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Mass spectrometry based proteomic analysis of human stem cells: a brief review

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Abbreviations: 2-DE, two-dimensional electrophoresis; BM, bone marrow; EGF, embryonic growth factor; FGF, fibroblast growth factor; hESCs, human embryonic stem cells; hMSCs, human mesenchymal stem cells; hNSCs, human neuronal stem cells; ICAT, isotope-coded affinity tag; iTRAQ, isobaric tags for relative and absolute quantification; LC/MS/MS, liquid chromatography tandem mass spectrometry; MALDI-TOF, matrix assisted laser desorption/ ionization time-of-flight; MPCs, mesenchymal progenitor cells; MSCs, mesenchymal stem cells; PCNA, proliferating cell nuclear antigen; PIP2, phosphatidylinositol bisphosphate; PMF, peptide mass fingerprinting; pRB, retinoblastoma protein; PRDX 1, peroxiredoxin 1; PRDX 4, peroxiredoxin 4; PTM, posttranslational modification; SELDI-TOF, surface enhanced laser desorption/ ionization time-of-flight; SILAC, stable isotope labeling by amino acids in cell culture; SPITC, 4-sulfophenyl isothiocyanate; TAGL2, transgelin-2; TCTP, translationally controlled tumor protein; UCB, umbilical cord blood

Abstract

Stem cells can give rise to various cell types and are capable of regenerating themselves over multiple cell divisions. Pluripotency and self-renewal potential of stem cells have drawn vast interest from different disciplines, with studies on the molecular properties of stem cells being one example. Current investigations on the molecular basis of stem cells pluripotency and self-renewal entail traditional techniques from chemistry and molecular biology. In this mini review, we discuss progress in stem cell research that employs proteomics approaches. Specifically, we focus on studies on human stem cells from proteomics perspective. To our best knowledge, only the following types of human stem cells have been examined via proteomics analysis: human neuronal stem cells, human mesenchymal stem cells, and human embryonic stem cells. Protein expression serves as biomarkers of stem cells and identification and expression level of such biomarkers are usually determined using two-dimensional electrophoresis coupled mass spectrometry or non-gel based mass spectrometry.

Keywords: adipocytes; electrophoresis, gel, two-dimensional; embryonic stem cells; human neuronal stem cells; mass spectrometry; mesenchymal stem cells; proteomics

Introduction

Stem cells have various unique characteristics; they can divide and renew themselves over many generations and have multi-potentiality. Differentiation and maturation of stem cells comprises complex serial events, which guide the undifferentiated cells to different lineages via distinctive developmental programs (Chung *et al.,* 2005; Hoffrogge *et al.,* 2006; Kwak *et al.,* 2006). Such regulatory changes can be studied systematically with transgenic technology and microarrays (Pazman *et al.,* 2000; Böttcher *et al.,* 2003; Gurok *et al.,* 2004; Pahnke *et al.,* 2004). The process of differentiation entails changes in types and amount of proteins expressed by the stem cell. Therefore, protein expression pattern can provide important clues about the progression differentiation process for stem cells at various stages.

Proteomics, the large-scale study of proteins, holds great promise in unraveling the molecular basis of stem cell differentiation. Proteomics has emerged as a robust method for comparing protein expression under different conditions that supplements high-throughput gene expression analysis at the RNA level (Baharvand *et al.,* 2006b). Proteomics approaches have been applied to create the molecular map of protein expression in stem cells. Proteome maps, or profiling of proteomes, have greatly contributed to the current body of knowledge on the biomarkers of stem cells. Functional proteomic analysis at a certain biological system

requires global quantification of all the protein machinery (Ma *et al.,* 2007).

Early efforts on stem cell proteomics heavily relied on two-dimensional electrophoresis (2-DE) and MS analysis for protein identification. Nowadays, several new techniques empowered with both qualitative and quantitative aspects are available: chemical isotope tagging techniques to isotope-coded affinity tag (ICAT), isobaric tags for relative and absolute quantification (iTRAQ), and 4-sulfophenyl isothiocyanate (SPITC) (Gygi *et al.,* 1999; Lee *et al.,* 2004; Ross *et al.,* 2004). In addition, all-around enzymatic isotope labeling techniques have also been applied, such as stable isotope labeling by amino acids in cell culture (SILAC) for *in vivo* labeling (Ong *et al.,* 2002), and enzymatic labeling of ^{18}O to C-terminal of peptides by proteases in the presence of ^{18}O water ($H_2{}^{18}O$) (Stewart *et al.,* 2001).

Issues such as loss of 3-D structure information upon linearization of proteins, incomplete post-translational modification mapping, and concentration dynamics problem due to lack of effective analyte enrichment technology (Aebersold and Cravatt, 2002; Hood, 2003) pose challenges to proteomics. Proteomics analysis should overcome the hurdles ahead in order to clear itself of the charge of being the bottleneck for systems-level analysis of biological information hierarchy. Nevertheless, to date current state-of-art proteomics approaches provide powerful tools for investigating the molecular basis of stem cell differentiation.

Human neuronal stem cells

Human neural stem cells (hNSCs) are self-renewing and multipotent precursors that can lead to neuronal and glial progenitor cell, which is in order to differentiate into neurons and astrocytes or oligodendrocytes, respectively (Ma *et al.,* 2007). The first proteomics studies based on 2-DE gel methodology have been reported in 1999, where Pearce and Svendsen (1999) showed first differential 2-DE gel approach to fetal human brain cells treated with both embryonic growth factor (EGF) and fibroblast growth factor (FGF). After Pearce and Svendsen's pioneering work, proteomic profiling has mainly been applied for compiling protein inventory in the adult nervous system of normal versus diseased individuals (Rohlff, 2000, 2001; Husi and Grant, 2001; Lubec *et al.,* 2003; Choudhary and Grant, 2004; Kim *et al.,* 2004; Taylor *et al.,* 2004). However, protein analysis of human fetal neuronal stem cell differentiation has not much progressed yet. Recently proteomics analysis on hNSC was carried out by Hoffrogge *et al.* (2006). hNSCs differentiated upon removal of growth factors from culture medium, evidenced by clear morphological changes. 500 μg of protein samples were loaded onto 2D SDS PAGE gels (25 cm × 22.5 cm × 0.5 cm) and stained with colloidal coomassie blue. After in-gel digestion peptide mixtures were analyzed by MALDI-TOF MS (Reflex III mass spectrometer, Bruker Daltonics) and MALDI-TOF MS/MS (4700 proteomics analyzer, Applied Biosystems) and protein identification searches were performed by MASCOT search engine (Matrix Science).

956 spots were excised from 2-DE gel slab and 412 spots were identified by MALDI-TOF MS. Specific functions of the identified proteins were inferred from the gene ontology database. Circa 21% of the proteins were related to protein synthesis, metabolism, processing and degradation; less than 11% were related to cytoskeleton proteins, stress response proteins, the functional group of RNA and other nucleic acids metabolisms and transport, signal transduction and others (Hoffrogge *et al.,* 2006).

According to Hoffrogge *et al.* (2006) peroxiredoxin 1 (PRDX 1) and transgelin-2 (TAGL2) were up-regulated and proliferating cell nuclear antigen (PCNA) and peroxiredoxin 4 (PRDX 4) were down-regulated in stem cells upon differentiation. PRDX 1 belongs to a family of anti-oxidative proteins and is known to be overexpressed when cells are exposed to proliferative signals or oxidative stress (Prosperi *et al.,* 1998). Transgelin-2, a protein of the calponin family, is the second protein whose expression level increased upon differentiation of hNSCs. Transgelin has direct interaction with actin filaments; saturation of transgelin-binding occurs at the transgelin/actin monomers ratio of 1:6, which causes rapid gelation of transgelin-actin complexes within min (Shapland *et al.,* 1993).

A down-regulated protein, PCNA is an autoantibody to a nuclear antigen in proliferating cells (Miyachi *et al.,* 2005) and correlates to the proliferative status of cells (Takasaki *et al.,* 1984). PRDX 4 is related with NF-κB activation. Crowly-Weber *et al.* (2002) has found earlier that NF-κB, PRDX 4 and Grp 78 are up-regulated in apoptosis resistant cells. Decreased PRDX4 levels in differentiated NSC may indicate that a pro-apoptotic situation is supported during differentiation.

Human mesenchymal stem cells

Human bone marrow stromal cell

Human bone marrow stromal cells, also known as human mesenchymal stem cells (hMSCs) or mesenchymal progenitor cells (MPCs), are the main element of the bone marrow microenvironment and support in promoting differentiation and proliferation of hematopoietic cells. hMSCs are characterized by their high plasticity, which is reflected in the manifold changes in transcription level and protein expression of these cells as they follow different lineages. Thus, proteome profiling of bone marrow stromal cells may provide important insights into the mechanism of normal and dysregulated hematopoiesis, as it was demonstrated in Seshi's work (2006). The proteome of hMPCs from normal versus leukemic individuals was analyzed using iTRAQ technology and 2D LC/MS/MS. 400 μg of protein sample was iTRAQ labeled for quantification and analyzed using 2D LC/MS/MS (2D LC; LC Packing, MS/MS; QStar Pulsar i, Applied Biosystems). For protein identification and quantification Pro Quant software (Applied Biosystems MDS SCIEX) was used. 73 out of approximately 900 proteins were differentially expressed in leukemic stromal cells compared with hMPCs.

Human mesenchymal stem cells derived from umbilical cord blood

While mesenchymal stem cells (MSCs) possess the desirable characteristics of stem cells, they are easy to obtain and culture, making these cells attractive candidates for tissue engineering approaches in mesenchymal tissue regeneration (Feldmann *et al.,* 2005). Their main resource is the bone marrow (BM). BM-MSCs exhibit multipotent differentiation potential and can induce mesodermal originated tissue. However, several complications arise when MSCs are obtained from the bone marrow: the invasive collection procedure, high chance of viral exposure (Amos and Gordon, 1995), sub-optimal proliferation rate *in vitro* condition, and limited differentiation capacity over multiple passages in tissue culture (Mueller and Glowacki, 2001; Stenderup *et al.,* 2003). Human umbilical cord blood (UCB) is an alternative source of hMSCs that is relatively free of such disadvantages.

Feldmann *et al.* (2005) compared the difference between BM-MSCs and UCB-MSCs. Immunological phenotype determined by surface proteins of UCB-MSCs was analyzed using FACS flow cytometry. The results confirmed that BM-MSCs and UCB-MSCs displayed same phenotype. As a further investigation on UCB-MSCs, cytosolic protein expression was analyzed via 2-DE. 500 μg of protein samples were loaded onto 2D SDS PAGE gels (20 cm × 18 cm × 0.4 cm) and visualized by silver staining. After automated spot excision by Flexys (Genomic Solutions) and in-gel digestion peptide mixtures were analyzed by MALDI-TOF/TOF MS (Ultraflex TOF/TOF, Bruker Daltonics) and MASCOT query (Matrix Science) was used for protein identification.

As a result, 2037 spots were excised from 2-DE gel slab and 205 spots were identified by MALDI-TOF-MS. Cellular functions of the resolved proteins were identified using the gene ontology database. The major group consisted of proteins that belong to cellular metabolism. The metabolic protein group encompassed numerous biochemical pathways such as Krebs cycle, amino acid metabolism, cellular housekeeping, and protein biosynthesis. Feldmann *et al.* (2005) identified the presence of protein vimentin in differentiating stem cells, which is normally absent in undifferentiated forms. Vimentin is the most omnipresent intermediate filament protein and is one of the first proteins to be expressed during the differentiation of the cell. Gelsolin was another one to be identified in differentiating MSCs. Gelsolin is an actin binding protein and displays biphasic responses. Under low calcium concentrations, gelsolin induces nucleation actin polymerization; however, gelsolin causes severing of actin filaments at high calcium concentrations. Gelsolin also binds phosphatidylinositol bisphosphate (PIP_2), a membrane-bound molecule, thereby functioning as an anchor to actin organization and signal transduction. Gelsolin expression is down-regulated as stem cell is under differentiation (Wang *et al.,* 2004). Such down-regulation may also affect cell shape and migratory capacity, and potentially the homing of stem cells (Evans *et al.,* 2004). The protein prohibitin has been known for key functions in progenitor cells; two prohibitin proteins, Phb1p and Phb2p (BAP37), are involved in various functions such as cell cycle regulation, mitosis and proliferation, apoptosis, assembly of mitochondrial respiratory chain enzymes, and aging. Since prohibitins participate in G1/S phase regulation in cell cycle and interact with the retinoblastoma protein (pRB), prohibitins are considered as latent tumor suppressors (Feldmann *et al.,* 2005).

Adipocyte differentiated from human mesenchymal stem cells

Adipocytes are cells from connective tissues that

have been characterized in the synthesis and storage of fat. Adipocytes serve important roles in mammalian physiology: maintenance of proper energy balance, storage of energy in lipid forms, mobilization of energy sources in response to hormonal stimulation, and signal transduction (Camp *et al.,* 2002). Better information regarding the molecular events in innate adipocyte differentiation will obviously enhance the treatment of adipocyte-related metabolic diseases such as obesity and diabetes (Nuttall and Gimble, 2000; Sekiya *et al.,* 2004).

Lee *et al.* (2006) have investigated the protein expression inherent to adipogenic differentiation using 2-DE, MALDI-TOF, and RT-PCR. For the 2-DE and MALDI-TOF, 90 μg of protein samples were loaded onto 2D SDS PAGE gels (26 cm $\times$ 20 cm) and visualized with silver staining. After in-gel digestion and desalting with Ziptip C18 (Millipore Corp.), peptide mixtures were analyzed by MALDI-TOF MS (Voyager DESTR mass spectrometer, Applied Biosystems), and protein identification searches were performed by Ms-Fit (http://prospector.ucsf.edu). The study focused on differential proteome expression in hMSCs versus adipocytes. Lee *et al.* (2006) performed 2-DE analysis for the cytosolic proteins extracted from both hMSCs and adipocytes differentiated from hMSC. Thirty-two protein spots showed differential proteomic results. Eight proteins were confirmed to be up-regulated by MALDI-TOF/MS: sintaxin binding protein 3, OSBP-related 3, phosphodiesterase, glycophorin, immunoglobulin kappa chain variable region, peroxisome proliferative activated receptor gamma, bA528A10.3.1, and T cell receptor V-β 4. Four proteins (syntaxin-3, OSBP-related protein 3, PPAR-γ and glycophorin) were shown to be related with adipogenesis. The differentially expressed proteins between hMSCs and adipocytes might be considered as cytosolic biomarker proteins for adipogenesis (Lee *et al.,* 2006).

Human embryonic stem cells

Human embryonic stem cells (hESC) consist of a population of undifferentiated pluripotent cells with both self-renewal and multi-lineage differentiation characteristics (Baharvand *et al.,* 2006b). Hayman and Przyborski (2004) reported surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) technology to explore potential biomarkers of human embryonal carcinoma (EC) stem cells. They identified prominent biomarkers that showed unique expression pattern in pre-versus post-differentiation human EC cells. Markers that were constitutively expressed in both populations were also investigated.

Also, Baharvand *et al.* (2006b) have applied 2-DE and MALDI TOF-TOF MS/MS (4700 Proteomics Analyzer, Applied Biosystems) to profile global map of proteome expressed in hESC. Cell line samples were analyzed in triplicate using 2-DE and 1,250 $\pm$ 150 spots were detected. For MS analysis, 120 μg of protein samples were loaded onto 2D SDS PAGE gels (24 cm strips) and stained with colloidal CBB G 250. 844 spots were collected for mass spectrometry based proteomic analysis and 685 proteins were identified by combining peptide mass fingerprinting (PMF) and tandem mass spectral data. Out of 685 proteins 434 proteins corresponded to unique proteins and 251 proteins corresponded to isoforms or post-translational modification (PTM). There are other reports that have also profiled plenty of isoforms or PTM (Maurer *et al.,* 2003; Elliott *et al.,* 2004). Careful analysis on all isoforms of a given protein is very important because many of the interesting regulatory steps, especially those involved in cell proliferation, migration and differentiation, count on protein PTM more than they do on protein expression levels (Levchenko, 2005). Baharvand *et al.* (2006b) identified a large number of proteins involved in protein synthesis, processing, and trafficking. Such finding suggests that hESCs are able to maintain the undifferentiated state until signals of lineage determination is received, upon which these cells quickly change phenotype and produce necessary proteins. Seven out of sixty proteins were assigned as up-regulated genes in transcriptomic analysis of hESC: cofilin1, DJ-1 protein, translationally controlled tumorprotein (TCTP), enolase 1, heat shock congnate 71-kDa protein, latate dehyrogenase B, and necleophosmin 1 (Baharvand *et al.,* 2006b). A large number of proteins, particularly the highly abundant ones, were identified as chaperones, heat shock proteins, ubiquitin/proteasome, and oxidative stress responsive proteins; expression of such proteins at high level implies the ability of hESCs to resist oxidative stress and increase life span. Several proteins involved in cell proliferation and differentiation belonged to the group of up-regulated proteins. Baharvand *et al.* (2006b) further identified 30 proteins among 1010 mESC- and stemness-specific proteins identified by Nagano *et al.* (2005). Baharvand *et al.* (2006a) have successfully identified the following proteins that may be able to serve as SC-specific biomarkers: hepatoma-derived growth factor, guanine nucleotide-binding protein beta, and CRABP1. However, the authors were unable to detect the products of many

transcripts that are characteristic to hESC, due to technical limitation of 2-D SDS-PAGE based systems and the discrepancy between the cellular mRNA levels versus protein levels in eukaryotic cells. 2-DE based analysis is obviously confined to examining only the highly abundant and hydrophilic proteins. Furthermore, silver staining method may not be the optimal method to prepare 2-DE gels for MS, especially when the coloration is saturated. Moreover, purifying SC to absolute homogeneity is beyond the limit of current technology.

Discussion

Stem cells are capable of both self-renewing process and giving rise to diverse tissue types. There is increasing public interest on clinical trial of stem cells. Proteomics, large scale studies of proteins, can be used to analyze protein contents of stem cells in a cost-effective and efficient way. Through 2-DE coupled mass spectrometry or non-gel based mass spectrometry, the basic analytical tools in proteomics, proteins of several types of stem cells such as hNSCs, hMSCs, and hESC have been analyzed. These works using proteomics approach is, however, limited to analyzing highly abundant proteins only. Therefore, new mass spectrometry based proteomic analysis techniques for stem cell proteins must be developed in correlation with other state-of-the-art analytical tools.

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