The Third International Symposium on Optimization and Systems Biology (OSB'09)
Zhangjiajie, China, September 20–22, 2009

Understanding the Biological Functions of DCF1 Based on Molecular Interaction Network

Xing-Ming Zhao[1,*] Renhong Qian[1,2] Xiangchun Ju[1,2]
Tieqiao Wen[1,2,†]

[1]Institute of Systems Biology, Shanghai University, Shanghai 200444, China
[2]The Laboratory of neural molecular biology, School of Life Sciences, Shanghai University, Shanghai 200444, China

Abstract It is important to understand the neural dendrite formation at molecular level. Unfortunately, the regulation mechanisms underlying neural dendrite formation remain unclear. In our previous work, it is found that dentritic cell factors (DCF1) express differentially between undifferentiated and differentiated neural stem cells (NSCs). In this study, we compared whole-genome gene expression data for DCF1-knockout and wild type mice. By integrating gene expression data and protein-protein interaction data, a connected subnetwork that consist of differentially expressed genes is identified. Analyzing the affected subnetwork, we find that glycolysis is enriched for down-regulated genes in the extracted subnetwork while biosynthetic process is enriched for up-regulated genes, which give hints that DCF1 is possibly related to these functions and provides guidelines for future experiment verification.

Keywords DCF1, Molecular interaction network, Neural dendrite formation

1 Introduction

Neural stem cells (NSCs) are multipotent progenitor cells that have self-renewal activity as well as the ability to differentiate into neurons, astrocytes, and oligodendrocytes [1]. These characteristics have made NSCs research hotspot from the aspects of both basic developmental biology and clinical therapeutic applications to the damaged brain [2, 3, 4]. However, the mechanisms about how NSCs differentiate into different kinds of cells are largely unknown. A number of external cues, as well as intrinsic cellular programs, are thought to regulate maintenance or differentiation of NSCs [5]. It is still not very clear how many genes affect the differentiation process.

In our previous work, we reported an EST termed as SHD11 using mRNA differentiation display and reverse Northern blot analysis, and found that this EST only expresses in undifferentiated NSCs [6]. Analysis of the ORF of this EST revealed a homology (with 98% sequence identity) with dendritic cell factor 1 (DCF1) which is a signaling molecule involved in differentiation and migration of the dendritic cell [6, 7]. The regulation of neural dendrite is important for the formation of functional neural networks. Ohkawa et al.

*Corresponding author.
†Corresponding author.

[8] showed that stathmin, a microtubule destabilizing factor, is down-regulated at both expression level and activity level during cerebellar development, and this down-regulation contributes to dendritic arborization. Later, we successfully cloned and expressed DCF1, and employed C17.2 [9] cells and primary NSCs to identify DCF1 dendrite formation function by RNAi. When DCF1 was over-expressed, NSCs maintain undifferentiated status whereas silence of DCF1 initiate NSCs differentiation [10].

Protein-protein interaction (PPI) networks are key to understanding complex cellular processes [11]. In this study, we compared whole-genome gene expression data for DCF1-knockout and wild type mice. By integrating gene expression data and protein-protein interaction data, a connected subnetwork that consists of differentially expressed genes was identified. Analyzing the affected subnetwork, we found that glycolysis is enriched for down-regulated genes in the extracted subnetwork while biosynthetic process is enriched for up-regulated genes, which give hints that DCF1 is possibly related to these functions and provides guidelines for future experiment verification.

2 Materials and methods

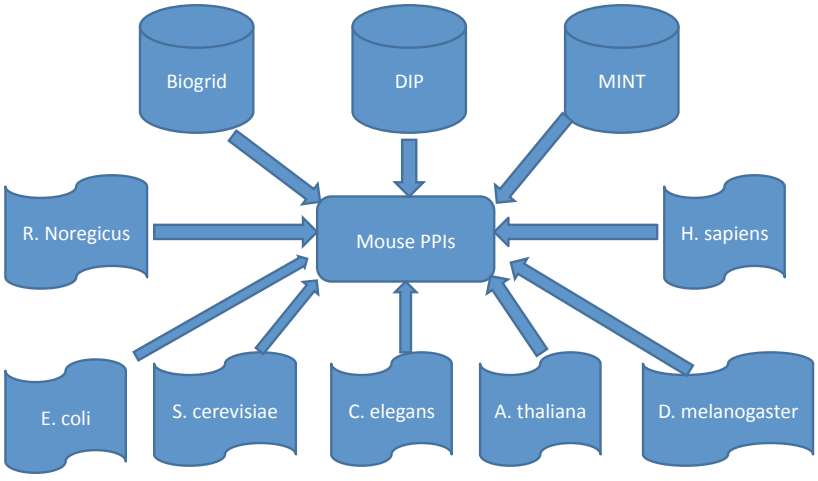

Figure 1: Integration of mouse protein-protein interaction data.

Six wild and DCF1 knockout mice were separately immersed in 75% alcohol for disinfection. By taking the hippocampals in PBS under aseptic conditions, we make them into single cell suspension through mechanical whipping. The supernatant was discarded after 900rmp, 5min centrifugation, and the hippocampals were then resuspended

Table 1: The top 5 most enriched functions for differentially expressed genes.

Functions	$P-$values
carbohydrate metabolic process	$7.1E-4$
cellular carbohydrate metabolic process	$7.2E-4$
alcohol metabolic process	$2.0E-3$
negative regulation of cell proliferation	$2.0E-3$
hexose metabolic process	$2.1E-3$

in medium (DMEM/F12 culture medium with B27, EGF and bFGF). Finally, they were cultured in a glass bottle in CO_2 incubator (5% CO_2, 37 degree). The gene expression data were measured 4 days later.

Since a limited number of protein interactions are available for *Mus musculus*, we integrate various data sources to construct an interaction map for mouse. Figure 1 shows the integration of various data sources for mouse protein-protein interactions. In this work, we used the predicted PPI data presented by Yellaboina et al [12]. The PPIs were first predicted by interologs, and interactions were further predicted based on the co-occurrence of mouse orthologs in predicted bacterial operons. Finally, the phylogenetic profiles were used to filter possible false-positives. In the predicted interactome data, there are 41109 interactions. In addition, we integrated all interaction data from public database, including MINT (4-8-2008 release) [13], BIOGRID (version 2.0.44) [14], and DIP (10-14-2008 release) [15]. Generally, different databases use different gene identifiers. In this work, we mapped distinct identifiers to the same gene names. As a consequence, there are 67339 interactions among 10383 genes in total.

3 Results

3.1 Function enrichment for differentially expressed gene in PPIN

To understand the biological functions of gene DCF1, we investigated the genes that differentially expressed due to knockout of gene DCF1. There are 627 genes that differentially expressed with more than 2-fold change after knockout of DCF1. We first investigated the function enrichment of these differentially expressed genes. Table 1 shows the top 5 most enriched functions for differentially expressed genes. From function enrichment analysis, we can see that the affected genes due to knockout of DCF1 are mostly related to metabolic process and cell proliferation. Therefore, it is reasonable to assume that DCF1 is possibly related to metabolic process. To test the hypothesis, we investigated pathways in which differentially expressed genes are enriched. Table 2 shows the pathway extracted from KEGG database that is enriched for differentially expressed genes. From enriched pathway, it can be found that differentially expressed genes are indeed related to metabolic pathways, which also prove that DCF1 gene may be related to metabolic process to some extent.

Despite differentially expressed genes can give hints on functions of DCF1, the differentially expressed genes were considered independently. In practice, some genes cooperated in concert to approach some specific function, e.g. genes in the same pathway. These cooperative genes generally interact with each other in the molecular interaction

Table 2: Enriched pathway for differentially expressed genes.

Pathway	$P-$values
Glycolysis Gluconeogenesis	3.9E-3

network. On the other hand, some differentially expressed genes do not interact, whereas they differentially express due to some indirect and complex regulation. Therefore, the differentially expressed genes should be put onto the molecular interaction network to investigate their roles in biological process. In this work, the 627 differentially expressed genes were mapped to mouse interactome, and the largest connected component consist of differentially expressed genes was obtained. Figure 2 shows the subnetwork identified from protein interaction networks consist of differentially expressed genes before and after DCF1 knock out, where red color means the expression of corresponding genes are up-regulated compared against those from wild-type mice, and vice versa for green ones. The PPI network consists of 52 proteins, where 26 gene are up-rgulated and 26 down-regulated.

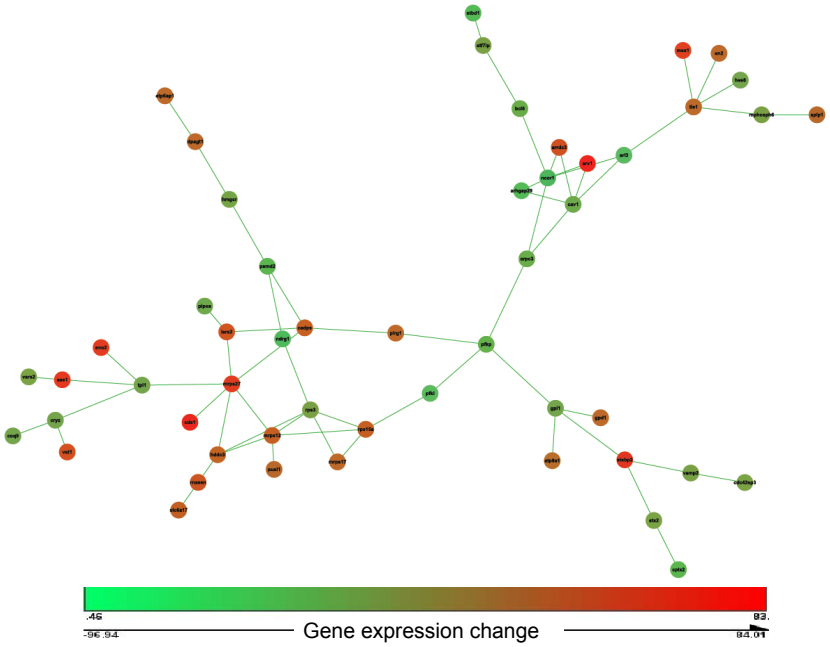

Figure 2: The subnetwork identified from protein interaction network that consists of differentially expressed genes before and after DCF1 knock out.

From the PPI network, it is interesting to see that up-regulated proteins are generally clustered together and similar for down-regulated genes. Furthermore, we investigated up-regulated genes and down-regulated genes respectively. Table 3 lists the top 5 most

Table 3: The top 5 functions enriched for up-regulated genes.

Function	$P-$values
cellular biosynthetic process	3.2E-5
biosynthetic process	3.4E-5
macromolecule biosynthetic process	4.5E-4
primary metabolic process	2.1E-3
cellular metabolic process	2.1E-3

Table 4: Top 5 functions enriched for down-regulated genes.

Function	$P-$value
glycolysis	1.0E-4
glucose catabolic process	1.5E-4
hexose catabolic process	1.6E-4
monosaccharide catabolic process	1.6E-4
alcohol catabolic process	1.8E-4
cellular carbohydrate catabolic process	2.9E-4

enriched functions for up-regulated genes and corresponding $P-$values. It can be seen from table 3 that most up-regulated genes are related to biosynthetic process, which is very different from the conclusions obtained from all differentially expressed genes.

Furthermore, we investigated the down-regulated genes. Table 4 lists the most enriched functions for down-regulated genes. We can see from enrichment analysis that down-regulated genes are significantly different from up-regulated genes with respect to functions. Most of down-regulated genes are related to metabolic process. In addition, we investigated pathways in which down-regulated genes involved. Table 5 shows the most enriched pathways for down-regulated genes. It can be seen that down-regulated genes are enriched in metabolic pathways, which is consistent with function analysis. We further investigated tissues that DCF1 occurs in. Table 6 lists the tissue in which down-regulated genes are enriched. From the analysis, it can be seen that down-regulated genes are more possibly related to NSC, which indicts that DCF1 is possibly related to glycolysis and catabolic process.

4 Discussions and conclusions

In this work, we analyzed the gene expression data obtained before and after knocking out DCF1. We investigated the affected subnetworks based on protein-protein interaction data and gene expression data. We identified a connected subnetwork from the protein-

Table 5: Pathways enriched for down-regulated genes.

Pathway	$P-$value
Glycolysis Gluconeogenesis	4.0E-4
Pentose phosphate pathway	2.3E-3
Fructose and mannose metabolism	7.3E-3

Table 6: Tissue in which down-regulated genes are enriched.

Tissue	$P-$value
Bone marrow	4.1E-3

protein interaction network that consists of genes with significant expression changes before and after knocking out DCF1. We further investigated enriched functions and pathways of the subnetwork, and found that glycolysis is the most enriched function in down-regulated genes while glucose catabolic process is the most enriched pathway for down-regulated genes. Surprisingly, up-regulated genes have distinct functions as down-regulated genes, where up-regulated genes are enriched with cellular biosynthetic process function. We also investigated the tissues in which the subnetwork occur most frequently. We found that most enriched tissues are related to brain, which supports previous study that DCF1 is possibly related to neural stem cell differentiation. In summary, we believe that DCF1 is possibly related to glycolysis and catabolic process. Although the exact function of DCF1 is unknown, the affected subnetwork can provide insight into the functions and biological processes in which DFC1 is involved.

Acknowledgements

Xing-Ming Zhao was partly supported by Innovation Funding of Shanghai University, Key Project of Shanghai Education Committee (09ZZ93), Open Funding of National Key Laboratory of Plant Molecular Genetics, and SRF for ROCS, SEM. Tieqiao Wen was partly supported by Major State Basic Research and Development Program of China (973 program, Grant No.2006CB500702), the National Natural Science Foundation of China (Grant No.30570590, 30770695), and the Shanghai Commission of Education Science and Technology Innovation Fund (Grant No.08 ZZ41).

References

[1] Temple, S. The development of neural stem cells. *Nature*, 414:112-117, 2001.

[2] Gage, F.H., et al. Cell therapy. *Nature*, 392(Suppl.): 18-24, 1998.

[3] Seiler, M.J., et al. Intact sheets of fetal retina transplanted to restore damaged rat retinas. *Invest. Ophthalmol. Vis. Sci.*, 39: 2121-2131, 1998.

[4] Arenas, E., et al. Stem cells in the treatment of Parkinson's disease. *Brain res bull*, 57: 795-808, 2002.

[5] Edlund,T., et al. Progression from extrinsic to intrinsic signaling in cell fate specification: a view from the nervous system. *Cell*, 96: 211-224, 1999.

[6] Wen, T., Gu, P., Chen, F. Discovery of two novel functional genes from differentiation of neural stem cells in the striatum of the fetal rat. *Neuroscience Letters*, 329: 101-105, 2002.

[7] Dietz, A.B., Bulur, P.A., Knutson, G.J., Matasic, R. and Vuk- Pavlovic, S. Maturation of human monocyte-derived dendritic cells studied by microarray hybridization. *Biochem. Biophys. Res. Commun.*, 275731-738, 2002.

[8] Ohkawa, N., Fujitani, K., Tokunaga, E., Furuya, S., Inokuchi, K. The microtubule destabilizer stathmin mediates the development of dendritic arbors in neuronal cells. *J Cell Sci*, 120(8):1447-1456, 2007.

[9] Snyder, E.Y., et al. Multipotent neural cell lines can engraft and participate in development of mouse cerebellum. *Cell*, 68: 33-51, 1992.

[10] Wang, L., Wang, J., Wu, Y., et al. A Novel Function of DCF1 During the Differentiation of Neural Stem Cells In vitro. *Cell Mol Neurobiol*, 28: 887-894, 2008.

[11] Patrick, A., Robert, B.R. InterPreTS: protein Interaction Prediction through Tertiary Structure. *Bioinformatics Applications Note*, 19: 161-162, 2003.

[12] Yellaboina, S., Dudekula, D., Minoru, K. Prediction of evolutionarily conserved interologs in Mus musculus. *BMC Genomics*, 9(1): 465, 2008.

[13] Chatr-aryamontri, A., Ceol, A., Palazzi, L., Nardelli, G., Schneider, M., Castagnoli, L., Cesareni. G. MINT: the Molecular INTeraction database. *Nucl. Acids Res.*, 35(suppl 1): D572–574, 2007.

[14] Breitkreutz, B., Stark, C., Reguly, T., Boucher, L., Breitkreutz, A., Livstone, M., Oughtred, R., Lackner, D., Bahler, J., Wood, V., Dolinski, K., Tyers, M. The BioGRID Interaction Database: 2008 update. *Nucl. Acids Res.*, 36: D637–D640, 2008.

[15] Salwinski, L., Miller, C., Smith, A., Pettit, F., Bowie, J., Eisenberg, J. The Database of Interacting Proteins: 2004 update. *Nucl. Acids Res.*, 32(suppl 1): D449–451, 2004.