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Cancer: A proteomic disease

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The development of cancer is a pathological process involving multiple environmental carcinogenic factors and genetic alterations. For decades, cancer researchers have focused on genomic and transcriptomic analyses. The completion of the Human Genome Project has opened the door to the post-genome era and oncoproteomics. Proteins play a critical role in tumorigenesis and influence the differences between normal cells and malignant cells. This report proposes the concept that cancer is a proteomic disease. This concept is based on examining protein expression profiles, post-translational modifications, and protein-protein interactions in carcinogenesis using recent advances in comparative, functional and structural proteomics. This approach provides a new way of viewing carcinogenesis, presents new clues in biomarker discovery for cancer diagnosis and therapy, and reveals important scientific findings and their significance to clinical applications.

cancer, proteomic disease, protein profiling, modification-specific proteome, protein-protein interaction

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The development of tumors is a multi-step process involving various environmental and genetic factors. Cancer is characterized by uncontrolled control cell growth, invasion and metastasis. Cancer studies using genomic and transcriptomic approaches have been used for years [1]. All genetic information is contained in the genome as an original template that guides protein biosynthesis, and proteins drive almost all the functions of the cell and determine phenotypes.

Likewise, the differences between cancer cells and normal cells are in large part determined by differential protein expression. Therefore, it is reasonable to study the mechanisms of tumorigenesis at the proteome level [2]. Along with the extensive application of proteomics theory and relevant cancer research techniques, oncoproteomics has been an active area of research in medical research, enabling many breakthroughs. Based on combining this new perspective with previous achievements in the field, we view cancer as a proteomic disease rather than a genetic disease.

1 Cancer development and proteome profiling

A key task of oncoproteomics is to select differentially expressed proteins and determine their function. Recent studies revealed distinct differences between cancer and normal cells at the proteome level, and that oncoproteome components vary at different points in time or under different conditions. These observations suggest that alterations in the proteome are involved in cancer development and in the regulation of malignancy.

A two-dimensional proteome map of human nasopharyngeal squamous cell carcinoma first published by Li *et al.* [3,4] found 28 proteins differentially expressed between normal nasopharyngeal epithelial tissue and nasopharyngeal carcinoma (NPC). Thirty-six proteins differentially expressed between normal nasopharyngeal epithelial tissue and nasopharyngeal carcinoma were laser-microdissected. Among the 36 proteins, the expression of stathmin, 14-3-3σ,

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annexin I and cathepsin D was associated with TNM (Tumor, Node, Metastasis) stage of nasopharyngeal carcinoma [5]. Hsieh *et al*. [6] analyzed proteome dynamics using two-dimensional gel electrophoresis coupled to mass spectrometry (2-DE) and reconstructed the associated cell cycle networks in human hepatoma cells that give rise to Mahlavu hepatocellular carcinoma (HHCM). They identified 2665 protein spots in all and the degree of global protein change was phase dependent, with the greatest change occurring in transitional phases at S/G2, G2/M, and G1/S. Further examination revealed that SP1, c-Myc, p53, YY1 and c-Jun acted as key transcription factors in G0/G1, G1/S, S, and G2/M, respectively, resulting in cell-cycle progression or arrest. To study the cellular and molecular mechanisms of Ras-mediated oncogenic transformation of ovarian epithelial cells, Young *et al*. [7] used a proteomic approach using 2-DE and mass spectrometry to profile two ovarian epithelial cell lines, one immortalized and the other transformed with an oncogenic rasV12 allele. More than 30 protein targets showed significant changes between immortalized and transformed cell lines, where the Ras-signaling pathway was found to be involved in an enhanced anti-oxidation state. This enhanced anti-oxidation state might constitute a common mechanism for tumor cells to evade oxidative stress induced apoptosis. Smad4 is a tumor-suppressor gene lost or mutated in 50% of pancreatic carcinomas. A study using 2-DE-MS examined proteomic changes in Smad4 knockdown (S4KD) pancreatic cancer cell lines with and without TGF-β stimulation. Five proteins were up-regulated and seven proteins were down-regulated, 10 of which were novel targets for TGF-β. These proteins functioned in processes such as cytoskeletal regulation, cell cycle progression, and oxidative stress [8].

Subcellular organelles are specially related to the corresponding cellular functions, so it is essential to separate and identify cancer cell organelle proteomes under various conditions to help identify tumorigenesis mechanisms. Qattan *et al.* [9] adopted quantitative, tandem mass spectrometry-based shotgun proteomic techniques to investigate the spatial distribution of proteins in MCF-7 breast cancer cells. A total of 2184 proteins were identified in four major organellar compartments: the cytosol, plasma membrane, endoplasmic reticulum, and mitochondrion. Of these proteins, 481 were found to have a unique subcellular location and 454 were ubiquitous. The remaining 1249 proteins were distributed over multiple, but not all, subcellular locations. These results indicate that multiple subcellular locations exist for proteins in processes related to breast cancer. Leth-Larsen *et al.* [10,11] identified 13 membrane proteins that were over-expressed and three that were under-expressed in a metastatic versus non-metastatic cell line from a total of 1919 protein entries. High ecto-5′-nucleotidase and integrin β1 expression correlated with tumor spread or distant recurrence, and poor outcome within a 10-year follow-up [10]. In addition, NDRG1, HLA-DRα, HLA-DRβ, and CD74 were associated with the ER(−)/PR(−) phenotype.

The degree of cancer differentiation is not only correlated with pathological phenotypes, clinical symptoms and prognosis, but also with proteomic alteration. Recently, Xiao *et al*. [12] performed quantitative proteomic analysis of flash frozen paraffin embedded (FFPE) NPC with various degrees of differentiation using isobaric tag for relative and absolute quantitation (iTRAQ) labeling, and two-dimensional liquid chromatography, tandem mass spectrometry (2D LC-MS/MS). Interestingly, 730 unique proteins were acquired from FFPE tissues. Of these, 531 are associated with various cellular processes, including proliferation, cell death, defense response, DNA repair and carbohydrate metabolism. One hundred and forty-one proteins were up-regulated, 140 down-regulated and 157 fluctuated in direction.

At multiple steps during cancer development and progression, the proteome keeps changing. Habermann *et al*. [13] analyzed the sequential alterations of the genome, transcriptome, and proteome during colorectal cancer progression and found that the expression of nine proteins decreased, and 32 proteins increased in proportion to cancer malignancy. The proteins identified played a role in cell cycle regulation, apoptosis and signal transduction (Figure 1). Wu *et al*. [14] performed a comparative proteomic study on 32 cases of human bronchial epithelia, including normal, metaplasia, dysplasia, and carcinoma tissues. Differentially expressed proteins were identified between the normal and metaplasia groups (33 proteins), the metaplasia and dysplasia groups (42), and the dysplasia and carcinoma groups (38). Some proteins are known to be involved in regulating cell proliferation, differentiation and signal transduction, whereas some are involved in cell-cell interaction and others are oncogene or anti-oncogene products. In another study, gastric cancer was induced in 12 Wistar rats by oral administration of N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) followed by evaluation of the protein expression patterns of matched tissues and corresponding metastases. Twenty-five proteins served as landmarks for comparison between tissues. Over-expression of HSP27 was confirmed by immunohistochemical analysis of human gastric cancer specimens and its expression correlated with the process of carcinogenesis and metastases [15]. Choong *et al.* [16] conducted proteomic analysis of the MCF10AT breast cancer model, composed of four isogenic xenograft-derived human cell lines that mimic different stages of breast cancer progression, using iTRAQ 2D-LC-MS/MS. Of more than 1200 proteins detected, 98 represent at least 20 functional groups, including kinases, proteases, adhesion, calcium binding and cytoskeletal proteins exhibited significant expression changes. These results suggest that the MCF10AT model of breast cancer progression models major re-programming in metabolism, or the "Warburg effect". We also validated that HIST1H2BM is down-regulated as breast cancer lesions progress.

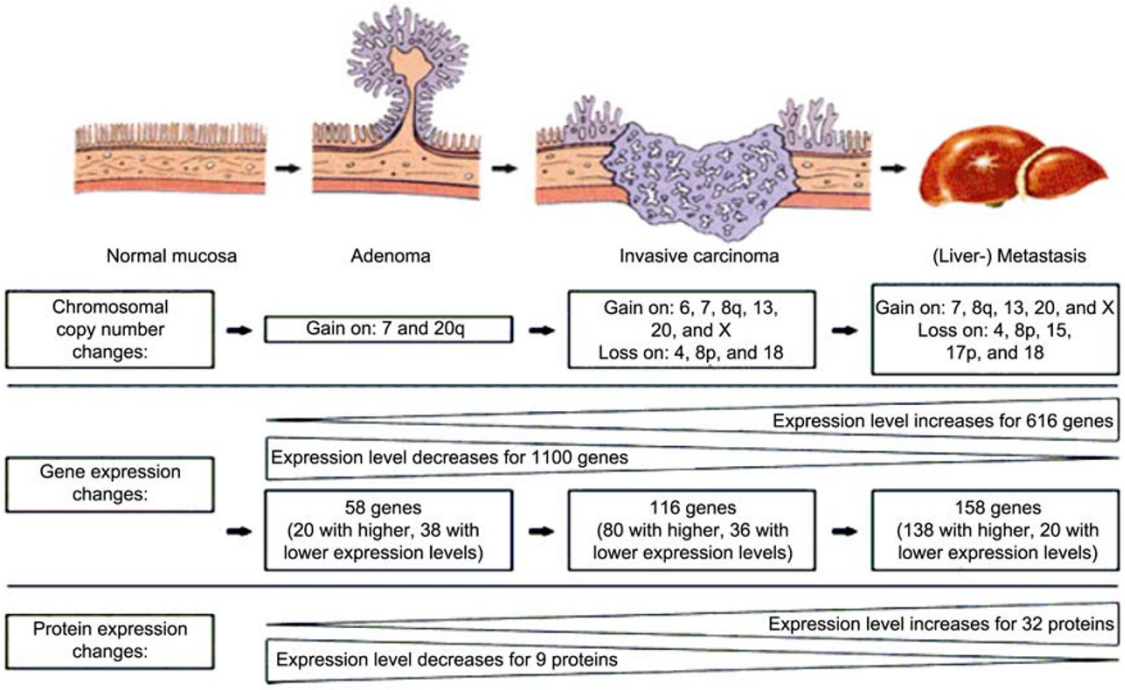

Figure 1 Summary figure of genomic, transcriptional, and proteomic changes along the "adenoma-carcinoma-sequence" [13].

Clinical proteomics has been used to discover many cancer-related protein markers that are involved in tumorigenesis and used in prognosis. Serological proteome analysis was performed on human lung squamous carcinoma (hLSC) to identify tumor-associated antigens. Fourteen hLSC-associated antigens were identified and six of those were validated to be up-regulated in hLSC tissues, suggesting potential application of these antigens in the diagnosis and therapy of hLSC [17]. Liu *et al*. [18] assessed protein expression differences between primary lung adenoma cancer with (LNM AdC) and without (non-LNM AdC) lymph node metastasis using a quantitative proteomic approach. Among the 20 differentially expressed proteins identified, statistical analysis indicated that over-expression of annexin A1, A2, and A3 was associated with increased relapse rate and decreased survival rate. Previous clinical proteomic studies have demonstrated that a single protein is insufficient as a cancer diagnostic marker and that a fingerprint composed of several proteins greatly enhances the sensitivity and specificity of diagnosis. For example, to detect early stage invasive epithelial ovarian cancer, the sensitivity of a multivariate model combining three biomarkers with CA125 (74% (95% CI, 52%–90%)) was higher than that of CA125 alone (65% (95% CI, 43%–84%)) at a matched specificity (97% (95% CI, 89%–100%)). When compared at a fixed sensitivity (83% (95% CI, 61%–95%)), the specificity of the model (94% (95% CI, 85%–98%)) was significantly better than that of CA125 alone (52% (95% CI, 39%–65%)). This demonstrates that combining biomarkers has the potential to improve detection of early stage ovarian cancer [19,20].

Studying cancer development and progression, and identifying markers at different stages using whole cell and organelle proteomics helps reveal how cancer develops, and may lead to new approaches for early diagnosis and improved therapy.

2 Tumor and proteomics of post-translational modifications

Post-translational modifications (PTMs) such as glycosylation, phosphorylation, and ubiquitination induce structural changes and play an important role in the genesis and development of cancer. Because of the broad dynamic range of protein expression, accurate detection of post-translationally modified proteins still presents a significant technical challenge. Nevertheless, development and integration of different methods, such as affinity-based enrichment methods, multi-dimensional separation technologies, and mass spectrometry show promise for post-translational proteomics at a large scale [21].

Glycoproteins contain oligosaccharides (glycans) covalently attached to polypeptide side-chains. Whelan *et al*. [22] chose the hydrazide method to specifically target, enrich, and identify glycosylated proteins from breast cancer cell membrane fractions using a LTQ Orbitrap mass spectrometer. Twenty-seven N-linked glycosylation sites in 25 different proteins were identified, including sites on epidermal growth factor receptor (EGFR), CD44, and the breast cancer

1, and early onset isoform 1 (BRCA1), which mediate breast cancer invasion and metastasis by regulating cell differentiation and proliferation. Chaerkady *et al*. [23] used lectin affinity chromatography and ^{18}O tag quantitative proteomics to profile matched cancer and non-cancer hepatocellular carcinoma (HCC) samples. Using this approach, they identified and quantitated over 200 glycoproteins. One protein of interest was clusterin isoform 2 (apolipoprotein J), a sulfated glycoprotein involved in apoptosis, cell death and complement activation, which is a potential biomarker involved in HCC metastasis. To assess the usefulness of a lectin microarray approach for identifying glycan profiles related to metastatic potential, cell lines L02 (control), Hep3B (no metastasis) and HCCLM3 (high metastatic potential) were evaluated. Comparison of L02 with Hep3B showed an increase of branched complex oligosaccharide, high-mannose sugars, terminal fucose, T antigen of mucin, reduced N-acetyl glucosamine or sialyl-Tn. These structural changes may have implications in the characteristic change of hepatocellular carcinoma invasion. HCCLM3 demonstrated increased levels of core fucose, sialic acid (mainly α2-3 link), N-acetyl glucosamine, bisecting N-acetylglucosamine and reduced β1-4 linked galactose. These structural changes may have implications in hepatocellular carcinoma metastasis [24].

Protein phosphorylation plays an important role in regulating many processes life. Li *et al*. [25] identified a total of 247 phosphoproteins containing 281 phosphotyrosine (pTyr) sites in Hep3B and MHCC97H (a highly metastatic HCC cell line). Bioinformatics analysis identified over-expressed phosphoproteins involved in cell motility and migration, protein autophosphorylation, cell-cell communication, and anti-apoptosis functions. These proteins play a role in a variety of signaling pathways, including EGFR signaling, cytokine- and chemokine-mediated signal transduction, and the PI3K and JAK-STAT cascades that are activated during HCC metastasis. Using 2-DE, 2-D Western blotting, and mass spectrometry, Ruan *et al*. [26] identified and quantified the tyrosine phosphorylation levels of 16 proteins in TGF-α-treated CNE2 human NPC cells. These results may provide new insights into EGFR phosphorylation signaling and have implications in molecular cancer therapy of NPC. Akashi *et al*. [27] measured the expression of 393 proteins in 39 human cancer cell lines (JFCR-39). Using an integrated approach allowed us to identify two peaks at 11.6 and 11.8 kD that showed significant correlations with sensitivity to the PI3K inhibitor, LY294002. In addition, based on results from A549 cancer cells, Akashi *et al*. [28] predicted the presence of novel multi-phosphorylated forms of 4E-binding protein 1 (4E-BP1), which is a downstream component of the PI3K/Akt/mTOR pathway and regulates protein synthesis. They also investigated the effect of ZSTK474, a novel phosphatidylinositol 3-kinase (PI3K) inhibitor that has little inhibitory effect on mTOR, on 4E-BP1 phosphorylation using phospho-specific antibodies. Interestingly, ZSTK474 inhibited phosphorylation of Ser65, Thr70 and Thr37/46 in 4E-BP1, sites shown to play an important role in cancer development.

Ubiquitination plays an important role in protein translocation, metabolism, function, regulation and degradation, in turn regulating cell growth, proliferation, apoptosis, differentiation, and tumor metastasis. Using a combination of immunoaffinity purification and liquid chromatography-tandem mass spectrometry (LC-MS/MS), Vasilescu *et al*. [29] were able to identify 70 ubiquitinated proteins from the human breast cancer cell line MCF-7 after treatment with the proteasome inhibitor MG132, implicating them in the progression of breast cancer. Meierhofer *et al*. [30] used stable Hela cell lines expressing a tandem hexahistidine-biotin tag (HB-tag) fused to ubiquitin in a two-step purification of the ubiquitinated proteome under fully denaturing conditions. They identified 669 ubiquitinated proteins, including 44 with all seven possible ubiquitin chain-linkage types. Quantitative mass spectrometry using the stable isotope labeling with amino acids (SILAC) strategy in cell culture was used to profile cells treated with the proteasome inhibitor MG132. This approach should help identify ubiquitinated proteins targeted to the proteasome for degradation and detect ubiquitination changes in response to growth conditions and cellular stress, all of which may play a role in cancer.

In summary, various proteomic differences have been shown to play a role in glycosylation, phosphorylation, ubiquitination and other post-translational modification related to cell cycle progression, cellular proliferation and migration, and apoptosis during tumor development.

3 Cancer development and protein-protein interactome dynamics

Protein-protein interactions play a central role in regulating the function of cells and organisms. Studies aiming to elucidate interactome dynamics at different times and conditions promise to be a major step towards modeling cellular function and behavior. A shift from a static to a dynamic network analysis should help elucidate the mechanisms of tumorigenesis [31].

Jonsson *et al*. [32] found that proteins in cancerous cells demonstrated an increase in the number of interacting partners and contained a higher number of highly promiscuous structural domains (i.e., domains with a high propensity for mediating protein interactions). Moreover, an underlying evolutionary distinction existed between the two groups of proteins, reflecting the central roles of proteins whose mutations lead to cancer. Alpha fetoprotein (AFP), for example, has been implicated in the development of HPC and is considered to be a diagnostic and prognostic tumor marker. The protein expression profiles of nine AFP-producing cell lines and seven non-AFP producing cell lines were generated by

fluorescence 2-dimensional difference gel electrophoresis (2D-DIGE). Multivariate analysis of six up-regulated and five down-regulated proteins was used to distinguish AFP-producing cell lines from non-AFP producing cell lines. Understanding their interaction could provide insight into the biology of AFP-producing hepatocellular carcinoma cells [33]. More than 50% of human cancers have a *p53* gene mutation. Hu *et al.* [34] and Sun *et al*. [35] revealed several p53 interacting proteins in nasopharyngeal carcinoma cells using RNA interference, immunoprecipitation and proteomic techniques. These proteins took part in the regulation of cell cycles and gene expression programs, and covered several signaling pathways. As p53 interacting proteins, they provided important clues regarding the mechanisms of p53 accumulation and inactivation in NPC. NDRG1 is known to play important roles in both androgen-induced cell differentiation and inhibition of prostate cancer metastasis. Tu *et al*. [36] identified 58 proteins that interacted with NDRG1 in prostate cancer cells using co-immunoprecipitation and mass spectrometry analysis. The results showed that NDRG1 bound directly to β-catenin and E-cadherin. Moreover, the interactome map provided the first road map for understanding the functions of NDRG1 in prostate cancer, which progresses from an androgen-dependent curable stage to an androgen-independent incurable stage. Application of protein-level and peptide-level sample fractionation combined with LC-MS/MS analysis enabled identification of 2235 unmodified proteins representing a broad range of functional and compartmental classes. Nine interacting proteins (SLC3A2, PXN, ITGB1, ITGB2, FLNB, ITGB4, ITGAV, ITGA3, EGFR) were clustered according to their gene expression profiles across four cell lines. The epidermal growth factor receptor (EGFR) shared a similar transcript profile with several of the integrin subunits across all four cell lines. EGFR receptor activation is governed by integrin mediated phosphorylation during cell adhesion. The up-regulation and down-regulation of integrin receptors may play a role in cancer invasion and metastasis by altering the ability of cells to adhere to surrounding cells and the extracellular matrix [37] (Figure 2).

A series of events in the development of cancer, including premalignant lesions, dysplasia, invasive and distant metastasis, are the result of multiple factors and their interactions. Therefore, studies focused on the interactome and tumor-associated gene networks as a whole may facilitate a better understanding of the mechanisms of tumorigenesis, and thus prevention.

In summary, the development and progression of cancer is closely related to abnormal protein expression, altered posttranslational modification, and protein interactions. In addition, aberrant protein alterations in cells can lead directly to malignant characteristics and oncogenic transformation. A proteomic approach to studying protein expression and signaling pathways may help scientists understand the mechanisms of tumorigenesis, identify new diagnostic markers and drugs, and provide a clinical basis for early diagnosis and treatment.

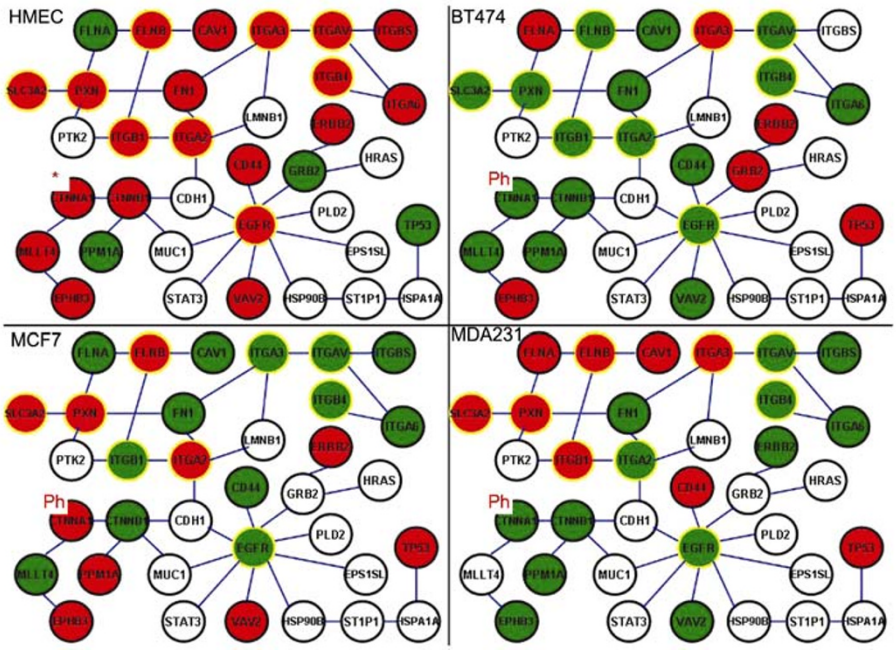

Figure 2 Protein-protein interaction network of nine modified proteins from breast cancer [37]. Red indicates increased abundance and green indicates reduced abundance relative to the reference panel of cell lines. The "Ph" superscript indicates phosphorylation preferentially detected in the cancer cell lines (MDA231, BT474, MCF7) and absent in HMEC (*).

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