (30-35). However, the areas with decreased DEPs, include: i) the actin cytoskeleton, ii) lamellipodia, iii) the Arp2/3 protein complex, iv) the proteasome complex, v) the mitochondrial matrix, and vi) the mitochondrial intermembrane space. The function of actin cytoskeleton, lamellipodia, and the Arp2/3 protein complex helps maintain cell morphology and regulates vesicle trafficking using actin filament dynamics (36, 37) while the proteasome complex contributes to the degradation of proteins (38). Cancer stem-like properties have low proteasomal activity in colorectal cancer, lung cancer, and prostate cancer (39-41). The mitochondrial matrix and mitochondrial intermembrane space play a major part of ATP synthesis in cells (42, 43). Mitochondrial ATP synthase is down-regulated in colon cancer treated with 5-fluorouracil resistance (44). This result suggests that liver metastasis from colon cancer reduces actin dynamics, protein degradation, and ATP synthesis.

In conclusion, our study identified DEPs between synchronous solitary liver metastasis and primary colon cancer using mass spectrometry analysis from five patient samples. Analysis of DEPs determined up-regulation of 7 proteins and down-regulation of 51 proteins. Protein-protein interactions among DEPs shows that: i) ACTC1, ii) WDR1, iii) HSPB1, iv) SDHA, v) PRDX6, vi) TP11, and vii) ALDH1A1 are connected with other proteins. Further studies are required to
investigate the functional mechanism of these identified genes in liver metastasis progression of colon cancer. This study provides a set of useful biomarkers for diagnosis of liver metastasis, which may prove to be potential new targets for anti-metastatic liver cancer treatment in colon cancer.

Conflicts of Interest

The Authors declare that there are no conflicts of interest.

Authors’ Contributions

Conceptualization, EKK, WSL and HHJ; Investigation, EKK and MJS; Resources, YJJ, WSL and HHJ; Data Curation, EKK and MJS; Writing, EKK, WSL and HHJ; Funding Acquisition, WSL and HHJ.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (NRF-2017R1D1A1B03035453) to WSL.

References

1 Jung KW, Park S, Kong HJ, Won YJ, Lee JY, Seo HG and Lee JS: Cancer statistics in Korea: Incidence, mortality, survival, and prevalence in 2009. Cancer Res Treat 44(1): 11-24, 2012. PMID: 22500156. DOI: 10.4143/crt.2012.44.1.11
2 Lee WS, Kim MJ, Yun SH, Chun HK, Lee WY, Kim SJ, Choi JL, Nishikawa H, Yamaguchi S and Otsubo T: Protein pattern difference in the colon cancer cell lines examined by two-dimensional gel electrophoresis. J Clin Invest 119(6): 1420-1428, 2009. PMID: 19487818. DOI: 10.1172/JCI39104
3 Curley SA: Outcomes after surgical treatment of colorectal cancer liver metastases. Semin Oncol Surg Today 36(12): 1085-1093, 2006. PMID: 17123137. DOI: 10.1007/s00595-006-3301-y
4 Naba A, Clauser KR, Whittaker CA, Carr SA, Tanabe KK and Hynes RO: Extracellular matrix signatures of human primary metastatic colon cancers and their metastases to liver. BMC Cancer 14: 518, 2014. PMID: 25037231. DOI: 10.1186/1471-2407-14-518
5 Lamouille S, Xu J and Derynck R: Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 15(3): 178-196, 2014. PMID: 24556840. DOI: 10.1038/nrm3758
6 Kalluri R and Weinberg RA: The basics of epithelial-mesenchymal transition. J Clin Invest 119(6): 1420-1428, 2009. PMID: 19487818. DOI: 10.1172/JCI39104
7 Katayama M, Nakano H, Ishiuchi A, Wu W, Oshima R, Sakurai J, Nishikawa H, Yamaguchi S and Otsubo T: Protein pattern difference in the colon cancer cell lines examined by twodimensional dimensional in-gel electrophoresis and mass spectrometry. Surg Today 36(12): 1085-1093, 2006. PMID: 17123137. DOI: 10.1007/s00595-006-3301-y
8 Naba A, Clauser KR, Whittaker CA, Carr SA, Tanabe KK and Hynes RO: Extracellular matrix signatures of human primary metastatic colon cancers and their metastases to liver. BMC Cancer 14: 518, 2014. PMID: 25037231. DOI: 10.1186/1471-2407-14-518
9 Brody EB, Ottey F and Lagranade J: Early sex education in relationship to later coital and reproductive behavior: Evidence from jamaican women. Am J Psychiatry 133(8): 969-972, 1976. PMID: 942015. DOI: 10.1176/ajp.133.8.969
10 Oakley BR, Kirsch DR and Morris NR: A simplified unsensitivie silver stain for detecting proteins in polyacrylamide gels. Anal Biochem 105(2): 361-363, 1980. PMID: 6161559. DOI: 10.1016/0003-2697(80)90470-4
11 Fernandez J, Ghanahdagi F and Mische SM: Routine identification of proteins from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (sds-page) gels or polyvinyl difluoride membranes using matrix assisted laser desorption/ionization-time of flight-mass spectrometry (maldi-tof-ms). Electrophoresis 19(6): 1036-1045, 1998. PMID: 9638950. DOI: 10.1002/elps.1150190619
12 Babicki S, Arndt D, Marcu A, Liang Y, Grant JR, Maciejewski A and Wishart DS: Heatmapper: Web-enabled heat mapping for all. Nucleic Acids Res 44(W1): W147-153, 2016. PMID: 27190236. DOI: 10.1093/nar/gkw419
13 Verhaak RG, Sanders MA, Bijl MA, Delwel R, Horsman S, Moorhouse MJ, van der Spek PJ, Lowenberg B and Valk PJ: Heatmapper: Powerful combined visualization of gene expression profile correlations, genotypes, phenotypes and sample characteristics. BMC Bioinformatics 7: 337, 2006. PMID: 16836741. DOI: 10.1186/1471-2105-7-337
14 Szklarczyk D, Franceschini A, Wyder S, Forsslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ and von Mering C: String v10: Protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 43: D447-452, 2015. PMID: 25352553. DOI: 10.1093/nar/gku1003
15 Montojo J, Zuberi K, Rodriguez H, Bader GD and Morris Q: Genemania: Fast gene network construction and function prediction for cytoscape. F1000Res 3: 153, 2014. PMID: 25254104. DOI: 10.12688/f1000research.4572.1
16 Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, Maitland A, Mostafavi S, Montojo J, Shao Q, Wright G, Bader GD and Morris Q: The genemania prediction server: Biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res 38: W214-220, 2010. PMID: 20576703. DOI: 10.1093/nar/gkq537
17 de Serres F and Blanco I: Role of alpha-1 antitrypsin in human health and disease. J Intern Med 235(6): 759-766, 2002. PMID: 12035031. DOI: 10.1046/j.1365-2796.2002.00002.x
18 Boone CD, Pinard M, McKenna R and Silverman D: Catalytic and proton transfer. Subcell Biochem 41: 311-335, 2014. PMID: 25254104. DOI: 10.12688/f1000research.4572.1
19 Rangarajan ES, Ruane KM, Proteau A, Schrag JD, Valladares R, Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, Maitland A, Mostafavi S, Montojo J, Shao Q, Wright G, Bader GD and Morris Q: The genemania prediction server: Biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res 38: W214-220, 2010. PMID: 20576703. DOI: 10.1093/nar/gkq537
20 Gunter TE, Gerstner B, Gunter KK, Malecki J, Gelein R, Valentine WM, Aschner M and Yule DI: Manganese transport via the transferrin mechanism. Neurotoxicology 17(1): 118-127, 2006. PMID: 16836741. DOI: 10.1186/1471-2105-7-337
21 Fonseca RB, Schulick RD, Lillemoe KD, Yeo CJ and Cameron JL: Trends in long-term survival following liver resection for hepatic colorectal metastases. Langenbecks Arch Surg 359(6): 759-766, 2002. PMID: 12035031. DOI: 10.1046/j.1365-2796.2002.00002.x
the importance of diet, and the role of chemoprevention. World J Clin Oncol 6(5): 133-141, 2015. PMID: 26468449. DOI: 10.5306/wjco.v6.i5.133

22 Markman B, Javier Ramos F, Capdevila J and Tabernero J: Egfr and kras in colorectal cancer. Adv Clin Chem 51: 71-119, 2010. PMID: 20857619.

23 Heinemann V, Stintzing S, Kirchner T, Boeck S and Jung A: Clinical relevance of egfr- and kras-status in colorectal cancer patients treated with monoclonal antibodies directed against the egfr. Cancer Treat Rev 35(3): 262-271, 2009. PMID: 19117687. DOI: 10.1016/j.ctrv.2008.11.005

24 Steele G Jr., Bleday R, Mayer RJ, Lindblad A, Petrelli N and Weaver D: A prospective evaluation of hepatic resection for colorectal carcinoma metastases to the liver: Gastrointestinal tumor study group protocol 6584. J Clin Oncol 9(7): 1105-1112, 1991. PMID: 2045852. DOI: 10.1200/JCO.1991.9.7.1105

25 Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, Maroun JA, Ackland SP, Locker PK, Pirodda N, Elfling GL and Miller LL: Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan study group. N Engl J Med 343(13): 905-914, 2000. PMID: 11006366. DOI: 10.1056/NEJM2000092834313102

26 Guiney J, Dienstmann R, Wang X, de Reyries E, Schlicker A, Heinemann V, Stintzing S, Kirchner T, Boeck S and Jung A: The importance of diet, and the role of chemoprevention. World J Clin Oncol 6(5): 133-141, 2015. PMID: 26468449. DOI: 10.5306/wjco.v6.i5.133

27 Yin X, Zhang Y, Guo S, Jin H, Wang W and Yang P: Large scale systematic proteomic quantification from non-metastatic to metastatic colorectal cancer. Nat Med 21(11): 1350-1356, 2015. PMID: 26457759. DOI: 10.1038/nm.3967

28 Parks SK, Chiche J and Pouysegur J: Disrupting proton dynamics and energy metabolism for cancer therapy. Nat Rev Cancer 13(9): 611-623, 2013. PMID: 23966902. DOI: 10.1038/nrc3579

29 Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell 144(5): 646-674, 2011. PMID: 24664755. DOI: 10.1007/978-1-4614-3209-8_82

30 Abbritti RV, Polito F, Cucinotta M, Lo Giudice C, Caffo M, Tomasello C, Germano A and Aguennouz M: Meningiomas and proteomics: Focus on new potential biomarkers and molecular pathways. Cancer Genomics Proteomics 13(5): 369-379, 2016. PMID: 27566655.

31 Da Costa GG, Gonigk TH, Kaviski R, Santos Sousa K, Kuoljol C, De Lima RS, De Andrade Urban C, Cavalli IJ and Ribeiro EM: Comparative proteomics of tumor and paired normal breast tissue highlights potential biomarkers in breast cancer. Cancer Genomics Proteomics 12(5): 251-261, 2015. PMID: 26417028.

32 Perez-Holanda S, Blanco I, Menendez M and Rodrigo L: Serum concentration of alpha-1 antitrypsin is significantly higher in colorectal cancer patients than in healthy controls. BMC Cancer 14: 355, 2014. PMID: 24886427. DOI: 10.1186/1471-2407-14-355

33 El-Akawi ZJ, Abu-Awad AM, Sharara AM and Khader Y: The importance of alpha-1 antitrypsin (alpha1-at) and neopterin serum levels in the evaluation of non-small cell lung cancer patients. Neuro Endocrinol Lett 31(1): 113-116, 2010. PMID: 20150872.

34 El-Akawi ZJ, Al-Hindawi FK and Bashir NA: Alpha-1 antitrypsin (alpha1-at) plasma levels in lung, prostate and breast cancer patients. Neuro Endocrinol Lett 29(4): 482-484, 2008. PMID: 18766166.

35 de Sa SV, Correa-Giannella ML, Machado MC, Krogh K, de Almeida MQ, Albergaria Pereira MA, Coelho Siqueira SA, Patzina RA, Ibiuki FS, Sogayar MC, Machado MC and Giannella-Neto D: Serpin peptidase inhibitor clade a member 1 as a potential marker for malignancy in insulinomas. Clin Cancer Res 13(18 Pt 1): 5322-5330, 2007. PMID: 17855650. DOI: 10.1158/1078-0432.CCR-06-1477

36 Zhou K, Sumigray KD and Lechler T: The arp2/3 complex has essential roles in vesicle trafficking and transcytosis in the mammalian small intestine. Mol Biol Cell 26(11): 1995-2004, 2015. PMID: 25833710. DOI: 10.1091/mbc.E14-10-1481

37 Henson JH, Yeterian M, Weeks RM, Medrano AE, Brown BL, Geist HL, Pais MD, Oldenbourg R and Shuster CB: Arp2/3 complex inhibition radically alters lamellipodial actin architecture, suspended cell shape, and the cell spreading process. Mol Biol Cell 26(5): 887-900, 2015. PMID: 25568343. DOI: 10.1091/mbc.E14-07-1244

38 Bard JAM, Goodall EA, Greene ER, Jonsson E, Dong KC and Martin A: Structure and function of the 26s proteasome. Annu Rev Biochem 87: 697-724, 2018. PMID: 29652515. DOI: 10.1146/annurev-biochem-062917-011931

39 Munakata K, Uemura M, Tanaka S, Kawai K, Kitahara T, Miyo M, Kano Y, Nishikawa S, Fukusumi T, Takahashi Y, Hata T, Nishimura J, Takemasa I, Muzushima S, Ikenaga M, Kato T, Murata K, Carethers JM, Yamamoto H, Doki Y and Mori M: Cancer stem-like properties in colorectal cancer cells with low proteasome activity. Clin Cancer Res 22(21): 5277-5286, 2016. PMID: 27166935. DOI: 10.1158/1078-0432.CCR-15-1945

40 Della Donna L, Lagadec C and Pajonk F: Radioresistance of prostate cancer cells with low proteasome activity. Prostate 72(8): 868-874, 2012. PMID: 21932424. DOI: 10.1002/pros.21489

41 Pan J, Zhang Q, Wang Y and You M: 26s proteasome activity is down-regulated in lung cancer stem-like cells propagated in vitro. PLoS One 5(10): e13298, 2010. PMID: 20949018. DOI: 10.1371/journal.pone.0013298

42 Stuart RA, Gruhler A, van der Klei I, Guiard B, Koll H and Neupert W: The requirement of matrix atp for the import of precursor proteins into the mitochondrial matrix and intermembrane space. Eur J Biochem 220(1): 9-18, 1994. PMID: 8119302. DOI: 10.1111/j.1422-1347.1994.tb18593.x

43 Glick BS, Wachtler C, Reid GA and Schatz G: Import of cytochrome b2 to the mitochondrial intermembrane space: The tightly folded heme-binding domain makes import dependent upon matrix atp. Protein Sci 2(11): 1901-1917, 1993. PMID: 8268801. DOI: 10.1002/pro.5560021112

44 Shin YK, Yoo BC, Chang HJ, Jeon E, Hong SH, Jung MS, Lim SJ and Park JG: Down-regulation of mitochondrial f1f0-atp synthase in human colon cancer cells with induced 5-fluorouracil resistance. Cancer Res 65(8): 3162-3170, 2005. PMID: 15833846. DOI: 10.1158/0008-5472.CAN-04-3300

Received June 12, 2019
Revised July 12, 2019
Accepted July 15, 2019