Research Article

Identification of key miRNA biomarkers by miRNA-gene interactions network regulating breast cancer in human

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Abstract

Objective: The objective of this work is to identify the key miRNA by using interaction network approach, which helps for identification of biomarkers for breast cancer. **Material and Methods**: The publicly available database is used for interaction studies where the genes associated with somatic and germline cancer is obtained from databases. This gene dataset was used to develop interaction network using miR Target Link tool. These miRNA-genes interactions are classified into strong and weak depending on the number of validated experiments. These miRNA-gene interaction-based network analysis are one of the latest methods which provides in depth understanding of miRNA and RNA interference system in different cancer type. These interaction networks followed by miRNA expression analysis in normal and cancerous breast tissue provides support to our finding for key miRNAs as biomarkers for detection of somatic and germline breast cancer. **Results**: In this study the miRNA-RNA interaction networks reveals, that miRNA hsa-miR-146a-5p, hsa-miR-15a-5p are key players in germline and hsa-miR-182-5p, hsa-miR-19b-3p, hsa-miR-194-5p, hsa-miR-125b-5p, hsa-miR-375, hsa-miR-100-5p are major contributor somatic breast cancer. These miRNAs may act as potential biomarkers for breast cancer which can identify and differentiate different cancer at initial stages by using specific technique. **Conclusion:** The role of miRNA in gene regulation is well known and they are used as biomarkers for detection of various diseases. Network based biomarkers identification for a specific cancer can act as a valuable tool for early diagnosis of various cancer types.

***Keywords*:** Interaction networks, somatic, germline, biomarkers, miRTargetLink

Introduction

According to International Agency for Research on Cancer, World Health Organization (WHO) there are nearly 14.1 million cases for cancer reported in 2012. Among the reported cases men are 7.4 million and women are 6.7 million. The total number of cancer cases were expected to be about 24 million by 2035 (Ferlay et al., 2013). This data provided does not include non-melanoma skin cancer. Non-reported cases of cancer from developing and under developed countries will more than the data provided. Lung cancer have highest number of reported cases with about 1.8 million patients followed by breast cancer with 1.7 million cases. This provided a base to carry this research on breast cancer.

Network is a term of information technology which represents interactions between all components to perform some task. The flow of information form one component to another in a specific controlled manner performs some task. So, biological networks like, protein network, gene network are well known introduced in last decade. The interaction network between biological molecules had gained interest among scientific community for providing a broader insight to a system. To understand the entire system of gene regulation mediated via miRNA for a specific disease condition it is important to go through the network of miRNA interaction networks.

Various non-coding sequences are reported to have role in controlling gene expression. MicroRNA and Untranslated Regions are such important noncoding sequences having

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very important role in gene regulation at transcription and translation level (Maute et al., 2014). A poor correlation was observed between number of mRNA molecules with its corresponding translated proteins. This reveals that number of factors are involved to regulate translation of any protein molecule. miRNA based gene expression control is reported in many cases, where these tiny molecules of ~22 nucleotide (Bartel, 2004) interacts with messenger RNA (mRNA). Initially it is stated that translation regulation is controlled by miRNA interaction at 3'UTR of mRNA. But later on they are also found interacting with 5' UTR (Lee et al., 2009) and coding sequence (Rigoutsos, 2009) of mRNA. These interactions are well defined and supported by various experimental techniques by which miRNA targets are identified. These techniques are based on protein arrays, RNA profiling, proteome profiling, ribosomal mapping. Various bioinformatics based algorithms using predictive methods were also used for finding the interactions. These predictive methods are based on machine learning and filtering algorithms. Most used predictive tools for miRNA interactions studies are Target Scan, miRanda, PicTar, Target Minner, mir Target (Kim et al., 2006) and Mir Mark. These algorithms work by finding the molecular architecture of MRE (miRNA response elements) which is composed of 'seed' of 6-8 base pairs of miRNA starting from second position of at 5' end and secondary structures. By using additional factors like base-paring, thermodynamic properties, cooperativity and conservations had had increased he optimality of an algorithms. The optimality of any prediction method is determined by high specificity and sensitivity which represents small number of false positive and false negative predictions respectively. These methods of predictions were cross validated with experimental dataset. These miRNA-mRNA interactions are stored in various databases like miR Tar Base and miRecords (Xiao et al., 2009). Most updated database miRwalk2, which is a multilayered data repository having information for validated and predicted miRNA targets for a specific miRNA (Dweeep and Gretz, 2015). It also provides information for pathways which are influenced by these interactions miRNA to the various parts of mRNA. These predictions were carried out by 13 different tools used for the interaction studies.

Material and methods

Gene data

The genes data for study of breast cancer were taken from Catalogue Of Somatic Cancer genetics (COSMIC) which have a repository of 616 curated gene responsible for cancer (Forbes et al., 2016). This database classifies genes in to various categories like germline, somatic, tissue type, Cancer syndrome, molecular genetics, role in cancer etc. The somatic and germline data for breast cancer was obtained from cosmic database. There were total 575 somatic genes, out of which 31 are reported to cause breast cancer. While in germline 101 gene entries were found out of which 8 are responsible for causing breast cancer. The comparison with all cancerous genes represented in figure 1 (Hamberg et al., 2016).

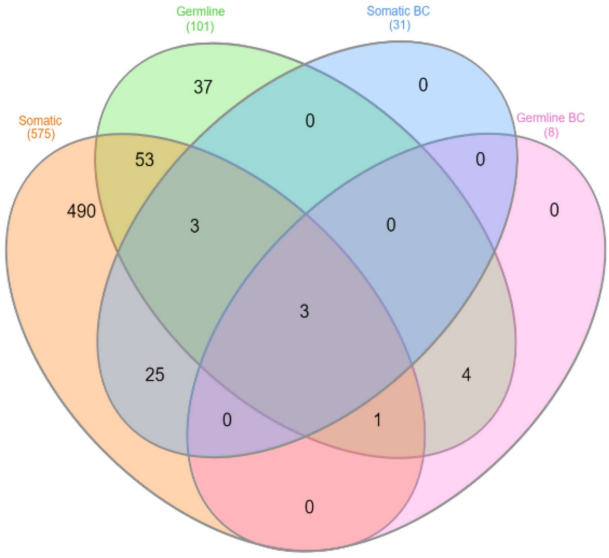

Figure 1. Number of somatic and germline genes causing breast cancer.

Interaction Network

miR Target Link is tool (Heberle et al., 2015) represents the miRNA-gene interactions and provides network which helps in the understanding the miRNA interaction patterns in a set of genes provided. It classifies results in to strong and weak interactions depending upon validation experiments and literature evidences. The strong interactions are those which have more than two experimental references for the interactions. Weak interactions are those which have less than two experimental evidences, or poor prediction values.

Interaction Sites

miRwalk 2 database (Dweep et al., 2014) was used to identified interaction sites of the identified miRNA. miRwalk2 database stores information of miRNA interactions from various validated data sources. It also has interaction profile of each identified miRNA at specific positions of mRNA in various organism including humans. About various diseases it stores 6727 gene ontologies, 4980 OMIM disorders, and 2035 disease ontologies.

miRNA Expression data

The miRNA expression pattern helps in determining specific functions and contributes for understanding overall outline of gene regulation studies. The miRNA expression

data is taken from miRmine database (Bharat et al., 2017). Specific miRNA identified from miRNA-gene interaction networks are searched for expression profile in various tissues and disease conditions. The RPM (Reads Per Million) value is taken for finding expression change in normal and cancerous tissue. The miRNA expression data used in this study is publicly available (Zu et al., 2014) with SRA experiment ID SRX513286 for normal and SRX513284 for cancerous breast tissue.

Motif Identification

MEME software suite (Trimothy and Charlse, 1994) was used for motif identification in various miRNA interacting to mRNA sites. All mature miRNA sequences were taken from Mirbase database and then processed for motif identification. Minimum motif width was taken as 6 and maximum width was 24 the average length of the sequences was found to be 23.5 nucleotide. The minimum e-value parameter was used for finding most appropriate motif.

Results

From COSMIC database which contains information for all gene contributing to somatic and germline cancer. The genes which are involved in breast cancer were taken from database and further classified in to two class somatic and germline genes. Total 31 genes were found for somatic breast cancer and 8 genes entries regulating germline breast cancer.

Tp53, CDH1, BAP1, CDKN1B, AKT1, ARID1A, CASP8, CCND1, ERBB2, ESR1, and NOTCH1 genes are having strong interactions with various miRNAs. miRNA interaction networks were developed for each class of genes i.e. somatic miRNA-gene interactions and germline miRNA-gene interactions. The miRNA interactions were classified in to strong and weak type. Strong interactions are those which are validated and have at least two references to one type of interaction studied thorough various methods. All miRNA gene interactions obtained are through genetic network are represented in table 1.

Table 1. miRNA-gene interaction types for somatic and germline cancer

Interaction Type	Somatic miRNA	Germline miRNA	Common miRNA in Somatic + Germline
Strong	54	4	3
Weak	179	17	13
Total Interactions	233	21	16

The miRNA gene interaction networks results showing that only 4 miRNAs have strong interactions with genes involved in germline breast cancer and 54 miRNAs are involved with somatic breast cancer. On finding the site of interaction on mRNA i.e. at which part of mRNA these miRNAs are binding to control expression, it was found that, all miRNAs for germline breast cancer it was observed are interacting to either 5'UTR or 3'UTRs preferably. This is an interesting fact that motivates us to find the motifs in miRNA whether these miRNAs have some specific pattern which drags them to bind to UTR regions. We are reporting two prominent motifs in miRNAs as represented in Figure 3a and 3b.

Later, finding interaction of all miRNAs in somatic breast cancer genes it was observed that out of total 54 strong

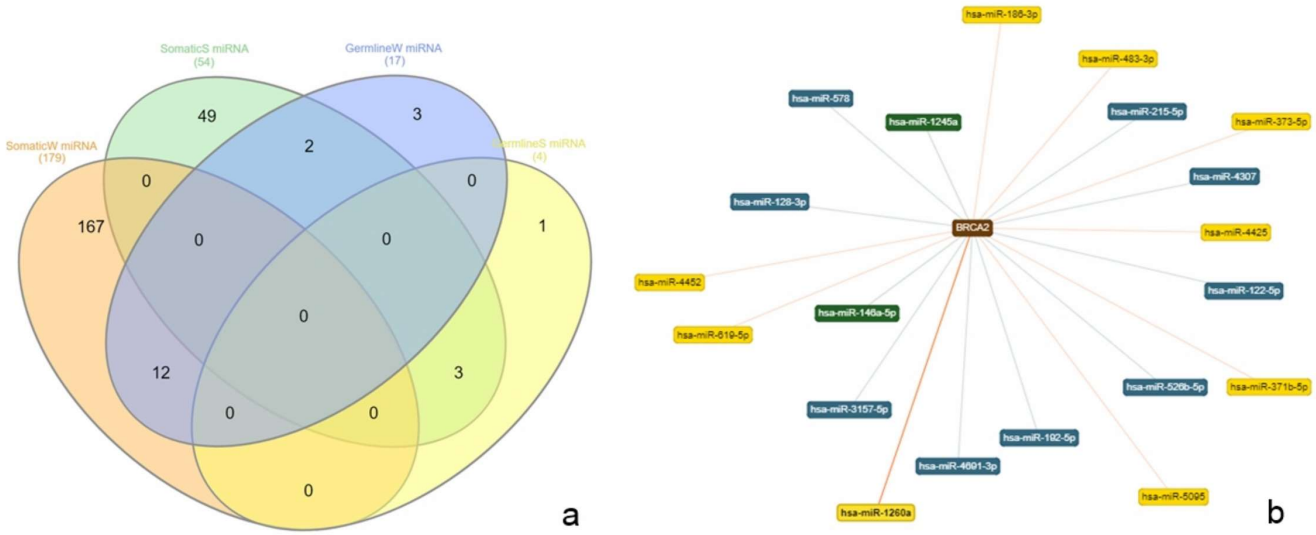

Figure 2. (a) Number of genes participating in miRNA based interactions in somatic and germline breast cancer. (b) A sample miRNA interaction of BRCA2 is represented

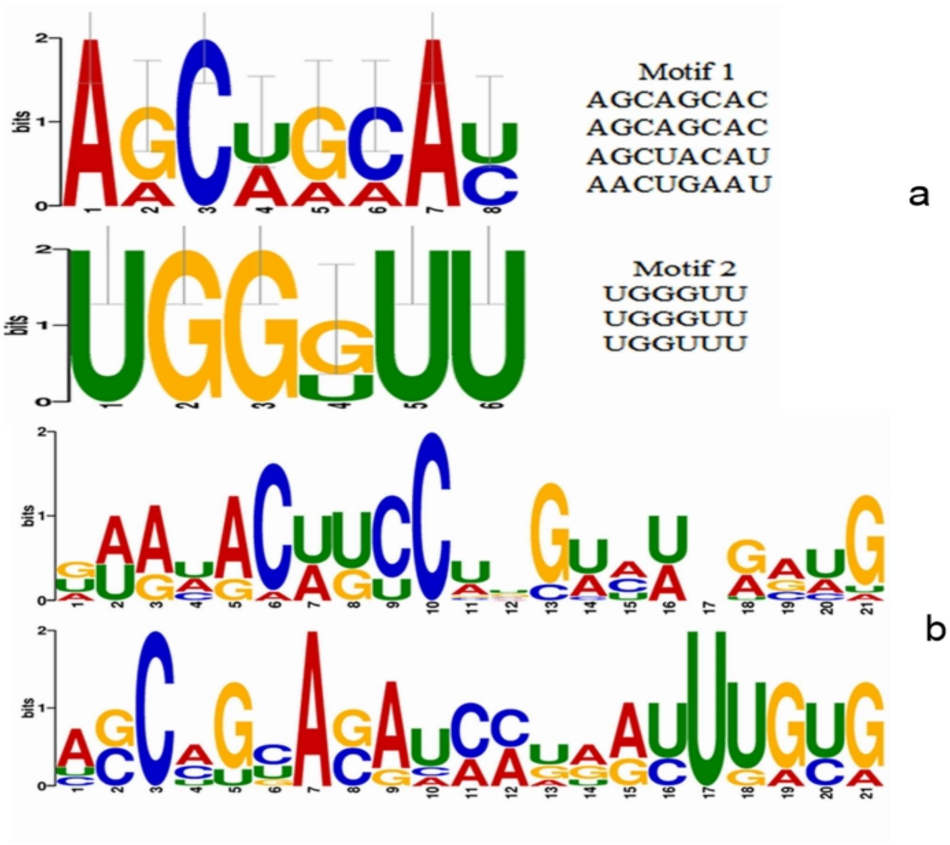

Figure 3. (a) miRNA Motif targeting genes of germline breast cancer and (b) miRNA Motif targeting genes of somatic breast cancer

miRNAs interactions only 3 miRNAs (hsa-miR-125b-5p, hsa-miR-125a-5p and hsa-miR-452-5p) are have affinity toward both 5'UTRs and 3'UTRs, while all other are having strong binding on specifically to 5' UTRs only. This provides an evidence that how these UTRs of mRNAs are responsible for binding of miRNA. It is well reported fact that the composition of UTR is responsible for mRNA translation to protein and controlled by miRNA interaction (Kumar, et al., 2016). On finding motifs in miRNA sequences using MEME software we found 2 motifs represented in Figure 4, with E= 4.7e-023, and E= 1.3e-004 respectively.

miRNA Expression in breast cancer

The expression value of each strongly interacting miRNA is represented Table 3 in reads per million (RPM) values with corresponding fold change in normal and breast cancer tissues. Up and Down expression of miRNA is considered if the difference in expression in cancerous and normal tissue is more than 5%. The expression pattern shows that hsa-miR-146a-5p is over expressed in germline while expression of hsa-miR-194-5p, hsa-miR-125b-5p, hsa-miR-375, hsa-miR-100-5p, hsa-miR-200b-3p, hsa-miR-452-5p has changed more than 1.5-fold in somatic cancer. Whereas, hsa-miR-16-5p, hsa-miR-15a-5p in germline and hsa-miR-181a-5p, hsa-miR-101-3p, hsa-miR-150-5p, hsa-miR-25-3p, hsa-miR-425-5p, hsa-miR-10b-5p, hsa-miR-15b-5p, hsa-miR-139-5p, hsa-miR-182-5p and hsa-miR-19b-3p are under expressed in somatic breast cancer. This provides an experimental support to our study for finding miRNA biomarker through miRNA-gene interaction network.

Discussion

In the current work, difference between somatic and germline breast cancer had been studied through miRNA gene interaction networks and expression profile to find important biomarker for detection of specific breast cancer. The miRNA and their role in controlling gene translation is well known to all (Budak and Akpinar, 2015; Zhang, 2015), but exact understanding of mechanism and their specificity is still under development. The interaction networks between various genes and proteins to understand the system are well stablished. Here, we used miRNA interaction networks and their expression, which reveals how different miRNA are acting together with various gene responsible for breast cancer. We identified genes through COSMIC database and segregated them into genes for somatic and germline breast cancer. Among all germline and somatic cancer genes, total 31 and 8 genes were found to have key role in causing somatic and germline breast cancer respectively. Out of these 3 genes were found common between both type as represented in figure1. These

Table 2. Weak and strong interactions of miRNAs with somatic and germline breast cancer genes

Type of Interaction	miRNA involved
miRNA_Germilne Strong	hsa-miR-146a-5p, hsa-miR-16-5p, hsa-miR-15a-5p, hsa-miR-221-3p
miRNA_Germilne_Weak	hsa-miR-215-5p, hsa-miR-192-5p, hsa-miR-182-5p, hsa-miR-4465, hsa-miR-26b-5p, hsa-miR-26a-5p, hsa-miR-1297, hsa-miR-27a-3p, hsa-miR-128-3p, hsa-miR-454-3p, hsa-miR-130b-3p, hsa-miR-19b-3p, hsa-miR-19a-3p, hsa-miR-124-3p, hsa-miR-193b-3p, hsa-miR-34a-5p, hsa-miR-17-5p.
miRNA somatic Strong	hsa-miR-125b-5p, hsa-miR-125a-5p, hsa-miR-25-3p, hsa-miR-30d-5p, hsa-miR-15a-5p, hsa-miR-16-5p, hsa-miR-221-3p, hsa-miR-222-3p, hsa-miR-10b-5p, hsa-miR-150-5p, hsa-miR-375, hsa-miR-200a-3p, hsa-miR-30a-5p, hsa-miR-30b-5p, hsa-miR-30c-5p, hsa-miR-30e-5p, hsa-miR-19b-3p, hsa-miR-9-5p, hsa-miR-92a-3p, hsa-miR-138-5p, hsa-miR-199a-5p, hsa-miR-204-5p, hsa-miR-200b-3p, hsa-miR-200c-3p, hsa-miR-141-3p, hsa-miR-429, hsa-miR-181a-5p, hsa-miR-24-3p, hsa-miR-196a-5p, hsa-miR-148a-3p, hsa-miR-194-5p, hsa-miR-182-5p, hsa-miR-452-5p, hsa-miR-302a-3p, hsa-miR-302b-3p, hsa-miR-302d-3p, hsa-miR-100-5p, hsa-miR-708-5p, hsa-miR-1-3p, hsa-miR-101-3p, hsa-let-7a-5p, hsa-let-7b-5p, hsa-miR-15b-5p, hsa-miR-520b, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-490-3p, hsa-miR-206, hsa-miR-559, hsa-miR-21-5p, hsa-miR-193a-3p, hsa-miR-22-3p, hsa-miR-145-5p, hsa-miR-139-5p
miRNA Somatic Weak	hsa-miR-128-3p, hsa-miR-215-5p, hsa-miR-192-5p, hsa-miR-578, hsa-miR-3157-5p, hsa-miR-4691-3p, hsa-miR-4307, hsa-miR-34a-5p, hsa-miR-485-5p, hsa-miR-27a-3p, hsa-miR-454-3p, hsa-miR-324-5p, hsa-miR-20a-5p, hsa-miR-18a-5p, hsa-miR-106b-5p, hsa-miR-106a-5p, hsa-miR-17-5p, hsa-miR-6825-5p, hsa-miR-6883-5p, hsa-miR-6785-5p, hsa-miR-4728-5p, hsa-miR-149-3p, hsa-miR-4644, hsa-miR-4306, hsa-miR-185-5p, hsa-miR-223-3p, hsa-miR-1296-3p, hsa-miR-6749-3p, hsa-miR-660-3p, hsa-miR-5193, hsa-miR-5196-3p, hsa-miR-19a-3p, hsa-miR-193b-3p, hsa-miR-26b-5p, hsa-miR-34c-5p, hsa-miR-1229-3p, hsa-miR-877-3p, hsa-miR-218-5p, hsa-miR-98-5p, hsa-miR-548x-3p, hsa-miR-548j-3p, hsa-miR-548aq-3p, hsa-miR-548am-3p, hsa-miR-548aj-3p, hsa-miR-548ah-3p, hsa-miR-548ae-3p, hsa-miR-548z, hsa-miR-548h-3p, hsa-miR-548d-3p, hsa-miR-548bb-3p, hsa-miR-548ac, hsa-miR-4789-5p, hsa-miR-4672, hsa-miR-6768-5p, hsa-miR-448, hsa-miR-153-3p, hsa-miR-155-5p, hsa-miR-1827, hsa-miR-6511a-5p, hsa-miR-1910-3p, hsa-miR-335-3p, hsa-miR-30e-3p, hsa-miR-30d-3p, hsa-miR-30a-3p, hsa-miR-142-3p, hsa-miR-152-3p, hsa-miR-148b-3p, hsa-miR-4652-3p, hsa-miR-4743-3p, hsa-miR-449a, hsa-miR-3140-3p, hsa-miR-329-5p, hsa-miR-4464, hsa-miR-4748, hsa-miR-6734-3p, hsa-miR-5088-3p, hsa-miR-1185-2-3p, hsa-miR-1185-1-3p, hsa-let-7f-2-3p, hsa-miR-98-3p, hsa-let-7f-1-3p, hsa-let-7b-3p, hsa-let-7a-3p, hsa-miR-302c-3p, hsa-miR-7852-3p, hsa-miR-4676-3p, hsa-miR-892c-3p, hsa-miR-130b-3p, hsa-miR-124-3p, hsa-miR-93-5p, hsa-miR-192-3p, hsa-miR-496, hsa-miR-365a-3p, hsa-miR-3191-5p, hsa-miR-6824-3p, hsa-miR-6764-3p, hsa-miR-330-5p, hsa-miR-326, hsa-miR-518c-5p, hsa-miR-188-5p, hsa-miR-296-3p, hsa-miR-484, hsa-miR-331-3p, hsa-let-7e-5p, hsa-miR-203a-3p, hsa-miR-144-3p, hsa-miR-765, hsa-miR-19b-1-5p, hsa-miR-4649-3p, hsa-miR-485-3p, hsa-miR-4768-3p, hsa-miR-5692a, hsa-miR-5583-5p, hsa-miR-3129-3p, hsa-miR-92b-3p, hsa-miR-32-5p, hsa-miR-363-3p, hsa-miR-367-3p, hsa-miR-29c-3p, hsa-miR-29b-3p, hsa-miR-29a-3p, hsa-miR-665, hsa-miR-6735-3p, hsa-miR-6884-5p, hsa-miR-4478,hsa-miR-4419b, hsa-miR-3929, hsa-miR-940, hsa-miR-6893-5p, hsa-miR-6808-5p, hsa-miR-6077, hsa-miR-8066, hsa-miR-758-5p, hsa-miR-374a-5p, hsa-miR-4691-5p, hsa-miR-6792-3p, hsa-miR-4709-3p, hsa-miR-20b-5p, hsa-miR-593-3p, hsa-miR-4261, hsa-let-7c-5p, hsa-let-7g-5p, hsa-miR-7845-5p, hsa-miR-9500, hsa-miR-6873-5p, hsa-miR-574-5p, hsa-miR-6818-5p, hsa-miR-6867-5p, hsa-miR-223-5p, hsa-miR-4746-3p, hsa-miR-18b-5p, hsa-miR-615-3p, hsa-miR-3662, hsa-miR-567, hsa-miR-181d-5p, hsa-miR-23b-3p, hsa-miR-181b-5p, hsa-miR-7109-5p, hsa-miR-6885-5p, hsa-miR-328-5p, hsa-miR-6887-5p, hsa-miR-6795-5p, hsa-miR-4422, hsa-miR-548e-5p, hsa-miR-129-5p, hsa-miR-7855-5p, hsa-miR-3121-5p, hsa-miR-1277-5p, hsa-miR-190a-3p, hsa-miR-5011-5p, hsa-miR-410-3p, hsa-miR-1252-3p, hsa-miR-153-5p, hsa-miR-3607-3p, hsa-miR-7-5p, hsa-miR-3185, hsa-miR-509-3p, hsa-miR-1226-3p, hsa-miR-335-5p

genes were then studied to find the miRNA interaction network networks through miRTargetLink for both type of cancer. The interactions were studied based on interaction evidences if any interaction have more than two relevant evidence then that is called as strong interaction if less than that are called as weak interactions. The interacting miRNA are listed in table 2 with weak and strong pattern. Weak interactions are much larger than strong interactions we used only strongly interacting miRNAs with respective gene type. By analyzing interaction networks, it was observed that total 4, 54 strong and 17, 179 weak interactions for germline and somatic breast cancer. Out of all interactions 16 miRNA interactions were common where 3 strong, 13 weak are common for somatic and germline breast cancer. This created a question whether these interactions have any common features among them this drives us to work on motif identification and interactions site detection. For motif finding all strong interacting mature miRNA sequences were taken to find common motif using MEME server shown in Figure 3a and figure 3b. The interaction sites were identified from MirWalk2 database where we found one interesting fact that most of the miRNAs have their preferable interactions sites at UTRs where only few are interacting at coding region. This is followed by miRNA expression analysis of where miRNA hsa-miR-146a-5p, hsa-miR-15a-5p for germline and hsa-miR-182-5p, hsa-miR-19b-3p, hsa-miR-194-5p, hsa-miR-125b-5p, hsa-miR-375, hsa-miR-100-5p are most influenced in breast cancer hence supports our ineraction network data. This provides a concept that miRNA-mRNA interaction networks can be used for identification of miRNA biomarkers.

Conclusion

It is well known fact that most of miRNA are interacting on either 5' or 3' UTR sequences of mRNA. In the current study, we are using a novel method to find putative biomarkers based on pattern of miRNA interactions through miRNA-interaction network in breast cancer. By interaction networks studies it is identified that miRNA hsa-miR-146a-5p upregulated by 1.068 fold and hsa-miR-15a-5p downregulated by 0.456 fold, are key players in germline breast cancer. However, somatic cancer hsa-miR-182-5p and hsa-miR-19b-3p are downregulated by 0.652 and 0.572, whereas hsa-miR-194-5p, hsa-miR-125b-5p, hsa-miR-375, and hsa-miR-100-5p are upregulated by 4.07, 2.501, 1.762 and 1.674 fold, are major contributor in somatic breast

Table 3. Expression of various miRNA in normal and cancerous breast tissues with fold change in expression (Zu et al., 2014)

Breast cancer	miRNA with strong	Normal (Tissue)	Breast cancer (Tissue)	Fold change	Type of Expression	Pubmed ID
Germline	hsa-miR-146a-5p	10922.3	11659.7	1.068	Up	24904649
	hsa-miR-221-3p	8765.6	8962.3	1.022	NA	
	hsa-miR-16-5p	6411.9	5423.8	0.846	Down	
	hsa-miR-15a-5p	5913.3	2697.4	0.456	Down	
Somatic	hsa-miR-194-5p	730.5	3219.5	4.407	Up	
	hsa-miR-125b-5p	904.4	2262	2.501	Up	
	hsa-miR-375	1531	2697	1.762	Up	
	hsa-miR-100-5p	4313.3	7222.1	1.674	Up	
	hsa-miR-200b-3p	660.9	1000.7	1.514	Up	
	hsa-miR-452-5p	347.8	522.1	1.501	Up	
	hsa-miR-148a-3p	8313.5	11790.2	1.418	Up	
	hsa-miR-30d-5p	9600.5	12094.8	1.260	Up	
	hsa-miR-125a-5p	2644	3220	1.218	Up	
	hsa-miR-24-3p	4869.8	5612.3	1.152	Up	
	hsa-miR-200a-3p	765.3	870.1	1.137	Up	
	hsa-miR-200c-3p	660.9	739.6	1.119	Up	
	hsa-miR-92a-3p	11212.1	12428.3	1.108	Up	
	hsa-miR-21-5p	17253	18968.8	1.099	NA	
	hsa-miR-222-3p	5913	6352	1.074	NA	
	hsa-miR-22-3p	10296.2	11050.6	1.073	NA	
	hsa-miR-30e-5p	12661.5	13182.4	1.041	NA	
	hsa-miR-199a-5p	2295.8	2320.3	1.011	NA	
	hsa-miR-141-3p	695.7	696.1	1.001	NA	
	hsa-miR-145-5p	695.7	696.1	1.001	NA	
	hsa-let-7a-5p	5556.2	5409.3	0.974	NA	
	hsa-let-7b-5p	12110.7	11355.2	0.938	NA	
	hsa-miR-181a-5p	6156.8	5329.5	0.866	Down	
	hsa-miR-101-3p	5982.9	5177.3	0.865	Down	
	hsa-miR-150-5p	7931	6526	0.823	Down	
	hsa-miR-25-3p	13890.5	11398.7	0.821	Down	
	hsa-miR-425-5p	5913.3	4698.7	0.795	Down	
	hsa-miR-10b-5p	5113.3	4046.1	0.791	Down	
	hsa-miR-15b-5p	2435	1827	0.750	Down	
	hsa-miR-139-5p	1460.9	957.1	0.655	Down	
	hsa-miR-182-5p	4939.4	3219.5	0.652	Down	
	hsa-miR-19b-3p	2156.6	1232.7	0.572	Down	

cancer. These miRNAs may help in early detection of germline and somatic breast cancer. These interaction networks followed by miRNA expression analysis in normal and cancerous breast tissue provides support to our finding for key miRNAs as biomarkers for detection of somatic and germline breast cancer. This study reveals that most of the interacting miRNA which regulate breast cancer are binding preferentially at 5' or 3' UTR region of mRNA. The current methods help, in finding the key miRNA through miRNA- RNA interactions and they may be used as biomarkers for detection of various diseases.

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Conflict of interest

The authors confirm that this article content has no conflict of interest.

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