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Cancer Proteomics and the Elusive Diagnostic Biomarkers

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Despite progress in genomic and proteomic technology and applications, the validation of cancer biomarkers of use as clinical early detection diagnostics has remained elusive. As described in this brief viewpoint, there are now recognized to be many types of clinical biomarkers and proteomic analyses, particularly when combined with other 'omic analyses, have been effective in many such biomarker identifications. However, in the area of early diagnosis of cancers, the problems associated with the conversion from identification to diagnostic have largely not been overcome. Notably, the Clinical Proteomic Tumor Analysis Consortium (CPTAC) of the National Cancer Institute (NCI), has been particularly successful in refining the analytical steps needed to tackle this challenging issue and has provided positive insight into how to solve many of the underlying problems. The potential for developing clinical diagnostics for early detection of highly lethal cancers and possible new therapeutic strategies through proteomic analyses, as seen through these CPTAC successes, is more promising than ever.

1. Introduction

Biomarkers have generally become the translational face of 'omics research, driven in large part by the popularization of "precision medicine." This term was seemingly introduced in a National Research Council (NRC) report in 2011[1] but the concept, at least in crude form, had earlier origins (and designations), such as P4 (predictive, personalized, preventive, participatory) medicine[2] among others,[3] which emphasized that the direction of medical care was moving toward treatments designed to reflect the uniqueness of humans as individuals. Indeed, "personalized medicine," as it was generally known in the 10 years before the NRC report, had its origins much earlier in the twentieth century when such basic observations as the existence of difference blood groups and the identification of familial traits (often associated with pathologies) pointed to the importance of genetic heterogeneity in the clinical management of disease; the subsequent appreciation was that not all patients with the same apparent malady would necessarily react similarly to a selected treatment.[3] The realization that this individuality was also influenced by environment and the past history of the patient enhanced this awareness, but it was not until the initial determination of the human genome was achieved, around the start of the new millennium,[4] that the value of detailed genetic information in patient care through increasingly individualized treatments began to be truly appreciated and it was another decade before it really began to be manifested.[5]

Although the terms "precision medicine" and "personalized medicine" are commonly used interchangeably they are not defined identically. "According to the National Research Council, 'personalized medicine' is an older term with a meaning similar to 'precision medicine.'" However, there was concern that the word "personalized" could be misinterpreted to imply that treatments and preventions are being developed uniquely for each individual. In precision medicine, the focus is on identifying which approaches will be effective for which patients based on genetic, environmental, and lifestyle factors. The Council therefore preferred the term "precision medicine" to "personalized medicine." However, some people still use the two terms interchangeably."[6] A more extensive explanation behind the decision to favor "precision" over "personalized" has been described.[5]

2. The Evolution of the Biomarker Concept

Inherent in the development of the analyses that are needed to exploit the promise of precision medicine are biomarkers, a term derived from the condensation of the longer descriptor, biological markers. These entities, which represent medical signs as opposed to medical symptoms (that are reported by the patients themselves)[7] may range from metabolites to macromolecules, or from functions like cardiac ejection volume to blood pressure and they can be used to detect, monitor, or assess a variety of clinically relevant conditions. In short, they may represent measurements that have been used in medicine for years or they may be related

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to recently discovered phenomena that are a manifestation of a pathological condition. What they have in common is that they are subject to quantitative and reproducible determination and, if they are to have practical application, under conditions found in germane clinical settings.

At the end of the last century, the National Institutes of Health (NIH) composed the Biomarkers Definitions Working Group, who proposed the first widely accepted definition of a biomarker:[8]

A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

This definition, interestingly, did not include genetic analyses (they did not change with respect to normal, pathological, or pharmacological processes) and the principal applications of these measurements were to monitor disease progression or responses to clinical intervention, for example, drug-induced changes. Although monitoring disease progression and the responses induced by therapeutics is clearly important, it would soon become evident that this definition was much too limited to deal with the technological advances that were waiting in the wings.

Since the introduction of the definition of a biomarker by the Biomarkers Definitions Working Group,[8] and an array of related terms such as medical signs, symptoms, surrogate endpoints, and clinical endpoints, there has been a continued discussion directed toward improving and extending their meaning and use. This has in part been driven by the growing realization that they have broader applicability than monitoring disease progression and its treatments. For example, in 1993, some 10 years before the Working Group definition was coined, the World Health Organization (WHO) International Programme on Chemical Safety produced[7] "an even broader definition[that] takes into account not just incidence and outcome of disease, but also the effects of treatments, interventions, and even unintended environmental exposure, such as to chemicals or nutrients." In their report on the validity of biomarkers in environment risk assessment, the WHO has stated that a true definition of biomarkers includes "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction."[9]

Much of these discussions of the past two decades has related to the importance of biomarkers as surrogate endpoints of treatment modalities and how they are related to clinical endpoints, but they also arose in part because the importance of including the rapidly increasing amount of genetic data as predictive biomarkers and the all-important use of biomarkers as diagnostics, particularly those that have been identified and can be assessed by proteomic technology. In 2015, the Food and Drug Administration (FDA)-NIH Joint Leadership Council identified the need to broaden and redefine the terms used in "translational science and medical product development as a priority need, with a focus on terms related to study endpoints and biomarkers" and created the FDA-NIH Biomarker Working Group,[10] which ultimately lead to the publication of Biomarkers, EndpointS, and other Tools (BEST) Resource glossary in January 2016.[11] This definitive publication lays out a proposed terminology for biomarkers and endpoints and as such is a "working dictionary" of present-day precision medicine. The authors stress that this is a "living" document and that it is intended that it be periodically updated.

The BEST Glossary[11] defines a biomarker as "a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers. A biomarker is not an assessment of how an individual feels, functions, or survives." This definition emphasizes that a wide range of physiologic measurements can be, and are, used to predict, detect, and treat disease but information derived from 'omics data is largely of the molecular type. **Table 1** summarizes the classes and definitions of biomarkers, as found in the BEST report including an example of each category. The inclusion of such biomarker types as predictive, prognostic, and susceptibility encompass (and to a considerable degree depend on) genomic data while those classed as diagnostic, monitoring, and pharmacological arise from the production of a variety of molecules such as RNA, proteins, and metabolites. Thus genomics (and epigenomics), transcriptomics, proteomics, microbiomics, and metabolomics—the principal components of 'omic technology—are the sources of biomarker identifications. The two additional types of biomarkers, safety and susceptibility/risk may be derived from several different classes of measurements but are primarily of interest to developers of drugs and other therapeutics and will not be considered further here. They do illustrate the comprehensiveness of the BEST Glossary.

It is also important to note that molecular biomarkers need not be single molecules but can be composed of multiple substances, usually referred to as panels.[3] One of the earliest attempts to use mass spectrometric-based measurements to determine a biomarker, identified as a panel of unidentified masses, was made from ovarian cancer samples[12] but systematic errors in sample management and more rigorous statistical analyses established that the data were flawed and thus meaningless as a diagnostic.[13] Unfortunately, this had the effect of making panels composed of multiple components unattractive as biomarkers and they were largely shunned for a number of years. This situation was not helped by the attitude of the authorities who were clearly biased against such an approach. However, it was also recognized early in the proteomic-based diagnostic discovery game that single peptide/protein biomarkers for complex diseases such as cancer, was likely to be a challenge that panels might overcome.[14] Improvements in technology and the attractive possibilities of adding new biomarkers to existing validated markers to make an even better diagnostic or combining determinations from different 'omic approaches to make a 'mixed' biomarker suggests that panels as biomarkers may well yet be valuable additions to biomarker identification.[3]

3. Challenges in Developing Diagnostics

When the Human Genome Project published the results of its progress in 2001—a 90% complete sequence of the three billion base pairs in the human genome—it had two major consequences: it marked the beginning of a flood of human genomic data that could be interrogated to reveal extensive

Table 1. The categories and definitions of clinical biomarkers as defined by the BEST Glossary.[11]

• Diagnostic Biomarker
A biomarker used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease.
Example: Blood sugar or hemoglobin A1c (HbA1c) may be used as a diagnostic biomarker to identify patients with Type 2 diabetes mellitus.
• Monitoring Biomarker
A biomarker measured serially for assessing status of a disease or medical condition or for evidence of exposure to (or effect of) a medical product or an environmental agent.
Example: Hepatitis C virus ribonucleic acid (HCV-RNA) level may be used as a monitoring biomarker when assessing treatment response in patients with chronic hepatitis C.
• Prognostic Biomarker
A biomarker used to identify likelihood of a clinical event, disease recurrence or progression in patients who have the disease or medical condition of interest.
Example: C-reactive protein (CRP) level may be used as a prognostic biomarker to identify patients with unstable angina or a history of acute myocardial infarction with a greater likelihood of recurrent coronary artery disease events.
• Predictive Biomarker
A biomarker used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent.
Example: Certain cystic fibrosis transmembrane conductance regulator (CFTR) mutations may be used as predictive biomarkers in clinical trials evaluating treatment for cystic fibrosis, to select patients more likely to respond to particular treatments
• Pharmacodynamic/Response Biomarker
A biomarker used to show that a biological response has occurred in an individual who has been exposed to a medical product or an environmental agent.
Example: Serum LDL cholesterol may be used as a pharmacodynamic/response biomarker when evaluating patients with hypercholesterolemia, to assess response to a lipid-lowering agent or dietary changes.
• Safety Biomarker
A biomarker measured before or after an exposure to a medical product or an environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse effect.
Example: Hepatic aminotransferases and bilirubin may be used as safety biomarkers when evaluating potential hepatotoxicity.
• Susceptibility/Risk Biomarker
A biomarker that indicates the potential for developing a disease or medical condition in an individual who does not currently have clinically apparent disease or the medical condition.
Example: Apolipoprotein E (APOE) gene variations may be used as susceptibility/risk biomarkers to identify individuals with a predisposition to develop Alzheimer's disease.

new insights into human disease and it provided the basis for interpreting mass spectrometric-based proteomic analyses on an hither to unknown scale. The latter, of course, was paired with the earlier development of two new ionization techniques (matrix-assisted laser desorption ionization or MALDI, and electrospray ionization or ESI) that transformed mass spectrometry by allowing peptides and proteins (among many other things) to be analyzed and identified by this technology that lead to the unbiased analyses of complex, unfractionated samples with the concomitant identification of thousands of proteins in a single run.[15] The possibilities for biomarker discovery of diagnostics for a broad spectrum of diseases, particularly those that were very difficult if not impossible to diagnose in early stages, was highly attractive and became an intense focus of earlier proteomic studies. Unfortunately, these well-hyped expectations did not turn out to be the case.[16] There were a number of reasons why the expected tsunami of new FDA-approved diagnostics did not materialize,[17] but it was not from the failure of proteomic technology to identify candidates. The number of 'identified' biomarkers is enormous, perhaps as much as a quarter of all human proteins.[18] But the conversion of these putative biomarkers into useful tests has not followed suit. As has long been recognized, the problems begin with validating germane candidates and multiply as the arduous task of creating an approved and useful test is carried forward through the process.[19] Some key considerations when developing clinical diagnostics include defining the clinical intended use of a biomarker,[20] careful study design to avoid/minimize systematic bias,[21] strong analytical rigor and reproducibility[22–24] and knowledge of regulatory requirements for a clinical assay.[25,26] Simple cost analyses also quickly reveal how difficult it is to devise a biomarker-based test that will have broad enough application to be financially viable and, accordingly, analyses of the success rates for achieving such goals over the past several years are discouraging,[16,27] despite the desirability of the goals. A number of reviews, commentaries, and editorials have been written regarding the problems associated with this discovery to validation gap.[28–30]

One could then reasonably ask why so much time and money has been spent in what has been a very unrewarding exercise, if one accepts that the goal of this work was not to "get grants and publish papers" but rather to attack the seemingly intractable problems of early stage detection of highly lethal cancers? The answer is probably that there is a lack of any really effective therapies for afflictions such as advanced ovarian or pancreatic cancer and that 'omic technology should have the potential to solve this problem; this is very much the essence of the promise of "precision medicine."

It is also noteworthy that there will still be very practical limitations for the use of such assays, even if they are devised. In the United States , and these values are shared by most Western countries, approximately a third of the population is over 50 years of age (≈107 million) and the incidence of pancreatic and ovarian cancer is ≈50 000 and ≈20 000 per year, respectively. About 75% of both classes will die per year. To be effective in treating either of these diseases by early detection, the goal of a biomarker-based diagnostic, it would require regularly screening very large segments of the population routinely to find the relatively small number (on a percentage basis) of new cases. Although not impossible it will require a substantial change in the way medicine is practiced in the United States and the world but then again, so will be the wide-scale introduction of precision medicine.

4. The Clinical Proteomic Tumor Analysis Program

There is no question that biomarkers are particularly important in the fight against cancer. Currently, there are over 20 FDA-approved/cleared protein biomarkers for a variety of cancers

Table 2. List of FDA-approved protein tumor markers (and year approved).

Biomarker[a,b]	Clinical Use	Type	Year
Pro2PSA	Discriminating cancer from benign disease	Prostate	2012
ROMA (HE4+CA-125)	Prediction of malignancy	Ovarian	2011
OVA1 (multiple proteins)	Prediction of malignancy	Ovarian	2009
HE4	Monitoring recurrence or progression of disease	Ovarian	2008
Fibrin/fibrinogen degradation product (DR-70)	Monitoring progression of disease	Colorectal	2008
AFP-L3%	Risk assessment for development of disease	Hepatocellular	2005
Circulating Tumor Cells (EpCAM, CD45, cytokeratins 8, 18+, 19+)	Prediction of cancer progression and survival	Breast	2005
p63 protein	Aid in differential diagnosis	Prostate	2005
c-Kit	Detection of tumors, aid in selection of patients	Gastrointestinal stromal tumors	2004
CA19-9	Monitoring disease status	Pancreatic	2002
Estrogen receptor (ER)	Prognosis, response to therapy	Breast	1999
Progesterone receptor (PR)	Prognosis, response to therapy	Breast	1999
HER-2/neu	Assessment for therapy	Breast	1998
CA-125	Monitoring disease progression, response to therapy	Ovarian	1997
CA15-3	Monitoring disease, response to therapy	Breast	1997
CA27.29	Monitoring disease, response to therapy	Breast	1997
Free PSA	Discriminating cancer from benign disease	Prostate	1997
Thyroglobulin	Aid in monitoring	Thyroid	1997
Nuclear Mitotic Apparatus protein (NuMA, NMP22)	Diagnosis and monitoring of disease	Bladder	1996
Alpha-fetoprotein (AFP)[c]	Management of cancer	Testicular	1992
Total PSA	Prostate cancer diagnosis and monitoring	Prostate	1986
Carcino-embryonic antigen	Aid in management and prognosis	Not specified	1985
Human hemoglobin (fecal occult blood)	Detection of fecal occult blood (home use)	Colorectal	1976

[a] Taken in part from ref. [17]; [b] While hCG is commonly used as a tumor marker, it has not been cleared/approved for this application by the FDA; [c] AFP is a Class III analyte because of its non-cancer intended use (aid in prenatal diagnosis of birth defects).

(**Table 2**),[17,31] which is not an impressive number when one considers the large number (thousands) of candidate protein biomarkers reported in the literature the past few decades.[32,33] Clearly there is a well-documented shortfall between the number of candidate biomarkers identified and those cleared or approved by the FDA for clinical use.[27,34] Too often, biomarkers identified in initial discovery studies have not shown reproducible activity during subsequent validation. This deficit is a major contributor to the high morbidity of many types of cancer and developing new diagnostic assays based on the identification of novel biomarkers is a priority.

Recognizing this huge unmet need, the NCI was quick to capitalize on the technological developments in genomics and proteomics. In 2006, the NCI initiated two programs: The Cancer Genome Atlas (TCGA; https://cancergenome.nih.gov) to understand the molecular basis of cancer [in collaboration with the National Human Genome Research Institute] and the Clinical Proteomic Tumor Analysis Consortium (CPTAC; https://proteomics.cancer.gov) as part of the Clinical Proteomic Technologies for Cancer initiative.

The application of genomics and proteomics to the understanding and management of cancer was the beginning of the broader use of 'omics technologies for this and related purposes.[3] Both depended on biomarkers but of quite different types and applications. The TCGA was more readily developed because of the analytical advancements and comparative ease in data collection and it has contributed significantly to the identification of biomarkers for cancer susceptibility, prevention, and diagnosis and helped to identify novel targets for therapeutic drug development. It has also been pivotal in refining and accelerating the clinical understanding of patient stratification for therapy, while simultaneously showing that molecular drivers of cancer are derived not just from DNA alterations alone, but from protein expression, modification and activity at the metabolic level. The application of proteomics to these latter manifestations was addressed by the founding of CPTAC.

The first goal of CPTAC was to standardize proteomics methods to insure reproducibility of datasets between laboratories that included: 1) standardization of comprehensive untargeted (discovery) protein analyses;[35] 2) standardization and best practices of targeted protein analyses (targeted proteomics);[36–39] 3) adoption of selective/multiple reaction monitoring (S/MRM) mass spectrometry assays (thyroglobulin) by clinical reference laboratories;[40,41] 4) development of an open-source computational tool for designing MRM assays that is supported by all major instrument vendors (Skyline software);[42] 5) development of mock 510(k) device clearance documents on the regulatory approval of multiplexed protein-based In Vitro Diagnostics (IVD) assays/platforms (mass spectrometry and protein array) done in coordination with the U.S. FDA and the American Association for Clinical Chemistry (AACC);[30] and 6) development of

proteomic data sharing policies (Amsterdam Principals) that are supported by peer-reviewed journals.[43]

CPTAC began its second round in 2011. The goal of this pilot study was to establish a coordinated effort between the CPTAC laboratories that would apply the standardized workflows to three (colorectal, breast, and ovarian) genomically-characterized tumors from TCGA. The aim was to systematically identify proteins that were derived from mutations in the various cancer genomes and related biological processes, that would extend the understanding of the molecular basis of these cancers beyond that elucidated through genomics. CPTAC's "proteogenomics" approach (comprehensive proteomics combined with genomics), was designed to demonstrate the scientific benefits of integrating proteomics with genomics to produce a more unified understanding of cancer biology, while creating improved resources that would be freely used by the global cancer community.[44–46] CPTAC's public resources include a Data Portal (https://cptac-data-portal.georgetown.edu/cptacPublic), Assay Portal (https://assays.cancer.gov), and Antibody Portal (https://antibodies.cancer.gov). These represent some of the largest repositories of proteomics/genomics data across these three cancer types.

These successes by CPTAC investigators of integrating proteomics with genomics (proteogenomics) led NCI to renew CPTAC in late 2015 and expanded its efforts on the comprehensive proteogenomic characterization of additional cancer types. More recently, CPTAC has helped initiate two new Cancer Moonshot strategic science and technology initiatives—the International Cancer Proteogenome Consortium (ICPC; https://icpc.cancer.gov) and the Applied Proteogenomics OrganizationaL Learning and Outcomes (APOLLO; https://apollo.cancer.gov) program. ICPC encourages international cooperation in proteogenomic cancer research, as well as the adoption of standardized proteogenomic workflows to ensure data quality. The cancer types studied is determined by each member country, with data collected made public (Data Sharing Pledge). Presently, ICPC consists of 31 research institutions spanning 12 countries with on-going projects on 13 cancer types. APOLLO is a U.S.-based effort (collaboration among the NCI, the Department of Defense, and the Department of Veterans Affairs) to incorporate proteogenomics into patient care as a way of looking beyond the genome, to the activity and expression of the proteins that the genome encodes.

5. Conclusion and Future Perspectives

The National Cancer Institute's CPTAC program took a risk and in doing so pioneered the emerging field of cancer proteogenomics, while perhaps more importantly restoring interest and enthusiasm in the use of proteomics in the pursuit of biomarkers that had not been particularly successful. Its integrated proteogenomic approach has already revealed new insights into cancer and a better understanding of cancer-relevant pathways through posttranslational modifications. The CPTAC program has demonstrated that proteogenomic approaches enable a more complete characterization of the biological pathways associated with tumor development and metastasis and have the potential to better match a cancer patient's individual tumor to targeted therapies. Undoubtedly, as technology improves, the rate of new diagnostics arising from a broad spectrum of laboratories will begin to increase and the CPTAC program has insured that proteomics will contribute its share to the 'omics-driven adoption of precision medicine.

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Conflict of Interest

The authors declare no conflict of interest.

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