# Enriching protein-protein and functional interaction networks in human embryonic stem cells

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**Abstract.** Human Embryonic Stem Cells (hESCs) have a great therapeutic potential in regenerative medicine, but the precise molecular mechanisms by which hESCs maintain or regulate their characteristics remain largely unknown. Since protein-protein interaction is vitally important in regulating hESCs, we utilized a network-based bioinformatics analysis in order to learn what and how specific proteins interact with each other. By combining protein-protein interaction data and a collection of genes over-expressed in hESCs, we constructed a protein interaction network using a breadth-first search algorithm. This scale-free network which is significantly larger than networks generated by random samplings, illustrates how these hESC-enriched proteins might interact with each other in hESCs. Of the top 5% highly connected nodes (corresponding to 21 proteins including MYC, H2AFX, RUVBL1, DDX18, CDC2, HDAC2 and HIST1H4C) presumably critical for determining the fate of hESCs, nearly half are known to be regulated by NANOG/SOX2/MYC. This underscores importance of these transcription factors in hESCs. In addition, *in silico* cis-element analysis suggests that NF-Y may be an important transcription factor regulating many of these hub proteins (high connected nodes) in hESCs. To further abstract the functional significance, directly connected proteins were matched to and grouped by gene ontology (GO) terms in molecular function category. Sixty-six interacting GO-GO terms paired through protein inter-actions were found over-represented in hESCs. This functional enrichment may be essential for understanding molecular characteristics in hESCs. Collectively, we analyzed hESC-enriched genes based on protein-protein interaction data, from which an hESC-enriched protein interaction network was constructed and a network of molecular functional terms was also identified. The results of this analysis, on the systems level, may shed new light to further our understanding of hESCs.

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## Introduction

Human Embryonic Stem Cells (hESCs) are pluripotent cells isolated from the inner cell mass of the blastocyst (1). They can differentiate into all tissue types *in vivo*, and into the three primary germ layers as well as extra-embryonic tissues *in vitro*. Because of these unique characteristics, these cells are potentially invaluable in regenerative medicine (2-5). Efforts on this have identified some important genes in hESC lines, such as OCT3/4, NANOG, REX1, SOX2 and FOXD3 (6-10). However, our knowledge concerning the many characteristics of hESCs is still limited. One main reason may be that the vast majority of studies have focused on individual genes/proteins, without considering the possible role of interactions. It is well known that most proteins do not function in isolation, but rather interact with one another to form molecular networks, and larger protein complexes are, in turn, part of a more extensive biological web.

With the evolution of high-throughput technologies in the post-genomics era, studies have shifted from characterization of single protein to investigation of the entire interactome. Nowadays, biological knowledge is often represented by networks, such as regulatory and metabolic networks. Construction and analyses of these networks have revealed interesting characteristics within the framework of inter-actome. For example, hub proteins in networks tend to be more conserved (11,12). There are an increasing number of studies focusing on the transcriptional networks, which emphasize the roles of transcription factors, in regulating human ES cells. As a result, a number of important tran-scription factors responsible for self-renewal and pluripotency have been identified (13-16). In recent years, the network-based approach has gained popularity and been successfully applied for analysis of protein-protein interaction networks in many species and diseases (17-19). For instance, one study using affinity purification of NANOG under native conditions followed by mass spectrometry generated a mouse ES protein interaction network including only 37 proteins (20). However, the picture is far from complete.

Microarray technology provides us a unique opportunity to examine gene expression patterns in hESCs. However,

heterogeneity of hESCs gene expression data could exist across different laboratories or different cell lines, which could be partly circumvented by meta-analysis to give a more robust result. A 'consensus hESCs gene list' was produced (21), and some of the genes on the list are also expressed in certain other tissues. The authors explain that these proteins may not be highly specific to hESCs individually, however, they act together with other proteins to function specifically in hESCs (21). Based on these observations, we postulate that there may be an enriched protein interaction sub-network(s) among a collection of those on or off the list to maintain or to modify properties of hESCs.

In this study, two questions were raised: i) Do the hESC-enriched genes/proteins interact directly with each other more frequently than expected by chance alone? In other words, does an hESC-enriched protein interaction network exist at all? ii) Can any enriched functional interaction patterns be identified to be important for maintaining characteristics of hESCs? To address these questions, we performed network-based analysis of both enriched genes/proteins and ontological terms to propose the existence of an hESC-enriched molecular interaction network.

## Materials and methods

*Gene list*. We collected genes over-expressed in hESCs in at least 3 independent studies, re-affirmed by a meta-analysis of human embryonic stem cells transcriptome - Amazonia (http://amazonia.montp.inserm.fr/) (21). We only collected those with known entrez gene id from the original 'consensus hESC gene list' called by the authors. The resulting list includes 1029 unique gene ids. We converted these gene ids to 1020 UniProtKB/Swiss-Prot accession numbers using UniProt ID mapping (http://www.uniprot.org/jobs) and named them hESC enriched proteins (hESPs).

*Protein-protein interaction data*. To obtain protein-protein interaction data, we downloaded the i2d database for human (http://ophid.utoronto.ca/ophidv2.201/) (12), which contains a new release of the Online Predicted Human Interaction Database (OPHID), and human protein-protein interactome assembled from other databases complemented by homolog interactions identified in other organisms (22). It now includes 138554 protein interaction pairs for 13560 proteins in UniProtKB/Swiss-Prot. We wrote a Perl script to remove reciprocally redundant pairs (for example: proteins A and B can form pairs A-B and B-A with one of them removed). We obtained 92545 unique protein interaction pairs (UPI). To the best of our knowledge, this is perhaps the largest protein interaction network for humans.

*Tissue specificity/selectivity*. Tissue-specific/selective gene expression is believed to be of physiological importance (23). To verify whether hESC-enriched genes are tissue-specific/selective or not, we compared them with 3904 tissue-selective genes surveying 97 tissue types (stem cell and other related tissue or cell types not surveyed) (24).

*Network construction and analysis*. Network for hESPs was constructed using a Perl script (named Max Network Program,

MNP). The program took as input human protein-protein interaction data downloaded from the i2d database, and mapped interaction of hESPs within the entire protein-protein interaction network using a breadth-first search algorithm. The resulting subnet, named as Max Network Protein Interaction Pairs or MNPIP, was visually rendered by Cytoscape program (http://www.cytoscape.org/) (25). To assess the significance of MNPIP, 1000 simulations were done with equal number of input proteins randomly selected. The free statistical package R (http://www.r-project.org/) was used to compute Wilcoxon signed rank test statistics for difference between MNPIP and sub-networks randomly generated, with a $p<0.05$ considered as significant.

*Hub analysis*. We classified nodes in the network according to the degree of connectivity. The top 5% of the most connected proteins in this network were regarded as hubs. Promoter sequence (from 1200 bp upstream to 200 bp downstream of the transcription start site, TSS) analysis was done using Gather (http://gather.genome.duke.edu/) and TFM-Explorer (http://bioinfo.lifl.fr/TFME/).

*Enriched functional interaction pairs and network*. Interacting partners are likely to be functionally related. To identify functional enrichment in hESCs, we assigned GOSlim function terms to human UiprotKB/Swiss-Prot proteins in i2d database whenever possible. A total of 42 GOSlim function terms from QuickGO (www.ebi.ac.uk/ego) were used. Proteins assigned with the same GOSlim term were considered functionally similar. One protein could have multiple GOSlim assignments if it is involved in various functional aspects. Based on the underlying protein-protein interactions, pairs of interacting GOSlim terms were formed to represent interacting functional groups. This was done with the help of a Perl script (named Interaction Patterns Analysis or IPA). We calculated an enrichment score for each GOSlim-GOSlim interaction pair originated from all human interacting proteins in i2d database or from hESC-enriched proteins. We based our enrichment score on EASE, a modified Fisher Exact p-value used by DAVID (http://david.abcc.ncifcrf.gov/home.jsp) (26).

## Results

*Tissue specificity/selectivity*. Previous study has generated 3904 tissue-selective unique protein-coding genes (from the latest Affymetrix annotation, express in 5 or less human tissues) for 97 normal human tissue types (24). When comparing 1029 hESC-enriched genes (see Materials and methods) with the tissue-selective ones, 274 genes (Fig. 1) were found intersected. This suggests that some of the hESC-enriched genes are not strictly specific to hESCs, which is not uncommon as described (21).

*Protein interaction network for enriched genes*. Previous studies have suggested that hESC-enriched proteins (hESPs) may act in a cooperative manner rather than in isolation. In this study, we found evidence to support this. First, out of the 1020 hESPs (see Materials and methods), 784 proteins were found in i2d interaction database, which formed 1765 binary protein interaction pairs (BPIP). With 1000 random samplings
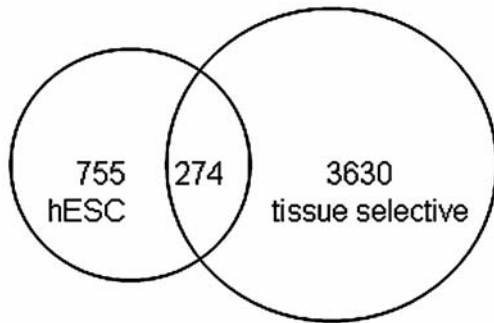
Figure 1. Venn diagrams of the hESC-enriched genes and tissue selective genes.

of the same size (i.e. 784 random proteins) from all non-redundant proteins recorded in i2d database, significantly less BPIP were found (484±50 BPIP, p<2.2e-16, estimated by one-sided Wilcoxon signed rank test with continuity correction). Next, we searched for the most dense sub-network consisting of interacting hESPs. Remarkably, a significant fraction of the interacting hESPs (403 out of 784 proteins, 51.4%) forms a large subnetwork connecting each other. However, 1000 random samplings of 784 proteins from i2d yielded significant smaller subnetworks consisting of 8.5-33.3% of input proteins (as compared to 51.4% for hESPs, p<2.2e-16, one-sided Wilcoxon signed rank test with continuity correction, Table I). Thus, the above results
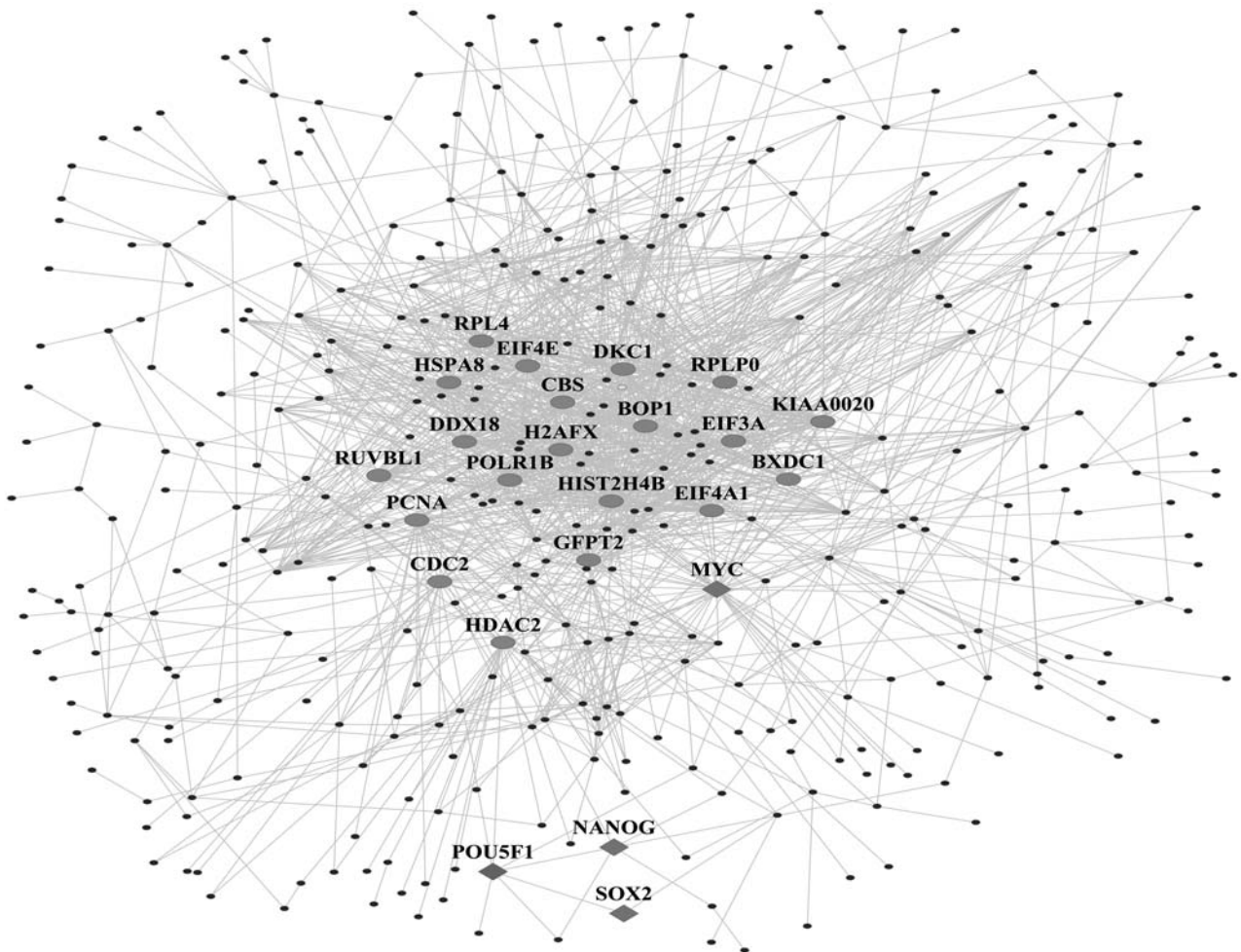


Figure 2. The hESC-enriched protein interaction network. Twenty hubs are labelled and shown as ovals and 4 known important transcription factors in the network are shown as diamonds (MYC is both a hub and a transription factor).

Table I. Significant analysis of BPIP and MNN using hESPs and randomly selected proteins.

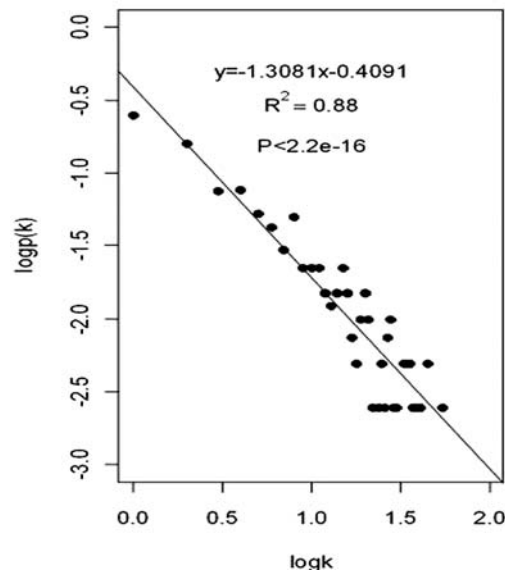| | hESCs | Random | P-value[a] |
|---|---|---|---|
| Number of binary protein interaction pairs (BPIP) | 1765 | 484±50 | P<2.2e-16 |
| Number of max network nodes (MNN) | 403 | 179±32 | P<2.2e-16 |

[a]One-sided Wilcoxon signed rank test with continuity correction.

Figure 3. The log plot of P(k) against k illustrating scale-free characteristics (γ=1.3081) of hESC-enriched protein interaction network (P(k)~k$^{-γ}$). The plot was generated using the free software R.

indicate that a significant portion of the hESPs may cooperate directly or indirectly by means of interaction to form the hESC-enriched protein interaction network (Fig. 2).

*Hubs analysis of hESC-enriched protein interaction network.* Most biological networks follow power law distribution, P(K) ~ K$^{-γ}$ according to the Barabasi-Albert model (27). A network with γ<3 is a typical scale-free network which possesses a small number of highly connected nodes/hubs in contact with a large number of nodes of low connectivity. The hESC-enriched protein interaction network (γ=1.3081, Fig. 3) in which a few hubs are heavily connected with most nodes of low connectivity, is a scale-free network by definition. Since it is hard to draw a line as to what a hub is, we focused on the top 5% most connected nodes in the network and called them hubs. This accounts for a total of 21 nodes (Table II), each of which having more than 28 partners.

Some of the transcription factors identified previously, e.g. OCT4, SOX2, NANOG and c-MYC, are very important for generating induced Pluripotent Stem (iPS) cells, and therefore are believed to be important regulators for hESCs

Table II. List of 21 hubs in hESC-enriched protein interaction network.

| Swiss-Prot Accession | Gene symbol | NANOG/SOX2/ MYC target[a] | Involvement in cancer[b] | Biocarta pathway[c] (part of cell growth) |
|---|---|---|---|---|
| P62805 | HIST1H4C | B, C | - | - |
| P01106 | MYC | - | Y | MAPKinase/p38 MAPK/WNT signaling pathway |
| P60842 | EIF4A1 | A | Y | Internal bibosome entry pathway mTOR signaling pathway |
| Q9NVP1 | DDX18 | B | Y | - |
| P16104 | H2AFX | B, C | Y | - |
| Q15397 | KIAA0020 | - | - | - |
| P36578 | RPL4 | - | - | - |
| P12004 | PCNA | B | Y | p53 signaling pathway |
| Q9H9Y6 | POLR1B | - | - | - |
| P11142 | HSPA8 | A | Y | - |
| O60832 | DKC1 | A | Y | - |
| P06493 | CDC2 | B, C | Y | Cyclins and cell cycle regulation |
| P06730 | EIF4E | A | Y | Internal ribosome entry pathway mTOR signaling pathway |
| Q9H7B2 | BXDC1 | - | - | - |
| Q14137 | BOP1 | - | Y | - |
| P05388 | RPLP0 | - | Y | - |
| Q14152 | EIF3A | - | Y | - |
| Q9Y265 | RUVBL1 | - | Y | - |
| Q92769 | HDAC2 | C | Y | Mechanisms of transcriptional repression by DNA methylation |
| O94808 | GFPT2 | - | - | - |
| P35520 | CBS | A | - | - |

[a]A, targets of MYC; B, targets of NANOG; C, targets of SOX2. [b]Y, involvement in cancer from current knowledge. [c]http://www.biocarta.com/genes/index.asp.

Table III. List of molecular function-GO-GO term interaction pairs enriched in human embryonic stem cells.

| GO term1 | GO term2 | GO term1 function | GO term2 function | EASE score |
|---|---|---|---|---|
| GO:0003677 | GO:0016787 | DNA binding | Hydrolase activity | 3.3e-46 |
| GO:0000166 | GO:0003677 | Nucleotide binding | DNA binding | 7.9e-40 |
| GO:0003677 | GO:0005515 | DNA binding | Protein binding | 1.5e-36 |
| GO:0005515 | GO:0016787 | Protein binding | Hydrolase activity | 8.4e-26 |
| GO:0003677 | GO:0003824 | DNA binding | Catalytic activity | 2.7e-24 |
| GO:0000166 | GO:0000166 | Nucleotide binding | Nucleotide binding | 4.4e-23 |
| GO:0003723 | GO:0005515 | RNA binding | Protein binding | 4.8e-17 |
| GO:0003824 | GO:0005515 | Catalytic activity | Protein binding | 5.6e-17 |
| GO:0000166 | GO:0016787 | Nucleotide binding | Hydrolase activity | 3.2e-16 |
| GO:0000166 | GO:0005515 | Nucleotide binding | Protein binding | 4.9e-16 |
| GO:0003824 | GO:0016787 | Catalytic activity | Hydrolase activity | 1.2e-14 |
| GO:0005515 | GO:0005515 | Protein binding | Protein binding | 2.1e-14 |
| GO:0003723 | GO:0016787 | RNA binding | Hydrolase activity | 5.0e-14 |
| GO:0003824 | GO:0003824 | Catalytic activity | Catalytic activity | 2.6e-13 |
| GO:0016787 | GO:0016787 | Hydrolase activity | Hydrolase activity | 3.6e-12 |
| GO:0003723 | GO:0003723 | RNA binding | RNA binding | 4.9e-12 |
| GO:0004518 | GO:0005515 | Nuclease activity | Protein binding | 9.9e-09 |
| GO:0003723 | GO:0003824 | RNA binding | Catalytic activity | 2.5e-07 |
| GO:0004518 | GO:0004518 | Nuclease activity | Nuclease activity | 3.4e-07 |
| GO:0005515 | GO:0008135 | Protein binding | Translation factor activity, nucleic acid binding | 2.2e-6 |
| GO:0005488 | GO:0016787 | Binding | Hydrolase activity | 2.4e-6 |
| GO:0004518 | GO:0016787 | Nuclease activity | Hydrolase activity | 3.7e-6 |
| GO:0003677 | GO:0005215 | DNA binding | Transporter activity | 5.9e-6 |
| GO:0003677 | GO:0016740 | DNA binding | Transferase activity | 9.0e-6 |
| GO:0003824 | GO:0004518 | Catalytic activity | Nuclease activity | 1.0e-5 |
| GO:0003723 | GO:0009055 | RNA binding | Electron carrier activity | 1.6e-5 |
| GO:0003676 | GO:0003677 | Nucleic acid binding | DNA binding | 2.1e-5 |
| GO:0016740 | GO:0016787 | Transferase activity | Hydrolase activity | 4.6e-5 |
| GO:0003723 | GO:0008135 | RNA binding | Translation factor activity, nucleic acid binding | 6.9e-5 |
| GO:0003677 | GO:0004518 | DNA binding | Nuclease activity | 6.7e-5 |
| GO:0003824 | GO:0008135 | Catalytic activity | Translation factor activity, nucleic acid binding | 1.4e-4 |
| GO:0000166 | GO:0008135 | Nucleotide binding | Translation factor activity, nucleic acid binding | 1.9e-4 |
| GO:0000166 | GO:0004518 | Nucleotide binding | Nuclease activity | 2.3e-4 |
| GO:0003723 | GO:0004518 | RNA binding | Nuclease activity | 3.0e-4 |
| GO:0003677 | GO:0005488 | DNA binding | Binding | 4.1e-4 |
| GO:0008135 | GO:0016787 | Translation factor activity, nucleic acid binding | Hydrolase activity | 4.8e-4 |
| GO:0003682 | GO:0005515 | Chromatin binding | Protein binding | 4.8e-4 |
| GO:0000166 | GO:0003676 | Nucleotide binding | Nucleic acid binding | 5.0e-4 |
| GO:0003677 | GO:0003682 | DNA binding | Chromatin binding | 6.6e-4 |
| GO:0003824 | GO:0016740 | Catalytic activity | Transferase activity | 7.5e-4 |
| GO:0003682 | GO:0016787 | Chromatin binding | Hydrolase activity | 0.002 |
| GO:0000166 | GO:0016740 | Nucleotide binding | Transferase activity | 0.002 |
| GO:0003676 | GO:0016787 | Nucleic acid binding | Hydrolase activity | 0.002 |
| GO:0003676 | GO:0003723 | Nucleic acid binding | RNA binding | 0.002 |

Table III. Continued.

| GO term1 | GO term2 | GO term1 function | GO term2 function | EASE score |
|---|---|---|---|---|
| GO:0003677 | GO:0005198 | DNA binding | Structural molecule activity | 0.005 |
| GO:0030234 | GO:0030234 | Enzyme regulator activity | Enzyme regulator activity | 0.006 |
| GO:0003676 | GO:0005515 | Nucleic acid binding | Protein binding | 0.006 |
| GO:0000166 | GO:0003682 | Nucleotide binding | Chromatin binding | 0.007 |
| GO:0003677 | GO:0008135 | DNA binding | Translation factor activity, nucleic acid binding | 0.007 |
| GO:0008135 | GO:0009055 | Translation factor activity,nucleic acid binding | Electron carrier activity | 0.007 |
| GO:0003723 | GO:0005488 | RNA binding | Binding | 0.007 |
| GO:0003824 | GO:0005488 | Catalytic activity | Binding | 0.008 |
| GO:0003676 | GO:0003824 | Nucleic acid binding | Catalytic activity | 0.010 |
| GO:0005488 | GO:0008135 | Binding | Translation factor activity, nucleic acid binding | 0.011 |
| GO:0003677 | GO:0016301 | DNA binding | Kinase activity | 0.012 |
| GO:0004518 | GO:0016301 | Nuclease activity | Kinase activity | 0.014 |
| GO:0016740 | GO:0016740 | Transferase activity | Transferase activity | 0.015 |
| GO:0005198 | GO:0005215 | Structural molecule activity | Transporter activity | 0.016 |
| GO:0005198 | GO:0009055 | Structural molecule activity | Electron carrier activity | 0.016 |
| GO:0003723 | GO:0016740 | RNA binding | Transferase activity | 0.017 |
| GO:0003824 | GO:0004721 | Catalytic activity | Phosphoprotein phosphatase activity | 0.017 |
| GO:0003700 | GO:0016787 | Transcription factor activity | Hydrolase activity | 0.018 |
| GO:0003723 | GO:0004721 | RNA binding | Phosphoprotein phosphatase activity | 0.020 |
| GO:0003676 | GO:0008135 | Nucleic acid binding | Translation factor activity, nucleic acid binding | 0.023 |
| GO:0003676 | GO:0003676 | Nucleic acid binding | Nucleic acid binding | 0.030 |
| GO:0008135 | GO:0008135 | Translation factor activity, nucleic acid binding | Translation factor activity, nucleic acid binding | 0.030 |

(6,7,9,28,29). In the hESC-enriched protein interaction network, transcription of many hub protein-coding genes were regulated by these transcription factors (30,31) (Table II). Interestingly, nearly half of the hub proteins are known to be involved in tumorigenesis or associated with poor cancer prognosis (32-45) (Table II). Among the top 10-ranked transcription factor binding sites predicted by both Gather and TFM explorer, those for transcription factor NF-Y were found in common within the proximal promoter sequences of nine hub protein-coding genes.

*Enriched functional interactions*. Cellular behavior is a consequence of the complex interactions between its numerous constituents. To gain more biological insights by studying functional interactions, we first annotated each protein in the hESC-enriched protein protein interaction pairs with a subset of Gene Ontology terms. In this study, 42 GOSlim terms from the molecular function category of the Gene Ontology were used. Our goal was to identify significantly enriched functionally interacting GO-GO pairs. This resulted in 66 GO-GO interaction pairs (Table III) enriched in hESCs. Except for GO:0030234, most of them were found in a functionally interacting network (Fig. 4). The top four most connected GOSlim terms in this network are: GO:0003677, GO:0016787, GO:0003723 and GO:0003824.

**Discussion**

We studied the interactions of a list of genes over-expressed in hESCs. A protein-protein interaction subnetwork formed
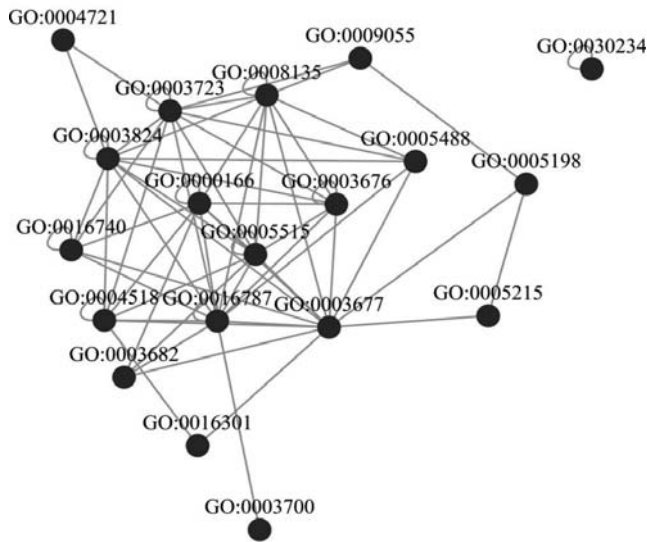
Figure 4. Enriched 'GOSlim-GOSlim interaction' network in hESC (molecular function interaction patterns).

network are likely to be essential for growth and the degree of connectivity correlates with other phenotypes in addition to essentiality (46-48). Scale-free networks, albeit more tolerant to random removal of nodes, are vulnerable to loss of highly interactive hubs (49).

MYC, one of the hubs in our hESC-enriched protein interaction network, is a key factor for inducing Pluripotent Stem (iPS) cells (29), and for regulating self-renewal and pluripotency in mouse ES cells (mESCs) (50). A recent study showed that H2AFX/H2AX, also a hub in the hESC-enriched protein interaction network, participates in a critical signaling pathway different from that of somatic cells to control stem cell proliferation (51). RUVBL1/TIP49a, an important hub identified in this study and a common hub in co-expression networks found in both hESCs and mESCs (52), plays a critical role in c-MYC and WNT signaling pathways (32,53). RUVBL1/TIP49a was also found to be evolutionarily highly conserved and essential for viability in yeast, flies and worms (54). Furthermore, WNT, mTOR and MAPK pathways are pivotal for regulating hESCs, as evidenced by the involvement of several hub proteins in these pathways (55-57). We anticipate that some other hubs or nodes identified in this study may turn out to be important factors in future investigations of hESCs.

To our surprise, several important transcription factors, namely NANOG, SOX2, OCT4, are not among the most connected hubs in this network whereas some of their targets are (Table II). These factors also share some common targets (30), which suggests that they work closely in a regulatory network. In addition, OCT4 interacts with NANOG and SOX2 in the hESC-enriched protein interaction network.

with a significant number of hESC-enriched genes was identified by integrating gene expression and protein interaction data. It is full of hESC-enriched genes and is very likely to be responsible for maintaining characteristics of hESCs. This scale-free network has a few dominant hubs heavily connected with most nodes of low connectivity. A series of studies have shown that hub proteins in a scale-free
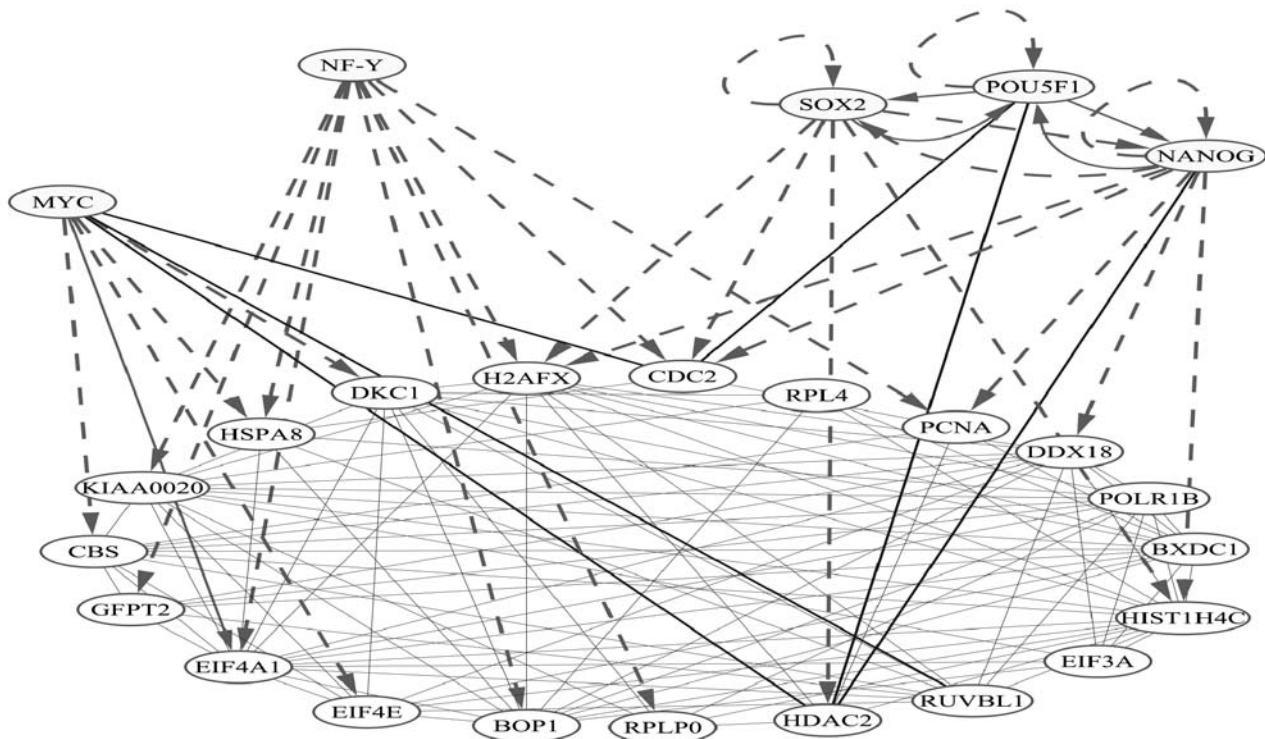


Figure 5. The relationship between hubs and important transcription factors in hESC-enriched protein interaction network: solid line, protein-protein interaction; solid line with arrows, protein-protein interactions with regulatory potential and direction (as indicated by arrows) and dash line with arrows, with regulatory potential and direction only. Important transcription factors (SOX2, POU5F1, NANOG, MYC and NF-Y), their targets (ovals) and regulatory circuits (arrows) are indicated.

Regulatory circuits formed by some of these factors and their targets, as reported previously based on experimental findings (20), may be used to fine-tune characteristics of the ES. Furthermore, the directed or self-directed regulatory loops of OCT4, NANOG and SOX2 can be used to maintain proper and stable expression level (30) through a robust synergistic and sustained network topology (through regulation of many hubs) whose stabilization is vitally important for hESC (Fig. 5). Any imbalance of a regulator with its targets or with itself in the network may produce unexpected outcomes. This also suggests that synergistic hubs target activation by their regulators is a very important mechanism by which these tran-scription factors, albeit not hubs, control self-renewal and pluripotency in hESCs.

In addition to the above important transcription factors, NF-Y was predicted by *in silico* analysis of promoter sequences to regulate close to half of the 21 hubs in the hESC-enriched protein interaction network. It has recently been shown that NF-Y binding site is rather conserved and over-represented in promoter regions of genes preferentially expressed in human and mouse pluripotent cells (58). In fact, NF-Y was down-regulated during differentiation (58). Taken all these observations together, NF-Y seems to be an important sustaining factor for the maintenance of hESCs by regulating its hub targets in the hESC-enriched protein interaction network (Fig. 5). In line with this, NF-Y also influences hematopoietic stem cell (HSC) self-renewal and differentiation (59).

Functional categories such as gene ontology terms (GO term) for genes enriched under certain condition(s) can facilitate functional interpretation and derive biologically meaningful conclusions. Previous studies mainly focused on the enrichment of GO terms for selected genes/proteins, largely ignored the interactions across various functional groups. Therefore, it makes more sense to identify biological meanings by investigating enriched functional interactions with protein interaction considered. To this end, we found 66 functional interaction pairs enriched in hESCs, which formed an interaction network. Interestingly, most of these interacting pairs, particularly those formed with GO:0003677 (DNA binding), GO:0003723 (RNA binding), are involved in transcription and translation. This is consistent with the need for self-renewal and unrestrained proliferation.

Human embryonic stem cell-derived therapy renews our hope for regenerative medicine, but we must first overcome several hurdles, one of which, perhaps the biggest, is that hESC-therapies may spur tumor formation (60,61). There are some apparent parallel traits such as self-renewal and differentiation capacity between stem cells and cancer cells, which prompt us to the hypothesis that tumors often arise from undifferentiated stem/progenitor cells, or cancer stem cells are derived from normal stem cells (62,63). Out of the 21 hubs in the hESC-enriched protein interaction network, 14 (67%) are involved in tumorigenesis or poor cancer prognosis. In addition, a recent study showed that an embryonic stem cell-like gene expression signature (part of what we used here), potentially contributing to stem cell-like phenotypes shown by many tumors, has been found in poorly differentiated aggressive human tumors (64). These observations can expand our knowledge in hESC biology and tumor formation.

In conclusion, this study has identified an enriched protein interaction network formed by 403 hESC-over-expressed gene-coding proteins. Enriched molecular functional inter-action network were also found in hESCs. The existence of these interaction networks beyond randomness suggests that they are important and very likely to be responsible for the maintenance of hESCs. The hubs governing the hESC-enriched protein interaction network, such as MYC, H2AFX, RUVBL1, DDX18, CDC2, HDAC2, HIST1H4C and so on, which are possibly critical in determining the fate of hESCs, deserve more attention in future investigations. It is worth noting that some hESC-associated proteins for self-renewal and pluripotency, for instance, KLF4 (14) and possibly other factors, are missing in the hESC-enriched protein interaction network. Despite this, our findings represent a step in the right direction, on the system levels, to gain more significant biological information in stem cell research. When more data become available, it is possible to refine the network and make it more informative for studying hESCs. It is also hoped that continuous research on hESCs will speed up its therapeutic applications.

## Acknowledgements

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