# Chapter 19

# Use of MicroRNAs in Personalized Medicine

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## Abstract

Personalized medicine comprises the genetic information together with the phenotypic and environmental factors to yield healthcare tailored to an individual and removes the limitations of the "one-size-fits-all" therapy approach. This provides the opportunity to translate therapies from bench to clinic, to diagnose and predict disease, and to improve patient-tailored treatments based on the unique signatures of a patient's disease and further to identify novel treatment schedules.

Nowadays, tiny noncoding RNAs, called microRNAs, have captured the spotlight in molecular biology with highlights like their involvement in DNA translational control, their impression on mRNA and protein expression levels, and their ability to reprogram molecular signaling pathways in cancer. Realizing their pivotal roles in drug resistance, they emerged as diagnostic targets orchestrating drug response in individualized therapy examples.

It is not premature to think that researchers could have the US Food and Drug Administration (FDA)-approved kit-based assays for miRNA analysis in the near future. We think that miRNAs are ready for prime time.

**Key words** miRNAs, Personalized medicine, Pharmacogenomics

## 1 Introduction

Pharmacogenomics investigations have emphasized genes that contribute to an individual patient's drug sensitivity, resistance, and toxicity. It has also designated the causes of interindividual variations in the expression and function of many of the genes, including the roles of microRNAs, DNA methylation, copy number variations, and single-nucleotide polymorphisms.

In this chapter, we focus on miRNAs, 19–24-nucleotide noncoding RNAs that function as gene regulators and have roles in countless cellular processes. We discuss how miRNAs, arranging the expression of pharmacogenomic-relevant genes, play a considerable role in drug efficacy and toxicity and have potential clinical reflections for personalized medicine.

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## 2 MicroRNA Pharmacogenomics

The National Cancer Institute of the National Institutes of Health, USA, defined "personalized medicine" in 2011 as a form of healthcare that considers information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease [1]. Developments in the field of pharmacogenomics are not only due to better sequencing technology but also stem from gene expression alterations on the account of regulatory elements and epigenetic variations that occur in response to the environment. In the realm of RNA, these modulations usually result from two comprehensive components: first, the direct regulatory impact of miscellaneous forms of noncoding RNA (hnRNA, microRNA, etc.) that have the capacity to modulate expression by interacting with DNA regulatory sequences in promoters or in different regions of target genes and, second, the environmentally stimulated chemical modulation of DNA nucleotides, principally by cytosine methylation.

The application of genomics in personalized medicine includes its potential to improve risk assessment, diagnosis, prognosis, and treatment. Classical examples of current genomic investigation in personalized medicine are (1) BRCA1/2 testing for risk assessment of breast cancer, (2) gene expression profiles to diagnose breast cancer subtypes [2], (3) number of trinucleotide repeats predicting the seriousness of Fragile X syndrome [3], and (4) Herceptin® (trastuzumab) management that is restricted using a companion diagnostics to women protecting from HER2-positive breast cancer [4, 5] and CYP2C19 variants associated with minimized clopidogrel response [6].

Metabolism of xenobiotics (drugs, environmental chemicals, carcinogens) and endobiotics (steroids, bile acids, and fatty acids) are catalyzed by essential enzymes called P450s that are transcriptionally regulated by nuclear receptors.

Cytochrome P450s and nuclear receptors, such as CYP1B1, CYP2A3 (rat), CYP2E1, CYP3A4, CYP24A1, pregnane X receptor (PXR), vitamin D receptor (VDR), peroxisome proliferator-activated receptor γ (PPARγ), RXRα (rat), hepatocyte nuclear factor 4α (HNF4α), estrogen receptor α (ERα), and human glucocorticoid receptors (GR) that are regulated by miRNAs, were studied in a wide range of studies [7].

## 3 Diagnostic and Prognostic Implementations: MicroRNA Signatures in Solid Tumors

Potential links to the etiology of a disease have been obtained from sets of differentially expressed mRNAs and miRNAs (expression signatures) acquired via expression profiling of whole tumor samples (Table 1).

**Table 1**
**Diagnostic and prognostic applications of miRNA signatures in solid tumors**

| Signature in breast cancer | Clinical application(s) | References |
|---|---|---|
| miR-7, -128a, -210, -516-3p | Disease progression (distant metastasis) of estrogen receptor+ lymph node– cases | [8] |
| miR-30a-3p, -30c, -182 | Response to adjuvant tamoxifen in advanced ER+ cases | [9] |
| miR-128a, -135a, -767-3p, -769-3p | Recurrence-free survival in ER+ cases | [10] |
| miR-27b, -30c, -144, -150, -210, -342 | Recurrence-free survival in ER– cases | [10] |
| miR-21, -181a | Recurrence-free and overall survival in all-comers | [11] |
| miR-21, -210, -221, -222 | Recurrence-free and overall survival in ER–, progesterone receptor–, human EGF receptor 2– cases | [11] |
| mir-21 | Response to neoadjuvant trastuzumab treatment, recurrence-free survival in stage I/II all-comers, overall survival in all-participants | [12–14] |
| miR-205 | Disease recurrence and overall survival in ER–, progesterone receptor–, HER2– cases | [15] |
| miR-210 | Disease progression (distant metastasis) in ER– lymph node– cases, disease progression (distant metastasis) in ER– PR– HER2– LN– cases, recurrence-free and overall survival in all attendants, response to tamoxifen in ER+ cases | [8, 16, 17] |

In colorectal cancer, 18 miRNA signatures (*see* **Note 1**) are responsible for subtype classification like microsatellite stable (MSS) vs. microsatellite instable (MSI-high) [18, 19]. Additionally, miR-320 and -498 signatures are accountable for recurrence-free survival in stage II MSS cases [18].

The prevailing oncomir in colorectal cancer is miR-21 involved in diagnostic and prognostic applications. This worthy miRNA plays a key role in response to neoadjuvant chemoradiotherapy in advanced rectal cancer [20], recurrence-free survival in stage II colon cases [21], recurrence-free survival in all-comers [22], recurrence-free and overall in all-contributors [23], and overall survival in all-contributors [24].

In lung cancer, miRNA signatures are miscellaneous. There are 34 miRNA signatures (*see* **Note 2**) responsible for prediction of subtype classification into adenocarcinoma (AdCa) vs. squamous cell carcinoma (SCC) in male smokers [25]. Signatures of the miRNAs let-7a and miR-221, 137, -182, and -372 are essential for

recurrence-free and overall survival in non-small-cell lung cancer (NSCLC) cases [26].

In the literature, it was shown that nineteen miRNA signature (*see* **Note 3**) can play a major role in prediction of overall survival in SCC cases [27], and let-7e and miR-34a, -34c-5p, -25, and -191 signatures have a role in estimation of overall survival in male smoker SCC cases [25].

According to the studies concerning lung cancer let-7a has prognostic factors alone like overall survival in NSCLC [28] and AdCa cases [29], miR-21 has prognostic value in overall survival in NSCLC [30] and SCC cases [31], and miR-34a is an important marker for recurrence-free and overall survival in NSCLC cases [32]. The miRNA miR-155 has crucial role in overall survival in AdCa cases [29], and miR-205 distinguishes AdCa from SCC [33–35].

These abovementioned issues contain important data for applications of miRNA signatures especially in solid tumors, but they are just preclinical data, and further validation and understanding of what these signatures reflect are required for converting them to approved approaches.

## 4 Correlation of Drug Resistance with Deregulation of MicroRNA Expression

Aberration of miRNA expression in malignant tumor cells is considerably observed and can be triggered by three distinct mechanisms:

1. Location of miRNAs in cancer-associated tissue.
2. Genomic region or at fragile sites, epigenetic regulation of miRNA genes.
3. Abnormalities in miRNA processing genes and proteins [36].

Changes in expression levels of miRNAs affect numerous target mRNAs and for this reason multiple proteins pioneering the diversity in the chemosensitivity of cancer cells through miscellaneous cellular processes. Cellular response to anticancer agents shifted by most of the miRNAs by means of survival signaling pathways and programmed cell death response modulation [37]. Additionally, there are reports about miRNAs affecting mechanisms such as drug targets and DNA repair systems [38, 39]. Ultimately, miRNAs also have duties in the regulation of drug metabolism by regulating the expression of drug-metabolizing enzymes and drug transporters [40].

Numerous studies have shown the effect of specific microRNAs on factors encompassed in apoptosis and survival pathways impressing anticancer drug sensitivity and resistance (Table 2).

**Table 2**
**Some important miRNAs and drug resistance/effect**

| Expression change | miRNA | Resistance/ sensitivity | Drug | Target gene | Cancer or cell line | Reference |
|---|---|---|---|---|---|---|
| Upregulation of expression | Let-7a | Resistance | IFNγ<br>Doxorobicin<br>Paclitaxel | Caspase-3 | Hepatocellular carcinoma<br>Human squamous | [41] |
| Overexpression | Let-7a/b | Radio-sensitization | - | RAS | Lung cancer | [42] |
| Overexpression | Let-7g | Resistance | - | RAS | Lung cancer | [42] |
| Increased expression | miR-1 | Sensitivity | Doxorobicin | Mcl-1 | NSCLC cells | [43] |
| Overexpression | miR-15b/16 | Sensitivity | Several drugs | Bcl-2 | Gastric cancer | [44] |
| Increased expression | miR-27a | Resistance | Paclitaxel | HIPK2 | Ovarian cancer | [45] |
| Overexpression | miR-27a/451 | Resistance | Vinblastine Doxorubicin | MDR1 | Ovarian cancer Cervical cancer | [46] |

Examples could be multiplied according to recent studies in literature due to the loss of, increased, or decreased expressions of 22 specific miRNAs (*see* **Note 4**) targeting genes (*see* **Note 4**) involved in apoptosis and survival pathways affecting anticancer drug sensitivity in various cancer types including breast, cervical, prostate, colorectal, colon, cholangiocarcinoma, T-cell leukemia, and osteosarcoma [7].

The mRNA, miR-21, is important in chemoresistance. Increased expression of miR-21 prevents gemcitabine-induced apoptosis targeting PTEN in cholangiocarcinoma [47] and unknown molecules in pancreatic cancer [48]. Overexpression of miR-21 triggers increased resistance to VM-26 in glioblastoma [49] and topotecan in breast cancer [50] by targeting LRRFIP1 and Bcl-2, respectively. Contrariwise, the expression inhibition of miR-21 increased the sensitivity to TRAIL-induced apoptosis in glioma [51]. Interestingly, it increased the expression protected against temozolomide-induced apoptosis via targeting Bax in glioblastoma [52] and arsenic trioxide-induced apoptosis by targeting PDCD4 in chronic myeloid leukemia [53] and also prevented apoptosis induced by arabinosylcytosine by targeting PDCD4 in acute myeloid leukemia [54].

**Table 3**
**MicroRNAs and drug relationships**

| miRNA | Change | Target gene | Cancer or cell line | Result | Reference |
|---|---|---|---|---|---|
| miR-24 | C to T | DHFR 3′UTR | DG44 cells | Resistance to methotrexate | [39] |
| miR-206b | C to T | ER-alpha | Breast cancer | Sensitivity to endocrine therapy | [58] |
| miR-519c | Deletion | ABCG2 3′UTR | Colon cancer | Inhibition of repression/ multidrug resistance | [59] |
| pri-miR-26a1/ pri-miR-100 | Several SNPs | – | Colon cancer | Longer time to progression after 5-fluorouracil or irinotecan treatment | [60] |

## 5 Drug Resistance miRNA Pharmacogenetics Association

The expression levels of mature miRNAs may be affected by two pathways: sequence variations in miRNA regions and miRNA-processing pathways [55, 56]. This issue is discussed in more detail in Chapter 18. The pharmacogenetic analysis of miRNAs may represent a revolutionary area of investigation for predicting treatment response or chemoresistance [57]. These alterations are owing to single-nucleotide polymorphisms (SNPs [56]). SNPs can occur in three different ways: (1) polymorphisms affecting miRNA biogenesis, by modulating the transcription of pri-miRNA or pre-miRNA processing and maturation; (2) polymorphisms in miRNA target sites; and (3) polymorphisms altering epigenetic regulation of miRNA genes [56] (Table 3).

## 6 MicroRNAs in Cancer Stem Cells: Association with Drug Resistance

Recently, it has become clear that miRNAs can play a pivotal role in the drug resistance of cancer cells and cancer stem cells. Drug resistance in cancer stem cells inserts another viewpoint to the challenge of drug resistance in cancer. Cancer stem cell hypothesis states that when not all cancer stem cells from a tumor are extirpated, the tumor will always reoccur [61] (Table 4).

The fact that cancer stem cells are often found to be resistant to one or multiplexed drugs increases the complexity for successful cancer cures. For this reason, it is extremely important to explore occasions to selectively target the cancer stem cell population of a tumor. Selective killing of cancer stem cells would properly enhance patient outcome by preventing metastasis and recurrence of the

**Table 4**
**miRNAs in cancer stem cells: Association with drug resistance**

| miRNA | Characteristics | Targets | Tumor model | Reference |
|---|---|---|---|---|
| miR-34 | Regulated by p53, downregulated in most tumors/drug resistance | Notch, HMGA2,Bcl-2 | Cancer stem cells lacking p53 gene expression | [62] |
| miR-125b | Decreased sensitivity of ATRA-induced apoptosis via upregulation | Unknown | Glioma | [63, 64] |
| miR-140 | Methotrexate and 5-FU resistance increased via upregulation | HDAC4 | Colon and osteosarcoma stem cells | [65] |
| miR-215 | Methotrexate and tomudex resistance increased by downregulation of DHFR and TS | DHFR, TS | Osteosarcoma and colon cancer stem cells | [66] |

primary tumor. In the last years, some encouraging studies have been succeeded in this research field, with distinct molecules developed to specifically kill cancer stem cells, such as monoclonal antibodies targeting leukemic stem cell marker CD44 [67] or drugs like nigericin and abamectin that inhibit cancer stem cell growth [68] or gene therapy [69, 70].

There are a lot of studies concerning with this issue. The miRNA, miR-34, is directly regulated by p53 and downregulated in most tumors, which suggests a common drug resistance via targeting Notch, HMGA2, and Bcl-2 in cancer stem cells that lack p53 expression [62].

Upregulation of miR-125b increases the rate of proliferation and decreases sensitivity to ATRA-induced apoptosis by targeting Bmf in glioma stem cells [102].

Overexpression of miR-140 elevates the resistance against methotrexate and 5-FU HDAC4 osteosarcoma and colon cancer stem cells [65].

Upregulation of miR-215 downregulates dihydrofolate reductase (DHFR) and thymidylate synthase (TS) which in turn leads to increased resistance against methotrexate and tomudex in osteosarcoma and colon cancer stem cells, respectively [65, 71].

## 7 MicroRNAs as Drugs

Rukov et al. reported that attempts are in progress to improve miRNA-based drugs, either in the form of miRNA mimics, amplifying the effect of a miRNA, or miRNA inhibitors, fundamentally suppressing the effect of a miRNA [7]. MicroRNA drugs have the

benefit that one miRNA may target and modify the expression of several genes with different roles in the same pathway. The most developed miRNA drug to date is a miRNA inhibitor targeting miR-122 in liver to treat hepatitis C virus (HCV [72]). One issue as such drugs approach clinical use is testing for interactions between the novel miRNA drugs and traditional drugs already in the market. The connection from miRNAs to drugs allows Pharmaco-miR to predict default interactions between novel miRNA drugs and more traditional drugs, which can then be tested experimentally [7]. For example miR-122 is estimated to target estrogen receptor 1 (ESR1), whose gene product is necessary for the important drug families of estrogens (e.g., estradiol) and antiestrogens (e.g., tamoxifen). If this predicted target is functional, treating patients for HCV with miR-122 may cause adverse drug effects if the patient is also undergoing treatment with estrogens or antiestrogens.

In recent years, an increasing number of papers describe a link between miRNAs and drug function through deregulation of pharmacogenomic-relevant genes. These studies are mainly performed in cancer cell lines and mainly describe chemoresistance. Drug toxicity studies and studies on drug metabolizers are remarkably rare. Also, many studies report miRNA deregulation in drug-resistant cells but fail to identify the miRNA target effector genes. The lack of such studies highlights how elusive it can be to link miRNA expression with the connection between genes and drug efficacy/toxicity. Pharmaco-miR is a web server designed to help in defining such interactions between miRNAs, target genes, and associated drugs by complementary of the pioneering resources on miRNA targeting and pharmacogenomics. The outcome usually comprises a miRNA pharmacogenomic set consisting of a miRNA, a target gene, and a drug commented in the literature as being linked with the target gene. Pharmaco-miR is thus a useful tool when predicting the effect of miRNAs on drug efficacy and toxicity or when developing hypothesis within miRNA pharmacogenomics. Identification of miRNA pharmacogenomic sets makes it possible to outline potential mechanisms for miRNA–drug interactions when planning experiments and assists in the interpretation of results. As the field of miRNA pharmacogenomics matures, Pharmaco-miR can be extended to collect relevant information within the field and allow searches specifically for miRNA pharmacogenomic sets, where the full set has been investigated in a pharmacogenomic context [7].

According to the National Center for Toxicological Research's 2011–2012 annual report, new biomarkers of liver damage are needed to improve detection of injury in animals and humans. A genomics approach was used on urine samples of rats treated with drugs and chemicals that cause liver injury. Several urinary miRNAs (also called epigenetic biomarkers) were identified that may serve as predictive biomarkers of hepatotoxicity.

MicroRNAs are differently expressed in diseases, and they have crucial responsibilities in diverse biological pathways. Notwithstanding alteration of DNA methylation or histone modifications, deregulated miRNA expression patterns of tumor cells have been described as colliding with drug response. Approaches to arrange the expression of chosen miRNAs have partly led to intriguing developments of chemotherapy response.

MicroRNAs are potential targets of therapeutics. The arrangement of miRNA levels covers a wide spectrum of technologies from gene therapy to antisense therapy. Targeting miRNAs for therapy could be an emerging field, although there are many obstacles to be overcome: stability, convenient in vivo delivery systems, and selectivity. An individual miRNA could regulate several genes and pathways simultaneously suggesting that miRNA modulation could be powerful. However, attention must be paid to the possibility that miRNA manipulation may cause adverse influences, for the reason that each miRNA target has not been identified. Chiefly there are two strategies to target miRNA expression: by blocking the expression of an oncomiR or by re-expression of a tumor-suppressor miRNA, or by targeting the genes involved in their transcription and processing. Anti-miRNA oligonucleotides (AMOs) [73–76], locked nucleic acids (LNA) [77–81], small-molecule inhibitors (SMIRs), miRNA sponges or decoys [71, 82, 83], nanoparticles [84–86], and miRNA mimics and adenovirus-associated vectors (AAV) [87–90] are the current ways to target miRNAs as drugs.

There is growing evidence that diagnostic and prognostic miRNA signatures are indispensable in tumor classification and treatment protocols. Differences in individual gene expressions stimulated the interest of global miRNA expression studies in human diseases that exposed modest variations between normal and tumor tissues [64, 91–93]. For instance, in tumor tissues, miR-125b and miR-145 are mostly determined at lower levels while miR-21, miR-155, and miR-210 at higher levels [50, 94–96]. But of course, there are slight differences in miRNA expressions, and further studies are needed to detect alterations in the signaling pathways and enlighten treatment response [95, 97]. There are high numbers of studies demonstrating miRNA signatures and their prognostic value correlation [53, 91, 98, 99].

Studies in CRC cell lines declared that mRNA and miRNA signatures could improve prediction of treatment response over K-Ras mutation status alone and thus inform patient eligibility to anti-EGFR-based treatments [100, 101]. Ragusa et al. reported on using cetuximab-sensitive and -resistant CRC cell lines to profile miRNA expression, and they suggested three miRNA signatures for estimating treatment response to cetuximab [101]. Two of these miRNAs, let-7b and let 7e, were found to be negative regulators of K-Ras expression. Several studies showed the importance of let-7's role in a poor outcome of cetuximab-treated metastatic

CRC patients [102, 103]. Also, let-7a-mediated regulation of K-Ras expression was first described in lung cancer [104].

The most important problem in the majority of the cancer cases is a lack of targeted therapy. MicroRNA signature analyses are indispensable in every step of therapy approach like tumor aggressiveness and resistance to treatment in almost every type of cancer affecting different gene expressions like EGFR and K-Ras and in several signaling pathways [105–107]. These studies also identified the organ site of carcinomas of unknown primary origin [108, 109].

Additionally, miRNA signatures are crucial in blood samples of patients to detect diseases earlier and monitor progression of diseases in a noninvasive way after treatment [110–113].

Global miRNA profiling pioneered to improve disease management from bench to clinical applications.

## 8 Conclusion

MicroRNAs are potential providers of drug response prediction. MicroRNAs are also the headliners of drug resistance and pharmacological drug response dilemma and conducives of drug dosage tailoring and inhibitors of adverse drug reactions that procreate personalized therapy.

Complicated dynamic natured networks between diagnostic, prognostic, and therapeutic miRNAs and their targets are complex, and in vitro studies alone may be improper to estimate miRNA significance related with early detection, progression, recurrence, and treatment response in vivo.

In the light of these novel findings, more studies should be conducted in order to demonstrate the utilization of these tiny but indispensable molecules in diagnosis and treatment of various diseases: a dream could be realized. Individualizing current anticancer regimens by predicting the potential intrinsic/acquired resistance and future therapeutic strategies to get over resistance, including specific targeting of miRNA (via mimics or antagomirs) is very important. Also these regimens target synergistic interaction with anticancer agents. These regimens use the modulation of expression of key proteins in different molecular mechanisms involved in drug activity.

## 9 Notes

1. In colorectal cancer, miR-142-3p, -144, -151, -212, -17, -20, -25, -32, -92, -93, -106a, -125a, -155, -191, -192, -203, -215, and -223 are responsible for subtype classification like MSS vs. MSI-high.

2. MicroRNA signatures such as let-7a, -7b, -7c, -7d, -7e, -7f, -7g, and -7i and miR-16, -17, -19b, -20a, -26a, -26b, -29a, -29b, -29c, -30b, -30d, -98, -103, -106a, -106b, 107, -146b-5p, -181a, -191, -195, -453, -491-5p, -498, -509-3p, -654-5p, and -663 are responsible for the subtype classification of AdCa vs. SCC.
3. MicroRNA signatures, such as let-7e and miR-17-5p, -20a, -20b, -21, -93, -106a, -106b, -126, -146b, -155, -182, -183, -191, 200a, -200c, -203, -210, and -224, have a major role in the prediction of the overall survival in SCC cases.
4. Specific miRNAs such as miR-27b, -29a/181a/221, -34a, -98, -122, -125b, -140, -143, -148a, -155, -192/215, -199a-3p, -200c, -204, -205, -212, -214, -221/222, -320, -328, -451, and -512 targeting genes (such as CYPIB1, SIRT1, HMGA2, Cyclin G1, BAK1, HDAC4, ERK5, MSK1, PXR, FOXO3a, TS, mTOR, C-Met, TUBB3, Mcl-1, HER3, PED, PTEN, P27, ERα, Bcl-2, ABCG2, and CSA) are involved in apoptosis and survival pathways affecting anticancer drug sensitivity in various cancer types, including breast, cervical, prostate, colorectal, colon, cholangiocarcinoma, T-cell leukemia, and osteosarcoma.

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