Quantitative proteomics characterization of cancer biomarkers and treatment

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Cancer accounted for 16% of all death worldwide in 2018. Significant progress has been made in understanding tumor occurrence, progression, diagnosis, treatment, and prognosis at the molecular level. However, genomics changes cannot truly reflect the state of protein activity in the body due to the poor correlation between genes and proteins. Quantitative proteomics, capable of quantifying the relatively different protein abundance in cancer patients, has been increasingly adopted in cancer research. Quantitative proteomics has great application potentials, including cancer diagnosis, personalized therapeutic drug selection, real-time therapeutic effects and toxicity evaluation, prognosis and drug resistance evaluation, and new therapeutic target discovery. In this review, the development, testing samples, and detection methods of quantitative proteomics are introduced. The biomarkers identified by quantitative proteomics for clinical diagnosis, prognosis, and drug resistance are reviewed. The challenges and prospects of quantitative proteomics for personalized medicine are also discussed.

INTRODUCTION

Cancer accounted for 16% of global deaths according to the 2018 American Association for Cancer Research (AACR) Cancer Progress Report. Global cancer incidence will continue to increase with the aging of the population. By 2040, the number of new cancer cases is expected to reach 27.5 million worldwide, and the number of deaths is expected to reach 16.3 million. The Cancer Genome Atlas (TCGA) database has published the genomic landscapes of 33 tumor types from 11,000 patients, which could help to promote the understanding of tumor occurrence, progression, diagnosis, treatment, and prognosis at the molecular level. As the direct executor of life activities, protein participates in almost all life processes, such as heredity, development, reproduction, material and energy metabolism, and stress. However, the correlation between genomics changes and protein abundance is very poor, especially for low-abundance proteins. Therefore, proteomics could be the bridge between genome information and functional proteins that helps to further the understanding of cancer.

Proteomics is based on the protein composition and changing of cells, tissues, or organisms. Proteomics studies the characteristics of proteins on a large scale, including protein expression levels, post-translational modifications (PTMs), and protein-protein interactions, to gain a comprehensive understanding of disease occurrence, cell metabolism, and other processes at the protein level. Quantitative proteomics provides comprehensive information on the protein interactions, signal pathways, and biomarkers of human disease by detecting the relative changes in protein abundance in diseased tissue samples. In this review, we discuss the application of quantitative proteomics in cancer research and the discovery of tumor biomarkers, as well as its potential significance in early clinical diagnosis, prognosis, and targeted therapy.

DEVELOPMENT OF QUANTITATIVE PROTEOMICS

The concept of the proteome was first put forward by Australian scientist Mark Wilkins in 1994, and the concept of proteomics was put forward in 1997 as a science that studies the composition and changes of proteins in cells, tissues, or organs. In 2001, the International Human Proteome Organization (HUPO) officially announced the promotion of proteomics research. In the past 20 years, proteomics technology has improved continuously, which has enabled the application of quantitative analysis methods in proteomics. In 2014, Nature published two papers on human proteome for the first time. The application of proteomics has extensively promoted the progress of natural science research (Figure 1).
Currently, there are four main quantitative proteomics methods, that is, labeling, label-free, targeted, and PTM, widely used in cancer research (Figure 2). Stable isotope labeling by amino acids in cell culture (SILAC) technology, isotopic tags for relative and absolute quantification (iTRAQ) technology, and tandem mass tags (TMTs) technology are the main methods used for the labeling quantitative proteomics.23–26 SILAC technology is suitable for analyzing living cells in culture with accurate quantification and good repeatability.27 SILAC removes the false positives in protein-interaction studies, reveals the large-scale kinetics of proteomes, and directly uncovers the important points in the cellular signaling pathways as a quantitative phosphoproteomics technology. The triple-label SILAC proteomic profiles have been used to reveal the deregulation of key cell cycle regulators in long intergenic non-coding RNA-nucleotide metabolism regulator (lincNMR)-depleted cells, such as the key 2'-deoxy-nucleoside 5'-triphosphate (dNTP) synthesizing enzymes RRM2, TYMS, and TK1, which implicated lincNMR in regulating nucleotide metabolism.28 The iTRAQ/TMT technology has high sensitivity, high throughput, and good reproducibility.29,30 Keller et al.31 reported that secretome analysis using iTRAQ proteomics revealed the caspase-1-mediated secretion of other leaderless proteins with known or unknown extracellular functions. Without labeling processing, label-free quantification is simple to conduct, but it requires high stability and repeatability of experimental operations. It is suitable for large-scale quantitative comparison and experimental design that cannot be realized with labeling quantification.32 Wepr et al.33 presented a label-free mass spectrometry-based strategy for the absolute quantification of protein complex components isolated through affinity purification and quantitatively analyzed the interaction stoichiometries in the human protein phosphatase 2A network.

Targeted quantitative proteomics is essentially a mass spectrum scanning mode based on the selection of specific target protein ion and product ion pairs.34–36 Targeted quantitative proteomics could detect the relative or absolute quantities of various target proteins in complex samples (Table 1). PTM is an important component of protein activity regulation.39–42 Phosphorylation modification is the most common and most important PTM regulating the protein kinase and other protein activities.43 Quantitative phosphoproteomics is widely used for proteinomic stratification and drug target identification. Jiang et al.43 adopted proteomic and phosphoproteomic profiling and characterized 110 paired tumor and non-tumor tissues of clinical early-stage hepatocellular carcinoma (HCC) related to hepatitis B virus (HBV) infection. The quantitative proteomics data highlighted the heterogeneity in early stage HCC. Many analytical methods of proteomics have been developed for different samples, including cell lines, clinical samples, and body fluids.13,27,28 Each type of sample has advantages and disadvantages (Figure 3). The choice of sample type depends on the purpose of the research.

**Quantitative Proteomics Classification of Tumor Subtype**

In clinical practice, there is an urgent need for the early detection of cancer and the differentiation of tumor subtypes to improve the existing treatment. Proteome-informed classification could distinguish the clinical features of early-stage non-smoker lung adenocarcinoma.44,45 Mass spectrometry-based proteomic profiling could classify the pancancer molecular subtypes of 532 cancers.46 Quantitative proteomics could identify and quantify the specific signaling pathways from the tumor tissues and corresponding para-tumor tissues of 24 patients at different stages of triple-negative breast cancer (TNBC).47 Quantitative proteomics could be used for the accurate classification of TNBC subtypes.48 Furthermore, the sub-network identified through quantitative phosphoproteomics was highly correlated with clinically identified breast cancer subtypes.49–51 SWATH/DIA-MS (state-of-the-art sequential windowed acquisition of all theoretical fragment ion/data-independent acquisition mass spectrometry) presented a promising complement for the stable classification of ovarian cancer subtypes.52,53 Quantitative proteomics of reverse-phase protein array (RPPA) could be used to classify diffuse large B cell lymphoma.54–56

**Identifying Potential Biomarkers with Quantitative Proteomics**

With the development of mass spectrometry, quantitative proteomics has become an important method to discover tumor biomarkers. Increasing amounts of tumor biomarkers have been discovered by quantitative proteomics.57–59 Samples from tumor tissue and paired adjacent tissue or patients and healthy people were prepared, digested into peptides, and then analyzed with liquid chromatography-tandem
mass spectrometry (LC-MS/MS). After quantification and filtration, tumor biomarkers were identified (Figure 4).

The comprehensive classification of lung adenocarcinoma provided bioinformatics resources for clinical treatment, drug development, and precision medicine.60–62 Quantitative analysis of control, HBV, cirrhotic, and HCC tissue showed CD14 as a promising biomarker.63 The DIA quantitative proteomics analysis of 10 paired tumor and non-tumor samples verified three oxidative phosphorylation biomarkers (UQCRQ, NDUFB7, and UQCRC2) in gastric cancer.37 By the quantitative analysis of tumor tissues against normal adjacent tissues (NATs), AQR, DDX5, DPEP1, and TNC were identified as biomarkers in colorectal cancer. Through the proteomics approach, triosephosphate isomerase 1 (TPI1) was identified as a biomarker for predicting the recurrence of intrahepatic cholangiocarcinoma.64 Quantitative proteomics is increasingly adopted for identifying biomarkers of early pancreatic cancer, such as actinin-4, annexin A2, Bcl-2, H1.3, IGFBP2, IGFBP3, and galectin-1 (Figure 5).65–71

**DISCOVERING DRUG TARGETS WITH QUANTITATIVE PROTEOMICS**

Quantitative proteomics is a promising tool for revealing the molecular mechanisms of drug action.58,63–75 Proteomics drug maps have greatly promoted the discovery of drug targets. After the quantitative analysis for 10,000 proteins and 55,000 phosphorylation sites (p-sites) from 125 cancer cell lines, the proteome activity landscapes were obtained. Adenylate kinase isoenzyme 1 (AK1) was discovered as a promising drug target for acute myeloid leukemia patients.76 The anaplastic lymphoma kinase (ALK) inhibitor ceritinib was found to be capable of modulating the protein-trafficking and degradation-related process of autophagy after the quantitative analysis of five lung cancer cell lines in response to more than 50 drugs.77 The proto-oncogene serine/threonine-protein kinase PIM3 has been widely used as a drug target. Quantitative phosphoproteomics revealed that PIM3 activated RhoA to promote migration and invasion of hepatoma cells.78 By comprehensive phosphoproteomics characterization of 110 tumors and 101 matched NATs, three candidate drug targets were identified for lung adenocarcinoma (LUAD), including SOS1 inhibition in KRAS mutant, PTPN11/Shp2 inhibition in both ALK fusion and EGFR mutant tumors, and STK11 mutation in neutrophil degranulation.62 Quantitative proteomics was adopted to characterize 200 paired EGFR-positive and EGFR-negative glioma tissues of all pathological types, and EGF-like domain multiple 7 (EGFL7) was identified as a potential diagnostic biomarker and therapeutic target.79

**DISCOVERING DRUG RESISTANCE BIOMARKERS WITH QUANTITATIVE PROTEOMICS**

Drug resistance and recurrence are the main obstacles to the long-term survival of cancer patients. It is crucial to understand the mechanisms and identify the biomarkers of drug resistance. Quantitative proteomics is a powerful tool for identifying drug resistance biomarkers.80–85 Proteomics drug maps have been widely used to identify drug resistance biomarkers.
proteomics could help to identify the proteins related to drug resistance.80–82 Tamoxifen resistance is one of the unsolved problems in breast cancer treatment. Through proteomics analysis of tumor tissues from tamoxifen therapy-sensitive and tamoxifen therapy-resistant breast cancer patients, high expression of ectonucleotide pyrophosphatase/phosphodiesterase-1 (ENPP1) and extracellular matrix metalloproteinase inducer (EMMPRIN) were found relevant to tamoxifen resistance.83 However, low expression of eukaryotic translation initiation factor 3 subunit 6/E (EIF3E) and guanine nucleotide-binding protein b subunit 4 (GNB4) were relevant to tamoxifen resistance.84 Moreover, EMMPRIN-negative tumors were more sensitive to neoadjuvant chemotherapy in bladder cancer (BC).84 Using label-free quantitative proteomics analysis of trastuzumab-resistant MKN45/R cells and parental MKN45 human gastric cancer cells, WNT signaling was identified as a potential target in trastuzumab-resistant cancer. Quantitative proteomics analysis of the anti-HCC efficacy of dihydroartemisinin (DHA) combined with sorafenib may help to understand the related molecular mechanism of anti-HCC.85 The drug resistance of ovarian cancer cell lines was evaluated with iTRAQ LC-MS/MS, and 28 biomarkers that might lead to cisplatin resistance were identified.86 Radio resistance biomarkers in several cancers, such as breast cancer, prostate cancer, and lung cancer, were identified with MS-based proteomics approaches.87

### Table 1. A comparison of detection methods for quantitative proteomics

| Methods of label | Applicable samples | Clinical samples | Advantages | Disadvantages | Application | Ref. |
|------------------|--------------------|------------------|------------|---------------|-------------|------|
| SILAC            | in vivo metabolic incorporation of lysine or arginine | tissue culture cells | no | high sensitivity, high accuracy | high cost limited to living samples | biomarker screening in cell lines | 27,28 |
| iTRAQ / TMT      | in vitro N terminus and lysine side chains of peptide | non-living samples | yes | high sensitivity, high repeatability, closely reflect the state of samples | poor to low-abundance proteins | biomarker screening | 29–31 |
| Label-free proteomics | no | non-living samples | yes | low cost, simple manipulation, not limited by samples, high throughput, closely reflect the state of samples | poor stability and repeatability | biomarker screening | 32,33 |
| Targeted proteomics | no | non-living samples | yes | high accuracy, high repeatability, wider dynamic range | poor to higher protein complexity and complex analysis | intestinal flora screening | 34–36 |
| PTM proteomics   | no | non-living samples | yes | closely reflect the state of samples, high requirements for peptide enrichment | kinase target screening | biomarker and drug target screening | 13,37,38 |

Protein kinases are primary molecular drug targets, and phosphorylation regulation is a key mechanism in cancer drug resistance. Through integrated proteomics and phosphoproteomics analysis of cisplatin-sensitive (T24S) and cisplatin-resistant (T24R) T24 human BC cell lines, CDK2 was identified as a potential chemoresistance biomarker in BC.88 Through phosphoproteomics analysis of lapatinib-sensitive (SKBR3) and lapatinib-resistant (SKBR3-LR) breast cell lines, p21-activated kinase 2 (PAK2) was identified as an effective therapeutic target to overcome acquired lapatinib resistance in HER2-positive breast cancer.89 The success in identifying cancer drug resistance biomarkers could help to develop biomarker-guided targeted therapy.

### THE APPLICATION OF QUANTITATIVE PROTEOMICS IN CLINICAL DIAGNOSIS AND TREATMENT

Liquid biopsy is increasingly recognized as a promising non-invasive identification method of clinical biomarkers. Many studies have shown that exosomes, i.e., 40- to 100-nm vesicles containing nucleic acids, proteins, and lipids, could be used as tumor biomarkers.90–92 Various biomarkers for different types of cancer have been identified with exosome proteomics (Figure 5). Thrombospondin-1 (THBS1), fibulin-1
(FBLN1), and fibrinogen gamma chain (FGG) were identified as clinical biomarkers for liver cancer.93–95 Leucine-rich alpha-2-glycoprotein 1 (LRG1), basigin (BSG), carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6), and integrin beta-1 (ITGB1) were identified as clinical biomarkers for lung cancer.96–99 Plasma or serum is an important component for liquid biopsy. The plasma protein level of HSP90α was validated as a potential prognostic biomarker in LUAD after a comprehensive proteomics analysis of 103 cases in China.60 The serum amyloid A protein was identified as a biomarker for renal cancer by comparing 119 patients with clear cell RCC and 69 healthy controls. BCAS3, IRX1, IRX4, and IRX5 were identified in breast cancer plasma samples through label-free quantitative proteomics.100–103 S100P and aldehyde oxidase were identified as potential liver cancer biomarkers from human serum through quantitative proteomics (iTRAQ).104 SOD2 was identified as a potential salivary biomarker in liver cancer through iTRAQ-based proteomics.105

CONCLUSIONS

With the development of mass spectrometry technology, quantitative proteomics has been widely applied for studying cancer mechanisms. Many biomarkers of different cancers identified with quantitative proteomics could help in the early diagnosis, prognosis, and drug resistance analysis.106,107 Three types of samples, including cell lines, clinical samples, and body fluids, are used in quantitative proteomics research. Clinical samples and body fluids are widely used in cancer research. Several biomarkers of 12 types of cancers identified from clinical samples and body fluids are listed in Figure 5. Liquid biopsy is increasingly recognized as a promising non-invasive identification method of clinical biomarkers. Many tumor-related biomarkers have been found in serum, urine, saliva, and exosomes.100–103 Due to high protein complexity and wide dynamic range, quantitative proteomics for liquid biopsy face significant challenges. Future research may focus on developing mass spectrometry technology with wider coverage and dynamic range.

A series of proteomics technologies have been developed for the comprehensive understanding of cancer occurrence and development mechanisms, including PTM proteomics, spatiotemporal proteomics, single-cell proteomics, and multi-omics. Since the functional diversity of proteins is achieved through PTMs, many protein kinases have been identified as drug targets through quantitative phosphorylation proteomics.108–110 Spatiotemporal proteomics allows the identification of proteins that change subcellular localization under different experimental conditions using quantitative proteomics.111 As a topic of frequent discussion in the past decade, single-cell proteomics evaluates the heterogeneity and rare types of cells based on cell types and the state of a single cell.112,113 Multi-omics approaches have become promising.
in the study of human diseases. HSP90β was identified as a potential prognostic biomarker for lung cancer through integrative analysis of proteome, phosphoproteome, transcriptome, and whole-exome sequencing data. A complicated regulatory map of the SLC2A2 gene with 16 candidate enhancers was identified for HCC by coupling transcriptome and proteome. The effective integration of all of these technologies eventually promotes accurate diagnosis and personalized medicine.

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AUTHOR CONTRIBUTIONS
X.-L.Y., W.L., Y.-S.M., and D.F. designed the study; X.-L.Y., Y.S., D.-D.Z., R.X., T.-M.W., H.-M.W., P.-Y.W., and D.F. conducted the study; X.-L.Y., J.-B.L., Y.-S.M., and D.F. collected data; X.-L.Y., J.-B.L., W.L., Y.-S.M., and D.F. performed the statistical analyses and interpreted the data. X.-L.Y., Y.-S.M., and D.F. wrote the manuscript. All authors contributed to the final version of the manuscript and approved the final manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

REFERENCES
1. Kristensen, V.N., Lingjærde, O.C., Russnes, H.G., Vollan, H.K., Frigessi, A., and Berresen-Dale, A.L. (2014). Principles and methods of integrative genomic analyses in cancer. Nat. Rev. Cancer 14, 299–313.
2. Borrebaek, C.A. (2017). Precision diagnostics: Moving towards protein biomarker signatures of critical utility in cancer. Nat. Rev. Cancer 17, 199–204.

3. Du, R., Shen, W., Liu, Y., Gao, W., Zhou, W., Li, J., Zhao, S., Chen, C., Chen, Y., Liu, Y., et al. (2019). TGFÎ² promotes the progression of lung adenocarcinoma by bridging EGFR/RAS/ERK signaling to cancer cell stenosis. Signal Transduct. Target. Ther. 4, 60.

4. Min, L., Zhu, S., Wei, R., Zhao, Y., Liu, S., Li, P., and Zhang, S. (2020). Integrating SWATH-MS proteomics and transcriptome analysis identifies CHISL1 as a plasma biomarker for early gastric cancer. Mol. Ther. Oncolytics 17, 257–266.

5. Xu, W., Xu, M., Wang, L., Zhou, W., Xiang, R., Shi, Y., Zhang, Y., and Piao, Y. (2019). Integrative analysis of DNA methylation and gene expression identified cervical cancer-specific diagnostic biomarkers. Signal Transduct. Target. Ther. 4, 55.

Karczewski, K.J., and Snyder, M.P. (2018). Integrative omics for health and disease. Nat. Rev. Genet. 19, 299–310.

7. Yang, K., Zeng, L., Ge, A., Pan, R., Xiao, L., Tong, Q., Yuan, M., Zhu, X., Ge, L., and Huang, Z. (2020). Integrating systematic biological and proteomics strategies to explore the pharmacological mechanism of danshen yin modified on atherosclerosis. J. Cell. Mol. Med. 24, 13876–13898.

8. Zhu, M., Dang, Y., Yang, Z., Liu, Y., Zhang, L., Xu, Y., Zhou, W., and Ji, G. (2020). Comprehensive RNA sequencing in adenoma-cancer transition identified predictive biomarkers and therapeutic targets of human CRC. Mol. Ther. Nucleic Acids 20, 25–33.

9. Pang, B., Zhu, Y., Ni, J., Thompson, J., Malouf, B., Bucci, I., Graham, P., and Li, Y. (2020). Extracellular vesicles: the next generation of biomarkers for liquid biopsy-based prostate cancer diagnosis. Theranostics 10, 2309–2326.

10. Yan, M., Sun, L., Li, J., Yu, H., Lin, H., Yu, T., Zhao, F., Zhu, M., Liu, L., Geng, Q., et al. (2019). RNA-binding protein KHSRP promotes tumor growth and metastasis in non-small cell lung cancer. J. Exp. Clin. Cancer Res. 38, 478.

11. Cher, N.T., Lohse, I., and Brothers, S.P. (2019). mRNA-to-protein translation in hypoxia. Mol. Cancer 18, 49.

12. Zhao, M., Zhang, Y., Jiang, Y., Wang, K., Wang, X., Zhou, D., Wang, Y., Yu, R., and Zhou, X. (2021). YAP promotes autophagy and progression of gliomas via upregulating HMGBl. J. Exp. Clin. Cancer Res. 40, 99.

13. Fernandes, E., Sores, J., Cotton, S., Peixoto, A., Ferreira, D., Freitas, R., Reis, C.A., Santos, L.L., and Ferreira, J.A. (2020). Esophageal, gastric and colorectal cancers: Hypoxia and angiogenesis. Mol. Cancer 19, 1874–1884.

14. Wilmut, M., Schlegl, I., Hahne, H., Gholami, A.M., Lieberenz, M., Savitski, M.M., Ziegler, E., Butzmann, L., Gesualt, S., Marx, H., et al. (2014). Mass-spectrometry-based draft of the human proteome. Nature 509, 582–587.

15. Choi, S., Goswami, N., and Schmidt, F. (2020). Comparative proteomic profiling of 3T3-L1 adipocyte differentiation using SILAC quantification. J. Proteome Res. 19, 4884–4900.

16. Wang, J., Zhu, S., Meng, N., He, Y., Li, R., and Yan, G.R. (2019). ncRNA-encoded peptides or proteins and cancer. Mol. Ther. 27, 1718–1725.

17. Griss, J., Vinterhalter, G., and Schwämmle, V. (2019). IsoProt: A complete and reproducible workflow to analyze iTRAQ/TMT experiments. J. Proteome Res. 18, 1751–1759.

18. Warrier, S., Patil, M., Bhansali, S., Varier, L., and Sethi, G. (2021). Designing precision medicine panels for drug refractory cancers targeting cancer stemness traits. Biochim. Biophys. Acta Rev. Cancer 1875, 188475.

19. Li, N., Li, J., Desiderio, D.M., and Zhan, X. (2021). SILAC quantitative proteomics analysis of ivermectin-related proteomic profiling and molecular network alterations in human ovarian cancer cells. J. Mass Spectrom. 56, e4659.

20. Gandhi, M., Grof, M., Holler, J.M., Coggins, S.A., Patil, N., Leupold, J.H., Munschauer, M., Schone, M., Hartigan, C.R., Alagavy, H., et al. (2020). The IncRNA lincNMR regulates nucleotide metabolism via a YBX1-RM2 axis in cancer. Nat. Commun. 11, 3214.

21. Tang, Q., Xu, Y., Deng, C., Cheng, C., Dai, Z., Yang, Z., Chen, X., Liu, C., and Su, J. (2021). Differential proteomic analysis to identify proteins associated with apoptosis in Bodmeria tricuspid (Hance) Makino using an iTRAQ-based strategy. J. Proteome Res. 20, 661–669.

22. Di Meo, A., Sohaei, D., Batruch, I., Alexandrou, P., Prassas, I., and Diamandis, E.P. (2021). Proteomic profiling of the human liver and biological fluid proteome. J. Proteome Res. 20, 444–452.

23. Wepf, A., Glatter, T., Schmidt, A., Aebersold, R., and Gstaiger, M. (2020). Quantitative interaction proteomics using mass spectrometry. Nat. Methods 6, 203–205.

24. Ong, S.E., Blagove, B., Kratcharimova, I., Kristensen, D.B., Steen, H., Pandey, A., and Mann, M. (2002). Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol. Cell. Proteomics 1, 376–386.

25. Heller, L., Thinard, R., Chevalier, M., Arpag, S., Jing, Y., Greferath, R., Heller, R., and Niculai, C. (2020). Secretion of proteins and antibody fragments from transiently transfected endothelial progenitor cells. J. Cell. Mol. Med. 24, 8772–8778.

26. DeSouza, L., Diehl, G., Rodrigues, M.I., Guo, J., Romashkin, A.D., Colgan, T.J., and Sui, K.W. (2005). Search for cancer markers from endometrial tissues using differentially labeled tag iTRAQ and cLCAT with multidimensional liquid chromatography and tandem mass spectrometry. J. Proteome Res. 4, 377–386.

27. Kim, M.S., Pinto, S.M., Getnet, D., Niruogi, R.S., Manda, S.S., Chaerkady, R., Madugundu, A.K., Kekar, D.S., Isserlin, R., Jain, S., et al. (2014). A draft map of the human proteome. Nature 509, 575–581.

28. Wilmut, M., Schlegl, I., Hahne, H., Gholami, A.M., Lieberenz, M., Savitski, M.M., Ziegler, E., Butzmann, L., Gesualt, S., Marx, H., et al. (2014). Mass-spectrometry-based draft of the human proteome. Nature 509, 582–587.
43. Jiang, Y., Sun, A., Zhao, Y., Ying, W., Sun, H., Yang, X., Xing, B., Sun, W., Ren, L., Hu, B., et al.; Chinese Human Proteome Project (CNPHP) Consortium (2019). Proteomics identifies new therapeutic targets of early-stage hepatocellular carcinoma. Nature 567, 257–261.

44. Stewart, P.A., Welsh, E.A., Slebos, R.J.C., Fang, B., Izumi, V., Chambers, M., Zhang, G., Cen, L., Pettersson, F., Zhang, Y., et al. (2019). Proteogenomic landscape of squamous cell lung cancer. Nat. Commun. 10, 5578.

45. Zhuo, H., Zhao, Y., Cheng, X., Xu, M., Wang, L., Lin, L., Lyu, Z., Hong, X., and Cai, J. (2019). Tumor endothelial cell-derived cadherin-2 promotes angiogenesis and has prognostic significance for lung adenocarcinoma. Mol. Cancer 18, 34.

46. Chen, F., Chandrashekar, D.S., Varambally, S., and Creighton, C.J. (2019). Pan-cancer molecular subtypes revealed by mass-spectrometry-based proteomic characterization of more than 500 human cancers. Nat. Commun. 10, 5679.

47. Velloso, F.J., Campos, A.R., Sogayar, M.C., and Correa, R.G. (2019). Proteome profiling of triple negative breast cancer cells overexpressing NOD1 and NOD2 receptors unveils molecular signatures of malignant cell proliferation. BMC Genomics 20, 152.

48. Lastiri-Pancaro, G., Mercado-Hernández, J.S., Kim, J., Jiménez, J.L., and Utrela, J. (2020). A quantitative method for proteome reallocation using minimal regulatory interventions. Nat. Chem. Biol. 16, 1026–1033.

49. Noblejas-López, M.D.M., Nieto-Jiménez, C., Galán-Moya, E.M., Tobar-García, D., Montero, J.C., Pandiella, A., Burgoz, M., and Ocaña, A. (2021). MZ1 co-operates with trastuzumab in HER2 positive breast cancer. J. Exp. Clin. Cancer Res. 40, 106.

50. Yim, H.E., Kim, J.H., Ahn, M.S., Jung, Y., Roh, J., Park, S.H., Kim, T.G., Choi, J.H., and Kang, S.Y. (2021). Clinicalpathological and molecular analysis of 45 cases of pure mucinous breast cancer. Front. Oncol. 10, 58876.

51. Thomas, R., Al-Khadairi, G., and Deock, I. (2021). Immune checkpoint inhibitors in triple negative breast cancer treatment: Promising future prospects. Front. Oncol. 10, 600573.

52. Thomas, S.N., Friedrich, B., Schnaibleb, M., Chan, D.W., Zhang, H., and Aebersold, R. (2020). Orthogonal proteome platforms and their implications for the stable classification of high-grade serous ovarian cancer subtypes. iScience 23, 101079.

53. Poulos, R.C., Hains, P.G., Shah, R., Lucas, N., Xavier, D., Manda, S.S., Anees, A., Koh, J.M.S., Mahboob, S., Wittman, M., et al. (2020). Strategies to enable large-scale proteomics for reproducible research. Nat. Commun. 11, 3793.

54. Suzuki, M., Muroi, A., Nojima, M., Numata, A., Pettersson, F., Zhang, Y., et al. (2019). Proteogenomic landscape of squamous cell lung cancer using iTRAQ quantitative proteomics. Oncotarget 8, 62011–62028.

55. Guo, J., Jang, R., Zhong, J.H., Dong, X., Liu, Y.X., Liu, Y.K., Huang, T.R., and Zhang, C.Y. (2017). Identification of CD4 as a potential biomarker of hepatocellular carcinoma using iTRAQ quantitative proteomics. Oncotarget 8,62011–62028.

56. Guo, Z., Wang, X., Yang, Y., Chen, Z., Wang, K., Teng, B., Huang, C., Zhao, Q., and Qu, Z. (2020). Hypoxic tumor-derived exosomal long noncoding RNA UCA1 promotes angiogenesis via miR-96-5p/AMOTL2 in pancreatic cancer. J. Proteomics 231, 105004.

57. O’Rourke, M.R., Sahni, S., Samra, J., Mettall, A., and Molloy, M.P. (2021). Data independent acquisition of plasma biomarkers of response to neoadjuvant chemo-therapy in pancreatic ductal adenocarcinoma. J. Proteomics 231, 105998.

58. Melchiolla, R., Spada, S., Di Modugno, F., D’Andrea, D., Carlo, A., Panetta, M., Mileo, A.M., Sperduti, I., Antoniani, B., Gallo, E., et al. (2020). The actin modulator HmEL regulates GAS6-AXL axis and pro-tumor cancer/stromal cell cooperation. EMBO Rep. 21, e50787.

59. Coleman, O., Henry, M., O’Neill, F., Roche, S., Swan, N., Geoghegan, J., Lordon, K., McVey, G., Moriarty, M., Mealey, P., and Clynes, M. (2020). Proteomic analysis of cell lines and primary tumors in pancreatic cancer identifies proteins expressed only in vitro and only in vivo. Pancreas 49, 1109–1116.

60. Flick, K.F., Yip-Snedecker, M.T., Sublette, C.M., Simpson, R.E., Colgate, C.L., Wu, H., Souli, M., Dewitt, J.M., Mosley, A.L., Ceppa, E.P., et al. (2020). A quantitative global proteomics approach identifies candidate urinary biomarkers that correlate with intraductal papillary mucinous neoplasm dysplasia. Pancreas 49, 1044–1051.

61. Guo, Z., Wang, X., Yang, Y., Chen, W., Zhang, K., Teng, B., Huang, C., Zhao, Q., and Qu, Z. (2020). Hypoxic tumor-derived exosomal long noncoding RNA UCA1 promotes angiogenesis via miR-1844-5p/AMOTL2 in pancreatic cancer. Mol. Ther. Nucleic Acids 22, 179–195.

62. Vinaiphat, A., Low, J.K., Yeoh, K.W., Chng, W.J., and Sze, S.K. (2021). Application of advanced mass spectrometry-based proteomics to study hypoxia driven cancer progression. Front. Oncol. 11, 559822.

63. Chang, J.W., Ding, Y., Tahir Ul Qamar, M., Shen, Y., Gao, J., and Chen, L.L. (2019). A deep learning model based on sparse auto-encoder for prioritizing cancer-related genes and drug target combinations. Cancerogenesis 40, 624–632.

64. Guan, N.N., Zhao, Y., Wang, C.C., Li, J.Q., Chen, X., and Piao, X. (2019). Anticancer drug response prediction in cell lines using weighted graph regularized matrix factorization. Mol. Ther. Nucleic Acids 17, 164–174.

65. Li, Y., Leon, J., and Park, J.H. (2020). Hypoxia-responsive nanoparticles for tumor-targeted drug delivery. Cancer Lett. 490, 31–43.

66. Feijno, M., Meng, C., Ruprecht, B., Osterlisch, T., Scheich, S., Kleigrewe, K., Drexoll, E., Samaras, P., Hogrebe, A., Helms, D., et al. (2020). Proteome activity landscapes of tumor cell lines determine drug responses. Nat. Commun. 11, 3639.

67. Ruprecht, B., Di Bernardo, J., Wang, Z., Mo, X., Ursu, O., Christopher, M., Fernandez, R.R., Zheng, L., Dill, B.D., Wang, H., et al. (2020). A mass spectrometry-based proteome map of drug action in lung cancer cell lines. Nat. Chem. Biol. 16, 1111–1119.

68. Deng, Y., Jiang, N., Wang, H., Chen, X., Gao, Y., Zhang, X., Qin, G., Li, Y., and Chen, R. (2020). Proto-oncogene serine/threonine kinase PIM3 promotes cell migration via modulating Rho GTPase signaling. J. Proteome Res. 19, 1298–1309.
81. Zhou, X.T., Ding, J., Li, H.Y., Li, H.Y., Zuo, J.L., Ge, S.Y., Jia, H.L., and Wu, J. (2020). A novel chemokine in the occurrence and metastasis of hepatocellular carcinoma. J. Cell. Mol. Med. 146, 1318–1323.

82. Lin, Q., Zhou, C.R., Bai, M.J., Zhu, D., Chen, J.W., Wang, H.F., Li, M.A., Wu, C., Li, X.J. (2020). Protein expression analysis in breast cancer: roles, mechanisms, and applications. Mol. Cancer 19, 187–200.

83. Umar, A., Kang, H., Timmermans, A.M., Look, M.P., Meijer-van Gelder, M.E., den Bakker, M.A., Jaitly, N., Martens, J.W., Luider, T.M., Foekens, J.A., and Pasa-Tolic, L. (2019). Proteomic and phosphoproteomic analysis of the anti-hepatic carcinoma effect of combined dihydroartemisinin and sorafenib. Biomed. Pharmacother. 126, 109862.

84. Hemdan, T., Malmström, P.U., Jahnson, S., and Segersten, U. (2015). Emmprin regulates TNFα-induced GM-CSF production by breast cancer MDA-MB-231 cells. Biomolecules 9, 555.

85. Fujiyuki, T., Amagai, Y., Shoji, K., Kuraishi, T., Sugai, A., Awano, M., Sato, H., Hattori, S., Yoneda, M., and Kai, C. (2020). Recombinant SLAMblind mesiales virus is a promising candidate for nectin-4 positive triple negative breast cancer therapy. Mol. Ther. Oncolytics 19, 127–135.

86. Cao, H., Zhu, X., Chen, X., Yang, Y., Zhou, Q., Xu, W., and Wang, D. (2020). Quantitative proteomic analysis to identify differentially expressed proteins in the persistent atrial fibrillation using TMT coupled with nano-LC-MS/MS. Am. J. Transl. Res. 12, 5032–5047.

87. Munagala, R., Aqil, F., Jeyabalan, J., Kandimalla, R., Wallen, M., Tyagi, N., Wälcher, S., Yan, J., Schultz, D.J., Spencer, W., and Gupta, R.C. (2021). Exosome-mediated delivery of RNA and DNA for gene therapy. Cancer Lett. 505, 58–72.

88. Li, M.Y., Liu, L.Z., and Dong, M. (2021). Progress on pivotal role and application of exosome in lung cancer carcinogenesis, diagnosis, therapy and prognosis. Mol. Cancer 20, 22.

89. Wang, Z.Y., Hung, A.C., Lo, S., and Yuan, S.F. (2021). Adipocytokines visfatin and resistin in breast cancer: Clinical relevance, biological mechanisms, and therapeutic potential. Cancer Lett. 498, 229–239.

90. Thomas, R., Al-Rashed, F., Akhter, N., Al-Mulla, F., and Ahmad, R. (2019). ACSL1 regulates TNFα-induced GM-CSF production by breast cancer MDA-MB-231 cells. Mol. Cancer 18, 564–568.

91. Guo, W., Li, Y., Pang, W., and Shen, H. (2020). Exosomes and cancer EMT and metastasis. Am. J. Transl. Res. 12, 5032–5047.