Identifying the Common Target Genes for miR-21 Using Functional Enrichment Analysis

Sim-Hui Tee

***Abstract*—miR-21 is a well-studied microRNA which has been implicated in many cancers. However, it is still no consensus pertaining to its target genes. This study employed a functional enrichment analysis to identify the common target genes for miR-21. Genomic data were compared using three different algorithms to identify the common target genes for miR-21. Our results enhance the understanding of the target genes and the relevant cellular pathways for the miR-21-related diseases.**

***Index Terms*—Bioinformatics, database, functional enrichment analysis, genes, microRNA.**

I. Introduction

MicroRNAs (miRNAs) are small non-coding RNAs that regulate the expression of messenger RNA (mRNA) genes [1]-[2]. In view of the fact that miRNAs play a significant role in cellular processes [3]-[5], the deregulation of miRNAs will lead to aberrant cellular activities which contribute to diseases [6]-[7]. Despite an enormous number of experiments has been carried out at molecular and computational level, most of the miRNA-target pairs are still remained invalidated [8].

This study probes into miR-21, which is a miRNA that implicated in many cancers [7], [9], to identify its target genes. miR-21 has been observed to over-express in breast cancer, lung cancer, colon cancer and brain cancer [10]. It is a well-known regulator of apoptosis [10], which is a mechanism of programmed cell death. Deregulation of miR-21 will contribute to the proliferation of cell resulting in tumor formation. The identification of the target genes for miR-21 is thus important in the molecular therapy. In this study, the target genes for miR-21 are identified using functional enrichment analysis. Genomic databases were used to compare the data that derived from different algorithms. Our results enhance the understanding of the target genes and the relevant cellular pathways for the miR-21-related diseases.

II. Methods

We used miRGator [11], an integrated miRNA database, to mine the target genes for miR-21 and the respective physiological pathways. Three different algorithms, which are miRanda, TargetScanS, and PicTar-5Way, were used to identify the common target genes. miRanda provides the information of conserved miRNAs across two species; whereas TargetScanS and PicTar-5Way are algorithms that provide the conserved miRNAs across five species. We filtered the non-common target genes that obtained from these three different algorithms. UniGene was used to study the Expressed Sequence Tag (EST) profile of the common target genes of miR-21. In addition, UCSC Genome Browser [12] was used to visualize the chromosomal position of the common target genes of miR-21.

Manuscript received June 9, 2012; revised July 5, 2012.

Sim-Hui Tee is with Multimedia University, Cyberjaya 63100, Malaysia (e-mail: shtee@mmu.edu.my).

III. Results and Discussion

miRGator was used to identify the target genes for miR-21. Using the functional enrichment analysis with a P-value setting to <=0.01 for three different algorithms (miRanda, TargetScanS, and PicTar-5Way), we obtained a list of target genes with the information pertaining to the binding sites for miR-21. The number of predicted pathways identified using miRanda, TargetScanS and PicTar-5Way are 17, 11, and 3, respectively. To identify the common target genes for miR-21, we narrow down the functional pathway enrichment analysis to find the common pathways among three different algorithms. Sprouty Regulation of Tyrosine Kinase Signal (SRTKS) is the only molecular pathway that is common to the three algorithms. Table I shows the details for this common pathway.

TABLE I: Common Pathway Derived From Three Algorithms (Miranda, TargetScans, And PicTar-5way)

Pathway name	Frequency in target genes	P-value	Fold ratio	Algorithm used
Sprouty regulation of tyrosine kinase signal (SRTKS)	40%	0.0001	110.5	PicTar-5Way
	3.26%	0.0039	9.0082	miRanda
	9.38%	0.0002	25.8984	TargetScanS

Sprouty is an inhibitor of Epidermal Growth Factor Receptor (EGFR) family signaling, where EGFR is one of the subfamilies of Tyrosine Kinase [13]. Deregulation of SRTKS will induce cancers as a result of overexpression or mutation of EGFR [13]. As shown in Table 1, the P-values obtained using the three algorithms display a consistent significance. The fold ratio, which is a measurement of expression level, is high, indicating that SRTKS is a major pathway of miR-21 target genes.

There are two genes that involved in SRTKS pathway, which are RASA1 and SPRY2. RASA1 is a Ras-related gene

DOI: 10.7763/IJFCC.2012.V2.108

which has been reported as a miR-21 target gene in the regulation of apoptosis, a deregulation of which will induce metastasis in colorectal cancer [14]. SPRY2 is a gene that encodes a protein belonging to the Sprouty family member [15]. Using UniGene, we have observed a wide variation of the transcript count in the gene expression pattern for both RASA1 and SPRY2. Of 26 expression pattern profiles for cancers, both RASA1 and SPRY2 are implicated in most of the tumorigenesis. However, we observed the non-expression of these genes in a few cancers, as listed in Table II These data indicate that the deregulation of miR-21 on RASA1 and SPRY2 will not have any impact to cause the cancers as listed in Table 2. Compared to SPRY2, RASA1 is implicated in more cancers implying that it is a more important gene in the cellular processes.

TABLE II: NON-EXPRESSION OF THE COMMON TARGET GENES OF MIR-21 IN CANCER TISSUE

RASA1	*SPRY2*
Bladder carcinoma Prostate cancer	*Adrenal tumor* Bladder carcinoma Cervical tumor Esophageal tumor Leukemia Lung tumor Ovarian tumor Prostate cancer Retinoblastoma

UCSC Genome Browser was used to visualize the chromosomal position of RASA1 and SPRY2, as shown in Figure 1 and 2, respectively. RASA1 displays the mutation in the form of insertion (blue bar) and deletion (red bar); whereas SPRY2 shows only deletion (red bar) in its codon sequences.

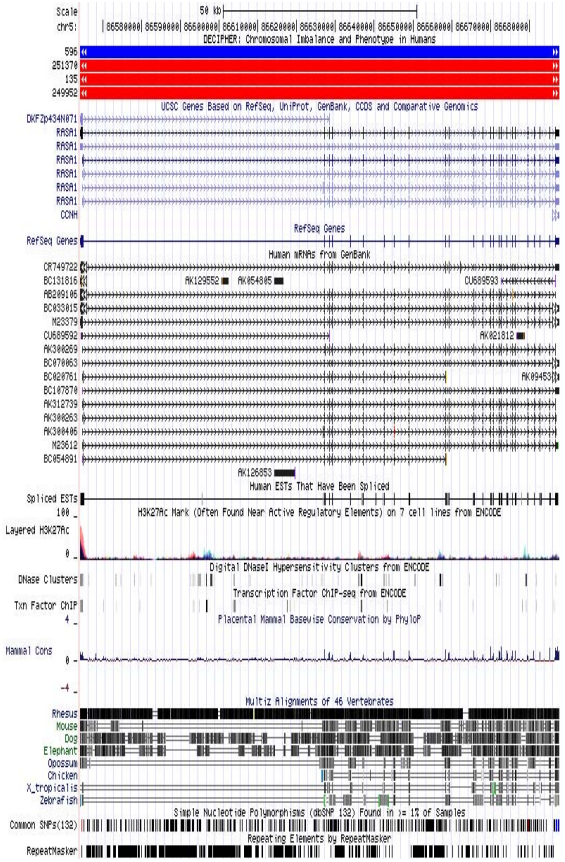

Fig. 1. Chromosomal position of RASA1

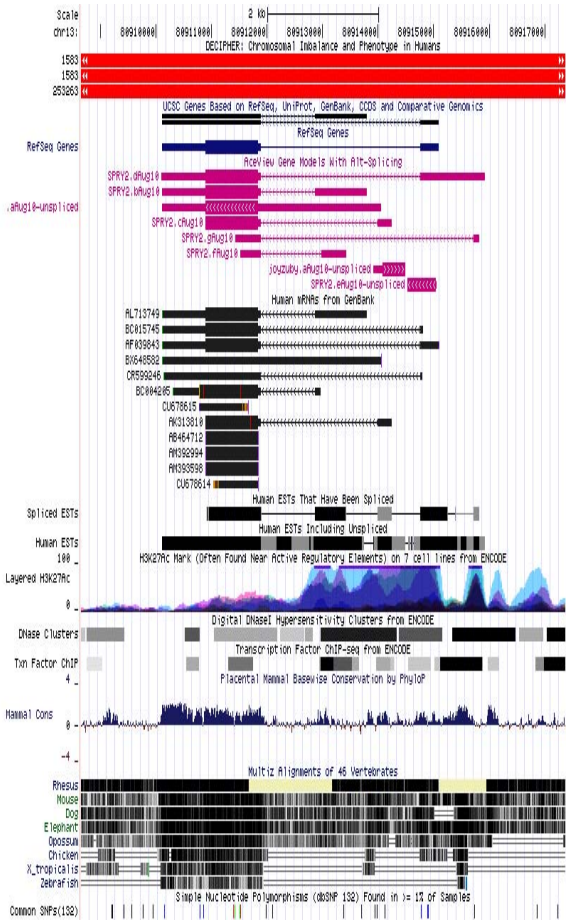

Fig. 2. Chromosomal position of SPRY2

IV. CONCLUSION

It is important to identify the target genes for miR-21 in cancer therapies. Due partly to the limitation of technology, however, different prediction algorithms yield different predicted target genes. This study carried out a functional enrichment analysis, using three algorithms (miRanda, TargetScanS, and PicTar-5Way), to identify the common target genes for miR-21. Our results show that RASA1 and SPRY2 are two common target genes for miR-21, as they are involved in the common cellular pathway, which is SRTKS. These findings enhance the understanding of the target genes for miR-21 which may lead to the further studies on the binding sites and binding machinery on the target genes.

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Sim-Hui Tee is a lecturer at Multimedia University, Malaysia. His research interests lie in Software Engineering and Bioinformatics. He has published numerous papers in both fields in the recent years. Besides, he has served as reviewers and editorial board members for several conferences and journals.