Pathway study and systematic evaluation of microRNAs involved in anthracycline- induced cardiotoxicity in breast cancer patients

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Review

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Abstract

Background

Anthracycline treatment often causes cardiotoxicity in breast cancer patients. Imaging and cardiac biomarkers are now used as criteria for cardiotoxicity. However, new biomarkers are needed for early diagnosis. Gene expression may be controlled, in part, by little non-coding RNA molecules called microRNAs (miRNAs). Several microRNAs (miRNAs) have been linked to cardiovascular illness and are being studied as indicators for cardiotoxicity caused by cancer treatments.

Methods

We conducted a comprehensive search of the literature until April 2020 using the following databases: Medline/PubMed, Cochrane Central Register of Controlled Trials, Scopus, Lilacs, Web of Science, and Embase. Anthracycline-induced cardiotoxicity and non-cardiotoxicity patients with breast cancer who participated in cohort studies reporting miRNA biomarkers were considered. Moreover, we examined the miRTarBase for experimentally confirmed miRNA-target interactions.

Results

Only five of the 209 studies that were found met the requirements for inclusion. Two population-based cohorts confirmed the validity of Let-7f, miR-1, miR-20a, miR-126, and miR-210. Epirubicin-cardiotoxicity dramatically down-regulated the pro-angiogenic miRNAs let-7f, miR-20a, miR-126, and miR-210, compared to the non-cardiotoxicity group. Although alterations in miR-1 levels have been debated in doxorubicin-treated breast cancer patients with cardiotoxicity, they have been demonstrated to give diagnostic and prognostic information in the context of myocardial infarction. Target genes for let-7f, miR-1, miR-20a, miR-126, and miR-210 were used to compile a cardiotoxicity-related reactome pathway.

Conclusion

Anthracycline-based cardiotoxicity during breast cancer treatment seems to be linked to let-7f, miR-1, miR-20a, miR-126, and miR-210.

Keywords: Anthracycline, cardiotoxicity, doxorubicin, epirubicin, microRNAs, and breast cancer.

1. Introduction

Female breast cancer accounts for the majority of all cancer diagnoses and deaths in women [1]. Breast cancer was responsible for the deaths of an estimated 627,000 women in 2018, or 15% of all female cancer fatalities, according to the World Health Organization [2]. Treatment with anthracyclines like doxorubicin and epirubicin for breast cancer has greatly increased disease-specific survival [3]. However, this chemotherapy schedule has been linked to a higher risk of cardiovascular complications and death, particularly in women of advanced age. Thus, early detection and prevention of cardiovascular disease [4] depend on accurate risk factor classification.

Recent studies have suggested using cardiac imaging and cardiac biomarkers to detect myocardial damage early in cancer patients receiving anthracycline and/or anti-HER2 (human epithelial growth factor receptor 2) treatment [5,6]. A reduction in left ventricular ejection fraction (LVEF) is the most generally known echocardiographic characteristic to cardiotoxicity assessment. However, LVEF has a low detection threshold for mild myocardial impairment [7]. During patient follow-up, circulating markers of cardiac disease onset such as troponins and brain natriuretic peptides have shown promise as useful biomarkers for identifying patients at risk for developing myocardial dysfunction [8,9]. However, when tissue damage has occurred, only then can circulating levels of these biomarkers rise.

The role of microRNAs (miRNAs) in anthracyclines-induced toxicity has been studied using in vitro and in vivo models [10]. Post-transcriptional regulation of gene expression is achieved by miRNAs, a family of short noncoding RNAs (21-25 nucleotides) that may either repress translation of mRNA or hasten its destruction [11]. They play a role in a wide variety of vital biological processes, including embryonic development, cell signaling, cell division, intercellular communication, and even apoptosis. It is important to note that aberrant miRNA expression has been linked to the onset and development of clinical disorders, such as heart illnesses [12].

Increased plasma levels of miR-1, miR-133a, and miR-208 were seen in rats that had been treated with doxorubicin [13,14]. Specifically, miR-133a levels quickly rose during acute myocardial infarction and were shown to be more sensitive than cardiac troponin T [15]. Additionally, 25 breast cancer patients who had received anthracycline-based chemotherapy showed increased plasma levels of miR-34a and miR-122 after treatment [16]. MiRNAs have been found to be promising indicators for cardiac disorders, but their likely participation in anthracycline-induced cardiotoxicity [17] still needs further investigation. Despite the clinical significance, most of the investigations have relied on non-human animal models, which have shortcomings as human biology predictors [18].

The purpose of this review was to determine whether or not anthracycline-induced cardiotoxicity is linked to the differential expression of circulating miRNAs in breast cancer patients. Pathway analysis was another goal, with the intention of uncovering the biological processes associated with these miRNAs.

2. Substances and Techniques

The methods used in this review were those recommended in the Cochrane Handbook [19]. Results were reported in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [20]. The PRISMA checklist is included in Additional file 1. The present study's protocol has been submitted to PROSPERO (http://www.crd.york.ac.uk/ PROSPERO, number CRD42020177833) at the International Prospective Register of Systematic Reviews.

2.1. technique for locating

P (participants) = women with breast cancer who received anthracycline treatment; E (exposure) = cardiotoxicity; C (control) = women with breast cancer who had anthracycline treatment without cardiotoxicity; O (outcome) = microRNA expression levels; PECO question set search technique. Medline was searched through PubMed (Medical Literature Analysis and Retrieve System Online), CINAHL EBSCO (Cumulative Index to Nursing and Allied Health Literature), Scopus, LILACS through Virtual Health Library (VHL) (Latin American and Caribbean Health Sciences),

Scielo (Scientific Electronic Library Online), ISI Web of Science (Core Collection), and Embase (Electronic Medical Library) for relevant articles. Mesh phrases "breast neoplasms," "cardiotoxicity," and "microRNA" were used in conjunction with the entry terms to find relevant articles. The PubMed search approach is outlined in the second supplementary file.

The same phrases were used to search for clinical studies in Google Scholar, www.scholar.google.com/ and OpenGrey, www.opengrey.eu/. We searched the databases of the Federal University of Minas Gerais (https://repositorio.ufmg.br/), the University of So Paulo (https://www.teses.usp.br/), the Oswaldo Cruz Foundation - Fiocruz (https://portal.fiocruz.br/repositorio-institucional-arca), the University of Brasilia (https://repositorio. No restrictions were placed on the reports' original language or publication date since any reports that could be relevant were taken into account. Additional relevant references were found by searching the reference lists of the featured papers.

Titles and abstracts of all publications retrieved were reviewed by two writers (J.D.P. and M.T.A.) working separately to determine inclusion in this research. In the event of a dispute, a third researcher (K.B.G. or J.A.G.T.) helped to reach a conclusion.

Studies were deemed to be eligible if they compared the miRNA expression levels of patients with breast cancer who underwent anthracycline cancer treatment with or without cardiotoxicity. Studies that did not include a non-cardiotoxicity control group, used animal models or cell lines, or failed to provide the results of interest were disregarded. Therefore, we omitted reviews and meta-analyses.

2.3. Methods for Choosing Studies and Extracting Data

Studies were obtained from many electronic databases, compiled into a single digital library, and then duplicates were eliminated using the EndNote® bibliographic management system. The data was compiled by two reviewers (J.D.P. and M.T.A.) using a common form. In cases where two reviewers could not agree on how to extract the data, a third reviewer (K.B.G. or J.A.G.T.) was brought in to settle the dispute. Data extraction included the following categories of information: 1) research details (such as author and publication year); 2) assessed sample type (plasma, serum, or tissue); 3) miRNAs measured; 4) miRNA detection technique; and 5) miRNA expression in each study group. Information was also gathered from the control and exposure groups.

2.4. Evaluation of the reliability of each research

Using the Newcastle-Ottawa quality evaluation scale (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp), two reviewers (J.D.P. and M.T.A.) evaluated the included studies' risk of bias and methodological quality. This scale has eight items, including the following: exposure cohort sample representativeness, unexposed cohort selection, exposure measure (e.g., secure records, structured interviews), outcome assessment method, study follow-up duration sufficient to test hypothesis, and adequate cohort follow-up. Each task is worth a certain number of stars, with nine being the maximum. If the study scores over 6, it is of good methodological quality. We examined the expression of miRNAs between the cardiotoxicity and non-cardiotoxicity groups, and we chose a p-value 0.05 to represent a statistically significant difference.

We searched the miRTarBase to find target genes for each of the five miRNAs identified in this systematic review, and considered their different names or aliases, as follows: let-7f (hsa-let-7a-5p), miR-1 (hsa-miR-1-3p), miR-20a (hsa-miR-20a-3p and hsa-miR-20a-5p), miR-126 (hsa-miR-126-3p and hsa-miR-126-5p), and miR-210 (hsa-miR-210-3p). miRTarBase was created to give complete information on experimentally proven miRNA-target interactions [21]. Only target genes that had at least one experimental validation technique (reporter assay, western blot, or qPCR) that provided strong evidence according to miRTarBase [21] were selected for the pathway analysis. Target genes for each

miRNA were manually extracted (Table 3), and Enrichr [23] was used to search for important well-curated signaling pathways based on these genes, with results ordered by p-value ranking 0.5.

3. Results

3.1. Cases for Research

In Fig. 1, we depict the process we followed to determine which papers to include in our meta-analysis. 1. From the original pool of 209 studies found, 53 were disqualified as duplicates or non-qualifiers. Studies that did not examine anthracycline treatment for breast cancer were not included, nor were experimental studies, review articles, or meta-analyses. Only the titles and abstracts of the remaining 156 articles were read and scored. At this juncture, J.D.P. and M.T.A.'s Kappa coefficient of agreement was 0.862.

After reading the titles and abstracts, 133 research were removed for not satisfying the inclusion criteria, and 23 possibly suitable publications were chosen. Reasons for excluding studies were: participants aged <18 years old, patients who were not treated with chemotherapy with anthracyclines, studies conducted on animal models or cell lines, studies which did not evaluate microRNA levels, those which did not present a proper non-cardiotoxicity group, review papers and meta-analyses. MiRNA expression was assessed in breast cancer patients with and without anthracycline-cardiotoxicity (cases and controls, respectively) in studies meeting the inclusion criteria. However, after reviewing the entire texts of these papers, we found that 18 were ineligible because they either (1) did not assess anthracycline-based cancer treatment, (2) did not offer a non-cardiotoxicity group, (4) were done on animal models/cell lines, or (5) were not main studies. Finally, five papers [[24], [25], [26], [27], [28]] met the inclusion criteria for this systematic review.

3.2. Methodology and evaluation of study quality

One of the five investigations was conducted on an Italian population [24], two on Chinese populations [25,28], one on Brazilians [26], and one on Americans [27]. Since the results of one research [24] were presented using two distinct anthracyclines (doxorubicin and epirubicin), we counted them as two separate reports. Table 1 displays the primary features of these items.

The studies used cohort designs and compared miRNA expression in plasma samples between groups with and without anthracycline-induced cardiotoxicity. Two studies in particular looked at miRNA expression as potential circulating markers of cardiotoxicity; Rigaud et al. [26] examined data from the CECCY study (NCT01724450), while Gioffre et al. [24] evaluated findings from the ICOS-ONE clinical trial (NCT01968200). One of the studies analyzed only triple negative breast cancer [28] and one excluded HER-2 positive breast cancer patients [26]. There were four studies where cardiac damage was measured at both baseline and after a year of medication. One research [27] was taken into account for the definition of a brief follow-up (after the first infusion).

Four studies included LVEF (measured by echocardiography) as a criterion for cardiotoxicity. One research [24] looked at cardiac troponin (troponin I or T) to determine the presence of cardiotoxicity. In the papers that were considered, sample sizes varied from 32 up to 363. There were a total of 708 people studied throughout all of these trials, with an average age of 45.38 years (cardiotoxicity, n = 76) and a wide range of ages (non-cardiotoxicity, n = 632).

In some of the trials we looked at, anthracycline was taken in tandem with other cytotoxic drugs. Both Todorova et al. [27] and Rigaud et al. [26] combined doxorubicin (cumulative dosage of 240 mg/m2) and cyclophosphamide (600 mg/m2), then administered paclitaxel (80 mg/m2 or docetaxel 75 mg/m2) thereafter. Patients on cardio-protective medications such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers, or beta-blockers were excluded from all but one study [Rigaud et al., 2016]. Epirubicin (100 mg/m2), cyclophosphamide (600 mg/m2), and docetaxel (75-100 mg/m2) were used as neoadjuvant chemotherapy in another research [28]. Qin et al. [25] used

the same neoadjuvant chemotherapy regimen in their trial, which included HER2 positive patients who were treated with trastuzumab on demand (6 mg/kg, following docetaxel). One research compared the two anthracyclines described above [24]. According to the clinical study, epirubicin and doxorubicin had a median cumulative dosage of 360 [270-360] and 240 [240-240] mg/m2, respectively. Breast cancer patients who participated in the study received taxanes in 63% of cases, trastuzumab in 22.5% of cases, and imatinib in 2% of cases [29].

Two studies [24,27] analyzed the miRNA profiles in the plasma of individuals receiving anthracycline treatment. Only Gioffre et al. [24] hand-picked miRNA candidates for single qPCR validation of miRNA array data. Two research [25,26] used a literature search to identify miRNAs for further analysis by RT-qPCR, whereas a third study [28] did not reveal the candidate miRNA selection procedure.

Supplemental material 3 displays the results of the quality evaluation of the included studies using NOS for cohort studies. The typical level of NOS was an 8. All studies had a low risk of bias (scoring 6), indicating that they were of excellent quality.

3.3. MicroRNAs that are differentially expressed between anthracycline cardiotoxicity and non-cardiotoxicity in breast cancer patients

Two studies solely looked at miRNA expression initially [25,28]. One research, however, analyzed miRNA expression before and after the first dosage of the medicine [27], whereas the other studies analyzed miRNA expression before and after therapy at least twice [24,26].

Different investigations have reported varying numbers of miRNAs with differential expression between individuals with cardiotoxicity and those without, from 3 [24] to 32 miRNAs [27]. Forty microRNAs were found to have significantly varied expression levels throughout the five trials (p 0.05; Table 2). Four microRNAs (let-7f, miR-20a, miR-126, and miR-210) exhibited consistent down-regulation in the cardiotoxicity group compared to the non-cardiotoxicity group in two investigations [25,28]. Only miR-1 exhibited inconsistent findings, with reports of down-regulation in one research [27] and up-regulation in another [26] among individuals with cardiotoxicity.

In particular, one research examined people with and without cardiotoxicity and found that 26 miRNAs were up-regulated in the cardiotoxicity group [27]. Another research [24] found that three miRNAs had higher expression levels in individuals with cardiotoxicity. Additionally, three miRNAs were down-regulated in patients with cardiotoxicity who were treated with anthracyclines for breast cancer in two separate investigations [25,27], and one miRNA was down-regulated in patients with cardiotoxicity who were treated with anthracyclines in a third study [28].

The levels of 11 miRNAs (let-7b, miR-17-3p, miR-18a, miR-19b-1, miR-130a, miR-146a, miR-148a-3p, miR-208a, miR-208b, miR-296, miR-423-5p) were not different between the cardiotoxicity and non-cardiotoxicity groups ($p > 0.05$, data not show).

3.4. Five miRNAs showed differential expression, and their target genes were analyzed for pathways.

We employed a strategy centered on the target genes reported in the miRTarBase for the five miRNAs (let-7f, miR-1, miR-20a, miR-126 and miR-210) or their aliases. Notably, let-7f and miR-1 each had a different number of target genes, ranging from 46 to 80 (Table 3). We then used the target genes for each miRNA to conduct a pathway analysis and look for potential biological pathways involved in anthracycline-induced cardiotoxicity. Each of the five miRNAs' reactome pathways is shown in Fig. 2. For example, the "Signal transduction R-HAS-162582" pathway is shared by let-7f, miR-1, miR-20a, and miR-126 (Fig. 2), while the "Cellular responses to stress R-HAS-2262752" route is shared by let-7f, miR-20a, and miR-210 (Fig. 2).

4. Discussion

The diagnosis of acute myocardial damage prior to the established malfunction [17] makes the hunt for new biomarkers for the early detection of cardiotoxicity therapeutically important. Researchers in this area have found evidence that miRNAs have a significant role in regulating anthracycline-induced heart damage [[13], [14], [15], [16]]. Because they are persistent in circulation, resistant to degradation by nucleases, and may be identified prior to the development of clinical signs [30], circulating miRNAs have the potential to serve as non-invasive biomarkers. The determination of the optimal miRNA candidates for cardiotoxicity evaluation is complicated by the fact that studies have showed conflicting findings regarding miRNA expression patterns [31]. Furthermore, considerable variation was also detected in various studies related to cardiotoxicity criteria, the number of patients involved and the number of miRNAs studied [32]. We conducted this meta-analysis to systematically analyze all studies that investigated the differential expression of miRNAs in breast cancer patients because of the relevance of miRNAs as diagnostic biomarkers in anthracycline-induced cardiotoxicity. Five microRNAs (let-7f, miR-1, miR-20a, miR-126, and miR-210) were shown to be considerably downregulated in two groups of breast cancer patients experiencing anthracycline-induced cardiotoxicity.

The let-7 family includes the pro-angiogenic miRNA let-7f [27]. This molecule has angiogenic and endothelial action and alters the clinical outcome for ischemic stroke in young people [13]. Directly affecting TGF- and vascular endothelial growth factor (VEGF) [27], Let-7f aids the vascular system. In dilated cardiomyopathy, decreased LVEF was also observed to be associated with reduced let-7f expression [27]. Therefore, let-7f may protect patients receiving anthracycline treatment from cardiotoxicity [27], lowering the risk of cardiac dysfunction. Breast cancer patients who had anthracycline-induced cardiotoxicity had lower levels of let-7f compared to those who did not experience cardiotoxicity, according to two Chinese studies included in this systematic review [25,28]. Based on their hypothesized pro-angiogenic function, 14 miRNAs were chosen for evaluation in both investigations using RT-qPCR. The validation of miRNAs on an independent cohort of participants helps to strengthen the use of miRNAs as minimally invasive screening and triage tools prior to further diagnostic assessment, even when miRNA candidates were picked from the literature or based on past evidences. In addition, the authors included a 12-month follow-up period and epirubicin (dosage of 100 mg/m2) chemotherapy patients in their study. In all trials, cardiotoxicity was defined as a reduction in LVEF of 10% from baseline, with an endpoint of 53% or less. The reliability of comparing findings is ensured by the consistency in research design and miRNA detection technology. Furthermore, let-7f was identified in plasma miRNA expression profiles from Chinese women with breast cancer in these two investigations, limiting generalizability. Validation of these results requires more research using samples from other demographics.

According to the results of the pathway study, one of let-7a-5p's target genes is LIN28A (Lin-28 Homolog A). The importance of Lin28a in pathological cardiac hypertrophy in a mouse model was recently shown [33]. Lin28a and Lin28b, two closely related RNA-binding proteins, play important roles in pluripotency, organismal development, tissue healing, and oncogenesis via their ability to block microRNA let-7 maturation or by directly binding to mRNAs to control their abundance and translation [34,35]. The miR-17 family, of which miR-20a is a part, is part of the miR-17/92 cluster, a group of genes with an oncogenic function that shows differential expression in breast cancer, particularly in estrogen receptor-negative tumors [36]. Notably, the miR-17/92 cluster was disrupted in cardiovascular, immunological and neurodegenerative illnesses [37]. Studies have revealed that miR-20a regulates angiogenesis in breast cancer and causes vascular mesh defects [38]. In addition, cardiotoxicity patients were shown to have lower plasma levels of miR-20a compared to patients who did not experience cardiotoxicity, suggesting that miR-20a might serve as a circulating marker of cardiotoxicity caused by cancer therapy [25,28].

In the work by Mojdeh Mahmoudian and others, it was found that some microRNAs were expressed more strongly in BC tumors compared to the nearby tissues. In particular, hsa-miR-25-3p, -29a-5p, -105-3p, and -181b1-5p were turned up, while hsa-miR-335-5p and -339-5p were turned down. Most of these potential microRNAs, except for hsa-miR-339-5p, were linked to different TNM stages by either going up or down in levels. In addition, all but hsa-miR-105-3p of the potential microRNAs were linked to HER-2 status. The study of ROC curves also showed that these six microRNAs

could work together as a measure to tell the difference between breast tissue samples from people who don't have tumors and those who do.

Cancers and autoimmune illnesses benefit greatly from miR-126's involvement in angiogenic and inflammatory processes [39]. Tumors have been demonstrated to have low levels of miR-126, despite the fact that this microRNA may block cancer cell proliferation, adaptability, migration, and invasion. Prognostic patterns for survival in neoplastic patients have been developed using miR-126 levels [40]. Furthermore, miR-126 has been linked to reduced myocardial damage after episodes of acute myocardial infarction. MiR-126 may be a measure of cardiotoxicity risk, since its levels were lower in individuals with cardiotoxicity compared to those without cardiotoxicity in two investigations [25,28]. In contrast, a study comparing the pre- and post-chemotherapy levels of miR-126 in 25 breast cancer patients reported that miR-126 was substantially up-regulated following neoadjuvant chemotherapy (cyclophosphamide or fluorouracil with epirubicin followed by docetaxel or paclitaxel) [16]. Notably, the link between miR-126 levels and indicators of cardiotoxicity [16] was not investigated in this investigation. This discrepancy may be because to the reduced or non-existent cardiac toxicity. Furthermore, miR-126's mechanisms inducing cardiotoxicity remain elusive.

The endothelial cell response to hypoxia is regulated by miR-210, which also has powerful anti-hypoxia properties. Capillary network development, as well as endothelial cell migration and differentiation, were all shown to be improved by miR-210 [41]. Overexpression of miR-210 protected cells against damage caused by hypoxia in vitro [42]. Furthermore, it has been revealed that miR-210 is positively regulated in cardiac stem cells under hypoxia to inhibit apoptosis and stimulate cell migration [43]. In cells of breast cancer lineage, while in a hypoxic environment, miR-210 stimulated metastasis, proliferation and self-renewal [44]. Importantly, a connection between miR-210 levels and the decrease in LVEF was identified in a sample of 97 breast cancer patients under anthracycline therapy; twelve developed cardiotoxicity with a drop in baseline LVEF [45]. Patients with cardiotoxicity have lower miR-126 levels compared to healthy controls [25,28]. Taken together, our findings imply that the differential control of miR-126 may affect cardiotoxicity.

The miR-1 is encoded by two separate genes, miR-1-1 and miR1-2, which are found on chromosomes 20 and 18, respectively. Following their export to the cytoplasm by the Exportin 5 protein, the two precursors are processed into mature versions of miR-1 that are otherwise indistinguishable. miR-1 has been linked to several cancers, including breast cancer [46]. In contrast to other tissues, cardiac muscle seems to have an increased level of miR-1 [47]. persons who have suffered from acute myocardial infarction have greater plasma levels of miR-1 compared to healthy persons. Due to its high levels of expression in skeletal muscle, miR-1 has been hypothesized to be secreted by dead cardiac myocytes [48]. Patients with breast cancer who were treated with doxorubicin and had cardiac dysfunction after cycles 2, 3, and 4 had higher plasma levels of miR-1 [26], whereas miR-1 was down-regulated after the first dosage of doxorubicin [27]. Both investigations looked at doxorubicin-treated patients, although they used different dosing and combination regimens [26,27]. Their results may vary because of the chemotherapy cycles (dosage, duration, and periodicity) and drug combinations, since cardiotoxicity is linked to both the peak plasma concentration and cumulative dose of anticancer medications [49]. Different molecular subtypes of breast cancer may also lead to varying clinical outcomes, which may account for the discrepancy in results. It's crucial to note that various sample sizes and techniques of detection were used. Rigaud et al. [26] specifically chose 6 potential miRNAs based on the literature to test for aberrant cardiac function in the plasma of 56 breast cancer patients. However, Todorova et al. [27] used a miRNome PCR panel to profile plasma from 20 individuals suffering from cardiotoxicity related to breast cancer. Results may vary and be difficult to compare when using multiple commercial kits and methods. Finally, in a study comparing doxorubicin with epirubicin for the treatment of breast cancer, Gioffre et al. [24] found no statistically significant variations in miR-1 levels between the two groups. Because LVEF didn't decline, cTnT and cTnI levels were used to determine how anthracycline affected these patients [24]. Two additional investigations, however, used LVEF to evaluate cardiotoxicity [26,27]. Using an OpenArray screening, miR-1 was not observed to be differently expressed

at baseline, throughout therapy and at follow-up, perhaps because few patients developed cardiac toxicity [24]. This work is unique in that it validated the expression of miRNAs in the same plasma samples using a second RT-qPCR approach, this time using TaqMan assays.

We searched the miRTarBase for target genes for the five miRNAs (Table 3) and identified significant Reactome pathways (Fig. 2) related to anthracycline-induced cardiotoxicity. Target genes for microRNAs let-7f, miR-20a, and miR-210 were used to identify the "Cellular responses to stress R-HAS-2262752" pathway. Accordingly, redox cycling and oxidative stress are among the well-known molecular mechanisms connected to doxorubicin-induced cardiotoxicity [[50], [51], [52], [53]]. Specifically, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) have been shown in recent research to have a significant role in both the pathogenic process of oxidative stress and the response of cells to oxidative stress [54,55]. Recent research, however, has shown that redox cycling is not the only cause of doxorubicin's cardiotoxicity. Anthracycline-dependent regulation of important signaling pathways affecting DNA damage response, cardiomyocyte survival, cardiac inflammation, energy stress, and gene expression modification [56] is one new hypothesis. Target genes for microRNAs let-7f, miR-1, miR-20a, and miR-126 were used to identify the "Signal transduction R-HAS-162582" pathway. Reactive oxidative stress, interference in apoptosis/growth/metabolism, and angiogenic imbalance are all implicated in anthracycline-associated cardiomyopathy, as reviewed by a recent molecular breakthroughs review [52].

Predicting which patients exposed to anthracyclines would go on to develop cardiomyopathy and heart failure has been difficult [52]. Predictive genetic indicators of functional significance for doxorubicin-induced cardiotoxicity and heart failure were previously established utilizing human Induced Pluripotent Stem Cells-derived cardiomyocytes [57]. Long-term doxorubicin treatment downregulates genes involved in apoptosis, DNA damage, and the oxidative stress response. After two and six days of treatment with 156 nM doxorubicin, several groups of genes were found to be down-regulated (sarcomere, myofibril, contractile fiber, and regulation of heart contraction genes) or up-regulated (stress response, p53 signaling pathway, and apoptosis genes), returning to control levels after the drug was removed [57,58]. The originality of this review and route analysis was its greatest asset. This is the first systematic study that we are aware of that specifically addresses the function of miRNAs in anthracycline-induced cardiotoxicity and how it affects the prognosis of breast cancer patients. We also demonstrated that two pathways, "Signal transduction R-HAS-162582" and "Cellular responses to stress R-HAS-2262752," are common to many of the miRNAs.

The present research contains several flaws, despite the fact that the literature search was performed in accordance with conventional recommendations. There were just five qualifying papers in this systematic review. Although we were able to identify five miRNAs that are linked to anthracycline-based cardiotoxicity, evaluating their potential application for early cardiotoxicity monitoring during chemotherapy was challenging due to the limited number of papers reporting on these miRNAs. Some studies do not adequately describe the population they studied (e.g., histological classification of breast cancer type, number of patients, age), the treatment they used (e.g., total or cumulative anthracycline dose), or the methods they used to assess cardiotoxicity (e.g., echography, troponins, etc.). Notably, nearly no research included or excluded individuals who were using cardioprotective medicines, despite the fact that these treatments have been shown to have positive benefits on cardiovascular health. Although the authors did not examine potential confounding variables or compensate for them in the study, the presence of comorbidities is an important factor that might hasten cardiotoxicity. Studies that chose just previously published miRNAs (such miR-1) were also included, which is a constraint. Indeed, the studies demonstrated substantial heterogeneity in the management of breast cancer, with regards to both the use of additional concurrent medicines known to have cardiotoxic effects and the duration of follow-up. Therefore, in this systematic review, the small number of studies with the same differentially expressed miRNA makes it difficult to undertake a quantitative analysis of the data (meta-analysis). Moreover, the majority of research merely reported whether or not the miRNA was substantially up- or down-regulated, rather than including the raw or normalized miRNA expression data. Five microRNAs were

identified in this systematic review as potentially being involved in anthracycline-induced cardiotoxicity in breast cancer patients. However, large prospective studies are required to corroborate this. Additional research into miRNAs linked to other pathways in the cardiotoxic process is needed, and studies using screening methods such as microarrays and/or RNAseq approaches should be conducted.

5. Conclusion

Five microRNAs (let-7f, miR-1, miR-20a, miR-126, and miR-210) were identified via our systematic review as having the ability to predict anthracycline-induced cardiotoxicity in breast cancer patients. These miRNAs and their targets engage in pathways of recognized importance for cardiotoxicity pathophysiology, such as pro-angiogenesis and myocardial infarction. It is possible that cellular responses to stress and signal transduction pathways are involved in anthracycline-induced cardiotoxicity, as shown by analysis of the target genes discovered for the five miRNAs. Considering their therapeutic potential as early prediction tools and prognostic indicators, we believe this is the first comprehensive study to examine the differential expression of circulating miRNAs in breast cancer patients impacted by anthracycline cardiotoxicity.

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