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Opportunities and Challenges for Nutritional Proteomics in Cancer Prevention[1,2]

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Abstract

Knowledge gaps persist about the efficacy of cancer prevention strategies based on dietary food components. Adaptations to nutrient supply are executed through tuning of multiple protein networks that include transcription factors, histones, modifying enzymes, translation factors, membrane and nuclear receptors, and secreted proteins. However, the simultaneous quantitative and qualitative measurement of all proteins that regulate cancer processes is not practical using traditional protein methodologies. Proteomics offers an attractive opportunity to fill this knowledge gap and unravel the effects of dietary components on protein networks that impinge on cancer. The articles presented in this supplement are from talks proffered in the "Nutrition Proteomics and Cancer Prevention" session at the American Institute for Cancer Research Annual Research Conference on Food, Nutrition, Physical Activity and Cancer held in Washington, DC on October 21 and 22, 2010. Recent advances in MS technologies suggest that studies in nutrition and cancer prevention may benefit from the adoption of proteomic tools to elucidate the impact on biological processes that govern the transition from normal to malignant phenotype; to identify protein changes that determine both positive and negative responses to food components; to assess how protein networks mediate dose-, time-, and tissue-dependent responses to food components; and, finally, for predicting responders and nonresponders. However, both the limited accessibility to proteomic technologies and research funding appear to be hampering the routine adoption of proteomic tools in nutrition and cancer prevention research. J. Nutr. 142: 1360S–1369S, 2012.

Introduction

The adoption of "omic" technologies defined as the collection and analysis of large-scale measurements related to the organization and regulation of biological systems sparked new enthusiasm for the prevention of chronic diseases, including cancer (1). One of the central tenants of the Human Genome Project was to provide a blueprint to categorize cancers and develop biomarkers of cancer susceptibility based on genetic information (2). However, variations in epidemiologic trials of nutrients for cancer prevention and susceptibility informed that gene-environment interactions have the potential for influencing a person's risk for cancer (3) or response to dietary intervention (4). For example, in BRCA-1 mutation carriers, the risk of breast tumors is either reduced by higher intake of fruits and vegetables (5) or increased by polymorphisms in the methyl-tetrahydrofolate reductase gene (6). The example of BRCA-1 illustrates that in the postgenomic era, there is a need for new investigative tools to explain how diet modifies the risk of cancer (4,7).

The field of proteomics is concerned with the systematic study of all proteins in cell compartments, tissues, and biofluids. Of the ~20,000 protein-coding human genes discovered through the genome project, ~8000 (38%) reportedly lack experimental evidence at the protein level (8). Up to one million different protein molecules have been estimated to originate from the combined effects of alternative splicing, protein modifications, and pathological and physiological conditions. Therefore, it seems reasonable that proteomic tools should be adopted to

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make a direct assessment of all the proteins that influence biological processes associated with cancer (9).

Historically, large differences in chemical properties of proteins and the wide dynamic range of protein concentrations have made profiling proteins challenging (10). However, recent improvements in technologies allow the identification and quantitation of proteins, analysis of protein-protein interactions, and characterization of posttranslational modifications (11). Therefore, assessing the dynamic changes of protein profiles brought about by dietary components may offer new opportunities for the development of proteomic signatures for each bioactive food component or diet and predictive models of cancer risk.

Articles presented in this supplement are from talks proffered in the "Nutrition Proteomics and Cancer Prevention" session at the American Institute for Cancer Research Annual Research Conference on Food, Nutrition, Physical Activity and Cancer held in Washington, DC on October 21 and 22, 2010. This session originated from the concept that future progress in the implementation of nutritional strategies for cancer prevention requires knowledge of how dietary components influence protein targets that govern the transition from normal to malignant phenotype. Proteomic studies presented by Zhen Xiao et al. (12) highlighted that isothiocyanates (ITC)[5] commonly found in cruciferous vegetables may target the microtubule network. They proposed that the antiproliferative effects of ITC may be related to covalent modifications of cysteine residues of tubulin leading to loss of tubulin polymerization, a process that is necessary for maintaining cell structure. The article presented by Baukje de Roos (13) addressed the benefits of using proteomic approaches to assess the influence of dietary fatty acids on mechanisms involved in carcinogenesis and discovery of new protein biomarkers of cancer risk. Angela Betancourt et al. (14) used proteomic technologies to discover proteins that modulated the response to the hormonally active chemical bisphenol A and the soy component genistein. Unequivocally, these studies offer compelling evidence that the future of nutritional proteomics in cancer prevention remains bright. However, efforts are needed for its incorporation into diagnostic tools for predicting benefits from dietary changes.

Protein Networks as Targets for Bioactive Food Components

Protein networks

Implicit in the adoption of proteomic tools is the concept that the composition and functionality of protein networks determine disease risk (15). The value of targeting protein networks rather than individual proteins or protein modifications stems from the fact that protein inter-relationships regulate biological processes such as proliferation, apoptosis, autophagy, DNA repair, inflammation, and angiogenesis. For example, various food components that possess anticarcinogenic properties have been shown to activate the tumor suppressor protein, P53 (16–21), a highly connected nodal protein that regulates a vast number of signaling pathways (22) (**Fig. 1**). One important consequence of P53 activation is the halting of transition through G1/S phase by the stimulated expression of the tumor suppressor protein P21, which then interferes with the formation of cyclin-dependent kinase complexes necessary for cell cycle progression (23). In addition, P53 has been reported to block G2/M phase transition by inducing expression of 14–3-3σ, which anchors CYCLIN B1-cyclin-dependent kinase 1 in the cytoplasm, and of GADD45, which dissociates CDC2 from CYCLIN B1 and P21. Moreover, the P53 protein has been shown to repress the *CYCLIN B1* and *CDC2* genes, further reinforcing its effects on cell cycle arrest (24,25). In addition to halting cell cycle progression, P53 induces apoptosis through inhibition of the antiapoptotic protein BCL-2, thus releasing BCL-2's inhibition on the proapoptotic BAX and BAK. The latter proteins stimulate the release of cytochrome-c from mitochondria, the repression of inhibitors of apoptosis proteins, and hampering of AIP's repression on caspase-9, leading to apoptosis (26). Also, P53 has been implicated in the regulation of proteins that participate in DNA repair (XPC, DDB2, P53R2), autophagy (DRAM, MAP-LC3II), inflammation (IKKβ/NFκB), and angiogenesis (MASPIN, TSP1, BAI1, VEGF, COLLAGEN VIIIα1) (22). Proteins regulated by P53 may in turn alter the expression levels, posttranslational modifications, DNA binding, protein-protein interactions, and localization of other proteins comprised in subnetworks further amplifying the duration and amplitude of the signal initiated by food components. Moreover, the P53 protein itself is extensively regulated through positive (transcription factor E2F) and negative (murine double minute-2) regulators, post-translational modifications that affect its levels, subcellular localization, DNA binding, and transactivation potential (27). The example of the P53 network provides an excellent proof-of-principle that proteomic tools are necessary to perform the measurement of quantitative and qualitative influences of food components on complex protein networks and subnetworks, the study of which is largely impractical using traditional protein methodologies.

Post-translational modifications

Factors that contribute to increasing the complexity of protein networks are post-translational modifications such as phosphorylation, acetylation, methylation, glycosylation, myristoylation, nitrosylation, sumoylation, palmitoylation, and ubiquitination. Proteins can also be modified through oxidation, nitration, or binding to lipid moieties (28). Post-translational modifications influence protein structure, stability, and localization. Phosphorylation of the ERK, JNK, and P38 kinases are necessary for their translocation to the nucleus and activation of transcription factors (29). The activation of phosphatidylinositol 3-kinases by point mutations, receptors, small GTPASE rat sarcoma (RAS), and AKT induce system-wide protein responses leading to cell transformation (30). Hence, proteomics offers attractive opportunities for the qualitative and quantitative analysis of how food components influence post-translational modifications associated with growth stimulation (31) or metabolic stress (32).

The human kinome comprises over 500 protein kinases, which transiently phosphorylate predominantly serine and threonine residues, although a subgroup (~90 tyrosine kinases) phosphorylates tyrosine residues on receptors, including EGFR, IR, and FGFR as well as nonreceptor proteins (i.e., tyrosine kinase SRC and others). About one-half of the protein tyrosine kinases are linked to human cancers through constitutive activation (33). ERK alone can phosphorylate >80 substrates in the cytoplasm and the nucleus (34). Interestingly, quantitative phosphoproteomic studies illustrated that a cluster of tyrosine kinases mediated the invasive effects of SRC (35), which is overexpressed in ~80% of human colorectal cancers (36). Therefore, a proteomic overview of the kinome in colonic cells may help

[5] Abbreviations used: 2D, 2-dimensional gel electrophoresis; ESI, electrospray ionization; ITC, isothiocyanate; LC, light chromatography; MALDI, matrix-assisted laser desorption/ionization; MS/MS, tandem MS; Q, quadrupole; RAS, rat sarcoma; SILAC, stable isotope-based labeling; TOF, time-of-flight.

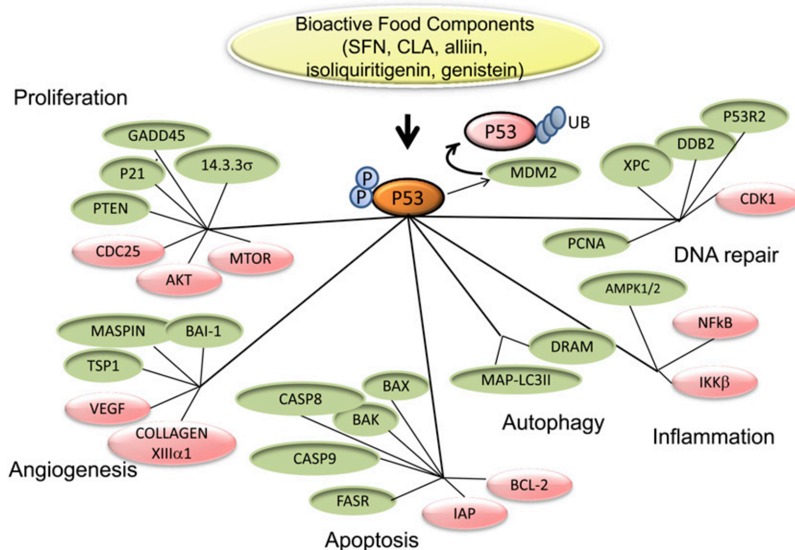

FIGURE 1 Protein networks as targets for bioactive food components. A simplified scheme of how food components reported to induce p53, selected as a prototype interconnecting protein node, may influence proteins that belong to neighboring networks controlling biological processes. Stimulatory effects of p53 are shown in green and repressive effects are shown in red. Proteomic tools are needed to learn about the topology and dynamic behavior of protein networks that impinge on cancer risk and assess quantitative and qualitative influences of food components.

identify dysregulated protein networks and assist in the generation of working hypotheses for targeting of SRC and other tyrosine kinases with food components. Other studies that used proteomic approaches suggested that the anticarcinogenic properties of genistein may be related to inhibition of the tyrosine kinase activity of SRC, EGFR, PDGFR, and IR (37) as well as activation of phosphatases, which reverse phosphorylation. Proteomic studies of peripheral blood mononuclear cells from postmenopausal women revealed that supplementation with soy isoflavones increased the levels of protein tyrosine phosphatases (38).

An example of a post-translational modification that influences subcellular distribution and protein-protein interactions is palmitoylation, which enhances hydrophobic anchoring of proteins to the fatty acid chain into the lipid bilayer. Palmitoylation regulates trafficking and function of many transmembrane proteins, including receptors, SRC family kinases, and RAS proteins. Proteomic analyses have the potential to assess how dietary modulators of palmitoylation influence redistribution of certain proteins to various cellular compartments and regulate processes associated with carcinogenesis (39). For example, palmitoylation of the estrogen receptor-α is necessary for its association with the plasma membrane and interactions with caveolin-1 and for the nongenomic activation of ERK- and AKT-regulated pathways (40). Therefore, proteomics may be useful to investigate how food components influence cellular relocation of proteins involved in growth stimulation. Studies with mouse colonocytes reported that supplementation with DHA disrupted RAS signaling by displacing H-RAS from caveolae while excluding EGFR from lipid rafts (41). Given the large number of cancers with abnormal RAS signaling, the adoption of proteomic approaches may accelerate the development of preventive strategies based on supplementation with DHA and other food components.

State of Development of Proteomic Technologies and Challenges

An in-depth discussion of the evolution and state of proteomic technologies is beyond the scope of this manuscript. Therefore, we refer to excellent reviews of the scientific principles of various proteomic platforms (42–44). The following paragraphs offer some considerations about the dynamic range and versatility of MS-based proteomics and examples of applications in nutrition and cancer prevention research. **Table 1** provides a list of preclinical and clinical investigations that have used various MS platforms to assess the impact of various food components and dietary mixtures on biological processes that impinge on cancer.

Top-down and bottom-up proteomics

Of the several proteomic methods that have been developed, all involve protein digestion, fractionation, and MS analysis of peptide ions, from which it is possible to derive the amino acid sequence and post-translational modifications and calculate the amount of selected peptides. In top-down proteomics, separation and analysis are performed directly on intact proteins, followed by digestion and MS analysis. The top-down approach starts with the intact protein and it draws inferences about amino acid composition, post-translational modifications, and protein functionality. Conversely, in bottom-up proteomics, protein samples first undergo proteolytic digestion followed by separation of peptides and analytical measurement by MS. The concept behind the bottom-up approach is to use information about amino acid and post-translational modifications to reconstruct the protein of interest and gain knowledge about its functionality. The latter method is more sensitive, but it has the drawback of not capturing all information about small proteins (<30 kD), because they generate fewer peptides (42).

Protein separation techniques

Two-dimensional (2D) SDS-PAGE has been widely used to detect differentially expressed proteins based on mass and charge. 2D electrophoresis utilizes isoelectric focusing prior to gel separation, which can be followed by gel excision of proteins of interest, digestion, and MS analysis. 2D gel electrophoresis separation followed by in-gel trypsin digestion and MS analysis have been used to identify protein targets of various food components, including grape resveratrol in lymphoma cells (45), cruciferous ITC and indole compounds in colon (46) and prostate (47,48) cancer cells, and the soy isoflavone genistein in developing normal mammary tissue (49) (Table 1). However, 2D gels have a bias against membrane proteins, large proteins, and low-abundance proteins. Also, proteins with an extreme isoe-

TABLE 1 MS platforms and proteomic studies of food components in cancer prevention research[1]

Food component	Model	Proteomic platform	Combined effects of food components on biological processes	Reference
Cruciferous				
BITC, PEITC, SFN	Lung, A549	2D-nano RPLC-MALDI-TOF/TOF	Disruption of cytoskeleton organization	48
BITC, PEITC, SFN	Breast, Ras-MCF10A	LC-ESI-Q-TOF	Induction of apoptosis	69
SFN	Breast, MCF10, MCF12A	SILAC, LC-QTOF	Upregulation of hydroxysteroid metabolism	59
SFN	Prostate, LNCaP	2D-MALDI-TOF/TOF	Induction of apoptosis	47
SFN	Colon, Caco-2	2D-MALDI-TOF	Decrease of neurotransmitter receptors	46
SFN	Liver, huh-7	2D-MALDI-TOF	Induction of apoptosis	70
PEITC	Liver, HepG2	2D-MALDI-TOF/TOF	Proapoptotic, antiinflammatory	71
PEITC	Plasma, TRAMP mice	2D-MALDI-TOF/TOF	Induction of autophagy	72
PEITC	Cervix, Hela	ESI-LTQ	Inhibition of proinflammatory cytokine (MIF)	73
Indol-3-carbinol	Lung, A/J mice	iTRAQ-SCX-LC-TOF/TOF	Detoxification, antiproliferative	60
Brussels Sprouts	PBMC, human	2D-LC-nanospray-MS	Growth arrest, proapoptotic, antioxidant	74
Brassica Sp.	Serum, human	MALDI-TOF	Inhibition of receptor tyrosine kinase	75
Brassica Sp.	Serum, human	MALDI-TOF	Reduced lipolysis in GSTM1^{+} subjects	52
Vegetable diets				
Mixture	Colon, C57BL6 mice	2D-MALDI-TOF	Maintenance of intracolonic pH,	76
Antioxidant	Brain, canine	2D-MALDI-TOF	Reduced protein oxidation	77
Isoflavones				
Genistein	Breast, Sprague-Dawley rat	2D-MALDI-TOF	Increased differentiation	78
Genistein	Breast, Sprague Dawley rat	2-D-MALDI-TOF/TOF,LC-ESI-MS/MS	Reduced EGFR signaling	49
Genistein	Stomach, SGC-7901	SILAC-SCX-LC-trap/Orbitrap	Impaired signaling, cell growth, invasion	37
Genistein	Leukemia, HL-60	2D-MALDI-TOF/TOF	Proapoptotic	79
Genistein	Stomach, SGC-7901	SILAC, SCX-LC-LTQ-Orbitrap	Induction of G2/M arrest and apoptosis	53
Genistein	Endothelial, EA.hy 926	2D-MALDI-TOF	Increased detoxification-GST	80
Soy isoflavones	PBMC, human	2D-MALDI-TOF	Antiinflammatory	38
Red clover isoflavones	Liver, Sprague Dawley rat	2D-MALDI-TOF	Reduced levels of 3a-hydroxysteroid-dehydrogenase	81
Flavonoids				
Quercetin	Colon, HT29	2D-MALDI-TOF	Proapoptotic, disruption of cytoskeleton	82
Quercetin	Colon, F344 rat	MALDI-FT-ICR MALDI-TOF/TOF	Reduced glycolysis, increased fatty acid oxidation, proapoptotic	83
Quercetin	Colon, SW480	2D-MALDI-TOF/TOF	Antiangiogenic	84
Quercetin	Liver, HepG2	SILAC-NanoHPLC-ESI-Q-TOF	Disruption of cytoskeleton, antiangiogenic	85
Quercetin	Neuroblastoma, SJ-N-KP	HPLC-ESI-Q-TOF	Antiproliferative, proapoptotic	86
Quercetin	Ovary, 2774	2D-SELDI-TOF	Proapoptotic	87
Quercetin	Prostate, PC-3	2D-NanoHPLC-ion trap	Proapoptotic, reduced glycolysis	88
Flavone	Colon, HT-29	2D-MALDI-TOF	Antiproliferative, proapoptotic	89
Fatty acids and cyclopentenone PG				
Fish oil	Serum, human	2D-MALDI-TOF, NanoLC-Q-Trap	Inhibition of acute-phase response, antiinflammatory	90
Fish oil	Liver, APOE3*Leiden Mice	2D-MALDI-TOF	Inhibition of hypoxia, enhanced fatty acid oxidation	91
t10,c12-CLA	Liver, E3*Leiden, mice	2D-MALDI-TOF	Increased lipid storage	91
	Liver, APOE$^{-/-}$, mice	2D-LC-ESI-MALDI/TOF	Inhibition of TCA cycle, stimulation of gluconeogenesis	92

(Continued)

TABLE 1 *Continued*

Food component	Model	Proteomic platform	Combined effects of food components on biological processes	Reference
c9,t11-CLA	Liver, APOE$^{-/-}$, mice	2D-LC-ESI-MALDI/TOF	Antiproliferative, antiinflammatory	92
Butyrate	Colon, HCT-116	iTRAQ/ICAT-2D-LC-MALDI-MS	Antiproliferative, proapoptotic, inhibition of glycolysis, increased oxidative phosphorylation	93
Butyrate	Colon, HT-29	2D-MALDI-TOF	Proapoptotic	94
Butyrate	Colon, HT-29	2D-MALDI-TOF/TOF	Antiproliferative, proapoptotic, disruption of glycolytic pathways	95
PGA_1	Fibroblast, NIH-3T3	NanoHPLC-ESI-Q/TOF	Disruption of cytoskeleton, proapoptotic	96
Grape compounds				
Grape seed extracts	Brain, Sprague Dawley rat	2D-MALDI/-TOF 2D-RPLC-ESI-D-TOF	Neuroprotective	97
Resveratrol	Lymphoma, Jeko-1	2D-Nano RP-HPLC-ESI–ion trap, Q-TOF	Proapoptotic	45
Resveratrol	Colon, HCT-116(Bax$^{+/-}$)	2D-MALDI-TOF/TOF	Induced proapoptotic signaling	98
Resveratrol	Prostate, LNCaP	2D-MALDI-MS	Reduced glycolysis	99
Garlic				
Diallyl-trisulfide	Stomach, BGC823	2D-MALDi/TOF, 2D-LC-ion trap	Stimulation of apoptosis	100
Diallyl-trisulfide	Bone, Saos-2	2D-MALDI-TOF	Impaired signaling, disruption of cytoskeleton, proapoptotic	101
Tea compounds				
Green tea extracts	Lung, A549	2D-HPLC-ESI-Q-TOF	Inhibition of cell motility	102
EGCG	Neuroblastoma, SH-SY5Y	2D-RPLC-ion trap	Neuroprotective	103
Theobroma cacao				
Procyanidin	Breast, MDA-MB-231,-436,-468; SKBR3	Antibody protein array	Antiproliferative, proapoptotic	104
Selenium				
Selenomethyl-selenocysteine	Plasma, Wistar rat	2D-MALDI-TOF	Stimulation of acute-phase response	105
Vitamins				
Retinoic acid	Breast, MCF-7	2D-MALDI-TOF	Cell cycle arrest, antiproliferative, proapoptotic	106
Retinoic acid	Neuroblastoma, SH-SY5Y	2D-MALDI-TOF/TOF, iTRAQ-LC-NanoESI- Q-TOF	Alteration of mRNA splicing and translation	107
L-Ascorbic acid	Leukemia, NB4	2D-MALDI-MS	Alteration of contractile system	108
Folate	Plasma, human	2D-NanoLC-Q-Trap	Activation of immune function	109
Folate deficiency	Colon, NCM460	2D-NanoLC-Q-Trap	Increased DNA damage, metastasis reduced phase II enzymes	110

[1] BITC, benzyl isothiocyanate; EGCG, (-)-epigallocatechin gallate; ESI, electrospray ionization; FT-ICR, Fourier transform-ion cyclotron resonance; ICAT, isotope-coded affinity tag; iTRAQ, isobaric tag for relative and absolute quantification; LTQ, linear trap quadrupole; MALDI, matrix-assisted laser desorption/ionization; PEITC, phenetyl isothiocyanate; RPLC, reverse-phase-LC; SELDI, surface-enhanced laser desorption/ionization; SFN, sulforaphane; SILAC, stable isotope-based labeling; SXC, strong cation exchange; TOF, time-of-flight; 2D, 2-dimensional gel electrophoresis; Q, quadrupole.

lectric point (10 < isoelectric point < 3) are not effectively resolved (50).

An alternative method to 2D separation is affinity chromatography, which can be used to capture proteins of interest. For example, in human plasma, ~99% of the protein mass is due to ~22 proteins, and their removal through a chromatographic approach (e.g., matrix immobilized antibody) is advantageous prior to MS to enhance the detection of less abundant proteins (51). The preliminary precipitation of albumin and Ig through affinity chromatography prior to MS analysis has been used successfully in clinical studies that examined the effects of the glutathione-*S*-transferase-M1 phenotype on the serum peptidome following supplementation with cruciferous vegetables (52). Other studies that investigated the anticarcinogenic properties of ITC utilized affinity chromatography with streptavidin-Sepharose beads to purify cysteine-containing protein targets of ITC (50). In the latter study, MS analysis of bound proteins identified macrophage migration inhibitory factor, a proinflammatory cytokine, as a primary binding target for ITC.

To by-pass challenges related to protein separation, the entire proteome from a biological sample (e.g., cell, tissue, biofluid) can be first digested, typically by trypsin. Then, the resulting peptides can be separated using various techniques, including ion exchange chromatography, isoelectric focusing, ion-pairing reversed-phase HPLC, and phosphopeptide chromatography. To investigate the effects of genistein on the phosphoproteomic profile of gastric cancer SGC-7901 cells, hydrophilic interaction chromatography methods with the metal oxide TiO^2 were utilized for the enrichment of phosphopeptides prior to light chromatography (LC)-tandem MS (MS/MS) analysis (53). These studies led to the identification of novel phopshoprotein targets for genistein, including receptors, signal adaptors, protein kinases, protein phosphatase regulatory subunits, and transcription regulators. Principles and applications of global and site-specific quantitative phosphoproteomics are reviewed elsewhere (54).

Ionization of peptides

For MS analysis, peptides first need to be ionized. Two main ionization techniques are commonly used and include matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). In MALDI, the peptide mixture is cocrystallized with a matrix that upon excitement with a UV laser leads to the ionization of peptides through gain of a proton. The ionized peptide molecule is usually referred to as $[M+H]^+$ (55). ESI utilizes a solvent system to dissolve the peptide mixture, which is then electro-sprayed into a vacuum chamber. Then, through solvent evaporation or extraction methods, the peptides are ionized. With ESI, most peptide ions gain more than one proton charge ($[M+nH]^{n+}$) (56). Regardless of the technique used for ionization, peptide ions are analyzed based on their *m/z* (42–44). Several examples of studies that utilized MALDI or ESI methods in nutrition and cancer prevention research are reported in Table 1.

MS

In MS, 3 types of information are necessary for each peptide and include mass, peptide ion intensity, and list of peptide ion fragments (44). An MS method for peptide ion mass determination is time-of-flight (TOF), in which travel distance of peptide ions is calculated based on the square root of *m/z*, i.e., peptides with a high *m/z* travel slower compared with those with a lower *m/z*. Then, the *m/z* values of unknown peptide ions are calculated against TOF of internal peptide ion standards. A second MS method utilizes quadrupole (Q) chambers that measure "spiraling" trajectories of peptide ions of preselected *m/z* values. Because of their sensitivity, triple Q have been used for quantitative measurement of single or multiple fragment ions. In single/multiple reaction monitoring, the first Q chamber is used to select the peptide ion of interest; in the second Q, the peptide ion is fragmented; and in the third Q, one or a few peptide ions are collected. Therefore, multiple Q are used as mass filters that allow the passage of ions of selected *m/z* ratios (57). A third approach for MS determination is based on ion traps, which eject peptide ions of different *m/z* values onto the MS detector. In general, ion traps are useful, because they accumulate ions of interest but have limited resolution (500–2000) compared with TOF analyzers (>10,000). A fourth group of MS known as Orbitraps and Fourier transform-ion cyclotron resonance separate ions based on oscillation frequencies and have a mass resolution >60,000 (44).

Platforms for proteomic analysis need to combine an ionization technique with an MS platform. Widely used combinations are MALDI-TOF for 2D electrophoresis and ESI-ion trap/Orbitrap for LC-MS. MS platforms commonly used today include a chromatographic technique (e.g., nano HPLC) followed by ESI-MS/MS analysis. Because the on-line nanoHPLC-ESI combination operates in the liquid phase, it eliminates losses due to separation and collection steps. Conversely, when combining LC with MALDI, eluted peptides need to be mixed with the appropriate matrix for subsequent MALDI analysis. The latter solution is more time-consuming compared with the LC-based platforms (42).

After peptide mass determination, a second goal in MS is to determine the amino acid sequence of the peptides of interest. This is accomplished through fragmentation of the peptide and recording of the *m/z* values of the fragments in a tandem mass spectrum. This approach relies on the use of 2 distinct (tandem) MS analyzers or the sequential use of the same MS analyzer. Examples of tandem platforms include Q-TOF, triple Q, and TOF-TOF (44). Sequential platforms utilize ion traps or Fourier transform-ion cyclotron resonance analyzers (42). The fragmentation of peptide ions can be accomplished through collision with gas molecules such as He, N_2, or Ar, which cause preferential cleavage of peptide bonds and weak modifications such as glycosylation and phosphorylation linkages (42). An alternative fragmentation technique utilizes electron transfer, in which positively charged peptide ions react either with an electron donor (e.g., fluoranthene) or electrons generated by heat, leading to the gain of an unpaired electron and peptide bond cleavage. Compared with collision methods, the electron transfer approach appears to be more accurate for the analysis of large peptides or peptides with post-translational modifications (54).

The selection of MS peaks for sequencing is commonly carried out using 3 strategies. In shotgun or discovery proteomics, a full scan of the peptide ions entering the MS is performed. Then, peptide ions are selected for fragmentation and determination of the amino acid sequence. This strategy has a bias for more intense protein signals. A second protein identification approach involves 2 separate MS analyses for quantification and sequencing. This method improves quantitative measurements in favor of less abundant proteins. A third approach is targeted proteomics, which focuses on determination of the spectrum of fragment ions from a preselected list of peptides (43,57).

When determining the amino acid sequence of the peptides of interest, the fragmentation spectrum of a peptide is compared with theoretical fragmentation patterns of peptides contained in databases. Then, the fidelity of the predicted amino acid sequence is scored using different computational tools. An

example of a database of consensus spectra is available through the PeptideAtlas project (58). A main issue in proteomic experiments is that of discriminating true- from false-positive matches. In de novo sequencing, the fragment ion spectrum is used to determine the peptide sequence (44).

Quantitative assessment and prediction of protein networks

Several LC-MS/MS–based methods have been developed for quantitative proteomics and include label-free methods (42), and stable isotope methods such as metabolic stable isotope-based labeling (59), enzymatic isotope-coded affinity tag (42), and chemical isobaric tag for relative and absolute quantification (60) labeling. In label-free methods, the MS is used directly for quantitation based on signal intensity of peptides or spectral counting. In MS methods that use stable isotope labeling, quantitation is based on the mass increase of the label. For example, linear ion trap-Orbitrap and quantitative stable isotope-based labeling analyses were adopted in shotgun proteomics to identify in gastric cancer SGC-7901 cells the phosphoproteins and their regulatory sites in signaling pathways targeted by genistein (53). These investigations helped to identify proteins that mediated genistein-induced G2/M phase arrest and apoptosis. Specifically, phosphorylation of BCLAF1 at Ser-512 was identified as the regulatory event involved in the repression of Bcl-2 expression in response to genistein (Table 1). These proteomic studies suggested that specific phosphosites rather than the whole protein should be examined to learn about the impact of food components on the regulation of protein networks. Similarly, proteomic studies that adopted a Q-TOF approach (45) revealed that the grape compound resveratrol induced apoptosis in lymphoma cells through upregulation of Ser-3 phosphorylated cofilin, which functions in mitochondria as a checkpoint for programmed cell death (61).

Results of shotgun proteomic studies suggest this is the method of choice when no prior knowledge is available and for measurements of relative and absolute protein abundance (62). One of the limitations of the shotgun approach is that repeated analyses of the same samples may generate different, partially overlapping proteomes. This problem can be overcome with repeated analysis and prefractionation or use of the last generation of MS-Orbitrap or Q-TOF (59).

A main objective of proteomic studies in nutrition and cancer prevention research is to develop predictive models of how pathways and protein complexes relay signals from food components. However, the cross-talk among pathways renders the dynamic prediction of protein network response to food components challenging. Sophisticated computational tools are now available to study protein-protein interaction networks (63) and for the proteomic-based analysis of cancer processes (64). Proteomic workflows should also include validation steps with various biochemical assays (65). Useful tools for the validation of MS data are protein microarrays, including forward- and reverse-phase protein arrays, which offer the advantage of high throughput. Some drawbacks of protein microarrays may be inability to fully inform about protein-protein interactions and complexity of spotting the complete proteome under study (66).

Future Areas of Proteomic Research and Needs

The complexity of protein wiring is a major challenge in the design of cancer prevention strategies based on individual bioactive components or food associations. Thousands of compounds present in the diet likely induce synergistic or opposing effects. Proteomic approaches are welcome to make an important paradigm shift. Specific research questions that should be addressed using proteomic approaches include: *1*) how the timing and dose of exposure to bioactive compounds influence the activity of protein networks that contribute to cancer processes; *2*) which are the protein networks and protein modifications that mediate the cell- and tissue-specific response to food components [global proteomic studies suggest that tissue specificity may be achieved by precise regulation of protein levels and modifications in space and time (67)]; *3*) whether food components lead to sustained regulation of protein networks even after the original food exposure has been removed; and *4*) which are the qualitative and quantitative proteomic modifications that discriminate between responders and nonresponders. Ideally, the systematic adoption of proteomic tools rather than a classical protein-by-protein approach should help isolate groups of proteins that can be targeted with individual food components or associations. However, the integration of proteomics with other complementary, high-throughput, "omic" approaches, such as genomics, epigenetics, and metabolomics, may offer the best insight into the mechanisms that determine the switch from normal to cancer phenotype and response to food components (68). This need for integration is perhaps best underlined by studies showing that interactions between inter-individual genotypic differences in metabolism and disposition influence the proteomic response to cruciferous vegetables (52).

To date, ~30,000 proteomic publications are available through a PubMed search. However, only ~6000 have reported on the use of proteomics in cancer research, and of the latter studies, only a small number (~120) focused on the effects of food components and diet. Also, many of the published nutrition proteomic and cancer studies do not report a comprehensive analysis of protein networks. It is clear that the adoption of proteomics tools in nutrition and cancer prevention research is lagging behind other research areas such as pharmacology, for which >5400 studies are available through PubMed. Several factors appear to be hindering the wide adoption of proteomic tools in nutrition and cancer prevention research and include: *1*) limited accessibility to proteomic technologies; *2*) insufficient preanalytical, sample handling, instrumentations, and sample processing training; and *3*) insufficient cross-training in postanalytical bioinformatics, computational biology, structural biology, and system biology analyses. Progress in these areas may be accelerated by pre- and postdoctoral training, early-career awards, workshops, and conferences. The widespread utilization of proteomic tools could be facilitated by the availability of low-cost platforms. It is important that professional organizations and funding agencies develop targeted initiatives, foster collaborations, and support new funding mechanisms to support and encourage collaborative efforts among proteomic, nutrition, and cancer scientists.

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