Resistance May Not Be Futile: microRNA Biomarkers for Chemoresistance and Potential Therapeutics

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Abstract
Chemoresistance to many commercially available cancer therapeutic drugs is a common occurrence and contributes to cancer mortality as it often leads to disease progression. There have been a number of studies evaluating the mechanisms of resistance and the biological factors involved. microRNAs have recently been identified as playing a role in the regulation of key genes implicated as cancer therapeutic targets or in mechanisms of chemoresistance including EGFR, MDR1, PTEN, Bak1, and PDCD4 among others. This article briefly reviews chemoresistance mechanisms, discusses how microRNAs can play a role in those mechanisms, and summarizes current research involving microRNAs as both regulators of key target genes for chemoresistance and biomarkers for treatment response. It is clear from the accumulating literature that microRNAs can play an important role in chemoresistance and hold much promise for the development of targeted therapies and personalized medicine. This review brings together much of this new research as a starting point for identifying key areas of interest and potentials for future study.

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Background
Chemoresistance: defining the problem
Across the globe today, one of the leading causes of death is cancer, and it is the second most common cause of death in the United States (1). Although progress has been made in the field of cancer treatment, no method is 100% effective for success. One major obstacle in cancer treatment is resistance of cancer cells to anticancer drug therapies. In fact, drug resistance is currently the most common factor in tumor recurrence (2). Resistance to therapy can be classified by 2 categories: intrinsic and acquired. Intrinsic resistance implies that prior to receiving the intended therapy, there already exists (de novo) factors that would make the intended therapy ineffective. Acquired resistance develops during the course of treatment, in which tumors that are not initially resistant to a particular drug develop resistance, often quickly. This is because of prevailing selection and overgrowth of drug-resistant variants in the tumor cells (3, 4), resulting in the futility of that treatment. Although correctly selected targeted treatments may improve tumor response rates, the majority of patients will eventually develop progressive disease even if they initially showed response to treatment (5).

Employing methods that tailor therapy to an individual’s cancer to improve clinical benefit—so-called personalized medicine—is certainly warranted. Deciphering the mechanisms involved in chemoresistance is critical to improve understanding of these complex pathways and to develop more effective targeted treatments. On development of chemoresistance, the alternative treatment used is another systemic therapy that is Food and Drug Administration–approved for that cancer, is an investigational agent under the umbrella of a clinical trial, or is an off-label use of a prescription drug. There is a burgeoning field of research to identify biomarkers that will either guide selection of these alternate treatments by predicting the potential causes of acquired resistance or better classify the disease to improve primary treatment options and circumvent intrinsic resistance.

A brief overview of the mechanisms of resistance
There are a number of mechanisms known to be involved in cancer drug resistance. These include genetic changes and variability, increased expression of target proteins, alteration of drug target, failure of the drug to reach or enter the target cell, ejection of the drug from the cell, drug inactivation, increased repair of DNA damage, reduced apoptosis, altered metabolism of the drug, random drug-induced mutational events, drug-induced mutational alterations of gene function, and drug-induced karyotypic changes (reviewed in detail in refs. 3, 6, and 7). See Fig. 1 for a summary of the general mechanisms of chemoresistance.

Chemoresistance is most often exhibited across a diverse panel of structurally and functionally unrelated drugs, known as multidrug resistance (MDR; reviewed in
Overcoming chemoresistance where MDR is involved can be a challenge as rational treatment options greatly decrease. The key mechanisms responsible for MDR in tumors can be categorized into nonclassical MDR and transport-based classical MDR (8). The nonclassical phenotype results from (a) altered activity of enzyme systems that decrease the cytotoxic activity of drugs, such as glutathione S-transferase and topoisomerase, or (b) reduced chemosensitivity through changes in the balance of proteins that control apoptosis (reviewed in detail in ref. 8). The classical phenotype involves either (a) decreased activity in the cellular uptake of the drug or (b) ejection of the drug from the cancer cells, reducing the drug’s ability to kill the cancer cells (5). Three proteins commonly known for their role in classical MDR are P-glycoprotein (P-gp; from MDR1 and ABCB1 genes), MDR-associated protein (MRP1; from ABCC1 gene), and breast cancer-resistant protein (BCRP; from ABCG2 gene; ref. 5). All 3 of these proteins contain hydrophobic compounds that are similar in their chemistry, and thus overlap in the substrate specificities that facilitate drug transport across the cell membrane (5).

In addition to the above mechanisms for MDR, there are clinical studies that connect expression or polymorphisms of certain genes with response to chemotherapy drugs. These include (a) high expression of the MDR1 and ERCC1 genes in association with reduced response to cisplatin therapy in advanced bladder cancer (9); (b) high expression of ERCC1, LRP, and MRP1 genes in non-small cell lung carcinoma (NSCLC) patients predicting response to cisplatin-based therapies (10); (c) EGFR, ERCC1, MDR1, XRCC1, XRCC3, XPA, and XPD gene polymorphisms as predictive biomarkers for treatment response to epidermal growth factor receptor (EGFR) kinase inhibitors and platinum-based therapies in NSCLC patients (11, 12); (d) low levels of dehydropyrimidine dehydrogenase and thymidylate synthase mRNA expression in colorectal and gastric cancers predicting sensitivity to 5-fluorouracil (13, 14); (e) genotyping of CYP2D6 in breast cancer patients to identify those most likely to respond well to adjuvant tamoxifen therapy (15). Thus, there are a number of mechanisms for drug resistance. In all cases, discovering ways to better define drug resistance is the first step toward designing methods to circumvent resistance and offer more viable treatment options for patients.

miRNA and Chemoresistance

miRNAs Defined and Their Involvement in Chemoresistance

To date there are a number of research findings, both laboratory and clinic based, that have reported the implications of miRNAs (miRNA) in chemoresistance. Although their involvement is apparent, the study of the specific pathways they are involved in and the mechanisms they help regulate is just beginning.

miRNAs are short (~22 nucleotide), non–protein-coding RNAs that are known to alter gene expression at a posttranscriptional level (16, 17). More than 1,200
valuated human miRNAs have been identified to date
(www.mirbase.org), and they are predicted to regulate
one third of the human genome with involvement
in development and progression of many diseases
(18–20). Recent studies have uncovered broad implica-
tions of miRNAs, demonstrating that a single miRNA
can impact hundreds of targets (21) and also that a
single target can be affected by multiple miRNAs
(22). The result is that miRNAs can affect many cellular
pathways; most notably those pathways controlling
developmental and oncogenic processes (23, 24).
Approximately half of the genes known to be regulated
by miRNAs are located in cancer-associated genomic
regions or fragile genomic sites (25). In addition, muta-
tion or aberrant expression of many miRNAs has been
found in cancer patients, leading to the study of
miRNAs as regulators of oncogenes and tumor sup-
pressor genes (26, 27). Defects in miRNA processing
have also been shown to enhance tumorigenesis (28),
supporting the hypothesis that miRNAs may truly be
posttranscriptional regulators of oncogenes.

Review of the regulatory function of microRNAs

The effector mechanism for the miRNA pathway is the
RNA-induced silencing complex (RISC), a ribonucleoprotein
complex that contains mature miRNAs and Argonaute
proteins (29, 30). The RISC complex mediates posttrans-
criptional gene silencing by targeting mRNAs (16). RISC
assembly and processing of miRNA in the cytoplasm is
mediated by the RISC-loading complex. The RISC complex
is guided to its target miRNA through complementary base
pairing with the single-stranded miRNA included in the
complex. Once bound to the complex, the miRNA is
degraded, affecting protein transcription and ultimately
silencing the target gene. See Fig. 2 for a diagram of the
RISC-mediated miRNA pathway.
The Era of Targeted Therapy: Promise, Resistance, and microRNAs That May Bridge the Gap

Research over the past several years has narrowed the focus of targeted therapy to the specific biological condition of a patient's tumor that provides the optimum treatment for that tumor's individual genetic profile. With equal interest, specific pathways have been studied to determine whether/how they integrate into the era of targeted treatments and personalized medicine. Although targeted treatment has improved the benefit to the patient, resistance to therapy still ultimately develops in most patients (5). In response to the need to overcome this resistance to chemotherapy drugs, much new research is focused on mechanisms of resistance, and ways to circumvent it. This section will lay the groundwork for the involvement of miRNAs in the chemoresistance story by looking at a few of the target genes and relevant pathways that are known to be involved in predicting patients at risk for resistance to certain chemotherapy drugs, and that are also suspected targets of specific miRNAs.

Genes affected by a miRNA pathway are often involved in cellular response to cancer drugs (6), including changes in drug transport, apoptosis, and cell death. Specific examples of miRNAs implicated in chemoresistance are discussed below. The findings in all of these examples are the beginning to a vast field of possibilities that require further clinical validation, using miRNAs first as biomarkers to predict drug resistance to better direct patient treatment, and second as possible drug targets to reverse resistance once it has developed in a tumor. As enumerated in Table 1, a number of studies have shown that aberrant miRNA expression can be involved in anticancer drug resistance. Mechanisms of drug resistance are often tied to changes in relevant proteins such as PTEN, PDCD4, P-gp, and MDR1. Protein changes can, in turn, be directly related to the mutation, aberrant expression, or displaced processing of a miRNA-coding gene, affecting the miRNA of interest and resulting in altered function of the target mRNA, which affects expression of the target proteins and fundamentally silences the target gene (6). Refer to Fig. 1 for a summary of the miRNA coding pathway and the subsequent affect on target genes.

miRNA and Chemoresistance

As the medical field has become accustomed to specific genes pointing the way to the most useful therapy, or directing the discovery of new therapies, miRNAs are also growing in the same application. The discovery of biological molecules—biomarkers—as determinants for optimal treatment course, treatment outcome, and reduction of health care costs (by only offering treatments if they are shown to be efficacious) is a growing area of interest in the medical research field (see detailed review on this topic in ref. 31). miRNAs have, thus far, shown much promise as credible biomarkers that are relatively easy to collect and measure (32), and current research has been building to support the hypothesis that over- or underexpression of a certain miRNA can be directly tied to a patient's response to chemotherapeutic agents. These short nucleotide strands are more stable than mRNA and even some proteins, and can thus be measured from formalin-fixed paraffin-embedded samples and bodily fluids (e.g., blood and sputum samples), the latter allowing for less invasive sample acquisition and analysis (32). miRNAs circulating in the body are also expected to provide higher levels of specificity and sensitivity over other presently measured biomarkers (32). Discussed below are some of the current findings that relate miRNA expression levels and their implications in chemoresistance.

Ranade et al. (33) recently reported increased tumor expression of miR-92a-2*, -147, and -574-5p as potential biomarkers predictive of de novo chemoresistance in patients with small cell lung cancer (SCLC). If validated in independent clinical sample sets, these miRNA biomarkers may assist in treatment stratification for SCLC clinical trials. A number of miRNAs have also been shown to be differentially expressed in docetaxel-resistant NSCLC, specifically increased levels of miR-98, -192, -424, and decreased levels of miR-194, -200b, -212 (2). Further validation of these miRNAs is warranted, and in some cases is currently in progress, to verify these miRNAs as biomarkers that confirm resistance in lung cancer treatment.

In two studies by Song et al. (34, 35), miR-140 and -215 were found to be overexpressed in drug-resistant colorectal cancer cell lines. On the basis of their findings, these miRNAs correlated with resistance to the anticancer drugs methotrexate, 5-fluorouracil, and tomudex. In addition, an analysis of possible protein targets for these miRNAs pointed to HDAC4 and DHFR, respectively (34, 35).

Ovarian cancer is also increasing its portfolio of miRNAs implicated as chemoresistance biomarkers. miRNAs that have been found, to date, to confer resistance when aberrantly expressed in ovarian cancer include miR-30c, -130a, -335 (36), -214, and -let-7i (2). Further, miR-30c, -130a, and -335 have been shown to have an effect on M-CSF (36), whereas miR-214 has been shown to affect PTEN (2). PTEN is a known tumor suppressor gene, and downregulation of PTEN can thus lead to unchecked cell growth (discussed in more detail later in this article).

As the examples above show, the field of miRNAs as predictors for chemoresistance is burgeoning. There are additional miRNAs implicated as chemoresistance biomarkers reported in acute lymphoblastic leukemia (ALL) and other cancers including pancreatic, gastric, cervical, and prostate. See Table 1 for a complete list of miRNAs that have been shown to increase resistance to specific chemotherapy drugs when aberrantly expressed compared to normal controls.

Although the concept of using miRNAs as biomarkers to predict treatment response in patients still clearly...
Table 1. Summary of miRNAs involved in chemoresistance: their intracellular levels, target proteins that they are known to regulate (where applicable), and target drug that is ultimately affected.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>miRNA intracellular levels</th>
<th>Protein target</th>
<th>Drug</th>
<th>Biomarker (B) or confirmed involvement in resistance (R)</th>
<th>Sample source</th>
<th>Cancer type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10a, -146a, -200b, -200c, -221/222, -345</td>
<td>Differentially expressed</td>
<td>MDR1 (for -345)</td>
<td>Cisplatin</td>
<td>B</td>
<td>in vitro</td>
<td>Breast</td>
<td>(52)</td>
</tr>
<tr>
<td>-15b, -16</td>
<td>Decreased</td>
<td>BCL-2</td>
<td>Multiple</td>
<td>B</td>
<td>in vitro</td>
<td>Gastric</td>
<td>(2)</td>
</tr>
<tr>
<td>-21</td>
<td>Increased</td>
<td>N/A</td>
<td>Gemcitabine</td>
<td>B</td>
<td>in vitro and Human sample</td>
<td>Pancreatic</td>
<td>(50)</td>
</tr>
<tr>
<td>-21</td>
<td>Increased</td>
<td>PTEN</td>
<td>Trastuzumab</td>
<td>R</td>
<td>in vitro</td>
<td>Breast</td>
<td>(46)</td>
</tr>
<tr>
<td>-21</td>
<td>Increased</td>
<td>PDCD4</td>
<td>Multiple</td>
<td>R</td>
<td>in vitro</td>
<td>Breast</td>
<td>(45)</td>
</tr>
<tr>
<td>-21</td>
<td>Increased</td>
<td>PTEN</td>
<td>N/A</td>
<td>B</td>
<td>in vitro and Human sample</td>
<td>Lung (NSCLC)</td>
<td>(51)</td>
</tr>
<tr>
<td>-21, -23b</td>
<td>Increased</td>
<td>TKR</td>
<td>Erlotinib, Sunitinib, Vandetanib</td>
<td>R</td>
<td>in vitro</td>
<td>Lung (NSCLC)</td>
<td>(42, 43)</td>
</tr>
<tr>
<td>-21, -106a, -206, -214</td>
<td>Increased</td>
<td>PTEN, RB1, Er (respectively)</td>
<td>Docetaxel</td>
<td>R</td>
<td>in vitro</td>
<td>Breast</td>
<td>(44)</td>
</tr>
<tr>
<td>-21, -200b</td>
<td>Increased</td>
<td>PTEN/P1-3K p85a, PTPN12/Src (respectively)</td>
<td>Gemcitabine</td>
<td>B</td>
<td>in vitro, in vivo, and Human sample</td>
<td>Cholangiocarcinoma</td>
<td>(53)</td>
</tr>
<tr>
<td>-27a, -130a, -214, -451, let-7</td>
<td>Differentially expressed</td>
<td>N/A</td>
<td>Multiple</td>
<td>B</td>
<td>in vitro and Human sample</td>
<td>Ovarian</td>
<td>(54)</td>
</tr>
<tr>
<td>-30c, -130a, -335</td>
<td>Decreased</td>
<td>M-CSF</td>
<td>Paclitaxel/cisplatin</td>
<td>B</td>
<td>in vitro</td>
<td>Ovarian</td>
<td>(2)</td>
</tr>
<tr>
<td>-92a-2*, -147, -574-5p</td>
<td>Increased</td>
<td>N/A</td>
<td>Platinum</td>
<td>R</td>
<td>in vitro</td>
<td>Lung (SCLC)</td>
<td>(33)</td>
</tr>
<tr>
<td>-98, -192, -424</td>
<td>Increased</td>
<td>N/A</td>
<td>Docetaxel</td>
<td>B</td>
<td>in vitro</td>
<td>Lung (NSCLC)</td>
<td>(2)</td>
</tr>
<tr>
<td>-101</td>
<td>Increased</td>
<td>Magi-2/AKT</td>
<td>Tamoxifen</td>
<td>R</td>
<td>in vitro</td>
<td>Breast</td>
<td>(47)</td>
</tr>
<tr>
<td>-125b</td>
<td>Increased</td>
<td>Bak1 (in breast)</td>
<td>Paclitaxel</td>
<td>B</td>
<td>in vitro</td>
<td>Breast and others</td>
<td>(49)</td>
</tr>
<tr>
<td>-128a</td>
<td>Increased</td>
<td>TGFbetaRI</td>
<td>Letrozole</td>
<td>B</td>
<td>in vitro</td>
<td>Breast</td>
<td>(55)</td>
</tr>
<tr>
<td>-128b, -221</td>
<td>Decreased</td>
<td>MLL, AF4, MLL-AF4, AF4-MLL, and CDKN1B</td>
<td>Glucocorticoid</td>
<td>B</td>
<td>in vitro</td>
<td>ALL</td>
<td>(2)</td>
</tr>
</tbody>
</table>

(Continued on the following page)
### Table 1. Summary of miRNAs involved in chemoresistance: their intracellular levels, target proteins that they are known to regulate (where applicable), and target drug that is ultimately affected (Cont’d)

<table>
<thead>
<tr>
<th>miRNA</th>
<th>miRNA intracellular levels</th>
<th>Protein target</th>
<th>Drug</th>
<th>Biomarker (B) or confirmed involvement in resistance (R)</th>
<th>Sample source</th>
<th>Cancer type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>-140</td>
<td>Increased</td>
<td>HDAC4</td>
<td>Methotrexate, 5-fluorouracil</td>
<td>B</td>
<td>in vitro</td>
<td>Colorectal</td>
<td>(34)</td>
</tr>
<tr>
<td>-143</td>
<td>Decreased</td>
<td>PK5,Bcl-2</td>
<td>5-fluorouracil</td>
<td>R</td>
<td>in vitro</td>
<td>Colorectal</td>
<td>(56)</td>
</tr>
<tr>
<td>-148a</td>
<td>Increased</td>
<td>MSK1</td>
<td>Paclitaxel</td>
<td>B</td>
<td>in vitro</td>
<td>Prostate</td>
<td>(57)</td>
</tr>
<tr>
<td>-155</td>
<td>Increased</td>
<td>FOXO3a</td>
<td>Multiple</td>
<td>B</td>
<td>in vitro and Human sample</td>
<td>Breast</td>
<td>(58)</td>
</tr>
<tr>
<td>-181a</td>
<td>Decreased</td>
<td>Multiple, including TGF-beta, Wnt, MAPK, VEGF, and mTOR</td>
<td>Fulvestrant</td>
<td>B</td>
<td>in vitro</td>
<td>Multiple</td>
<td>(59)</td>
</tr>
<tr>
<td>-193b</td>
<td>Increased</td>
<td>N/A</td>
<td>Cisplatin/oxaliplatin</td>
<td>B</td>
<td>in vitro</td>
<td>Nasopharyngeal, gastric</td>
<td>(60)</td>
</tr>
<tr>
<td>-194</td>
<td>Decreased</td>
<td>N/A</td>
<td>Docetaxel</td>
<td>B</td>
<td>in vitro</td>
<td>Lung (NSCLC)</td>
<td>(2)</td>
</tr>
<tr>
<td>-214</td>
<td>Increased</td>
<td>PTEN/AKT</td>
<td>Cisplatin</td>
<td>B</td>
<td>in vitro</td>
<td>Ovarian</td>
<td>(2)</td>
</tr>
<tr>
<td>-221, -222</td>
<td>Increased</td>
<td>DHFR/T5</td>
<td>Methotrexate, raltrexed</td>
<td>B</td>
<td>in vitro</td>
<td>Colorectal</td>
<td>(35)</td>
</tr>
<tr>
<td>-221, -222</td>
<td>Increased</td>
<td>KiT, p27kip1</td>
<td>TRAIL</td>
<td>B</td>
<td>in vitro</td>
<td>Lung (NSCLC), liver</td>
<td>(62)</td>
</tr>
<tr>
<td>-424</td>
<td>Decreased</td>
<td>ErbB</td>
<td>Fulvestrant</td>
<td>B</td>
<td>in vitro</td>
<td>Multiple</td>
<td>(59)</td>
</tr>
<tr>
<td>-449a/b</td>
<td>Decreased</td>
<td>N/A</td>
<td>Erlotinib, vandetanib</td>
<td>R</td>
<td>in vitro</td>
<td>Lung (NSCLC)</td>
<td>(42)</td>
</tr>
<tr>
<td>-451</td>
<td>Decreased</td>
<td>N/A</td>
<td>Tamoxifen</td>
<td>B</td>
<td>in vitro</td>
<td>Breast</td>
<td>(48)</td>
</tr>
<tr>
<td>-let-7i</td>
<td>Decreased</td>
<td>N/A</td>
<td>P-glycoprotein (MDR1 gene)</td>
<td>R</td>
<td>in vitro</td>
<td>Human sample</td>
<td>(44)</td>
</tr>
</tbody>
</table>

NOTE: Confirmed chemoresistance implies that the miRNA has been confirmed to increase IC_{50} values toward drug resistance in cell lines or increase resistance to the therapy in an in vivo model.

Abbreviations: ALL, acute lymphoblastic leukemia; N/A, not available; NSCLC, Non-small-cell lung cancer; SCLC, Small-cell lung cancer; TS, thymidylate synthase.
needs further study including use of high-quality samples and validation in independent cohorts, miRNAs may be more promising as biomarkers than earlier reported biomarker possibilities. The current stage of research is laying the foundation for future adoption and adaptation that will allow for use of in vivo miRNA levels as predictive biomarkers. If the patient presents with a miRNA biomarker that is predictive of de novo resistance, alternative methods could be explored that are more likely to result in a beneficial response—this is the application of personalized medicine.

**MicroRNA Pathway Targets for Chemoresistance**

**EGFR and miR-21, -23b, and -424**

EGFR is a tyrosine kinase receptor in the ErB/HER family, and its activation signals downstream pathways that regulate cell proliferation, differentiation, and survival. Abnormal expression of EGFR can thus lead to development of tumors, and it has also been shown to be a prominent indicator of treatment response in some NSCLC patients (37). One common effective class of agents against aberrant expression of EGFR is the EGFR tyrosine kinase inhibitor (TKI; refs. 37, 38). TKIs can be dramatically effective for advanced NSCLC patients whose tumor harbors an EGFR mutation associated with drug sensitivity (e.g., G719X, exon 19 deletion, and L858R): 70% will experience tumor regression when initiated (37), but acquired resistance, attributed largely to an increased selection in tumor cells for TKI-resistant EGFR mutations, is quite common (37, 38).

V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) is an oncogene that encodes for the protein KRAS, which is involved in regulating cell division. Mutations of KRAS (found in 35%–40% of metastatic colorectal cancer and ~10%–20% of NSCLC) are involved in drug resistance that decreases the effectiveness of both EGFR monoclonal antibodies (e.g., cetuximab and panitumumab) and EGFR-TKIs (e.g., erlotinib and gefitinib; refs. 39, 40). KRAS mutations result in activation of the Ras/Raf/MEK/ERK pathway with the loss of EGFR signaling control, rendering EGFR inhibitors ineffective. More studies are evaluating how to circumvent this resistance mechanism, including combinatorial approaches involving TKIs and new inhibitors such as MEK, Braf, Hsp90, and mTOR (41).

Some preliminary studies in NSCLC cell lines, exploring miRNA biomarkers associated with TKI resistance in vitro, reported that increased levels of both miR-21 and miR-23b were indicative of increased sunitinib resistance in NSCLC cell lines (42, 43) whereas decreased miR-424 levels were indicative of increased resistance to both erlotinib and vandetanib (42). As with most of these newly discovered biomarkers, more research is currently under way to validate these miRNAs, translate the findings to an in vitro study, and explore ways to apply any validated findings as legitimate tools for predicting treatment response.

**MDR1 and miR-451**

Activation of the MDR1/ABCG1 gene is a commonly known pathway for chemoresistance and results in the overexpression of P-gp, a multidrug transporter glycoprotein that belongs to the ATP-binding cassette superfamily (7). When P-gp is overexpressed in cells, they become resistant to a variety of structurally and functionally diverse chemotherapy drugs (7).

In a study of MCF-7/DOX, doxorubicin-resistant breast cancer cells, miR-451 was found to be decreased in expression in the resistant cell line compared with control cells that were not doxorubicin-resistant (44). Further, the study showed that miR-451 regulates the MDR1 showed gene. Increasing the cellular levels of miR-451 decreased MDR1 expression, and more importantly, increased cell sensitivity to doxorubicin (44). This resulted in reversal of doxorubicin resistance observed in the presence of MDR1 expression and helped to circumvent the MDR1 resistance pathway.

**PTEN and miR-21, -101, and -449a/b**

PTEN is a tumor suppressor gene that is known to regulate apoptosis and cell invasion (45). In a recent preliminary report by Sumaiyah et al. (46), upregulation of miR-21 in breast cancer cells correlates with decreased expression of PTEN and an increased resistance to the HER2 monoclonal antibody trastuzumab. PTEN loss had been previously conferred with trastuzumab resistance in HER2 overexpressing breast cancer. After identifying miR-21 as a player in this pathway, miR-21 levels were measured in patients with HER2-positive breast cancer (46). miR-21 was found to be significantly increased in patients who showed poor response to trastuzumab treatment and disease progression (46), tying miR-21 into the story of decreased PTEN expression leading to trastuzumab resistance. Further research is warranted to determine whether miR-21 can be used clinically as a biomarker for trastuzumab resistance.

Another suppressor mechanism for PTEN, leading to tamoxifen resistance in breast cancer, has been discovered through Magi-2, a scaffold protein that activates PTEN through phosphorylation (47). Tamoxifen is a widely known anticancer drug used in treatment of breast cancer. An estrogen antagonist that vies with estrogen for binding at estrogen receptor (ER) sites, it inhibits gene transcription and tumor growth that is dependent on estrogen. Resistance to this form of therapy is often seen in tumor cells that become estrogen independent, and are thus also uninhibited by tamoxifen (48). Two preliminary studies recently identified miRNAs that seem to be involved in tamoxifen resistance. The first study examined the role miRNAs play in estrogen-independent growth of breast cancer cells. The authors report that miR-101 transfected into MCF-7 cells was able to promote estrogen-independent growth, which also led to tamoxifen resistance (47). Other miRNAs are known to confer tamoxifen resistance work by targeting ER, but miR-101 had no affect on ER
expression or activity (47), begging the question of what pathway or mechanism was affected by miR-101. The answer is that miR-101 activated AKT through suppression of Magi-2 (47), identifying a new pathway that is a possible therapeutic target for overcoming tamoxifen resistance, as well as a potential biomarker (miR-101) to identify breast cancer patients who may be intrinsically resistant to tamoxifen treatment. The second study reported that miR-449a and miR-449b were decreased in resistant breast cancer cell lines (48). The authors examined frozen breast tumor tissues and noted an inverse correlation with miR-449a/b and tumor grade, suggesting that these miRNAs may be involved in tamoxifen resistance in breast cancer.

As mentioned earlier, miR-21 has been implicated in several different cancer activation pathways and lung cancer is proving to be no exception. miR-21 has been shown to repress the tumor suppressor PTEN in NSCLC, affecting cell growth and invasion (2). Elevated miR-21 levels have been shown to stimulate growth and invasion in cancer cells, making it a plausible target for future therapies.

**Bak1 and miR-125b**

Another common drug used to treat breast (and other) cancers is paclitaxel. Zhou et al. (49) recently reported miR-125b to be upregulated in paclitaxel-resistant tumor cells, demonstrating that it causes inhibition of cytotoxicity and apoptosis, both of which are induced by the drug. On further exploration, they also found that miR-125b suppresses Bak1 in breast cancer cells (49), identifying Bak1’s potential involvement in the mechanism of paclitaxel uptake into cells and a possible therapeutic target for treating paclitaxel resistance. As paclitaxel is used to treat multiple tumor types, this may prove to be an important finding for further studies in overcoming paclitaxel resistance in multiple tumor types.

**miR-21: More to the Story?**

As noted in Table 1, miR-21 is listed numerous times in relation to different cancers, treatments, and resistance pathways. miR-21 is also mentioned several times in the earlier discussion on pathway targets as being involved in different cancers including pancreas (50), lung (51), and breast (45). Increased miR-21 expression has been shown to be indicative of chemoresistance through 2 pathways. The first is by increased cell proliferation and decreased expression programmed cell death 4 (PDCD4). PDCD4 is a tumor suppressor protein that, when present, plays an important role in decreasing oncogenesis. Recent studies have also shown that introducing a miR-21 inhibitor successfully inhibits the targeted functions of the miRNA, increasing levels of PDCD4, and thus decreasing oncogenesis and expression of IAP (inhibitors of apoptosis proteins) and MDR1/P-gp (45). The second pathway is by repression of the tumor suppressor PTEN in NSCLC, which is involved in regulation of cell growth and invasion (51). When functioning correctly, PTEN aids in apoptosis, and in cancers, it helps keep the tumor cells from growing abnormally (45). Elevated miR-21 levels repress this critical role of PTEN and instead lead to cancer cell growth and invasion. See Fig. 3 for a summary of the resistance mechanism resulting from increased miR-21 expression.

As the list of miRNAs involved in chemoresistance is compiled, it is interesting to note certain recurring miRNAs in the literature and miR-21 is certainly one of them. It has already been shown to be significantly involved in 4 different cancers and one cannot help but wonder what other pathways or cancers this small nucleotide strand may be involved with. Additional studies exploring miR-21 involvement in chemoresistance and other cancer-related pathways is much deserved. This is just one small aspect to the miRNA story that is evolving. As research continues, hopes are high that more of these supertargets that tie in to multiple diseases and prognoses will be discovered.

**Future Directions**

The field of study with miRNAs is vast and has many areas of application. A single miRNA can regulate tens to hundreds of gene targets, and it is essential that this library of information continues to be built up for further investigation of miRNA effects on resistance mechanisms, and potential ways to reverse or alter these effects. If additional studies continue to validate specific miRNAs as biomarkers for chemoresistance, they will enable the scientific community to advance patient care through improved stratification for clinical trials (leading to new drug treatments), and through increased development of targeted treatments much the way genes have. Current cancer treatments are selected by evidence from clinical trials directed at specific cancer populations as well as patient/physician convenience for known toxicity. Although there are currently several methods (discussed earlier) used to identify patients who may be at risk for resistance, there exist few standardized reliable methods to identify cancers that may be resistant to the selected course of systemic therapy. It is imperative that the scientific community pursue research avenues to both predict and overcome chemoresistance, to improve treatment options and outcomes for patients.

There is particular promise for the future in targeting miRNAs as therapeutics to guide personalized medicine. One potential use for miRNAs is to serve as predictive biomarkers to direct therapies that are currently available on the market, and thus improve treatment response and outcomes. They also hold promise in the field of drug development to give direction for new therapies that are currently emerging on the research front, particularly for therapeutic development as miRNA mimics or antagonists.

The critical step to developing miRNAs for human use is to create stable and effective targeted delivery with
minimization of other potential and probable target and off-target effects. A phase II study is currently underway (www.clinicaltrials.gov, study no. NCT01200420). The study tests a proprietary nucleic acid (miraversen) that captures miR-122, a miRNA important for hepatitis C viral replication. Originally tested in chimpanzees, the molecule has so far shown to be effective with no evidence of resistance or side effects in the treated animals. Miraversen is the first miRNA-targeted drug therapy to go into human clinical trials. The study is expected to be completed in December 2011 and could lead to the beginning of an exciting and rapidly expanding aspect of miRNA targets as therapeutics for personalized medicine.

In summary, miRNAs hold promise as biomarkers for chemoresistance that will help guide patient treatments for optimum results with minimum toxicity to the patient, as well as for the development of therapeutic treatments that precisely and directly target the biological area of interest—the developing field of personalized medicine.

Conclusions

As the research discussed earlier has shown, miRNAs have fast become a field of interest, particularly for their discovered involvement in a number of different oncogenic pathways and the potential they bring for a deeper look into the mechanisms of chemoresistance and future therapies that can be used to circumvent this resistance. miRNAs are already showing their potential as biomarkers to predict treatment response in a number of different cancers, and continued clinical validation is needed to hone in on the most promising of these predictions. There has also been preliminary research showing the involvement of miRNAs in key pathways regulating cancer cell growth, proliferation, invasion, and so on, and this pushes them toward use as novel targets for new anticancer treatments. Again, further research is warranted to validate involvement in these and other pathways, and to continue pursuing miRNA delivery systems that can be translated to therapeutic treatments.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

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