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Brief Overview of The Applications of Proteomics in Theranostics

ABSTRACT

Theranostics is an emerging field of medicine that uniquely combines drugs and/or techniques to simultaneously or sequentially diagnose and treat medical conditions. Proteomics, the large-scale characterisation of proteins, is now being applied in theranostics. The proteome is the entire set of proteins that is produced by an organism. It varies temporally and spatially according to the distinct needs of the organism. Major breakthroughs in human proteomics are seen in its theranostics applications as biomarkers (e.g. in cancer, Alzheimer's disease, various other neurodegenerative disorders, autism, cardiovascular diseases) and in therapeutics (e.g. personalised treatment).

INTRODUCTION

Proteomics is the analytical study of all proteins transcribed by a genome.[1] It was Anderson and Anderson in 1977 who pioneered in this field by studying human plasma proteins. They even predicted that someday all proteins in the human body could be identified.[2]

Human proteomics is catalysing a new research field that has the potential to be translated into the clinical setting. This 'clinical proteomics' is trying to understand what, how much and when, certain proteins are expressed in health and disease. Thus, it has the potential to help in the discovery of novel biomarkers of disease processes and help to improve their diagnosis and prognosis.[3-5] Also proteomic profiles have great potential to unveil pathophysiological circuits associated with pathology of disease and these may provide targets for new treatments or prevention. They can also offer novel ways for personalised therapeutics.

Amongst the main crucial technologies that are used to identify, quantify and characterise the proteins (and hence describe the proteomic signatures or profiles) of normal and disease processes, a combination of mass spectrometry,[6] two-dimensional electrophoresis[7] and bioinformatics is applied. These powerful technologies, especially quantitative MS and bioinformatics, have made great advances and today it is possible to analyse complex mixtures of proteins in a more rapid, accurate and quantitative way. The field of bioinformatics has advanced to meet the need for data acquisition, interpretation and presentation. These great advances are helping to discover the function of proteins and the underlying pathological mechanisms of disease.

1. PROTEOMICS IN CARDIOVASCULAR DISEASES

Arrell et al.[8] found that in patients with dilated cardiomyopathy (DCM) there are 88 myocardial proteins which have decreased expression. They proposed that this low myocardial protein profile can be used as a diagnostic and prognostic signature for DCM.

Borozdenkova et al.[9] studied proteomic signatures as potential markers for rejection after heart transplants. Of the 100 proteins that they found to be overexpressed, 13 were specific to heart tissue. Of these 13, two proteins, tropomyosin and alpha beta-crystallin, were measurable in the serum of patients having rejection after 3 months.

As mentioned, proteomic bio-profiles can reveal pathophysiological circuits associated with pathology (causation and progression) and these can provide targets for new treatments or prevention. In keeping with this Ferreira et al.[10] initially identified 252 proteins in the plasma of heart failure patients. After factoring a number of variables, the number of circulating plasma proteins associated with heart failure pathophysiology was decreased to 38. These were specifically linked by the authors to apoptosis, inflammation, vascular physiology, remodelling of matrix, control of blood pressure, and cholesterol metabolism [ClinicalTrials.gov Identifier: NCT02556450].

Further to this, Lind et al.[11] have worked on a proteomic chip to discover new biomarkers for AF. Using this chip they discovered that four other proteins, specifically FABP4, IL-6, TIM-1 and AM, besides the already known ones (i.e. NT-pro-BNP, FGF-23, GDF-15) are linked to the development of AF.

2. PROTEOMICS IN MENTAL ILLNESS

The underlying pathophysiology of mental illness remains unclear. Proteomics is one tool that has the potential to objectively help this situation and thus aid in their theranostics.

Xu HB et al.[12] analysed the plasma proteomes of patients with major depressive disorders and healthy controls. They showed expression of altered proteins (A2M and isoform-1 of VDP) involved in immunoregulation and lipid metabolism. They proposed that disruption in immunoregulation and lipid metabolism might be implicated in the pathological mechanisms of major depressive disorders.

In a similar but more recent proteomic study, Smirnova et al.[13] also showed differences in the serum proteomes of schizophrenia and bipolar disorder.[14] Specifically, they discovered 27 proteins for schizophrenia, and 18 proteins for BD. In schizophrenia, the proteins were linked to the immune response, cell growth and maintenance, cell communication, and to the regulation of metabolism of proteins and of nucleic acids. In BD, the proteins were linked to the immune response, cell membrane transportation, communication of cells and their growth, and to neurons and oligodendrocyte development.

As with other proteomic studies in other areas such results will eventually surely unveil the intricate pathways of psychotic disorders with beneficial outcomes in management within the clinical setting.

3. PROTEOMICS IN AUTO-IMMUNE DISEASES

Proteomics is also being applied in auto-immune disease. Wu et al.[15] discovered potential biomarkers for systemic lupus erythematosus (SLE). In this study, SLE serum autoantibodies were validated using serum samples from 306 participants. Four peptides (SLE2018Val001, SLE2018Val002, SLE2018Val006, and SLE2018Val008) were identified as being able to differentiate SLE patients from healthy controls. Wu et al. propose that their approach could be implemented to identify autoantibodies in other diseases.

Xu et al.[16] investigated 106 proteins in patients with psoriasis which are involved in several biological signalling pathways relevant in the disease. Following a comparison with a healthy cohort, the authors found that, of these 106 proteins,58 are only found in psoriatic patients. From these 58, 21 proteins have been identified as markers for treatment outcomes. Furthermore, 3 proteins showed a reliable correlation with the severity of the disease.

In rheumatoid arthritis, serum amyloid A (SAA) and S100 have already been identified as potential biomarkers. Nys et al.[17] furthered the research and found that there is a negative relationship between the subtypes of SAA, SAA1α and SAA1β for early-onset RA. They also found that isoform SAA2 and S100A8/S100A9 proteins are overexpressed irrespective of the RA phase. They propose that these findings might be indicators of other unknown pathways of the disease.

Another proteomic study in RA patients was done by Chen et al.[18] They analyzed biomarkers to distinguish responders to triple therapy (methotrexate, leflunomide and infliximab). They analysed 51 proteins that were expressed differently in responders and non-responders. Such proteins in proteomics are called differentially expressed proteins (DEPs). Of these DEPs 5 were significantly up-regulated whilst another 5 were down-regulated. This study shows how proteomics can be used as a tool to predict clinical response.

4. PROTEOMICS IN MULTIPLE SCLEROSIS

Malekzadeh et al.[19] studied DEPs during the progression of MS. The study was spread over 4 years and involved a cohort of healthy individuals and 3 cohorts of MS patients with 3 different rates of progression. Importantly they found 8 potential biomarkers, including LGLAS8, CCL3, RGMA, C3, FGF9, and EHMT2. These proteins are associated with complement pathways, activation of the immune system, and cell-cell and cell-matrix adhesions. The authors of the study propose that these proteins are involved in the progression of MS and they envisage further research to use them in the clinical setting.

5. PROTEOMICS IN INFLAMMATORY BOWEL DISEASE (IBD)

Ning L et al.[20] compared proteomic profiles of intestinal tissue taken from healthy individuals, and patients with IBD, specifically Crohn's disease (CD) and ulcerative colitis. They found several DEPs, like angiotensin converting enzyme 2 (ACE2) and angiotensin converting enzyme 1 (ACE), being overexpressed. Such overexpression was more marked in CD. Most importantly they found overexpression of CD38 in inflamed tissue. This is a protein which is intricate in the nicotinamide adenine dinucleotide (NAD)[10] metabolism. They proposed that this finding might need to be followed further to study the function of CD38 and NAD metabolism in intestinal inflammation.

On the other hand Lehmannet al.[21] compared fecal samples taken from patients with CD and UC via a metaproteomic approach. Importantly they found that CD and UC patients showed underexpression of human IgA and the protein RprY from Bacillus fragilis. However, in CD they found an overexpression of the enzyme sucrose-isomaltase. The authors concluded that that, following validation, their fecal metaproteomic approach could be used as a non-invasive way in the diagnosis of CD and UC.

6. PROTEOMICS IN AUTISM

Various causes have been implied in the pathophysiology of autism, but the exact mechanisms remain elusive. Proteomic studies might prove to be useful to uncover these. Junaid et al.[22] used a proteomic profiling method on autopsied brains of Autism Spectrum Disorder patients. They discovered an abnormal protein pattern as a result of an aberrant gene expression in their grey matter. Specifically, they found reduced glyoxalase 1 expression and propose that this gene might be a possible aetiological factor.

In another proteomic analytical study to unveil the underlying mechanisms in autism, Corbett et al.[23] compared protein profiles from two groups of children, one group comprising autistic children and the other comprising a cohort of normal children as control. The study showed that in the autistic group, there was an increased expression of Apolipoprotein B-100,

Complement Factor H Related Protein (FHR1), Complement C1q and Fibronectin 1 (FN1). The authors proposed that these differences might be aetiological factors in the abnormal brain development in autistic children.

7. PROTEOMICS IN CANCER

Proteomic studies are also being integrated with other studies like genomics and transcriptomics. This augments the discovery of the molecular players involved in the pathophysiology of diseases, including cancer. A case in point is the study carried out by Wu et al.[24] Here, analysis of proteomic and transcriptomic profiles showed that the long non-coding RNA molecule HOTAIR (HOX Transcript Antisense Intergenic RNA), which has been implicated in human tumorigenesis, also shows dysregulation in hepatocellular carcinoma (HCC). Specifically, HOTAIR inhibition was found to be associated with dysregulation of several transcripts and proteins. Functional bioinformatic studies of the data collected showed that these transcripts and proteins relate to biological circuits in cancer. Furthermore, the study showed that HOTAIR caused cell proliferation partly by its regulation of OGFr expression (opioid growth factor receptor), the latter being known to have a negative regulation on cell proliferation in HCC.

HOTAIR is also dysregulated (specifically it is overexpressed) in breast cancer. This over-expression is responsible in metastasis through HOTAIR recruitment of another complex molecule called Polycomb repressive complex 2 (PRC2), which then silences additional genes, besides the HOXD gene cluster. In 2016 Meredith et al.[25] carried out a proteomic analysis and found that other proteins are associated with HOTAIR's action. One such significant interaction is that between HOTAIR and hnRNP (heterogeneous nuclear ribonucleoprotein) A2/B1. This interaction is central to chromatin structure regulation in cells of breast cancer. Indeed, the authors found that knocking down A2/B1 reduced PRC2 activity and also cell invasion.

8. PROTEOMICS IN OBSTETRICS AND GYNAE DISORDERS

Tarca et al.[26] carried out a study comparing plasma proteins bio-profiles in 90 normal pregnant women and 33 who had early pre-eclampsia. They discovered that a specific proteomic signature preceded pre-eclampsia. Specifically, at 16-22 weeks matrix metalloproteinase-7 and glycoprotein IIb/IIIa complex were overexpressed and could be used as reliable predictors. Predictors from 22-28 weeks were increased levels of sialic acid binding immunoglobulin-like lectin 6 (siglec-6) and activin-A, and decreased levels of isoform 121 (VEGF-121), placental growth factor (PlGF) and vascular endothelial growth factor A. From 28 weeks to 32 weeks, the best biomarkers were activated leukocyte cell adhesion molecule, siglec-6, and VEGF-121.

In 2019 Eckert et al.[27] used proteomics to characterise pivotal molecules in ovarian cancer, studying the latter phenotype in-situ and its progression in metastasis. They discovered that methyltransferase nicotinamide N-methyltransferase (NNMT) and the proteins that it regulates underpins metastasis. Specifically, they revealed that over-expression of NNMT leads to ovarian cancer cells to migrate and proliferate, causing also a reduction of histone methylation and S-adenosyl methionine. These epigenetic changes caused a global change in gene expression associated with the ovarian cancer phenotype behaviour.

9. PROTEOMICS IN NEURODEGENERATIVE DISORDERS

Mining large data from proteomics, Deolankar et al.[28] propose a new pathway to diagnosis and treat Alzheimer's disease. Specifically, they used spectral data to sieve for protein post-translational modifications, and indeed, found proteins modified post-translationally. They propose that these may be used as biomarkers for diagnosis or as molecular targets for treatment in AD. Of the many novel proteins found, 13 of them showed high expression.

Mallah et al.[29] employed a micro-proteomic platform to find spatiotemporal signatures of protein markers after traumatic brain injuries (TBI) in a rat model. Specifically, they analysed different brain regions at 1 day, 3 days, 7 days, and 10 days, post-injury. They found that there was an over-expression of proteins that are similarly expressed in Parkinson's disease. Amongst these were GPR158, HGMB1, Synaptotagmin and Glutamate Decarboxylase in ipsilateral substantia nigra. The authors propose that their study shows a possible link for PD or Parkinsonism post-TBI.

Amyotrophic lateral sclerosis[14] and frontotemporal dementia (FTD) are two neurodegenerative disorders whose pathophysiology is yet not clear even though the culprits may be the aggregation of abnormal proteins in neurons which lead to their degeneration. The proteins that have been implicated are tau, superoxide dismutase 1 (SOD1) and TAR DNA binding protein of 43 kDa (TDP-43). In this regard, Hedl et al.[30] discuss new technologies and approaches like SILAC (stable isotope labelling by amino acids in cell culture), IP-MS (immunoprecipitation mass spectrometry) and BioID (biotin identification), the latter two being PPI (protein-protein interaction) techniques. These proteomic approaches are high-throughput, quantitative and unbiased. Moreover, they are novel avenues that can be used to unveil the mechanisms of pathology, biomarkers and therapeutic targets for ALS and FTD.

10. PROTEOMICS IN OBESITY

It is a well-known fact that, following bariatric surgery, those obese diabetic patients showing insulin resistance, have their glucose levels controlled, notably after the biliopancreatic diversion, BPD. ClinicalTrials.gov Identifier:

NCT01151917 (2009) conducted between 2009 and 2012employed a proteomic platform to investigate the proteins and peptides that underlie the mechanisms involved in the glycaemic restoration after BPD.

Indeed, Nicolai et al.[31] used mass spectrometry and identified a low-abundance peptide that regulates glucose and appetite. Specifically, they discovered that the secretion of oxyntomodulin, a gut hormone, is increased 10-fold after the gastric bypass in type 2 diabetic patients. They also found that oxyntomodulin is co-secreted with glucagon-like peptide-1 (GLP-1), another gut hormone. Moreover, oxyntomodulin acts using the same receptor as GLP-1 and is deactivated by the same protease that breaks down the latter. Thus, they proposed that oxyntomodulin and GLP-1 may regulate glucose metabolism and appetite.

Such discoveries of low-abundance regulatory peptides show again that the proteomic approach has great potential in therapeutic translational research, namely that of providing new ways to treat insulin resistance.

11. PROTEOMICS FOR THE DISCOVERY OF NOVEL DRUG TARGETS

Proteomics is fast becoming a requisite of the discovery of drugs. This is made possible by the fact that proteomic technologies like MS but also mapping of protein-protein interaction have matured into reliable methods. Indeed, hot spots on suspected culprit proteins are being discovered and offer potential targets for novel therapeutic drugs.

CONCLUSION

In the future, the introduction of new biomarkers into medical practice can influence patients' health in many ways. However, more input is needed before research is turned into a diagnostic test that saves lives.

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