Epigenetic Regulation of miRNAs and Breast Cancer Stem Cells

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Abstract MicroRNAs (miRNAs) have emerged as important targets of chemopreventive strategies in breast cancer. We have found that miRNAs are dysregulated at an early stage in breast cancer, in non-malignant ductal carcinoma in situ (DCIS). Many dietary chemoprevention agents can act by epigenetically activating miRNA-signaling pathways involved in tumor cell proliferation and invasive progression. In addition, many miRNAs activated via chemopreventive strategies target cancer stem cell signaling and prevent tumor progression or relapse. Specifically, we have found that miRNAs regulate DCIS stem cells, which may play important roles in breast cancer progression to invasive disease. We have shown that chemopreventive agents can directly inhibit DCIS stem cells and block tumor formation in vivo, via activation of tumor suppressor miRNAs.

Keywords Chemoprevention · MicroRNAs · Breast cancer · DCIS · Epigenetics · Cancer stem cell

Introduction

Breast cancer is a heterogeneous disease, which occurs via multiple genetic and epigenetic alterations in gene expression. Breast cancer is not a single disease but consists of numerous histological stages and molecular subtypes. Approximately 70 % of breast cancers are dependent on estrogen receptor (ER) signaling which promotes tumor cell proliferation and

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survival [1]. Estrogen signaling pathway promotes breast tumorigenesis through direct control of gene expression via nuclear ER signaling and via genotoxic metabolites of estrogen hormone [2].

Obstacles to improving clinical outcomes include better understanding of disease recurrence, overcoming drug resistance, and preventing metastasis. Despite improvements in early detection and the development of targeted therapies for some breast cancer types, breast cancer remains the second leading cause of cancer deaths among women [3].

Nearly all breast cancers arise from breast epithelial cells of the terminal ductal lobular unit [4]. Based on the site of origin, if the tumor arises in the milk ducts or the lobular glands, breast cancers are classified as ductal or lobular carcinomas. Majority of invasive tumors are infiltrating ductal carcinoma (IDC). Breast tumors may begin as atypical hyperplasia, benign lesions that possess some but not all characteristics of carcinoma whose presence is viewed as a risk factor for breast cancer. Breast cancer is first apparent as carcinoma in situ, premalignant non-invasive lesions. Next, it is thought that these lesions may undergo a series of critical progressions towards incurable metastatic disease.

DCIS and the Transition to IDC

Ductal carcinoma in situ (DCIS) is the most common type of non-invasive early stage breast cancer in women, accounting for 80 % of cases. It is characterized by the abnormal growth of epithelial cells in the ducts, confined within the ductal area, maintenance of the basement membrane and lack of stromal invasion [5]. With the advancements in diagnostic technologies, the disease can be diagnosed at a very early stage and appropriate therapeutic strategies be adopted that may reduce the chances of progression to other malignant forms.

Detection of ductal carcinoma in situ (DCIS), an early non-invasive stage of breast cancer was rare prior to widespread mammography but now accounts for 25 % of newly detected breast cancer cases, where it is typically observed as microcalcifications on mammograms [6, 7]. The standard of care for DCIS patients involves surgery and radiation and, for some patients, targeted hormonal therapy [8]. About 15 % of patients with DCIS possess recurrent disease following therapy [9].

Studies have indicated that DCIS is a precursor lesion for invasive forms of breast carcinomas. In some cases, DCIS is transformed to IDC, characterized by penetration of cancer cells into the stromal area. It was reported in the cell-based assays that there are few genetic differences between the DCIS and IDC components [10–14], indicating that the epithelial cells in the DCIS are pre-invasive in nature. The progression of invasion is a complex and dynamic process, and the underlying molecular mechanisms are still poorly understood.

Evidence suggests that cancer stem-like cells serve as malignant precursor cells in DCIS lesions and that these cells possess enhanced migratory capacity and are primed for invasive progression [15, 16•, 17•]. Currently, due to dearth of information and lack of available diagnostic technologies, clinicians are unable to predict which patients are at greater risk for progression to invasive disease. Furthermore, for patients without ER+ lesions, there are no available molecularly targeted treatments to supplement surgery and radiation. Cancer stem-like cells in DCIS lesions may serve as important therapeutic targets for chemopreventive strategies.

MicroRNAs

MicroRNAs (miRNAs) are short non-coding RNA molecules approximately 22 nucleotides in length. miRNAs function by targeting untranslated regions of protein coding messenger RNAs (mRNAs) via seed sequence complementarity. This miRNA targeting results in mRNA degradation or translational inhibition [18].

Pioneering work from Dr. Carlo Croce and Dr. George Calin revealed that miRNAs are dysregulated in nearly every type of human cancer when compared to normal tissue [19]. Furthermore, they found that miRNAs were frequently located at fragile sites, sites of amplifications, and common breakpoint regions [20]. Moreover, it has also been found that numerous miRNAs are subject to gain or loss of expression via dysregulation of epigenetic programs [21–24]. Functionally, miRNAs have been found to regulate every hallmark of tumorigenesis.

In breast cancer, they function as oncogenes (e.g., miR-21) or tumor suppressor genes (e.g., miR-34) [22–24]. Expression profiling has revealed unique signatures of miRNA dysregulation in different histological types and molecular subtypes of breast cancer. A study that included the miRNA expression patterns of 51 human breast cancer cell lines revealed differential miRNA profiles among different subtypes of breast cancer. It has been found that more than hundred miRNAs were differentially expressed within the luminal and basal subtypes of breast cancer cell lines. 40 miRNA were found to be differentially expressed within basal-like and normal-like/claudin low breast cancer cell lines while 39 miRNAs were associated with the ERBB2 overexpression and 24 miRNAs were associated with E-cadherin mutations within the luminal group. On the other hand, 31 miRNAs were associated with the E-cadherin promoter hypermethylation within the breast cancer cell lines that have no luminal origin. Furthermore, 30 miRNAs were associated with p16INK4 status and a few miRNAs were associated with BRCA1, PIK3CA/PTEN, and TP53 mutation status. Finally, 12 miRNAs were associated with DNA copy number variation of the respective locus [25]. Another study investigated the miRNA expression profiles of primary breast tumors from the patients who were disease-free 5 years after the surgery or developed either early or late recurrence. It has been observed that miR-149, miR-10a, miR-20b, miR-30a-3p, and miR-342-5p were downregulated in tumors from patients who had early recurrence compared to the patients with no recurrence. These data confirmed the potential role of miRNAs to be used as prognostic markers for the different types and status of breast tumors [26]. Furthermore, it has been shown that miRNA dysregulation is an early event in breast cancer since DCIS lesions also possess dysregulated miRNA expression [27•]. miRNAs are also dysregulated within subpopulations of breast cancer cells as some miRNAs demonstrate significantly different expression in cancer stem-like cells when compared with non-stem cancer cells [28]. Moreover, we have recently identified miR-140, which is downregulated in DCIS lesions, as a critical regulator of DCIS stem cells that may serve as chemopreventive target for inhibiting progression to invasive disease [17•].

Epigenetics

It is well known that alterations in the epigenetic landscape of tumor cells play important roles in breast tumorigenesis. Epigenetic alterations in breast cancer include global DNA hypomethylation and localized hypermethylation in CpG islands of tumor suppressor genes in addition to altered histone acetylation and methylation [29, 30]. Even early stage breast cancers demonstrate altered epigenetic profiles, as altered DNA and histone methylation have been observed in DCIS lesions.

Members of the polycomb group (e.g., BMI-1, EZH2) are frequently overexpressed in breast tumors [31–33]. Polycomb group proteins are involved in silencing differentiation genes in embryonic stem cells. Differentiation genes are marked

with H3K27me3 via PRC2 complex, which is often guided via long non-coding RNA. This mark is recognized by PRC1 complex which catalyzes H2A mono-ubiquitination resulting in the tightening of chromatin and repression of gene expression [34]. In breast cancer, these proteins may play oncogenic role, preventing differentiation and promoting stem-like characteristics in cancer cells.

Numerous miRNAs are dysregulated via epigenetic mechanisms in breast cancer, i.e., miR-200 [24, 35], miR-335 [36], miR-195 [37], miR-34 [22, 23], miR-375 [38], etc. Cellular specialization and terminal differentiation often involves changes in epigenetic profile as well as changes in miRNA expression. Vrba et al. found that 10 % of miRNAs in mammary tissues were subject to cell type-specific epigenetic regulation (either DNA methylation of trimethylation of histone 3 lysine 27 (H3K27me3)) [39]. As cancer is akin to loss of differentiation, it is unsurprising that this involves altered epigenetic machinery and as such, dysregulated miRNA expression.

In addition, miRNAs have been found to directly regulate epigenetic enzymes, and dysregulation of miRNA expression may alter the epigenetic landscape. miR-29b was found to regulate DNMT3a/b and to be lost in some breast cancers [40, 41]. Dr. Michael Clarke and Dr. Kevin Struhl's work revealed that the miR-200 family regulates multiple components of polycomb group including BMI-1 and Suz12 [42, 43]. Furthermore, they found a critical role of miR-200 and polycomb group in regulating breast cancer stem cells. The miR-200 family has also been implicated in regulating SIRT1, a class III histone deacetylase overexpressed in breast cancers [35]. As miR-200 family members are frequently downregulated in advanced breast cancer via epigenetic mechanisms, this creates a negative feedback loop promoting a stem cell-like state in cancer cells [44].

Several other miRNAs have been implicated in the regulation of polycomb group proteins in breast cancer. miR-214 and miR-26a are both downregulated in breast cancer and have been shown to target the 3′ untranslated region (3′ UTR) of EZH2 mRNA in breast cancer cells [45, 46]. Restoration of these miRNAs resulted in EZH2 downregulation and inhibition of breast cancer growth in vitro and in vivo.

Since epigenetic mechanisms frequently underlie miRNA dysregulation in breast cancer, this provides a potential therapeutic window for restoring miRNA expression. Multiple epigenetic drugs have been developed that are effective in targeting breast tumor cells including DNMT and HDAC inhibitors. Furthermore, there is ongoing drug development for therapeutics to target polycomb group including EZH2 inhibitors, which would potentially target cancer stem cells. Epigenetic therapy impacts global heterochromatin resulting in very nonspecific targeting, but one mechanism of anti-tumor activity is through activation of silenced tumor suppressor miRNAs. We have found that treatment with epigenetic

therapy can reactivate multiple miRNAs in early stage breast cancer, specifically silenced miR-140, which can target DCIS stem cells and inhibit tumor growth [17•].

Role of miRNAs in Cancer Prevention and Chemosensitivity

Cancer chemoprevention is the incorporation of chemical agents that are found naturally in food and/or administration of them as pharmaceuticals to prevent, delay, or inhibit the process of carcinogenesis. Growing research implies the role of dietary factors in the regulation of miRNA expression and their targets [47]. Given that regulation of miRNAs is associated with tumor cell proliferation, apoptosis, differentiation, angiogenesis, invasion, metastasis as well as pathways in stress response, it stands to reason that some nutrients and bioactive food compounds involved in the alteration of miRNA expression might prove effective to protect against cancer.

The potential link between dietary chemoprevention and miRNA regulation was first demonstrated with studies showing that miRNAs that are associated with diabetes and obesity were also associated with carcinogenesis. For instance, lower levels of adiponectin and higher levels of leptin that are involved in insulin resistance have also been associated with abnormally regulated miRNAs in carcinogenesis such as let-7, miR-27, and miR-143, reporter tumor suppressor miRNAs [48].

Retinoids/Vitamin A

Retinoids are promising chemopreventive agents for breast cancer [49]. All-trans retinoic acid (ATRA) is the major metabolite of vitamin A, which is an essential dietary factor associated with cell proliferation and differentiation. ATRA decreased the proliferation of estrogen receptor (ER)-positive (luminal) breast cancer cells but did not have the same effect on ER-negative (basal) breast cancer cells [50]. miRNAs were found to be involved in the mechanism of action of ATRA against breast cancer. It was found that in ER-positive breast cancer cells, ATRA increased the expression of miR-21, which antagonized the anti-proliferative effect of ATRA but reduced the cell motility of cancer cells [50]. Further experiments showed that the retinoid-driven upregulation of miR-21 occurred through the enhanced transcription of miR-21 gene via the ligand-dependent activation of the nuclear retinoid receptor RARα. Knockdown of miR-21 brought back the ATRA-dependent growth inhibition and senescence while the suppression of cell motility was prevented. Moreover, upregulation of miR-21 caused the retinoid-dependent inhibition of maspin, whose function was associated with ATRA-induced growth inhibition and ATRA-dependent anti-motility

responses. Additional research was carried out to identify the genes differentially expressed in ER-positive and negative breast cancer cells upon the treatment with ATRA. These studies revealed pro-inflammatory cytokine IL1B, the adhesion molecule ICAM-1, and PLAT (tissue-type plasminogen activator) as novel targets of miR-21 [50].

Resveratrol

Resveratrol (3,4′,5-trihydroxy-trans-stilbene) is a dietary polyphenol and chemopreventive agent found in grapes, berries, and peanuts. Resveratrol acts on multiple discrete stages of carcinogenesis including initiation, promotion, and progression by regulating cell division and growth, apoptosis, inflammation, angiogenesis, and metastasis [51]. In mice, resveratrol in the diet significantly decreased the incidence and multiplicity of 7,12-dimethylbenz(a)anthracene(DMBA)-initiated mammary tumors [52]. In rats, dietary resveratrol inhibited DMBA-induced mammary cancer via the maturation of the mammary gland and decrease of cell proliferation [53]. Xenograft studies in nude mice showed that resveratrol was able to inhibit the growth of basal MDA-MB-231 tumor explant, increase apoptosis, and decrease angiogenesis [54]. The spontaneous tumorigenesis in HER-2/neu transgenic mice was delayed when their water was supplemented with resveratrol [55]. Resveratrol was reported to be associated with the decrease of expression of oncogenes miR-155 and miR-21 and with the increase of the expression of tumor suppressor miR-663 [56]. Overexpression of oncogenes miR-155 and miR-21 has been identified in many solid tumors including breast cancer [57]. The mechanism through which resveratrol regulates miRNA expression is not currently understood.

EGCG/Green Tea

In several cancers including breast cancer the chemopreventive effects of polyphenols such as epigallocatechin-3-gallate (EGCG) and other tea catechins have been observed [58]. Polyphenols induce apoptosis, cell cycle arrest, and inhibits angiogenesis. miRNAs are shown to be involved in mechanism of action of the EGCGs. Combined polyphenols resveratrol, quercetin, and catechin administered by gavage have been reported to reduce the primary growth of xenografts of basal MDA-MB-231 breast cancer cells in nude mice [59, 60]. miRNA expression profile of luminal MCF7 breast cancer cells were determined upon the administration of green tea polyphenols. Treatment of MCF7 cells with low concentrations of green tea extract polyphenol-60 significantly modified the miRNA expression profile. Forty-eight-hour post-treatment with 10 μg/ml polyphenon-60 altered the expression of 23 miRNAs, including miR-21 and miR-27, both of which were downregulated [61].

Vitamin D

miRNA expression patterns in several cancers including breast cancer were also associated with vitamin D and its metabolites 1,25-dihydroxyvitamin D3 (1, 25D3) and 25-hydroxyvitamin D3 (25(OH)D3). 25(OH)D3 protects the breast epithelial cells against cellular stress, which is among the key factors for the early carcinogenic process, via the regulation of miR-182 expression [62].

Chemosensitization via miRNAs

In addition to their potential chemopreventive role in breast cancer, numerous studies demonstrated the potential significance of miRNAs as cancer therapeutics. One potential therapeutic approach would be to target the appropriate miRNAs involved in drug resistance to sensitize cancer cells to the chemotherapy [47].

Among the miRNAs that are deregulated in breast cancer, miR-221 and miR-222 expressions are increased in ERα-negative cells and were found to directly interact with the 3′-untranslated region of ERα. MiR-221 and/or miR-222 transfection made ER-positive (luminal) MCF7 and T47D breast cancer cells resistant to tamoxifen. On the other hand, silencing miR-221 and/or miR-222 sensitized ER-negative (basal) MDA-MB-468 cells to tamoxifen-induced cell growth arrest and apoptosis [63].

MiR-200c is another potential target for developing novel therapeutics to treat aggressive and chemoresistant breast cancers. miR-200c expression was reported to be associated with a less aggressive phenotype of breast cancer and with the enhanced sensitivity of breast cancer cells to microtubule-targeting agents [64]. The function of miR-200c was investigated in several breast cancer cell lines including relatively well-differentiated luminal breast cancer cell lines that are ER positive and express the epithelial marker E-cadherin (MCF7, T47D, BT474, ZR75) and less differentiated basal breast cancer cell lines that are ER and E-cadherin negative (MDA-MB-231 and BT-549). The mechanism of action of miR-200c was found to be through suppressing its direct target ZEB1, which is a transcription factor that is able to repress E-cadherin and is associated with epithelial to mesenchymal transition [65]. Another direct target of miR-200c is TUBB3, which is also associated with resistance to microtubule-binding chemotherapeutic agents [64]. miR-200c suppresses ZEB1 and TUBB3, reduces the invasive capacity of cancer cells by restoring E-cadherin expression, and increases their chemosensitivity to microtubule-targeting agents.

In another study, microRNA array was performed to compare the miRNA expression profiles of tumor tissues from patients with triple-negative breast cancer (TNBCs) to normal breast tissues and the cell viability following treatment with doxorubicin was investigated to assess the association of the

expression profiles of miRNAs with chemosensitivity [66]. Five miRNAs (miR-155-5p, miR-21-3p, miR-181a-5p, miR-181b-5p, and miR-183-5p) were up regulated and six miRNAs (miR-10b-5p, miR-451a, miR-125b-5p, miR-31-5p, miR-195-5p, and miR-130a-3p) were downregulated in TNBCs. The data showed that overexpression of miR-130a-3p or miR-451a enabled TNBC cells to be chemosensitive to doxorubicin. Another study confirmed the association of miR-451 with chemoresistance to doxorubicin in luminal breast cancer cells. MCF7 cells transfected with miR-451 reduced the MDR1 gene product, p-glycoprotein (P-gp), and increased sensitivity of MCF7 cells to doxorubicin [67].

Other studies performed on luminal breast cancer cell models revealed miR-205 and miR-125b as additional potential targets for chemosensitivity [68–70]. miR-205 was reported to be downregulated in breast tumors compared to normal counterparts. HER3 receptor is one of miR-205 direct targets, which inhibits the activation of Akt. Transfection of miR-205 to SKBR3 breast cancer cells reduced their clonogenic potential and sensitized them to tyrosine-kinase inhibitors Gefitinib and Lapatinib, preventing the HER3-mediated resistance. These findings introduced miR-205 as a potential tumor suppressor in breast cancer and showed its potential as a novel therapeutic for chemosensitization [68].

Blood serum samples from 56 breast cancer patients with IDC that were pre-operative neoadjuvant chemotherapy were investigated for the profiles of their circulating miRNAs prior to any treatment [70]. Several miRNA expressions were further tested in surgical samples to determine the effect of the chemotherapy on cancer cell proliferation and apoptosis. miR-125b was found to be significantly associated with therapeutic response as it showed a higher expression level in non-responsive patients. Furthermore, breast cancers with upregulation of miR-125b had a higher level of proliferating cells and lower level of apoptotic cells compared to the samples received after neoadjuvant chemotherapy. The data was confirmed in vitro with MCF7 breast cancer cells, where overexpression of miR-125b increased the chemoresistance and downregulation of miR-125b sensitized breast cancer cells to chemotherapy. E2F3 appeared as a novel and direct target of miR-125b in breast cancer cells as overexpression of miR-125b significantly reduced E2F3 protein level in MCF7 cells [70].

Association of miRNAs with Breast Cancer Stem Cells

In addition to finding miRNAs dysregulated in tumors compared to normal tissues, researchers have found that miRNA expression also varies within heterogeneous tumor samples where different subpopulations of tumor cells possess unique miRNA signatures. Breast cancer stem cells (BCSCs) were found to possess a unique miRNA signature.

miRNA expression in human $CD44^+/CD24^{-/low}$ lineage-BCSCs were compared to the lineage-non-stem cancer cells (NSCC). As many as 37 miRNAs were found to be differentially expressed in BCSCs compared to NSCCs [42]. Among these, three clusters including miR-200c-141, miR-200b-200a-429, and miR-183-96-182 were downregulated in human BCSCs, normal human and murine mammary stem/progenitor cells and embryonal carcinoma cells. miR-200c was found to regulate the expression of BMI1 and Suz12, which is involved in stem cell self-renewal. Furthermore, miR-200c suppressed the clonogenicity of the BCSCs in vitro and the tumorigenicity of human BCSCs in vivo [42].

The miR-200a family is involved in epithelial to mesenchymal transition (EMT) in breast cancer [65]. Our lab showed that epigenetic silencing of miR-200a expression was associated with the transformation of normal mammary epithelial cells through the overexpression of SIRT1 [35]. MiR-200a is also downregulated in MDA-MB-231 breast cancer cells compared to non-tumorigenic MFC10A cells. Reintroduction of miR-200a was able to prevent the transformation. These results further confirmed miR-200a as a tumor suppressor in breast cancer.

Similar results were observed in a synthetic model in which mammary epithelial cells were transformed via engineering with an ER-Src oncogene and induction of SRC via tamoxifen treatment [28]. The isolated cancer stem cells emerged from transformed cells showed differential expression of miRNAs compared to non-stem cancer cells. In particular, miR-200 family, let-7 family, and miR-145 downregulation were observed.

In CSCs isolated from breast cancer cell lines, let-7 miRNAs were also significantly downregulated. Let-7 lentivirus-infected BCSCs were less proliferative, and formed less mammospheres, indicating let-7 can negatively regulate self-renewal of BCSCs [71]. Furthermore, Let-7 infection led to decreased tumor formation and metastasis in vivo. Reducing let-7 expression, on the other hand, enhanced the self-renewal capacity of non-tumor-initiating cells in vitro. The targets of let-7, H-RAS, and HMGA2 decreased with the let-7 infection as expected. Silencing H-RAS reduced the self-renewal capacity of BCSCs but had no effect on differentiation, whereas silencing the HMGA2 enhanced differentiation without having any effect on self-renewal. These results demonstrate that let-7 regulates the stem cell properties of BCSCs by silencing more than one target.

Yu and colleagues extended their research to investigate the role of miR-30 on stem cell-like behaviors in BCSCs [72]. miR-30 was reduced in CSCs where its target genes Ubc9 and ITGB3 were upregulated. Restoration of miR-30 in CSCs inhibited their self-renewal capacity by decreasing Ubc9 and increased apoptosis by decreasing ITGB3. Similarly, silencing miR-30 in differentiated breast cancer cells increased their

ability to self-renew. Their in vivo studies showed that ectopic expression of miR-30 in breast tumor-initiating xenografts reduced tumorigenesis and metastasis, whereas silencing miR-30 had the opposite effect.

Yu and colleagues also linked miR-34c dysregulation to the functions of BCSCs in luminal breast cancer models [22, 23]. Their data showed that ectopic expression of miR-34c decreased the self-renewal capacity of breast tumor-initiating cells (BT-ICs), inhibited EMT, and decreased the tumor cell migration through the repression of NOTCH4. They identified a hypermethylated CpG site in the promoter of miR-34c, which had a role in the downregulation of miR-34c in BCSCs by preventing DNA binding activities of SP1. This data confirms the importance of epigenetic regulations of miRNAs in BCSC and presents miR-34c as a potential target for eliminating CSCs.

In a similar study, miR-181 was implicated in the regulation of cancer stem cells [73]. Treatment with TGF-β, which is involved in miR-181 regulation, induced their mammosphere formation ability. The expression of miR-181 family members was increased in tumor-initiating mammospheres, which suggested the importance of miR-181 regulated by TGF-β for maintaining the BCSC phenotype.

Breast Cancer Stem Cells and Chemoresistance

The role of miRNAs in the chemoresistance of tumor-initiating cells was recently investigated. miR-128 was found to be significantly downregulated in chemoresistant BCSCs [74]. BM1 and ABCC5 were found to be upregulated in these CSCs and were discovered to be direct targets of miR-128. Introduction of miR-128 to BCSCs decreased the expression of BM1 and ABCC5 and increased the apoptotic rate and DNA damage upon treatment with doxorubicin. This suggests that miR-128 restoration might be a good therapeutic strategy to target BCSCs. Another group confirmed the potential of miR-128 as a therapeutic target in BCSCs [75]. They found that overexpression of miR-128 decreased the mammary carcinoma stem cell-like behavior in MDA-MB-231 breast cancer cells, reduced the percentage of CD44$^+$/CD24$^{-/low}$ subpopulation in vitro, and decreased the tumor-initiating ability in vivo.

miR-16 expression was also found to be significantly reduced in mammary tumor stem cells [76]. Overexpression of miR-16 or inhibition of its target WIP1 decreased the self-renewal ability of mammary tumor stem cells in mice and sensitized MCF7 cells to doxorubicin.

A novel BCSCs subpopulation was isolated that have a PROCR$^+$/ESA$^+$ phenotype. miRNA profiling revealed miR-495 upregulation [77]. Ectopic expression of miR-495 in breast cancer cells increased their ability to form colonies in vitro and tumors in vivo. E-cadherin was identified to be a direct target of miR-49; E-cadherin expression was decreased via miR-495 targeting, contributing to cell invasion.

REDD1 was identified as another direct target of miR-495 and its suppression by miR-495 resulted in enhanced cell proliferation in hypoxia conditions. E12/E47 transcription factor was significantly upregulated in BCSCs and also turned out to be directly regulated by miR-495. These results showed that miR-495 plays a key role in the maintenance of stem cell-like phenotypes of BCSCs and might be a potential target for the development of novel therapeutics.

miRNA Targeting of DCIS CSCs

We have examined the role of miRNAs in DCIS and identified miR-140 downregulation as a reproducible marker of DCIS, and even a more dramatic decrease was observed in IDC samples [17•, 78]. Furthermore, our lab provided a direct link between BCSC maintenance and miR-140 expression, which implies a role for BCSCs in DCIS to IDC transition through miR-140 regulation. miR-140 is a potential tumor suppressor that is downregulated in breast cancer including early stage DCIS. Our research revealed that upon the stimulation with estrogen, miR-140 expression decreases in ER-positive luminal breast cancer cells [78]. We also found that E2 stimulation significantly increased the CD44^{high}/CD24^{low} subpopulation. When miR-140 was overexpressed following estrogen stimulation, however, the percentage of CD44^{high}/CD24^{low} subpopulation significantly decreased. We identified the stem cell regulator SOX2 as a novel target of miR-140.

Next, we found that miR-140 is significantly downregulated in DCIS stem cells, which is suspected to play an important role in the progression of DCIS to IDC [17•]. We identified important pathways regulated by miR-140 in DCIS stem cells, including SOX9 and ALDH1, which are highly activated in DCIS stem cells. Moreover, restoration of miR-140 via molecular approach or through the dietary compound sulforaphane (SFN) decreased SOX9 and ALDH1 expression in ER-negative/basal-like DCIS model as well as reducing tumor growth in vivo. All together, these results suggest that miR-140 might be a strong candidate to be included in preventive strategies for patients with basal-like DCIS.

We further characterized a CD49f$^+$/CD24$^-$ stem cell-like subpopulation with high ALDH1 activity in DCIS cells [16•]. These studies revealed that DCIS stem cells are more migratory compared to non-stem cancer cell counterparts. We also found that DCIS stem cells could be targeted with the chemopreventive agent sulforaphane (SFN), which reduced ALDH1 expression (at least in part through miR-140 activation), decreased mammosphere formation and decreased progenitor colony formation capacity. Moreover, we found that exosomes from DCIS stem cells differentially expressed several miRNAs including miR-140, miR-29a, and miR-21 and SFN was able to reprogram DCIS stem cell intercellular signaling as evidenced via changes in exosomal miRNA secretion.

Conclusions

Despite improvements in detection and therapeutic management, breast cancer remains the second leading cause of cancer-related death among women. Furthermore, advanced metastatic breast cancer remains an incurable disease. Therefore, new prevention strategies are needed to curb disease formation and progression.

Currently, patients with early-detected tumors are candidates for adjuvant hormonal therapy if their disease is ER positive. However, for patients with ER-negative disease, there are no available molecularly targeted agents. Dietary agents are generally well-tolerated, and high-risk healthy individuals and patients with early non-malignant lesions may be ideal candidates for chemopreventive strategies with the most promising compounds. The potential is supported by epidemiological studies, and the results from in vivo preclinical models have so far been very promising.

We find that miRNAs, in particular, miR-140 can be activated via epigenetic therapy or dietary compounds and can target DCIS stem cells, thereby preventing disease relapse or progression to invasive carcinoma.

We have summarized the modulation of miRNAs in response to many well-known dietary chemopreventive agents. The fact that a single miRNA can regulate multiple genes and a single gene can be regulated by multiple miRNAs challenges the elucidation of specific gene-miRNA interactions, but such research is crucial to better identify chemopreventive strategies to inhibit the signaling pathways critical to disease formation and progression.

Compliance with Ethics Guidelines

Conflict of Interest   Nadire Duru, Ramkishore Gernapudi, Gabriel Eades, Richard Eckert, and Qun Zhou declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent   This article does not contain any studies with human or animal subjects performed by any of the authors.

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