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Original Paper

Identification of Novel MicroRNAs and Their Targets in Leukemia Cancers: A Computational Approach

Adham Fani Maleki[1], Balal Sadeghi[2*], Mohammad Hadi Sekhavati[3], Habib MotieGhader[4]

ABSTRACT

MicroRNAs (miRNAs), one of the most abundant groups of regulatory non coding RNAs in multicellular organisms, play important roles in many fundamental cellular processes. More than four hundred miRNAs have been identified in humans and the deregulated expression of miRNA has been also shown in many cancers. Despite the postulated involvement of miRNAs in tumourigenesis, there are only a few examples where an oncogene or a tumour suppressor has been identified as a miRNA target. Here, we present an in silico analysis of potential miRNA- MYC interactions. We showed evidence for the regulation of c-MYC, one of the most potent and frequently deregulated oncogenes, via the predicted binding site in transcriptional and post transcriptional regions. In this work, bioinformatics approach for the prediction and validation of possible targets for miRNAs has been used. A list of putative targets is available and validation of which would be experimentally validated.

Key words: MicroRNAs, MYC, Leukemia Cancers, Computational Approach

Author Information:

1Embryonic and Stem Cell Biology and Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran.

2Department of Food Hygiene and Public Health, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

3Department of Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran.

4Laboratory of Systems Biology and Bioinformatics (LBB), Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran.

Currently MicroRNAs (miRNAs) considered as outstanding class of sSmall regulatory non-coding RNAs (1). MiRNAs are single-stranded consist of 18 -24 nucleotides (nt) in length transcripts cleaved from 70- to 100-nt hairpin precursors by RNase III-type enzyme and are encoded in the genomes (2). The mature miRNA has ability to regulate gene expression by inhibiting translation and/or by inducing degradation of target messenger RNAs (3). MicroRNAs are important to cell function because of their ability to control mRNA translation (4).

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Corresponding Author E-Mail: Sadeghi.balal@gmail.com

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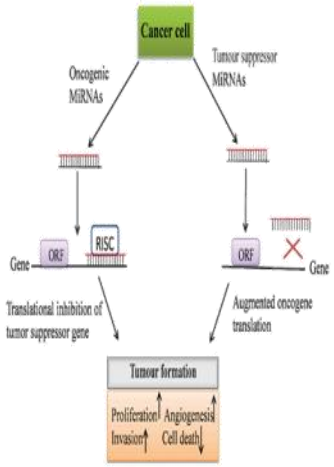
According to various studies, miRNAs has critical role(s) in various regulatory processes such as embryonic development, protein secretion, viral infection, cell self-renewal/differentiation, cell division and cell proliferation (4). Several scientific reports stated that many miRNAs, referred to as onco/tumor suppressor miRNAs, play role (s) in the development of various human malignancies (5-8). Aberrant expression of miRNAs involved to carcinogenesis by promoting the expression of proto-oncogenes or by inhibiting the expression of tumor suppressor genes (3). In addition, it is about 50% of genes that encoding miRNAs are located in cancer associated genomic regions (CAGR) or fragile sites (2). Various types of leukemia including: acute lymphoblastic leukemia (ALL), Acute myeloid leukemia (AML) and Chronic lymphocytic leukemia (CLL) has been researched extensively from miRNAs point of view and currently it has been identified several miRNAs involved in all types of leukemia (3). miR-15a-16-1 was the first miRNAs identified as regulator of CLL (9). Currently there are several miRNAs identified and verified in different type of leukemia cancers (10). miRNAsMiRNAs not only arouse interest among researchers studying molecular mechanism and also gene regulation in different type of malignancies but also they theoretically represent therapeutic targets.

A



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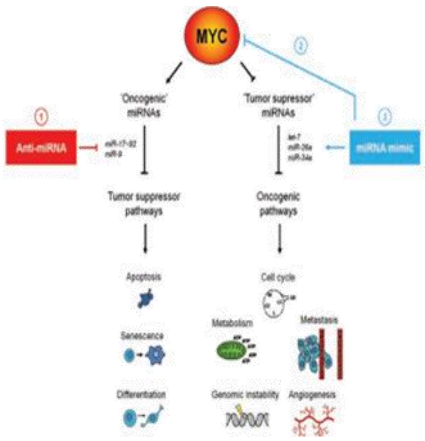

Figure 1: A) MicroRNAs as oncogenes or tumour suppressor genes B) Strategies to target the MYC miRNA interplay in cancer.

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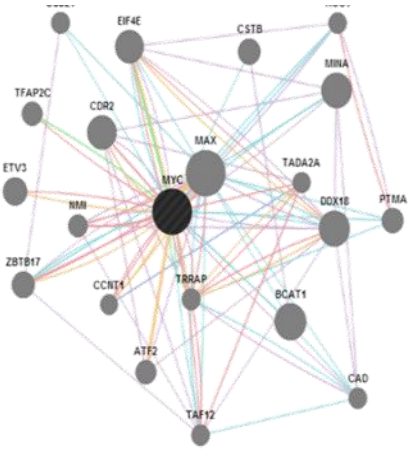

Figure 2: Interactions between MYC-associated miRNAs and their potential mRNA targets

Material and Methods

A: Computational approach to miRNAs gene finding

The UTR sequences were retrieved from GenBank. The current GeneBank format was converted to a FASTA file. In order to identify potential and possible hairpin structures of miRNAs genes in MYC within the area of interest (5' UTR, 3' UTR, iIntronic and promoter), SCC profiler (13) and miPRED (14) online classifier (http://www.bioinf.seu.edu.cn/miRNA) programs were employed.

For the identification of putative miRNAs precursors in MYC introns CID-miRNA was used along with the prediction of Dorsha processing sites using Microprocessor SVM program (15, 16).

Hence, search for new miRNAs with computational approaches could be useful for better understanding of molecular pathways as well as possible new drugs (11).

MYC plays a role as transcription factor that involved in cell cycle and cell growth by acting downstream of many ligand membrane receptor complexes and signal transduction pathways. (12). MYC is also a proto-oncogene that contributes to the genesis of many human cancers (Figure 1) (11).

Currently there are strong evidences that MYC has critical roles in molecular pathways involved in Hodgkin lymphomas and also chronic lymphocytic leukemia (CLL) (12). Over expression of MYC in B lymphocytes is to be sufficient to increase cell growth that could be unlinked from cell proliferation. In addition, its central role in the growth programs of T lymphocytes. These evidences demonstrate necessity of study on common miRNAs in different leukemia types as possible regulator of MYC. In this study, at the first phase we evaluated MYC genome; 3' UTR, 5' UTR and also promoter to find possible new miRNAs (s). In the second phase, potential of common miRNAs in different leukemia types for possible interaction with MYC has been evaluated.

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miRBase (http://www.mirbase.org/index.shtml) database was also used to determine the degree of conservation of miRNAs and its precursor sequence along with blat search against human genome (17). The folding temperature was set to 37°C. We used a cut-off value -18 kcal/mol.

B: Computational approach to target prediction

DIANA-microT web server was employed in order to find potential target MYC for miRNAs (18, 19).

The prediction was also performed by using RNAhybrid (20, 21), targetscan (http://www.targetscan.org/vert_61/), mirtarbase (http://mirtarbase.mbc.nctu.edu.tw/), ElMMo (http://www.mirz.unibas.ch/ElMMo3/index.php), mirdb (http://mirdb.org/miRDB/), MatureBayes(18), pmirp (22) and mirz (23) online tools. Diana-mirpath (24) and geneset2miRNA (25) online tools used to find the pathways in which MYC-miRNAs is involved. Also, for extraction of common miRNAs in different types of leukemia cancers miRCancer and dbDEMC were developed (25, 26). The basis of the assumption that the epistatically interacting genes operated in the same pathway, to search for the co-expression of miRNA target genes with miRNA host gene, GENEMANIA online tool was used (27).

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Results and Discussion

The goal of the bioinformatics analysis was to find potential binding sites in the set of human MYC oncogene. In humans, as in other animals, the miRNA regulation is accomplished by binding the miRNAs predominantly to the 3' untranslated region (UTR) of the messenger RNA (28-30). For this reason the investigation and scanning for miRNA:mRNA binding sites was limited to this sequences of Homo sapiens. In first, we could not find any region of Myc gene with potentials change to miRNA genes. After the scanning and filtering of the MYC dataset and miRNA genes, positive results for binding sites in sequences.

(MYC genes) from this set were obtained. Supplementary material presents an arbitrary selection of known MYC-genes with their predicted miR-regulators of the analysis. The result was then graphed using GENEMANIA to show the interactions between MYC-associated miRNAs and their potential mRNA targets. The nodal nature of the targets and their overlaps suggests that MYC-associated miRNAs work in concert. We have checkedchecked all the human MYC potential interactions and found that they are all located in the 2/3-th region of the 3'UTR. However, there is no obligatory region that has been proven so far for the localization of the miRNA binding sites within the 3' UTR. miRNAsMiRNA sites also have preferences to the of 3'UTRs sequence composition. Our *in* silico analysis revealed also an additional interaction of potential interest (Supplementary material).

Deregulation of MYC contributes to the development a wide variety of human tumors, including leukemia and neuroblastoma. Counteracting the tumor-promoting activity of MYC is therefore an appealing anticancer strategy. Approaches targeting different levels of the MYC pathway are currently being explored. With the characterization of the interplay between MYC and miRNAs, yet another layer of targeting opportunities has emerged. Thus the rapidly growing understanding of the biology of miRNAs in cancer as well as the development in RNA-mediated therapeutic approaches in recent years promises new, exciting modes of specific targeting in cancer.

More than 28000 human miRNAs have been identified so far and bioinformatics analyses predict that the total number may be increased. More than 2000 miRNAs have been already correlated with cancer. Moreover, the expression profile of miRNAs from cancer tissues has been suggested to be prognostic concerning the speed of cancer development and its malignancy. A correlation between the expression of particular miRNAs and their effects on target oncogenes followed by tumourigenesis is beginning to gain evidence. Of particular interest is the possibility that the growth of cancer cells can be repressed by artificial modulation of the level of miRNAs. Despite the accelerated research interest in the miRNA-regulation, there are only a few examples where an oncogene or a tumour suppressor has been identified as a miRNA target and the regulatory

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connection validated. Our results indicate that the correlation of the MYC gene, which is one of the critical oncogenes, is modulated by numbers of miRNAs, expanding the numbers of validated oncomirs.

Since the significant increase of miRNA targets, all previous validations (including some "oncomirs") have been gathered by Sethupathy et al, in a database called TarBase (31).

Although now there are many programmesprograms established for the computational prediction of miRNA targets(32), the large number of predicted targets makes it difficult to choose the best candidates for further experimental verification in a biological system. The advantage of our bioinformatics approach is the possibility of relatively fast, manual scanning of the secondary structures formed by analysed pairs of putative interactors through the use of the RNAfold format. It is worth noticing, that in the results list of our scanning important oncogenes can be found, that have already been proven as targets for particular miRNA (see Additional file). Based on our analysis it is not possible to specify which member(s) of the miRNAs family is (are) responsible for the down-regulation of *MYC*.

MYC is one important oncogene with the ability to induce both cell proliferation and apoptosis (33). Moreover, *MYC* regulates the transcription of miRNAs from miR-17 cluster and two of them (miR-17-5p and miR-20) regulate transcription factor E2F1 at the translational level (34), which is also transcriptionally activated by *MYC* (35). A large number of potential miRNA target sites in human oncogenes has been proposed and presented here based on an *in silico* analysis. Targets for different oncomirs, experimentally validation of which would be of special interest, are needed. We must design the "gold-standard" method to experimentally validate miRNA::target interactions. The miRNAs that are encoded by miRNAs family are conserved between mammalian species, both at the sequence level and at their temporal expression patterns, which probably indicates their general role in gene regulation (36). The MYC, on the other hand, is one of the most potent and frequently deregulated oncoproteins in human cancers (37, 38); therefore its deregulation is regarded as a hallmark of many cancers (39).

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