LC-MS proteomic profiling of trace FFPE samples in gastrointestinal cancer

Chunmei Guo
Human Phenome Institute, Fudan University
Lingling Li
Human Phenome Institute, Fudan University
Hui Liu
Henan Normal University
Chen Ding (chend@fudan.edu.cn)
Human Phenome Institute, Fudan University

Research Article

Keywords: Proteomics, Trace FFPE sample, AOPs, gastrointestinal cancer

Posted Date: April 20th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1556405/v1

Abstract

Background: Early screening and diagnosis of gastrointestinal cancer would be benefit for patients' prognosis. However, the complexity of cancer progression and the extreme trace amount of tissue samples in different stages have limited in portraying the characterization of early-stage cancer. Here, We disclosed the biological pathways and built an integrated proteomic map of trace FFPE samples in gastrointestinal cancer.

Methods: In this study, a quantitative proteomic method with chromatography with mass spectrometry (LC-MS/MS) was used to analyse the different amounts of peptides between the trace duodenum and gastric cancers.

Results: We performed a comprehensive proteomic landscape of trace formalin-fixed, paraffin-embedded (FFPE) samples with 5 different amounts of peptides (AOPs) in 40 gastrointestinal cancer samples. Proteomic analysis disclosed the different features of high/low proteins in duodenum and gastric cancers, and revealed the gradually decreased proteins number with the dilutions of AOP. In addition, we found the high coverage of identified proteins when the input of AOPs were no less than 0.5 μg. Proteomic methods represented the loss of tumor heterogeneity when the input of AOPs were no more than 0.5 μg. We also illustrated the dominant pathways in duodenum cancer and gastric cancer, and found the loss function when the input of AOPs was no more than 0.5 μg. Collectively, our study built an integrated proteomic map of trace FFPE samples in gastrointestinal cancer, and provided a valuable resource for clinical researches, especially for early-stage cancer.

Conclusion: We disclosed the biological pathways of the trace samples, and revealed the high coverage (> 60%) of identified proteins when the input of AOPs was no less than 0.5 μg, keeping the primary functions of tissues. These findings provided a new insight in early-stage cancer in the clinic.

Introduction

Cancer is a major health problem worldwide and leads nearly 10 million deaths every year [1, 2]. Early screening and diagnosis of disease is approved by World Health Organization (WHO) and has been prominent nowadays, especially in gastrointestinal cancer. The major events disclosed in advanced-stage cancer, appeared to be identified in early-stage cancer, the surveillance of which leading to better prognosis for patients [3, 4]. However, the complexity of cancer progression and the extreme trace amount of tissue samples in different stages have limited in portraying the characterization of early-stage cancer.

Advances in medical science and the great progress in mass spectrometry-based proteomics provided chances for researchers exploring FFPE biospecimens, providing a valuable resource for clinical and biomarker researches. In addition, FFPE tissue biopsies showed a high degree of the proteome pattern similarity between histological regions samples collected for 1 and 15 years, and facilitates tumor stratification [5]. Therefore, biobanked FFPE samples were employed for many types of cancers and presenting proteomic characterization. For example, proteomic analysis of colon rectal cancer (CRC)

revealed decreased T cell infiltration and increased glycolysis in CRC [6]; *Sai Ge* et.al, described a proteomic landscape of diffuse-type gastric cancer, and illustrated the overrepresentation of immune response in the third subtypes with the worst survival [7]. However, the proteomic profiling of trace samples and the probable peptides amount of input for LC-MS remain largely unknow.

Here, we performed proteomic analysis of clinical FFPE samples in duodenum cancer and gastric cancer, presented proteomic landscape of the trace samples with 5 different amounts of peptides (AOPs), disclosed the biological pathways of the trace samples, and revealed the high coverage (> 60%) of identified proteins when the input of AOPs was no less than 0.5 μg, keeping the primary functions of tissues. These findings provided a new insight in early-stage cancer in the clinic.

Materials And Methods

Processing of formalin-fixed, paraffin-embedded (FFPE) specimens

All the FFPE specimens of duodenum/gastric cancer were prepared and provided by Zhongshan Hospital, Fudan University, and the study was carried out in compliance with the ethical standards of Helsinki Declaration II and approved by the Institution Review Board of Fudan University Zhongshan Hospital (B2019-200R). For clinical sample preparation, slides (10 μm thick) from FFPE blocks were macro-dissected, deparaffinized with xylene and washed with ethanol. All the selected specimens were evaluated and confirmed by two or three experienced and board-certified gastrointestinal pathologists, and materials were aliquoted and kept in storage at -80 ℃ until further processing.

Protein extraction and digestion

Nearly 1 mg samples were lysed in a buffer (0.1 M Tris-HCl, pH 8.0, 0.1 M DTT, and 4% SDS) supplemented with protease inhibitors and phosphatase at 99°C for 30 mins. The crude exact was then clarified by centrifugation at 12,800 × g for 10 mins and the supernatants were loaded into 10 kD Microcon filtration devices (Millipore) and centrifuged at 12,000 × g for 20 mins and washed twice with Urea lysis buffer (8 M Urea, 100 mM Tris-HCl pH 8.0), and twice with 50 mM NH_4HCO_3. Then the samples were digested using trypsin at an enzyme to protein mass ratio of 1:25 overnight at 37°C. Peptides were extracted, and the concentration was measured by Nanodrop (Thermo Fisher, cat: ND-LITE-PR). As well as 4 gastric cancer cases, 4 duodenum cancer cases were diluted into 5 different AOPs: 2 μg, 1 μg, 0.5 μg, 0.25 μg, and 0.125 μg, respectively. Thus, a total of 40 FFPE samples were acquired and dried (SpeedVac, Eppendorf).

Proteome analysis in LC-MS/MS Analysis

For the proteome profiling samples, peptides were analyzed on a Q Exactive HF-X Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, Rockford, IL, USA) coupled with a high-performance liquid chromatography system (EASY nLC 1200, Thermo Fisher). Dried peptide samples re-

dissolved in Solvent A (0.1% formic acid in water) were loaded to a 2-cm self-packed trap column (100-μm inner diameter, 3 μm ReproSil-Pur C18-AQ beads, Dr Maisch GmbH) using Solvent A and separated on a 150-μm-inner-diameter column with a length of 15 cm (1.9 μm ReproSil-Pur C18-AQ beads, Dr Maisch GmbH) over a 75 min gradient (Solvent A: 0.1% Formic acid in water; Solvent B: 0.1% Formic acid in 80% ACN) at a constant flow rate of 600 nL/min (0–75 min, 0 min, 4% B; 0–10 min, 4–15% B; 10–60 min, 15–30% B; 60–69 min, 30–50% B; 69–70 min, 50–100% B; 70–75 min, 100% B). The eluted peptides were ionized under 2 kiloVolts and introduced into mass spectrometry. Mass spectrometry was operated under a data-dependent acquisition mode. For the MS1 Spectra full scan, ions with m/z ranging from 300 to 1,400 were acquired by Orbitrap mass analyzer at a high resolution of 120,000. The automatic gain control (AGC) target value was set as 3E6. The maximal ion injection time was 80 ms. MS2 Spectra acquisition was performed in the ion trap mode at a rapid speed. Precursor ions were selected and fragmented with higher energy collision dissociation (HCD) with a normalized collision energy of 27%. Fragment ions were analyzed by the ion trap mass analyzer with the AGC target at 5E4. The maximal ion injection time of MS2 was 20 ms. Peptides that triggered MS/MS scans were dynamically excluded from further MS/MS scans for 12 s.

All the proteomic raw data had been uploaded to the iProx Consortium (https://www.iprox.org/) with the subproject ID (IPX0002843001). All the data of 40 trace samples were search against the same database with MaxQuant. As well as in gastric cancer samples, the raw data of the samples with AOPs in duodenum cancer were on the basis of the match between runs (MBRs) algorithm [8].

Principal component analysis (PCA) of trace samples

We performed PCA on a total of proteins identified in 20 duodenum/gastric cancer samples to illustrated the global proteomic difference among the samples with 5 different AOPs in gastrointestinal cancer. The PCA function under the scikit-learn R package was implemented for unsupervised clustering analysis with the parameter ‘n_components = 2’ on the expression matrix of global proteomic data. A colored ellipse represented the 95% confidence coverage for each group.

Consensus clustering analysis of trace samples

The protein expression matrix of the 20 trace samples of duodenum cancer (n = 4,334) or gastric cancer (n = 4,582) was used to identify the proteomic characteristics of trace samples. Consensus clustering was performed using Consensus ClusterPlus R package (version 1.46.0) [9], and the parameters were shown as following: reps = 1,000, pItem = 0.8, pFeature = 1, clusterAlg = ‘hc’, distance = ‘euclidean’, plot = ‘PDF’. Integrative analysis of the results disclosed that clustering by k = 4 appeared to have the clearest cut and revealed the significance of the consistent input of AOPs in clinic research.

Pathways enrichment analysis

To investigate the dominant signaling pathways of the overlap proteins and each concentrated gradient of trace samples, we used gens sets of molecular pathways in David [10]. For this analysis, pathways

from the GO database were considered. Statistical significance was considered when *P* value was less than 0.05 and FDR *q* value was no more than 0.1.

Results

Overview of proteomic landscape of trace FFPE samples in duodenum cancer and gastric cancer

We performed proteomic profiling of trace 40 samples collected from 4 duodenum and 4 gastric cancer patients who had not experienced prior chemotherapy or radiotherapy. The amounts of peptides were diluted from each patient samples as 2.0 μg (D1/G1), 1.0 μg (D2/G2), 0.5 μg (D3/G3), 0.25 μg (D4/G4), and 0.125 μg (D5/G5), respectively (**Fig. 1** and **Supplementary Fig. S1a**). With the dilution of the AOPs, the number of identified proteins slightly decreased from ~ 4,000 in the D1/G1 to ~ 2,000 in the D5/G5 (Fig. 2a).

Proteomic analysis was performed using a label-free quantification strategy [7, 11]. Protein abundance of all samples was firstly calculated by intensity-based absolute quantification (iBAQ) [12, 13] and then normalized as a fraction of the total (FOT). The nearly same number of identified proteins in samples with the same AOPs indicated the stability and the highly quality control of MS platform. As well as in gastric cancer, we found the top proteins extensively expressed in all samples in duodenum cancer, including HBA2, HBB, AGR2, HIST2H4B, HIST2H2BF, etc. (Fig. 2a, **Supplementary Table S1a**). However, the lower proteins detected in the samples with 5 different AOPs were distinctive. For example, the low abundance proteins of NIPBL, GTF3C1, HEATR5A, MDN1, and CLASP2 were observed in the D1, and of SAMD9, NBAS, C6, ABCC1, and PRRC2A were disclosed in D2 (Fig. 2a). Likewise, the top proteins identified all gradients samples in gastric cancer, such as ALB, HBB, KRT8, HIS2H4B, HIS2H2BF, etc. (**Supplementary Table S1b**). The differential low expressed proteins were identified in 5 AOPs samples groups. For example, ITPR2, CAPN15, and ABCB1 were detected in G1, and UBR2, VAV2, and WDR7 were observed in the G2 (**Supplementary Fig. S1b**). Taken together, we built a proteomic landscape of trace FFPE sample in duodenum cancer and gastric cancer for the first time, and disclosed the different features of high/low proteins in duodenum and gastric cancers with different AOPs.

Proteomic characterization of trace FFPE samples with 5 different AOPs

To explore the coverage in different gradients, we applied Venn diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/) to the D1 to the D5, in which the D1 was regarded as the basal. As well as in the gastric cancer samples, we found the overlapped proteins were decreased with the dilution of peptides in duodenum cancer samples ranging from 3,228 in overlap 1 (D-3,228, D1 & D2) to 1,638 in overlap 4 (D-1,638, D1&D2&D3&D4&D5) (Fig. 2b and **Supplementary Fig. S1c**). In addition, when the input of AOPs of the trace samples was no less than 0.5 μg, the coverage of identified proteins was over 60% in duodenum cancer and 70% in gastric cancer (Fig. 2b and **Supplementary Fig. S1c**).

To characterize the proteomic profiles of the trace samples with different AOPs, we performed principal component analysis (PCA) and found the clearly discrimination among trace samples when the input of AOPs was no less than 0.5 μg in duodenum cancer and gastric cancer (Fig. 3a and **Supplementary Fig. S2a**). Consensus clustering identified 4 clusters in duodenum cancer and 3 clusters in gastric cancers, which further revealed the gathering of trace samples with the same input of AOPs (Fig. 3b and **Supplementary Fig. S2b**). Especially, the cluster 1 in gastric cancer contained the G1, G2, and G3, indicating the similarity of the trace samples (**Supplementary Fig. S2b**). As well as in gastric cancer, Spearman's correlation analysis illustrated that the higher correlation coefficients were detected in the trace samples with the same input of AOPs in the duodenum cancer (Fig. 3c and **Supplementary Fig. S2c**). These findings implied the key role of the same input in cancer cohort researches, and revealed the minimum standard of the input of AOPs (0.5 μg) in trace samples for proteomic profiles, providing a new insight for early-stage cancers.

Biological pathways in 5 AOPs trace samples

Previously, we showed that Wnt signaling and extracellular matrix organization were the driver pathways in gastric cancer [7]. Indeed, we also detected Wnt signaling ($p < 0.05$, FDR < 0.1) and extracellular matrix organization ($p < 0.05$, FDR < 0.1) in all samples of gastric cancer (Fig. 4a). In addition, visualization of the Gene Ontology (GO) disclosed that the overlapped proteins participated in the metabolic machinery (e.g., tricarboxylic acid cycle and glycolysis) ($p < 0.05$, FDR < 0.1), immune response (e.g., antigen processing and presentation, NF-kappaB signaling) ($p < 0.05$, FDR < 0.1), and canonical cancer-related pathways (e.g., cell cycle and Mapk signaling) ($p < 0.05$, FDR < 0.1), suggesting the potential carcinogenesis in gastric cancer (Fig. 4a, **Supplementary Table S1c**). These biological pathways ($p < 0.05$, FDR < 0.1) were also overrepresented in duodenum cancer, indicating their potential cancer driven effects in duodenum cancer.

To investigate the loss functions with the dilutions of AOPs, we integrated the differential expressed proteins (DFP, fold change ≤ 0.5), in which the D1 was the basal. We then performed GO analysis of the DEPs and found antigen processing and presentation was decreased when the input of AOPs were no more than 0.5 μg in duodenum cancer, which was also observed in gastric cancer when the input of AOPs of trace samples was no more than 0.25 μg. The loss functions of cell cycle, Wnt signaling, and NF-kappaB signaling were detected when the input of AOPs were no more than 0.25 μg in duodenum cancer (Fig. 4b, **Supplementary Table S1d**). Anti-EGFR strategy is prevalent for gastrointestinal cancer, for example panitumumab to anti-EGFR in colorectal cancer [14, 15], and was employed in treating duodenal adenomatous [16, 17]. However, compared with the D1, EGFR signaling ($p = 2.93E-4$, FDR = 0.022), was notably attenuated in the D5 (Fig. 4b). In gastric cancer, we found the reduction of complement cascade when the input of AOPs was no more than 0.5 μg, and the loss function of NF-kappaB signaling ($p = 1.15E-7$, FDR = 4.22E-7), Wnt signaling ($p = 1.24E-5$, FDR = 1.27E-3), and cell cycle ($p = 3.91E-6$, FDR = 4.91E-4), indicating the useless when the input was in significantly light amounts. Together, we revealed the driver pathways for the carcinogenesis of duodenum cancer and gastric cancer, disclosed the loss

functions with the dilutions of AOPs, and indicated the significantly light amounts was useless for scientific researches.

Discussion

Cancer is one of leading causes of death worldwide, and the early screening and diagnosis provided better outcomes for patients. FFPE tissue samples represent gold standard for archiving pathology samples, keeping the tissue stability, and are available for clinical research [18]. In addition, high-throughput proteomic analysis of FFPE tissues samples enables to explore the molecular characterization of cancers, including CRC [6], gastric cancer [7], breast cancer [19], and so on. However, Whereas, the proteomic profiling of trace samples and the probable input of AOPs for LC-MS remain largely unknow.

In this study, we performed proteomic analysis of 20 duodenum cancer and 20 gastric cancer samples with 5 different AOPs, and described proteomic landscape of trace FFPE samples in duodenum/gastric cancer. We identified gradually decreased proteins number with the dilutions of AOPs, ranging from ~ 4,000 proteins in D1/G1 to ~ 2,000 protein in D5/G5. In addition, we found the high expressed proteins (e.g., HBB, HBA, etc.) were consistent in D1–D5, and G1–G5. In contrary, the low expressed proteins were distinctive both in duodenum cancer and gastric cancer.

Surprisingly, the proteomic methods (e.g., PCA and CCP) proved that the loss of tumor heterogeneity was notably observed when the input of AOPs was less than 0.5 μg. Compared to the D1/G1, (Spearman's) correlation coefficients were no more than 0.7 when the input of AOPs was less than 0.5 μg. These results suggested the importance of the consistent input of AOPs and provided a new insight for clinical research.

GO analysis of overlapped identified proteins revealed the biological pathways in duodenum cancer and gastric cancer. Among of the dominant pathways detected in our study, Wnt signaling and extracellular matrix organization are the driver pathways in gastric cancer [7]. In addition, we detected metabolic processes (e.g., TCA cycle, glycolysis), and immune responses (e.g., antigen processing and presentation, NF-kappaB signaling), and canonical cancer-related pathways (e.g., cell cycle and Mapk signaling), which were also identified in the duodenum cancer for the first time. Furthermore, we found the loss functions of these dominant pathways (e.g., Wnt signaling, cell cycle, etc.) when the input of AOPs were no more than 0.5 μg.

In brief, our study, for the first time, demonstrated the proteomic map of trace samples with different concentration gradient of peptide. We discovered the high coverage of identified proteins and tumor heterogeneity stability when the input of AOPs was no less than 0.5 μg. We also revealed the biological pathways of duodenum cancer and gastric cancer, and found the loss functions when the input of AOPs was no more than 0.5 μg. We believe this study provides new insights into the understanding proteomic characterization of the trace samples, and represents valuable resource for early-stage cancer and other clinical researches.

Conclusion

In this study, we performed proteomic analysis of 20 duodenum cancer and 20 gastric cancer samples with 5 different AOPs, and described proteomic landscape of trace FFPE samples in duodenum/gastric cancer. Proteomic analysis disclosed the different features of high/low proteins in duodenum and gastric cancers, and revealed the gradually decreased proteins number with the dilutions of AOPs, ranging from ~ 4,000 in 2 μg peptides to ~ 2,000 in 0.125 μg peptides. In addition, we found the high coverage of identified proteins when the input of AOPs were no less than 0.5 μg. Proteomic methods represented the loss of tumor heterogeneity when the input of AOPs were no more than 0.5 μg. We also illustrated the dominant pathways in duodenum cancer and gastric cancer, and found the loss function (e.g., Wnt signaling and cell cycle) when the input of AOPs was no more than 0.5 μg. Collectively, our study built an integrated proteomic map of trace FFPE samples in gastrointestinal cancer, and provided a valuable resource for clinical researches, especially for early-stage cancer.

Declarations

Authors' contributions

C.M.G. and C.D. conceived and designed the analysis. L.L.L. and H.L. collected the data. L.L.L., H. L, contributed analysis tools. C.M.G. performed the analysis. C.M.G. and C.D. wrote the paper. All authors read and approved the final manuscript.

Funding

This work was supported by Chinese Ministry of Science and Technology (2016YFA0502500), and Key Projects of Strategic International Scientifc and Technology Innovation Cooperation of Chinese Ministry of Science and Technology (2020YFE0201600).

Availability data and materials

All the proteomic raw data and the results files of duodenum cancer and gastric cancer had been uploaded to the iProx Consortium (https://www.iprox.org/) with the PXD identifers (PXD026756).

Ethics approval and consent to participate

The study was carried out in compliance with the ethical standards of Helsinki Declaration II and approved by the Institution Review Board of Fudan University Zhongshan Hospital (B2019-200R).

Consent for publication

No applicable.

Competing interests

The authors declare that they have no competing interest.

Author details

[1]State Key Laboratory of Genetic Engineering and Collaborative Innovation Center for Genetics and Development, School of Life Sciences, Institute of Biomedical Sciences, Human Phenome Institute, Fudan University, Shanghai 200433, China. [2]State Key Laboratory Cell Diferentiation and Regulation, Overseas Expertise Introduction Center for Discipline Innovation of Pulmonary Fibrosis, (111 Project), College of Life Science, Henan Normal University, Xinxiang 453007, Henan, China. [3]Academy of Medical Science, Zhengzhou University, Zhengzhou 450052, China.

References

1. F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J Clin 68 (2018) 394–424. 10.3322/caac.21492.
2. R.L. Siegel, K.D. Miller, H.E. Fuchs, A. Jemal, Cancer Statistics, 2021, CA Cancer J Clin 71 (2021) 7–33. 10.3322/caac.21654.
3. D. Jiang, X. Li, H. Wang, C. Xu, X. Li, A. Sujie, H. Zeng, Y. Hou, Y. Zhong, A retrospective study of endoscopic resection for 368 patients with early esophageal squamous cell carcinoma or precancerous lesions, Surg Endosc 31 (2017) 2122–2130. 10.1007/s00464-016-5208-z.
4. W.C. Song, X.L. Qiao, X.Z. Gao, A comparison of endoscopic submucosal dissection (ESD) and radical surgery for early gastric cancer: a retrospective study, World J Surg Oncol 13 (2015) 309. 10.1186/s12957-015-0724-1.
5. Y. Zhu, T. Weiss, Q. Zhang, R. Sun, B. Wang, X. Yi, Z. Wu, H. Gao, X. Cai, G. Ruan, T. Zhu, C. Xu, S. Lou, X. Yu, L. Gillet, P. Blattmann, K. Saba, C.D. Fankhauser, M.B. Schmid, D. Rutishauser, J. Ljubicic, A. Christiansen, C. Fritz, N.J. Rupp, C. Poyet, E. Rushing, M. Weller, P. Roth, E. Haralambieva, S. Hofer, C. Chen, W. Jochum, X. Gao, X. Teng, L. Chen, Q. Zhong, P.J. Wild, R. Aebersold, T. Guo, High-throughput proteomic analysis of FFPE tissue samples facilitates tumor stratification, Mol Oncol 13 (2019) 2305–2328. 10.1002/1878-0261.12570.
6. S. Vasaikar, C. Huang, X. Wang, V.A. Petyuk, S.R. Savage, B. Wen, Y. Dou, Y. Zhang, Z. Shi, O.A. Arshad, M.A. Gritsenko, L.J. Zimmerman, J.E. McDermott, T.R. Clauss, R.J. Moore, R. Zhao, M.E. Monroe, Y.T. Wang, M.C. Chambers, R.J.C. Slebos, K.S. Lau, Q. Mo, L. Ding, M. Ellis, M. Thiagarajan, C.R. Kinsinger, H. Rodriguez, R.D. Smith, K.D. Rodland, D.C. Liebler, T. Liu, B. Zhang, C. Clinical Proteomic Tumor Analysis, Proteogenomic Analysis of Human Colon Cancer Reveals New Therapeutic Opportunities, Cell 177 (2019) 1035–1049 e1019. 10.1016/j.cell.2019.03.030.
7. S. Ge, X. Xia, C. Ding, B. Zhen, Q. Zhou, J. Feng, J. Yuan, R. Chen, Y. Li, Z. Ge, J. Ji, L. Zhang, J. Wang, Z. Li, Y. Lai, Y. Hu, Y. Li, Y. Li, J. Gao, L. Chen, J. Xu, C. Zhang, S.Y. Jung, J.M. Choi, A. Jain, M. Liu, L. Song, W. Liu, G. Guo, T. Gong, Y. Huang, Y. Qiu, W. Huang, T. Shi, W. Zhu, Y. Wang, F. He, L. Shen, J.

Qin, A proteomic landscape of diffuse-type gastric cancer, Nat Commun 9 (2018) 1012. 10.1038/s41467-018-03121-2.

8. S. Tyanova, T. Temu, J. Cox, The MaxQuant computational platform for mass spectrometry-based shotgun proteomics, Nat Protoc 11 (2016) 2301–2319. 10.1038/nprot.2016.136.
9. M.D. Wilkerson, D.N. Hayes, ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking, Bioinformatics 26 (2010) 1572–1573. 10.1093/bioinformatics/btq170.
10. M. Kanehisa, S. Goto, KEGG: kyoto encyclopedia of genes and genomes, Nucleic Acids Res 28 (2000) 27–30. 10.1093/nar/28.1.27.
11. Y. Jiang, A.H. Sun, Y. Zhao, W.T. Ying, H.C. Sun, X.R. Yang, B.C. Xing, W. Sun, L.L. Ren, B. Hu, C.Y. Li, L. Zhang, G.R. Qin, M.H. Zhang, N. Chen, M.L. Zhang, Y. Huang, J.N. Zhou, Y. Zhao, M.W. Liu, X.D. Zhu, Y. Qiu, Y.J. Sun, C. Huang, M. Yan, M.C. Wang, W. Liu, F. Tian, H.L. Xu, J. Zhou, Z.Y. Wu, T.L. Shi, W.M. Zhu, J. Qin, L. Xie, J. Fan, X.H. Qian, F.C. He, Y.P. Zhu, Y. Wang, D. Yang, W.L. Liu, Q.M. Liu, X.M. Yang, B. Zhen, Z.Y. Wu, J. Fan, H.C. Sun, J.Y. Qian, T. Hong, L. Shen, B.C. Xing, P.Y. Yang, H.L. Shen, L.J. Zhang, S.J. Cheng, J.Q. Cai, X.H. Zhao, Y.L. Sun, T. Xiao, Y.S. Mao, X.M. Chen, D. Wu, L. Chen, J. Dong, H.T. Deng, M.J. Tan, Z.X. Wu, Q.C. Zhao, Z.Y. Shen, X.G. Chen, Y.H. Gao, W. Sun, T. Wang, S.Q. Liu, L. Lin, J. Zi, X.M. Lou, R. Zeng, Y. Wu, S.J. Cai, B. Jiang, A.Q. Chen, Z.J. Li, F.Q. Yang, X.L. Chen, Y.N. Sun, Q.L. Wang, Y. Zhang, G.S. Wang, Z.C. Chen, W.S. Qin, Z.S. Li, C.H.P.P. C, Proteomics identifies new therapeutic targets of early-stage hepatocellular carcinoma, Nature 567 (2019) 257-+. 10.1038/s41586-019-0987-8.
12. B. Schwanhausser, D. Busse, N. Li, G. Dittmar, J. Schuchhardt, J. Wolf, W. Chen, M. Selbach, Global quantification of mammalian gene expression control (vol 473, pg 337, 2011), Nature 495 (2013) 126–127. 10.1038/nature11848.
13. B. Schwanhausser, D. Busse, N. Li, G. Dittmar, J. Schuchhardt, J. Wolf, W. Chen, M. Selbach, Global quantification of mammalian gene expression control, Nature 473 (2011) 337–342. 10.1038/nature10098.
14. L.A. Diaz, Jr., R.T. Williams, J. Wu, I. Kinde, J.R. Hecht, J. Berlin, B. Allen, I. Bozic, J.G. Reiter, M.A. Nowak, K.W. Kinzler, K.S. Oliner, B. Vogelstein, The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers, Nature 486 (2012) 537–540. 10.1038/nature11219.
15. J.Y. Douillard, K.S. Oliner, S. Siena, J. Tabernero, R. Burkes, M. Barugel, Y. Humblet, G. Bodoky, D. Cunningham, J. Jassem, F. Rivera, I. Kocakova, P. Ruff, M. Blasinska-Morawiec, M. Smakal, J.L. Canon, M. Rother, R. Williams, A. Rong, J. Wiezorek, R. Sidhu, S.D. Patterson, Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer, N Engl J Med 369 (2013) 1023–1034. 10.1056/NEJMoa1305275.
16. H. Tsang, T. Yau, P.L. Khong, R.J. Epstein, Bevacizumab-based therapy for advanced small bowel adenocarcinoma, Gut 57 (2008) 1631–1632. DOI 10.1136/gut.2008.153866.
17. N.J. Samadder, D.W. Neklason, K.M. Boucher, K.R. Byrne, P. Kanth, W. Samowitz, D. Jones, S.V. Tavtigian, M.W. Done, T. Berry, K. Jasperson, L. Pappas, L. Smith, D. Sample, R. Davis, M.K. Topham,

P. Lynch, E. Strait, W. McKinnon, R.W. Burt, S.K. Kuwada, Effect of Sulindac and Erlotinib vs Placebo on Duodenal Neoplasia in Familial Adenomatous Polyposis A Randomized Clinical Trial, Jama-J Am Med Assoc 315 (2016) 1266–1275. 10.1001/jama.2016.2522.

18. J. Zhong, Z. Ye, C.R. Clark, S.W. Lenz, J.H. Nguyen, H. Yan, K.D. Robertson, G. Farrugia, Z. Zhang, T. Ordog, J.H. Lee, Enhanced and controlled chromatin extraction from FFPE tissues and the application to ChIP-seq, Bmc Genomics 20 (2019) 249. 10.1186/s12864-019-5639-8.
19. J.Y. Xu, C. Zhang, X. Wang, L. Zhai, Y. Ma, Y. Mao, K. Qian, C. Sun, Z. Liu, S. Jiang, M. Wang, L. Feng, L. Zhao, P. Liu, B. Wang, X. Zhao, H. Xie, X. Yang, L. Zhao, Y. Chang, J. Jia, X. Wang, Y. Zhang, Y. Wang, Y. Yang, Z. Wu, L. Yang, B. Liu, T. Zhao, S. Ren, A. Sun, Y. Zhao, W. Ying, F. Wang, G. Wang, Y. Zhang, S. Cheng, J. Qin, X. Qian, Y. Wang, J. Li, F. He, T. Xiao, M. Tan, Integrative Proteomic Characterization of Human Lung Adenocarcinoma, Cell 182 (2020) 245–261 e217. 10.1016/j.cell.2020.05.043.

Figures

Fig. 1

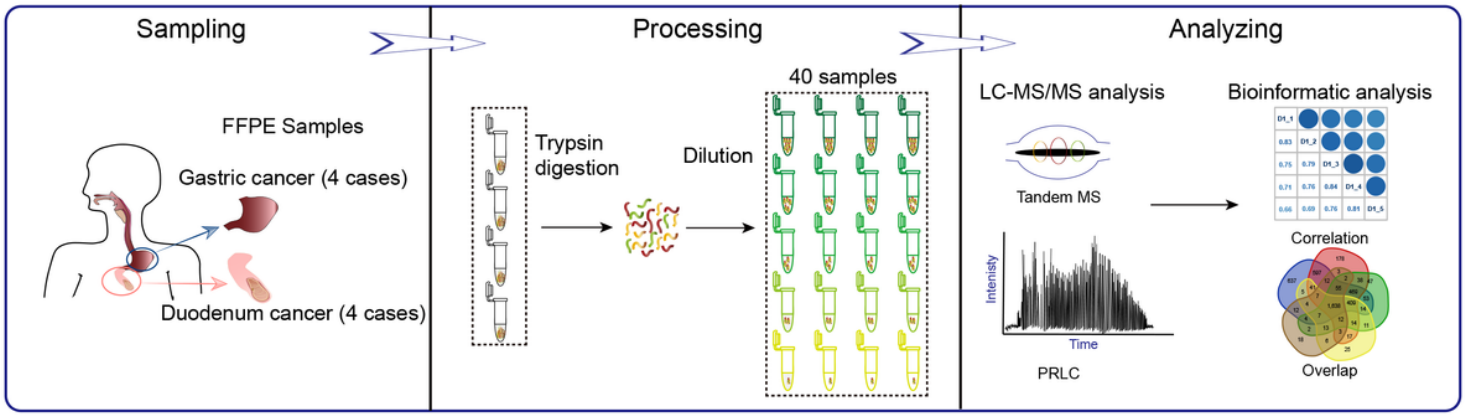

Figure 1

A brief workflow of experimental design. Refer to Supplementary Fig. S1a.

Fig. 2

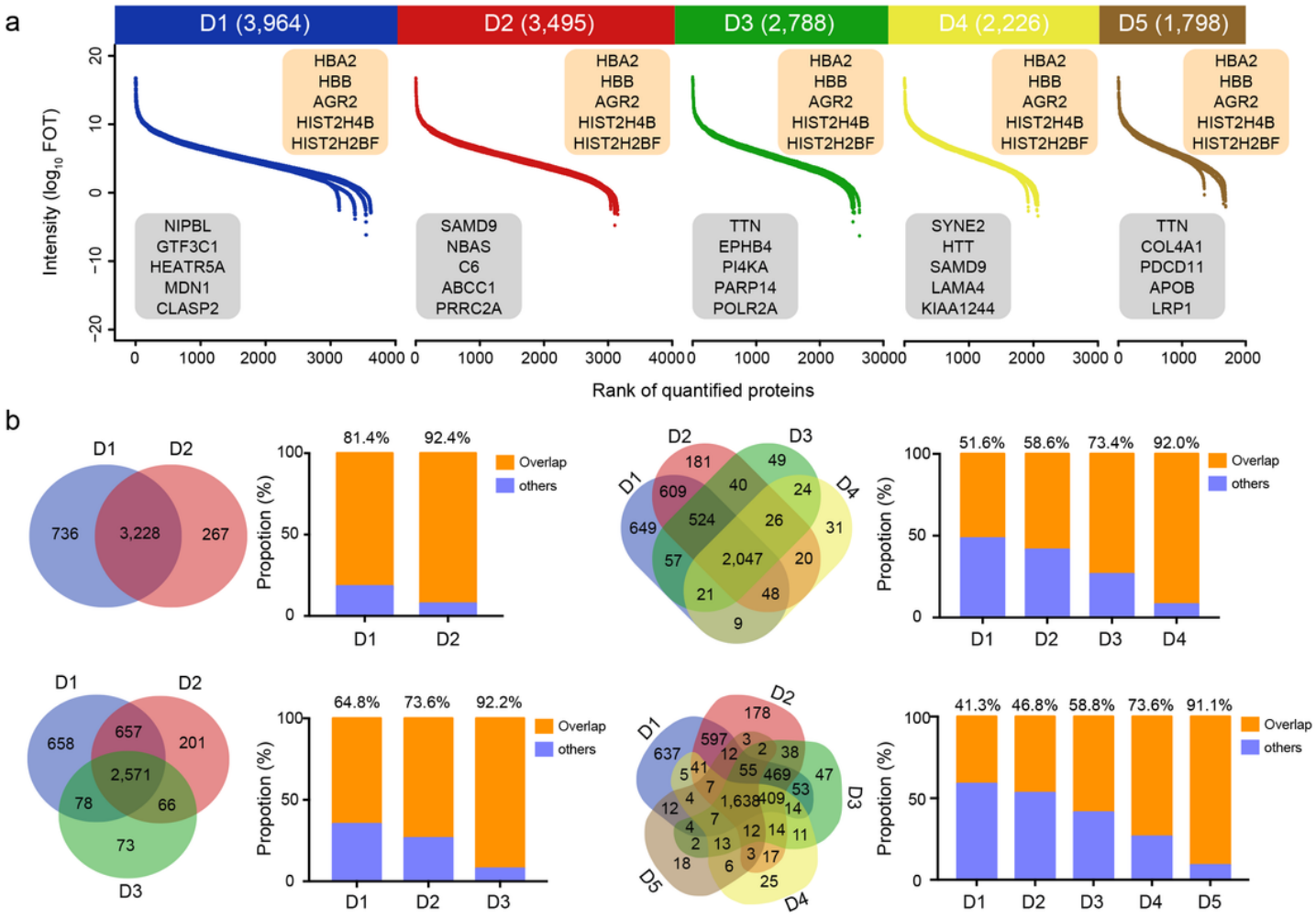

Figure 2

a: Overview of the proteomic profiles of duodenum cancer samples with 5 different AOPs. The highest-abundance and lowest-abundance proteins were shown in the box. b: Overview of the overlapped proteins and the coverage of overlapped proteins in duodenum cancer samples with 5 different AOPs.

Refer to Supplementary Fig. S1b–c, and Supplementary Table S1a–1b.

Fig. 3

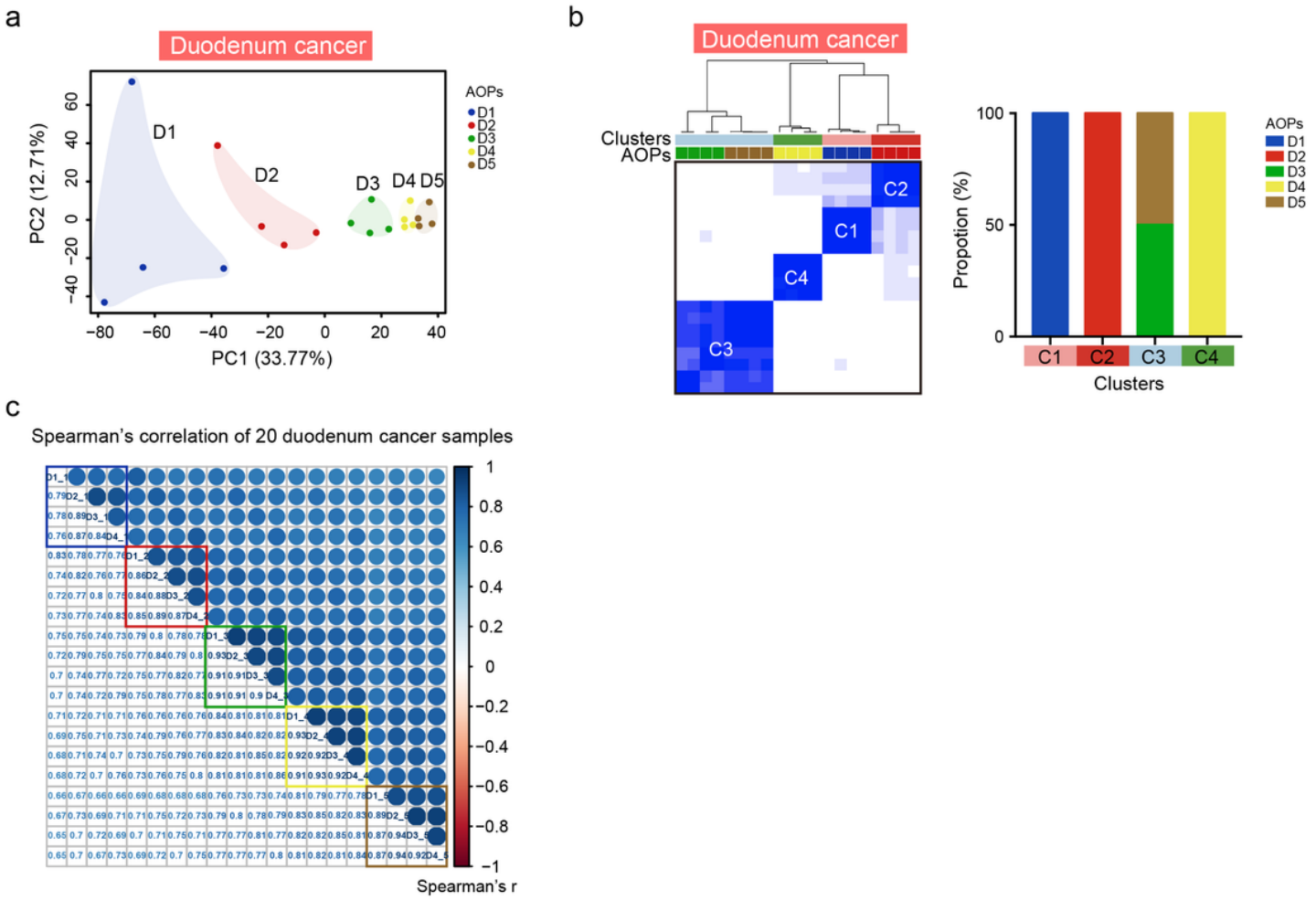

Figure 3

a: Principal component analysis (PCA) of proteomic data in 20 duodenum cancer samples. b: Consensus clustering analysis of proteomic data in 20 duodenum cancer samples (left) and the distribution of the samples with 5 different AOPs in 4 clusters (right). c: (Spearman's) correlation analysis of 20 duodenum cancer samples. Refer to Supplementary Fig. S2.

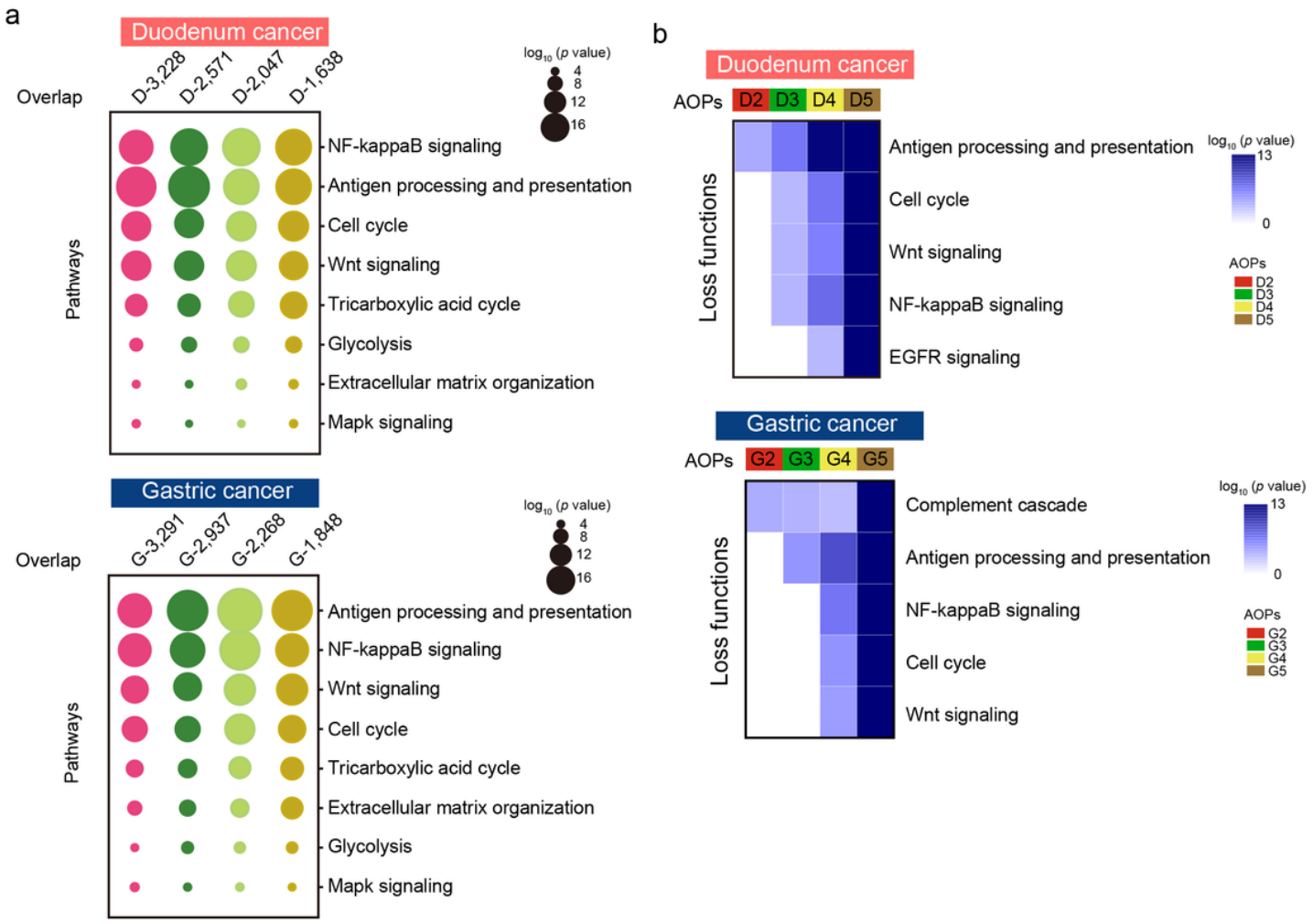

Figure 4

a: The GO term of overlapped proteins of duodenum cancer (top) and gastric cancer (bottom). b: The loss functions of duodenum cancer (top) and gastric cancer (bottom) samples with 5 different AOPs. Refer to Supplementary Table S1c–1d.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Graphicalabstract.pdf
- SupplementaryTableS1.xlsx
- SupplementaryFigure.pdf
- SupplementaryInformation.docx