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Quantitative proteomics analysis highlights the role of redox hemostasis and energy metabolism in human embryonic stem cell differentiation to neural cells

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ABSTRACT

Neural differentiation of human embryonic stem cells (hESCs) is a unique opportunity for in vitro analyses of neurogenesis in humans. Extrinsic cues through neural plate formation are well described in the hESCs although intracellular mechanisms underlying neural development are largely unknown. Proteome analysis of hESC differentiation to neural cells will help to further define molecular mechanisms involved in neurogenesis in humans. Using a two-dimensional differential gel electrophoresis (2D-DIGE) system, we analyzed the proteome of hESC differentiation to neurons at three stages, early neural differentiation, neural ectoderm and mature neurons. Out of 137 differentially accumulated protein spots, 118 spots were identified using MALDI-TOF/TOF and LC MS/MS. We observed that proteins involved in redox hemostasis, vitamin and energy metabolism and ubiquitin dependent proteolysis were more abundant in differentiated cells, whereas the abundance of proteins associated with RNA processing and protein folding was higher in hESCs. Higher abundance of proteins involved in maintaining cellular redox state suggests the importance of redox hemostasis in neural differentiation. Furthermore, our results support the concept of a coupling mechanism between neuronal activity and glucose utilization. The protein network analysis showed that the majority of the interacting proteins were associated with the cell cycle and cellular proliferation. These results enhanced our understanding of the molecular dynamics that underlie neural commitment and differentiation.

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Biological significance
In highlighting the role of redox and unique metabolic properties of neuronal cells, the present findings add insight to our understanding of hESC differentiation to neurons. The abundance of fourteen proteins involved in maintaining cellular redox state, including 10 members of peroxiredoxin (Prdx) family, mainly increased during differentiation, thus highlighting a link of neural differentiation to redox. Our results revealed markedly higher expression of genes encoding enzymes involved in the glycolysis and amino acid synthesis during differentiation. Protein network analysis predicted a number of critical mediators in hESC differentiation. These proteins included TP53, CTNNB1, SMARCA4, TNF, TERT, E2F1, MYC, RB1, and AR.

1. Introduction

Neural development is a complex, multistep process. Despite the known extrinsic signaling molecules BMP, WNT and FGF, other relevant developmental cues are largely unknown [1–3]. Neural differentiation of human embryonic stem cells (hESCs) is a unique opportunity for in vitro analysis of neurogenesis and for the study of central nervous system (CNS) diseases [4–7].

Several protocols for neural differentiation have been developed that generate neurons, astrocytes, and immature oligodendrocytes in a manner similar to normal fetal development [8]. However, prior to the application of these cells for therapeutic purposes, it is a major goal to generate uniform populations of various neural subtypes [9,10]. Therefore, application of these cells depends on progress in defining the mechanisms that control their differentiation into different subtypes as well as the development of distinct biomarkers for their isolation. The advent of 'omics' technologies has paved the way for the discovery of a broad spectrum of genes and molecular mechanisms involved in hESC differentiation to neural cells. Various platforms such as high-throughput sequencing and DNA microarrays have been used to study molecular mechanisms at the transcript level [11,12]. However, changes at the mRNA level do not necessarily correlate with protein abundance [13]. Proteomics has proven to be a powerful, direct method for simultaneous identification and quantification of proteins in addition to further defining the molecular mechanisms involved in pluripotent stem cell differentiation. Traditional two-dimensional electrophoresis (2-DE) techniques have been the primary method to quantify changes in the abundances of proteins during embryonic stem cell (ESC) proliferation and differentiation [8,14]. A quantitative proteomics profile that uses 8-plex iTRAQ has been used to monitor the temporal dynamics of protein abundance during hESC differentiation into motor neurons and astrocytes. In this study, Chaerkady et al. used embryoid body formation followed by Noggin and FGF administration for neural induction. They monitored protein dynamics in neural progenitors, motor neurons and astrocytes [15]. These studies showed the strength of proteomics approach in clarifying the molecular control behind cell differentiation, although functional analysis of proteins remains to be further investigated for describing pathways in that proteins connected to the intercellular and intracellular signaling.

In the current study, we applied a two-dimensional differential gel electrophoresis (2D-DIGE) approach coupled with mass spectrometry-based protein identification to study alteration of hESC proteome undergoing neural differentiation induced by Noggin, Sonic hedgehog (SHH, a ventralizing factor) and retinoic acid (RA, a caudalizing factor). After normalization against hESC, 118 differentially expressed proteins were evaluated by pathway analysis and GO mining to explore their potential role associated with neural differentiation. Through further validation by Western blot, we confirmed that differentially accumulated proteins involved in oxidation–reduction, transport, RNA processing, metabolism and protein folding may play a role in regulating neural differentiation.

2. Materials and methods

2.1. Human embryonic stem cell (hESC) culture and neural induction

Starting hESC Royan H6 (passages 25–35) [16] was maintained under feeder-free conditions on Matrigel in DMEM/F12 medium (Gibco; 21331-020) supplemented with 20% knock-out serum replacement (KSR, Gibco; 10828-028), 2 mM L-glutamine (Gibco; 25030-024), 0.1 mM β-mercaptoethanol (Sigma; M7522), 1% nonessential amino acids (Gibco; 11140-035), 100 units/mL penicillin and 100 μg/mL streptomycin (Gibco; 15070-063), and 100 ng/mL basic fibroblast growth factor (bFGF) [17]. The medium was changed daily and cells passaged once weekly with collagenase/dispase at a ratio of 1:2 (R&D Systems). Human ESCs showed normal karyotype (46XY) and expressed the key pluripotency markers, Oct4 and Nanog.

Neural differentiation was performed as previously described [18]. Briefly, differentiation of hESCs was induced by DMEM/F12 medium supplemented with 2% N2, 5% KOSR, 20 ng/mL bFGF, 500 ng/mL Noggin (R&D Systems), 50 ng/mL SHH (R&D Systems), 2 μM RA (Sigma) and 10 ng/mL LIF (R&D Systems). At two days after induction, we removed SHH and RA. After an additional seven days, neural ectoderm (rosette-like structures) formed. Neural rosettes that grew for six days without Noggin resulted in neural tube formation. The neural-like tube structures were separated manually with a sterile pulled-glass pipette and dissociated into single cells by 0.008% trypsin (27250_018; Gibco) and 2 mM disodium EDTA (108454; Merck, Darmstadt, Germany), then replated on laminin (5 mg/mL; L2020; Sigma) and poly-L-ornithine (15 mg/mL; P4957; Sigma)-coated tissue culture dishes in neurobasal medium (21103_ 049; Gibco) supplemented with 2% N2 (17502_048; Gibco), 2% B27 (17504_044; Gibco), 2.5% fetal bovine serum (FBS; 16141_079; Gibco), 200 mM ascorbic acid (AA; A8960; Sigma), fibronectin (5 mg/mL; F0635; Sigma), laminin (1 ng/mL; L2020; Sigma), BDNF (20 ng/mL; 248 BD/CF; R&D), GDNF (20 ng/mL; G1777; Sigma) and db-cAMP (1 ng/mL; D0260; Sigma) for up to 14 days.