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Research Article

New Insight in Neuron Regeneration: Induction of Glia Cell to Neuron Cell

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ABSTRACT

Induction neuron from a veriety of cell resource were remaining challeng in regenerative medicine, so finding the convenient method to reprogram different cells to neuron could be helpful. In this study, we analysis the transcriptome of glia and neuron cells to determine the gene expression in neuron that different when compare to glia cells. Then based on this transcriptom data seek the transcription factor and miRNA. Data extract from transcriptome database of mouse cells comprise cerebral cortex that generated by RNAseq technique. By comparison neuron against glia cells (astrocyte, oligodenderocyte and microglia) determined different gene expression in neuron. By using genetrail2 database determined transcription factor and miRNA associated with neuron gene expression.Result determined the 500 genes with different expression in neuron in comparison with glia cells. 2 significant TF families, DLX and MSX, 3 TF, Sp1, Ctcf and Pax1, 85 miRNA release from analysis this 500 gene. Analysis the gene target of all identified miRNA represent the important biological process related to neurogenesis neurodevelopment, in addition to most important proteins like Dnm1, Gad1 and Grin1 were obtained by functional and structural of network analysis. Dnm1 and Grin1 regulated by Sp1. In sum up, since one of the methods to reprogramming resident glia cells to induce neuron is applying TF and miRNAs, TF like Sp1 lonely or in combination with other factors can be experimentally approved.

Keywords: neuron cell, glia cell, differentiation, transcription factor, miRNA

INTRODUCTION

The major cell types in the central nervous system (CNS), neurons and glia cells are derived from multipotent neural precursor cells (NPCs). Limited ability of NPCs to regenerative main cells in adult cerebral cortex in pathophysiological conditions such as degenerative disorders or physical damages is crucial neurological challenges. Todays, tissue engineering and cell therapy methods overcome the limited capacity to regenerate neural tissue after brain injuries [1, 2]. Several investigations revealed that the reprogramming of resident glial cells and even non-residential cells in vitro and in vivo to generate functional synapse-forming neurons, spinal motor neurons, functional glutamatergic and dopaminergic neurons[3-5].

Generally, induction mechanism is related the present of specific factors such as cocktails of transcription factors (TF) and neuron-specific microRNAs that influence gene expression and epigenetic conditions and finally induce specific

signal transduction or involves cross-talk between distinct signaling pathways [5].

Recently Aravantinou-Fatorou et.al reports a synergistic action of CEND1 and NEUROG2 in reprogramming of mouse postnatal cortical astrocytes and embryonic fibroblasts toward neuronal precursor and differentiated neuron phenotype [5]. Niu et. al. (2015) report the action of SOX2 that reprograms resident astrocytes into neural progenitors after screening 12 transcription factors related to differentiation [6]. In previous study from this research group revealed that neurotropic factors BDNFand nogginsufficient for programing neuron survival and neuroplasticity *in vivo* [7]. Ressel C.Addis et al differentiated astrocyte to dopaminergic neuron in Parkinson models by 3 transcription factors NURR1, LMX1B and ASCL1 [4]. Karow et al use a synergetic effect of SOX2 and ASCL1 to reprograming isolate pericyte to induce neurons [8]. Troper et al 2013, caiazzo 2011 induced dopaminergic neuron from astrocyte and fibroblast by apply transcription factors ASCL1, Lmx1 a/b and Nurr1 (ALN) [9, 10].

Here, we use the RNAseq result of all cells comprise brain to determine the difference gene expression in neuron against glial cells (astrocyte, oligodenderocyte and microglia) to find transcriptional factors and microRNA related neuron phenotype.

METHODS:

Data:

All rat brain cell consist of glia cell (astrocyte, oligodenderocyte and microglia) and neuron cell were characterized by RNAseq technique and generated a transcriptome database (http://web.stanford.edu/group/barres_lab/brain_rnaseq.html)[11]that ability to gene enrich in variant condition. In this study we enrich genome neuron in contrast to genome of astrocyte, oligodenderocyte and microglia.

Statistical analysis of molecular signatures:

To identify transcription factor enrichment related to gene expression in neuron compared to glia cells use GenTrail2 Database (http://genetrail2.bioinf.uni-sb.de/)[12]. In GenTrail2 algorism by applying TRANSFAC database determine significant TF related gene enrichment, TF complexes and TF families. To find the miRNA enrichment related genome expressed in neuron in comparison to glial cell use TRANSFAC database in GenTrail2 algorism. GenTrail2 algorism also determines the important protein or genes related to all these miRNA.

Network analysis:

For deep analysis on miRNA protein targets use network analysis to find out the most important protein in structural and functional analysis. So apply cytoscape software [13] to reconstruct network and analysis the network to find hub and bottleneck proteins. Protein –protein network reconstruct base on several databases include InnattDB-IMEx, Intact, MatrixDB, InnateDB, BIND, Reactom Fls, Mentha, MINT, Bhf-ucl, BioGrid, MBinfo, BAR, InnateDB, Uniprot and APID. For functional analysis use Bingo App [14] that run on cytoscape software. Other structural analysis performed by MCODE on cytoscape software to find the motifs.

RESULTS:

Gene enrichment of neuron cell against glia cells in tanscriptome database determined most abundant first genes according to highest expression which represented in table1. Supplementary table 1 represents the all 500 proteins and their fold changes in neuron cells against glia cells. Statistical analysis on the genes expressed in neuron by GenTrail2 based on TRANSFAC determined Transcription factor families which 2 of 23 according table 2 were significant. Table 3 represents the Transcription factors which 3 of 337 were enriched significantly. The Validated targets of these 3 TF were represented in table 4.

Table 5 represent 20 first important miRNAs enriched based on TRANSFAC database. This database recognized 85 significant miRNAs of all 183 miRNAs that link to the genes expressed in

neurons. All significant miRNAs enriched were representing in supplementary table 5. Protein that regulated by these miRNA combination represented in supplementary table 6.

Network analysis on these proteins determined the most important structural and functional proteins. Functional analysis by BINGO on these proteins determines the biological process related to development and differentiation that represent in table 7. Table 8 represented the highest degree and betweenness centrality protein in network from these proteins. Result of MCODE analysis represented in figur1. DNM1 is a seed of this motif.

Table 1: Gene expression in neurons when compare to glia cells

GENE	FOLD CHANGE
Reln	1774
Nhlh2	659.77
Slc17a6	560.94
Trp73	527.31
Nxph4	470.63
Npy	434.84
Sst	402.61
A930038C07Rik	346.24
Islr2	327.11
Lhx5	311.56
5330417C22Rik	279.67
Snhg11	268.36
Clstn2	242.62
1500016L03Rik	232.89
Ecel1	222.66
Tmem130	219.26
Dlx6os1	209.97

Table2: Transcription factor families enriched based onTRANSFAC database

Type	Rank	Name	Number of hits	Expected number of hits	q-value
enriched	1	DLX	2	0.014	0.0123
enriched	1	MSX	2	0.014	0.0123

Table3: Transcription factors enriched based on TRANSFAC

Type	Rank	Name	Number of hits	Expected number of hits	q-value
enriched	1	Ctcf	5	0.147	0.0020
enriched	2	Sp1	10	1.442	0.0036
enriched	3	Pax6	4	0.189	0.0449

Table4: Validated targets related to 3 transcription factors enriched based on TRANSFAC

Targets of transcription factor ctcf		
name	**score**	**GO biological process**
PCDHA2	20.035	homophilic cell adhesion via plasma membrane adhesion molecules (GO:0007156)
PCDHA3	23.309	
PCDHA5	24.429	
PCDHA6	17.759	
PCDHA7	14.741	
Targets of transcription factor Sp1		

Name	score	GO biological process
ABCC8	45.341	single-organism transport (GO:0044765)
ACHE	48.019	
ATP1A3	49.096	
CXADR	22.378	
DNM1	14.358	
GRIN1	19.815	
NEUROD1	34.234	
NTRK1	61.321	
PRTN3	24.067	
SLC17A6	560.940	
Targets of transcription factor Pax6		
name	**score**	**GO biological process**
HTR3A	41.063	pancreatic A cell differentiation(GO:0003310)
L1CAM	99.263	
NEUROD1	34.234	
PCSK1	160.970	

Table 5: significantly miRNAs enriched based on TRANSFAC

Type	Rank	Name	Number of hits	Expected number of hits	q-value
enriched	1	mmu-miR-15a-5p	32	4.227	1.79e-15
enriched	1	mmu-miR-15b-5p	32	4.227	1.79e-15
enriched	1	mmu-miR-16-5p	32	4.248	1.79e-15
enriched	1	mmu-miR-195a-5p	32	4.234	1.79e-15
enriched	5	mmu-miR-128-3p	31	3.982	1.99e-15
enriched	6	mmu-miR-103-3p	25	3.205	1.47e-12
enriched	6	mmu-miR-107-3p	25	3.184	1.47e-12
enriched	6	mmu-miR-1907	25	3.177	1.47e-12
enriched	6	mmu-miR-322-5p	25	3.191	1.47e-12
enriched	6	mmu-miR-497a-5p	25	3.191	1.47e-12
enriched	11	mmu-miR-34a-5p	25	3.394	4.16e-12
enriched	11	mmu-miR-449a-5p	25	3.387	4.16e-12
enriched	13	mmu-miR-153-3p	21	2.429	2.31e-11
enriched	14	mmu-miR-101a-3p	24	3.394	2.47e-11
enriched	14	mmu-miR-101b-3p	24	3.387	2.47e-11

Table 7: GO analysis based on BINGO in cytoscape.

GO-ID	Biological process	p-val
7268	synaptic transmission	1.0620E-11
48667	cell morphogenesis involved in neuron differentiation	2.5590E-11
48812	neuron projection morphogenesis	4.6015E-11
7409	axogenesis.	1.1208E-10
22008	neurogenesis	1.2284E-10
48731	System development	1.6916E-9
48666	neuron differentiation	1.0666E-8

Table 8: the most important proteins derived from network analysis of protein targets of miRNAs

Uniprot name	Symbol name	Between nesscentrality	Degree
P48318	Gad1	6.55E-04	5
Q80U57	RIMS3	1.77E-04	2
P35438	Grin1	0.553224	825

P60879	SNAP25	0.14458	172
P39053	Dnm1	0.11358	120
Q9R171	Cbln1	0.076631	40
P28652	Camk2b	0.075635	41
Q62277	SYP	0.07082	62
P05532	KIT	0.062539	39
P62823	Rab3c	0.05548	30
Q91XM9	Dlg2	0.039772	56
O08599	Stxbp1	0.036204	41
Q60867	Neurod1	0.035886	33
P23818	Gria1	0.03563	46
P35235	Syp	0.031522	31
P11627	L1cam	0.030439	46
P46096	SYT1	0.028635	32
P97792	Cxadr	0.015583	40

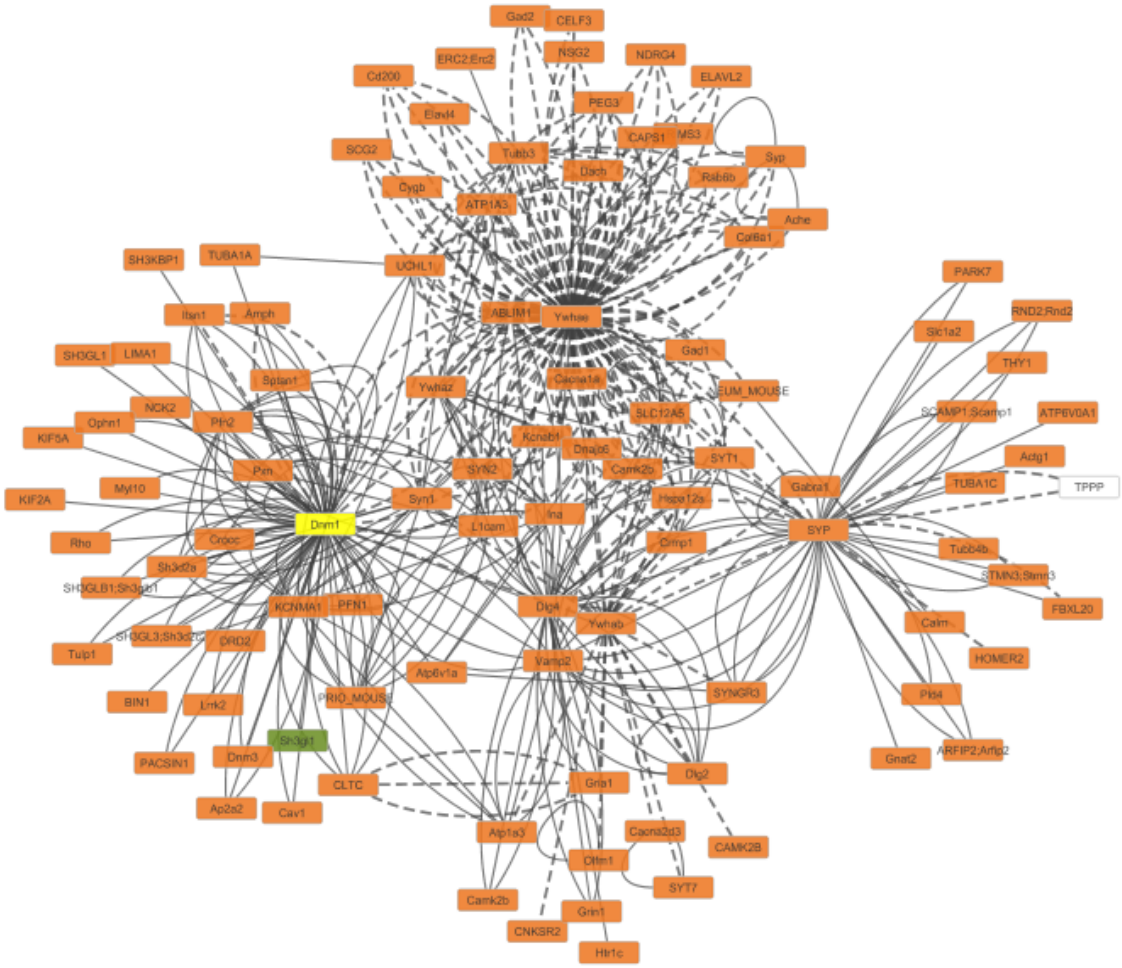

Figure1: Structural analysis by MCODE analysis in cytoscape software. Yellow node is related to seed protein.

DISCUSSION

A number of reprogramming approaches in cell induction approved and are in applicable stage in clinic. For neuron inductions and then apply in clinic, it has been encountered to a lot of challenges. In this study focus on seeking molecular factors with induction ability that induce glial cells to neurons. Based on transcriptome analyzing by comparing neuron with glial cells (astrocyte, oligodenderocyte and microglia) (table1) enriched 2 transcription factor families that related to DLX and MSX. DLX family involved in development and differentiation of neuron in several part of brain [15]. MSX family is general TF in cell morphogenesis in different cells [16]. However in reprograming different origin cells to neuron used a variety kind of TFs such as SOX2, BDNF,ASCL1, LMX1B, NURR1, Nurr1, Lmx1 a/b, Pax6 ،Neurog2 ،Ascl1 ،DLX2 ،NeuroD1, NeuroD4,Insm1,Prox1 andSOX11[5-10 and 17-19], here determined these two TF family may induce brain resident cells to neurons. The most significant TFs are Ctcf, Sp1 and Pax6. All proteins that regulate by these 3 TFs determined in table 4. The biological process related to Ctcf having contrary activity from positive regulatory to negative especially in gene expression and epigenetic regulation. In addition it can be involved in stem cell differentiation [20, 21]. Sp1 is TF with a variety of functions from activator to repressor activity [22-24]. Sp1 involved in development of a number of tissues. Finally Pax6 links to development of the eye, nose, central nervous system and pancreas and its isomers 5a functions as a molecular switch that specifies target genes [25]. Functions of all of these TFs were associated to functional neurons generation. So combination of them may induce glia cells to neuron cells.

In addition the present of TFs in differentiation, miRNAs as other regulatory molecules are capable to induce glia cell to neuron. Analyzing the transcriptome related the proteins expressed in neuron determined the significant miRNAs, as seen in table 5 and supplementary table6, the significant miRNAs that regulate expression of specific genes have critical roles. So that GO analysis base on cytoscape by BINGO (table 7) determined the important molecular function related to neurogenesis, synaptic transmission, cell morphogenesis involved in neuron differentiation, neuron projection morphogenesis and axogenesis.

Network analysis also identify the hubs, the node with the most degree that related to Grin1, SNAP25 and Dnm1. Grin1 with 825 interactions to other proteins can be valuable molecule that regulate or expressed by TF Sp1. According to supplementary table 6, the significant miRNA regulate Grin1. Since Grin1 connect to the most amount of proteins involved in differentiation, the significant miRNAs 16, 15b, 195a, 15a, 322, 107, 1907, 497, 103 and 153could be the most important reprograming factors to induce neuron cells.

Network analysis (table 8) also released Gad1 with the highest betweenness centrality but low node degree. Gad1 involved in several biological processes contain neurotransmitter synaptic transmission, biosynthetic process and neurotransmitter secretion [26]. According to supplementary table 7, expression of Gad1 controls by the present of miR-128, 101a, 19b, 673, 101b and 19a. So these miRNAs can be proposed for reprograming glia cell to neuron cells.

Network analysis by MCODE on miRNA targets revealed only one motif that its seed is Dnm1. Its function includes as microtubule-associated protein involved in receptor-mediated endocytosis and producing microtubule bundles. In addition epileptic encephalopathy is caused by its mutations [27]. Most important mRNA that regulates Dnm1 is miR-1897, 128, 673 and 672. This protein is expressed by transcription factor Sp1, so Sp1 also may be key molecule by this reprogram the neurogenesis.

CONCLUSION:

Result determined regulator proteins that are transcriptional factors and of these Sp1 is most valuable that regulate the expression of two most important proteins Grin1 and Dnm1. The miRNA related to proteins Grin1, Dnm1 and Gad1 could be factors for reprograming the neurons that needs experimentally investigate.

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REFERENCES

1. Asano, H., et al., Astrocyte differentiation of neural precursor cells is enhanced by retinoic acid through a change in epigenetic modification. Stem Cells, 2009. 27(11): p. 2744-2752.
2. Guo, Z., et al., In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. Cell stem cell, 2014. 14(2): p. 188-202.
3. Heinrich, C., et al., Generation of subtype-specific neurons from postnatal astroglia of the mouse cerebral cortex. Nature protocols, 2011. 6(2): p. 214-228.
4. Addis, R.C., et al., Efficient conversion of astrocytes to functional midbrain dopaminergic neurons using a single polycistronic vector. PloS one, 2011. 6(12): p. e28719.
5. Aravantinou-Fatorou, K., et al., CEND1 and NEUROGENIN2 Reprogram Mouse Astrocytes and Embryonic Fibroblasts to Induced Neural Precursors and Differentiated Neurons. Stem cell reports, 2015. 5(3): p. 405-418.
6. Niu, W., et al., SOX2 reprograms resident astrocytes into neural progenitors in the adult brain. Stem cell reports, 2015. 4(5): p. 780-794.
7. Niu, W., et al., In vivo reprogramming of astrocytes to neuroblasts in the adult brain. Nature cell biology, 2013. 15(10): p. 1164-1175.
8. Karow, M., et al., Reprogramming of pericyte-derived cells of the adult human brain into induced neuronal cells. Cell stem cell, 2012. 11(4): p. 471-476.
9. Roper, S.N. and D.A. Steindler, Stem cells as a potential therapy for epilepsy. Experimental neurology, 2013. 244: p. 59-66.
10. Caiazzo, M., et al., Direct conversion of fibroblasts into functional astrocytes by defined transcription factors. Stem cell reports, 2015. 4(1): p. 25-36.
11. Zhang, Y., et al., An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. The Journal of Neuroscience, 2014. 34(36): p. 11929-11947.
12. Stöckel, D., et al., Multi-omics Enrichment Analysis using the GeneTrail2 Web Service. Bioinformatics, 2016: p. btv770.
13. Shannon, P., et al., Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome research, 2003. 13(11): p. 2498-2504.
14. Maere, S., K. Heymans, and M. Kuiper, BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. Bioinformatics, 2005. 21(16): p. 3448-3449.
15. Pleasure, S.J., et al., Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. Neuron, 2000. 28(3): p. 727-740.
16. Bensoussan-Trigano, V., et al., Msx1 and Msx2 in limb mesenchyme modulate digit number and identity. Developmental Dynamics, 2011. 240(5): p. 1190-1202.
17. Wagner, J., et al., Induction of a midbrain dopaminergic phenotype in Nurr1-overexpressing neural stem cells by type 1 astrocytes. Nature biotechnology, 1999. 17(7): p. 653-659.
18. Dai, C., et al., PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces

oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. Genes & development, 2001. 15(15): p. 1913-1925.
19. Cornell-Bell, A.H., et al., Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. Science, 1990. 247(4941): p. 470-473.
20. Phillips, J.E. and V.G. Corces, CTCF: master weaver of the genome. Cell, 2009. 137(7): p. 1194-1211.
21. Ohlsson, R., R. Renkawitz, and V. Lobanenkov, CTCF is a uniquely versatile transcription regulator linked to epigenetics and disease. TRENDS in Genetics, 2001. 17(9): p. 520-527.
22. Infantino, V., et al., Identification of a novel Sp1 splice variant as a strong transcriptional activator. Biochemical and biophysical research communications, 2011. 412(1): p. 86-91.
23. Bonello, M.R. and L.M. Khachigian, Fibroblast growth factor-2 represses platelet-derived growth factor receptor-α (PDGFR-α) transcription via ERK1/2-dependent Sp1 phosphorylation and an atypical cis-acting element in the proximal PDGFR-α promoter. Journal of Biological Chemistry, 2004. 279(4): p. 2377-2382.
24. Yu, H.T., et al., Transcriptional regulation of human FE65, a ligand of Alzheimer's disease amyloid precursor protein, by Sp1. Journal of cellular biochemistry, 2010. 109(4): p. 782-793.
25. Singh, S., et al., Modulation of PAX6 homeodomain function by the paired domain. Journal of Biological Chemistry, 2000. 275(23): p. 17306-17313.
26. Chessler, S.D. and Å. Lernmark, Alternative splicing of GAD67 results in the synthesis of a third form of glutamic-acid decarboxylase in human islets and other non-neural tissues. Journal of Biological Chemistry, 2000. 275(7): p. 5188-5192.
27. Study, T.D.D.D., Large-scale discovery of novel genetic causes of developmental disorders. Nature, 2015. 519(7542): p. 223-228.