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Identification of a membrane proteomic signature for human embryonic stem cells independent of culture conditions

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Abstract Proteomic profiling of human embryonic stem cells (hESC) can identify cell fate determination and self-renewal biomarkers. Employing Fourier transform LC-ESI-MS/MS and MS^3 mass spectrometry, we obtained a membrane proteomic signature overlapping between hESC cultured on mouse embryonic fibroblast (MEF) feeders and those grown under MEF-free culture conditions. We identified 444 transmembrane or membrane-associated proteins, of which 157 were common between both culture conditions. Functional annotation revealed CD antigens (10%), adhesion proteins (4%), proliferation-associated proteins (4%), receptors (41%), transport proteins (21%), structural proteins (5%), and proteins with miscellaneous functions (15%). In addition, 15 CD antigens and a number of surface marker molecules not previously observed in hESC at a proteome level, e.g., Nodal modulator 1, CD222, transgelin-2, and CD81, were identified. In conclusion, we describe the first membrane proteome profile of hESC that is independent of culture conditions. These data can be used to define the phenotype of hESC.

Introduction

One of the major issues facing the understanding of the biology of human embryonic stem cells (hESC) and their use in therapy is the absence of well-defined hESC-specific markers that can be employed to ensure quality control of hESC, to compare cell lines obtained from different laboratories, and to identify differences between hESC lines propagated under different culture conditions. Previous studies have tried to identify a "molecular signature" of hESC based on gene expression profiling (Abeyta et al., 2004; International Stem Cell Initiative (ISCI), 2007; Laslett et al., 2007; Sato et al., 2003). The first global transcriptional profile of a single hESC line, the Wisconsin H1 cell line, grown

Abbreviations: hESC, human embryonic stem cell; LC-ESI-MS/MS, liquid chromatography electrospray ionization mass spectrometry[2]; LC-FTICR, liquid chromatography Fourier transform ion cyclotron resonance; MEF, mouse embryonic fibroblast; MG, Matrigel; TMHMM, transmembrane helix prediction based on a Markov model.

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on mouse feeder cells identified the presence of 918 genes enriched in undifferentiated cells compared with differentiated cells (Sato et al., 2003). Also, a comparison of H1 cells to undifferentiated mouse embryonic stem cells showed several genes expressed in common, suggesting the presence of a core pluripotency program (Sato et al., 2003). In another study, transcriptome analysis using Affymetrix oligonucleotide microarrays of various, independently derived hESC grown on mouse embryonic fibroblast feeders demonstrated that the majority of genes expressed in hESC lines were similar, but a small number showed a unique expression profile, which could be explained only by either culture conditions or intrinsic genetic differences (Abeyta et al., 2004). A recent microarray study focusing on gene expression profiles of early lineage committed hESC has reported that in addition to genes associated with pluripotency, lineage-specific transcription factors were coexpressed during early differentiation stages (Laslett et al., 2007). Recently, the International Stem Cell Initiative (ISCI, 2007) employed low-density arrays and identified a common expression profile for 59 hESC lines. While these studies are useful, the identified markers cannot provide a complete picture of the functional proteins being expressed.

Mass spectrometry-based proteome analysis is a powerful tool for identification of proteins by sequencing of peptides from complex mixtures and has been employed to describe the biological nature of a variety of cell types, including adult stem cells (Adachi et al., 2007; Cox and Mann, 2007; Foster et al., 2005). However, application of this technology to hESC has been limited. Two studies have reported proteome analyses of human embryonic stem cells using whole-cell lysates. The first, a study by Baharvand et al. (2006) employed 2D gel electrophoresis followed by MALDI TOF/TOF mass spectrometry. The second study, by van Hoof et al. (2006), employed 1D gel separation, in-gel digestion, and LC-FTICR-MS/MS analysis. While these studies have provided interesting data regarding the pluripotent proteome profile of the cells, they were not directed toward identification of new surface markers, since no specific procedure was performed to enrich for membrane proteins.

Applying proteome analysis for the identification of novel cellular surface markers to a reasonable proteome coverage has, traditionally, been hampered by the poor probability of obtaining peptides from low-abundance plasma membrane (PM) proteins in a complex mixture. A previously published PM isolation procedure from our group (Foster et al., 2005) demonstrated its suitability for identifying novel low-abundance surface markers of human mesenchymal stem cells (hMSC). The aim of the current study was therefore to apply the same methodology with modifications to obtain low-abundance membrane proteins of hESC. Since hESC are currently cultured under different *in vitro* conditions, we wanted to identify a common proteome profile of hESC independent of culture conditions. We employed hESC populations obtained from two different but standard and commonly employed culture conditions, feeder-containing and feeder-free cultures, and we carried out membrane proteome analysis using a method based on LC-ESI-FTICR-MS^2 and MS^3. Our data present a "membrane proteome profile" of hESC, which provides insights into self-renewal programs characteristic of hESC and identifies novel surface markers characteristic of hESC.

Results and discussion

Characterization of hESC-OD3

hESC-OD3 cells were grown successfully both on inactivated mouse feeder cells and under feeder-free culture conditions, forming colonies that expressed the pluripotency markers Oct-4, Nanog, Sox2, Tra1-81, and alkaline phosphatase (ALP), visualized by immunocytochemistry. Also, hESC-OD3 cells expressed Tra1-60, Tra1-81, and SSEA4 as demonstrated by FACS analysis (Supplementary Fig. 1). In addition, the cells expressed Oct4, Sox2, Nodal, ALP, and hTERT mRNA as evidenced by RT-PCR. Similar expression levels were observed in cells grown without feeders (data not shown) and in hESC-HUES9 grown on inactivated feeders (Supplementary Fig. 2). Analysis of Giemsa-banded hESC-OD3 chromosomes revealed a karyotype of 46XX inv(9)(q20) in 20 metaphase spreads. In addition, hESC-OD3 were able to form embryoid bodies (EB) that contained cells from all three germ layers as evidenced by immunocytochemical positive staining for albumin and CK18 (endoderm), TUJ-1 (ectoderm), and CD31 and CD34 (mesoderm). hESC-OD3 obtained from MEF feeder-cell cultures were able to form teratomas when implanted under the kidney capsule and subcutaneously in NOD-SCID mice (Prokhorova et al., 2008). Data showing the characterization from colony staining, flow cytometry, karyotyping, and EB can be found in Supplementary Fig. 1. Side-by-side comparison of hESC-OD3 with a commonly employed hESC line, HUES9 hESC, revealed similar phenotypic characteristics (Supplementary Fig. 2).

Proteome analysis and functional classification of the membrane fraction

The Mascot search engine initially matched sequenced peptides to 3133 protein identifications before data validation. Following validation by the MSQuant program (for criteria see Materials and Methods), we obtained a total of 1075 proteins (539 nonredundant proteins detected in the hESC–MEF and 536 in the hESC–MG populations). Filtration of the 1075 proteins for membrane and cell surface proteins resulted in 240 proteins selective for the hESC–MEF fraction and 204 proteins selective for the hESC–MG fraction, of which 157 were found under both culture conditions (Fig. 1). Manual clustering for cellular compartmentalization using the SwissProt/UniProt and Ensembl databases of the unfiltered dataset provided information about the efficiency of the membrane-enrichment procedure.

Seventy-seven percent of the proteins shared between the hESC–MEF and the hESC–MG growth conditions were integral membrane proteins, proteins with known or predicted membrane anchors, or membrane-associated proteins, whereas the percentage of nuclear or nuclear membrane-bound proteins was lower than 7% (Fig. 2a).

Common proteins detected under the hESC–MEF and hESC–Matrigel conditions

We considered the group of 157 proteins shared between hESC–MEF and hESC–MG as a potential profile of membrane proteins independent of culture conditions. Functional