Variations of human genetic and proteomic (proteins) profiles imply that the same medical treatment may not be suitable for patients even if they have the same diagnosis.

Personalized medicine uses new techniques of molecular analysis, such as genomics, proteomics, and metabolomics, combined with predictive modeling to better manage diseases. The goal is to achieve optimal medical outcomes for a patient through risk assessment, prevention, diagnosis, therapy, treatment response monitoring based on individual patient’s genetic, proteomic, or metabolomic profile. Similar to genomics, which examines the activity of a large number of genes (genome), proteomics and metabolomics focus on proteins (proteome) and metabolic products (metabolome) as we are entering into the post-genomic era. In the cascade from genes, transcripts, proteins and metabolites, proteins and metabolites are closer to disease phenotype and bridge the gap between genotype and phenotype (Figure 1).

Recent developed technologies in proteomics and metabolomics, show that it is possible to compare the proteome or metabolome from disease samples and control samples to identify the differences between these groups. This kind of differences is often called “biomarker”. According to the National Institutes of Health, USA, “Biomarker” can be defined as molecules that can be objectively measured and evaluated as indicators of normal or disease processes and pharmacologic responses to therapeutic intervention [1]. A good biomarker is able to distinguish between healthy and disease states with a high degree of accuracy. Moreover, these biomarkers should be measureable in readily accessible body fluids (e.g. blood, urine, tear fluid, saliva, cerebrospinal fluid, etc.). Biomarkers can be used to improve molecular diagnosis/prognosis, better understand disease mechanisms, monitor therapeutic response, provide targets for developing new drugs and treatments, and evaluate drug efficacy in a clinical trial. Furthermore, biomarkers can also be used to stratify patients into disease sub-types, determine the severity of the disease or different phases in a chronic disease, tracking disease progression and assess the risk of disease recurrence.

Proteomics/metabolomics methods based on mass spectrometry [2] hold promise for the discovery of novel biomarker because these new technologies are high throughput and allow identification and quantification of large number of proteins or metabolites simultaneously. However, not like tools for genomics, the technologies for proteomics and metabolomics are still immature. The core of such technology is based on an expensive machine called “mass spectrometer (MS)”, which measures the molecular weight. We are dealing with complex biological/clinical samples (body fluids, tissues, cells). Compared to 25,000 human genes, there are maybe hundreds of thousands of human proteins and more than 7900 human metabolites [3]. Both diversity and dynamic range (9~12 order of magnitude) challenge the analytical technology. Despite the most advanced protein/metabolite separation methods (for example, multi-dimensional nano-flow liquid chromatography, ultra-performance liquid
chromatography, etc.) and MS technology (Orbitrap, Fourier transform ion cyclotron resonance MS, and very recent TripleTOF), we are only able to identify and quantify a fraction of the entire human proteome and metabolome. The sensitivity of the mass spectrometer is not sufficient to pick up many low-abundant proteins or metabolites, such as, cytokines. The advance of proteomics/metabolomics is largely dependent on development of the MS.

The typical pathway [4] to develop a new disease biomarker includes following five stages: discovery, verification, validation, clinical evaluation and disease control. In the discovery phase, candidate biomarkers can be generated from a pilot study involving limited number of patients with controls (often 10s). Appropriate study populations must be carefully selected. Precise definition of the diseases or clinical conditions and accurate clinical assessment determine the quality of the clinical samples. Mass spectrometry based platforms are typically the method of choice in the discovery stage. Large number of candidate biomarkers (can be 100s) might be found in the discovery stage. The goal for verification phase is to narrow down to the most promising candidates from the initial list. Both targeted MS method (MRM, multiple reaction monitoring, [5]) and conventional antibody based methods (such as, Enzyme-linked immunosorbent assay, ELISA and Western blot) can be used in this stage. Considering clinical and biological variation, candidate biomarkers must be validated using a large number of samples (100s) in the validation phase before using them in the clinical practice. Validation of the new biomarkers is often very challenging. Considering the diversity of proteomic/metabolomic profiles in human populations, the concept of population proteomics/metabolomics is introduced [6]. Population proteomics/metabolomics can be defined as the investigation of human proteins/metabolites across and with populations to define and understand protein/metabolite differences. Many factors including age, sex, race, diet, and location/climate have to be taken into consideration. Pooling of results from multi-sites and cross validation of the potential biomarkers are extremely useful for population proteomics/metabolomics studies. Sophisticated statistical tools and modeling algorithms are needed to mine the data. Sensitivity and specificity of the biomarker panel will be determined in the clinical evaluation phase. And the effect of the disease management will be defined in the disease control phase. These last two phases involve large number of patients (1000s) and can be considered as population proteomics/metabolomics studies.

A single biomarker may be ideal in clinical use but it hardly exists due to the fact that many diseases are multi-factorial diseases. One good example is from the dry eye study carried out in Singapore eye research institute [7]. Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance and tear film instability with potential damage to the ocular surface. It is accompanied by increase osmolarity of the tear film and inflammation of the ocular surface. Dry eye disease is a world-wide clinical problem which affects millions of people with a prevalence estimated to be as high as 5-24% of the general population. The fundamental clinical problem of dry eye is a disconnection between the signs and symptoms coupled with the lack of objectively measurable end points. The biomarker candidates we identified reflect the characteristics of the disease, i.e. tear secretion deficiency, inflammation of the ocular surface and elevated osmolarity of the tear film. One of the best single tear biomarker may differentiate patients with or without dry eye with 80% accuracy. However, a combination of four tear biomarker (biomarker panel) can significantly increase the diagnostic accuracy to 95% [7]. These tear biomarkers were initially identified from patients in Singapore (patients: 82% Chinese, 11% Indian and 7% Malay). Subsequently, similar results were obtained using patients from Tianjin, China where all were Chinese. Our results suggested [7] the stability of tear biomarkers across population and geographic location (average temperature in Singapore is between 25 °C and 30 °C and 85% humidity while average temperature in Tianjin is between -4 °C and 27 °C and humidity from 50% to 77%).

The last step to use biomarker for personalized medicine is to develop inexpensive doctor's office device, also called point-of-care (POC) device. POC device requires reliable, sensitive (some require sub pg/ml concentration), specific, automated, cheap, be able to measure multiple biomarkers (multiplex), easy to operate and fast. Ideally, it can be used in a clinical setting and on-the-spot diagnosis. Although, liquid chromatography-mass spectrometry (LC-MS) method can cover from biomarker discovery to verification and validation stages, it is still too expensive to reach doctor's office. It is also technically demanding. ELISA is another option and has often been used in biomarker verification as an independent technique. It is also an important method for clinical protein determinations. However, conventional ELISA is not ideal for POC device because it requires technical expertise and has many limitations including cost, sample size, analysis time and multiplex analysis. New technologies based on optical methods, electrochemical immunoassays, microfluidic device, biochip, and protein arrays have been developed and demonstrated their promise for future POC device [8].

No doubt, biomarker is a crucial part of personalized medicine (Figure 2). Proteomics/metabolomics offers high throughput technologies for biomarker discovery. Before personalized medicine becomes a reality, the advances in proteomics/metabolomics platform, standardized procedures in biomarker development workflow, establishment of population proteomics/metabolomics, and joint effort involving clinicians, proteomics/metabolomics scientists, biochemical/molecular scientists, bioinformaticians and statisticians all play an important role.
Figure 1.
“Omics” cascade: Recently significant technologies have been developed to study proteome and metabolome.

“Omics” Cascade

[Diagram showing the cascade from Genome to Transcriptome to Proteome to Metabolome to Phenotype]

Figure 2.
The role of biomarkers in personalized medicine.

[Diagram showing the critical role of biomarkers in personalized medicine, linking body fluids, tissues, cells, genomics, proteomics, metabolomics, imaging, system biology, bioinformatics, disease mechanism, disease diagnosis & prognosis, targets for drug development, statistics, clinical trials, and leading to personalized medicine]
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