

PROTEOMICS RESEARCH: IMPLICATIONS FOR CLINICAL MEDICINE

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In 1953 James Watson, PhD, and Francis Crick, PhD, unveiled their double-helix model of DNA.¹ On the day of their breakthrough, Dr Watson walked into an English pub and announced they had “found the secret of life.”² Their work revolutionized how scientists, physicians, and the general public view biology, and massive efforts were subsequently launched to decode the human genome. In 1990, almost a half century after Drs Watson and Crick’s discovery, the US Department of Energy and the National Institutes of Health coordinated the Human Genome Project. The project finished in 2003 after successfully sequencing the 3 billion base pairs of the human genetic code. Yet, despite the vast amounts of clinically relevant information both these milestones yielded, in many respects Dr Watson’s statement was naive.

The genome is, indeed, the blueprint for biological life; however, post-translational modifications of amino acid sequences create many more proteins than the genes coding them. A surprising finding of the Human Genome Project is that there are far fewer protein-coding genes in the human genome than there are proteins (30,000–40,000 protein-coding genes³ vs approximately 500,000 proteins, respectively⁴). With the human genome sequenced, research has shifted in the direction of a functional analysis of gene products.⁵

Researchers now face the challenge of understanding the expression, function, and regulation of non-gene proteins encoded by an organism—an area of study called proteomics.⁶ More than 200 modifications that alter cell signaling and cellular processes have been identified.⁷ Such alterations, which change the 3-dimensional structure of proteins and their activities, occur either during or after translation. Techniques that identify these modifications and their locations can help provide an understanding of biological pathway function and regulation,⁶ which, in turn, might improve disease screening, prevention, and treatment techniques. This article examines the state of the research in proteomics and emphasizes clinical implications.

Proteomics

The entire complement of proteins expressed in a cell⁸ or bodily fluid (eg, urine⁹) by a genome is called its proteome, which changes in response to environmental stimuli and disease states. As important as genetics are to health, genes code for protein sequences, they do not explicitly

code for the protein-to-protein or protein-to-other-molecule interactions that generate function at the level of the cell or organelles. The physiology governing health and pathology is a dynamic process resulting from interactions between the proteins within cells, organs, and systems.

This said, genotypes do predispose genomes to express certain proteins and not others, and many other aspects of cellular microenvironments determine genetic expression and the resultant proteome. These include factors such as pH levels, diet, hypoxia, and drug administration.^{4, 10, 11} The ultimate 3-dimensional structure of proteins and their functions depends on post-translational modifications such as phosphorylation and glycosylation, lipid attachments, and peptide cleavage.⁴

Whereas genomics decodes sequence information and provides a “parts catalog,” proteomics strives to define the functions and relationships of individual “parts” and predict the outcomes of their interactions.¹²

Researching the proteome—rather than the genome exclusively—may provide a more clinically useful understanding of subcellular structure and function. Studies of protein structures is called “profiling” proteomics, whereas research into how proteins function is called, appropriately, “functional” proteomics.¹³

Proteomic profiling is used to describe all the proteins in a sample from an organism, organ, tissue, cell, or organelle. In short, profiling proteomics catalogs the proteins researchers find. This type of profiling is being extensively studied for its potential to detect diseases earlier than is possible with current methodologies. Two areas of research that are receiving most of the funding in profiling proteomics are cancer and diabetes research.

On the other hand, functional proteomics is helping researchers move beyond a reductionist approach—traditionally taken by scientists to help them understand biological processes—to studying physiology. In this new paradigm, proteins are studied to discover how they modulate functions of cells and tissues, and how proteins and their activities are regulated. Functional proteomics has yielded many incredible insights into how biological systems function, such as the physiology of muscle contractions and bone resorption.

Proteomics Research

Proteomics research is beginning to yield important

new biomarkers that may lead to earlier detection of diseases as well as yielding novel methods for determining responses to therapies and predicting reactions to toxic drugs.

Cancer

Cancer has amassed the most research data, and proteomics' potential to affect clinical outcomes prompted the National Cancer Institute's Board of Scientific Advisors to approve a 5-year, \$104 million Clinical Proteomic Technologies Initiative in June 2005.¹⁴

According to authors who wrote on serum proteomics in cancer diagnosis in the February 2004 issue of the *Annual Review of Medicine*, "Cancer has traditionally been thought of as a genetic disease, but functionally it is a proteomic affliction."¹⁵ Altered protein networks and signal pathways direct cancer growth, cell survival, tumor invasion, and distant metastasis. Although histological examination of tissue is the current diagnostic criteria for cancer, in fact, tissue derangement occurs relatively late in cancer progression. Alterations in protein expression and function are the earliest signs of cancer. Creating sensitive and specific methodologies to establish "biosignature" profiles that discriminate against disease states is one of proteomics' goals.¹⁶⁻¹⁸

Current cancer screening methods lack sensitivity and specificity, and histological derangement is a relatively crude method for diagnosis. For example, although cancer antigen 125 (CA125) is the most widely used biomarker for detecting ovarian cancer, it is elevated in only 50–60% of patients with stage I ovarian cancer, and it has a positive predictive value of less than 10%. More than 80% of women with ovarian cancer are not diagnosed until the cancer has reached a late stage, at which time their 5-year survival rate plummets to 35%.¹⁶ In contrast, successful detection of stage I ovarian cancer is routinely cured by surgery and is associated with a greater than 90% 5-year survival rate.¹⁹⁻²²

Such early detection may be possible with a proteomics tool developed to identify early-stage ovarian cancer. One test on 116 masked serum samples from women with and without ovarian cancer showed the tool to be 100% sensitive (95% CI 93–100) and 95% specific (95% CI 87–99), including the successful identification of all 18 stage I cases. In addition, the tool had a positive predictive value of 94% (95% CI 84–99).¹⁶ This new technology uses mass spectroscopy to characterize the analyte and, according to the report in the *Lancet*, requires "only a small serum sample that could be obtained by fingerstick, and results are obtained in less than 30 minutes."¹⁶

Proteomics research is also being applied to describe gene-environment interactions—including the effects of food and dietary supplements—in cancer cells. Flavonoids are a class of more than 6,000 compounds present in plants that include quercetin, myricetin, catechin, and gallic acid. They are found in variable concentrations in common foods, such as onions (*Allium cepa*), apples (*Malus sylvestris*)

tea (*Camellia sinensis*), and grapes (*Vitis spp.*). Flavonoids contain antioxidants and their consumption has been shown to decrease the risk of cardiovascular disease.^{23,24} In an in vitro proteomics study, quercetin was analyzed for its effects on caspase-3 expression in HT-29 human colon cancer cells.²⁵ Caspase-3 is a protein involved in apoptosis (programmed cell death), which is frequently underactive in cancer cells. In the study, cancer cell proliferation was dose-dependently inhibited by quercetin with an EC50 value of $81.2 \pm 5.5 \mu\text{M}$; and the activity of alkaline phosphatase, a marker of differentiation, increased more than 300% at the maximum quercetin dose. Half-maximal stimulation of differentiation occurred at $74.4 \pm 8.2 \mu\text{M}$ quercetin.

Quercetin also has been shown to increase an annexin family protein and decrease type 2 cytoskeletal 8 keratin, nicotinamide adenine dinucleotide (NADH) dehydrogenases iron-sulphur (Fe-S) protein 3, and an unidentified protein in vitro in SW480 human colon cancer cells.²⁶ The pattern changes caused by quercetin in the identified proteins are indicative of cells becoming more "normal."

Diabetes

Treatment of metabolic and cardiovascular diseases, as well as toxicity studies of drugs and industrial chemicals, are also benefiting from proteomics technology.²⁷ For example, according to a 2004 analysis in *Clinical Science*, early detection of kidney damage in type 2 diabetes using a current diagnostic method such as microalbuminuria "is not specific for diabetic renal damage, because it may also reflect generalized vascular injury or renal damage from other causes, such as hypertension."²⁸ A proteomics approach to specifically identifying early renal damage in patients could potentially lead to earlier diagnosis and improved outcomes.

Recently, proteomics profiling in patients with non-insulin dependent diabetes was used to attempt identification of early renal damage.²⁸ In this clinical trial, urine samples from 112 nonsmoking patients who'd had type 2 diabetes for at least 3 years were compared to samples from 39 nonsmoking healthy controls. "Diabetic" and "normal" polypeptide patterns were determined by comparing urine samples from the diabetic and control groups, respectively. The "diabetic pattern" of protein excretion contained insulin-like peptide 3 (INSL3), uromodulin (ie, Tamm-Horsfall protein), and an albumin fragment in 35% of those samples from diabetic volunteers with albuminuria concentrations > 100 mg/L (normal is 12.5 mg/L) compared to only 4% of samples in the control group.

Unfortunately, the detection of the diabetic pattern was not sensitive enough to identify early-stage diabetic renal damage, but research continues with the hope of this methodology being further refined so earlier diagnosis becomes possible. That said, one unexpected result did come out: all the diabetic volunteers with retinopathy

exhibited the urinary polypeptide pattern indicative of diabetic nephropathy. However, diabetic retinopathy was not the primary outcome measure. Additional research should clarify whether this methodology is applicable to early-stage diabetic nephropathy and whether it might be a better screening method for diabetic retinopathy than current ophthalmic examinations.

On the more definitive side, urinary proteomics analyses have shown a 100% sensitivity and 90% specificity for detecting immunoglobulin A (IgA) nephropathy;²⁹ and a 72% sensitivity and 100% specificity for detecting urolithiasis.³⁰ It is also being investigated for the detection of renal and bladder cancers.³¹ Urine proteomics is a promising area of research for earlier detection and treatment of urological pathologies.

Other Research

Because minute changes in protein concentrations can be detected, proteomic-based biomarkers may also prove useful in tracking response to therapies, so that regimens can be modified earlier in nonresponsive patients. Confirmation of these findings and validation of the methodology in future experiments may lead to less expensive screening methods and to earlier detection of diseases.

Most of the published research in functional proteomics focuses on defining research agendas and future directions. Therefore, the promising clinical implications of this new area of research are mostly theoretical. Potential benefits exist for drug therapies and nutritional medicine. For example, folic acid is crucial for 1-carbon metabolism, and low folic-acid concentrations lead to an increase in serum homocysteine, an independent risk factor for cardiovascular disease. An *in vivo* rat study in which the animals were fed a folate-depleted diet found that low folate alters the concentrations of 9 liver proteins. As a result, two antioxidant enzymes—glutathione peroxidase 1 (GPx 1) and peroxiredoxin 6 (Prdx 6)—more than doubled in the livers of folate-deficient rats compared to controls, which indicates increased oxidative stress. Extending this research to humans might lead to providing targeted antioxidant therapies for people with folate deficiencies.

Vitamin D has multiple effects *in vivo*, and the recommended daily allowance (RDA) of vitamin D is now considered by many to be inadequate.³² Vitamin D is a fat-soluble vitamin that influences gene expression, promotes signal-transduction pathways, and activates protein kinases and protein phosphorylation.³³ The proteomic effects of vitamin D depletion and repletion have not yet been defined, however. A functional proteomics approach to the study of vitamin D has been proposed by researcher James C. Fleet, PhD, an associate professor of foods and nutrition at Purdue University, Lafayette, Ind, who suggested an experimental design in which the proteomes from vitamin D-replete and vitamin D-depleted volunteers would be

analyzed.³⁴ The proteome would then be analyzed in vitamin D-depleted volunteers who were given vitamin D supplementation to define how the proteome changed over time with the addition of vitamin D. While this research has yet to be conducted, it is an example of how proteomics research may continue to expand our definitions of the roles foods, vitamins, and minerals play in the body.

Conclusion

Proteomics is a relatively new and still-maturing field of study. Clinical benefits remain on the horizon, but earlier detection of cancers and other diseases using proteomics technologies will likely be common in 5 to 10 years. The post-genomic era is providing a wealth of biological data from proteomics and other “-omics” research. A new paradigm for how we diagnose and treat disease is emerging.

The ‘-omics’ Research Emphases

Proteomics is just one of many new “-omics” fields of studies. Although there may be some overlap in research agendas, such as with proteomics and nutrigenomics, the different -omics investigate specific aspects of alterations in phenotypes. Each emphasizes a different aspect of physiology. A partial list of these areas of research is provided below.

-omics	Definition
Glycomics	The study of sugars within organisms, their structure, function, and interaction; it encompasses identification, analysis, and management of glycol information.
Metabolomics	The determination and study of low-molecular-weight compounds in a sample. These compounds are the substrates and by-products of enzymatic reactions and have a direct effect on the phenotype of a cell.
Proteomics	The study of the way proteins work inside cells, and how they interact with each other.
Pharmacogenomics	The study of patients’ genotypes and their responses to drugs.
Nutrigenomics	The study of how genes and dietary components interact to alter phenotype.
Transcriptomics	The study of the complete set of RNA messages coded from the DNA within a cell.

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