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MicroRNAs mediate therapeutic and preventive effects of natural agents in breast cancer

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Abstract

MicroRNAs (miRNAs) are a set of non-coding small RNA molecules that play a critical role in regulation of protein coding genes in cells. MiRNAs have been extensively studied as novel biomarkers, therapeutic targets, and new drugs in various human diseases. Breast cancer is a one of the leading tumor types significantly affecting women health worldwide. Over the past decade, a number of natural agents, such as paclitaxel and curcumin, have been applied for treatment and prevention of breast cancer due to their relatively low toxicity. However, the mechanisms of action have not been completely understood. Investigation on miRNAs is able to potentially provide a novel insight into better understanding the anticancer activities of these natural products. Given that a single miRNA can target multiple genes, theoretically, those genes involved in a certain phenotype can be clustered with one or a few miRNAs. Therefore, pleiotropic activities of natural agents should be interpreted by interactions between selected miRNAs and their targets. In this review, we summarize the latest publications related to the alterations of miRNAs by two natural agents (paclitaxel and curcumin) that are currently used in intervention of breast cancer, and conclude that the mechanism involving the regulation of miRNA expression is one of the keys to understand pleiotropic activities of natural agents.

Keywords

MicroRNA; Natural agents; Breast cancer; Cancer prevention; Therapeutics

Introduction

MicroRNAs (miRNAs) are a set of small non-coding RNA molecules in cells, which had been regarded as “junk” RNAs until their capability of regulating coding gene expression was uncovered a few decades ago [1]. According to the latest release from miRbase (Version 21, <http://www.mirbase.org/>), the total number of miRNAs in all species is 28 465, consisting of 1 881 human miRNAs, which is equivalent to 10% of human protein-coding genes (< 19 000) in accordance to the latest study on human genome annotation [2]. The regulatory mechanism of miRNA on gene expression is based on the complementary binding of miRNA to the 3′-UTR sequences of target genes, which leads to suppression at

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the post-transcriptional and translational levels. Given the short length of miRNA (18–22 nucleotides) and the simple sequence match principle, one miRNA is able to target multiple genes; whereas one gene can be triggered by different miRNAs. Results from multiple studies have also reported that miRNAs have the capability of regulating over 30% human protein coding genes [3]. Therefore, through regulation of target gene expression, miRNAs are involved in numerous cellular events, including normal and pathological processes related to various human diseases [4].

Breast cancer remains the leading cause of cancer deaths in American women [5]. The recent cancer statistical data by American Cancer Society reports that the 5-year survival rate of patients with primary breast cancer is 99%, but this figure reduces to 25% in the individuals with distant metastases [6]. Therefore, early detection and prevention are extremely important to reduce the mortality of breast cancer patients. Natural agents can be simply defined as chemical compounds or small molecules produced from a biological resource that can be found in nature [7]. Those agents with pharmacological activity to stop, retard or reverse the disease progression can be used for either therapy or prevention, or both. Many natural agents have been studied in basic and clinical studies, such as paclitaxel (Taxol), which was isolated from the Pacific yew, *Taxus brevifolia*, and has been broadly used to treat a spectrum of human tumors, including breast cancer [8].

When searching Pubmed by using key words “breast cancer and natural agents” on December 7th, 2016, there are 2018 returned results in which 70% were published within last 10 years. This result supports an increasing interest in studying natural agents in breast cancer. In this review article, we will summarize the studies on two leading natural agents, paclitaxel and curcumin, which have shown the potential in treatment and prevention of breast cancer. In particular, we will focus on the publications in the past two years reporting alteration of miRNAs in response to each agent. We hope this summary can help improve the understanding of the underlying mechanisms responsible for their anticancer activity.

Paclitaxel

Paclitaxel was first discovered when the National Cancer Institute of the United States screened natural agents in 1960's. However, its structure was not published until 1971 because of difficulty in characterization of this agent [9]. Over the past decades, paclitaxel has been broadly used for the treatment of a variety of human tumors, including breast cancer.

Zhang *et al* have reported that miR-100 is globally downregulated in human breast tumor samples [10]. When the individual subtypes including luminal A, luminal B, HER2 and basal-like are further analyzed, miR-100 is shown to have the lowest expression in luminal A breast cancer specimens. MCF-7 is a luminal A subtype breast cancer line; forced expression of miR-100 results in sensitizing MCF-7 cells to paclitaxel, and the cells show the phenotypes with improved cell cycle arrest, multinucleation, and apoptosis. However, these phenotypes are compromised by knockdown of miR-100 in MBA-MB-231 cells, which were characterized as basal-like breast cancer cells showing relatively higher expression of miR-100. The molecular mechanism involved in miR-100 modulation of paclitaxel sensitivity in breast cells is partially attributed to alteration of mammalian target of

rapamycin (mTOR), a putative target of miR-100. Suppression of mTOR by miR-100 has been documented to promote cell death and inhibit cell growth. In addition, by mining the TCGA data, miR-100 is found to be not only a tumor suppressor in breast cancer, but also a biomarker for paclitaxel treatment, based on the result that downregulation of miR-100 is correlated with the declined overall survival rate in patients [10].

Compared to normal human mammary epithelial cell line MCF-10A, the miR-101 expression is downregulated in a triple negative breast cancer (TNBC) cell panel consisting of eight lines. In addition, in two different cohorts of TNBC samples, miR-101 is consistently found to be downregulated in tumor tissues, compared to the normal tissues [11]. Transfecting miR-101 to MDA-MB-435 cells significantly increases the sensitivity of tumor cells to paclitaxel. MCL-1 is further identified as the direct target of miR-101 to interact with miR-101 in TNBC cells [11]. MCL-1 is an anti-apoptotic gene showing oncogenic roles in variable human tumors [12]. In MDA-MB-435 cells, MCL-1 overexpression can attenuate the effects on apoptosis induced by either paclitaxel alone, or the combination of paclitaxel and miR-101 mimics. This study supports that the miR-101/MCL-1 pathway is involved in the inhibitory activity of paclitaxel in TNBC cells [11]. Intriguingly, Gao *et al.* reported that MCL-1 could be targeted by miR-26a in breast cancer cells [13]. MiR-26a is downregulated in both breast cancer specimens and cell lines, and restoring miR-26a could improve the paclitaxel sensitivity in breast cancer cells in which downregulation of MCL-1 is involved [13].

Distinct from miR-100 and miR-101, miR-520h has been reported as an oncomir in lung cancer [14]. In breast cancer MDA-MB-231 and MDA-MB-468 cells with stably expressed miR-510h, resistance to paclitaxel is significantly increased when compared to the cells transfected with vehicle vectors. After searching the global putative targets of miR-520h and sorting those associated to apoptosis, DAPK2 is picked based on its documented role as a pro-apoptotic gene [15]. MiR-520h targeting DAPK2 is verified by using Western blotting and luciferase reporter assays, respectively. As expected, restoring DAPK2 in both vehicle control cells and miR-520h overexpressed cells significantly improves paclitaxel sensitivity. A log-rank analysis of 95 patients with relatively higher expression of miR-520h versus 51 individuals with relatively lower expression of miR-520h shows that miR-520h expression is correlated with the poor prognosis and tumor progression in breast cancer patients [16]. Therefore, miR-520h is a therapeutic target of paclitaxel in breast cancer, and its interaction with DAPK2 is involved in the chemoresistance to paclitaxel [16].

Paclitaxel/carboplatin (PTX/CBP) is an efficacious neoadjuvant chemotherapy regimen for breast cancer patients [17]. A recent study reported that the elevated expression level of miR-621 is highly correlated with the improved pathological complete response and disease free survival in a cohort of breast cancer patients treated with PTX/CBP [18]. When analyzing a panel of breast cancer cell models in response to paclitaxel or carboplatin individually, MCF-7 cells show more resistant than MDA-MB-231 cells to both drugs. This result is consistent with the study by Zhang *et al.* [10]. Similar to miR-100 [10], miR-621 has a lower expression in MCF-7 cells than MDA-MB-231 cells. In order to study if the differential expression of miR-621 is involved in chemosensitivity of breast cancer cells, miR-621 expression is up- or down-regulated by transfection of the precursors or sponge

inhibitors into MCF-7 cells. The results show that alteration of miR-621 is obviously correlated with the sensitivity of MCF-7 cells to paclitaxel/carboplatin treatment. Moreover, the elevated miR-621 could remarkably enhance P53 transcriptional activity to upregulate p21 and DR5, which ultimately promotes apoptosis of tumor cells. To address the interaction between miR-621 and p53, a putative target of miR-621, FBXO11 is identified. It is reported increased p53 neddylation resulting in suppression of P53 activity by abrogating the acetylation at Lys320 [19]. Therefore, when miR-621 is upregulated by paclitaxel/carboplatin treatment, in turn, FBXO11 is suppressed, and P53 transcriptional activity is enhanced, leading to upregulation of p21 and DR5 in promotion of apoptosis of breast tumor cells [18].

MiR-34 is a well-known tumor suppressor miRNA, which has been developed to a drug that is investigated in a Phase I study for treatment of patients with advanced solid tumors [20]. In a recent study, miR-34a is reported to be downregulated in breast cancer samples and its expression levels are correlated with tumor progression and metastasis [21]. In particular, miR-34a could increase the sensitivity of MCF-7 cells to paclitaxel. MiR-34a and paclitaxel show a synergistic effect on reduction of viable cell numbers. When studying the molecular mechanism involved in miR-34a sensitizing breast cancer cells to paclitaxel, Notch1 is of particular interest as the direct target of miR-34a. Aside from showing inverse expression to miR-34a in breast cancer samples, Notch1 is an important player to maintain stemness of breast cancer stem cells [21]. Cancer stem cells have been known to be responsible for chemoresistance [21]. Therefore, downregulation of Notch1 can promote differentiation of breast cancer stem cells and eventually improve the sensitivity to paclitaxel [21].

Studies have reported that paclitaxel resistant (PR) breast and ovarian cancer cells could display more aggressiveness due to enhanced epithelial-mesenchymal transition (EMT) [22–23]. Yang *et al.* recently reported that miR-125b is dramatically downregulated in MCF-7 and SKBR3 PR cells, compared to the parental cells [22]. When miR-125b mimics are transfected to these PR cells, their motility is significantly reduced as measured by wound-healing and invasion assays. The mechanistic studies find that miR-125b could increase mesenchymal-epithelial transition (MET) in which its putative target, Sema4C, is, at least partially, involved in this process, based on the results that knockdown of Sema4C by siRNAs could mimic the effect resulted from elevation of miR-125b on reduction of PR breast cells motility [22]. However, miR-125b's signature is distinct from individual PR cell models. Together with miR-221/222 and miR-923, miR-125b is found to be upregulated in two PR cell lines derived from MDA-MB-435 cells [24]. Transfection of miR-125b to parental MDA-MB-435 cells could attenuate the inhibitory effect of paclitaxel through suppression of tumor cell apoptosis. Bak1 is identified as the direct target of miR-125b [24], which is known as an anti-apoptotic gene. In this study, Bak1 is found to be a key player in mediation of paclitaxel-induced apoptosis and miR-125 suppression of Bak1 is involved in chemoresistance to paclitaxel [24].

In a retrospective study, sixty-eight breast cancer patients who received neoadjuvant chemotherapy with the combination of epirubicin and paclitaxel were divided into two groups based on the patients' responses to chemotherapy [25]. MiRNA profiling results have implied that the expression levels of miR-19a and miR-205 in the patient serum could be correlated with their responses to the epirubicin and paclitaxel regimen. In a validation

cohort consisting of 56 breast cancer samples, the quantitative data of serum miR-19a and miR-205 expressions are integrated into a predictive model based on the logistic regression analysis to “predict” the patient responses to the neoadjuvant chemotherapy. The results show that the probability of this model is 89.71%, the specificity is 75.00%, and the sensitivity is 81.25% [25].

Curcumin

Curcumin is a bioactive compound derived from the turmeric that is known to have suppressive effects on inflammation, oxidative damage, and carcinogenesis [26]. Bisphenol A (BPA) is a chemical presenting in the manufacture of plastics and epoxy resins that are universal in the environment and daily life of humans [27]. Recent studies have reported that exposure of BPA could increase the risk of several human cancers, including breast cancer [27–28]. In the study by Li *et al*, curcumin is found to attenuate the oncogenic role of BPA in promoting growth of MCF-7 cells [27]. Oncogenic miR-19a and -19b are found to be involved in carcinogenesis led by BPA, whereas curcumin could trigger these two miRNAs in MCF-7 cells. In addition, curcumin could restore the expression of PTEN and P53 in BPA treated MCF-7 cells. The mechanism of action is attributed to miR-19a and -19b targeting PTEN, which in turn reduces phosphorylation of Akt and MDM-2, resulting in elevation of p53 [27]. In metastatic breast cancer MDA-MB-231 cells, miR-181b is found to be upregulated by curcumin treatment in the miRNA microarray analysis [29]. Cytokines, CXCL1 and CXCL2, are the direct targets of miR-181b [29]. In MDA-MB-231 cells and primary breast tumor cells isolated from patient samples, incubation with curcumin could lead to upregulation of miR-181b and downregulation of CXCL1 and CXCL2 [29]. In addition, miR-181b is found to be involved in mediation of anti-proliferative, anti-invasive, and pro-apoptotic activities of curcumin in breast cancer cells [29]. In the study by Guo *et al*, synergistic effects of curcumin with another natural agent emdodin on inhibition of breast cancer cell growth and invasion are demonstrated. In particular, miR-34 is found to be upregulated in MDA-MB-231 and MDA-MB-435 cells by both drugs, but the combination of two drugs show more significant induction in miR-34. Bcl-2 and Bmi-1 are the targets of miR-34 [30]. In both breast tumor cell lines, the combination of curcumin and emdodin could reduce the expression of Bcl-2 and Bmi-1, and this suppressive effect could be compromised by knockdown of miR-34. These results support that miR-34 targeting Bcl-2 and Bmi-1 is involved in anticancer activity of curcumin and emdodin in breast cancer [30]. Bcl-2 is also reported as the co-target of miR-15a and miR-16 that are downregulated by curcumin in MCF-7 cells [31].

MiRNAs targeted by paclitaxel and curcumin are summarized in Table 1 in which the target genes of individual miRNAs that are involved in anticancer activity of both drugs are also included.

Perspective and Conclusion

Our lab has been engaged in miRNA and cancer research since 2004, and we are the first group to report that the compelling stability of miRNA in formalin-fixed paraffin-embedded samples versus fresh frozen samples by using a systematic analysis [32]. This study sheds a light on adapting miRNA as new biomarkers in translational and clinical research, in

particular, when archived clinical samples are employed but the stability and integrity of mRNA are of concern. As described above, the total number of miRNA is about 10% of human protein coding genes, and one miRNA is able to regulate multiple target genes. Therefore, miRNAs are more advantageous as biomarkers than mRNAs, given a single miRNA that may cluster and carry messages from a number of mRNAs. Over the past decades, miRNA has been broadly studied as biomarkers in cancer [33]. However, using miRNAs to predict the response of breast cancer patients to chemotherapy including natural agents have not been intensively studied until recently, which, at least partially, is due to the lag of technology development to detect the miRNA from patients using non-invasive methods. At present, circulating miRNA has been stamped as stable blood-based biomarkers for cancer detection [34–35]. As mentioned earlier, Li *et al.* have developed a new model integrating serum miR-19a and miR-205 expression that can predict the response of breast cancer patients to the neoadjuvant chemotherapy with a combination of epirubicin and paclitaxel [25]. This study provides new insights into the application of miRNA into clinical studies, in particular for prediction of chemosensitivity to develop precision medicine.

Aside from biomarker, most of studies are focused on miRNA as therapeutic targets. In the publications as summarized above, alteration of miRNAs in response to the treatment of paclitaxel in breast cancer is intensively studied; and up/down-regulation of their targets along with miRNA fluctuation are interpreted as the mechanisms involved in drug anticancer activity. However, it remains largely unknown how drugs regulate miRNAs. This may be an important question to promote our efforts on new drug development. Our lab is one of a few pioneers who have been engaged in studying mechanisms in regulation of miRNA. After first reporting that p53 is involved in modulation of a panel of miRNAs at the transcriptional level in human colon tumor cells when treated with 5-FU [36], our research efforts are continued on miRNA and chemopreventive capacity of nonsteroidal anti-inflammatory drugs (NSAIDs) and natural agents. In particular, our current efforts are focused on the mechanisms involved in regulation of miRNA by NSAIDs in human breast and colon cancers. NSAIDs are well known for treating a variety of inflammatory conditions and pain associated with arthritis. However, studies have reported that the long-term use of NSAIDs can significantly reduce the incidence and risk of death from different forms of human cancers [37–40]. Cyclooxygenase-2 (COX-2) inhibition has been regarded to be responsible for antineoplastic activity of NSAIDs [41], whereas the potential gastrointestinal, renal and cardiovascular toxicities resulting from COX-2 inhibition fail to endorse the long-term use of NSAIDs in the clinic [42–44]. Studies have already demonstrated that COX-2 inhibition could not fully account for antineoplastic properties of NSAIDs; however, the mechanisms responsible for the full spectrum of NSAIDs activities have not been fully understood yet. In one of recent projects, we have found that the NSAID, sulindac sulfide (SS) at a non-cytotoxic concentration, could significantly inhibit the motility of both breast and colon cancer cells [45]. When studying the mechanism of action, we report a panel of oncogenic miRNAs including miR-10b, miR-17, miR-21, and miR-9, which could be downregulated by SS. Those oncogenic miRNAs have been exclusively reported to promote tumor cell motility [46–49]. In particular, we demonstrate that NF- κ B signaling is involved in regulation of miRNA expression by SS, showing the first evidence to understand the mechanism of action [45]. In our latest publication, we have reported that TGF- β mediates the suppressive

signal of SSA, a novel non-COX inhibitory derivative of SS in regulation of oncogenic miR-21 in breast cancer. In this study, we report that SSA could reduce the phosphorylation of smad2/3 and thereby block TGF- β signaling transduction into the nucleus, resulting in downregulation of miR-21 transcriptionally [50]. We are extending our research efforts to natural agents and tumor cell transformation as well as prevention of tumor metastasis in which regulation of miRNA by natural agents will be observed specifically, given the lack of similar studies to fill the knowledge gap between drug treatment and miRNA alteration. We hope that our research accomplishment can attract more efforts to study the mechanistic roles of miRNA in mediation of drug activities, especially for natural agents and other compounds with pleiotropic activities in treatment and prevention of human diseases.

As illustrated in Figure 1, aside from biomarker and therapeutic target, miRNAs have been developed as novel drugs to treat human diseases [20]. As mentioned earlier, miR-34 is studied in Phase I trial for treating a panel of patients with advanced solid tumors [20]. Miravirsin is a β -D-oxy-locked nucleic acid-modified phosphorothioate antisense oligonucleotide targeting miR-122 in liver [51]. In a Phase II study, Miravirsin shows a dose-dependent reduction in HCV RNA levels without evidence of viral resistance [52]. However, targeting multiple genes remains a concern on off-target effects when developing miRNA therapeutics. Recently, the achievements on nanomedicine provide new opportunities for targeted delivery of miRNA in a safer and more efficacious manner. For example, siRNA carried by DNAP particles could maintain the silent phenotype up to 14 days after a single administration, and these particles do not show obvious *in vivo* toxicity [53]. Likely, targeted delivery of tumor suppressor miRNA or anti-oncomiRs to the specific tissues can magnify the local efficacy of chemotherapy. Therefore, development of neoadjuvant therapy to combine nature agents, such as paclitaxel or curcumin, with miRNA therapeutics may hold a large promise to improve our treatment and prevention on human tumors including breast cancer.

In conclusion, miRNAs are a set of key molecules endogenously existing in human cells, which have been involved in diagnosis, treatment, and prevention of various human diseases. In particular, study of miRNA will provide invaluable insights into development of new strategies to manage breast cancer in the clinic.

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Fig. 1.
Applications of miRNA in human diseases

MiRNAs involved in therapeutic and preventative activities of paclitaxel and curcumin in breast cancer cells

Table 1

Drug	miRNA	Target(s)	Breast cell models	Endogenous expression of miRNA in referred cell models	References
Paclitaxel	miR-100	mTOR	MCF-7; MDA-MB-231	down	[10]
	miR-101	MCL-1	MDA-MB-435	down	[11]
	miR-26	MCL-1	MDA-MB-231, MCF-7, MDA-MB-435, MDA-MB-468	down	[13]
	miR-520h	DAPK2	MDA-MB-231; MDA-MB-468	up	[16]
	miR-621	FBXO11	MCF-7; MDA-MB-231	down	[18]
	miR-34	Notch1	MCF-7	down	[21]
	miR-125b	Sema4C	MCF-7; SKBR3	down	[22]
Curcumin	miR-125b	Bak1	MDA-MB-435	up	[24]
	miR-19a; miR-19b	PTEN	MCF-7	up	[27]
	miR-181b	CXCL1; CXCL2	MDA-MB-231	down	[29]
	miR-34	Bcl-2; Bmi1	MDA-MB-231; MDA-MB-435	down	[30]
	miR-15a, miR-16	Bcl-2	MCF-7	down	[31]