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Research Article

MicroRNAs in Molecular Technology to Address Global Disease Bench to Bedside Research

Noor UL Ain Akram[1], Zainab Shahzor[1], Iram Mushtaq[1], Ayesha Ishtiaq[1], Khadam Hussain[1], Iram Murtaza[1*]

[1] Signal transduction Laboratory, Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, 45320, Pakistan.

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Abstract

MicroRNAs a category of noncoding RNA dysregulations are involved in numerous pathological conditions including cancer, diabetes, heart diseases, immunological disorders, neurological diseases and many metabolic diseases. Advancement in the knowledge of microRNA applications led to the diagnosis of some early markers of diseases. Currently innovative molecular biotechnology is holding promising future in the field of application of microRNA silencers and inhibitors, replacers and over expressers for tackling life threatening diseases. RNA seq libraries of mega patients groups in the field of cancer include the expression profile of multiple microRNAs but there is need of time to develop such libraries of miRNAs in other diseases too. It will facilitate scientist in future to judge the perspective of microRNA applications in multiple clinical scenarios by simple bioinformatics analysis.

Keywords – microRNAs, diagnostic markers, applications

Küresel Hastalık Tezgâhından Başucu Araştırmasına Yönelik Moleküler Teknolojideki mikroRNA'lar

Öz

MikroRNA'lar, kodlamayan RNA düzensizliklerinin bir kategorisi, kanser, diyabet, kalp hastalıkları, immünolojik bozukluklar, nörolojik hastalıklar ve birçok metabolik hastalık dahil olmak üzere çok sayıda patolojik durumda yer alır. MikroRNA uygulamaları bilgisindeki ilerleme, bazı erken hastalık belirteçlerinin teşhisine yol açtı. Halihazırda yenilikçi moleküler biyoteknoloji, yaşamı tehdit eden hastalıklarla mücadele için mikroRNA susturucuları ve inhibitörleri, ikame ediciler ve aşırı ifade edicilerin uygulanması alanında gelecek vaat ediyor. Kanser alanındaki mega hasta gruplarının RNA dizi kütüphaneleri, çoklu mikroRNA'ların ekspresyon profilini içerir, ancak diğer hastalıklarda da bu tür mikroRNA kütüphanelerini geliştirmek için zamana ihtiyaç vardır. Gelecekte bilim adamlarının basit biyoinformatik analizi ile çoklu klinik senaryolarda mikroRNA uygulamalarının perspektifini yargılamasını kolaylaştıracaktır.

Anahtar Kelimeler: mikroRNA'lar, teşhis belirteçleri, uygulamalar.

[*] Prof. Dr. Iram Murtaza; (irambch@qau.edu.pk) orcid.org/0000-0001-8092-5211

Introduction

Non-coding RNAs are the highly functional and vibrant nucleic acids involved in various physiological and pathological processes of cellular differentiation, growth and cell cycle regulation, immunity, stress response, angiogenesis, nervous system development and acts as biomarkers in identification of many diseases [1]. These small RNA molecules are referred to as micro RNAs (mi-RNAs) regulate the process of RNA interference (RNAi) which results in gene silencing or translational repression and hence regulates the expression of genes [2]. Numerous researches have been conducted that show the association of microRNAs with several pathologies [3, 4]. Cancer and cardiovascular diseases are the leading cause of mortality worldwide. Despite of the vast basic and clinical research on respective fields, it still tops in causing morbidity and mortality [5, 6]. Cancer is characterized by uncontrolled cell division and proliferation [7]. Cardiac hypertrophy is a condition of abnormal cardiomyocyte enlargement accompanied by ventricular wall thickening [8]. Various bioinformatics tools are used for identification of novel molecular therapeutic interventions for the treatment these diseases [9, 10]. We have aimed to investigate the role of different micro RNAs in regulating the expression of different biomarkers involved in pathogenesis of diseases progression. National Centre of Biotechnology Information (NCBI) gene testing registry (GTR) was used to extract the names of the genes involved in cancer and cardiac hypertrophy. Target Scan human (7.2.2018) predicted the miRNA targets for all the genes. Some novel miRNA and gene relations have been predicted, directs us to develop new miRNA biomarkers for the diagnosis of cancer and cardiac hypertrophy.

Materials and Method

A- Selection of genes from National Centre of Biotechnology Information (NCBI) gene testing registry (GTR)

The National Centre for Biotechnology Information (NCBI) is a database which stores the information related to biomedicine and biotechnology. It gathers the information from many other databases including NCBI epigenomics and PubMed. NCBI houses the information regarding the diseases and the genes involved in the disease. We accessed NCBI webpage and searched for cancer and cardiac hypertrophy genes sequencing panel **(Figure 1A).**

B- Insilico prediction of micro-RNA target genes through Target Scan

The bases of miRNA target prediction vary in different softwares. miRTarBase, miRDB, miRWalk, miRGator [11], miRSystem [12], are the tools which are used for the prediction of mi-RNA targets and are most widely used softwares as they help in comparing the data from various other popular tools by detailed statistical analysis. SVMicrO [13], miRanda [14], PITA [15] and TargetScan [16] are the most widely used popular mi-RNA prediction tools.

Target Scan is one of the widely used softwares for predicting miRNA targets. It predicts the miRNA targets by searching the conserved sites **(Figure 1B)**. Amongst these conserved sites, 8mer, 7mer and 6mer sites are considered which match a specific region in the miRNA known as seed region. We filtered furthur based on conservation of miRNA among three species (human, rat and mouse) and 8 nt target analysis **(Figure 1C)**. It also provides with the percentile score and context ++ scores which help in predicting authenticity of the data.

The webpage of target scan softwares opens with the options of either entering the gene name or the miRNA name to search into the database for the relative targets. We used target scan software and entered the names of all the genes involved in the formation of cancer and cardiac hypertrophy as reported by NCBI. We selected the specie (human, rats and mouse) and then we proceeded by entering the names of our genes and obtained the details of miRNA targets for various sites. We also used the alternative method by entering the names of our miRNAs in the given second bar and from there on we got the detailed overview of the genes that have the same miRNA targets, and we found our respective genes from the whole given data and then found the details of the conserved sites and position of miRNAs.

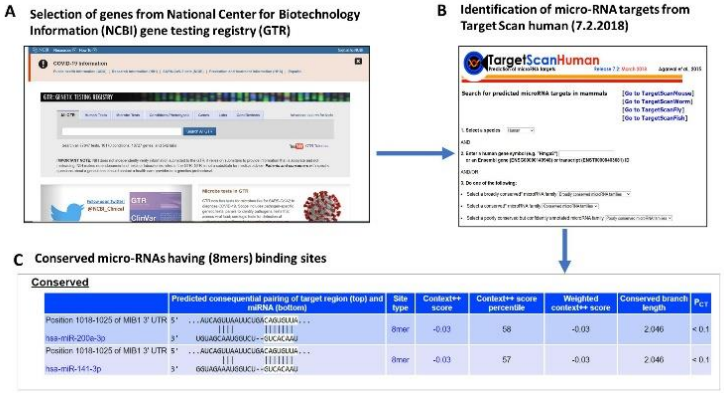

Fig. 1. Bioinformatic tool analysis A) Selection of genes from NCBI (GTR) B) Predicted miRNA through target scan C) Conserved miRNA having (8mers) binding sites.

Results

Commonly expressed miRNAs and regulated target genes involved in cancer and cardiac hypertrophy

We have examined 24 micro RNAs involved in both cancer and cardiac hypertrophy **(Figure 2)**. For Instance, miR-30-5p is thought to be major player in regulations of genes function in cancer and cardiac hypertrophy. TSC1(Tuberous Sclerosis 1 Protein) is a tumour suppressor gene, and play role in development of vascular tissue [17]. Its expression is regulated by miR-30-5p (Position: 500-506 3' UTR, Chr:6). ACTC1 (Actin Alpha Cardiac Muscle 1), Position: 105-112 3' UTR, Chr:6 [18], ABCC9 (ATP Binding Cassette Subfamily C Member 9) Position 2962-2969 3' UTR, Chr:6 [19], and MIB1(MIB E3 Ubiquitin Protein Ligase 1) Position 1370-1377 3' UTR, Chr:6 [20] are biomarkers involved in cardiac hypertrophy and are regulated by miR-30-5p. The miR-26-5p (Position: 1261-2626 3′UTR, Chr:10), which is a target for PTEN gene is associated with breast cancer [21].

The miR-218-5p (Position: 417-423 3′UTR, Chr: 10), which is a target of the RET gene is involved mainly gastric cancers [22], bladder cancer and cardiovascular disorders [23, 24]. Many others micro RNAs and their targets are enlisted in **(Figure 2).**

miRNAs and regulated target genes cardiac hypertrophy

We have examined 26 genes involved in progression of cardiac hypertrophy regulated by micro RNAs having 8-nt binding seed region. **(Figure 3A).**

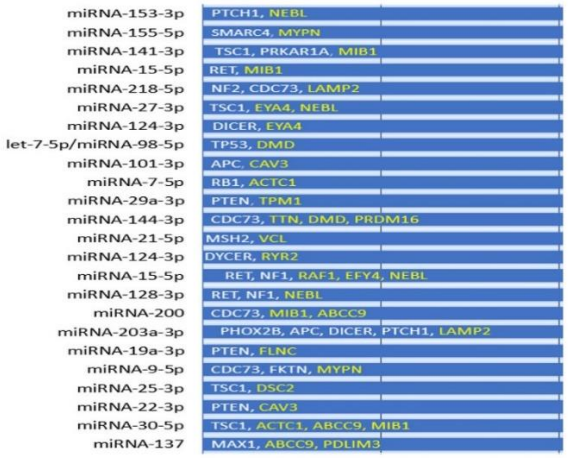

Fig. 2: List of common micro RNAs involved in cancer and cardiac hypertrophy. White color represents genes targets involved in cancer. Yellow color represents genes targets involved in cardiac hypertrophy.

M1B1 (E3 Ubiquitin-Protein Ligase MIB1) [25] expression is regulated by more than 12 micro RNAs, PRDM16 (MDS1/EVI1-Like Gene 1) [26] is regulated by 11 micro RNAs and NEBL is regulated by 10 micro RNAs. We have presented the graphical representation of all important genes and their respective 8-nt micro RNAs **(Figure 3A).**

We have also presented different genes targets regulated by more than one number of micro RNAs **Figure 3B), Table 1.** M1B1 expression is regulated by 10 micro RNAs, PRDM16 by 7 micro RNAs, and RYR2 by 7 micro RNAs.

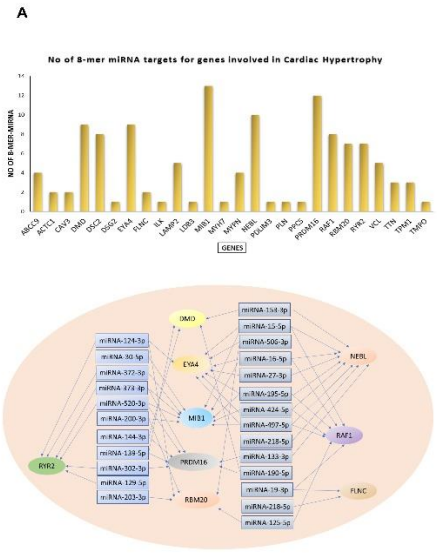

Fig. 3: miRNA targets of genes involved in cardiac hypertrophy A) Graphical representation of gene targets regulated by 8-nt miRNAs B) Genes regulated by more than one number of miRNAs

miRNAs and regulated target genes involved in cancer

We have examined 28 genes involved in cancer regulated by more than one micro RNAs having 8-nt binding sites with their respective target genes **(Figure 4A).** NF1[27], CDC 37 [28] APC [29] are regulated by 7 micro RNAs and PTEN [30] is regulated by 8 micro RNAs. We have also presented different genes targets regulated by more than one number of micro RNAs **(Figure 4B).** Different genes targets regulated by multiple micro RNAs are shown in **Table 2**. The miR-155-5p (Position: 251-257 3′UTR, Chr: 1), found as a target of the CDC73 gene promotes the process of autophagy in the case of cervical cancers [31]. The miR-190-5p (Position: 90-96 3′UTR, Chr: 12), found as a target of CDC73 is not reported to play any direct role in causing endocrine neoplasia, however, it seems to affect the metabolic pathways [32]. The miR-148 (Position: 1144-1150 3′UTR, Chr: 14), found as a target of MAX1 gene, upregulation of miR-148 leads to reduced inflammation [33]. The miR-34/449-5p (Position: 413-419 3′UTR, Chr: 1), which is a target of SDHC gene is involved in causing head and neck cancer [34]. The miR-802 (Position: 29-36 3′UTR, Chr: 11), which is a target of SDHD is reported to be linked with the suppression of gastric cancer [35]. The miR-17-5p (Position:515-522 3′UTR, Chr: 2), which is a target of TMEM127 gene is linked with the hepatocellular carcinoma [36]. The miR-122-5p (Position:200-206 3′UTR, Chr: 17), which is a target of TP53 gene is linked osteosarcoma [37], neuronal damage and many other cancers. The miR-223-3p (Position: 146-152 3′UTR, Chr: 3), which is a target of VHL gene is reported to be linked with thyroid carcinoma and it is a potent marker for identifying low grade thyroid carcinoma.

Table. 1 List of miRNAs targeting different genes involved in Cardiac hypertrophy

S. No	Putative Target Gene	miRNA
1	**ABCC9**	miRNA-137, miRNA-30-5p, miRNA-200-3p, miRNA-429
2	**ACTC1**	miR-30-5p, miR-7-5p
3	**CAV3**	miR-22-3p, miR-101-3p
4	**DMD**	miR-153-3p,miR-144-3p,miR-142-5p,miR-139-5p, miR-190-5p,let-7-5p,miR-98-5p,miR-4500,miR-4458
5	**DSC2**	miR-25-3p, miR-205-5p, miR-32-5p,miR-92-3p,miR-363-3p,miR-367-3p,miR-148-3p,miR-152-3p
6	**EYA4**	miR-506-3p, miR-145- 5p, miR-124- 3p, miR-27- 3p, miR-15- 5p, miR-16- 5p, miR-195- 5p, miR-424- 5p, miR-497- 5p
7	**FLNC**	miR-19-3p, miR-218- 5p
8	**ILK**	miR-542-3p
9	**LAMP2**	miR-203-3p, miR-183- 5p, miR-365- 3p, miR-218- 5p, miR-140- 5p
10	**LDB3**	miR-205-5p
11	**MIB1**	miR-200-3p, miR-141- 3p, miR-15- 5p, miR-16- 5p, miR-195- 5p, miR-424-5p, miR-497- 5p, miR-30- 5p, miR-302- 5p, miR-372- 3p, miR-373- 3p, miR-520- 3p, miR-30- 5p
12	**MYPN**	miR-9-5p, miR-24- 3p, miR-129- 5p, miR-155- 5p
13	**NEBL**	miR-128-3p, miR-15- 5p, miR-16- 5p, miR-195- 5p, miR-424- 5p, miR-497-5p, miR-133- 3p, miR-218- 5p, miR-27- 3p, miR-153- 3p
14	**PDLIM3**	miR-137
15	**PLN**	miR-425-5p
16	**PRDM16**	miR-133-3p, miR-144- 3p, miR-139- 5p, miR-96- 5p, miR-1271- 5p, miR-219-5p, miR-302- 3p, miR-372- 3p, miR-373- 3p, miR-520- 3p, miR-190- 5p,
17	**RAF1**	miR-15-5p, miR-16- 5p, miR-195- 5p, miR-424- 5p, miR-497- 5p, miR-19- 3p, miR-125- 5p, miR-7- 5p
18	**RBM20**	miR-125-5p, miR-124- 3p, miR-506- 3p, miR-217, miR-26- 5p, miR-200- 3p, miR-203- 3p
19	**RYR2**	miR-124-3p, miR-302- 3p, miR-372- 3p, miR-373- 3p, miR-520- 3p, miR-455-3p, miR-129- 5p
20	**VCL**	miR-21-5p, miR-590- 5p, miR-193- 5p, miR-103- 3p, miR-107
21	**TTN**	miR-144-3p, miR-451, miR-183- 5p
22	**TPM1**	miR-29-3p, miR-183- 5p, miR-142- 3p
23	**TMPO**	miR-139-5p

Table. 2 List of miRNAs targeting different genes involved in Cancer

S. NO	PUTATIVE TARGET GENE	MIRNAS
1	**MAX**	miRNA-137
2	**SDHC**	miRNA-365-3p
3	**MSH2**	miRNA-21-5p
4	**SMARCB1**	miRNA-1-3p
5	**SMARCA4**	miRNA-155-5p, miRNA-489-3p
6	**Tp53**	let-7-5p, miRNA-150-5p
7	**AIP**	miRNA-199-5p, miRNA-204-5p
8	**RET**	miRNA-15-5p, miRNA-128-3p
9	**SUFU**	miRNA-129-3p, miRNA-194-5p
10	**PHOX2B**	miRNA-203a-3p, miRNA-204-5p
11	**NF2**	miRNA-489-3p, miRNA-218-5p
12	**CDKN1B**	miRNA-142-3p, miRNA-221-3p, miRNA-24-3p
13	**SDHD**	miRNA-802, miRNA-204-5p, miRNA-23-3p
14	**VHL**	miRNA-204-5p, miRNA-142-5p, miRNA-181-5p
15	**SMARCE1**	miRNA-103-3p, miRNA-135-5p, miRNA-302-3p, miRNA-19-3p
16	**TSC1**	miRNA-141-3p, miRNA-25-3p, miRNA-30-5p, miRNA-27-3p, miRNA-130-3p
17	**TMEM127**	miRNA-17-5p, miRNA-202-5p, miRNA-212-5p, miRNA-181-5p, miRNA-140-3p
18	**RB1**	miRNA-199-3p, miRNA-26-5p, miRNA-132-3p, miRNA-7-5p, miRNA-192-5p
19	**MSH6**	miRNA-103-3p, miRNA-499a-5p, miRNA-216a-5p, miRNA-155-5p
20	**APC**	miRNA-29-3p, miRNA-135-5p, miRNA-142-3p, miRNA-129-5p, miRNA-203a-3p, miRNA-26-5p, miRNA-802, miRNA-101-3p
21	**NF1**	miRNA-103-3p, miRNA-137, miRNA-19-3p, miRNA-128-3p, miRNA-27-3p, miRNA-15-5p, miRNA-30-5p, miRNA-182-5p, miRNA-217
22	**DICER**	miRNA-182-5p, miRNA-124-3p, miRNA-29-3p, miRNA-190-5p, miRNA-103-3p, miRNA-221-3p, miRNA-203-3p
23	**PRKAR1A**	miRNA-155-5p, miRNA-141-3p, miRNA-183-5p, miRNA-96-5p, miRNA-499a-5p, miRNA-455-3p, miRNA-192-5p
24	**PTCH1**	miRNA-140-3p, miRNA-153-3p, miRNA-101-3p, miRNA-203a-3p, miRNA-141-3p
25	**PTEN**	miRNA-388-3p, miRNA-29-3p, miRNA-22-3p, miRNA-103-3p, miRNA-19-3p, miRNA-26-5p, miRNA-23-3p, miRNA-148-3p, miRNA-499a-5p
26	**CDC73**	miRNA-182-5p, miRNA-218-5p, miRNA-200, miRNA-144-3p, miRNA-181-5p, miRNA-9-5p, miRNA-101-3p, miRNA-130-3p

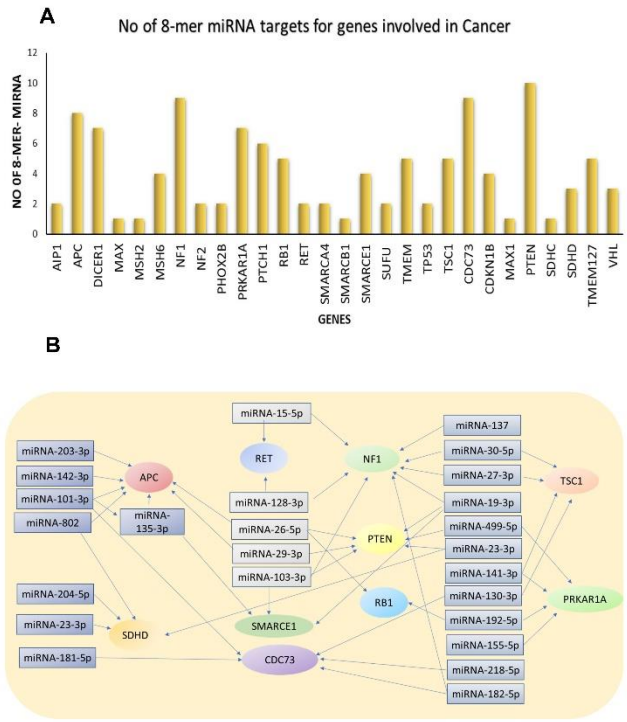

Fig. 4: miRNA targets for genes involved cancer A) Graphical representation of gene targets regulated by 8-nt miRNAs B) Genes regulated by more than one micro RNAs

Discussion

Exploration of miRNAs to identify their functioning and roles in diseases is being carried out extensively. MicroRNAs have proved to be essential gene expression regulators thereby manifesting in several diseases. Furthermore, they carry a potential to serve as emerging biomarkers for numerous diseases. In this study, we have described a computational strategy using bionformatic tools for the identification of candidate master miRNA regulators of genes involved in both cancer and cardiac hypertrophy through high-quality training datasets. We have performed target prediction analyses with the most stringent conservation criteria among (human , rat and mouse) and filtered 8-nt miRNAs regulates many target genes involved in cellular processes.

Our results predicted many master regulators which are common in both cancer and cardiac hypertrophy. Furthure more, we have expolered 8-nt binding teargets genes involved in cancer as well as in cardiac hypertrophy. TargetScan analyses showed NF1, RET and MIB1 genes as a target of miR-15. This microRNA has been reported to induce tumor inhibitory effects. Study conducted by Lu et al has shown that miR-15 lessens the proliferation and migration of thyroid cancer cells. It displays these effects by targeting BCL2 gene, which is highly expressed in cancer cells [38]. Researchers are working on miR-15 based therapeutic for chronic lymphocytic leukemia by targeted inhibition of BCL2 [39]. These studies make miR-15 an attractive microRNA to be used against brain cancer and cardiac hypertrophy by selectively targeting gene targets. The miR-155-5p (Position: 164-171 3′UTR, Chr: 17), is also related to PRKAR1A gene. miR-155-5p promotes the autophagy in cervical cancers [40].

Through literature survey, we identified several genes reported in cardiac hypertrophy **(Table 1)** and cancers **(Table 2)**. Various studies on MSH6, a DNA mismatch repair (MMR) protein coding gene showed its involvement in brain cancers. MSH6 together with MSH2 forms a protein complex, which is responsible for the DNA repair. A study conducted in glioblastoma reported mutation in 4th exon of MSH6, which led to alterations in its gene expression [41]. In a similar study conducted on glioma cell lines by Xie et al, mutation in MSH6 led to wide-ranging genome hyper-mutations and resistance to treatment [42] From TargetScan, we identified that miR-499-5p binds with MSH6 in the seed region as an 8mer target. This miRNA has been previously reported in study conducted by Yang et al, which showed its correlation with occurrence of gastric cancer [43]. Another study conducted on lung carcinoma reinforced the positive correlation of miR-499-5p with cancer. In this study, overexpression miR-499 was linked to tumor proliferation and migration. In vitro inhibition using anti-mir-499 oligonucleotides reduced tumor growth [44]. Role of miR-499 against brain cancers has yet to be recognized but implying its role in other cancers, it can be thought of as a potential therapeutic target. TMEM gene family is involved in several cell functionalities like apoptosis and signaling. Various studies on this gene show the role of TMEM family as tumor enhancers and are found to be upregulated in glioblastoma. In a study conducted by Xu et al, silencing of TMEM-168 using siRNA led to inhibition of tumor proliferation [45]. In a similar study, TMEM-48 knockdown led to reduction in tumor cell invasion and migration. Qiu et al showed that TMEM97 silencing using RNA interference induced anti-tumor effects by suppressing the cell cycle transition regulators CDK2 and cyclinD1 [46]. MiR-202 as predicted by TargetScan is an 8mer target of TMEM. Numerous Investigations performed on miR-202 revealed its anti-tumor properties. Study conducted on breast cancer cell lines elucidated the anti-proliferative activity of miR-202 against tumor cells. Harati showed that silencing of miR-202 leads to increase in brain invasive property of malignant breast cancer [47]. MiRNA induced TMEM silencing could be a potential therapeutic intervention for brain neoplasm. From NCBI database, SMARCE1 was identified to have a role in causation of brain neoplasm. This gene has previously been reported to be linked with cancer in several studies. In a study conducted by Liu et al, increased expression of SMARCE1 was observed the in gastric cancers where it activates the MAPK/ERK signaling pathway, leading to increased tumor proliferation [48]. In a similar study by Sokol et al., inhibition of SMARCE1 led to decreased tumor proliferation and invasion in breast cancer cell lines, thus exhibiting its oncogenic potential [49]. MiR-302 has been predicted by TargetScan as a possible 8mer target for SMARCE1. Several researches on miR-302 have revealed its anti-cancer properties. Two different studies, conducted in ovarian cancer cells and hepatocellular carcinoma cells, showed reduced expression of miR-302 in the said tumor cells. Administration of miR-302 led to a decrease in colony forming ability and proliferation of cancer cells [50, 51]. In addition to this, investigations to discover the role of miR-302 have been performed in glioblastomas. These cancers normally express low level of miR-302. Scientists engineered the glioma cells to express normal levels of miR-302. A decrease in proliferation was observed in neighboring non-engineered glioma cells which occurred because of paracrine mode of action of the said miRNA [52]. Regulating gene expression levels of SMARCE1 by miR-302 mediated gene silencing could prove to be a promising

therapeutic intervention. Numerous researches carried out on miRNA-103 have revealed its tumor suppressive potential. In a study conducted on glioma cells, low expression levels of miR-103 were detected. Administration of miR-103 in these cells led to decrease in tumor growth. MiR-103 targets brain derived neurotrophic factor (BDNF) thus inhibiting neuronal growth and maturation [53]. In a similar study conducted by Chen et al, miR-103 was shown to inhibit tumor proliferation by targeting and silencing gene expression of SALL4, a highly expressed gene in tumor cells [54]. Since a single miRNA can bind to multiple targets, miR-103 is predicted to control gene expression and silencing of several genes that include NF1, PTEN, SMARCE1, DICER 1 and MSH6. NF1 gene encodes for a protein called neurofibromin that controls cell growth. Loss of function was observed in 11% of glioblastoma cases. A genetic disorder called neurofibromatosis is caused due to inheritance of mutated NF1 (loss of functional mutation) which makes the individual five time more susceptible to brain malignancies [55].

RYR2 (Cardiac Muscle Ryanodine Receptor) gene encodes a ryanodine receptor, found in cardiac muscle sarcoplasmic reticulum, one of the components of a calcium channel, [56]. Defects in Ca^{2+} release from channels/ryanodine receptors (RyR2) accelerates pathological cardiac hypertophy. This gene is regulated by miR-133 [57] and miR-1 and its expression is found to be upregulated in cardiac hypertrophy [58].

Further in vivo analysis using techniques like RT-qPCR, NGS, luciferase reporter assay and western blotting is required to experimentally validate the potential therapeutic miRNAs targets predicted in this study. MiRNA based therapeutics are generally classified into two types; miRNA mimics and antagomiRs. The miRNA mimics restore normal concentration of a particular miRNA whose concentrations reduced as a result of pathology. On the contrary, antagomiRs cause silencing of overexpressed miRNAs involved in diseases. Modulating gene expression using microRNA mimics or antagonist depends on the role of target gene. Working on miRNA based therapeutic is being done by numerous biotechnological companies. Yet, the search of miRNAs as a prospective therapeutics for cancer and cardiac hypertrophy therapy is at the very beginning.

Conclusion

MicroRNAs can be employed as a treatment against many pathologies including cancer and cardiac hypertrophy. In this study, we limited ourselves to In silico bioinformatic analysis using NCBI databases as well as target Scan. Our findings suggested some possible therapeutic targets that may be employed in treatment of diseeases. Some of the targets have not been reported previously and these newly predicted targets can help us in future to design therapeutics and establish novel biomarkers for the better diagnosis and prognosis of pathologies. To confirm our findings, in vivo and in vitro experimentations are essential.

Acknowledgment

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