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Review

Proteomics advancements in fetomaternal medicine

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ABSTRACT

The study of interactions between genes and their environment, thus of molecules that form the “functional genome”, has been greatly enhanced throughout the course of post-genomic era by the expansion of the omic technologies. Proteomic technologies and mass spectrometry in particular, form a well-armored strategy towards high-throughput simultaneous analysis of thousands of proteins and protein-related molecules. Proteomics as in the past, are still currently offering novel perspectives in the understanding over the pathophysiology of malicious conditions as well as in the development of molecular-targeted therapeutics against them. This review examines the offerings of proteomics research over the unique physiological phenomenon of pregnancy and its related complications, which affect both maternal and perinatal health.

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Introduction

Human reproduction, understood as the totality of genital processes that determine reproductive success, is a multi-dimensional process, encompassing interactions which occur at different levels—molecular, cellular, tissue, organ and organismal. Despite the fact that current research applications aspire to attribute knowledge at any of these levels, our understanding over the molecular basis of most aspects of human reproduction can still be characterized as poor. Over the past years enormous effort to decipher features of human reproduction has been put to play by the two dominant fields of biosciences: study of genes and proteins including genomics and proteomics technologies, respectively [1].

Following conception, all succeeding stages, such as successful implantation, placentation, pregnancy progression, and delivery are highly dependent on a complex interaction of intracellular and extracellular factors, which include hormones, adhesion molecules, growth factors, and immunomodulators [1].

During pregnancy, maternal physiological processes undergo significant changes in order to adapt to the rapidly growing fetus. The placenta, a fetomaternal organ/structure, acts as a dynamic interface between maternal and fetal tissues, which supports the latter until capable of independent existence [2]. The orchestrated functions of maternal, fetal and placental tissues assist to non-problematic pregnancy progression and finally to smooth neonate delivery.

Pregnancy-related disorders such as intra-uterine growth restriction (IUGR), pre-eclampsia (PE), preterm labor (PTL), and intra-amniotic infection (IAI), being disorders of multifactorial etiology, contribute significantly to maternal and fetal mortality; while having a prevalence of 5–10%, they are often difficult to predict (WHO, 1987; Lumley, 2003) [3]. Decades of research have significantly contributed to a clearer understanding over the underlying pregnancy pathophysiology, yet there is still distance to be covered before scientists gain a full comprehension over the molecular roots responsible for phenomena of complications. Current diagnostic methods are deemed unable to accurately predict pathological pregnancy conditions prior to their clinical manifestation.

On this regard, the emergence of powerful mass spectrometry-based proteomic techniques has added a new dimension to the field of fetomaternal medicine. This review will provide an update on how proteomics-based technologies, as has done in the past, continue until this day to provide novel knowledge on the biochemical blueprint of pregnancy and its related complications.

Proteomics methods

Separation methods

Gel-based proteomics

Traditional two-dimensional (2D) SDS–PAGE is still considered to be the most robust and reliable protein separation technique in proteomics pregnancy research, although various new and improved techniques are nowadays available [4–6]. The separation of proteins by electrophoresis is based on the fact that charged molecules migrate through a matrix upon application of an electric field. Matrices such as polyacrylamide are routinely used for the separation of proteins, mainly those of <250 kDa, while the whole approach termed Gel Proteomics is ideal for serving various purposes/applications from expression profiling, to quantitative differential expression measurements, as well as for research of post-translational modifications [7].

Visualization of the separated proteins on polyacrylamide gels is performed either with staining for the visible range (colloidal Coomassie blue, zinc imidazole, and silver nitrate/diamine (Ag)), or the non-visible range [fluorescence staining (sypro ruby etc.)], with each staining technique exerting variable sensitivities.

While gel proteomics is one the most popular methods for protein expression analysis worldwide, this technique suffers serious limitations: i) not all proteins in a given biological sample are in sufficient quantities to be visualized on a SDS–PAGE (low abundant proteins); ii) limitations due to protein size, proteins above a certain size (approx. 250 kDa) cannot be detected; iii) inadequacy in limited-separation of hydrophobic proteins (e.g. membrane proteins), given their low solubility and buffer compatibility; and iv) reproducibility issues. Thus, many replicate experiments are required, in addition to background normalization in order to substantiate in-experiment reproducibility.

Shotgun proteomics

Shotgun proteomics consists of the analysis of proteomes in their original complex form, following an initial digestion step. In the whole aspect of shotgun proteomics, high-resolution liquid chromatography and mass spectrometry are employed in order to provide massive protein identification results. Peptides generated through enzyme digestion (usually using trypsin) are subjected to a first-dimensional separation, typically by strong cation exchange (SCX) chromatography, which separates peptides based on their net positive charge [8]. During a second separation stage which is usually reverse-phase chromatography, the SCX fractions are further fractioned based on their hydrophobicity [9]. Following this two-step separation, samples are finally transferred online to a tandem mass spectrometer, where twice-fractionated peptides are subjected to tandem mass sequencing. When compared to the 2D SDS–PAGE approach previously described, shotgun proteomics provides almost a two-order of magnitude greater amount of results (regarding instrumentation throughput), if one considers equal initial amounts of protein samples to be analyzed *via* each method.

Mass spectrometry

The ability of mass spectrometry (MS) to identify and furthermore, to precisely quantify thousands of proteins in complex samples has in fact broadly impacted on both biology and medicine. Mass spectrometry is at the heart of virtually all proteomics experiments as it provides the key tools for the analysis of proteins. Developments of technology and methodology in the field of mass spectrometry and proteomics have been rapid over the last decade, thus providing improved and novel strategies for the global understanding of cellular functions, for organ biology comprehension and systems biology studies.

Mass spectrometers consist of three basic components: an ion source, a mass analyzer and an ion detector. We first consider the ionization technology and subsequently the mass analyzer itself.

Ionization methods

MALDI. The operation principle of matrix-assisted laser desorption/ionization (MALDI) involves encapsulation of the proteins and

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