Review Article

Proteomics of rare diseases: A concise review

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Current status of proteomics and other omics technologies used for rare diseases

Rare diseases are those that affect a limited number of individuals. They are defined as a disease that affects no more than 1 in 2000 individuals and among them one third are children[1]. The number of disorders that fit this definition is very large, being over 5000 according to the World Health Organisation[2]. There are more than 7000 rare diseases that affect over 5% of individuals, worldwide[3]. As such, a rare disease is said to affect 1 in 13 of the world's population[4]. However, due to the lack of epidemiological evidence, its true burden is difficult to estimate[5]. With the introduction of high throughput sequencing techniques, such as next-generation sequencing, the diagnostic odyssey of a rare disease is slowly reducing. However, the main challenge is in its treatment and biomarker discovery approaches as most proteomic tools fail in the face of these orphan diseases due to the scarce numbers of diseased individuals of a particular disease or phenotype.

In recent years, a discipline that integrates proteomic and genomic data has emerged and it's known as Proteogenomics[6]. This new discipline allows us to identify biomarkers that are a reflection of biological processes involved in rare diseases. However, finding the best possible method is challenging. This is mainly because biomarkers in rare diseases show low specificity since body fluids might not always reflect the true nature of the disease[7]. Low specificity, coupled with low sample size, is the main obstacle faced by clinicians and scientists alike, to apply discovery-driven techniques in the field of proteomics. Hence, integrating protein data with the genomic data is the new frontier[8]. They are not only very rare but also have a variable expression, may have very long courses, and have incompletely known late effects[9].

Immunoassays

Thus far, the most robust technique is an immunoassay, which detects specific protein domains using antibodies to capture them. Immunoassay is commonly used in proteomics and biomarker validation owing to its high sensitivity[10]. Techniques such as ELISA immunoassay has shown promising results in some rare diseases such as pemphigoid and Emery–Dreifuss muscular dystrophy with dilated cardiomyopathy[11,12]. To overcome low sample amounts, several multiplex immunoassays are available which are capable of investigating multiple proteins simultaneously. For example, such a biomarker panel (SMA-MAP) is currently being used in patients with spinal muscular atrophy. It measures plasma protein levels associated with motor function. However, this commercial panel needs further validation to assess its application in other neuromuscular diseases[13]. An inexpensive, yet effective immunoassay, which has colloid nanocrystal tags with multiple targets, is also described in the literature. This assay can measure up to six antigens in a single run. However, since there can be non-specific binding, its precise application would require optimization[14]. The heterogeneity of rare diseases makes it difficult to measure specific antigens, especially if they are unknown. Hence, immunoassays have major limitations in proteomic studies related to rare or undiagnosed diseases. An affinity-based proteomics approach, using blood-derived samples of patients affected by muscular dystrophy, is also available. This compares levels of proteins in the patients' blood. Based on the protein profile it can differentiate the degree of severity in patients affected with Duchenne muscular dystrophy (DMD)[8]. This technique has the potential to be used in other forms of rare diseases that are in dire need of blood-based protein markers.

Mass-spectrometry

Mass spectrometry is another instrument that is widely used. Advanced MS-based instruments, such as linear ion trap-Orbitrap, can study up to 2000

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fractionated proteins. Some of the protein fractions are not described in the literature and can have a biological significance, especially in rare diseases. Some of the commonly described molecules include cytokines, cell surface proteins, transporter proteins, and even structural proteins. Rare metabolic diseases, such as Niemann–Pick, can benefit from MS-based protein assays especially in the development of screening tests. One such metabolite excreted in urine is sphingomyelinase. Due to the ease of access to urine samples, this may pave the way for rapid screening tests for rare metabolic diseases[15,16]. Employing advanced MS-based techniques on body fluids may enable us to understand the pathophysiological processes in rare diseases. However, there are still several drawbacks. There can be a high degree of variability depending on the instrument and it must be stressed that prior knowledge of proteins of interest is fundamental for its success as a screening tool.

The way forward

An integrated approach is needed for rare diseases when so little is still known. The way forward is the 'multi-omics approach' where a patient's genomic data is integrated with the protein profile. However, for this method to be successful, prior knowledge of proteins of interest will be required. This can only be achieved through strengthening basic science research in rare diseases with phenotypic input from clinicians.

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