

Study of miRNA mRNA Modules and their Relationship with Cancer Pathways

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Abstract

miRNAs (microRNAs) are small RNAs which regulates mRNA target genes and supposed to be involved in those small RNAs that not only recognize but also regulate mRNA target genes. Evidences are relevant enough to show that they are key regulators for various functions including development as well as diseases like cancer. Aim is to predict miRNA mRNA regulatory modules which consists of miRNAs and target genes that work together in gene regulation influencing biological processes. Consequently the method provides source for miRNA and target sets found to be closely related parts in the gene regulatory pathways. mRNA target genes are involved in cancer pathways and have roles both as tumor suppressor and oncogenes. The study includes the development of a link between miRNA mRNA modules and cancer pathways.

Keywords: microRNA, mRNA, Regulatory modules, Pathway.

1. INTRODUCTION

Lee and his co-workers gave an idea of microRNA in 1993, although the name was given in 2001 when it was discovered in *Caenorhabditis elegans* [1, 2]. MicroRNAs (miRNAs) are transcribed non coding RNA with a limited length of nearly 20 to 25 nucleotides found to be involved in numerous biological processes [3,4]. microRNA binds to 3' UTRs of mRNA targets and are responsible for gene regulation [5, 6] with mRNA destruction or translational inhibition. Apart from negative effect on gene expression, they are also found to be involved positively [7] with functions both in plants and animals from leaf development to larval development. They are responsible for many diseases affecting nearly every organ of human body and have major roles in tumor both as promoter and suppressor [8, 9]. There role in the scenario of cancer is well known, as around 50% of them are found on cancer related genomic sites thus showing their involvement in the disease [10]. Gene regulation by miRNAs is really noticeable as a single miRNA targets many genes. MicroRNAs (miRNAs) recognize and regulate their target genes. They regulate various functions related to development and disease, including cancer. Concept of miRNA regulatory modules was first told by Yoon and De Micheli. They are said to be combinations of miRNA and their target mRNAs having similar function and biological process. Probabilistic learning methods [11] and rule based methods [12] are well known methods that help in finding regulatory modules. Thus candidates are constructed for miRNA regulatory modules.

2. MATERIALS AND METHODS

2.1.) Problem Formulation

Goal was to find miRNA-mRNA modules on the basis of miRNA and mRNA expression profiles and the miRNA target binding score matrix.

- $A = \{a_1, a_2, \dots, a_{Pa}\}$, represents the set of miRNAs.

- $B = \{b_1, b_2, \dots, b_{Pb}\}$, represents the set of mRNAs

, where $P_a =$ number of miRNAs and $P_b =$ number of mRNAs.

- Subset (A' , B') was supposed to be a module, where $|A'| \leq |A|$ and $|B'| \leq |B|$.

- $P_a \times P_a$ ($P_b \times P_b$) matrices were formed representing the expression profiles of miRNAs and mRNAs respectively.

- $P_a \times P_b$ represents miRNA target binding scoring matrix.

Thus modules were selected which contained miRNA and mRNA subsets.

2.2.) Dataset Preparation

GCT files for mRNA expression genes and microRNA were studied here. Around 89 human tissue samples (68 tumor and 13 normal) for 11 tissues (colon, pancreas, kidney, bladder, prostate, ovary, uterus, lung, mesoderm, breast) were studied. miRNA data files and mRNA data files were obtained as matrices as following:

Figure 1 shows the expression profile data of mRNA and Figure 2 shows the expression profile data for miRNA for different samples.

i

) mRNA expression profile dataset sample:

mRNA Sample	Symbol	TC2	TP2	TK1	TBL1	TPR1	TO4	TUT1
A28102_at	GABRA3	5.16662	6.99321	7.04974	6.79776	6.49894	6.46628	6.17345
AB000114_at	OMD	5	6.06779	5	6.10229	6.69284	6.65208	5.6753
AB000115_at	C1orf29	7.69092	7.03985	7.11246	7.5488	5.82759	8.36482	6.0533
AB000220_at	SEMA3C	6.16463	6.65429	5.027	8.8278	9.37305	6.93015	6.71709
AB000381_s_at	GML	5.4516	5	5.9561	5	5	6.379	5

Figure 1: Sample of miRNA expression data

ii.) miRNA expression profile dataset file:

miRNA	NC3	TC3	NP1	TP1	NK1	TK1	NB1	TB1
hsa-let-7d	7.33001	9.77485	9.53409	7.75051	9.45428	8.14952	9.43228	9.32095
hsa-let-7e	7.16847	9.32795	7.99561	6.84044	9.55354	8.13731	8.22252	8.57449
hsa-miR-1	11.4035	11.3218	8.03782	5.56835	7.62402	5	7.69383	5.71716
hsa-miR-101	9.47113	8.94853	9.43007	9.23957	11.0572	7.53196	9.77271	10.0803
hsa-miR-103	8.83539	9.60971	8.75641	9.04829	10.8527	6.69919	9.58058	10.8846

Figure 2: Sample of miRNA expression profile dataset

iii.) miRNA target binding score matrix:

After constructing above matrices, a new matrix is constructed using PICTAR tool, which is the miRNA mRNA target site matrix

miRNA/mRNA	APC	COL1A2	ESR1	FGFR3	GCH1	JAG1	PKD2	PTEN	BLMH
hsa-let-7d	0	3.06	0	0	0	0	0	0	0
hsa-let-7e	0	2.53	0	0	0	0	0	0	0
hsa-miR-1	0	0	0	0	3.18	0	0	0	0
hsa-miR-101	0	0	0	0	0	0	0	0	0
hsa-miR-103	0	0	0	0	0	0	0	0	2.95
hsa-miR-106b	0	0	0	0	0	0	3.03	2.34	0

Figure 3: Sample of miRNA mRNA target binding score matrix

2.3.) Correlation

A correlation matrix developed using miRNA expression profile and mRNA expression profile helps to construct miRNA mRNA correlation matrix. From this matrix along with information gained from miRNA mRNA target binding score matrix, the final matrix called the miRNA mRNA module matrix is constructed. For further filtration of miRNA mRNA combinations the data is subjected to GSEA analysis. GSEA analysis was done which resulted in the relational representation between miRNA and target genes.

3.3) Pathway Analysis:

A biological system contains gene products which participate in a pathway constituting a biological task. [19] Metabolic pathways signal transduction pathways etc. are categories of such pathways. The mRNA target genes which were part of miRNA mRNA modules were considered and their relation with various pathways searched. The relations for the pathway databases were obtained from published PubMed abstracts although the pathways were constructed.

3. RESULTS AND DISCUSSION

3.1) miRNA mRNA modules:

S.no.	miRNA mRNA module
1	hsa-miR-143,hsa-miR-181a,NOVA1,ST8SIA4,ZFP36L1
2	hsa-miR-125b,hsa-miR-145,DAG1,NEDD9,YES1,BMPR2,PTPRF
3	hsa-miR-126,hsa-miR-181b,NOVA1,PCAF,EIF4A2
4	hsa-miR-212,hsa-miR-132,HIC1,OVCA2,BCL6
5	hsa-miR-27a,hsa-miR-143,NOVA1,CDH5,ADD3
6	hsa-miR-101,hsa-miR-19a,hsa-miR-221,ATXN1,CTCF,RAB1A
7	hsa-let-7e,hsa-miR-26a,ARID3A,TAF5,HAS2,NOVA1,AKAP6,DYRK1A
8	hsa-miR-149,hsa-miR-29a,BCL2L2,PLAG1,SP1,CBX1
9	hsa-miR-17-5p,hsa-miR-25,CIC,EDG1,SSFA2,PCAF,SALL1
10	hsa-miR-134,hsa-miR-15a,KPNA3,RUNX1T1,EPHA7

Figure 5: Some of the miRNA mRNA modules obtained

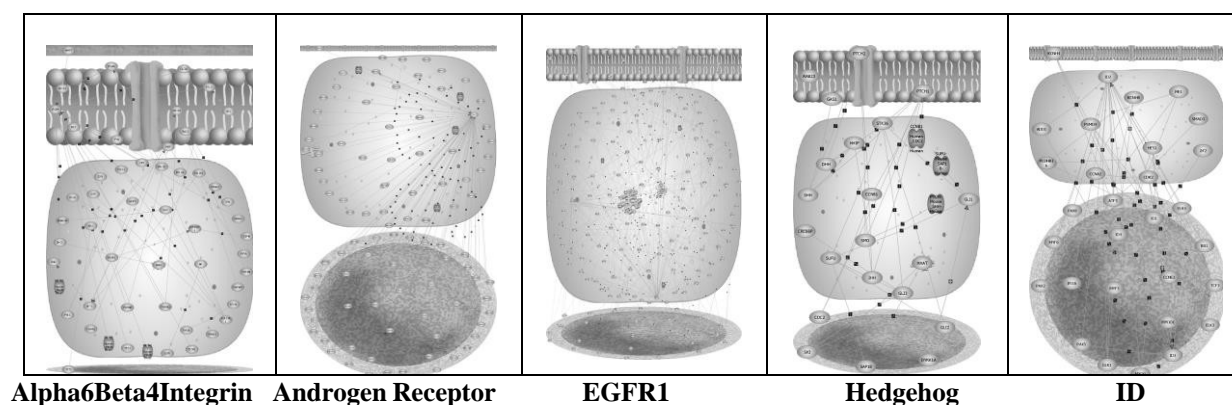


Figure 6: Some of the cancer pathways constructed

No.	Name	Detail	Few genes	Function
1	Alpha6Beta4Integrin	Integrin protein complex with α and β chain heterodimerises to form receptor	ABL1,EGFR, YES,BPAG	Hemidesmosomes formation, cell adhesion, attachment, apoptosis
2	Androgen Receptor	Binds to specific DNA sequences influencing androgen responsive gene transcription	AES,BRCA1, CDC2,CDK9	Regulates gene expression in glycolysis, growth, cell development, apoptosis, lipid metabolism
3	EGFR1	Uses EGF receptor with receptor tyrosine kinases leading to receptor dimerization, protein kinase activity	AKT1,BCAR1,CREB	Activates RAS and MAP kinase pathways; involved in apoptosis and cell cycle.
4	Hedgehog	Secretive proteins which includes Sonic, Desert, Indian which causes transmembrane binding on diffusion	CCNB1,DHH, PTCH,RAB23	Important in cellular processes, cell cycle proliferation, cell regulation and embryogenesis.
5	ID	Members of helix loop helix family with 4 members -Id1 to Id4. Consists of transcription factors and stimulation concerned with ligands	ADD1,CDK2, ID1,SMAD3	Regulate cell growth, differentiation, apoptosis, senescence and angiogenesis.

Figure 7: Cancer Pathways-genes-functions

Gene expression analysis in combination with perturbations, treatments, mutations were studied for processes like signal transduction and metabolism which helps to get knowledge about the molecular and functional effects on genes. Gene expression data helps in studying molecular pathways for the regulatory effect of genes. The pathway specifies the role of entities in a molecular reaction which is both participatory and regulatory. Gene interactions which may be activating or inhibitory, direct or indirect were all under pathway study. Reactants and products have participatory roles while catalysts act as regulators which could be effective both positively and negatively.

4. CONCLUSION

microRNAs have roles in cancer therapy as they behave both as tumor suppressors and oncogenes. Examples of miRNAs oncogenes : miR-21 and miR-17-92 and tumor suppressive genes : let-7 and miR-34. These miRNAs regulate mRNAs causing gene regulation. Thus,

combinations of miRNA and mRNA provide knowledge about how they are involved in various biological processes. miRNA connects and regulates pathways as well as act as switches for genome regulating gene products. Regulated genes are both oncogenic and tumor suppressive with roles of acting as drug targets by various pharmaceutical and biotechnology industry. miRNAs have high therapeutic role as they have multiple target genes. miRNA with their target mRNA studied for their integrated function by the formation of miRNA mRNA module. Pathways with target genes were also developed and thus miRNAs develop to be a part of regulatory network in relation with their target mRNAs with information obtained from miRNA mRNA modules. Gene regulatory pathways, cancer signalling and immune signalling were formed that showed relations among the genes whether its activation or inhibition. Now considering the functionally similar miRNA mRNA modules, miRNAs could be obtained which are related to the genes which are part of these pathways showing the same effect of miRNA as the genes

have. miRNAs are so small but they act just in a huge way to obtain such regulatory networks. Gene regulation caused by these miRNAs helps in targeting several tumor causatives. Thus, miRNAs are important in therapeutics helping to avoid various diseases including not only cancers but various heart disease like cardiac hypertrophy, neurological diseases like Alzheimer's syndrome, regulating cholesterol, causing immunodeficiency and regulating viral diseases.

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