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Proteomics of the Human Placenta: Promises and Realities

J.M. Robinson^{a,*}, W.E. Ackerman IV^b, D.A. Kniss^{b,c}, T. Takizawa^d, and D.D. Vandr^a

^aDepartment of Physiology and Cell Biology, Ohio State University, 304 Hamilton Hall, 1645 Neil Avenue, Columbus, OH 43210, USA

^bDepartment of Obstetrics and Gynecology (Laboratory of Perinatal Research and Division of Maternal-Fetal Medicine), Ohio State University, Columbus, OH 43210, USA

^cDepartment of Biomedical Engineering, Ohio State University, Columbus, OH 43210, USA

^dDepartment of Molecular Anatomy, Nippon Medical School, Tokyo, Japan

Abstract

Proteomics is an area of study that sets as its ultimate goal the global analysis of all of the proteins expressed in a biological system of interest. However, technical limitations currently hamper proteome-wide analyses of complex systems. In a more practical sense, a desired outcome of proteomics research is the translation of large protein data sets into formats that provide meaningful information regarding clinical conditions (e.g., biomarkers to serve as diagnostic and/or prognostic indicators of disease). Herein, we discuss placental proteomics by describing existing studies, pointing out their strengths and weaknesses. In so doing, we strive to inform investigators interested in this area of research about the current gap between hyperbolic promises and realities. Additionally, we discuss the utility of proteomics in discovery-based research, particularly as regards the capacity to unearth novel insights into placental biology. Importantly, when considering under studied systems such as the human placenta and diseases associated with abnormalities in placental function, proteomics can serve as a robust ‘shortcut’ to obtaining information unlikely to be garnered using traditional approaches.

Keywords

Placenta; Placental cell lines; Proteomics; Discovery-based research

1. Introduction

The human genome project was motivated by the notion that delivering the dictionary of the human genetic code would enable biomedical scientists to solve the most deeply held questions regarding human disease. While the completion of this intellectually and technologically challenging project has provided an essential lexicon, it is abundantly clear that this information alone is insufficient to decipher gene function at an organismal level. Ultimately, many important implications of the human genome project for biology, pathobiology, and human healthcare will be realised only when coupled with a more

*Corresponding author. Tel.: +1 614 292 9663; fax: +1 614 292 4888. robinson.21@osu.edu (J.M. Robinson).

complete understanding of the expression and function of terminal gene products, the proteins.

Broadly speaking, proteomics refers to the identification and quantification of all proteins derived from the genome. While the genome is relatively static at any point, the proteome is highly variable. This variability can be appreciated by considering that protein expression, both quantitatively and qualitatively, fluctuates markedly among different eukaryotic cell types. Moreover, protein expression in a given cell type also varies temporally during the course of differentiation, and in response to various environmental challenges. Adding to the complexity, many proteins can exist in multiple isoforms due to alternative splicing, RNA editing, alternative promoters, and post-translational modifications [1]. Further nuances can occur when comparing the normal proteome to the same proteome in a disease state, a major goal of proteomics research [2–6]. Thus the 25,000 or so genes in the human genome can give rise to far greater numbers of protein species than predicted solely by gene number (the number of possible protein species in the human has been estimated to be as high as one million [7]). Therefore global analysis of a proteome from an intact tissue, organ, or organism is a daunting proposition.

The prospect of high-throughput global proteome analysis is tantalising and highly desirable. However, traditional proteomics methodologies fall well short of this objective. A salient example lies in the technical challenges associated with identifying certain low abundance proteins that are of importance in disease processes. Considering the enormous dynamic range of protein expression (which can vary over 7e8 orders of magnitude in cells and over 9 orders of magnitude in serum [8,9]), this task is not trivial. Conventional two-dimensional gel electrophoresis (2-DGE) coupled with mass spectrometry (MS) is inherently biased toward the identification of relatively abundant proteins; low-copy proteins, frequently the most interesting regulatory components of the cell, are susceptible to falling below the limits of detection by 2-DGE [10,11]. Although the use of large amounts of starting material has enabled the identification of some low abundance proteins by 2-DGE [10], many remain difficult to detect due to a ‘swamping out’ effect from more abundant structural and housekeeping proteins [12]. Further technical issues related to sample preparation, particularly the degree of protein solubilization from a given biological sample, can also limit the repertoire of proteins available for analysis [11,13–22]. Beyond this, protein isoform usage under different conditions (e.g., differentiation state, healthy vs. disease states and other variables) can also have profound physiologic consequences [1], and must be suitably resolved if a given proteomics analysis is to be considered comprehensive. As summarised eloquently in the review by Rice and colleagues [23]: “Currently, there is no single analytical platform that can deliver proteome-wide coverage—the desired holistic, non-reductionist approach.” Until technological advances overcome these issues, it is crucial to bear these limitations in mind when contemplating proteomics studies.

The placenta is certainly not immune to any of the complications associated with proteomics analysis. How then are we to obtain meaningful and robust proteomics data from the placenta? Rather than attempting to solve the entire placental proteome *en masse*, two prevailing approaches have been adopted to simplify the system to be analysed. In the first, sometimes referred to as parallel profiling, a comparative analysis is conducted between two

(or among several) treatment groups or clinical cohorts. Using a common fractionation strategy (typically, 2-DGE), candidate proteins can be targeted for identification based upon criteria such as differential expression. While throughput is emphasised over comprehensiveness, this method presents an effective and practical means to parse large protein data sets into formats that provide meaningful information regarding clinical conditions (e.g., novel therapeutic targets, or biomarkers to serve as diagnostic and/ or prognostic indicators of disease). A second strategy for simplification is to focus on a defined sub-proteome [24]. For example, proteomic analysis of specific sub-cellular compartments or multi-protein complexes, once isolated in enriched form, could be conducted [25–27]. Alternatively, one could choose to analyse proteins readily solubilized with a well-defined detergent-buffer system, such as in the analysis of lipid raft components [28,29]. Here, as opposed to the comparative approach, the intent is usually to define the normal subproteome in its entirety, for which a combination of complementary methods for protein fractionation and identification may be employed. Examples of both strategies to study placental proteomes will be discussed in the following sections.

Placental proteomics is in its infancy since relatively few studies have been published to date. When *placenta* and *proteomics* were combined and used as the search terms to query the PubMed database at the National Institutes of Health in September, 2007 only 29 ‘hits’ were found, representing a combination of human and animal studies. Other proteomics studies related to pregnancy are found using other search terms; for example, proteomics studies of vaginal fluids [30,31] and amniotic fluid [32–34] have been presented. The emerging role for proteomics analysis in pregnancy research has been reviewed [35]. Progress made in the use of proteomics methodologies in the study of pre-eclampsia has been reviewed recently [36], and Rice and colleagues [23] have reviewed translational proteomics as applied to reproductive biology. In the present review we will discuss some of the studies found in our database search to illustrate strengths and weaknesses of the various approaches that have been employed to obtain proteomics data from placenta and related cells or cell lines. We purposefully focus this review on proteomics studies of human placenta, placenta-derived cells, cell lines related to placenta, and sub-cellular compartments derived from these cells or tissues.

2. Comparative studies of placenta and placental-derived cells

In an early study of its type, Hoang and colleagues used comparative proteomics to examine the effects of hypoxia on cultured cytotrophoblasts from first-trimester placentas [37]. This *in vitro* model was chosen to mimic a number of the phenotypical characteristics of trophoblasts that can occur during pregnancy associated with reduced placental perfusion, such as pre-eclampsia. These investigators employed 2-DGE and matrix assisted laser desorption ionization—time of flight mass spectrometry (MALDI–eTOF MS) for their analysis. They detected ~250 protein spots in the stained 2-DGE preparations, 43 of which were identified by MALDI–eTOF MS. Somewhat surprisingly, the intensity of only 6 spots changed in abundance by two-fold or greater when the cells were cultured under reduced oxygen tension. While this was a well-executed study inasmuch as the experiments were conducted four times to assess interassay variation, these results illustrate certain limitations of this approach. In particular, these results highlight the detection limits of 2-DGE

preparations, given that the 250 protein spots observed is a clear underestimate of the full protein repertoire of cytotrophoblasts (or any eukaryotic cell type for that matter). Indeed, a proteomics analysis of nucleoli isolated from human cells identified >250 proteins in that sub-cellular structure alone when a tandem mass spectrometry (MS/MS) analysis was applied to a complex mixture of peptides derived from protease digests of nucleoli [38]. The pros and cons of the 2-DGE approach have been discussed by a number of investigators [10,14,20,39], and will not be considered in this review. Nevertheless, it is worth noting the composition of the lysis buffer system used to extract the cytotrophoblasts [37]. They employed a commonly used lysis system (containing urea, thiourea, and CHAPS detergent) that, while compatible with the isoelectric focusing strips used in the first dimension of 2-DGE preparations, does not solubilize all cellular proteins. For example, integral membrane proteins are poorly solubilized by this lysis system [40]. Accordingly, none of the 43 proteins identified by Hoang and co-workers [37] were membrane proteins. Several studies have been devoted to devising lysis buffers that improve the extraction of membrane proteins and are also compatible with 2-DGE [13,16,17,22]. However, it has not been clearly established that any lysis buffer system compatible with 2-DGE yields a complete representation of all integral membrane proteins.

In another *in vitro* comparative study, Sawicki and colleagues [41] examined the effects of neurokinin B, a tachykinin family member implicated in the systemic manifestations of pre-eclampsia [42], on placental cells. For this study, primary cultures of term cytotrophoblast cells were utilised [41]. Using 2-DGE and LC/MS/MS, these authors found that in neurokinin B-treated cytotrophoblasts, 20 proteins displayed significant decreases in expression compared to control cells [41]. Notably, four of these proteins were involved in antioxidant defence pathways, while two inhibited intravascular anticoagulation. Interestingly, some of the targets of neurokinin B suppression were also found in the analysis of Hoang et al. [37] to be regulated in response to hypoxia.

In an analysis of clinical samples, Sun et al. [43] examined protein expression patterns in placental trophoblasts isolated from normotensive and pre-eclamptic pregnancies. Using 2-DGE, 34 proteins were determined to be differentially expressed between the two conditions. Seven of these proteins were subsequently identified by MALDI-TOF-MS. Three of these proteins displayed reduced expression (peroxiredoxin 2, disulfide isomerase ER-60, and Delta 3,5-Delta 2,4-dienoyl-CoA isomerase) while four proteins were up-regulated (TIM21-like protein, dihydrolipoyl dehydrogenase, endoplasmic reticulum resident protein, and protein disulfide isomerase precursor) [42].

Considering the more complex scenario of whole tissues, Mine and colleagues compared placentas derived from normal and pre-eclamptic patients using 2-DGE and MS [44]. High-resolution 2-DGE maps were obtained using a series of narrow pH ranges for isoelectric focusing to improve spot resolution. This approach, which requires more separate gel runs to cover the pH spectrum than using broad pH range isoelectric focusing, provides for greater coverage of the proteome. These authors also found a larger number of spots detected when 8M guanidine hydrochloride was substituted for 8M urea as a denaturing agent (450 spots vs. 314). This increased resolution confounded their attempts to detect differences in the proteomic maps using comparative techniques. To overcome this problem, these

investigators employed 2-dimensional immunoblotting to screen for circulating antibodies in the sera of pre-eclamptic women. From this analysis, antibodies to dynactin p-50 were identified which, in a retrospective analysis, were found to be elevated in pre-eclamptic patients and levels correlated positively with disease severity.

Using a slightly different approach, Webster and colleagues compared normal and pre-eclamptic placentas using immunoblots probed with an anti-nitrotyrosine antibody [45]. In this study, it was demonstrated that pre-eclamptic placentas showed increased protein nitration [45]. Post-translational modifications for one of the nitrated proteins, mitogen-activated protein kinase (MAPK), were analysed by MS. MAPK peptides whose mass matched theoretical peptides modified by phosphorylation and nitration were identified. This study illustrates the potential for using a proteomics approach to study a specific protein and its posttranslational modifications.

In another study that considered both complex tissues and clinical context, Liu et al. [46] compared placental villous tissue from indicated terminations and spontaneous early pregnancy loss by 2-DGE and MALDI-TOF MS. The gels from early pregnancy loss tissue contained 1735 ± 121 protein spots while the control group contained 1552 ± 153 spots. Changes in apparent concentration were noted in 30 spots, among which 13 decreased and 5 increased. A total of 12 of these spots were subsequently identified by MALDI-TOF MS. The discrepancy in the total spot numbers in the two conditions was not addressed. Three principal antioxidant enzymes (copper/zinc-superoxide dismutase, peroxiredoxin 3, and thioredoxin-like 1 protein) were down-regulated in the early pregnancy loss cases, suggesting that depletion of placental antioxidant defences may contribute to this common pregnancy complication. In addition, S100 calcium binding protein, galectin-1, chorionic somatomammotropin hormone 1, transthyretin, fas inhibitory molecule, eukaryotic translation elongation factor, RNA-binding protein, ubiquitin-conjugating enzyme E2N, and proteasome beta-subunit exhibited differential expression.

A comparative proteomics study of similar complexity was conducted using tissues from term and preterm deliveries [47]. In this case, the placentas were dissected to include both fetal and maternal membranes. This study combined high-resolution 2-DGE and MS/MS to search for differences between the two types of placenta in which only those proteins missing from one or the other condition were identified. Eleven proteins, absent from either term or preterm placentas, were identified. Fifty other proteins were found to vary significantly between term and preterm placenta but these proteins were not identified.

3. Comparative studies of placental surrogates

Cultured cell lines are often more convenient to work with than cells derived from the placenta proper. In addition to convenience, it is easier to obtain large quantities of these cultured cells than to obtain equivalent numbers of primary cells from the placenta. The latter point may be crucial when proteins of low abundance are of interest. Cells routinely used as surrogates for trophoblasts are the BeWo, JEG-3, and JAR choriocarcinoma cell lines.

To study cellular responses to supra-physiological oxygen tension, Vorum and colleagues [48] performed a proteomics analysis (2-DGE and MALDI-TOF MS) using JEG-3 cells. Comparison of 2-DGE maps from control and hyperoxic cultures revealed 101 protein spots common to all gels. While visual inspection of the figures in this paper reveals that the gels contain far more than 101 protein spots, presumably these other proteins were not consistently detected from gel-to-gel. In response to high oxygen tension, 13 of the 101 proteins were down-regulated. MALDI-TOF MS analysis of tryptic digests of those 13 protein spots resulted in the identification of nine different proteins, including those involved in oxidant defence (peroxiredoxin 1 and neuropolypeptide h3), glycolysis (glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate mutase), and cell structure/motility (villin 2, tubulin β , and profilin I). Notably, a number of the oxygen-responsive proteins identified in this study were independently found in other systems to be hypoxia-inducible (glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, and poly(rC)-binding protein 1).

Cell-cell fusion to form syncytia is a key event in placental biology. BeWo cells, which can be induced to fuse in culture, have been employed in a number of studies to investigate various aspects of syncytia formation. Hu and colleagues [49] conducted a proteomics analysis of the hypoxia-induced response in syncytia formation in BeWo cells. They reported that hypoxia inhibits syncytialization and that 20 proteins are either up-regulated or down-regulated as determined by 2-DGE; those proteins were subsequently identified by MS analysis. These hypoxia-responsive proteins included antioxidants (peroxiredoxin 1, peroxiredoxin 2 and peroxiredoxin 6), glycolysis pathway proteins (malate dehydrogenase and enolase), cytoskeletal components (keratin 1 and β -actin), annexins (annexin A2 and annexin A5), galectin-3, 14-3-3 tau, and several molecular chaperones.

In a second study of syncytialization, Nampoothiri et al. [50] performed proteomics profiling of BeWo cells grown in the presence or absence of forskolin. In 2-DGE preparations, 48 protein spots were observed in control cells and 63 spots in forskolin-treated cells. From the latter set, four protein spots were further analysed with MALDI-TOF-MS. These spots were identified as retinoblastoma susceptibility protein 1, inte-grase interactor protein 1, and phosphorylated and dephosphorylated forms of ras p21 [50]. While these results are interesting, the low number of protein spots indicates under-sampling of the BeWo proteome; in the study of Hu et al. [49], >600 protein spots were resolved by 2-DGE. It is also noteworthy that plasma membrane proteins, which might be expected to change dramatically during syncytialization, were under-represented in both this [50] and the prior [49] study, likely the result of the cell lysis buffer systems employed (see previous discussion of this issue).

An example of a proteomics-type study that did not rely on 2-DGE or MS analysis is the work of Ishioka and colleagues [51]. Using JAR cells to study protein alterations related to hypoxia, these investigators monitored a selected set of 40 apoptosis-related proteins by immunoblotting methods with the so-called Powerblot system. This approach has the advantage of monitoring a circumscribed set of proteins of interest; this same feature also sets the limits of this method, since only those selected proteins are monitored for change.

4. Studies of placental sub-proteomes

The studies discussed in the previous sections, whether with placental tissue or cultured cells, were comparative in nature. Comparisons were made between normal tissue and tissue derived from diseased cases, or between treated cells and controls. This focus is to be expected; many investigators are interested in finding clear cut differences in protein expression patterns, because such information may be of clinical significance. However, a common limitation in all of these parallel profiling studies is the inherent, if not requisite, bias attendant in each simplification strategy. While both practical and insightful, these early studies are limited in scope, focusing on selective differences in protein expression patterns (or protein modifications) within complex samples. This is less a critique of study design than an admonition of technological restrictions: As current methodologies are insufficiently developed to enable global protein profiling, much silent (but potentially valuable) information goes undetected, either the result of physico-chemical properties (e.g., insolubility in a defined buffer), falling below the detection limits of a given platform, or failing to meet an arbitrary threshold for significance based on differences in spot volume. As such, it remains essential that comparative profiling studies be interpreted in light of inherent confines of a given biological system and proteomics platform.

Since the entire protein repertoire of the human placenta is not presently known, detailed analyses of protein expression in healthy placentas remains indispensable. However, the inherent complexity of placental tissue makes it currently impractical to define the proteome of the tissue in its entirety. As stated previously, a major strategy used to address this problem is to focus on a defined sub-proteome [24]. Using such a simplified system, a variety of complementary techniques for solubilization, fractionation, and identification can be used to take comprehensive inventory of the full range of proteins therein.

Using such an approach, Paradela and colleagues examined the protein composition of a vesicular fraction enriched in placental alkaline phosphatase (PLAP), termed microvillous membranes (MVM) [52]. A total of 57 proteins were identified in this fraction from the assignment of 166 different peptides. Plasma membrane proteins accounted for 23% of the total, while 56% were cytoplasmic proteins. The number of proteins identified in this study seems low, and likely represents a significant underestimate of the MVM proteome (see below). In addition, the data presented by Paradela and colleagues [52] show that 38 of the 57 proteins (66%) were identified on the basis of a single peptide. It has been noted that protein identification based on a single peptide may be problematic [53,54]. Ideally independent methods should be used to validate such observations.

Inasmuch as the mitochondrion plays a pivotal role in oxidative metabolism, attempting to define the proteome of the human mitochondrion is a laudable goal. Rabilloud and colleagues have used placental mitochondria as the reference standard to define the mitochondrial proteome [55,56]. Placenta was their choice of human tissue since large amounts of mitochondria can be isolated from this source and tissue samples are generally available. While this analysis is ongoing, much progress has been made. The first of these studies identified a number of proteins, but none of them were membrane proteins. This was due to difficulties in identifying membrane proteins by 2-DGE using conventional sample

preparation techniques as previously noted. Improvements in sample preparation were evident since 10 membrane proteins were identified in their second paper in this series. There is cause for optimism that additional mitochondrial membrane proteins will be identified with 2-DGE and MS through continued advances in technology. A total of 86 mitochondrial proteins were identified in this 2-DGE-based mitochondrial proteome analysis of the 311 known mitochondrial proteins in the SWISS-PROT database at that time. Studies reporting on aspects of proteomics in the human placenta and related cells are summarised in Table 1.

5. Placental proteomics: a 'shortcut' to biological insight

A proteomics study can, in some cases, be likened to a fishing trip. Just as in a fishing trip where one sometimes catches fish, even big ones, discovery-based science like proteomics or transcriptomics (as in the use of DNA-microarray technology to monitor mRNA expression) can also have big rewards. Ideally, this discovery-based approach can lead to new findings that enable hypothesis-based research that would not have occurred in the absence of such data. A cogent comparison of discovery-based and hypothesis-based research, and their complementary roles in knowledge creation has been presented by Kell and Oliver [57]. To illustrate these complementary roles, in this section, we describe our experiences with proteomics that have led us to explore research questions that, until recently, we had never thought to ask.

The placenta is unusual in its reliance on a syncytial structure, the syncytiotrophoblast (STB), as the key element involved in the movement of metabolites, gases, and waste products to and from the developing fetus. The apical plasma membrane of the STB is the initial site where transport across the placenta occurs. As important as this plasma membrane is, it is not well understood at the molecular level. Several groups have developed methods for isolating membrane vesicle preparations enriched in markers for the STB apical plasma membrane, with an average enrichment of ~20-fold (range 14–37 fold) in 13 different studies [58]. However, the proteins of the STB apical plasma membrane are generally both low in abundance and resistant to solubilization, rendering this subproteome especially difficult to resolve by standard 2-DGE [40]. To define the *bona fide* sub-proteome of the STB apical plasma membrane, we reasoned that 20-fold enrichment may be inadequate for high-quality proteomics; thus, we developed an alternative method to generate more highly enriched preparations of this membrane.

Our approach was adapted from the procedure developed by Jacobson and colleagues [59,60]. Using cationic colloidal silica (CCS) particles that bind selectively cell surfaces through electrostatic interactions, we sought to alter the density of the STB apical plasma membrane so as to facilitate purification through homogenization and centrifugation procedures. In preliminary studies, we found that conventional CCS particles bound only to the tips of STB microvilli (MV), failing to coat the lateral or basal MV surfaces (Fig. 1 A, B). An alternative type of CCS, Ludox CL, was found to coat the entire apical surface of the STB (Fig. 1 C, D). Electron microscopic examination of the conventional CCS and the Ludox CL showed that the CCS particles were, on average, 3 times larger than the Ludox CL (hereinafter referred to as small CCS (smCCS)) (Robinson et al., submitted). Following

homogenization and centrifugation, the microvillous morphology of these isolated segments was largely retained (Fig. 2). Thus the smCCS STB fractions were very different from MVM fractions isolated by others [52], since they do not form vesicle-like structures and retain their resemblance to MV. By comparing the levels of PLAP in the isolated smCCS-coated fraction and the starting homogenate, we estimated that enrichment consistently ranged between 200- to 400-fold (Robinson et al., submitted), well in excess of the ~20-fold reported by others with vesicles isolated from the STB [52,58]. Using a combination of 2-DGE and immunoblotting we found that cytoskeletal proteins (i.e., ezrin, moesin, and actin) were enriched in the smCCS-coated fraction, while histones were dramatically depleted (Robinson et al., submitted). This fraction was highly enriched for cytoskeletal proteins characteristic of MV [61,62] as well as the plasma membrane marker, PLAP at this point in the procedure.

To further purify this fraction for proteomics analysis, we sought to deplete non-membrane proteins since, as noted earlier, plasma membrane proteins are typically low-abundance proteins [17,18,40]. The MV fraction was treated in a sequential manner with solutions of increasing stringency to disrupt protein-protein interactions: (a) low salt; (b) high salt; (c) high pH; (d) urea; and finally (e) boiling SDS (Robinson et al., submitted). The last step was intended to solubilize the remaining membrane proteins. It should be noted that because the smCCS remained associated with the membranes through all of the extraction steps, the samples could be quickly pelleted by centrifugation following each extraction step in a countertop microcentrifuge. We found that each treatment removed a relatively unique set of proteins as determined by 1-DGE and 2-DGE. Careful accounting of the protein concentrations following sequential extraction showed that the final pellet was depleted of an average of 80% of the proteins, while PLAP was essentially quantitatively recovered following the final extraction step. This further 5-fold enrichment, when coupled with the 200- to 400-fold enrichment of PLAP in MV fraction, represents a final enrichment of ~1000- to ~2000-fold for PLAP. To the best of our knowledge this represents the most highly enriched preparation of the apical plasma membrane of the STB yet achieved.

The sequentially extracted pellet of the smCCS-coated fraction was then used for proteomics analysis. The solubilized proteins were separated by 1-DGE; the gel lanes were subsequently sliced into 30 individual segments. The segments were subjected to in-gel trypsin digestion to generate peptides. By MS/MS, over 6000 unique peptides were detected and a large number of proteins (424) were positively identified with multiple unique peptides; a smaller number of proteins (100) identified by single peptides were also present in the proteomics dataset.

We will not discuss all of the proteins identified in the extracted STB fraction, but rather will focus on one of the proteins to illustrate the point that discovery-based proteomics analysis can have great value and lead to deeper biological understanding. Our proteomics analysis unambiguously identified the protein dysferlin [63], which had not been documented in the placenta heretofore. Prior studies of dysferlin focused on skeletal muscle where it is known to reside in the plasma membrane (sarcolemma) of muscle fibres [64]. Mutations in dysferlin have been associated with limb girdle muscular dystrophy type 2B and Miyoshi myopathy [65,66], and cellular studies have shown that dysferlin functions in

repair of the damaged sarcolemma [67,68]. More recently it has been reported that dysferlin plays a similar role in the heart and that dysferlin deficiency leads to cardiomyopathy in certain conditions [69]. Using immunochemical methods, we found that dysferlin in the placenta was concentrated in the apical plasma membrane of the STB, but not detected in CTBs *in situ* [63]. Immunofluorescence analysis of cultured primary CTBs showed that cells that had spontaneously fused to form syncytial structures expressed dysferlin while adjacent mononuclear CTBs did not [63]. We do not know if dysferlin functions in syncytial formation; however, we hypothesise that dysferlin functions in repair of the apical plasma membrane of the STB. There is likely a continuous need for repair of this STB membrane because large numbers of syncytial knots are shed throughout pregnancy [70,71]. The mechanisms for the shedding of syncytial knots and for repair of the plasma membrane following the shedding event are not known. Identification of dysferlin in the human placenta using discovery-based proteomics has nevertheless permitted us to begin addressing this novel hypothesis.

6. Conclusions

Placental proteomics research is in its early stages, but offers great promise. Establishment of a comprehensive proteomics database for the placenta will be instrumental in allowing detailed comparisons between normal placentas and those derived from disease states. In future studies, it will be important to move beyond protein identification to the analysis of isoform usage and more quantitative analysis of proteins in normal and diseased conditions. Although we now know that proteomics analysis is far more difficult than was envisioned in the early days of this technology (owing, first and foremost, to the great complexity of the eukaryotic proteome), technologies important for proteomics are continuously evolving, and there is reason to hope that these current limitations will ultimately yield to new research.

Current limitations notwithstanding, discovery-based proteomics can serve as a ‘shortcut’ to the elucidation of biological problems. While this discovery-based approach may not appeal to all scientists, it may nonetheless provide important insights not obtained using more focused methods, thereby enabling hypothesis-based research that would not have occurred in the absence of such data. Our work on the proteomics-based discovery of dysferlin expression in the human placenta illustrates this point: This discovery and subsequent biochemical and cell biological studies of dysferlin in the placenta and CTBs have provided insight into its potential role in placental biology [63]. Indeed this work now positions us to generate testable hypotheses concerning the function of dysferlin in the human placenta. The complementary roles of discovery-based and hypothesis-based approaches to biomedical research in the creation of new knowledge/information should be appreciated. A knowledge reservoir, consisting of both information and understanding, concerning a given topic such as the placenta, can lead to hypotheses that are tested by acquisition of data that can lead to the acceptance or rejection of the hypotheses and in so doing create new knowledge, primarily in the form of understanding. A complementary process is the acquisition of new data through discovery-based processes (e.g., proteomics) leading to new knowledge, primarily in the form of information, thus enhancing the knowledge base. Both infusion of new information and understanding in turn leads to additional testing of hypotheses.

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Fig. 1.

Coating the apical plasma membrane of the STB with cationic colloidal silica—analysis by electron microscopy. (A) Survey electron micrograph of placental tissue incubated with conventional CCS particles. (B) Higher magnification image of the region in the rectangle in panel A showing that the heterogeneously-sized CCS particles concentrated at the tips of the MV and not being bound to the lateral sides of the MV (arrows) or the planar basal portion of the apical plasma membrane (arrowheads). (C) Survey electron micrograph of placental tissue incubated with smCCS particles. (D) Higher magnification image of the region in the rectangle in panel C showing the small smCCS particles completely coating the tips of the MVs as well as the lateral sides of the MV (arrows) and the planar basal portion of the apical plasma membrane (arrowheads). Bars = 2 μ m.

Fig. 2.

Isolation of microvilli from the STB—analysis by electron microscopy. (A) Survey electron micrograph of the isolated STB MV coated with smCCS. Note the microvillous appearance of the smCCS-coated membranes (arrows). A relatively small number of uncoated membrane vesicles are also present (arrowheads). (B) Higher magnification image of the region in the rectangle in panel A. The extracellular face of the apical plasma membrane of the STB is coated with smCCS particles (arrows). While most of the

membrane surface is coated with smCCS a low level of contaminating uncoated membranes are present (arrowheads). Bars = 1 μ m.

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Table 1

Summary of human placenta-related proteomics studies reviewed

Cell or tissue	Condition	Methods of analysis	Ref.
Placenta	Pre-eclampsia	2-DGE and MS	[44]
Placenta	Pre-eclampsia	Immunoblot and MS	[45]
Placenta	Early pregnancy loss	2-DGE and MALDI-TOF MS	[46]
Placenta	Preterm labor	2-DGE and MALDI-TOF MS	[47]
Placenta	Normal (microvilli)	LC/MS/MS	[52]
Placenta	Normal (mitochondria)	2-DGE and MS	[55,56]
Cytotrophoblast	Hypoxia	2-DGE and MALDI-TOF MS	[37]
Cytotrophoblast	Neurokinin B-treated	2-DGE and LC/MS/MS	[41]
Cytotrophoblast	Pre-eclampsia	2-DGE MALDI-TOF MS	[43]
BeWo cells	Hypoxia	2-DGE and MS	[49]
BeWo cells	Forskolin treated	2-DGE and MALDI-TOF MS	[50]
JEG-3 cells	Hyperoxia	2-DGE and MALDI-TOF MS	[48]
JAR cells	Hypoxia	Immunoblot	[51]