Discovery and verification of gelsolin as a potential biomarker of colorectal adenocarcinoma in a Chinese population: Examining differential protein expression using an iTRAQ labelling-based proteomics approach

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OBJECTIVE: To identify and validate potential biomarkers of colorectal adenocarcinoma using a proteomic approach.

METHODS: Multidimensional liquid chromatography/mass spectrometry was used to analyze biological samples labelled with isobaric mass tags for relative and absolute quantitation to identify differentially expressed proteins in human colorectal adenocarcinoma and paired normal mucosa for the discovery of cancerous biomarkers. Cancerous and noncancerous samples were compared using online and offline separation. Protein identification was performed using mass spectrometry. The downregulation of gelsolin protein in colorectal adenocarcinoma samples was confirmed by Western blot analysis and validated using immunohistochemistry.

RESULTS: A total of 802 nonredundant proteins were identified in colorectal adenocarcinoma samples, 82 of which fell outside the expression range of 0.8 to 1.2, and were considered to be potential cancer-specific proteins. Immunohistochemistry revealed a complete absence of gelsolin expression in 86.89% of samples and a reduction of expression in 13.11% of samples, yielding a sensitivity of 86.89% and a specificity of 100% for distinguishing colorectal adenocarcinoma from normal tissue.

CONCLUSIONS: These findings suggest that decreased expression of gelsolin is a potential biomarker of colorectal adenocarcinoma.

Key Words: Biomarker; Colorectal cancer; Gelsolin; Proteomic

Approximately 1,529,560 cancer-related deaths are estimated to occur annually in the United States; of these, 142,570 are attributed to colorectal cancer (CRC) (1). Due to early detection, clinical prognoses have improved significantly; however, robust screening tests are needed for all types of gastrointestinal cancer. Although a wide variety of screening modalities for CRC exist, only 40% of cases are detected at early stages (2). The identification of novel protein biomarkers or therapeutic targets may ultimately improve patient care and survival.

Proteomics combined with mass spectrometry (MS) has become a powerful method for the global examination of proteins in the post-genomics era, enabling the discovery of cancer biomarkers and drug targets. Although transcriptomics provides the tools for unravelling gene expression networks, proteomics links these networks to protein products and provides additional insight into post-translational modifications that regulate cellular functions, thereby complementing genomic analyses. The identification of differentially expressed proteins in CRC using proteomics suggests that expression patterns of proteins may have some utility in the discovery of cancer biomarkers.

Differential tagging with isotopic reagents, such as isotope-coded affinity tags (3), or the more recent variation that uses isobaric tags for relative and absolute quantitation (iTRAQ) reagents followed by multidimensional liquid chromatography (LC) and tandem MS/MS analysis, is emerging as a powerful methodology in the search for disease biomarkers. Many recent studies (4-11) using iTRAQ reagents resulted in the identification and relative quantitation of proteins that led to the discovery of potential cancer markers for prostate, radiotherapy-resistant, renal cell, lung, oral, head and neck, breast and
TABLE 1
Gelsolin expression in colorectal cancer patients (n=61)

| Characteristics        | Cases (n=61) | Gelsolin expression | P     |
|------------------------|-------------|---------------------|-------|
|                        | Absent (n=53) | Reduced (n=8)      |       |
| Sex                    |             |                     | 0.975*|
| Male                   | 34          | 29                  | 5     |
| Female                 | 27          | 24                  | 3     |
| Age, years, mean ± SD  | 61.00±9.33  | 61.79±7.58          | 55.75±16.82 | 0.348†|
| Histological differentiation | 19         | 15                  | 4     |
| Poorly differentiated   |             |                     |       |
| Moderately differentiated | 42         | 38                  | 4     |
| Well-differentiated     | 0           | 0                   | 0     |
| TNM stage              |             |                     | 0.164‡|
| I                      | 13          | 12                  | 1     |
| II                     | 20          | 19                  | 1     |
| III                    | 18          | 14                  | 4     |
| IV                     | 10          | 8                   | 2     |
| Histological classification | 49         | 47                  | 2     |
| Adenocarcinoma         |             |                     |       |
| Mucinous adenocarcinoma| 10          | 6                   | 4     |
| Signet-ring cell carcinoma | 2          | 0                   | 2     |
| Undifferentiated cancer | 0           | 0                   | 0     |
| Tumour localization    |             |                     | 0.474*|
| Colon                  | 18          | 17                  | 1     |
| Rectum                 | 43          | 36                  | 7     |

Data presented as n unless otherwise indicated. *Determined using χ2 test (continuity correction); †Determined using t test; ‡Given the limited sample size, patients were combined: stage I and II as the early stage group, stage III and IV as the advanced stage group; §Mucinous adenocarcinoma, signet-ring size, patients were combined: stage I and II as the early stage group, stage III and IV as the advanced stage group; ††Determined using χ2 test.

Methods

Patients

Data from all CRC patients who underwent surgical resection at the 150th Hospital, PLA, Luoyang, China, between January and June 2010, were retrieved from the files of the department of pathology. The criteria for inclusion in the study were as follows: presence of sporadic tumours without evidence of polyposis or familial predisposition syndrome; availability of clinical information and tumour stage; availability of tissue material; and a diagnosis of colorectal adenocarcinoma. The criteria for exclusion from the study were the presence of palindromic or metastatic tumour(s), and patients receiving antitumour therapy including chemotherapy, radiotherapy or biotherapy. Of the patients fulfilling these criteria, 66 cases were randomly selected. The patient consent forms and tissue-banking procedures were approved by the Research Ethics Board of the 150th Hospital. Histological diagnosis of each sample was confirmed using microscopic examination of hematoxylin and eosin-stained sections obtained during radical correction surgery.

Tissue material

Paired tissue samples of primary tumours (obtained from surgical resections) and their normal mucosa (not less than 5 cm from the cancerous margin) were flash frozen in liquid nitrogen within 20 min of devitalization after radical correction surgery (n=5; used for iTRAQ and Western blot analysis) or fixed in buffered formalin and embedded in paraffin (n=61, used for IHC).

The frozen samples were sectioned and stored at −80°C. The tissue from the opposite face of the histological section was then washed three times in approximately 1 mL of phosphate buffered saline (PBS) containing a mixture of protease inhibitors as described previously (12) (1 mM 4-[2-aminoethyl] benzenesulfonyl fluoride, 10 μM leupeptin, 1 μg/mL aprotinin and 1 μM pepstatin). The washed tissues were then homogenized in 0.5 mL of PBS containing protease inhibitors using a handheld homogenizer. These homogenates were then flash frozen in liquid nitrogen and stored at −80°C until use.

Neoplastic lesions were classified histologically according to the Vienna classification of digestive epithelial neoplasia (13). The tumour, node, metastasis (TNM) stage of the adenocarcinomas was determined according to the American Joint Committee on Cancer classification (14). The following data were recorded: localization, histological classification and TNM stage for adenocarcinomas (Table 1).

Protein digest, iTRAQ labelling and strong cation-exchange fractionation

Cancerous and paired normal flash-frozen samples from five patients were thawed and clarified by centrifugation, and protein concentrations were determined with a Bradford-type assay using a protein quantification reagent (Bio-Rad, USA). Cancerous samples and paired normal samples were pooled in equal parts and precipitated.

The samples (100 μg each) were reduced, alkylated and digested with trypsin overnight at a protein/enzyme ratio of 20:1 at 37°C. Each digest was concentrated to a volume of 15 μL in a speed vacuum followed by the addition of 15 μL of 1 M TEAB. The iTRAQ reagent was dissolved in 70 μL of ethanol and added to the digest, and the mixture was incubated at room temperature for 1 h. Cancer and normal samples labelled with different iTRAQ reagents were mixed and dried to a volume of 50 μL. The combined peptide mixture was fractionated by strong cation-exchange (SCX) chromatography on an UltraMate high-performance liquid chromatography (HPLC) system (LC Packings, The Netherlands) using a polysulfoethyl A column (2.1×100 mm, 5 μm, 300 Å [PolyLC, USA]). The sample was subsequently dissolved in 1 mL of SCX loading buffer (25% v/v acetonitrile, 10 mM KH2PO4, pH 2.8), and pH was adjusted to 2.8 by adding 1 M phosphoric acid. The entire sample was loaded onto the column and washed successively for 30 min at a flow rate of 200 μL/min. Peptides were eluted with a linear gradient of 0 mM to 500 mM KCl (25% v/v acetonitrile, 10 mM KH2PO4, pH 2.8) over 30 min at a flow rate of 200 μL/min. The absorbance at 214 nm was monitored, and 15 fractions were collected along the gradient.

LC/MS analysis

Each SCX fraction was dried down, dissolved in 0.1% formic acid and analyzed by MS (Qstar Pulsar [Applied Biosystems-MDS Scieix, USA] interfaced with an Agilent 1100 HPLC system. Peptides were separated on a reverse-phase column packed with 10 cm of C18 beads (36,075 m, 5 μm, 120 Å, YMC ODS-AQ [Waters Associates, USA]) with an emitter tip (New Objective, USA) attached. The peptides retained were washed using a gradient ranging from 5% to 40% solvent B (98%
acetonitrile, 0.1% formic acid) in solvent A (0.1% formic acid) for 60
minutes at a flow rate of 300 nl/min. Survey scans were acquired from
a spectra of 400 m/z to 1200 m/z, with up to three precursors selected for
MS/MS using a dynamic exclusion of 45 s. Rolling collision energy was
used to promote fragmentation, with the collision energy range 20% higher
than that used for unlabelled peptides because of the presence of
tiTraq tags.

Data analysis
The MS/MS spectra data were extracted and searched for in the
Uniprot-Sprot database (version 4, total number of entries: 230,093,
entries for Homo sapiens: 14,515) using ProteinPilot version 3.0 soft-
ware (revision 114732 [Applied Biosystems, USA]) with the Paragon
method using the following search parameters: H sapiens as species,
trypsin as enzyme (one missed cleavage allowed), cysteine static modifi-
cation with methylmethanethiosulfate and iTraq (peptide labelled at
the N terminus and lysine) as sample type. Mass tolerance was set to
0.15 atomic mass units for the precursor, and 0.1 atomic mass units for
the fragment ions. The raw peptide identification results from the
Paragon algorithm (Applied Biosystems, USA) searches were further
processed by the Pro Group algorithm (Applied Biosystems, USA)
within the ProteinPilot software before final display. The Pro Group
algorithm uses the peptide identification results to determine the min-
imal set of proteins that can be reported for a given protein confidence
threshold. For each protein, Pro Group algorithm reports two types of
scores: unused ProtScore and total ProtScore. The total ProtScore is a
measurement of all the peptide evidence for a protein, and is analog-
gous to protein scores reported by other protein identification software
packages. The unused ProtScore, however, is a measurement of all
evidence for a protein that is not better explained by a higher ranking
protein. In other words, the unused ProtScore is calculated by using
the unique peptides (peptides that are not used by the higher ranking
protein) and is a true indicator of the presence of protein. This is how
a single-protein member of a multiprotein family was isolated.

The protein confidence threshold for a single-protein member was
calculated using a threshold of 0.05, which was consistent between
two independent biological experiments were manually validated and
quantified. Two peaks for each of the signature ions (113 and 114)
were obtained and corrected according to the manufacturer's instruc-
tions to account for isotopic overlap. Only signature ions with intensi-
ties <1500 counts were used for quantitation. When intensities of the
signature ions were >1500 counts, 1:1 ratios were generated due to
isotopic overlap. Only signature ions with intensities <1500 counts were
considered, resulting in a mean, SD and P values to estimate statistical
significance of the protein changes were calculated using Pro Group software. Differentially
expressed proteins (ratio >1.2 or <0.8) that were consistent between
two independent biological experiments were manually validated and
quantified. Two peaks for each of the signature ions (113 and 114)
were obtained and corrected according to the manufacturer's instruc-
tions to account for isotopic overlap. Only signature ions with intensi-
ties <1500 counts were used for quantitation. When intensities of the
signature ions were >1500 counts, 1:1 ratios were generated due to
detector saturation.

Western blot analysis
To verify the iTraq data, samples (1 μg) were separated by 12%
sodium dodecyl polyacrylamide gel electrophoresis and transferred to
polyvinylidene fluoride membranes. After blocking for 1 h at room
temperature with blocking buffer (20 mM Tris-HCl pH 7.5, 100 mM
NaCl, 0.1% Tween 20 [TBS/Tween 20]) and 5% nonfat milk powder,
membranes were incubated overnight at 4°C in a 1:25,000 dilution of
primary antibody (rabbit monoclonal antibody [Abcam (Hong Kong)
Ltd, Hong Kong, China) in blocking buffer. The membranes were
washed with TBS/Tween 20, incubated for 1 h at room temperature in
secondary antibody (donkey antirabbit immunoglobulin G, Santa Cruz
Biotech, USA) at a 1:2000 dilution in blocking buffer and, finally,
washed with TBS/Tween 20. Blots were developed using Immun-Star
TMAP Substrate Pack (BioRad, USA) and scanned on an Epson Scan
(Agilent Technologies, USA) scanner within the linear range of
detection.

Evaluation of immunohistochemical staining
A standard two-step indirect streptavidin-biotin method was applied to
sections (3 μm thick) of deparaffinized tissue (StreptABComplex/
HRP Duet, Mouse/Rabbit Amplification kit, ZhongShan Company,
China). After autoclave sterilizer antigen retrieval (5 min in sodium
citrate buffer [pH 7.3]) and peroxidase quenching with 3% hydrogen
peroxide for 15 min, a 1:150 dilution of antigelosin antibody
(Albarn (Hong Kong) Ltd, Hong Kong, China) was applied over-
night at 4°C. Biotinylated goat immunoglobulin G and streptavadin-
biotin complexes were then applied for 30 min each. Sections were
counterstained with Mayer’s hematoxylin. Primary antibody omission
served as a negative control. Smooth muscle cells and endothelial cells
served as an internal positive control.

To evaluate the percentage of gelosin-positive cells, the entire sec-
tion was first scanned to determine the overall distribution of gelosin
expression within the tumour and its degree of heterogeneity; then,
the percentage of gelosin-positive cells was determined in representa-
tive areas by examining 10 randomly selected microscopic fields
(magnification ×400) of each tissue section. Observations were made by
two different observers who were blinded to clinical outcome (ie, the
slides were coded, and the pathologists did not have previous
knowledge of the local tumour burden, lymphonodular spread and
grading of the tissue samples while scoring the immunoreactivity).
Sections were scored positive if epithelial cells showed immunoposi-
tivity in the cytoplasm, plasma membrane and/or nucleus when judged
independently by two scorers. A grading system was used to express
the proportion of positive cells in each case as follows: grade (G) 0,
negative; G1, <25% positive cells; G2, 25% to 50% positive cells; G3,
50% to 75% positive cells; and G4, >75% positive cells per lesion. In
each type of lesion, results were expressed as the percentage of cases of
each grade.

Statistical analysis
ProteinPilot software was used using a Paragon method was used to analyze
MS/MS spectra. Results of IHC were analyzed using the
ProtScore 2.0 (unused) with at least one peptide with 99% confidence.

The protein confidence threshold for the present study was
0.15 atomic mass units for the precursor, and 0.1 atomic mass units for
the fragment ions. The resulting peptides were fractioned using strong cation exchange chromatography and analyzed by liquid chromatography (LC)-tandem MS-MS
treatment of the cells was determined using the two-sided
test. Differences between two means with
P<0.05 were considered to be statistically significant.

RESULTS
Quantitative proteomics analysis of CRC and normal tissue
In the present study, iTraq-based stable isotope labelling of colorec-
tal tissues was performed to identify dysregulated proteins in CRC.
The experimental strategy is shown in Figure 1.

After labelling with iTraq reagents (113 for cancer samples and
114 for normal samples), the two samples were mixed and analyzed by
LC-MS/MS. The MS/MS fragmentation of the iTraq-labelled pep-
dides resulted in signature peaks (at 113.1 and 114.1) for quantitation,
while the fragmentation along the peptide backbone resulted in b-
and y-type fragments, which were used to identify the peptide
sequence. Only proteins with at least two peptides per protein and a
high annotation confidence (>95% ) were considered, resulting in a
total of 802 nonredundant proteins, which included structural pro-
teins, signalling components, enzymes, receptors, transcription fac-
tors and chaperones. Eighty-two altered proteins outside the
expression range of 0.8 to 1.2 were considered to be potential CRC-
specific proteins (Appendix). Among these 82 proteins, gelosin was
specifically identified as a potential biomarker given the following: gel-
solin, one of the major actin-binding proteins, is involved in the regula-
tion of actin cytoskeleton organization via its severing and capping
activity of actin filaments (15); and loss of gelosin, a tumour suppressor,

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TABLE 2
Peptide sequences, confidence, ratios, protein ratios and P values of gelsolin calculated by ProteinPilot* software

| Peptide sequence | Confidence, % | Ratio† | Protein ratio‡ | P   |
|------------------|--------------|--------|----------------|-----|
| AGALNSNDAVFLK    | 99           | 0.4295 | 0.5012         | <0.0001† |
| EVGQFESATFLGFK    | 99           | 0.2144 |                |     |
| HVVPNEVVQR        | 99           | 0.1181 |                |     |
| QTOQVSLPEGGETPLFK | 99           | 0.3839 |                |     |
| SEDCFILDHKGDK     | 99           | 0.2984 |                |     |
| TGAQELLRR         | 99           | 0.3147 |                |     |
| TPSAAYLVGTGASEAEK | 99           | 0.9661 |                |     |
| YIETDPANR         | 99           | 0.5898 |                |     |

*Applied Biosystems, USA; †Cancerous/normal; ‡Actual value 9.04×10^-5

Figure 2) Representative tandem mass spectrometry (MS/MS) spectra for peptides derived from gelsolin. For each MS/MS spectrum, b- and y-type fragment ions enabled peptide identification, whereas the peak areas of each of the iTRAQ signature ions (insets) enable quantification of the peptides and protein. A MS/MS spectrum of the peptide sequence AGALNSNDAVFLK from gelsolin. B MS/MS spectrum of the peptide sequence EPAHLMSLFGKPMIIYK from gelsolin. C Colorectal cancer and the paired normal sample were iTRAQ tagged by 113 and 114, respectively. The isobaric tag 115 and 116 were used in another experiment of colorectal cancer to examine the gelsolin peptide sequence EPAHLMSLFGKPMIIYK from gelsolin. D Colorectal cancer and the paired normal sample were iTRAQ tagged by 113 and 114, respectively. The isobaric tag 115 and 116 were used in another experiment of colorectal cancer to examine the gelsolin peptide sequence EPAHLMSLFGKPMIIYK from gelsolin. E Gelsolin in cancerous sample. F Gelsolin in paired normal sample. Gelsolin Isobaric tags for relative and absolute quantitation.

is one of the most frequently occurring molecular defects in ovarian, breast, colon and pancreatic cancers (16-19). Gelsolin peptide sequences, peptide confidence, peptide ratios, protein ratios and P values calculated by ProteinPilot are presented in Table 2.

According to the protein sequence coverage map, protein sequences with matching peptides could be divided into groups with the highest confidence, moderate confidence, lowest confidence and the portions of the protein sequence for which no peptides were identified. The MS/MS spectra of two matching peptides (AGALNSNDAVFLK and EPAHLMSLFGKPMIIYK) with the highest confidence are shown in Figure 2, with the peaks for the signature ions shown in the insets.

Western blot analysis
Western blot experiments were performed to validate the iTRAQ assay results. Based on a literature search, gelsolin was chosen. Gelsolin was detected by Western blot from the pooled sample used in a 2D LC-MS/MS experiment. Gelsolin is considered to be downregulated in CRC based on gray scale analysis of the protein strap, which was consistent with the discovery experiment (Figure 3).

Figure 3) Validation of gelsolin by Western blot. Western blot results using frozen tissue samples used in the discovery experiment. A β-actin in normal sample. B β-actin in cancerous sample. C Negative control. D Gelsolin in normal sample. E Gelsolin in cancerous sample. The gray scale in the cancerous sample (E) is 0.48 of that of the normal sample (D).

Immunohistochemical staining
Gelsolin expression was tested using IHC in 61 primary human colorectal adenocarcinomas and their paired normal mucosa for further validation. Gelsolin expression was detected in all normal colon samples examined (61 of 61 [100%]) (Figure 4A and 4D). In epithelial cells, gelsolin was detected in both enterocytes and goblet cells. Gelsolin showed cytoplasmic expression and, in part of the cytoplasm surrounding the mucus vacuoles, no nuclear labelling was detected. Compared with normal mucosa, gelsolin expression was decreased in all tumours analysed (61 of 61 [100%]) (Figure 4A and 4C), resulting either in a complete absence of detection in 86.89% (53 of 61) of samples (Figure 4A) or in focal staining of clusters of tumour cells in 13.11% (eight of 61) of samples (Figure 4C). Gelsolin achieved a sensitivity of 86.89% and specificity of 100% in distinguishing colorectal adenocarcinoma from normal tissue.

In cancer samples (reduced expression in eight cases) with clusters of gelsolin-positive cells, the mean (±SD) percentage of gelsolin-positive tumour cells was 21.18±7.73%. The percentage of gelsolin-positive cells was reduced in cancer compared with paired normal mucosa (P=0.034 [paired sample t test]) (Figure 4C and 4D). No adenocarcinoma was scored as G4 (>75% of positive cells) or G3 (50% to 75% of positive cells), whereas 37.5% of cases (three of eight) were classified as G2 (25% to 50% of positive cells) and 62.5% of the cases (five of eight) were classified as G1 (<25% of positive cells). Gelsolin-positive cells showed cytoplasmic expression in adenocarcinoma (three of eight samples [37.5%]); mucinous adenocarcinoma (three of eight samples [37.5%]); and signet-ring cell carcinoma (two of eight samples [25%]). Compared with tumours devoid of expression, gelsolin expression tended to be reduced in mucinous adenocarcinoma and signet-ring cell carcinoma (P<0.0001). This decrease in gelsolin expression in CRC was not correlated with age, sex, histological differentiation, TNM stage or tumour localization (Table 1).

DISCUSSION
Compared with traditional proteomic methods, such as 2D gel analysis followed by MS/MS identification, the iTRAQ-coupled 2D-LC-MS/MS
approach provides higher detection sensitivity and holds the promise of effectively depicting cellular protein profiles in tissues (9,11,20). In recent years, this approach has been used in prostate, radiotherapy-resistant, renal cell, lung, oral, head and neck, breast and hepatocellular cancers (4-11).

In the present study, we applied isobaric peptide tags (ie, iTRAQ) and multidimensional LC-MS/MS to identify proteins that are differentially expressed in CRC and paired normal mucosa samples. Differential gelsolin expression was validated by Western blot analysis and IHC. Because adenocarcinoma comprises more than 50% of CRCs, only colorectal adenocarcinoma was included in the present study.

Using a combination of subtractionistion, iTRAQ-based labelling, 2D-LC peptide separation, MS and database searches for human proteins, 82 proteins altered outside the range of 0.8 to 1.2 were considered as potential CRC-specific proteins. Downregulation of gelsolin was confirmed by Western blot analysis and IHC. These serial studies suggest that combined proteomics and bioinformatics analysis can generate valid candidates that may be further evaluated for their role in the progression from normal tissue to adenocarcinoma. Although the validation of a protein cannot be extrapolated to the full protein list, it did suggest that the iTRAQ results were reliable.

Abnormal expression of gelsolin has been reported in many types of tumours. The expression of gelsolin has been reported to be frequently silenced in various cancers (16,18,21-27). Conversely, overexpression of gelsolin has been found to be a negative prognostic predictor in a subpopulation of patients with nonsmall cell lung cancer, urachal cell, and EGFR/erb-B2 breast cancer (25,28,29). Gelsolin regulates the architecture and dynamics of cells by capping, severing and nucleating actin filaments. However, the precise molecular mechanism behind the reduction of gelsolin expression has not yet been clarified (22,30). Despite the high incidence of CRC, only a few contradictory studies regarding the expression of gelsolin in this type of malignancy and its association with clinicopathological features have been reported. The results from the current study suggest that combined proteomics and bioinformatics analysis can generate valid candidates that may be further evaluated for their role in CRC progression.

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AUTHORS’ CONTRIBUTIONS: Nai-jun Fan was responsible for the conception and design of this study, providing samples and clinical data, drafting and revising the article and performing the experiments. Chun-fang Gao contributed to the design of this study, Chuang-song Wang, Jian-Yin and Qing-yin Liu contributed to the validation of gelsolin. Jing Lv, Guang Zhao, Xin-hua Sheng, Xiu-li Wang and Dong-hui Li contributed to samples and clinical data collection. All authors have read and approved the final manuscript.

CONCLUSION

The results presented reinforce the idea that iTRAQ and 2D-LC-MS/MS represent an effective and reliable proteomic process in cancer biomarker discovery; the downregulation of gelsolin expression is a general mechanism during the transition from normal tissue to cancer; and that gelsolin may be a potential biomarker for colorectal adenocarcinoma in the Chinese population.

COMPETING INTERESTS: The authors have no conflicts of interest to declare.

APPENDIX

Dysregulated proteins (n=82) in colorectal adenocarcinoma identified using an iTRAQ-based proteomic approach

| Protein                                      | Expression level in cancer |
|----------------------------------------------|-----------------------------|
| Histone H3.3                                 | Increased                   |
| Neutrophil defensin 3                        | Increased                   |
| 40S ribosomal protein S19                    | Increased                   |
| Collagen alpha-(XII) chain                   | Increased                   |
| Histone H4                                   | Increased                   |
| Elongation factor 2                          | Increased                   |
| Histone H2B type 1-N                         | Increased                   |
| Annexin A2                                   | Increased                   |
| 60 kDa heat shock protein, mitochondrial     | Increased                   |
| Fibrinogen beta chain                        | Increased                   |
| 78 kDa glucose-regulated protein             | Increased                   |
| Protein disulfide-isomerase                  | Increased                   |
| Thymidine phosphorylase                      | Increased                   |
| Tenascin                                     | Increased                   |
| Flamin-B                                     | Increased                   |

Expression level in cancer: Increased

Sp|P84243|H33_HUMAN
Sp|P96666|DEF3_HUMAN
Sp|P30091|RS19_HUMAN
Sp|Q69715|COCA1_HUMAN
Sp|P62605|H4_HUMAN
Sp|Q13639|EFF2_HUMAN
Sp|Q96777|HGBN_HUMAN
Sp|P07355|ANX2_HUMAN
Sp|P10809|CH60_HUMAN
Sp|P02575|FIBB_HUMAN
Sp|P11021|GRPP78_HUMAN
Sp|P07237|PDIA1_HUMAN
Sp|P19971|TYPH_HUMAN
Sp|P24821|TENA_HUMAN
Sp|P75369|FLNB_HUMAN

Protein accession

Keratin4 type I cytoskeletal 1, sp|P05783|K1C18_HUMAN
Keratin2 type II cytoskeletal 8, sp|P05787|K2C8_HUMAN
Neutral alpha-glucosidase AB, sp|Q14697|GANAB_HUMAN
Serum albumin, sp|P02768|ALBU_HUMAN
40S ribosomal protein S17, sp|P08708|RS17_HUMAN
Protein disulfide-isomerase A3, sp|P30101|PDIA3_HUMAN
SH3 domain-binding glutamic acid-rich-like protein 3, sp|Q25991|SH3L3_HUMAN
Annexin A1, sp|P04083|ANX1A_HUMAN
Fibrinogen gamma chain, sp|P02679|FGB_HUMAN
Plastin-2, sp|P13796|PLSL_HUMAN
Myosin-9, sp|P35575|MYH9_HUMAN
Neutral alpha-glucosidase AB, sp|Q14697|GANAB_HUMAN
40S ribosomal protein S17, sp|P08708|RS17_HUMAN
Serum albumin, sp|P02768|ALBU_HUMAN
Plectin-1, sp|Q15149|PLEC1_HUMAN
Keratin4 type I cytoskeletal 1, sp|P05787|K2C8_HUMAN
Keratin4 type II cytoskeletal 8, sp|P05787|K1C18_HUMAN

Expression level in cancer: Increased

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### APPENDIX – CONTINUED

Dysregulated proteins (n=82) in colorectal adenocarcinoma identified using an iTRAQ-based proteomic approach

| Protein | Accession | Expression level in cancer |
|---------|-----------|---------------------------|
| Heat shock protein HSP 90-alpha | sp|P07900|HS90A_HUMAN | Increased |
| Fibronectin | sp|P02751|FN1C_HUMAN | Increased |
| Myosin-11 | sp|P35749|MYH11_HUMAN | Decreased |
| Hemoglobin subunit alpha | sp|P69905|HBA_HUMAN | Decreased |
| PDZ and LIM domain protein 7 | sp|Q09NR12|PDLZ1_HUMAN | Decreased |
| Collagen alpha-1(VI) chain | sp|P12109|COL6A1_HUMAN | Decreased |
| Neuroblast differentiation-associated protein AHNAK | sp|Q09666|AHNK_HUMAN | Decreased |
| Synaptophysin | sp|Q9UM36|SYNP2_HUMAN | Decreased |
| Alpha-actinin-1 | sp|P12814|ACTN1_HUMAN | Decreased |
| ATP synthase subunit beta, mitochondrial | sp|P06576|ATPB_HUMAN | Decreased |
| Collagen alpha-1(XIV) chain | sp|Q05707|COEA1_HUMAN | Decreased |
| Tensin-1 | sp|Q09HBL0|TENS1_HUMAN | Decreased |
| Decorin | sp|P07585|PGS2_HUMAN | Decreased |
| Collagen alpha-2(VI) chain | sp|P12110|COL6A2_HUMAN | Decreased |
| Talin-1 | sp|Q09490|TLN1_HUMAN | Decreased |
| Gelsolin | sp|P06396|GELS_HUMAN | Decreased |
| Myosin light chain kinase, smooth muscle | sp|Q15746|MYLK_HUMAN | Decreased |
| Desmin | sp|Q15061|DIHMN_HUMAN | Decreased |
| ATP synthase subunit alpha, mitochondrial | sp|P25705|ATPA_HUMAN | Decreased |
| Sorbin and SH3 domain-containing protein 1 | sp|Q98X66|SRBS1_HUMAN | Decreased |
| Ig gamma-1 chain C region | sp|P01852|IGHG1_HUMAN | Decreased |
| Desmplakin | sp|P05924|DESP_HUMAN | Decreased |
| Membrane primary amine oxidase | sp|P16853|AOGC3_HUMAN | Decreased |
| Lamin-A/C | sp|P02545|LMNA_HUMAN | Decreased |
| Lumican | sp|P51884|LUM_HUMAN | Decreased |
| Polymerase I and transcript release factor | sp|Q6NZI2|PTF1_HUMAN | Decreased |
| Peroxiredoxin-2 | sp|P32119|PRDX2_HUMAN | Decreased |
| Troponymosin beta chain | sp|P07951|TPM2_HUMAN | Decreased |
| Troponymosin alpha-1 chain | sp|P09432|TPM1_HUMAN | Decreased |
| Prolargin | sp|P06576|PRELP_HUMAN | Decreased |
| Myosin regulatory light polypeptide 9 | sp|P24844|MYL9_HUMAN | Decreased |
| Profilin-1 | sp|P07737|PROF1_HUMAN | Decreased |
| Vinculin | sp|P18206|VINC_HUMAN | Decreased |
| Creatine kinase B-type | sp|P12277|CKB_HUMAN | Decreased |
| Filamin-C | sp|P01834|FLNC_HUMAN | Decreased |
| Calponin-1 | sp|P51911|CNN1_HUMAN | Decreased |
| Memeic | sp|P07900|MBA_HUMAN | Decreased |
| Spectrin alpha chain, brain | sp|Q09490|SPTA2_HUMAN | Decreased |
| Ig gamma-2 chain C region | sp|P01859|IGHG2_HUMAN | Decreased |
| Glyceroldehyde-3-phosphate dehydrogenase | sp|Q04406|G3P_HUMAN | Decreased |
| Ig kappa chain C region | sp|P01854|IGKC_HUMAN | Decreased |
| Tranexin | sp|P01859|TGAL_HUMAN | Decreased |
| Phosphatidylethanolamine-binding protein 1 | sp|P01854|TAGL_HUMAN | Decreased |
| Peroxiredoxin-5, mitochondrial | sp|P09490|PRDX5_HUMAN | Decreased |
| EH domain-containing protein 2 | sp|Q09N24|EH2D_HUMAN | Decreased |
| Tenascin-X OS+Homo sapiens | sp|P22105|TENX_HUMAN | Decreased |
| Cysteine and glycine-rich protein 1 | sp|P05924|CGRP1_HUMAN | Decreased |
| Ig lambda chain C regions | sp|P09490|LAC_HUMAN | Decreased |
| Hemoglobin subunit beta | sp|P68871|HBB_HUMAN | Decreased |
| Heat shock 70 kDa protein 1 | sp|P08107|HSP71_HUMAN | Decreased |
| Filamin-C | sp|Q04406|FLNC_HUMAN | Decreased |
| Calponin-1 | sp|P01857|CNN1_HUMAN | Decreased |

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