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Breast Cancer Analysis Using miRNA Sequencing

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Breast cancer is one of the most common types of cancer among women and it is the leading cause of death in American women between the ages of 40 and 55. One out of 8 females in the United States will develop breast cancer at some point in their lives. MiRNAs are single stranded RNAs which have shown to play an important role in breast cancer progression by prevent some genes to express properly. Therefore, it is not surprising that the improper activity of miRNAs can cause various disease, including cancer. New studies suggest new ways for diagnostic, prognostic and predictive biomarkers using miRNAs analysis which can lead to designing miRNA-based anticancer therapies, with or without combination with current therapy methods to improve disease response and increase cure rates. In this survey, some of the newly conducted research, that use miRNA sequencing to analyze breast cancer, are reviewed.

Categories and Subject Descriptors: A.1 [**Introductory and Survey**]

Additional Key Words and Phrases: Breast Cancer, miRNA sequencing

Contents

1. INTRODUCTION

This survey concerns research which attempts to solve the problem of breast cancer analysis and identification using miRNAs. Since, miRNA are known to have an important role in breast cancer development, understanding the nature of their changes and the way they progress toward breast cancer development can lead us to design reliable diagnosis and better treatments of breast cancer tumors.

Relevant research papers were found by searching Google Scholar with the keywords *RNA sequencing,miRNA* and *breast cancer*. Various keywords and author names were used to search the ACM publication library, International Agancy for Research on Cancer(IARC), BMC biology,and BMC bioinformatics.

25 journal papers which are closely related to this survey were identified. They are listed in the bibliography.

10 papers were chosen as the basis of this survey. The papers and the reasons for choosing them are given below: [Iorio et al. 2005] was chosen as it identifies miRNAs whose expression was correlated with specific breast cancer features, such as estrogen and progesterone receptor expression. [Blenkiron et al. 2007] was chosen as it can be a suitable platform to classify breast cancer into molecular subtypes. [Lowery et al. 2009] was chosen as it describes use of artificial neural networks(ANN) to analyses microRNA expression profiles and predict the estrogen receptor (ER), progesterone receptor (PR) and HER2/neu receptor status of breast cancer samples. [Nygaard et al. 2009] was chosen as it addresses the identification and analysis of miRNAs in human breast cancer using deep sequencing. [Sun et al. 2010] was chosen as it identifies several miRNAs which may play an important role in identification of the biological characteristics of stem cells. [Buffa et al. 2011] was chosen as it describes the first large study integrating micro-RNA and mRNA global profiles in human breast cancer. [Farazi et al. 2011a] was chosen as it addresses the role of miRNA expression using RNA sequencing to analyze breast tumors. [Kim et al. 2012] was chosen as it describes a hierarchical support vector machine (SVM) to classify cancer subtypes based on their pathway. [Chang et al. 2012] was chosen as it describes and investigation of the role of differences in miRNA expression between normal and breast tumor tissues using RNA sequencing. [Volinia et al. 2012] was chosen as it describes and investigation of the role of microRNA regulation in breast cancer progression.

The remainder of this survey is structured as follows: Section 2 contains reviews of 3 papers ([Iorio et al. 2005], [Lowery et al. 2009] and [Buffa et al. 2011]). These papers are all concerned with role of miRNA in developing breast cancer tumors. Section 3 contains reviews of 2 papers ([Blenkiron et al. 2007] and [Kim et al. 2012]). These papers are all concerned with using miRNA in identification of breast cancer subtypes. Section 4 contains review of 4 papers([Nygaard et al. 2009], [Farazi et al. 2011a], [Chang et al. 2012] and [Volinia et al. 2012]). These papers are all concerned with using RNA sequencing for ana-

lyzing breast Tumors. Section 5 contains review of 1 paper ([Sun et al. 2010]). This paper is concerned with miRNA analysis using microarray technology .

2. ROLE OF MIRNA IN DEVELOPING BREAST CANCER

2.1. MicroRNA gene expression deregulation in human breast cancer

According to Iorio et al. [2005], it has been shown that miRNAs are usually expressed or mutated in cancer, suggesting that they may play an important role in activating tumor suppressor genes. The authors state that recognition of miRNAs that are differentially expressed between normal and tumor samples may help to identify those that are involved in human breast cancer and establish the basis to understand their pathogenic role. No article in this survey has been mentioned as previous work by the authors.

The authors state that the analyzed 76 breast cancer and 10 normal breast samples to identify miRNAs which their expression is significantly decreased in cancer versus normal breast tissues. Figure 1 shows the result obtained by the authors.

Table 2. Normal and tumor breast tissue class predictor miRNAs

miRNA name	Median expression		ANOVA* *P*	SVM prediction strength†	PAM score‡		Chromosome map
	Cancer	Normal			Cancer	Normal	
mir-009-1	1.36	1.01	0.0091	8.05	0.011	−0.102	1q22
mir-010b	1.11	1.70	0.0449	8.70	−0.032	0.299	2q31
mir-021	1.67	1.08	0.0047	10.20	0.025	−0.235	17q23.2
mir-034	1.67	1.09	0.0106	8.05	0.011	−0.106	1p36.22
mir-102 (*mir-29b*)	1.36	1.14	>0.10	8.92	0.000	−0.004	1q32.2-32.3
mir-123 (*mir-126*)	0.92	1.13	0.0940	9.13	−0.015	0.138	9q34
mir-125a	1.20	1.73	0.0033	8.99	−0.040	0.381	19q13.4
mir-125b-1	1.30	2.87	0.0265	14.78	−0.096	0.915	11q24.1
mir-125b-2	1.26	2.63	0.0233	17.62	−0.106	1.006	21q11.2
mir-140-as	0.93	1.10	0.0695	11.01	−0.005	0.050	16q22.1
mir-145	1.52	3.61	0.0040	12.93	−0.158	1.502	5q32-33
mir-155 (*BIC*)	1.75	1.37	0.0012	10.92	0.003	−0.030	21q21
mir-194	0.96	1.09	>0.10	11.12	−0.025	0.234	1q41
mir-204	0.78	0.89	0.0022	8.10	−0.015	0.144	9q21.1
mir-213	3.72	2.47	0.0108	9.44	0.023	−0.220	1q31.3-q32.1

*ANOVA (Welch *t* test in the Genespring software package) as calculated in Table 1.
†Support Vector Machine prediction analysis tool (from Genespring 7.2 software package). Prediction strengths are calculated as negative natural log of the probability to predict the observed number of samples, in one of the two classes, by chance. The higher is the score, the best is the prediction strength.

Fig. 1. [Iorio et al. 2005], page 7068

The authors claim that they could identify miRNAs whose expression was correlated with specific breast cancer features, such as estrogen and progesterone receptor expression. This

paper has been cited by [Blenkiron et al. 2007], [Sun et al. 2010], [Farazi et al. 2011a], [Chang et al. 2012] and [Volinia et al. 2012].

Table I represents some information about the papers discussed in this section.

Table I. List of papers presented in section 2

Year	Title	Authors	Papers referred to	Major Contribution
2005	MicroRNA gene expression deregulation in human breast cancer	Iorio et al.	[Blenkiron et al. 2007], [Sun et al. 2010], [Farazi et al. 2011a], [Chang et al. 2012] and [Volinia et al. 2012]	Identifying miRNAs which their expressions are significantly decreased in cancer versus normal breast tissues
2009	MicroRNA signatures predict estrogen receptor, progesterone receptor and HER2/neu receptor status in breast cancer	Lowery et al.	None	Identifying an optimum miRNA set using sequential selection and addition of miRNAs to the ANN
2011	microRNA-Associated Progression Pathways and Potential Therapeutic Targets Identified by Integrated mRNA and microRNA Expression Profiling in Breast Cancer	Buffa et al.	[Chang et al. 2012]	Using a cox regression for distant relapse-free survival to identify microRNAs that provide independent information.

2.2. MicroRNA signatures predict estrogen receptor, progesterone receptor and HER2/neu receptor status in breast cancer

According Lowery et al. [2009], in spite of new strategies which are increasingly being investigated and implemented, unpredictable response and the development of resistance to additional therapy remain major challenges in the clinical management of breast cancer patients. [Lowery et al. 2009] refer to [Blenkiron et al. 2007] as the previous work of their study. According to [Lowery et al. 2009], in [Blenkiron et al. 2007], there has been little overlap between breast cancer gene sets, which brings some uncertainty regarding their biological significance and reproducibility. The authors state that microarray technology is highly dependent on bioinformatics, mathematics and statistics to produce biologically relevant results. Moreover they state that the generation of high-complexity microarray data makes the development of new data analysis methodologies necessary. Moreover, they state that the current methods such as hierarchical clustering have shown some limitations for the modeling and analysis of high dimensionality data.

Artificial neural networks(ANN) is a machine learning technique that the authors used to analyze microRNA expression profiles and predict the estrogen receptor (ER), progesterone receptor (PR) and HER2/neu receptor status of breast cancer samples. The authors state that at each step of their model, additional miRNA transcripts were selected. Based on the authors claim, the addition of key miRNA transcripts can improve the predictive capabilities of the signature and model the data more accurately. As result, the miRNA transcript signatures predictive of ER, PR and HER2/neu status were generated from microarray data using the ANN model.

The authors claim that sequential selection and addition of miRNAs to the ANN successfully identified an optimum miRNA set. They state that their novel way to use ANN for analyzing miRNA expression identified biologically relevant miRNAs which are able to discriminate between various tumors with different hormone receptor condition. There is no citation to this paper by other papers in this survey.

2.3. MicroRNA-Associated Progression Pathways and Potential Therapeutic Targets Identified by Integrated mRNA and microRNA Expression Profiling in Breast Cancer

Buffa et al. [2011] state that several microRNAs previously linked with breast cancer subtype or progression in experimental models were not identified as independently prognostic. This agrees with previous results from prognostic studies. On the other hand using methods such as global expression analysis is challenging, as the microRNA- mRNA interaction network is complex and the effect on each individual mRNA is often small. Buffa et al. [2011] refer to [Blenkiron et al. 2007] as previous works of their study. Based on the authors opinion, despite previous efforts, integrated analysis of microRNA and mRNA global expression profiles has yet to be explored. They state that such analysis have the potential not only to identify microRNAs that are independent prognostic factors, but to identify interactions between microRNAs and targeted mRNAs for enhanced marker.

The authors state that to identify microRNAs that provide independent information, a Cox regression for distant relapse-free survival (DRFS) is proposed. They also used penalized least-square minimization with variable selection and regularization to prevent over fitting. They used a 2-step Cox analysis accounting for clinical, pathologic, and molecular features. They also state that a they use samples from 219 patients with early primary breast cancer. They state that matched microRNA and mRNA profiling was successfully obtained from 207 of 219 samples by using Illumina Human RefSeq-8 and miRNAv1 arrays. Figure 2 shows the result obtained by the authors.

The authors claim that their work is the first large study integrating micro-RNA and mRNA global profiles in human breast cancer. They state that these results explain the potential novel therapeutic targets, and could also be used to validate findings in independent studies. This paper has been cited by [Chang et al. 2012] in this survey.

Gene symbol	Function	Probeset used	HR[a]	P
TARBP1	DICER	GI_19743835-S	0.32	**0.0041**
DICER1	DICER	GI_29294648-I	0.44	**0.0388**
EIF2C4 (AGO4)	RISC	GI_29029592-S	0.45	**0.0420**
ESR1	p68 and p72 interaction	GI_4503602-S	0.49	0.0657
ARS2	Regulation of microprocessor	GI_33383230-A	0.74	0.4313
PRKRA	DICER	GI_32261293-S	0.86	0.7036
DGCR8	Microprocessor	GI_38488719-S	0.89	0.7642
EIF2C1 (AGO1)	RISC	GI_29171732-S	0.90	0.7667
RNASEN	Microprocessor	GI_21359821-S	1.06	0.8757
TARBP2	DICER	GI_19743837-A	1.24	0.5725
ADARB1	Editing of specific microRNAs	GI_7669476-I	1.27	0.5206
RAN	Transport	GI_6042206-S	1.34	0.4535
ESR2	p68 and p72 interaction	GI_10835012-S	1.51	0.2767
TRIM32	Binding to miRISC, enhancing microRNA activity	GI_15208649-S	1.78	0.1326
EIF2C3 (AGO3)	RISC	GI_29337285-A	2.46	**0.0244**
EIF2C2 (AGO2)	RISC	GI_29171733-S	2.63	**0.0128**
XPO5	Transport	GI_22748936-S	3.11	**0.0035**

[a]Cox univariate analysis. Gene expression considered as continuous variable, ranked, and normalized between 0 and 1. When more than one probeset mapped to the same gene, the probeset with the greater effect is shown. Probesets with $P < 0.05$ are highlighted in bold.

Fig. 2. [Buffa et al. 2011], page 5642

3. USING MIRNA IN IDENTIFICATION OF BREAST CANCER SUBTYPES

3.1. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype

According to Blenkiron et al. [2007], although both mRNA and miRNA are informative with regard to tumor subtype, their functional relationship remains unclear. The authors state that they are interested to investigate that changes in miRNA expression correlate with changes in mRNA levels as well or not. Blenkiron et al. [2007] cite [Iorio et al. 2005] as previous work in their study. As the authors mention in this article, many studies of miRNA expression in human cancer have focused only on the deregulation of miRNA expression. So, as the authors claim, their study represents the first integrated analysis of miRNA expression, mRNA expression and genomic changes in human breast cancer and may serve as a basis for functional studies of the role of miRNAs in breast cancer development. For generating a comprehensive set of miRNA expression profiles for breast cancer, they select 99 primary human tumors, 5 normal breast samples and 33 breast cancer cell lines for miRNA expression profiling. Figure 3 shows the result obtained by the authors.

MicroRNA/proximal probe correlations

		Host gene		Proximal probe		
miRNA	Chromosome position	Name	Pearson correlation	Name†	Pearson correlation	miRNA/probe distance (kb)
miR-101	1p31.1			*FLJ26232*	0.4337	35.99
miR-30e-5p	1p34.2	*NFYC*	0.4950			17.02
miR-181a	1q31.3			Hs.497310	0.4106	19.61
miR-205	1q32.2			*NPCA-5*	0.7936	0.00
miR-10b	2q31.1			*HOXD10*	0.4902	30.96
				HOXD8	0.4472	18.53
miR-149	2q37.3	*GPC1*	0.6567			11.68
miR-30a-3p	6q13			*BC040204*	0.6406	16.17
miR-30a-5p	6q13			*BC040204*	0.7091	16.17
				C6orf155	0.4995	11.12
miR-30c	6q13			*BC040204*	0.4566	10.30
miR-106b	7q22.1	*MCM7*	0.5157			1.02
miR-25	7q22.1	*MCM7*	0.4939			0.59
miR-93	7q22.1	*MCM7*	0.4988			0.79
miR-181a	9q33.3	*NR6A1*‡		*R08260*	0.5913	1.51
miR-181b	9q33.3	*NR6A1*‡		*R08260*	0.5634	2.78
miR-196a	12q13.13			*HOXC10*	0.4914	1.75
miR-342	14q32.2	*EVL*	0.7208			34.26
miR-10a	17q21.32			GI_30159691	0.8123	0.40
				HOXB6	0.8085	16.10
				HOXB5	0.7150	11.48
				HOXB3	0.6908	29.79
				HOXB2	0.7019	36.74
				HOXB4	0.6856	2.90
				HOXB8	0.5937	32.43
miR-199a*	19p13.2	*DNM2*‡		*TMED1*	0.4111	15.22
miR-99a	21q21.1	*C21orf34*	0.4766			67.87
miR-155	21q21.3			*BIC*	0.4688	0.73
let-7a	22q13.31			*FLJ27365*	0.5272	1.45
let-7b	22q13.31			*FLJ27365*	0.5732	2.38

†Gene symbol, accession number or Illumina probe identifier. ‡Gene lies on the opposite strand.

Fig. 3. [Blenkiron et al. 2007], page 9

The authors claim that their proposed profiling method can be a suitable platform to classify breast cancer into molecular subtypes. They state that they focus on miRNA expression analysis of a large set of breast tumors to identify signatures of tumor subtype. Also they claim that they identified 7 out of 24 miRNAs that had previously been associated with breast cancers. Also they state that, one miRNA, miR-155, is differentially expressed in ER- versus ER+ tumors, over expressed in breast tumors compared to normal controls and additionally other tumor types, suggesting that this miRNA may have diagnostic potential beyond breast cancer. This paper has been cited by [Lowery et al. 2009], [Nygaard et al. 2009], [Farazi et al. 2011a] and [Buffa et al. 2011] in this survey.

Table II represents some information about the papers discussed in this section.

Table II. List of papers presented in section 3

Year	Title	Authors	Papers referred to	Major Contribution
2007	MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype	Blenkiron et al.	[Lowery et al. 2009], [Nygaard et al. 2009], [Farazi et al. 2011a] and [Buffa et al. 2011]	Representing the first integrated analysis of miRNA expression, mRNA expression and genomic changes in human breast cancer.
2012	Pathway-based classification of cancer subtypes	Kim et al.	None	Introducing a new method to produce more stable pathway-based markers for discriminating breast cancer tumors

3.2. Pathway-based classification of cancer subtypes

Kim et al. [2012] state that the most significant gene markers are unstable (not reproducible) among data sets. One of the shortcomings of previous work, as authors of this article mention, is that the clinical implementation are slow, because the marker sets identified by independent studies rarely display substantial overlap. Using multi-level hierarchical feature, with individual genes at the first level and initial gene aggregates (pathways) at the next level, is an instance of a machine learning approach which organizes individual features into a hierarchical feature structure. Using this feature, the authors designed Gene Set Enrichment Algorithm (GSEA) to combine expression profiles from the cancer genome atlas and classify ovarian phenotypes based on ovarian cancer tissues.

The authors state that the present method produced more stable pathway-based markers for discriminating breast cancer tumors. Between two datasets for breast cancer metastasis,

the intersection of standard significant gene biomarkers totaled 7.47% of selected genes, compared to 17.65% using pathway-based markers. In addition, integrating pathway and gene information, The authors identified six (SQLE, E2F1, PTTG1, TSTA3, BUB1B, MAD2L1) known cancer genes significant for ovarian and breast cancer respectively.

Kim et al. [2012] claim that identification of canonical biomarkers improves clinical utility of high-throughput datasets for diagnostic and prognostic applications. There is no citation to this paper by other papers in this survey.

4. USING RNA SEQUENCING FOR ANALYZING BREAST TUMORS

4.1. Identification and analysis of miRNAs in human breast cancer and teratoma samples using deep sequencing

Since MiRNAs play important roles in various disease such as cancers, identifying the whole collection of miRNAs and understanding their expression patterns is an important goal. Nygaard et al. [2009] cite [Blenkiron et al. 2007] paper as the previous works of their research. The authors state that several papers have already described the usefulness of miRNAs as diagnostic molecules in diseases like cancer. Use of High-Throughput (HT) sequencing techniques are making miRNAs increasingly more popular for such discovery and profiling efforts. One of the shortcomings of the paper, as the authors addressed, is that the large amounts of data will be generated, and appropriate bioinformatics methods are needed to deal with the huge amount of data. The authors analyzed miRNA sequencing data from breast cancer samples, to evaluate the differential miRNA expression between breast cancer and normal adjacent breast, and to identify novel miRNAs. They state that they identified several differentially expressed miRNAs which increase the chance of miRNA involvement in breast cancer. They designed a novel combined method to analyze miRNA data for the purpose of identifying new miRNAs. They do it in two step: finding and quantifying expressed genomic regions giving rise to small RNA reads, and, as second step, scoring these regions as potential new miRNAs. Figure 4 shows the result of their experiment on breast cancer (BC) and their corresponding normal adjacent tissues (BN).

miRNA	BN	BC	Fold change
mir-200b	22.8 (1)	27122.2 (2325)	1189.6
mir-200c	45.5 (2)	44072.2 (3778)	968.6
mir-21	22.8 (1)	15363.4 (1317)	673.8
mir-378	68944.3 (3027)	466.6 (40)	-147.8
let-7a	2186.5 (96)	50313.2 (4313)	23.0
mir-320	136180.4 (5979)	19376.4 (1661)	-7.0
mir-23a	11319.9 (497)	44748.8 (3836)	4.0
mir-22	25646.3 (1126)	7150.9 (613)	-3.6

Fig. 4. [Nygaard et al. 2009],page 4

The authors claim that they identified five novel miRNAs, as well as two alternative precursors for known miRNAs. Several miRNAs were differentially expressed between the breast cancer and normal breast samples. Also they claim that the end variability was shown to be significantly different between miRNA and non-miRNA loci. There is no citation to this paper by other papers in this survey.

Table III represents some information about the papers discussed in this section

4.2. MicroRNA sequence and expression analysis in breast tumors by deep sequencing

In [Farazi et al. 2011a], the problem addressed by the authors is that mRNA expression signatures have been identified for some breast cancer types. The reason behind the aggressive nature of some types of breast cancer have not yet been identified completely. The authors mentioned papers [Iorio et al. 2005] and [Blenkiron et al. 2007] as the previous works of their research.

Table III. List of papers presented in section 4

Year	Title	Authors	Papers referred to	Major Contribution
2009	Identification and analysis of miRNAs in human breast cancer and teratoma samples using deep sequencing	Nygaard et al.	None	Evaluating the differential miRNA expression between breast cancer and normal adjacent breast, and identifying novel miRNAs.
2011	MicroRNA sequence and expression analysis in breast tumors by deep sequencing	Farazi et al.	[Chang et al. 2012] and [Volinia et al. 2012]	Designing an unsupervised hierarchical clustering model for their clinical samples using miRNAs
2012	Breast cancer signatures for invasiveness and prognosis defined by deep sequencing of microRNA	Volinia et al.	[Chang et al. 2012]	gathering detailed molecular analysis of the normal to offensive breast cancer alternation
2012	Comprehensive analysis of microRNAs in breast cancer	Chang et al.	None	Applying next generation sequencing data to perform miRNA analysis in breast cancer

The authors addressed the shortcoming of the previous works as the role of miRNa in breast cancer has been studied in several researches but only a few of them have focused on identifying the miRNA level or the variations in their sequence. Also, the relation between these miRNAs and their alteration has not been identified in large clinical studies yet.

Farazi et al. [2011] state that they found two miRNA variations among the other miRNAs. They also designed an unsupervised hierarchical clustering model for their clinical samples using miRNAs. They gathered samples from patients between 1985 and 2006. Those samples were then isolated and in the next step they were aligned to the genome to find their actual

position. Finally, as the authors state, using an analysis method and ImageGauge software, the expression level of each miRNA was measured. The authors identified various miRNA variations. Also, they found two single nucleotide polymorphisms miR-196a-2* and miR-146a which have been suspected before for their role in breast cancer. they claimed that, based on their experiments, the normal breast samples were separated from most non-offensive breast cancer types by miR-21 miRNA. In addition, the authors claim that the patients who went on to develop breast cancer showed an increase in mir-423 expression, and triple negative breast cancer was most distinct from other tumor subtypes because of up-regulation of the miRNAs mir-17 and mir-92.

This paper has been cited by [Chang et al. 2012] and [Volinia et al. 2012].

4.3. Comprehensive analysis of microRNAs in breast cancer

miRNA sequence reads exist with the name isomiRs, whose position and length are different from the reference miRNAs. Chang et al. [2012] state that there are different issues with isomiR, such as pattern preferences in specific libraries, target gene selection difference between different isomiRs and so on.

The authors refer to [Iorio et al. 2005], [Buffa et al. 2011], [Farazi et al. 2011a] and [Volinia et al. 2012] as previous work in this area of research. According to the authors, the previous work by Farazi et al. only focused on the relevance of specific miRNAs and the tumor malignancy type, without providing further experimental validation.

The authors apply their next generation sequencing data to perform miRNA analysis in breast cancer. They include differential miRNA expression, position shifts in isomiRs and changes in 3' end of chromosome. They gathered sequence reads from deferent datasets. Then they determined clean reads and mapped them to the genome. Then reverse transcription primers were specifically designed for the examined miRNAs. Finally they used miRNA target gene to identify significantly enriched pathways.

Figure 5 shows the comparison between normal and tumor samples using different types of RNAs conducted by the authors.

Category	miRNA	mRNA	tRNA	rRNA	snoRNA	scaRNA	snRNA	other ncRNA	repeat	unknown
Normal	75.24%	2.16%	0.47%	0.87%	0.84%	0.04%	0.12%	9.78%	0.0349%	10.45%
Tumor	84.79%	0.86%	0.37%	0.33%	0.35%	0.04%	0.14%	6.40%	0.0006%	6.70%

Fig. 5. [Chang et al. 2012], page 4

The authors claim that they identified 22 differentially expressed miRNAs in normal and cancer tissues which could be involved in breast cancer development. Also, they claim that based on their data analysis, the position shifts in isomiRs and 3' end changes were uniform. Also, There is no citation to this paper by other papers in this survey.

4.4. Breast cancer signatures for invasiveness and prognosis defined by deep sequencing of microRNA

The alternation from a normal breast cancer to its offensive type is the most important event in breast cancer development that is still not well understood. Volinia et al. [2012] refer to [Iorio et al. 2005] and [Farazi et al. 2011a] as previous works of their study.

Also, no shortcomings of previous work were mentioned by [Volinia et al. 2012]. The authors studied the relation between miRNAs and predicted some new miRNAs by using overall survival parameters. Also, they claim that they gathered detailed molecular analysis of the normal to offensive breast cancer alternation which has been done by only a very few laboratories at this level of detail. The authors state that they created miRNA profiles for offensive breast cancer and normal breast samples. They found 9 miRNAs which are differentially expressed during alternation. Figure 6 (table S2 in the original paper) shows those miRNAs.

Parametric *P* value	IDC (all)	DCIS (HER2+/ER–)	Fold change	miRNA
4e-07	1782.9	4307.6	0.41	miR-10b
5e-07	16213.2	41554.8	0.39	miR-143
5.2e-06	4601.1	1822.5	2.52	let-7d
1.59e-05	222.9	360.3	0.62	miR-218(2)
5.43e-05	282.2	639.3	0.44	miR-335-5p
7.13e-05	5898.2	12809.3	0.46	miR-126
0.0004	2978.2	1526.1	1.95	miR-221
0.0004	5669.2	1954.3	2.9	miR-181a(2)
0.0010	838.2	249.3	3.36	miR-210

Fig. 6. [Volinia et al. 2012], Supplementary material, page 5

The authors claim that they obtained very informative miRNAs for breast cancer. They claim that their results confirmed the miRNA deregulation reported in previous studies. They also claim that their work increases the knowledge of the miRNA role in progression of breast cancer.

This paper has been cited by [Chang et al. 2012].

5. MICRORNA ANALYSIS USING MICROARRAY TECHNOLOGY

5.1. Microarray-based analysis of microRNA expression in breast cancer stem cells

The breast cancer is one of the most common cancers in woman. Sun et al. [2010] state that previous research has shown that breast cancer stem cells (e.g. ESA+CD44+CD24-/low, BCSCs) possessing the stem cell properties of self-renewal and multi-directional differentiation are the most fundamental contributors to drug resistance, recurrence and development of breast cancer. The authors address [Iorio et al. 2005] as previous work of their study. The authors believe that there are some unresolved issues with regard to the molecular basis of cancer tumors. For example, what is the involvement of breast cancer stem cells in the molecular mechanisms of tumor development? or the role of miRNAs in the function of breast cancer stem cells. They investigated the miRNA expression profiles of ESA+ CD44+ CD24-/low BCSCs from the MCF-7 cell line, since the identification of cancer stem cell-related miRNAs would provide valuable information for a better understanding of cancer stem cell properties and even the molecular mechanisms of cancer tumors. The authors, as they mention in this paper, isolated ESA+ CD44+ CD24-/low BCSCs from MCF-7 cells using fluorescence-activated cell sorting (FACS). A human breast cancer xenograft assay was performed to validate the stem cell properties of the isolated cells, and microarray analysis was performed to screen for breast cancer stem cells (BCSC)-related miRNAs. These BCSC-related miRNAs were selected for bioinformatic analysis and target prediction using online software programs. Figure 7 shows the result obtained by the authors.

Based on the results obtained in this study, the authors claim that the miR-301, miR-296, miR-21 and miR-373 are expressed in development of human stem cells, indicating that

Name	E	CT(BCSCs)	CT(MCF-7)	ΔCT (BCSCs-MCF7)	RQ (BCSCs/U6)	RQ (MCF-7/U6)	RQ (BCSCs/MCF7)	Chip (BCSCs/MCF7)
U6 RNA	1.893 ± 0.087	18.307 ± 0.163	15.003 ± 0.227	3.303 ± 0.297			8.154 ± 0.516	
miR-122a	1.885 ± 0.098	23.650 ± 2.810	23.253 ± 2.812	0.397 ± 0.031	0.041 ± 0.007	0.006 ± 0.001	6.344 ± 0.402	50.414
miR-188	1.766 ± 0.036	31.103 ± 0.539	24.795 ± 0.508	6.308 ± 0.129	0.004 ± 0.003	0.015 ± 0.001	0.226 ± 0.513	0.207
miR-200a	1.900 ± 0.074	28.387 ± 0.261	21.253 ± 0.632	7.134 ± 0.652	0.002 ± 0.001	0.021 ± 0.017	0.086 ± 0.514	0.159
miR-21	1.899 ± 0.011	24.657 ± 1.325	17.263 ± 1.435	7.393 ± 0.195	0.016 ± 0.003	0.226 ± 0.051	0.071 ± 0.503	0.211
miR-224	1.683 ± 0.065	32.437 ± 0.400	33.497 ± 0.624	-1.060 ± 0.288	0.011 ± 0.001	0.001 ± 0.000	14.175 ± 2.033	14.491
miR-296	1.905 ± 0.025	27.237 ± 0.291	22.247 ± 0.468	4.990 ± 0.255	0.003 ± 0.001	0.009 ± 0.003	0.334 ± 0.587	5.242
miR-301	1.873 ± 0.017	27.487 ± 0.476	19.791 ± 0.619	7.696 ± 0.179	0.005 ± 0.004	0.081 ± 0.006	0.066 ± 1.008	0.205
miR-31	1.817 ± 0.027	27.397 ± 0.448	25.613 ± 0.634	1.783 ± 0.210	0.013 ± 0.001	0.005 ± 0.000	2.816 ± 0.328	10.700
miR-373*	1.902 ± 0.040	24.370 ± 1.438	24.060 ± 1.404	0.310 ± 0.096	0.019 ± 0.001	0.003 ± 0.000	6.684 ± 0.548	6.183
miR-200C	1.888 ± 0.053	24.513 ± 0.658	19.527 ± 0.938	4.987 ± 0.290	0.032 ± 0.042	0.100 ± 0.013	0.345 ± 0.531	1.720

Fig. 7. [Sun et al. 2010], page 5

these miRNAs may play an important role in identification of the biological characteristics of stem cells. There is no citation to this paper by other papers in this survey.

Table IV represents some information about the papers discussed in this section.

Table IV. List of papers presented in section 5

Year	Title	Authors	Papers referred to	Major Contribution
2010	Microarray-based analysis of microRNA expression in breast cancer stem cells	Sun et al.	None	Identifying of cancer stem cell-related miRNAs to provide valuable information for a better understanding of cancer stem cell properties and even the molecular mechanisms of cancer tumors.

6. CONCLUDING COMMENTS

This survey reviewed ten papers, which were studies of the role of miRNA's in developing breast cancer and using RNA sequencing to identify relevant miRNAs which can lead to developing more effective breast cancer treatment. It was surperising that all of the papers in this survey were journal papers. Also most of these references are relatively new.

Table V represents some information about the papers discussed in this survey. Future

Table V. List of papers presented in survey

Year	Title	Authors	Papers referred to
2005	MicroRNA gene expression deregulation in human breast cancer	Iorio et al.	[Blenkiron et al. 2007], [Sun et al. 2010], [Farazi et al. 2011a], [Chang et al. 2012] and [Volinia et al. 2012]
2007	MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype	Blenkiron et al.	[Lowery et al. 2009], [Nygaard et al. 2009], [Farazi et al. 2011a] and [Buffa et al. 2011]
2009	Identification and analysis of miRNAs in human breast cancer and teratoma samples using deep sequencing	Nygaard et al.	None
2009	MicroRNA signatures predict estrogen receptor, progesterone receptor and HER2/neu receptor status in breast cancer	Lowery et al.	None
2010	Microarray-based analysis of microRNA expression in breast cancer stem cells	Sun et al.	None
2011	microRNA-Associated Progression Pathways and Potential Therapeutic Targets Identified by Integrated mRNA and microRNA Expression Profiling in Breast Cancer	Buffa et al.	[Chang et al. 2012]
2011	MicroRNA sequence and expression analysis in breast tumors by deep sequencing	Farazi et al.	[Chang et al. 2012] and [Volinia et al. 2012]
2012	Breast cancer signatures for invasiveness and prognosis defined by deep sequencing of microRNA	Volinia et al.	[Chang et al. 2012]
2012	Comprehensive analysis of microRNAs in breast cancer	Chang et al.	None
2012	Pathway-based classification of cancer subtypes	Kim et al.	None

works are suggested by some of the authors of the papers. Blenkiron et al. [2007] state that further analysis of integrated datasets can help to solve miRNA dependent pathways in breast cancer. Lowery et al. [2009] suggest that finding the miRNA layer of genetic regulation can help targeted therapy in breast cancer by improving the understanding of molecular targets. Chang et al. [2012] state that the further study on the possible effects of miRNAs on breast cancer is needed. Buffa et al. [2011] suggest that the approach used in their study can be used in study of other biologically and clinically similar diseases.

7. ACKNOWLEDGMENT

I would like to gratefully thank Dr. Richard Frost for his useful detailed comments and notes on organizing the structure of this survey, and Dr. Luis Rueda for his support and advice.

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