International Journal of Oncology Research
www.oncologyjournal.in
Online ISSN: 2664-6668, Print ISSN: 2664-665X
Received: 26-03-2022, Accepted: 12-04-2022, Published: 27-04-2022
Volume 2, Issue 1, 2022, Page No. 21-28

Using MicroRNA expression levels to detect doxorubicin resistance in breast cancer: A systematic review

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DOI: https://doi.org/10.33545/2664665X.2022.v2.i1a.9

Abstract
Purpose: Aberrant expression of miRNA in tumor cells have been studied for almost a decade for their association with survival. This systematic review summarizes the miRNAs found to have increased expression in doxorubicin-resistant breast cancer cells, their roles in cell survival, and clinical utility of creating a miRNA panel to detect doxorubicin-resistance.
Methods: A search in PubMed and Google Scholar was performed to identify studies published until July 2021 on miRNAs associated with doxorubicin-resistance in breast cancer cells. Articles selected for inclusion were evaluated for risk of bias and assessed for quality by using MOOSE guidelines. The identified miRNAs and their roles in cell survival were summarized.
Results: A total of 8 studies were included in this systematic review. Most of the studies were primary research of miRNAs associated with doxorubicin-resistance as well as the function of the gene products they produce and their role in chemoresistance. Six different miRNAs with increased expression in doxorubicin-resistant cells relative to parental breast cancer cells (miRNA-200, miRNA-31, miRNA-141, miRNA-429, miRNA-21, miRNA-181) were described in more than one study.
Conclusion: This systematic review is limited by lack of homogeneity between the included studies owing to sparse data on the subject. Because of the multifactorial process of chemoresistance, several variables and their individual effects on miRNA expression levels are not accounted for consistently across studies due to limited data. More studies are needed to substantiate the increasing evidence that these miRNAs can characterize a chemoresistant phenotype. In the future, the identification of miRNA expression levels in chemoresistant cancer cells can aid in directing therapies to combat their respective mechanism of resistance.

Keywords: miRNA, biomarker, doxorubicin-resistance, chemotherapy, breast cancer, systematic review

Introduction

Breaast cancer is the second leading cause of cancer deaths in worldwide and in the United States. In the population over age 60 years, cancer incidence is higher and women make up most of this age group due to life expectancy. Early screening and improved treatments have contributed to cancer survivorship. There are several types of treatments available including surgical resection, radiation therapy, and various chemotherapeutic agents [1]. About 20-25% of all breast cancer diagnoses are triple-negative breast cancer (TNBC), which is characterized by the lack of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2). TNBC has a less favorable prognosis despite available treatments because these receptors are the targets for conventional therapy [2]. As such, treatment is typically limited to surgery, radiation, and cytotoxic therapy. Current standard therapy for triple-negative breast cancer is combination chemotherapy regimen including doxorubicin, an anthracycline. A major adverse effect of doxorubicin is dose-dependent cardiotoxicity which limits its use to a lifetime dose of 450-550mg/kg [3]. However, because it has been positively associated with pathologic complete response rates (pCR), it is used more frequently in patients with TNBC [4]. Although pCR is increased in TNBC compared to non-TNBC, patients who have residual disease after neo-adjuvant therapy have lower survival rates than those with non-TNBC [2]. In adjuvant and neo-adjuvant chemotherapy, effectiveness is limited by resistance developed in the tumor tissue by genetic and epigenetic changes in the cancer cells which can be intrinsic or acquired through exposure to chemotherapy agents [5]. The majority of patients with TNBC have residual disease after initial treatment which has a high risk of relapse, especially within the first three years of treatment. For patients who have breast cancer relapse, the possibility of resistance to conventional therapies limits potential chemotherapy options because standard regimens contain anthracyclines. Initiating rechallenge regimens containing anthracyclines have been suggested for patients with recurrence after adjuvant chemotherapy who have 6 to 12 months of disease free survival but there is few data to support their efficacy. Further studies are needed on the use of anthracylines in rechallenge regimens with consideration to resistance due to dose-limiting toxic effects that require close monitoring [3, 6] in order to provide an alternative to discontinuation.

The molecular changes that occur with resistance have been studied for over a decade and have allowed for the development of more targeted therapies [5]. MicroRNAs (miRNAs) have been studied as potential biomarkers for diagnosis, prognosis, and response to therapy using a technique called liquid biopsy [4]. miRNAs are noncoding RNAs composed of 21-25 nucleotides that regulate the expression of genes that modify translation of messenger RNA (mRNA) responsible for differentiation, apoptosis, and cell proliferation. Studies have shown that expression of miRNA can be altered by exposure to antineoplastic agents [7]. Researchers have been able to estimate sensitivity or resistance to certain chemotherapy agents by measuring miRNA expression and identifying the proteins they produce in samples with known resistance. From there, the mechanisms of resistance through the roles of these proteins have been investigated and innovative therapy of targeting their parent miRNAs has been proposed as a potential therapeutic strategy [5]. As we build the knowledge to make that technology possible, we can apply the fundamental information we do have to avoid unnecessary exposure to ineffective chemotherapeutics by detecting resistance. In this article, we performed a systematic literature review of miRNAs that are upregulated in doxorubicin-resistant breast cancer cells. Our findings show that there is sufficient agreement in the literature to warrant further investigation of the miRNAs cohesively with a larger powered study.

Materials and Methods

Search strategy

This systematic review adheres to the guidelines of Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) [9]. To obtain studies for this systematic review, we used two databases to search for relevant articles, Google Scholar and PubMed. We searched Google Scholar on June 9, 2021 for relevant primary research using the search terms “doxorubicin resistance,” “miRNA,” and “breast cancer” with exclusion terms of “sensitivity,” “sensitizes,” and “multi-drug.” PubMed was searched on March 17, 2021 for original research studies using the search terms “doxorubicin resistance” and “miRNA.” Primarily articles of genetic studies were returned in these databases. Of the results, only articles whose abstracts related specific miRNA associated with doxorubicin resistance in breast cancer were considered for evaluation. To further refine the results, studies were limited to those published between 2017 and 2021. Additional studies were located within the reference list of selected articles from the database searches. We screened titles and abstracts, reviewed full texts, and extracted data based on criteria outlined by MOOSE criteria including first author, study design, cell lines and/or preparation methods, miRNA expression levels and detection methods, quantitative outcomes, source of funding and reported conflicts of interests, and study limitations.

Eligibility criteria

Two individual investigators (JK and MP) independently assessed the eligibility of the retrieved articles. Discrepancies were resolved by consensus. Articles were excluded if they were case reports, letters, commentaries, conference records or reviews; included a combination of multiple miRNAs; included doxorubicin in combination with another drug; or lacked sufficient data for estimating HRs and 95% confidence intervals. Articles were considered eligible for inclusion in the systematic review if they met all the following initial inclusion criteria: focused on triple-negative breast cancer; measured miRNA expression levels in tumor or blood samples; clearly defined the utilized miRNA cut-off points; clearly described the utilized miRNA detection methods; analyzed the correlations between doxorubicin resistance and miRNA expression; and provided quantitative outcomes for miRNA expression. Data corresponding to the aforementioned selection criteria were extracted from articles meeting the exclusion and inclusion criteria.

Data extraction

The studies were evaluated for eligibility by the exclusion and inclusion criteria by both authors (JK and MP) and any discrepancies were presented to the corresponding authors for further evaluation. Data was extracted independently by 2 investigators (JK and MP), who used a spreadsheet designed based on PRISMA guidelines to retrieve predefined information from all studies qualifying for final inclusion. The following data were extracted after reviewing the full-text article and supplemental material: first author and publication year, miRNAs involved, cell lines studied and their origin, quantitative miRNA measurement platform, normalization method, unit of measurement and cut off value, and source of odds ratio with statistical significance. If quantitative outcome measurements (95% CIs) and P values could not be extracted from the original article, we estimated these values using the available data or the Kaplan–Meier curves presented in the articles.

Quality assessment

All of the included studies were assessed for quality using criteria derived from the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. The criteria determined by the authors to ensure quality control were clearly defined study design, description of the cells studied, definition of how resistance was determined, use of normalization and internal reference indicator, description of method of miRNA measurement, and comparison of miRNA expression levels relative to sensitive cell line. Two of the authors (JK and MP) critically reviewed the quality of eligible articles and selected only those who met all these criteria for final inclusion.

Publication bias

Two of the authors (JK and MP) individually assessed each included article for risk of bias. The year of publication, description of study method, and systems of measurement must be clearly defined in the article. Grey literature was included when available and effect size was calculated for these studies as well as those with statistically non-significant results and smaller studies by comparing them to larger studies and studies with statistical significance. A third author was used to resolve any disagreement on publication bias between the reviewers (JK and MP).

Ethical consideration

Ethical approval was not required for this study.

Results

Selection of studies

A flow diagram of the study selection process is shown in Figure 1. A total of 543 publications were identified in the initial search. After reviewing the titles and abstracts of these articles, we identified 35 articles evaluating the expression levels of specific miRNAs as they relate to doxorubicin resistance in TNBC. An additional 21 articles were selected for full-text evaluation by screening the reference lists of relevant articles. We then reviewed the full texts of these articles and excluded 26 articles. In total, 8 articles were eligible for inclusion in this systematic review.

Studies that could be included were selected using the following factors as guidelines for inclusion: an analysis of the association between miRNA expression and doxorubicin resistance, specific cell lines of human breast cancer, reported miRNA quantitative platform and normalization method, defined unit of measurement, and outcomes with statistical analysis. Some studies were not considered because of certain exclusion criteria such as studies that did not involve doxorubicin resistance in breast cancer, studies that focus on gene products of miRNA, and studies of samples exposed to multiple drugs. Additionally, articles that were not primary research and studies performed only in vitro were excluded.

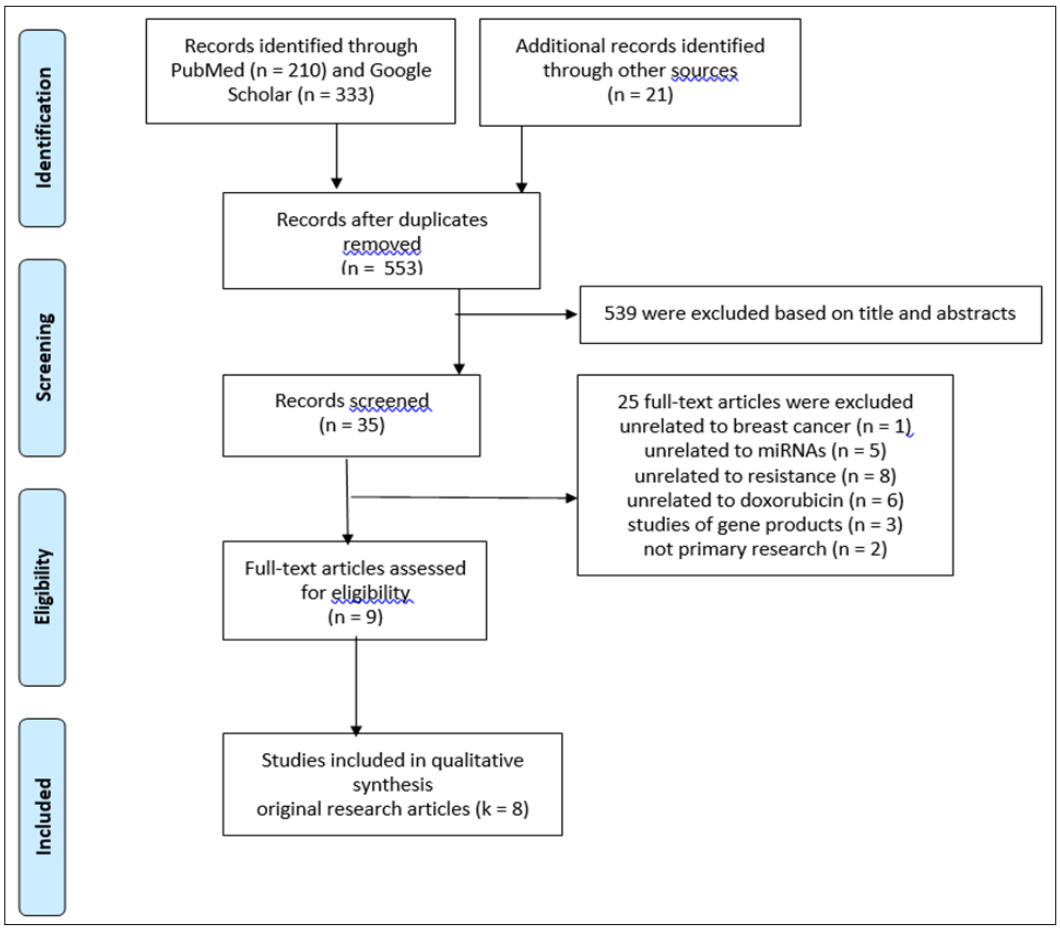

Fig 1: Flow diagram of the study selection process.

Characteristics of the included studies

Six of the included studies used the breast cancer cell line MCF-7 and resistant cell line MCF-7/Adr [9, 10, 13, 14, 17, 18] while three studies used MCF-MBA-231 and its resistant cell line [13, 15, 16]. All studies used reverse transcriptase-PCR to measure miRNA expression levels quantifiably. Most studies reported their results

as expression levels of miRNA by resistant cell line relative to parental cell line measured by folds of relative change. All of the included studies expressed this data as the mean +/- standard deviation and used a p-value of at least < .05 for significance. Five of the studies described only one particular miRNA while the remaining included studies reported several miRNAs (Table 1). In the included articles, the increased expression of 29 different miRNAs were associated with doxorubicin resistance. Among these, 6 miRNAs (miRNA-200, miRNA-31, miRNA-141, miRNA-429, miRNA-21, miRNA-181) were reported by more than one study [11, 13, 15, 16, 17]. We selected these 6 miRNAs for inclusion in this review (Table 2).

Table 1: Main characteristics of the eligible studies

Author, year	population	sample	miRNA quantification method	internal reference indicator	relative expression analysis method	comparison group	miRNAs	Source of HR	measurment units	cut-off value	P-value	reference
Bao 2012	MCF-7 and MDA-MB-231	human MCF-7 and MDA-MB-231, cultured MDA-MB-231 doxorubicin-resistant, MCF-7-Dox from laboratory of Debasis Mondal	miRNA northern blot assay kit (Signosis BioSignal Capture)	U6snRNA	(none)	resistant cells relative to parent cells	hsa-mir-31 hsa-mir-3021 hsa-mir-200a hsa-mir-200b hsa-mir141 hsa-mir-429 hsa-mir-373 hsa-mir-936 hsa-mir-597	measurement	folds of upregulation	two-fold perturbation in expression magnitude	N/A	13
Dai 2019	MCF-7 and MCF-7/Adr	MCF-7 and MCF-7-R purchased from Shanghai Gaining Biotechnology Co	qRT-PCR TaqMan MicroRNA Detection Kit (Thermo Fisher)	U6snRNA	2-ddCt	healthy vs breast cancer	mi-RNA-222	relative expression of miRNAs	expression, relative expression levels	(none)	p < 0.05	9
Kovalchuk 2008	MCF-7 and MCF-7/DOX	human, resistant cells synthesized in lab	SuperTaq Ploymerase (Ambion), mirVana qRTPCR miRNA Detection Kit (Ambion)	human 5s rRNA	2-ddCt	resistant cells relative to parent cells	miR-451 hsa-miR-106a hsa-miR-21 hsa-miR-206 hsa-miR-28	fold change of miRNA	fold change of miRNA level	(none); miRNA detection signal threshold was defined as twice the maximum background signal (180)	p < 0.01	14
Niu 2016	MDA-MB-231	MDA-MB-231 obtained from ATCC, tumor tissue obtained from patients with TNBC, MBA-MB-231 treated with 2 μg/mL DOX	quantified by NanoString nCounter, measured by qPCR	U6sncRNA	(none)	control vs treated	miRNA-181a miRNA-21 miRNA-125b miRNA-23a miRNA-96	relative expression of miRNAs, measurement	miRNA induction (fold)	(none)	p < 0.05	15
Ouyang 2014	MCF 10A, MDA-MB-231, BT-549, and Hs 578T	human TNBC tissue to identify miRNA expression signatures, MDA-MB-231/BT-549/Hs578T purchased from ATCC	qRT-PCR CFX96 (bioRad), NanoDrop 1000	U6	2-ddCt	TNBC compared to their adjacent normal tissues	miR-155-5p miR-21-3p miR0181a-5p miR-181b-5p miR-183-5p	relative expression of miRNAs	miRNA fold change	(none)	p <0.05	16
Sun 2018	MCF-7 and MCF-7/Adr	MCF-7 and MCF-7-R purchased from KeyGENE	qRT-PCR with ABI 7900 (ABI)	U6	2-ddCt	resistant cells relative to parent cells	miRNA-574	relative expression of miRNAs	relative expression of miRNA	(none)	p <0.05	10
Liu 2019	MCF-7 and MCF-7/DOX	MCF-7 and MCF-10A purchased from BeNA Culture Collection, MCF-7/DOX synthesized from MCF-7 in lab, human breast tissue samples	qRT-PCR by reverse transcription #BPI01030 (Beijing Protein Innovation) then subjected to PCR using UltraSYBR Mixture Kit	U6	2-ddCt	resistant and parental cells to normal breast tissue	miR-202-5p	relative expression of miRNAs	relative expression of miRNA, expression level	(none)	p < 0.05	18
Li 2020	BCSC1, BCSC2, and BCSC5	BCSC1 and BCSC2 from primary triple-negative breast tumors of patients who received NACT, BCSC5 from surgical patient, tumor tissue specimens from BCSC isolation obtained from Comprehensive Cancer Centre Freiburg	TaqMan Advanced MicroRNA Assay (Thermo Fisher)	(none)	(none)	BCSCs treated with chemotherapy to untreated control cells	hsa-miR-193a-5p hsa-miR-92a-3p hsa-192-5p hsa-miR-192-5p hsa-miR-375 hsa-miR-155-5p hsa-miR-21-3p	relative expression of miRNAs	relative expression of miRNA	(none)	p < 0.05	12
Lv 2014	MCF-7 and MCF-7/Adr	MCF-7 obtained from ATCC, MCF-7/Adr cells synthesized in lab	reverse transcribed using PrimeScript RTReagent Kit (Takra), PCR performed on ABI 7900 PCR System (Applied Biosystems) using SYBR Green PCR Master Mix (Applied Biosystems)	U6snRNA	2-ddCt	MCF-7 and MCF-7/Adr compared to control MCF-7	miR-200a miR-141 miR-31 miR-429 miR-196b	fold change relative to the control	folds of upregulation	10-fold change	P < 0.05	17

Table 2: Descriptive characteristics and relared data from included studies.

miRNAs	author, year	relative miRNA expression		cell line
		folds of change	P-value	
21	Kovalchuk, 2008	2.9	< 0.01	MCF-7/DOX
21-3p	Ouyang, 2014	2.05416956	< 0.05	human TNBC
28	Kovalchuk, 2008	7.6	< 0.01	MCF-7/DOX
31	Bao, 2012	81	N/A	MDA-MB-231-R
31	Lv, 2014	5 (SD 4.9-5.1)	< 0.05	MCF-7/DOX
106a	Kovalchuk, 2008	2.3	< 0.01	MCF-7/DOX
141	Bao, 2012	16	N/A	MDA-MB-231-R
141	Lv, 2014	14 (SD 12-15)	< 0.05	MCF-7/DOX
181a	Niu, 2016	2.5 (SE .7)	< 0.05	MDA-MB-231 treated with doxorubicin
181a-5p	Ouyang, 2014	5.06324165	< 0.05	human TNBC
181b-5p	Ouyang, 2014	5.60820896	< 0.05	human TNBC
183-5p	Ouyang, 2014	3.27145522	< 0.05	human TNBC
196b	Lv, 2014	2 (SD 2)	< 0.05	MCF-7/ADR
200a	Bao, 2012	28	N/A	MDA-MB-231-R
200a	Lv, 2014	32 (SD 30-36)	< 0.05	MCF-7/ADR
200b	Bao, 2012	3	N/A	MDA-MB-231-R
200c	Lv, 2014	19 (SD 18-20)	< 0.05	MCF-7/ADR
202-5p	Liu, 2019	4	< 0.05	MCF-7/DOX
206	Kovalchuk, 2008	4.8	< 0.01	MCF-7/DOX
222	Dai, 2009	3.75	< 0.05	MCF-7-R
302f	Bao, 2012	58	N/A	MDA-MB-231-R
373	Bao, 2012	2	N/A	MDA-MB-231-R
429	Bao, 2012	5	N/A	MDA-MB-231-R
429	Lv, 2014	7.5 (SD 7.2-7.7)	< 0.05	MCF-7/ADR
574	Sun, 2018	2 (SD .6)	< 0.05	MCF-7/Adr

miRNA-21 and doxorubicin resistance

Two articles described upregulation of miR-21 in doxorubicin-resistant breast cancer cells [14, 16]. Ouyang obtained triple-negative breast cancer cells from patients who underwent surgical resection and measured the levels of miR-21-3p expression. MiRNA expression levels of these cells were compared to those in normal adjacent breast tissue cells. The authors reported about a 2-fold increase in expression level of miR-21-3p in the breast cancer cells [16]. Similarly, Kovalchuk reported a 2.9-fold change in expression level of miR-21 in doxorubicin-resistant MCF-7 cells as compared to sensitive MCF-7 cells [14].

miRNA-31 and doxorubicin resistance

Bao compared the expression levels of miRNA-31 in a doxorubicin-resistant metastatic breast cancer cell line, MDA-MB-231-R, to the expression levels in its doxorubicin-sensitive parental cell line. The authors found an 81-fold increase in miRNA-31 expression levels in the doxorubicin-resistant cells relative to the doxorubicin-sensitive cells. [13] Lv reported a 5-fold increase in miRNA-31 expression levels in MCF-7/DOX cell line relative to its doxorubicin-sensitive parental cell line [17].

miRNA-141 and doxorubicin resistance

Bao measured the expression levels of miRNA-141 in MDA-MB-231-R cells to be 16-fold higher than their parental doxorubicin-sensitive cells [13]. Lv found a 5-fold increase in miRNA-141 expression levels in doxorubicin-resistant cells as compared to doxorubicin sensitive MCF-7 cells [17].

miRNA-181a/b and doxorubicin resistance

Ouyang compared the expression levels of miRNA-181b-5p in tissue cells collected from patients with confirmed triple-negative breast cancer to normal adjacent breast tissue of those patients and found a 5.6-fold increase in the TNBC cells. Ouyang found a 5.06-fold change between TNBC cells and normal adjacent tissues. Then they referenced literature to determine which deregulated miRNAs were associated with doxorubicin resistance and attempted to identify targets of their genes [16]. Niu found the relative expression of miRNA-181a to be 2.5-fold higher in MDA-MB-231 cells treated with doxorubicin than MDA-MB-231 cells without any exposure to doxorubicin [15].

miRNA-200a/b and doxorubicin resistance

Lv examined miRNA-200a expression in MCF-7/ADR cells and found a 32-fold increase in their expression levels relative to MCF-7 cells [17]. Bao found that miRNA-200a expression levels were 28-fold higher in MDA-MB-231-R cells than in MDA-MB-231 cells. They found that miRNA-200b expression levels were 3-fold higher in doxorubicin-resistant MDA-MB-231 cells than MDA-MB-231-R cells [13].

miRNA-429

miRNA-429 expression levels in MDA-MB-231-R cells were found to be 5-fold higher than the miRNA-429 expression levels in MDA-MB-231 cells as reported by Bao, *et al*. [13] Lv found a 7.5-fold increase in miRNA-429 expression levels in MCF-7/ADR cells relative to MCF-7 cells [17].

Discussion

Chemoresistance of tumor cells can be intrinsic, innate to the cells' morphology, and enhanced in response to chemotherapy exposure. The chemoresistance can occur through evasion of apoptosis or efflux of drugs from the cells by increasing the pumps that expel them from the tumor cells. These functions occur as a result of gene Products which are made through transcription from miRNAs. In searching the available studies of miRNAs associated with doxorubicin resistance, hundreds of miRNAs were reported with varied directions of deregulated miRNA expression levels. There were a large number of studies that explored miRNAs associated with doxorubicin sensitivity in order to identify their gene products as potential therapeutic targets. We included studies that discussed miRNAs associated with doxorubicin-resistance.

We conducted a systematic review to investigate the feasibility of creating a diagnostic panel of miRNAs capable of detecting doxorubicin-resistance in breast cancer patients. Many laboratory studies have isolated deregulated miRNAs of chemoresistant tumor cells in order to determine the mechanism of resistance their respective gene products are responsible for. The purpose of these studies is to identify potential targets for novel therapeutics such as nanoparticle delivery systems. Some deregulated miRNAs have decreased expression in doxorubicin-resistant cells relative to their unexposed parental cells. Considering the mechanism of laboratory testing and the affordability/mass-producible features/cost of testing, the most feasible means of testing is to confirm the presence of a miRNA in the sample, rather than quantify the amount of each miRNA in the sample. This excludes any downregulated miRNAs as candidates for inclusion in the proposed diagnostic panel. Using the folds of change in miRNA expression levels reported in the literature, we can establish cut-off values for each miRNA associated with doxorubicin-resistance.

Most of the miRNAs we found in the literature search were assessed only in a single study. Six miRNAs known to be associated with doxorubicin-resistance (miRNA-200, miRNA-31, miRNA-141, miRNA- 429, miRNA-21, miRNA-181) were evaluated in more than one study [11, 13, 15, 16, 17]. Therefore, we included these 6 miRNAs in our systematic review to determine if their expression levels were similar across studies. This review showed that in-

creased expression of miRNA-200, miR-31, miRNA-141, miRNA-429, miRNA-21, miRNA-181 were found by measurement or qt-PCR quantification, to be present in levels high enough to create a diagnostic panel. The miRNAs described were upregulated in MCF-7/ADR, MDA-MB-231-R, and human breast cancer tumor cells relative to their doxorubicin-sensitive counterparts.

MiRNA-21 is one of the most studied miRNAs because of its association with many cancer types. It is hypothesized to regulate various oncogenic processes such as tumor growth, spread, and invasion. Therefore, its increased expression can be associated with an increase in these processes. One way by which miRNA-21 serves as a key regulator of these processes is by inhibition of the proapoptotic phosphatase and tensin homolog (PTEN) tumor suppressor which has been shown to slow cell proliferation and, thus, apoptosis in breast cancer [19]. Aberrent expression of miRNA-31 has also been seen in various cancer types. It has been seen to have a tumor suppressor function in some cancer types and an oncogenic function in others. Interestingly, studies have shown that increased expression of miRNA-31 demonstrates tumor suppressor activity in highly aggressive breast cancers and promotion of metastasis in non-aggressive breast cancers. MiRNA-31 has been reported to have a role in mammary stem cell (MSC) activity regulation which is controlled by the Notch and Wnt pathways. Many studies have found that this miRNA regulates invasiveness and metastasis formation through activation of several oncogenic genes [20]. In triple-negative breast cancer cells, downregulated expression of miRNA-31 decreased levels of p65, downregulating protein kinase C episilon (PKCε) protein which participates in the regulation of BCL2 protein NF-κB pathway leading to chemosensitivity and apoptosis of tumor cells. However, in basal-like human breast cancer cells, upregulation of miRNA interfered with NF-κB signaling through targeting Smad3 and Smad4 and activated the Wnt/E-cadherin pathway, triggering tumorigenesis through β-catenin protein [21]. In this review, the expression levels of miRNA in doxorubicin-resistant MCF-7 breast cancer cells and MDA-MB-231-R cells were increased relative to their parental doxorubicin-sensitive cells [17, 13]. Neither study mentioned the presence of hormonal receptors of estrogen and progesterone which have been shown to alter levels of p65 in previous studies [21]. Because of this miRNA lack of tissue specificity and aberrant expression in breast cancer types, its utility as a biomarker of doxorubicin-resistance is limited. More studies are needed to explore the relationship of miRNA-31 overexpression and chemoresistance.

Similarly, miRNA-141 and miRNA-429 of the miRNA-200 family, have varied deregulation patterns and, therefore functions, depending on the breast tumor type and histological grade [22, 23]. The miRNA-200 family consists of 2 clusters, the miRNA-200b/200a/429 cluster or the miRNA-141/200c cluster [24]. Upregulation of some of the miRNAs in these clusters promote tumor invasiveness in certain breast cancer subtypes through the increased secretion growth factors such as vascular endothelial growth factor (VEGF) through deregulation of the phosphatidylinositol-4, 5-bisphosphate 3-kinase/Protein kinase B (PI3K/AKT) signaling pathway. VEGF is known to promote angiogenesis which permits migration and invasion of tumor cells. Multiple studies have reported overexpression of the miRNA-200 family in tumor cells with increased aggressiveness and invasion, even within the same triple-negative breast cancer, although these miRNAs are generally lower in triple-negative breast cancer compared to other subtypes [24]. MiRNA-141 is associated with poor prognosis and chemotherapy resistance [17]. Its increased expression has been seen in highly aggressive malignant breast tumors relative to benign tumors [22] and in doxorubicin-resistant cells relative to their doxorubicin-sensitive parental cells [17].

In triple-negative breast cancer cells, treatment with chemotherapy has been shown to cause increased expression of miRNA-181a which is associated with poor distant metastasis free survival (DMFS). DNA damage by chemotherapy activates signal transducer and activator of transcription 3 (STAT3) of the NF-κB pathway which mediates transcriptional activation of miRNA- 181 which can function in regulation of epigenetic regulation and as a transcription factor. With exposure to doxorubicin, its increased expression correlates with increased cancer cell survival and aggressiveness by targeting the pro-apoptotic gene BAX [15].

Limitations of study

The primary limitation of this systematic review is the lack of available data on new concepts in molecular chemotherapeutic studies. Although the role of miRNA in tumor growth and survival has been investigated for nearly a decade, it has not been validated through studies sufficiently to become widely accepted knowledge and therefore has yet to be applied for the purpose of any therapeutic value. With limited data, our review is confined to a small number of studies which are sparsely homogenous with variable methods and measurements of outcome. These differences contribute to the difficulty in determining whether results are reliable. For this reason, a systematic review was chosen over a meta-analysis. This deficit is highlighted to invite other researchers to conduct a meta-analysis as more data on the subject is published. We are still understanding the mechanisms of miRNA and how they are affected by insults to the tumor microenvironment which provides some uncertainty to the conclusions of any studies on the subject.

As most of the included studies were conducted in vitro, the results are further challenged by the unclear utility of clinical application as they do not take into account the interaction of the cells in their environment and the holistic response that occurs when the cells are manipulated. Chemoresistance is a multifactorial process, intertwining several mechanisms such as drug efflux, apoptosis, phenotype of cancer cells, the tumor microenvironment, and the various signaling pathways that are involved in the response to chemotherapy exposure. Because of all of these factors, chemoresistance must be studied from several different aspects. This is not achieved in studies that assess chemotherapeutic stress in vitro. It is not likely that the amount and concentration of chemotherapeutic agents that reach the tumor cells can be estimated in individual patients. So, studies typically imitate the chemotherapeutic stress on the cells with drug-specific sub-lethal doses in vitro and

delineate what response they will have In vivo with the other factors considered [12]. The limited availability of data is even further decreased when considering that the various factors that contribute to chemoresistance vary between cell lines, chemotherapy drugs, and individual patients can augment the heterogeneity of studies. More studies are needed to be able to conduct a meta-analysis with significant power. As more data becomes available, we can assemble a chemoresistant phenotype with an appropriate miRNA profile to detect it.

Conclusions

Specific miRNAs can potentially detect whether cancer cells have developed chemoresistance which can prevent unnecessary exposure to these toxic therapies. Due to the limited research available, the clinical application of these findings is not confirmed. Studies should be conducted to determine the sensitivity and specificity of a miRNA panel test for drug resistance.

HCA Healthcare Disclaimer

This research was supported (in whole or in part) by HCA Healthcare and/or an HCA Healthcare affiliated entity. The views expressed in this publication represent those of the author(s) do not necessarily represent the official views of HCA Healthcare or any of its affiliated entities.

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