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Gene expression regulation during differentiation from murine ES cells due to microRNA

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We have infered gene expression regulation via microRNA using both gene expression profile and target gene tables during the differentiation from embryonic stem cell to neuronal cells. Comparison between obtained list of microRNA which regulates gene expression significantly and that of miRNAs whose expression level changes significantly results in significant overlap.

1. Introduction

MicroRNAs (miRNAs) are the single strand RNAs with the length from 20 to 25 nucleotide in cells. It is a member of non-coding RNAs which are supposed to control gene expression. They are believed to suppress expression of target genes, by aligned to complimentary seed sequence with the length of eight nucleotide, which is typically located at 3' untranslated region (UTR) of target genes. Although it is possible to validate target genes experimentally[1)], it is a very time/cost consuming process. Thus, usually, computer oriented predictions are employed to list target genes of each miRNA.

Recently[2),3)], we have proposed MiRaGE method which estimates amount of contribution of each miRNA to target gene regulation. We have validated our algorithm by analysis of gene expression profiles of miRNA-transfected cells and computer-predicted potential miRNA target lists; our method mostly can correctly predict the transfected miRNA as only one significant miRNA after multiple comparison correction.

In this paper, we have applied MiRaGE method to the process during the differentiation from embryonic stem (ES) cells to neuronal cells. The estimated miRNAs which regulate genes significantly has overlaps with the list of miRNAs whose expression levels change during differentiation.

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2. Materials and Methods

2.1 Gene expression data

Gene expression data is downloaded from GEO by the accession number GSE11523[4)]. In this paper, data for the differentiation from ES cells to neuronal cells are used.

2.2 miRNA expression

miRNA expression data is taken from smiRNAdb[5)]. We have used "Sample Comparison" page to extract miRNA expression for "ESC-WT-frac-21" and "ESC-fem" set versus "Cerebellum", "Cortex", "Midbrain", "Brain-FMR-KO","Brain-WT" and "Hippocamp-HT22" set, i.e., differentiation from ES cells to differentiated brains. Then 96 miRNAs are selected as downregulated miRNAs.

2.3 MiRaGE method

Principal component analysis (PCA) is applied to gene expression profile during 6 days. Since there are two biological replicates for each day, thus in total $2^6 = 64$ combinations are obtained. PCA is applied to each of all combinations. miRNAs whose target gene expression is significantly upregulated are listed by MiRaGE method[2),3)] based upon the 1st principal components obtained for each of 64 combinations. Then miRNAs listed at least once as top 100 significant miRNAs are selected.

3. Results

First we have picked up overlap between miRNAs listed by MiRaGE and those by smiRNAdb. Since target genes and miRNAs are expected to have reciprocal relation, these two set should have significant overlap. Since smiRNAdb does not include miRNAs having names with "*", we have also shown the results when such miRNAs are excluded from the analysis via MiRaGE method (see Table1). It is clear that many biologically interesting miRNAs are listed[6)]. For example, mir-302 cluster is known as being expressed in ES cell. MiRaGE correctly detected that their target genes are upregulated. mir-200, mir-429 and mir-141 is expected to be induced by cMyc, which is ES cell marker genes. mir-294 and

Table 1 miRNAs which are selected as downregulated ones by siRNAdb and are selected by MiRaGE method as those whose target genes are upregulated. Freq. means number of times listed by MiRaGE as top 100 miRNAs whose target genes are upregulated. Left : overlaps with the list of downregulated miRNAs. Right: same as left, but miRNAs with "*" are excluded when top 100 are selected via MiRaGE method.

miRNAs	Freq.
mmu-miR-200b	64
mmu-miR-200c	64
mmu-miR-23a	64
mmu-miR-23b	64
mmu-miR-291a-3p	64
mmu-miR-429	64
mmu-miR-294	63
mmu-miR-295	63
mmu-miR-302a	63
mmu-miR-302b	62
mmu-miR-302d	62
mmu-miR-199a-5p	60
mmu-miR-141	44
mmu-miR-200a	44
mmu-miR-409-3p	43
mmu-miR-369-3p	23
mmu-miR-96	7
mmu-miR-674	5
mmu-miR-467b	4

miRNAs	Freq.
mmu-miR-200b	64
mmu-miR-200c	64
mmu-miR-23a	64
mmu-miR-23b	64
mmu-miR-291a-3p	64
mmu-miR-297a	64
mmu-miR-29a	64
mmu-miR-429	64
mmu-miR-467a	64
mmu-miR-467b	64
mmu-miR-467c	64
mmu-miR-467d	64
mmu-miR-467e	64
mmu-miR-669b	64
mmu-miR-669d	64
mmu-miR-294	63
mmu-miR-295	63
mmu-miR-302a	63
mmu-miR-302b	62
mmu-miR-302d	62
mmu-miR-199a-5p	60
mmu-miR-199b	58
mmu-miR-34a	53
mmu-miR-130a	44
mmu-miR-141	44
mmu-miR-200a	44
mmu-miR-409-3p	43
mmu-miR-369-3p	23
mmu-miR-96	7
mmu-miR-674	5
mmu-miR-467b	4

mir-295 belong to famous mir-290 cluster which is known as being expressive is murine ES cells. If we consider that miRNA expression list is taken from independent samples, this coincidence is remarkable enough.

4. Conclusion

In this paper, we have applied MiRaGE method to murine ES cell differentiation to neuronal cells. MiRaGE method correctly detected miRNAs' target gene regulation under the reciprocal relationship. Thus, we have succeeded in showing miRNAs expressive in ES cells surely regulate target genes.

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