



MicroRNAs and DNA-Damaging Drugs in Breast Cancer: Strength in Numbers

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MicroRNAs are a class of small non-coding regulatory RNAs playing key roles in cancer. Breast cancer is the most common female malignancy worldwide and is categorized into four molecular subtypes: luminal A and B, HER2+ and triple-negative breast cancer (TNBC). Despite the development of multiple targeted therapies for luminal and HER2+ breast tumors, TNBC lacks specific therapeutic approaches, thus they are treated mainly with radio- and chemotherapy. The effectiveness of these therapeutic regimens is based on their ability to induce DNA damage, which is differentially resolved and repaired by normal vs. cancer cells. Recently, drugs directly targeting DNA repair mechanisms, such as PARP inhibitors, have emerged as attractive candidates for the future molecular targeted-therapy in breast cancer. These compounds prevent cancer cells to appropriate repair DNA double strand breaks and induce a phenomenon called *synthetic lethality*, that results from the concurrent inhibition of PARP and the absence of functional BRCA genes which prompt cell death. MicroRNAs are relevant players in most of the biological processes including DNA damage repair mechanisms. Consistently, the downregulation of DNA repair genes by miRNAs have been probe to improve the therapeutic effect of genotoxic drugs. In this review, we discuss how microRNAs can sensitize cancer cells to DNA-damaging drugs, through the regulation of DNA repair genes, and examine the most recent findings on their possible use as a therapeutic tools of treatment response in breast cancer.

Keywords: breast cancer, DNA repair, DNA damage response, DNA-damaging drugs, microRNAs

GENERAL OVERVIEW

MicroRNAs

MicroRNAs (miRNAs) are endogenous, small non-coding RNAs that regulate gene expression at post-transcriptional level. Mature miRNAs are single strand molecules of ~18–25 nucleotides (nt), transcribed by RNA polymerase II/III as long primary transcripts with a hairpin structure, called pri-miRNAs. Pri-miRNAs are then cleaved in the nucleus into ~60 nt long molecules (pre-miRNAs) by the Microprocessor, a multi-protein complex comprising the RNase III enzyme DROSHA and its cofactor DGCR8 (1, 2). As pre-miRNAs, these molecules are specifically recognized by the nuclear export machinery, mainly composed of Exportin-5 and Ran-GTPase, and exported to the cytoplasm where their processing is completed. The dsRNA stem of pre-miRNAs is asymmetrically cleaved by the second multi-domain RNAase III enzyme DICER into a short nucleotide duplex (3). During this step, the transactivation-responsive RNA-binding protein

(TRBP) mediates the assembling of the miRNA-induced silencing complex (miRISC), favoring DICER and Argonaute protein (AGO1, AGO2, AGO3 or AGO4) interaction (4). The miRISC complex selects the mature miRNA (guide strand), which then guides the machinery to the target mRNA (5). MiRNA/mRNA interaction occurs by the recognition between the “seed” region at the miRNA 5'UTR and its complementary sequence on the 3'UTR of the designated mRNA. The result of the pairing is, either the translational repression or transcript degradation, in dependence of the complementary degree between the two sequences (6). After the discovery of the small RNA *lin-4* function in the larval development of *Caenorhabditis elegans* in 1993, many researchers had started to investigate the regulatory potential of these small molecules (7). To date, it is well-known that miRNAs participate in almost every biological process in mammals, including cancer (8). Several mechanisms alter miRNA expression in cancer such as genomic aberrations, epigenetic changes, dysfunction of the processing machinery, alteration of transcription factor expression, among others (9).

In cancer, miRNAs can act as tumor suppressors or oncogenes (oncomiR). Functionally, miRNAs with a tumor suppressor role target oncogenes and are generally downregulated in cancer cells (e.g., miR-205 and miR-34 in breast cancer). While, oncogenic miRNAs target tumor suppressor genes and are usually upregulated in tumor cells (e.g., miR-21, miR-155, and miR-221/222 in breast cancer) (10).

MiRNA capability to regulate several target genes involved in oncogenic mechanism such as proliferation, progression, metastasis, and therapy response, makes these small molecules fascinating candidates as therapeutic tools. In fact, recent studies have been focused on develop new strategies to make possible the miRNA-based therapy approach. Generally, miRNAs can be reintroduced in cancer cells using miRNA *mimics* or be inhibited by *anti-miRs*. During the last years, new methods to deliver and to stabilize miRNA *mimics* and *anti-miRs* have been developed, some of which are currently in clinical trials.

MiRNA *mimics* and *anti-miRs* can be delivered with lipid carriers, for instance the miR-34-based therapy MRX34 (Mirna Therapeutics) deliver miR-34 *mimic* sequence through the lipid carrier NOV40. MRX34 is the first miRNA-based therapy undergoing in a clinical trial for cancer treatment. During 2013, patients with lymphoma, melanoma, multiple myeloma and liver, small cell lung and renal carcinoma were enrolled in a phase I clinical trial. Unfortunately, despite the promising results obtained with the partial response of 3 patients and stable disease in other 14 patients, the trial was terminated in September 2016 due to severe and lethal immune-related adverse reactions occurred in some patients (clinicaltrials.gov:NCT01829971) (11, 12). Additionally, EnGeneIC Delivery Vehicle (EDV) nanocells (also called TargomiRs) coated with epidermal growth factor

receptor (EGFR)-specific antibodies are currently in a phase I trial to deliver miR-16 mimics in patients with malignant pleural mesothelioma and NSCLC (clinicaltrials.gov: NCT02369198), current preliminary results show that the treatment is well-tolerated. MiRNA *mimics* can be also conjugated to *N*-acetyl-D-galactosamine (GalNAc) particles, improving their uptake into cells through clathrin-mediated endocytosis. Moreover, RG-125 (Regulus Therapeutics), a GalNAc-conjugated containing an *anti-miR*-103/107 sequence, recently entered in clinical investigations to treat non-alcoholic steatohepatitis (NASH). In addition, a phase I trial in HCV-infected patients was initiated to evaluate the response of RG-101 (Regulus Therapeutics), a *N*-acetyl-D-galactosamine (GalNAc)-conjugated *anti-miR* targeting miR-122. Finally, in November 2015, a LNA-modified anti-miR-155 (MRG-106) has begun to test in a phase I clinical trial to treat patients with cutaneous T-cell lymphoma (clinicaltrials.gov: NCT02580552).

Breast Cancer

Breast cancer represents one of the most common malignancies worldwide and a leading cause of cancer-related death in women (13). Biological and genomic characterizations have described breast cancer as a highly heterogeneous disease, according to histological and molecular features, and responsiveness to therapy (14).

Clinically, breast cancers are firstly categorized, according to the expression of three receptors routinely assessed by immunohistochemistry assay in the following subtypes: estrogen and progesterone receptor positive (ER+, PR+), human epidermal growth factor receptor positive (HER2+) and triple-negative (ER-, PR-, HER2-) malignancies (15, 16). This classification provide valuable clinical information, mainly to choice the first line treatment, in addition to the histological grade, clinical stage, patient's age and menopausal status. The advent of high throughput technologies, such as microarray-based transcriptomic analysis, has provided new sources of information regarding breast cancer biology. Gene expression profiling of breast cancers identified five intrinsic molecular subtypes: hormone receptors positive luminal A and luminal B, HER2-enriched, basal-like and normal-like. These subtypes differ in incidence, prognosis and responsiveness to therapy (17–19). Luminal B (ER+, PR+, HER2-, Ki67+) usually present higher clinical grade than luminal A (ER+, PR+, HER2-, Ki67-) tumors, and some of them also express HER2 receptor. The HER2-enriched (HER2+, ER-, PR-) most frequently present high grade and node positive, whereas the basal-like (HER2-, ER-, PR-) subgroup mainly comprises triple-negative breast cancers (TNBCs) and frequently shows BRCA1 mutations, both germinal and sporadic (20).

An additional intrinsic subtype described more recently is the claudin-low subtype, sorted mainly from the TNBC subgroup and characterized by stem cell-like features (21, 22). A more detail classification based on molecular portrait of TNBCs have been provided in 2011 by Lehmann and colleagues through the identification of six subtypes with distinct gene expression patterns and response to treatment: basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M),

Abbreviations: DDR, DNA damage response; NER, nucleotide excision repair; BER, base excision repair; MMR, mismatch repair machinery; DSBs, Double Strand Breaks; SSBs, Single-Strand Breaks; NHEJ, non-homologous end joining; HR, homologous recombination; ER, estrogen receptor; PR, progesterone receptor; HER2, epidermal growth factor receptor 2; TNBC, triple negative breast cancer; miRNAs, microRNAs; IR, ionizing radiation.

mesenchymal stem-like (MSL), and luminal androgen receptor (LAR) subtype (23). High level of cell cycle and DNA damage response genes are expressed by BL1 and BL2 subtypes, which are preferentially sensitive to cisplatin. Epithelial-to-mesenchymal transition and growth factor pathways are enriched in the M and MSL subtypes, which respond to PI3K/mTOR and abl/src inhibitors. The LAR subtype, characterized by androgen receptor (AR) signaling and shorter relapse-free survival, is sensitive to bicalutamide (an AR antagonist). During the last years, next generation sequencing (NGS) has significantly improved the molecular characterization of breast carcinomas, providing data on gene mutations, DNA copy number variations, DNA methylation and miRNA expression patterns. Important examples of such studies are The Cancer Genome Atlas (TCGA) project, the International Cancer Genome Consortium (ICGC) and the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) (24, 25). Information gathered from these studies are particularly useful to the clinical practice, because they provide a list of new molecules, which can be potentially targeted or exploited for therapy interventions to improve drug efficacy (26).

The role of miRNAs in breast cancer was deeply investigated in the last decades. The first miRNA signature in breast cancer was described by Iorio et al. (27), followed by several studies that have demonstrated a functional role of miRNAs in the disease. One of the most studied miRNA in breast cancer is miR-21, which acts as an oncomiR mediating cell survival, proliferation, invasion, and metastasis (28). MiR-9, targeting E-cadherin and regulating the EMT process, is recognized as a metastamiR in breast cancer (29); as well as miR-10b, one of the first metastamiRs described in a breast cancer model (30). Conversely, many miRNAs have been identified as tumor suppressors in breast cancer. MiR-205 and miR-125a, for example, modulate the expression of HER3 and HER2 oncogenes, respectively (31, 32). Moreover, it has been reported that miR-205 and miR-200 family have an important anti-tumorigenic role by targeting ZEB1 and ZEB2, suppressing EMT process (33).

DNA Repair Mechanisms

Genomic instability is well-recognized as one of the hallmarks of cancer (34). Many studies have demonstrated that breast cancer cells have defective DNA damage response (DDR) mechanisms. In general, when DNA damage occurs, cells repair the errors and continue to proliferate; otherwise, the damage can cause mutations or chromosomal rearrangements, which induce tumorigenesis. The DDR is a complex system activated upon DNA damage, shaped by the activity of DNA damage signal transduction, DNA repair mechanisms, cell cycle checkpoints and apoptosis signaling pathways (35). DDR regulates DNA repair by inducing the following molecular processes: detection of damage sites, recruitment of repair factors and repair of DNA lesions. DDR machinery is divided into DNA damage sensors, signal transducers and effectors. DDR can use different mechanisms to repair DNA damage. In particular, two mechanisms are designated to remove damaged and modified nucleotides: (1) Nucleotide Excision Repair (NER) which works

on helix-distorting and transcription-blocking lesions (i.e., UV-induced pyrimidine dimers) and (2) Base Excision Repair (BER) which removes single nucleotides by methylation, alkylation, deamination, or oxidation (36, 37). Other DDR mechanism participates in the recognition of incorrect insertions or deletions of nucleotides during DNA synthesis, which can lead to microsatellite instability and cancer. These errors affect the canonic DNA sequence and induce base mismatches that cause the distortion of DNA helix. The Mismatch Repair Machinery (MMR) operates through MSH2 and MLH1, which form heterodimers with MSH3 or MSH6 and MLH3, PMS2, or PMS1, respectively (38, 39). Many environmental agents can cause other types of DNA damage such as: Double-Strand Breaks (DSBs) or Single-Strand Breaks (SSBs) (40). SSBs and modified bases are the most common DNA damage, approximately in 1 day occurs 20,000 events per cell, but are usually repaired via BER mechanism (41). Instead, DSBs induce the recruitment of MRE11–RAD50–NBS1 (MRN) complex, which activates the serine/threonine-specific kinases ATM which allows its auto-phosphorylation (pATM) and the phosphorylation of the Ser139 of histone H2AX (γ H2AX) in response to DNA damage signals. γ H2AX recruits additional pATM molecules and DDR proteins, such as p53-binding protein 1 (53BP1), at DSB site, to generate a nuclear foci (42, 43). In response to DSBs, ATM kinase promotes the phosphorylation of many proteins, in particular Chk2 kinase, one of the most important effectors of ATM (44, 45). On the other hand, ATR recruit Chk1 kinase after stalled replication-forks and SSBs induced by UV (46, 47). ATM and ATR induce the phosphorylation of multiple proteins to activate downstream DNA repair pathways and induce cell cycle arrest, apoptosis or senescence when damage repair was not efficient (48). DSBs are repaired by two mechanisms: (1) Non-Homologous End Joining (NHEJ), active during all phases of the cell cycle but its activation occur mainly in G0/G1 phases, or (2) Homologous Recombination (HR), which acts in the S/G2 phases of the cell cycle (49, 50). In particular, NHEJ resolve double-stranded DNA breaks by enhancing Ku protein binding, as well as the recruitment and activation of DNA-PK. NHEJ complex includes DNA ligase IV, XRCC4, and XLF/Cernunnos protein which promotes a direct ligation of the ends of DSBs. However, this mechanism can induce some alterations, such as deletions or mutations of DNA sequences at the DSB site or around it (40). HR comprehends a set of proteins, including BRCA1, BRCA2, RAD51, and PALB2 that allow the restoration of the original DNA sequence at the damage site. Briefly, the DNA sequence near to DSB is deleted and the sequence on the homologous sister chromatid is used as a template to synthesize new DNA at the DSB site (37, 49). Many studies have demonstrated that miRNAs regulate, at transcriptional and post-transcriptional levels, the DNA damage sensor, signal transducer and effector genes in cancer cells. For example, miR-182, miR-181a/b, miR-28, and miR-146 have been demonstrated to target BRCA1 in breast cancer cells. The DDR gene RAD51 is modulated by miR-155, miR-107, miR-221/222; whereas ATM is targeted by miR-181a/b and miR-18a. Moreover, in breast cancer cells, miR-125b and miR-34a are able to control the expression of TP53, the main cell cycle regulator

(51). **Figure 1** shows a schematic representation of DNA damage repair mechanisms and some relevant miRNAs involved in the modulation of DNA repair genes. Therefore, miRNAs represent an important regulatory mechanism of DNA repair pathways and a novel source to exploit DDR gene/miRNA interactions for clinical purposes as potential biomarkers and therapeutic tools.

In the next sections, we will review how miRNAs could influence the response to DNA-damaging drugs in breast cancer therapy. Indeed, several studies have reported a key role of miRNAs in the responsiveness to DNA-damage based therapies, by modulating the expression of genes involved in the initiation, activation and maintenance of DDR mechanisms.

DNA DAMAGING DRUGS IN BREAST CANCER THERAPY

The strategies of breast cancer treatment include surgery, radiation, chemotherapy, hormone therapy, and biological targeted therapy. Patients with hormone receptor positive (ER+, PR+) tumors receive hormone therapy (i.e., tamoxifen and aromatase inhibitor), whereas patients affected by HER2+ tumors receive anti-HER2 targeted therapy (i.e., trastuzumab and pertuzumab) (52). Thus, the biggest challenge is represented by the clinical management of TNBCs, mainly due to the lack of targeted drugs. Indeed, the standard therapy for these tumors still remains cytotoxic chemotherapy (53). In term of response to chemotherapy, luminal A tumors show lower responsiveness, luminal B are more responsive than luminal A but less than HER2-enriched and basal-like, which are the subgroups with the higher response rate.

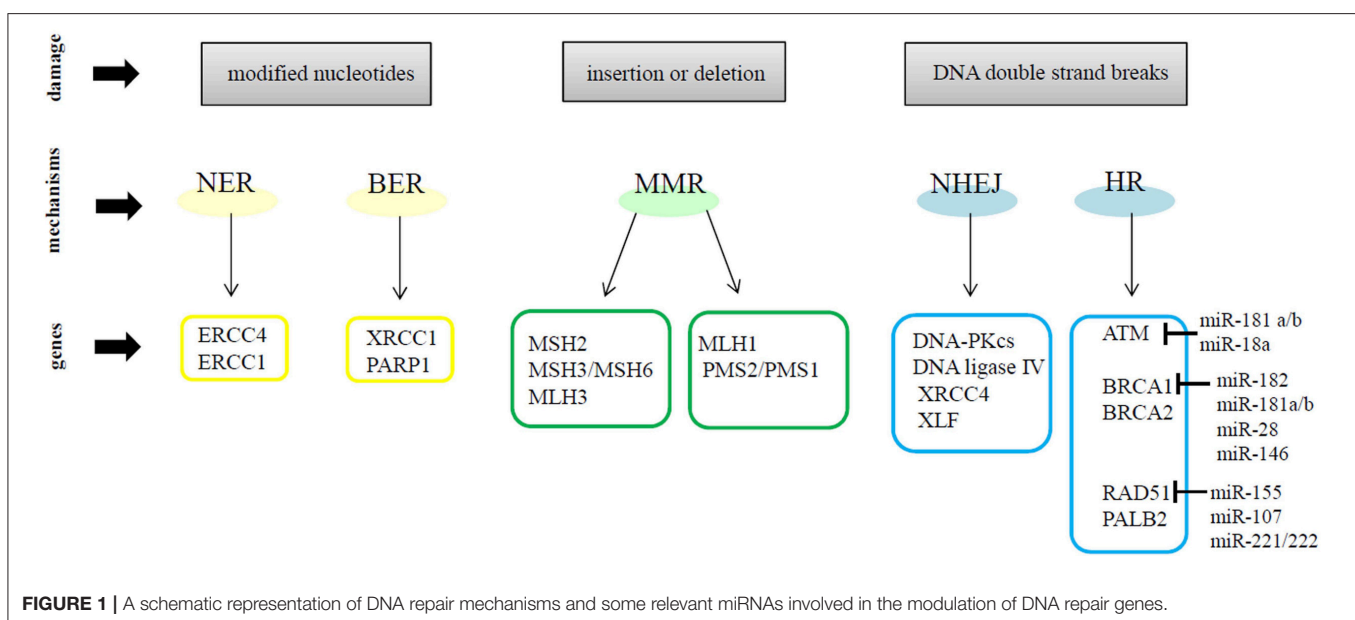
In breast cancer management, radio- and chemotherapy exert their effects by causing DNA damage, and are usually used as first-line drugs in combination with hormone and

target therapies. Ionizing radiation (IR), anthracyclines, platinum compounds, and taxanes usually induce DSBs and SSBs, the efficacy of current DNA-damaging drugs is correlated with the capabilities of cancer cells to resolve and repair DNA lesions. Cancer cells are highly proliferative in comparison with normal cells, this feature increases their susceptibility to DNA damage exposure in the S phase of the cell cycle (20). However, the main problem of radio- and chemotherapy is the development of acquired resistance along the drug administration.

Radiotherapy treatment, based on the administration of a specific amount of energy, induces the activation of multi-staged processes which enhance tumor cell death. In particular, DSBs promote chromosomal alterations and affect cell division, contributing to cell death or mutation (54). Ionizing radiation (IR), such as X-rays, can extend cell damage by direct DNA breaks or indirectly through the creation of free radicals (55, 56). Tumors receiving a large total dose of radiations sometimes develop radioresistance, which eventually leads to treatment failure. The acquired radioresistance can be associated with altered expression of cell cycle molecules and DDR effectors, such as the overexpression of cyclin D1 and the constitutive activation of DNA-PK and AKT, respectively (57).

Chemotherapy is the most common therapy for cancer. Chemotherapeutic agents promote tumor cell death by direct cytotoxicity, activation of host immune response, inhibition of cell proliferation and induction of apoptosis (58). After cytotoxic agents administration, DNA damage is the first event sensed by the cellular stress response machinery, triggering the activation of effector systems, such as apoptosis (59). Unfortunately, resistance to chemotherapy can occur at many levels including DNA repair, cell cycle regulation and evasion of apoptosis (60).

Recently, the identification of BRCA-associated DNA repair mechanisms, frequently impaired in TNBCs, led to the development of specific DNA damage target therapies.



BRCA genes, involved in DNA repair through HR after DSBs, are altered in sporadic and hereditary breast cancer; notably, in sporadic tumors BRCA1 mutations are rare (<5%) while ~10% of TNBC patients present germline mutations in BRCA1/2 which increase breast cancer risk about 60–70% (61, 62). In the last years, many groups have deep investigated the role of defective HR mechanisms in cancer: the so-called “BRCAness” status is defined as the presence in tumor cells of alternative mechanisms impairing BRCA1/2 functions, or the alteration in HR genes. BRCAness is a phenocopy of BRCA1/2 mutations, in fact, HR mechanisms result defective although tumor cells do not carry mutations in BRCA1/2 genes (63).

As above clarified, for patients affected by TNBCs, targeted therapies are not currently available and chemotherapy may lead the acquisition of resistance in the later stages of the disease (64). In the last years PARP inhibitors have emerged as a possible therapeutic approach, especially when cancer cells lack functional alleles of the genes BRCA1 or BRCA2 (65). Poly-[ADP-Ribose]-Polymerase-1 (PARP-1) is a crucial molecule involved in the activation of the DNA-damage response. PARP-1 is a nuclear protein implicated in various processes involving DNA-related transactions. PARP-1 recognizes DNA-damage sites and creates long chains of poly-ADP-ribose, required for the appropriate recruitment of DNA repair enzymes (66). When DNA damage occurs, PARP-1 is rapidly recruited to the altered DNA and converts nicotinamide adenine dinucleotide (NAD) into ADP-ribose polymers (PAR) by attracting XRCC1, a scaffold protein which stabilizes or stimulates compounds involved in single-stranded breakage (67). PARP-1 is composed of three functional domains: the amino-terminal DNA-binding domain composed of two zinc fingers, which is necessary to bind the DNA breaks; the automodification domain, which allows the enzyme to PARate itself, and the c-terminal catalytic domain where ADP-ribose subunits are transferred from NAD⁺ to proteins acceptors (67). PARP inhibitors are able to block the catalytic PARP domain competing with NAD, and impairing the single-stranded DNA breakage repair activity. This mechanism induces apoptosis through the accumulation of damaged DNA in the cells. Functionally, PARP inhibitors (e.g., olaparib, talazoparib, rucaparib, and veliparib) prevent cancer cells from appropriately repair DNA damages, consequently genotoxic stress results in cancer cell death (65). Cells with BRCA1/2 wild-type can still repair the damage through HR, whereas mutated BRCA1/2 cells strictly depend on PARP activity for DNA repair. The inhibition of PARP in absence of functional BRCA genes results in *synthetic lethality*. As well-known, chemotherapy alters pathways involved in DNA damage repair; for this reason, PARP inhibitors can sensitize tumor cells to chemotherapy and radiotherapy and can induce *synthetic lethality* in tumors from patients with hereditary or sporadic mutations in BRCA1/2 genes. Different PARP inhibitors have been evaluated in preclinical studies and in clinical trials as mono or combination therapies for breast cancer patients, particularly for TNBC. In a BRCA1-deficient breast cancer mouse model, the combination of a PARP inhibitor with cisplatin or carboplatin increases the recurrence-free and overall survival, indicating that PARP inhibitors can improve the efficacy of DNA-damaging compounds (68). Moreover, Hay T.

and colleagues have shown that daily treatment with olaparib for 28 days in mice with BRCA2^{-/-} mammary epithelium caused a significant regression of 46/52 tumors (69).

In clinics, different trials on breast cancer patients with BRCA1/2-defective tumors demonstrated that PARP inhibitors, such as olaparib, enhance the therapeutic response when administrated as single agents or in combination with platinum compounds.

In a first study of phase I, 60 patients, affected by different tumor types including breast cancer, were enrolled and treated with olaparib. Among these, 22 patients had BRCA1 or BRCA2 mutations. Results showed that olaparib has the capability to inhibit PARP with limited adverse effects in comparison with conventional chemotherapy, however an antitumor activity was observed only in patients carrying BRCA mutations (70). In a phase II study, two cohorts of 27 patients with confirmed BRCA1 or BRCA2 mutation-advanced breast cancer were enrolled and treated with two different doses of olaparib. The first cohort had an overall response rate (ORR) of 41% and in the second cohort an ORR of 22%; moreover no particular toxicity has been reported (71).

Furthermore, when olaparib had been combined with paclitaxel in a cohort of 19 patients with metastatic TNBC in phase I study, an ORR of about 30–40% has been observed. Particularly seven patients had a confirmed partial response and one patient remained stable with olaparib monotherapy without progression (72). To date, phase III trials are ongoing to investigate the use of olaparib in the metastatic and neoadjuvant setting, for patients with mutations in BRCA1/2 (62). Finally, a randomized phase II trial that recruited patients with TNBC and/or BRCA mutations, treated with cisplatin alone or in combination with rucaparib, showed that both treatment groups present a similar disease-free survival at 1 year follow-up (~76%), and rucaparib did not add significant toxicity to the cisplatin regimen (62, 73).

Results of clinical studies with PARP inhibitors have shown promising results in advanced breast cancer, but there is still an urgent need to identify suitable patients who may actually benefit from this treatment. Further investigations to find new strategies to efficiently impair DNA repair mechanisms in breast cancer patients could enhance the response to radio-, chemo-, and PARP inhibition therapies.

MICRORNAs REGULATE DNA REPAIR GENES AND RADIO-CHEMOTHERAPY RESPONSIVENESS

Alterations in DNA repair mechanisms and in miRNA expression are both features of cancer development and progression (28, 74). As reported in this review, genotoxic agents, causing DNA damage, are commonly used for radio- and chemo-therapeutic treatments in breast cancer. MiRNA up- or down-modulation is often involved in the regulation of DNA repair mechanisms (75) and it is currently known that miRNAs can regulate responsiveness to drugs (76). Thus, the alterations of miRNA expression involved in DDR mechanisms play an important role

in responsiveness to radio- and chemotherapy. Recently, our group has shown that miR-302b expression in breast cancer cell lines induces cisplatin sensitivity, reducing cell viability and proliferation in response to the treatment (77). E2F1, a master regulator of the G1/S transition, is directly targeted by miR-302b. Moreover, this miRNA, through the negative regulation of E2F1, indirectly downregulates ATM, the main cellular sensor of DNA damage, affecting cell-cycle progression following cisplatin treatment. As a result, miR-302b impairs the capability to repair damaged DNA upon cisplatin treatment, enhancing apoptosis in breast cancer cells (77).

Accordingly, another group has demonstrated that miR-302 family is able to sensitize breast cancer cells to radiotherapy; in particular Liang et al. showed that the decreased expression of miR-302a induces radiotherapy resistance and the reintroduction of miR-302a expression enhances radiotherapy sensitivity in *in vitro* and *in vivo* breast cancer models, abrogating the expression of AKT1 and RAD52 (78).

Gasparini et al. revealed that miR-155 overexpression reduced RAD51 levels in human breast cancer cells, which affects the response to IR and impairs the efficiency of HR repair enhancing IR sensitivity both in *in vitro* and *in vivo* models. Moreover, a series of TNBC patients with high levels of miR-155 and low expression of RAD51 revealed a significant association with a better overall survival. Thus, miR-155 expression can be considered as a prognostic biomarker which allows to identify TNBC patients who will likely be responsive to IR-based therapeutic approach (79). It was broadly demonstrated the over-expression of the oncomiR miR-21 in tumors with relevant consequences in cell cycle, DNA damage repair, apoptosis, autophagy, and hypoxia of cancer cells during irradiation. Indeed, cell cycle progression is influenced by miR-21 through the induction of DNA damage in G2 checkpoint and the miRNA inhibition (by anti-miR-21 administration) reduced the G2/M block and induced apoptosis following radiation treatment in breast cancer (80).

Of note, the overexpression of the miR-205, an oncosuppressive miRNA, increases the response to tyrosine

kinase inhibitors, lapatinib and gefitinib in preclinical breast cancer models (81). Recently, it has been described that enhanced expression of miR-205 sensitizes breast cancer cells to radiation by regulating ZEB1 and affecting DNA repair. Indeed, miR-205 directly targets Ubc13, a protein involved in the homologous recombination. Moreover, the authors demonstrated that the delivery of miR-205 mimics, by nanoliposomes in a xenograft model, has a therapeutic effect sensitizing tumor to radiation (82).

MiR-18a is upregulated in breast cancer cell lines and patient tissues, interestingly its ectopic expression downregulates ATM. This phenomenon in breast cancer cells reduced the DNA damage repair ability, the efficiency of HR and sensitized cells to radiation treatment (83). Wip1 is a regulator of DNA damage signaling pathways, in particular it inhibits the phosphorylation of some DNA repair factors, including ATM, Chk1, Chk2, p53, and others. Zhang et al have demonstrated that miR-16 targets Wip1, affecting DNA repair and sensitizing breast cancer cells to doxorubicin treatment (84). Furthermore, using a TNBC *in vivo* model, Wang et al. demonstrated that miR-96 reduces the expression of REV1 and RAD51 and consequently inhibits tumor growth after cisplatin treatment. Thus, miR-96 is a potent cisplatin sensitizer *in vivo* (85).

Finally, diverse studies have focused their attention on miRNAs directly targeting BRCA genes in breast cancer. BRCA1 and BRCA2 are tumor suppressor genes important for the HR mechanism, which is the process involved in the repair of DNA DSBs. For instance, miR-218 directly targets BRCA1 and that its restored expression in cisplatin resistant breast cancer cell lines sensitizes cells against the drug, affecting DNA damage (86). Moreover, miR-638 overexpression increases sensitivity to DNA-damaging agents, ultraviolet (UV) and cisplatin, and reduces proliferation, invasive ability, and DNA

TABLE 1 | miRNAs involved in the chemo- and radio-responsiveness, through the regulation of DNA repair genes.

microRNA expression	Gene target	Drug response
miR-302b overexpression	E2F1 and ATM	Cisplatin sensitivity (77)
miR-302a overexpression	AKT1 and RAD52	IR sensitivity (78)
miR-155 overexpression	RAD51	IR sensitivity (79)
miR-21 downregulation	G2/M block	IR sensitivity (80)
miR-205 overexpression	Ubc13	IR sensitivity (82)
miR-18a overexpression	ATM	IR sensitivity (83)
miR-16 overexpression	Wip1	Doxorubicin sensitivity (84)
miR-96 overexpression	REV1 and RAD51	Cisplatin sensitivity (85)
miR-218 overexpression	BRCA1	Cisplatin sensitivity (86)
miR-638 overexpression	BRCA1	UV and Cisplatin sensitivity (87)

TABLE 2 | miRNAs involved in the PARP inhibitors response, through the regulation of DNA repair genes.

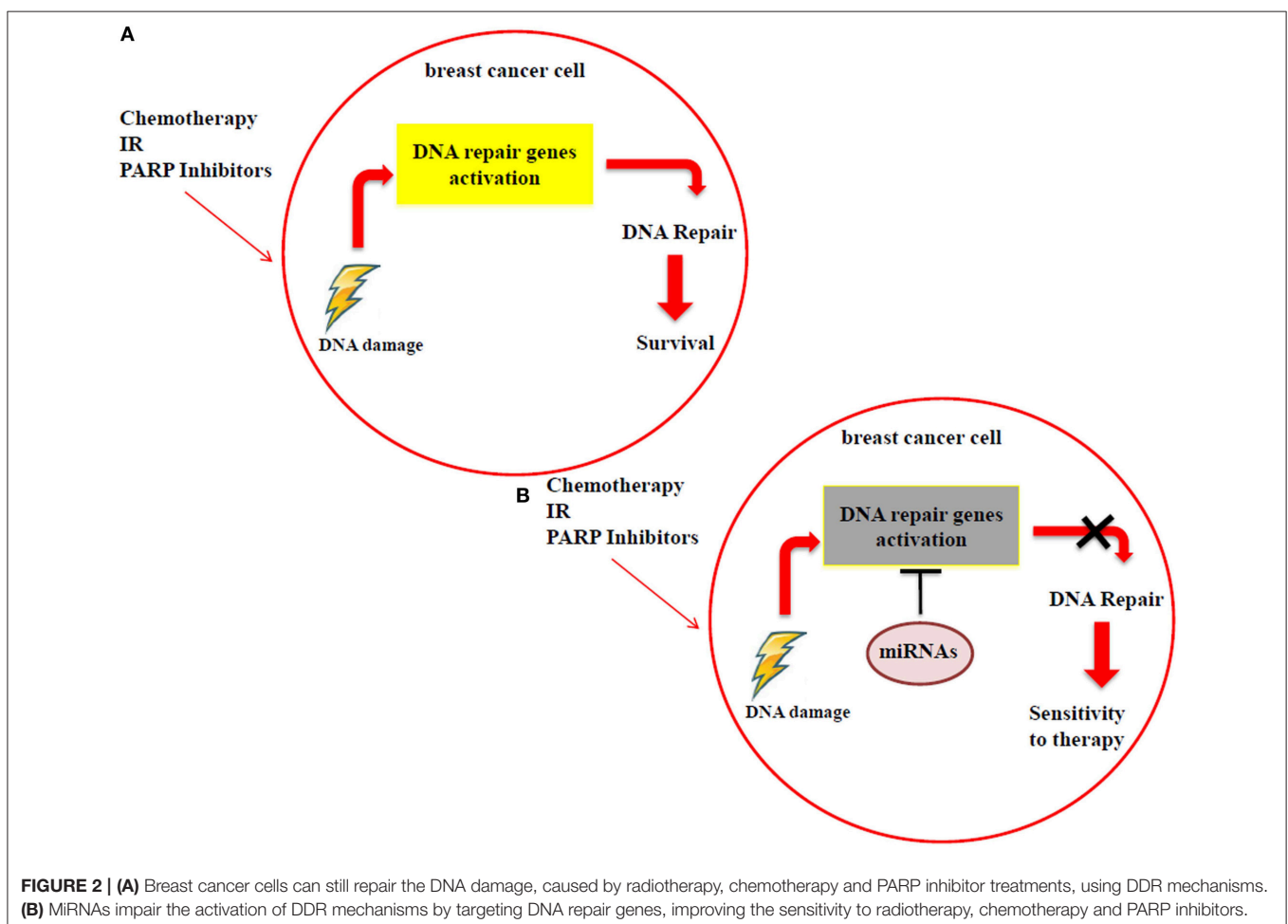
microRNA expression	Gene target	Drug response
miR-182 overexpression	BRCA1	IR and PARP inhibitors sensitivity (88)
miR-182 overexpression	CHEK2	PARP inhibitors sensitivity (89)
miR-107 and miR-122 overexpression	RAD51	PARP inhibitors sensitivity (90)
miR-103 and miR-107 overexpression	RAD51 and RAD51D	PARP inhibitors sensitivity (91)
miR-96 overexpression	RAD51 and REV1	PARP inhibitors sensitivity (85)
miR-181 overexpression	ATM and BRCA1	PARP inhibitors sensitivity (92)
miR-21 overexpression	MSH2	PARP inhibitors sensitivity (92)
miR-664b-5p overexpression	CCNE2	PARP inhibitors and chemo-sensitivity (93)
miR-151-5p overexpression	SMARCA5	PARP inhibitors sensitivity (94)

repair capabilities, by down-regulation of BRCA1 in TNBC cells (87). **Table 1** summarizes miRNAs involved in the chemo- and radio-responsiveness, through the regulation of DNA repair genes, in breast cancer.

MICRORNAs REGULATE DNA REPAIR GENES AND PARP INHIBITOR RESPONSE

As reported above, PARP inhibitors represent one of the most innovative approaches in the development of anti-breast cancer therapies. However, whether miRNAs could influence sensitivity to PARP inhibitors has not been deeply investigated yet. Indeed, few reports have described the role of miRNAs in the modulation of PARP inhibitor response. Here, we report the main results about this regulation in breast cancer. In 2011, Moskwa et al. have demonstrated that breast cancer cells overexpressing miR-182 are more sensitive to IR and PARP inhibitors via BRCA1 targeting and impairment of DNA repair. These results were also confirmed in *in vivo* models; mice injected with breast cancer cells overexpressing miR-182 showed a reduced tumor growth when treated with PARP inhibitor olaparib (88). Moreover, it has been demonstrated that CHEK2, another gene involved in the

HR, is a direct target of miR-182-5p. This regulation enhances the sensitivity to a PARP inhibitor in breast cancer cells (89). As reported by Neijenhuis et al. and Huang et al. miR-107, miR-222 and miR-103 regulate the DDR and sensitize tumor cells to PARP inhibitors in breast cancer cell lines, targeting RAD51 and impairing HR (90, 91). It is also known that miR-96 targets RAD51 and REV1, and that the overexpression of this miRNA in breast cancer in *in vitro* models results in improved sensitivity to PARP inhibitors (85). In this context, it has also been demonstrated that TGF β regulates DNA repair genes and responsiveness to PARP inhibitors. Liu et al. have shown that two TGF β -targeted DNA-repair genes, ATM and BRCA1, both regulated by miR-181, and MSH2 targeted by miR-21, contribute to TGF β -induced sensitivity to PARP inhibition (92). More recently, the role of miR-664b-5p has been investigated. This miRNA is a tumor suppressor and results upregulated upon PARP inhibitor plus chemotherapy treatments. Thus miR-664b-5p has an important role in the regulation of PARP inhibitors to increase chemosensitivity by targeting CCNE2 in BRCA1 not-mutated TNBC (93). Furthermore, an interesting mechanism involving miR-151-5p and its target SMARCA5, an ISWI family member with an important role in DSB repair (94), has been proposed. Indeed, Tommasi et al. reported the possibility of



considering the overexpression of PARP1 and miR-151-5p as predictive biomarkers, useful to correctly select sporadic breast cancers for treatment with PARP inhibitors.

Table 2 summarizes the miRNAs involved in the PARP inhibitor response, through the regulation of DNA repair genes, in breast cancer.

CONCLUSION

Many studies have reported that miRNA modulation in breast cancer, by using *in vitro* and *in vivo* models, can be exploited to achieve a higher response to the DNA-damaging drugs, as radiotherapy, chemotherapy and PARP inhibitors. These small RNAs have the ability to directly target DNA repair genes, thus resulting in the impairment of DNA repair mechanisms. When breast cancer cells are treated with radiotherapy, chemotherapy or PARP inhibitors, the resulting DNA damage could be still repaired through the activation of specific DNA repair genes, such as ATM, BRCA1/2, RAD51, DNA-PK etc., thus cells survive and continue to proliferate (**Figure 2A**). Specific miRNAs, targeting DNA repair genes, are able to impair the mechanisms involved in repairing the DNA damage and to promote a higher sensitivity to the treatments in breast cancer cells (**Figure 2B**). For this reason, miRNAs could be exploited as predictive biomarkers and therapeutic tools to increase the response to DNA-damaging drugs. Considering PARP inhibitors, they are currently not in clinical practice for breast cancer and the inclusion criteria to treat patients using these drugs in clinical trials are that tumor cells are BRCA 1/2 mutated. We can speculate that the miRNAs involved in the regulation of

DNA repair genes could represent a novel strategy to mimic the BRCAness phenotype, making tumor cells BRCA1/2 wild type more responsive to PARP inhibitors. However, in this context it is relevant to underline that currently the feasibility of a miRNA-based therapy in clinics has not been demonstrated yet, either alone or in combination with standard therapies. Technical and practical issues still need to be solved, such as the toxicity, off-target effects and systemic delivery. Indeed, for the clinical practice it will be necessary to identify the best strategy to deliver miRNAs directly to the tumor, i.e., by conjugation with antibodies or specific nanoparticles, thus avoiding unwanted off-target effects in healthy cells.

AUTHOR CONTRIBUTIONS

IP, GC, and AC wrote and edited the manuscript. IP revised the new strategies of breast cancer treatment. GC revised the breast cancer and microRNAs sections. AC revised DNA damage section and the recent findings about microRNAs and DNA-damaging drug responsiveness.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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