Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

A bioinformatics approach for identification of miR-100 targets implicated in breast cancer

R. Shamsi[1], M. Seifi-Alan[1], A. Behmanesh[2], M. D. Omrani[1], R. Mirfakhraie[1], S. Ghafouri-Fard[1*]

[1] Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Medical Informatics, School of Health Management and Information Sciences, Iran University of Medical Sciences, Tehran, Iran

Correspondence to: s.ghafourifard@sbmu.ac.ir

Received June 17, 2017; **Accepted** October 22, 2017; **Published** October 31, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.10.16

Abstract: MicroRNAs (miRNAs) are small endogenous non-coding RNAs with principal roles in regulation of protein expression via translation repression and mRNA degradation. Based on these roles they are implicated in tumourigenesis processes as well. Among them is miR-100 which can exert both tumor suppressor and oncogenic functions in various cancer types. In breast cancer, it has been shown to affect apoptosis, epithelial-mesenchymal transition as well as tumor-related signaling pathways. In the present study, we introduce a novel approach for identification of miR-100 target genes which are possibly implicated in breast cancer pathogenesis. We applied 14 online tools for prediction of miR-100 target genes and used gene expression data produced by DNA microarray technology. By combining these two sets of data we proposed a list of miR-100 target genes with possible involvement in breast cancer. Considering the role of miR-100 as a context-dependent chief regulator of the cancer-related signaling pathways and a potential target for therapeutic modalities, identification of its targets would pave the way for designing new approaches for cancer treatment or sensitization of cancer cells to standard treatments.

Key words: miR-100, Target prediction; Bioinformatics; Microarray; Breast cancer.

Introduction

MicroRNAs (miRNAs) are small endogenous non-coding RNAs with fundamental roles in regulation of protein expression which is exerted via translation repression and mRNA degradation. The function of miRNAs in gene expression regulation has been documented in nearly all biological pathways. Consequently, aberrant expressions of miRNAs are implicated in many human disorders including cancer. Approximately half of miRNA genes are located in tumor-associated genomic regions which implies their participation in carcinogenesis (1). In addition, bioinformatics predictions of miRNA target genes have shown that nearly one third of all human protein-coding genes are supposed to be regulated by miRNAs. Multiple of these protein-coding genes participate in tumorigenesis (2). More specifically, abnormal miRNAs expression profile has been shown in every aspect of cancer development including cancer initiation and progression as well as tumor-microenvironment interactions (3). miRNAs can be classified according to their function in tumorigenesis process into two groups including onco-miRs which are up-regulated in cancers and act as oncogenes and tumor suppressor miRNAs (ts-miRs) which are down-regulated in tumors (4). Among miRNAs whose expression has been assessed in various tumors is microRNA-100 (miR-100). miR-100 is an evolutionary conserved member of miR-99 family (5). The ability of miR-100 to modulate expression of many important molecules in tumorigenesis process has potentiate it to function as both a tumor promoter and a tumor suppressor (6). In breast cancer cell lines, miR-100 has been demonstrated as an epithelial-mesenchymal transition (EMT) inducer. Despite this function, it prevents the tumorigenicity, motility and invasiveness of mammary tumor cells. Its down-regulation has been detected in human breast cancer as a result of hypermethylation of its host gene *MIR100HG* (7). In addition, down-regulation of miR-100 in breast cancer cells results in over-expression of the proliferation- and survival-promoting oncogene insulin-like growth factor (IGF) 2 as well as other proteins of the IGF/mammalian target of rapamycin (mTOR) signaling cascade (8). However, a more recent study has shown the role of miR-100 in breast cancer tumorigenesis which is exerted through preventing the apoptotic activity of SK-BR-3 cells. Nevertheless, miR-100 has the opposite effect in regulating other types of breast cancer cells. The same study has assessed the effects of miR-100-mediated apoptosis regulation on breast cancer tumorigenesis *in vivo* and has shown significant decrease in tumor development in mice treated with anti-miRNA-100 oligonucleotide (AMO-miR-100). Consequently, miR-100 antagonism has been suggested as an inhibitory modality for the development of breast cancer (9). On the other hand, miR-100 has been shown to prevent maintenance and expansion of breast cancer stem cells in basal-like cancer via inhibition of Polo-like kinase1 (Plk1). Such action leads to differentiation of cancer stem cells changing a basal like phenotype into luminal phenotype which is responsive to hormonal therapy (10). Considering the distinctive role of miR-100 in breast cancer subtypes, in the present study we introduce a novel bioinformatics approach for identification

of miR-100 targets in each breast cancer subtype.

Materials and Methods

The bioinformatics pipeline employed in the current study is shown in Figure 1.

Bioinformatics analysis of miR-100 target genes

Fourteen online tools were applied for prediction of miR-100 target genes. TargetScan prediction is based on sequence complementarity to target sites with specific attention to perfect base-pairing in the seed region and sequence conservation (11). The miRBase tool considers sequence annotation such as genomic location, precursor sequences and literature citations (12). MiRmap combines thermodynamic, evolutionary, probabilistic and sequence-based characteristics of miRNAs to predict their targets (13). MiRWalk algorithm first examines perfect matches in the seed and then extends it till a mismatch is detected (14). DIANA-microT searches for 7-, 8- or 9-nucleotide long seed-complementaries, or 6-nt seeds with one G:U wobble and calculate the weighted quantity of conserved and non-conserved sites of a gene (14). PITA finds seed complementary of 6 to 8 nucleotides, permitting up to one G:U wobble in 7 and 8-mers and up to one mismatch in 8-mers (14). PACCMIT–CDS identifies potential miRNA targets within coding sequences (CDS) by exploring conserved motifs that are complementary to the miRNA seed region and also overrepresented in comparison with a background model preserving both codon usage and amino acid sequence (15). Rna22 is another prediction tool which does not depend on cross-species conservation and first searches for putative miRNA binding sites in the sequence of interest, then detects the targeting miRNA (16). miRTarBase has gathered more than three hundred and sixty thousand miRNA-target interactions (MTIs) by manually searching relevant literature (17). The MIRZA-G uses evolutionary conservation in predicting canonical miRNA target sites and in addition, it predicts non-canonical miRNA target sites (18). MBSTAR uses a multiple instance learning approach for predicting specific functional binding sites in mRNA targets (19). miRDB is a miRNA target prediction and functional annotation tool in which miRNA functional annotations are demonstrated with a major emphasis on mature miRNAs, which are the functional carriers of miRNA-mediated gene expression regulation (20). The microRNA.org predicts miRNA targets based on advancement of the miRanda algorithm and scores the targets for probability of mRNA down-regulation using mirSVR, a regression model that is trained on sequence and background characteristics of the predicted miRNA::mRNA duplex (21). Finally, the microRNA Data Integration Portal-mirDIP has been constructed through integration of prediction databases, comparison of predictions to in vitro data, and application of cross-database predictions to model the microRNA:transcript interactome (22). After application of all above mentioned tools we selected genes which have been predicted as miR-100 targets in at least two prediction tools.

Experimentally validated miR-100 target genes

Due to the pitfalls associated with the predictions of miRNA target sites with the computational algorithms (23), in order to find functionally relevant targets in breast cancer, we focused our search on researches which accomplished functional studies for identification of miR-100 targets.

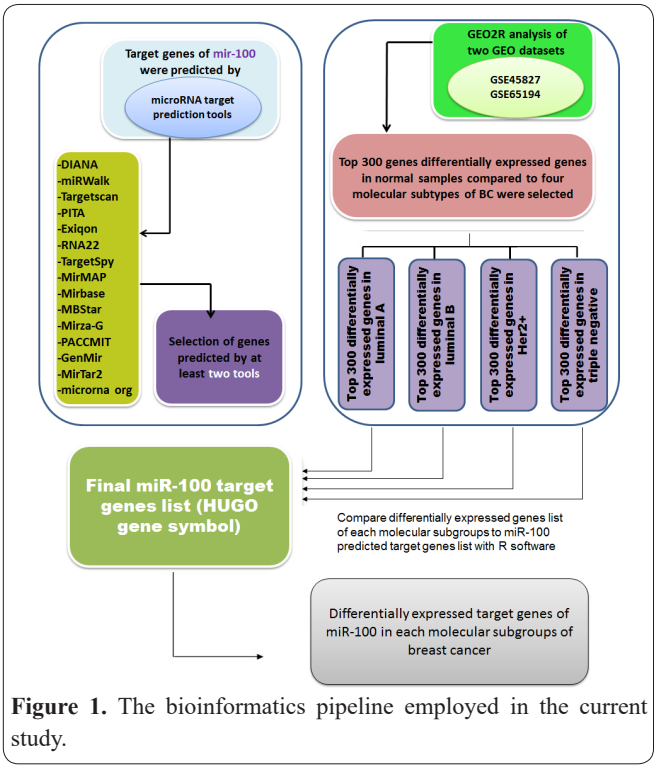

Figure 1. The bioinformatics pipeline employed in the current study.

Microarray data analysis

Using the Gene Expression Omnibus (GEO) repository at the National Center for Biotechnology Information (NCBI) archives (24), we got access to gene expression data produced by DNA microarray technology. By entering "breast cancer" and "transcriptome" key words and selection of "Expression profiling by array" as the filter and the minimum sample size of 100 for datasets, 10 datasets were retrieved. Two of them with similar array platforms (GPL570) and inclusion of four molecular subtypes of breast cancer (luminal A, luminal B, Her2+ and triple negative) have been selected for the current study (GSE45827, GSE65194). The pipeline used for selection of these datasets is shown in Figure 2.

Table 1 shows the summary of datasets used in the study.

Identification of differentially expressed genes in breast cancer subtypes

GEO2R web tool (https://www.ncbi.nlm.nih.gov/geo/info/geo2r.html) was used for comparison of expression profile of each breast cancer subtype samples with normal samples in order to identify genes that are differentially expressed across samples. Based on Log2-fold change between two experimental conditions (LogFC) and adjusted P values provided by the software, we selected 300 genes in each subtype with the most significant differential expression compared with normal samples.

Identification of miR-100 targets among differentially expressed genes in breast cancer subtypes

The R statistical program (25) was used for compa-

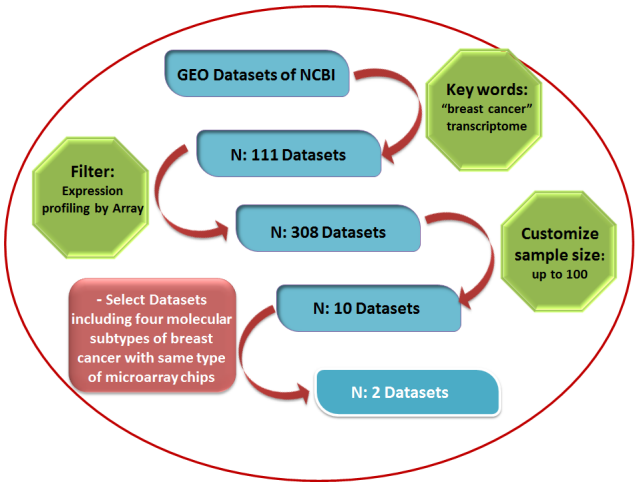

Figure 2. Flow chart of the protocol used for the search of breast cancer microarray datasets from the GEO database.

rison and identification of miR-100 target genes which are differentially expressed in each cancer subtype compared with normal samples.

Enrichment annotation analysis and network construction

For the network construction and the enrichment annotation step, we used FunRich analysis tool version 3 (26) which provides a schematic clusterization of the gene list with demonstration of the related pathway.

Results

Experimentally validated miR-100 target genes

In order to assess the sensitivity of miRNA target prediction tools and validation of data provided by these tools we compared the list of experimentally validated miR-100 target genes to target genes predicted by the

Table 1. Microarray datasets used in the current study.

GEO Number	Year	Samples	Chip type
GSE65194	2015	N:130 41 TNBC 30 Her2 30 Luminal B 29 Luminal A 11 normal breast tissue samples	Affymetrix Human Genome U133 Plus 2.0 Array
GSE45827	2016	N:155 11 normal 41 TN 30 Her2 29 Luminal A 30 Luminal B	Affymetrix Human Genome U133 Plus 2.0 Array

Table 2. Experimentally validated target genes of miR-100 in all cancers.

Target gene	Tools predicted the target gene	Disease	Reference
PLK1	Mirbase, EXIQONE Mirsearch	Lung adenocarcinoma Cervical cancer Non-small cell lung cancer Nasopharyngeal cancer	(27)
EGR2	MBStar, EXIQONE Mirsearch	Oral squamous cell carcinoma	(28)
ID1	3 tools	Oral squamous cell carcinoma	(28)
FGFR3	>10 tools	Pancreatic cancer cells	(29)
MMP13	3 tools	Oral cancer cells	(28)
ATM	>10 tools	Glioma	(30)
IGF1R	>10 tools	Head and neck squamous cell carcinoma Pancreatic cancer Adrenocortical cancer	(31, 32)
BMPR2	5 tools	Adipose derived mesenchymal stem cells	(33)
CTDSPL	>10 tools	Acute myeloid leukemia	(34)
HS3ST2	>10 tools	Gastric cancer	(35)
MTOR	>10 tools	Bladder cancer, Acute myelocytic leukemia, ovarian cancer, endometrial	(36)
RPTOR	3 tools	adrenocortical cancer	(28)
FOXA1	3 tools	Bladder cancer	(37)
AGO2	>10 tools	Prostate cancer	(38)
NCOR2	Microrna.org, mirwalk	Glioblastoma	(39)
Ciy61	RNA22, Mirwalk	Osteosarcoma	(40)
RAP1B	5 tools	Colorectal cancer	(41)
BAZ2A	>10 tools	Prostate cancer	(42)
THAP2	>10 tools	Prostate cancer	(42)
FKBP5	6 tools	Acute lymphoblastic leukemia	(43)

Table 3. Experimentally validated target genes of miR-100 in breast cancer.

Target gene	Tools predicted the gene	Reference
IGF2	MirMAP, Mirwalk, PITA	(8)
MTMR3	>10 tools	(9)
MTOR	>10 tools	(44)
SMARCA5	>10 tools	(10)
HOXA1	>10 tools	(7)
FZD8	>10 tools	(45)

mentioned bioinformatics tools. Tables 2 and 3 show the experimentally validated miR-100 target genes in all cancer types and breast cancer as well as the number of bioinformatics tools predicted each gene respectively. All of genes demonstrated in Table 2 have been predicted by miRTarBase as a target of miR-100. However, among those demonstrated in Table 3, only *MTOR* has been strongly predicted by miRTarBase as a target of miR-100.

miR-100 target genes implicated in breast cancer

Based on the novel approach of combining microarray data analysis and bioinformatics prediction tools we identified miR-100 target genes which are differentially expressed in breast cancer tissues compared with normal tissues and are possibly implicated in breast cancer (Table 4).

Network construction

Both functional enrichment and interaction network analysis were performed using FunRich tool. The final datasets were analyzed against three different background databases namely FunRich, UniProt and Custom. Figure 3 shows the schematic interaction diagram. Interaction network provided by this tool demonstrates that miR-100 regulates numerous genes participating in apoptosis, DNA repair and cell cycle control in breast cancer. Bioinformatics tools have also shown similar expression pattern for these genes in breast cancer tissues which implies a similar regulatory process for them. In addition, several of these genes have important interactions with others which complicate the network of miR-100 targets. Consequently, miR-100 is anticipated to directly or indirectly control expression of numerous genes which highlights its role in the development of breast cancer.

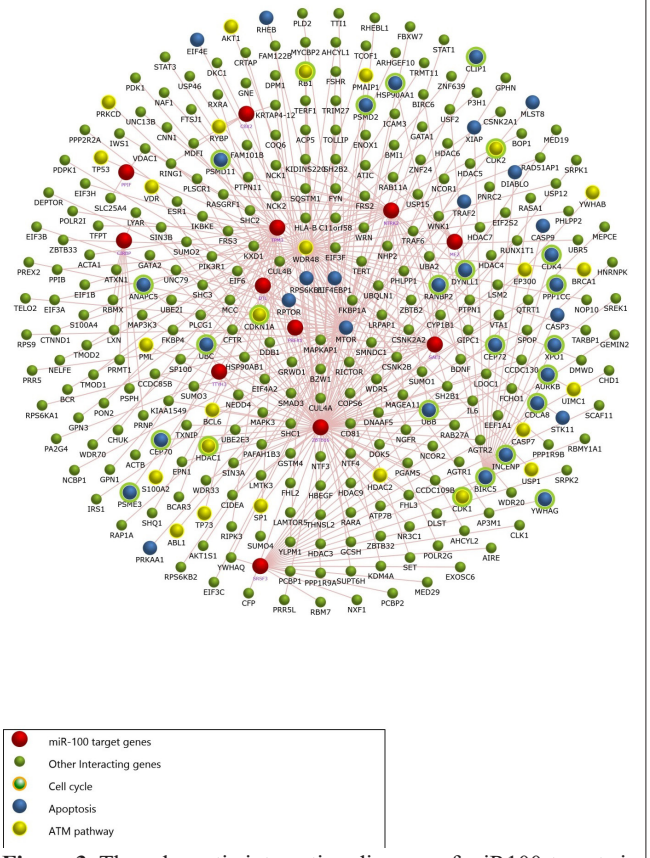

Figure 3. The schematic interaction diagram of miR100-targets in breast cancer. Direct targets of miR-100 interact with several genes implicated in cell cycle control (demonstrated by green cycles with orange shadow), apoptosis (demonstrated by blue cycles) and ATM pathway (demonstrated by yellow cycles).

Discussion

Breast cancer as the main cause of cancer death in women (46) has been accounted as an important area for identification of cancer biomarkers among both protein coding RNAs (47-49) and non-coding RNAs (50-53). miRNAs as a novel group of non-coding RNAs have also attained attention of researchers in the field of cancer biomarker discovery (6). miR-100 has been known as a significant player in the development of breast cancer. It has been shown to prevent the apoptotic activity of SK-BR-3 cells but induce apoptosis in other types of breast cancer cells (9). Another study has shown that the forced expression of this miRNA inhibits the replication capability of basal-like cancer stem cells (CSC) and alters an aggressive phenotype of cancer into a phenotype with a better prognosis (10). The role of miR-100 in inhibition of CSC self-renewal and induction of their differentiation in breast cancer has been emphasized by others as well (54). In addition, miR-100 reduced expression has been detected in both human breast cancer primary tumors and cell lines while its forced expression has been shown to promote the effects of paclitaxel in MCF-7 cells (44).

Considering the role of miRNAs in regulation of expression of multiple genes, different approaches have been evolved for prediction of their putative target. Experimental data provide valuable lines of evidence but they are time consuming. On the other hand, computational algorithms for the predictions of miRNA target sites have been associated with pitfalls (23). Consequently, in the present study we applied a novel approach for prediction of miR-100 targets in breast cancer based on both computational algorithms and experimental data available from previous microarray analyses. Although microarray studies are regarded inferior to RNA seq/Western blot, the high throughput nature of this technique as well as availability of its results in public databases were the initiatives for selection of this technique in the current study. Furthermore, we constructed an interaction network demonstrating genes and signaling pathways controlled by miR-100 in breast cancer. Experimental techniques such as microarray are frequently used to measure gene expression following up-regulation or down-regulation of a certain miRNA. However, based on the regulatory effect of miRNA on both mRNA expression and protein expression, such approach

Table 4. miR-100 predicted target genes with differential expression in breast cancer subtypes based on the log2-transformed fold change. The over- and under- expression are shown in green and red, respectively.

Genes	Luminal A	Luminal B	Her2+	TNBC	Function of the gene	Tools predicted the gene
SAE1					regulates protein structure and intracellular localization	>8 tools
SRSF3					a member of the serine/arginine (SR)-rich family of pre-mRNA splicing factors	>5 tools
HN1L					Jupiter microtubule associated homolog 2	>5 tools
CYYR1					cysteine and tyrosine rich 1	>5 tools
PPIF					part of the mitochondrial permeability transition pore	>5 tools
DESI2					inhibits proliferation through S phase arrest and apoptosis	>5 tools
TMEM30A					transmembrane protein 30A	>5 tools
ME2					catalyzes the oxidative decarboxylation of malate to pyruvate	>5 tools
NIPBL					role in developmental regulation	>3 tools
SULF1					an extracellular heparan sulfate endosulfatase	MBStar, MirMAP, PITA
FOXA1					forkhead class of DNA-binding proteins	DIANA, MirMAP, PITA
ARFGEF1					intracellular vesicular trafficking	MBStar, MirMAP, PITA
LARP4B					RNA metabolism and translation	Targetscan, MirMAP, PITA
TPM3					actin-binding protein	Mirbase, PITA, MBStar
GPRC5A					may play a role in epithelial cell differentiation	Targetscan, MirMAP
FIGN					microtubule severing factor	Targetscan, MirMAP
PDE2A					phosphodiesterase 2A	MirMAP, PITA
MUC3A					epithelial glycoprotein	Targetscan, MirMAP
NTRK2					phosphorylates members of the MAPK pathway	MBStar, MirMAP, PITA
DTL					E3 ubiquitin protein ligase homolog	microrna org, MBStar, PACCMIT
WDR48					interact with ubiquitin specific peptidase 1 (USP1)	microrna org, DIANA, PACCMIT
COG5					required for normal Golgi morphology and function	MBStar, MirMAP, PITA
PREX1					activate RAC1 by exchanging bound GDP for free GTP	DIANA, MirMAP, PITA
CELSR1					Positive Regulator of Endothelial Cell Migration and Angiogenesis.	MirMAP, PITA
ZNF595					transcription factors that can regulate developmental and cellular processes	microrna org, Mirbase, PITA
GABRP					multisubunit receptor chloride channel that mediates the fastest inhibitory synaptic transmission	Mirbase, PITA, MBStar
LGR6					glycoprotein hormone receptor that potentially functions as a tumor suppressor	MirMAP, PITA
ATP1A2					responsible for maintaining the electrochemical gradients of Na and K ions across the plasma membrane	MirMAP, PITA
BIRC5					inhibitor of apoptosis	Mirbase, PITA
CBX2					initial evidence of an oncogenic role	Mirbase, PITA
ZBTB16					involved in cell cycle progression	DIANA, PACCMIT
CIRBP					inhibits DNA damage-induced apoptosis by regulating p53	Exiqone Mirsearch, Targetscan, MirMAP
HSPA12B					involved in susceptibility to atherosclerosis	Targetscan, MirMAP, PITA
TTYH3					function as chloride anion channels	MirMAP, PITA

would leave out targets which are regulated at the level of mRNA translation into protein (23). Multiple databases have been evolved with the purpose of deposition and sharing experimental data among them is the GEO DataSets which stores original submitter-supplied records (Series, Samples and Platforms) as well as curated DataSets. The latter produces the groundwork of GEO's advanced data presentation and analysis characteristics such as tools to detect differences in gene expression levels and cluster heatmaps. The second strategy for identification of miRNA targets has been provided by computational algorithms of miRNA target prediction. The most commonly applied algorithms are *ab initio* algorithms and machine learning approaches. In the former approach, the high number of false positives is the most important limitation while for the latter it is the reduced number of negative interactions with experimental support (23). In the present study, we combined bioinformatics prediction tools as well as GEO based differential expression analyses to find the most relevant miR-100 targets in breast cancer. The documentation of shared features for targets and specific sites with significant experimental validation is necessary to reveal the rules of miRNA–mRNA interactions (23). The result of the present study is of practical value in this regard. In addition, our dataset provides more miR-100 targets with possible implication in breast cancer compared with available experimentally validated targets in breast cancer. As the experimental validation of miRNA targets by available techniques such as western blotting or luciferase assay are relatively expensive and cumbersome, the proposed approach in the current study would be an effective way for suggestion of possible miRNA targets in distinctive contexts. In addition, due to complex miRNA-mRNA interactions, miRNA regulation should be considered as a complex network composed of genes targeted by many miRNAs and often having several sites for the same miRNA. The context-dependent manner of such network makes it challenging to rebuild reliable miRNA-mRNA interactions in experiments carried out in vitro (55). So, computational analyses are helpful in this regard. Considering the role of mir-100 as a context-dependent chief regulator of the cancer-related signaling pathways and a potential target for therapeutic modalities (8), identification of its targets would pave the way for designing new approaches for cancer treatment or sensitization of cancer cells to standard treatments.

Acknowledgments
This article has been extracted from the thesis written by Mrs Roshanak Shamsi in School of Medicine Shahid Beheshti University of Medical Sciences. (Registration No: 102M).

Conflict of interest
None

References

1. Sevignani C, Calin GA, Siracusa LD, Croce CM. Mammalian microRNAs: a small world for fine-tuning gene expression. Mamm Genome 2006; 17(3):189-202.
2. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005; 120(1):15-20.
3. Nouraee N, Mowla SJ, Calin GA. Tracking miRNAs' footprints in tumor-microenvironment interactions: Insights and implications for targeted cancer therapy. Genes Chromosomes Cancer 2015; 54(6):335-52.
4. Cruz FD, Matushansky I. MicroRNAs in chromosomal translocation-associated solid tumors: learning from sarcomas. Discovery Medicine 2011;12(65):307-17.
5. Heimberg AM, Sempere LF, Moy VN, Donoghue PCJ, Peterson KJ. MicroRNAs and the advent of vertebrate morphological complexity. P Natl Acad Sci USA 2008;105(8):2946-50.
6. Qin C, Huang RY, Wang ZX. Potential role of miR-100 in cancer diagnosis, prognosis, and therapy. Tumor Biol 2015; 36(3):1403-9.
7. Chen DH, Sun YT, Yuan Y, Han ZB, Zhang PJ, Zhang JS, et al. miR-100 Induces Epithelial-Mesenchymal Transition but Suppresses Tumorigenesis, Migration and Invasion. Plos Genet 2014; 10(2).
8. Gebeshuber CA, Martinez J. miR-100 suppresses IGF2 and inhibits breast tumorigenesis by interfering with proliferation and survival signaling. Oncogene 2013; 32(27):3306-10.
9. Gong Y, He TL, Yang L, Yang G, Chen YL, Zhang XB. The role of miR-100 in regulating apoptosis of breast cancer cells. Sci Rep-Uk 2015;5.
10. Petrelli A, Carollo R, Cargnelutti M, Iovino F, Callari M, Cimino D, et al. By promoting cell differentiation, miR-100 sensitizes basal-like breast cancer stem cells to hormonal therapy. Oncotarget 2015; 6(4):2315-30.
11. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell 2003;115(7):787-98.
12. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 2006; 34(Database issue):D140-4.
13. Vejnar CE, Blum M, Zdobnov EM. miRmap web: Comprehensive microRNA target prediction online. Nucleic Acids Res 2013; 41(Web Server issue):W165-8.
14. Tabas-Madrid D, Muniategui A, Sanchez-Caballero I, Martinez-Herrera DJ, Sorzano CO, Rubio A, et al. Improving miRNA-mRNA interaction predictions. BMC genomics 2014;15 Suppl 10:S2.
15. Marín RM, Vaníček J. Optimal use of conservation and accessibility filters in microRNA target prediction. PloS one 2012;7(2):e32208.
16. Miranda KC, Huynh T, Tay Y, Ang Y-S, Tam W-L, Thomson AM, et al. A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. Cell 2006;126(6):1203-17.
17. Chou C-H, Chang N-W, Shrestha S, Hsu S-D, Lin Y-L, Lee W-H, et al. miRTarBase 2016: updates to the experimentally validated miRNA-target interactions database. Nucleic Acids Res 2016;44(D1):D239-D47.
18. Akhtar MM, Micolucci L, Islam MS, Olivieri F, Procopio AD. Bioinformatic tools for microRNA dissection. Nucleic Acids Res 2016; 44(1):24-44.
19. Bandyopadhyay S, Ghosh D, Mitra R, Zhao Z. MBSTAR: multiple instance learning for predicting specific functional binding sites in microRNA targets. Sci Rep 2015;5:8004.
20. Wang X. miRDB: a microRNA target prediction and functional annotation database with a wiki interface. RNA (New York, NY) 2008; 14(6):1012-7.
21. Betel D, Wilson M, Gabow A, Marks DS, Sander C. The microRNA.org resource: targets and expression. Nucleic Acids Res 2008; 36(Database issue):D149-53.
22. Shirdel EA, Xie W, Mak TW, Jurisica I. NAViGaTing the micronome–using multiple microRNA prediction databases to

identify signalling pathway-associated microRNAs. PloS one 2011;6(2):e17429.
23. Reyes-Herrera PH, Ficarra E. One decade of development and evolution of microRNA target prediction algorithms. Genomics Proteomics Bioinformatics 2012; 10(5):254-63.
24. Barrett T, Edgar R. Gene Expression Omnibus: Microarray Data Storage, Submission, Retrieval, and Analysis. Methods in enzymology 2006;411:352-69.
25. Team RC. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013.
26. Pathan M, Keerthikumar S, Ang CS, Gangoda L, Quek CY, Williamson NA, et al. FunRich: An open access standalone functional enrichment and interaction network analysis tool. Proteomics 2015;15(15):2597-601.
27. Liu J, Lu K-H, Liu Z-L, Sun M, De W, Wang Z-X. MicroRNA-100 is a potential molecular marker of non-small cell lung cancer and functions as a tumor suppressor by targeting polo-like kinase 1. BMC cancer 2012;12(1):519.
28. Henson BJ, Bhattacharjee S, O'Dee DM, Feingold E, Gollin SM. Decreased expression of miR-125b and miR-100 in oral cancer cells contributes to malignancy. Genes, Chromosomes and Cancer 2009;48(7):569-82.
29. Li Z, Li X, Yu C, Wang M, Peng F, Xiao J, et al. MicroRNA-100 regulates pancreatic cancer cells growth and sensitivity to chemotherapy through targeting FGFR3. Tumor Biol 2014;35(12):11751-9.
30. Ng WL, Yan D, Zhang X, Mo Y-Y, Wang Y. Over-expression of miR-100 is responsible for the low-expression of ATM in the human glioma cell line: M059J. DNA repair 2010;9(11):1170-5.
31. Chen Z, Jin Y, Yu D, Wang A, Mahjabeen I, Wang C, et al. Down-regulation of the microRNA-99 family members in head and neck squamous cell carcinoma. Oral Oncol 2012;48(8):686-91.
32. Huang J, Egger M, Grizzle W, McNally L. MicroRNA-100 regulates IGF1-receptor expression in metastatic pancreatic cancer cells. Biotechnic & Histochemistry. 2013;88(7):397-402.
33. Zeng Y, Qu X, Li H, Huang S, Wang S, Xu Q, et al. MicroRNA-100 regulates osteogenic differentiation of human adipose-derived mesenchymal stem cells by targeting BMPR2. FEBS letters 2012; 586(16):2375-81.
34. Zheng YS, Zhang H, Zhang XJ, Feng DD, Luo XQ, Zeng CW, et al. MiR-100 regulates cell differentiation and survival by targeting RBSP3, a phosphatase-like tumor suppressor in acute myeloid leukemia. Oncogene 2012;31(1):80-92.
35. Yang G, Gong Y, Wang QZ, Wang YM, Zhang XB. The role of miR-100-mediated Notch pathway in apoptosis of gastric tumor cells. Cell Signal 2015; 27(6):1087-101.
36. Sun J, Chen ZL, Tan XG, Zhou F, Tan FW, Gao YB, et al. MicroRNA-99a/100 promotes apoptosis by targeting mTOR in human esophageal squamous cell carcinoma. Med Oncol 2013 ;30(1).
37. Drayton RM, Peter S, Myers K, Miah S, Dudziec E, Bryant HE, et al. MicroRNA-99a and 100 mediated upregulation of FOXA1 in bladder cancer. Oncotarget 2014; 5(15):6375-86.
38. Wang M, Ren D, Guo W, Wang ZY, Huang S, Du H, et al. Loss of miR-100 enhances migration, invasion, epithelial-mesenchymal transition and stemness properties in prostate cancer cells through targeting Argonaute 2. Int J Oncol 2014; 45(1):362-72.
39. Alrfaei BM, Vemuganti R, Kuo JS. microRNA-100 Targets SMRT/NCOR2, Reduces Proliferation, and Improves Survival in Glioblastoma Animal Models. Plos One 2013; 8(11): e80865.
40. Huang JH, Gao KD, Lin J, Wang QG. MicroRNA-100 inhibits osteosarcoma cell proliferation by targeting Cyr61. Tumor Biol 2014; 35(2):1095-100.
41. Peng H, Luo J, Hao H, Hu J, Xie SK, Ren DL, et al. MicroRNA-100 regulates SW620 colorectal cancer cell proliferation and invasion by targeting RAP1B. Oncol Rep 2014; 31(5):2055-62.
42. Leite KRM, Morais DR, Reis ST, Viana N, Moura C, Florez MG, et al. MicroRNA 100: a context dependent miRNA in prostate cancer. Clinics 2013;68(6):797-802.
43. Li XJ, Luo XQ, Han BW, Duan FT, Wei PP, Chen YQ. MicroRNA-100/99a, deregulated in acute lymphoblastic leukaemia, suppress proliferation and promote apoptosis by regulating the FKBP51 and IGF1R/mTOR signalling pathways. Brit J Cancer 2013; 109(8):2189-98.
44. Zhang BT, Zhao RR, He Y, Fu X, Fu LY, Zhu ZM, et al. Micro RNA 100 sensitizes luminal A breast cancer cells to paclitaxel treatment in part by targeting mTOR. Oncotarget 2016; 7(5):5702-14.
45. Jiang Q, He M, Guan S, Ma M, Wu H, Yu Z, et al. MicroRNA-100 suppresses the migration and invasion of breast cancer cells by targeting FZD-8 and inhibiting Wnt/β-catenin signaling pathway. Tumor Biol 2016;37(4):5001-11.
46. Dianatpour M, Mehdipour P, Nayernia K, Mobasheri MB, Ghafouri-Fard S, Savad S, et al. Expression of Testis Specific Genes TSGA10, TEX101 and ODF3 in Breast Cancer. Iranian Red Crescent Med J 2012;14(11):722-6.
47. Seifi-Alan M, Shamsi R, Ghafouri-Fard S, Mirfakhraie R, Zare-Abdollahi D, Movafagh A, et al. Expression analysis of two cancer-testis genes, FBXO39 and TDRD4, in breast cancer tissues and cell lines. APJCP 2014; 14(11):6625-9.
48. Ghafouri-Fard S, Shamsi R, Seifi-Alan M, Javaheri M, Tabarestani S. Cancer-testis genes as candidates for immunotherapy in breast cancer. Immunotherapy 2014;6(2):165-79.
49. Kazemi-Oula G, Ghafouri-Fard S, Mobasheri MB, Geranpayeh L, Modarressi MH. Upregulation of RHOXF2 and ODF4 Expression in Breast Cancer Tissues. Cell J 2015;17(3):471-7.
50. Iranpour M, Soudyab M, Geranpayeh L, Mirfakhraie R, Azargashb E, Movafagh A, et al. Expression analysis of four long non-coding RNAs in breast cancer. Tumour Biol 2016;37(3):2933-40.
51. Tasharrofi B, Soudyab M, Nikpayam E, Iranpour M, Mirfakhraie R, Sarrafzadeh S, et al. Comparative expression analysis of hypoxia-inducible factor-alpha and its natural occurring antisense in breast cancer tissues and adjacent noncancerous tissues. Cell Bioch Func 2016; 34(8):572-8.
52. Soudyab M, Iranpour M, Ghafouri-Fard S. The Role of Long Non-Coding RNAs in Breast Cancer. Arch Iran Med 2016; 19(7):508-17.
53. Sarrafzadeh S, Geranpayeh L, Tasharrofi B, Soudyab M, Nikpayam E, Iranpour M, et al. Expression Study and Clinical Correlations of MYC and CCAT2 in Breast Cancer Patients. Iran Biomed J 2017; 21(5):303-311
54. Deng L, Shang L, Bai S, Chen J, He X, Martin-Trevino R, et al. MicroRNA100 inhibits self-renewal of breast cancer stem-like cells and breast tumor development. Cancer Res 2014; 74(22):6648-60.
55. Witkos TM, Koscianska E, Krzyzosiak WJ. Practical Aspects of microRNA Target Prediction. Curr Mol Med 2011;11(2):93-109.