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Pharmaco-miR: linking microRNAs and drug effects

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Abstract

MicroRNAs (miRNAs) are short regulatory RNAs that down-regulate gene expression. They are essential for cell homeostasis and active in many disease states. A major discovery is the ability of miRNAs to determine the efficacy of drugs, which has given rise to the field of 'miRNA pharmacogenomics' through 'Pharmaco-miRs'. miRNAs play a significant role in pharmacogenomics by down-regulating genes that are important for drug function. These interactions can be described as triplet sets consisting of a miRNA, a target gene and a drug associated with the gene. We have developed a web server which links miRNA expression and drug function by combining data on miRNA targeting and protein–drug interactions. miRNA targeting information derive from both experimental data and computational predictions, and protein–drug interactions are annotated by the Pharmacogenomics Knowledge base (PharmGKB). Pharmaco-miR's input consists of miRNAs, genes and/or drug names and the output consists of miRNA pharmacogenomic sets or a list of unique associated miRNAs, genes and drugs. We have furthermore built a database, named Pharmaco-miR Verified Sets (VerSe), which contains miRNA pharmacogenomic data manually curated from the literature, can be searched and downloaded via Pharmaco-miR and informs on trends and generalities published in the field. Overall, we present examples of how Pharmaco-miR provides possible explanations for previously published observations, including how the cisplatin and 5-fluorouracil resistance induced by miR-148a may be caused by miR-148a targeting of the gene KIT. The information is available at www.Pharmaco-miR.org.

Keywords: *microRNAs; pharmacogenomics; database; web server; miRNA pharmacogenomic set; Pharmaco-miR*

INTRODUCTION

MicroRNAs (miRNAs) are short (~22 nucleotides) regulatory RNAs that down-regulate gene expression at the posttranscriptional level by inhibiting translation or initiating mRNA degradation [1, 2]. The mature miRNA functions in the RNA-induced silencing complex (RISC) [3] and recognizes its mRNA targets by a system of partial complementarity between the miRNA and the 3′UTR of the target gene [1, 2, 4]. In the 'miRbase' database of miRNAs [5], 2042 human mature miRNAs are registered, although the role of many of these is still unclear. miRNA target genes are available from a variety of sources, including computational predictions [6–8], single gene studies [9, 10] and, most recently, large-scale experimental studies that combine cross-linking of loaded RISC complexes to target UTRs, immunoprecipitation and next-generation sequencing to identify miRNA targets [11–13].

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More than half of human genes are likely targets of evolutionarily conserved miRNA regulation [14]. miRNAs are therefore important regulators of gene expression, and many cellular processes, including differentiation, apoptosis and stress responses, are controlled by miRNAs [15]. miRNAs are essential for cell homeostasis and tissue identity, regulate many disease provoking genes and are as such implicated in many diseases, including diabetes [16], neurological diseases [17] and cardiovascular diseases [18]. They are significantly deregulated in cancer [19] and individual types of cancer can in fact be defined and identified by their miRNA profile [19–21]. miRNAs are therefore broadly recognized to have great potential, both within diagnostics and therapeutics.

A recent major observation involving a new role for miRNAs is their ability to determine the efficacy of drugs. These findings gave rise to the field of miRNA pharmacogenomics [22]. Traditionally, pharmacogenomics deals with how genomic variations, for instance sequence variation within specific genes, determine drug function. One ultimate aim of pharmacogenomics is to predict which drugs will be the most effective and safe for a patient based on individual genomic and transcriptomic features, thereby allowing personalized treatment strategies. Pharmacogenomics therefore has great clinical potential. The majority of pharmacogenomic research to date has focused on the role of single-nucleotide polymorphisms (SNPs), variations in unique DNA nucleotides in the genome and studying copy number variations (duplications or deletions of genes or larger genomic segments). Alternative mRNA splice patterns and differences in gene expression levels constitute other research strategies within pharmacogenomics.

A ROLE FOR miRNAs IN PHARMACOGENOMICS

Many drugs require the expression of specific genes to function, and drug function can be affected by changes in the expression level of these pharmacogenomic genes. miRNAs are essential for tissue identity and hardwired into cell-specific regulation. As such miRNAs regulate many genes involved in pharmacogenomics. Furthermore, the transition from healthy to disease tissue is accompanied by extensive changes in miRNA profiles. These changes may either be a direct cause of the disease or a secondary effect of other regulatory changes in the cell [20]. The change may in both cases be important for the expression of pharmacogenomic genes. Finally, tissues deriving from the same disease, for instance tumors of the same type, can vary in their miRNA profile between patients [19]. miRNA profiling is therefore used to sub-categorize cancers and other diseases [19–21]. These differences in miRNA profiles may be fundamental in miRNA pharmacogenomics, since different profiles will affect pharmacogenomics related genes differently, with extensive downstream consequences for drug effect. In support of this, there is evidence that variations in miRNA patterns in similar diseases do in fact lead to differences in gene expression [23, 24]. If genes affected by such differences are involved in drug function, the efficacy of the drug may also be affected.

It is therefore not surprising that a series of recent miRNA pharmacogenomic studies document how miRNAs are important for the ability of drugs to induce their effect (reviewed in [25]). In general, these studies identify miRNA targets in genes that are also known to bind or transport chemotherapeutic drugs. They then show that affecting gene expression with varying levels of miRNAs also affects the efficacy of the drugs known to depend on the gene for its function. One example of this is the importance of miR-125b for calcitriol (active vitamin D) efficacy in breast cancer MCF-7 cells. Calcitriol binds to the vitamin D receptor (VDR) and thereby induces the formation of an active transcription factor complex. Mohri *et al*. [26] have shown that VDR protein levels are lowered by miR-125b. When this occurs, the efficacy of calcitriol is similarly decreased, resulting in higher cancer cell proliferation (Figure 1A).

miRNA PHARMACOGENOMIC SETS: THE LINEAR RELATIONSHIP BETWEEN miRNAs, GENES AND DRUGS

As exemplified above, the principle of miRNA pharmacogenomics entails that increased miRNA expression down-regulates genes encoding proteins that promote drug efficacy. Conversely, lowered miRNA levels may result in up-regulated genes with products that inhibit drug function. Both these processes may affect drug function and therefore make miRNAs indirect, potentially potent regulators of drug efficacy. Thus, the role of miRNAs in pharmacogenomics can conceptually

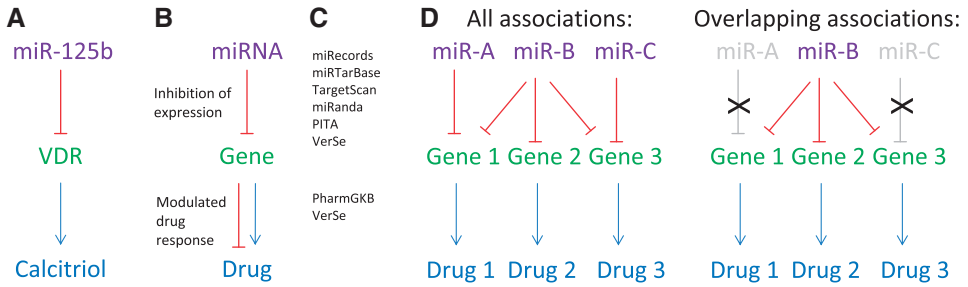

Figure 1: (**A**) An example of an experimentally verified miRNA pharmacogenomic set. miR-125 b inhibits vitamin D receptor (VDR) expression. VDR is a co-factor for calcitriol, and lower protein levels decrease calcitriol efficacy [26]. (**B**) The concept of miRNA pharmacogenomics. While increased levels of miRNA always inhibits gene expression, protein levels can either increase or decrease drug response, depending on protein function. Together, a miRNA, a target gene and a drug that interacts with the target gene's product forms a miRNA pharmacogenomic set. (**C**) Sources integrated in Pharmaco-miR. miRNA targets are fetched from VerSe, miRecords [10], miRTarBase [9], TargetScan [14], miRanda [7] and PITA [8], and gene–drug interactions are from VerSe or annotated by the Pharmacogenomics Knowledge Base [37]. (**D**) The conceptual difference between searching for 'all associations' and 'overlapping associations'. When searching for all associations, the output consists of all miRNA pharmacogenomic sets which contain at least one of the input parameters, while when searching for overlapping associations the output consist of miRNA pharmacogenomic sets that contain objects which are shared by all the search terms entered. In this example, by entering three genes as the search entries and choosing 'all associations', both miR-A and miR-C are included with one set each (miR-A–Gene 1–Drug 1 and miR-C–Gene 3–Drug 3, respectively), while miR-B occurs in three sets (miR-B–Gene 1–Drug 1, miR-B–Gene 2–Drug 2 and miR-B–Gene 3–Drug 3). However, when choosing 'overlapping associations', at least one component in every output set must be associated with all the search entries. In this example, only miR-B connects all three genes entered. Thus, only sets with miR-B occur in the output, while sets with miR-A and miR-C are not included. Notice that in this case, it is not necessary for any of the drugs to be associated with all three genes; it is sufficient that they occur in sets where miR-B is also present.

be viewed as collections of miRNA pharmacogenomic sets consisting of miRNA, gene and drug (Figure 1B). According to this model, a miRNA affects drug function via the regulation of a gene whose protein product interacts with that drug. For example, since 125b targets VDR and thereby affects calcitriol function, miR-125b–VDR–calcitriol constitutes a miRNA pharmacogenomic set (Figure 1A).

Pharmaco-miR VerSe: A DATABASE OF miRNA PHARMACOGENOMIC SETS

Here, we present a database of experimentally verified miRNA pharmacogenomic sets consisting of a miRNA, a target gene and a drug depending on the gene for its function. The database is named Pharmaco-miR Verified Sets (VerSe). It has been built as part of the Pharmaco-miR web server and is available for download on the server at Pharmaco-miR.org. While the Pharmaco-miR web server includes the VerSe database, it also offers parsing and data filtering, as will be described below. The miRNA pharmacogenomic sets in Pharmaco-miR VerSe are curated from published studies. As also exemplified above, two aspects can be said to characterize miRNA pharmacogenomic sets and have to be fulfilled for inclusion in Pharmaco-miR VerSe: (1) the miRNA must be shown to target the gene directly in the specified context (typically shown by luciferase experiments) and (2) the subsequent inhibition of gene expression must affect drug efficacy in the same context. Based on these criteria, we have collected miRNA pharmacogenomic sets described in the literature. The database consists of 269 pharmacogenomic sets annotated from 149 original articles. The sets encompass 105 miRNAs, 119 genes and 72 drugs (Table 1).

A GROWING FIELD

Looking at the articles in VerSe reveals a steady increase in the number of publications in miRNA pharmacogenomics. The first article to present a miRNA pharmacogenomic set (and therefore the earliest set in Pharmaco-miR VerSe) was published in 2007 by Mishra *et al.* [27]. This first example of miRNA pharmacogenomics is slightly atypical, since

Table 1: Databases included in Pharmaco-miR

		Conserved miRNAs[a]	Nonconserved miRNAs	miRNA–gene links	Genes	Gene–drug links	Drugs
miRTarBase		227		998	466		
miRecords		132		375	215		
Targetscan	Conserved targets	247	402	28 436	1615		
	Non-conserved targets	249	428	191 834	2300		
	Total	249	428	211 795	2280		
miRanda	High mirSVR	249	851	365 910	2307		
	Low miRSVR	249	851	674 011	2312		
	Total	249	851	884 911	2316		
PITA	Top predictions	673		30 752	1365		
	All predictions	677		526 624	2198		
VerSe		105		190	119	210	72
PharmGKB					2397	11 300	921
Total		**1154**		**1 026 667**	**2463**	**11 482**	**951**

[a]Includes broadly conserved TargetScan miRNAs.

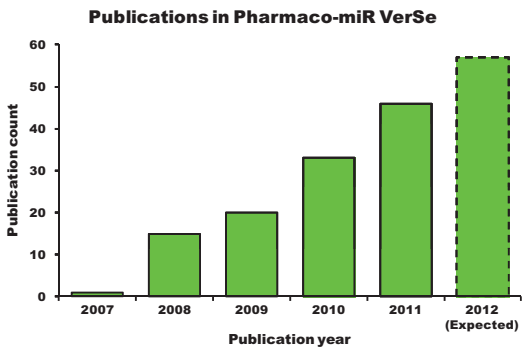

Figure 2: The increasing number of yearly papers included in Pharmaco-miR VerSe.

obstructed miRNA function occurs due to a SNP in a miR-24 target site in the gene dihydrofolate reductase, affecting methotrexate efficacy. Since then, miRNA pharmacogenomic papers have steadily increased in frequency, with 46 papers in 2011 and 57 expected for 2012 (Figure 2).

THE CENTRAL ROLE OF miR-21

One trend apparent in Pharmaco-miR VerSe is that many published miRNA pharmacogenomic sets contain miR-21. In fact, 24 of 269 unique sets in Pharmaco-miR VerSe include miR-21. It has, for instance, been shown to affect 5-flouruoracil (5-FU) action alone through targets in SPRY2 [28], PDCD2 [29], PTEN [29] and MSH2 [30]. While some of the many examples of miR-21 effect on drug function may partly be due to the large focus on miR-21 in the RNA community, it also fits well with the important role of miR-21 as an 'onco-miR', targeting many tumor suppressor genes, among them PTEN [29, 31, 32] and PDCD4 [32, 33].

Due to the important role for these genes in cancer, they are also central in chemotherapy. For example, miR-21 regulates drug efficacy and also has a part regulating tumorigenesis [30]. MSH2, for instance, encodes a DNA tumor suppressor protein involved in DNA repair, and its disruption leads to an increased mutational rate in cancer cells. The 5-FU, on the other hand, has an anti-cancer effect, since 5-FU metabolites are incorporated into and disrupt the DNA of proliferating cells. MSH2 and 5-FU therefore both have related to DNA replication and repair. Although MSH2 is a tumor suppressor, its gene product increases the efficacy of 5-FU, since it promotes incorporation of 5-FU metabolites into DNA. Therefore, during 5-FU treatment, decreased MSH2 levels, for instance after miR-21 targeting, attenuates the effect of 5-FU, and increased miR-21 levels leads to 5-FU resistance [30]. Thus, the same factors that make miR-21 central in cancer also provide miR-21 with the ability to strongly regulate drug efficacy, testifying to the link between cancer miRNAs and Pharmaco-miRs.

LIMITATIONS TO THE miRNA PHARMACOGENOMIC EXPERIMENTAL MODEL

The focus on cancer miRNAs and chemotherapeutics hints at a common structure of many miRNA pharmacogenomic studies. Drug-resistant cancer cell

lines are developed (often accompanied by miRNA deregulation as the cells become resistant), changes in miRNA and mRNA expression are monitored, a candidate miRNA (and consequently also its target gene) is perturbed and this interaction is shown to reinstate drug sensitivity. This last point is determined by two phenotypes that are typical for cancer: apoptosis and cell invasiveness after addition of the drug. While this model is both elegant and experimentally straightforward, it is by nature limited to chemoresistance studies. Testing non-chemotherapeutic drugs is more experimentally challenging, since they in most cases require other proxies for drug efficacy.

Similarly, toxicity studies are remarkably absent from miRNA pharmacogenomic literature, most likely due to the lack of an experimental model that can be broadly applied. As the field of miRNA pharmacogenomics develops, one challenge will be studying links between miRNAs and drug toxicity.

Another type of studies only rarely performed link drug metabolism and miRNA activity. One explanation for this may be that many drugs are primarily metabolized in the liver while they function in other tissues. In this case, standard stable cell line experiments do therefore not suffice.

Only two studies in the VerSe database (which describe full miRNA pharmacogenomic sets) include members of the cytochrome P450 families, the major group of drug metabolizing enzymes. Komagata *et al*. [34] showed that miR-125b affects calcitriol efficacy by targeting CYP24A1, a calcitriol metabolizer. However, this case is not typical, since the drug in this example is metabolized and performs its function (binding to the vitamin D receptor) in the same tissue (MCF-7 breast cancer cells). Similarly, Pan *et al*. [35] induced CYP3A4 in pancreas cancer cells which activated cyclophosphamide to produce cytotoxic agents. Introducing miR-27a down-regulated CYP3A4 expression and simultaneously attenuated the cytotoxic effect of cyclophosphamide. Thus, in both the above cases, drug metabolism and function occurred in the same tissue, unlike what is the case in most *in vivo* situations.

We have previously shown that miRNA targets are significantly underrepresented in genes that metabolize drugs when compared with genes in general [36]. However, considering the large number of drugs potentially metabolized by a few enzymes, the pharmacokinetic impact of a single miRNA target in one gene can be quite dramatic. CYP3A4 is thought to be involved in the metabolism of more than half of all drugs, and it is associated with 243 drugs and drug classes according to PharmGKB, a valuable source of accumulating knowledge within the pharmacogenomics field [37]. One future challenge, to identify the true scope of miRNA pharmacogenomics, is therefore to apply new experimental setups, for instance to animal models, so that miRNA targeting and drug action can be tested in different tissues.

These above examples illustrate how limitations in methodology and hypothesis building may limit the scope of the studies performed, and the pervasiveness of miRNA pharmacogenomics may be broader than work done to date within the research area indicates. In general, a major limitation within miRNA pharmacogenomics today is determining the mechanism of action linking miRNAs and drug response. In hindsight, the mechanism is often obvious as exemplified by Mohri *et al.* [26], but the *de novo* identification of such triplet sets of interdependent molecules (miRNAs, genes and drugs) is very challenging, since a plethora of miRNA targets and gene drug dependencies exist, creating a virtually endless number of possible combinations. It is thus a continuous challenge for miRNA pharmacogenomic studies to determine the exact associations between miRNAs, genes and drugs and thereby identify valid miRNA pharmacogenomic sets. For instance, miRNAs are often found to be perturbed in drug-resistant cells [26, 38–40]. There is, however, no necessary causal connection between such deregulated miRNAs and the drug resistance phenotype. On the other hand, identifying potential connections between perturbed miRNAs and the drug resistance phenotype would contribute important information about the mechanism of drug resistance and facilitate the use of miRNA profiling to predict drug responses. In particular, the challenge is to identify possible miRNA targets that may at the same time be effector genes generating drug resistance. In other cases, specific sets of genes may be deregulated in drug-resistant cells, but the underlying regulatory reason (for instance involving miRNA regulation) will be difficult to predict. In a clinical context, the challenge will be to predict which drugs may show unwanted efficacy and toxicity effects based on miRNA expression profiling alone.

Establishing candidate miRNA pharmacogenomic interactions as outlined above has demanded extensive searches in miRNA targeting databases (typically consisting of computational predictions; detailed

later in the text), drug databases such as the PharmGKB [37] as well as biomedical literature to produce the link between miRNA targeting and genes relevant for drug efficacy. Many studies therefore fall short of identifying the full drug resistance pathway, and some offer two out of the three components, usually drugs and miRNAs [38–40].

To assist in determining such complete miRNA-pharmacogenomic sets of miRNAs, their target genes and the associated drugs, we have constructed a web server named Pharmaco-miR (www.pharmaco-miR.org). Pharmaco-miR accepts miRNA, gene and drug names as input and provides the relevant associated miRNA pharmacogenomic sets as output. The database combines data from a variety of sources, including several miRNA target prediction databases, curated literature of experimentally validated miRNA target genes and published gene–drug interactions as annotated by PharmGKB or in the VerSe database that accompanies the web server. Thus, Pharmaco-miR allows researchers to quickly and uniquely determine which miRNAs, genes and drugs may interact in a pharmacogenomic context.

miRNA DATA INTEGRATION

Pharmaco-miR is designed to determine miRNA pharmacogenomic sets, consisting of a miRNA, a target gene and a drug described in literature as being associated with the target gene. To this end, a variety of databases have been integrated (Figure 1C). Two databases, miRecords [10] and miRTarBase [9], collect experimentally verified miRNA targets as described in the literature. Although the number of validated targets is growing fast, only a small fraction is thought to have been identified so far. Pharmaco-miR therefore also includes several databases of computational miRNA target predictions: TargetScan [6, 14], miRanda [7, 41] and PITA [8] (Table 1). The different database methodologies are described below, and the data files used in Pharmaco-miR are described in the Supplementary Materials accompanying this article.

COMPUTATIONAL miRNA TARGET PREDICTIONS

TargetScan was the first miRNA target database published [6]. It was originally launched as part of the first major target search, revealing the importance of the 'seed' region [6], but has been regularly updated and improved [14]. The database includes all potential target sites with minimum a 6mer seed match but does not allow for mismatches in the seed sequence. It permits stringent filtering based both on target conservation and conservation of the miRNA family. Target sites can furthermore be evaluated based on seed type and context contribution as well as depth of evolutionary conservation [6, 14]. The version of TargetScan included in Pharmaco-miR is 5.2.

The miRanda algorithm [7] uses less stringent criteria than TargetScan for prediction of target sites. miRNA are aligned to mRNAs to identify complementarity, but while seed match is weighed more strongly, G:U wobble base pairing and mismatches in the seed do not lead to exclusion from the set of predictions [7]. Similarly, the algorithm considers conservation throughout the potential miRNA matching region rather than only seed conservation. Targets are scored based on the free energy of the mRNA:miRNA heteroduplex. miRanda therefore includes de facto targets that may be missed by more stringent algorithms; however, this increase in sensitivity is likely accompanied by a significant loss of specificity. The version of miRanda used in Pharmaco-miR is the August 2010 release.

PITA [8] differs from the two above algorithms mainly by considering not only the miRNA:mRNA match but also structural features surrounding the putative target site and the energy cost to open and expose the target site to the miRNA-loaded RISC. Full seed match is required for 6mer sites, while 7mer and 8mer matches are allowed one wobble G:U pair. Catalog version 6 of PITA is used in Pharmaco-miR.

GENE–DRUG ASSOCIATIONS

Information on gene–drug associations is based on literature annotations from two different sources. The majority of drugs (921) derive from PharmGKB, which among other things include a list of genes and drugs which have been linked experimentally according to the literature. The PharmGKB drugs are linked to 2397 genes. Other gene–drug associations derive from the database VerSe build specifically for Pharmaco-miR. Both are built from literature annotations. The VerSe database contains 269 sets, linking 119 genes and 73 drugs (Table 1).

WORKFLOW

Pharmaco-miR searches are performed by entering the name of a gene, drug and/or miRNA of interest in the search field. The search page also contains an option to change query features and the databases searched. Using the default options, the Pharmaco-miR output consists of detailed miRNA pharmacogenomic sets of miRNAs, genes and drugs. Also, in the database features, sets and subsets of miRNA target predictions may be picked. These choices vary depending on the structure of the different databases. For TargetScan, conserved and non-conserved miRNA targets may be searched independently, and miRNAs with varying levels of conservation may be included in the search. Similarly, conserved and non-conserved targets may be selected for miRanda which also allows for searching different miRSVR scores, an indicator of the efficacy of the target site [41]. PITA targets are separated into top targets, which are the most likely to be functional miRNA binding sites, or can be expanded to include all predicted miRNA targets.

'DETAILED ASSOCIATIONS TABLE' AND 'REDUCED ASSOCIATIONS TABLE' IN QUERY FEATURES

As mentioned above, Pharmaco-miR output consists of detailed miRNA pharmacogenomic sets of miRNAs, genes and drugs, available when choosing the default 'detailed association table' option. A reduced output option ('reduced associations table') may also be chosen in the query features and results in a list of unique miRNA/gene/drug names, where each name occurs only once. Thus, this output option does not inform on the specific miRNA pharmacogenomic sets but shows the names of all objects in the sets without duplications. This feature is useful to get an overview of larger datasets.

RANKING THE PREDICTIONS

Setting the search options provides the initial filtering of Pharmaco-miR predictions. However, it will often be necessary to further sort the sets. Pharmaco-miR predictions derive from a variety of sources, and targets are generated and scored on the basis of different algorithms that cannot be easily aligned. Pharmaco-miR predictions can therefore be scored on the basis of the original ranking system for the different target predictors. This statistical information can be accessed by selecting the 'Associations Statistics' checkbox in the results page. For TargetScan, Context Scores are given, for miRanda the mirSVR score and for PITA the total free energy score. These scores are described in more detail on the web server. For the remaining databases, which are all based on literature annotations, the number of papers describing the associations is given as a proxy for the support for the association.

'ALL ASSOCIATIONS' AND 'OVERLAPPING ASSOCIATIONS' IN QUERY FEATURES

The output miRNA pharmacogenomic sets may be generated by two different methods: the default 'all associations' output provides all miRNA pharmacogenomic sets which contain at least one of the search input terms. While this gives the largest amount of information, it may in some cases be preferable to focus only on sets that share some components. For instance, if experiments reveal that some miRNAs are perturbed in drug-resistant cells, it may be interesting to know whether these miRNAs may have an additive effect on drug resistance, even though they do not target the same genes. In this case, 'overlapping associations' should be selected rather than 'all associations'. In the 'overlapping associations' analysis, all sets must contain at least one component that co-occurs with all the entered search terms (Figure 1D). If miRNAs are entered, for instance, all sets in the output will contain a gene that is targeted by all entered miRNAs and/or a drug which may be affected by all the miRNAs, possibly through the actions of different genes. This gene/drug in question will then be shown with bold letters in the output.

EXPORT AND LINKS

An export option allows the tables in Pharmaco-miR to be exported (as comma delimited .csv files) and automatically opened in Microsoft Excel.

Furthermore, a series of links couple the output with the background databases. Since the information in Pharmaco-miR largely consists of data integrated from other sources, it may be important to refer back to these sources to gain more detailed knowledge on parts of the Pharmaco-miR output. Also, since associations are partly based (when using computational miRNA target predictions) or fully based (when validated targets are used) on literature

annotations, links are also provided to PubMed entries for relevant papers.

USING Pharmaco-miR PREDICTIONS

A simple way of using Pharmaco-miR is looking for miRNA targets in genes relevant for pharmacogenomics. A search among such genes reveals that many genes have experimentally confirmed miRNA targets, but the target has not been studied in a pharmacogenomic context. It can therefore be hypothesized that the effect of these drugs may be influenced by perturbations in the predicted miRNAs. For instance, the anticoagulant warfarin has a narrow therapeutic window and is the leading cause of adverse drug event-related hospitalizations in the United States [42]. Warfarin is metabolized in part by CYP1A1, and the *CYP1A1* gene carries an experimentally confirmed miR-125b target. It is therefore plausible that the optimal dose of warfarin depends on, among other factors, the level of miR-125b.

The difficulty in identifying full miRNA pharmacogenomic sets is exemplified in the many studies that succeed in determining which miRNAs are deregulated in drug-resistant cells, but then fail to determine the relevant target genes and therefore the mechanism of action that generates drug resistance. For instance, Hummel *et al*. convincingly show that miR-148a induces sensitivity to cisplatin and 5-FU in previously resistant esophageal adenocarcinoma (EAC) and squamous cell carcinoma (SCC) cell lines, however, they do not identify relevant target genes [38]. Using default Pharmaco-miR search settings, five sets containing miR-148a and cisplatin are identified (as shown in Table 2) and may contain relevant effector genes for cisplatin resistance. Among them is the gene KIT which encodes a signaling protein with oncogenic potential. Interestingly, inhibition of KIT signaling has been shown to increase responsiveness to cisplatin in ovarian cancer cells [43]. It is therefore possible that a similar mechanism exists in EAC and SCC cells, so miR-148a down-regulates KIT and, by inhibiting its signaling activity, increases cisplatin efficacy. This is only one example of the usefulness of Pharmaco-miR in hypothesis building and experimental planning.

That said, it is important to note that both miRNA targeting of genes and gene–drug interactions vary in a context-dependent manner, and some of the output of Pharmaco-miR is based on computational predictions only. The predictions made by the web server, therefore, do not replace experimental verification but rather provide a significant and useful focus for such experiments. Stronger predictions can be achieved by using only experimentally verified targets or targets with strong statistical support, and more sets, although less likely to be functional, can be searched by adding low-value databases or databases with nonconserved miRNA targets.

Table 2: Examples of papers that identify deregulated miRNAs in drug resistance, but not the effector genes, and the genes suggested by Pharmaco-miR

Study	Resistance drug	Deregulated miRNA	Pharmaco-miR putative effector gene
Hummel *et al*. [38]	Cisplatin	miR-148a	ATP7A
	Cisplatin	miR-148a	KIT
	Cisplatin	miR-148a	ERBB3
	Cisplatin	miR-148a	PTEN
	Cisplatin	miR-148a	LRP2
Dai *et al*. [40]	Docetaxel	miR-130a	TGFBR2
	Docetaxel	miR-130a	CDKNIA
	Docetaxel	miR-130a	PTEN
	Docetaxel	miR-181d	MAPT
	Docetaxel	miR-181d	BCL2
Bian *et al*. [39]	Cisplatin	miR-451	CDKN2D

Moreover, Pharmaco-miR can be used to build hypothesis on which functionally important miRNA pharmacogenomic sets may not yet been identified. For instance, six genes very important in pharmacogenomics (named VIP genes by PharmGKB) contain miRNA targets that have previously been validated experimentally (Table 3). These genes are in turn associated with the function of dozens of drugs, some of which are listed in Table 3. Only very few of these sets (such as miR-125b–VDR–calcitriol) have been tested in a pharmacogenomic context (and is therefore included in VerSe). However, the strict criteria for target selection (experimental testing) and the well-studied gene–drug relationships (as VIP genes) increase the likelihood that these sets are relevant for miRNA pharmacogenomics and therefore worth studying experimentally.

For instance, in the folate cycle, thymidylate synthetase (TYMS) catalyzes the methylation of dUMP to dTMP and is essential for the synthesis of dTTP and hence for de novo DNA synthesis.

Table 3: PharmGKB VIP genes with experimentally validated miRNA targets and selected associated drugs, as identified by Pharmaco-miR

Gene	miRNAs	Drugs (selected)
AHR	miR-124, miR-375	GS-9350, omeprazole
BRCA1	miR-146a	Mifepristone, tamoxifen
NR1I2	let-7a	GS-9350, amlodipine, amoxicillin, ampicillin, antineoplastic agents, artemisinin and derivatives, aspirin, bexarotene, budesonide, carbamazepine, cefadroxil, cefuroxime, celecoxib, chlorpromazine, cyclophosphamide, cyclosporine, dexamethasone, diclofenac, diethylstilbestroldocetaxel, doxorubicin, econazole, erythromycin, estradiol, estriol, etoposide, flurbiprofen, fluvastatin, glibenclamide, griseofulvin, hydrocortisone, ifosfamide, isradipine, lansoprazole, lovastatin, meloxicam, methadone, miconazole, mifepristone, montelukast, nafcillin, nevirapine, nifedipine, ondansetron, oxiconazole, paclitaxel, penicillin, phenobarbital, phenytoin, pravastatin, progesterone, protease inhibitors, rabeprazole, reserpine, rifampin, ritonavir, saquinavir, simvastatin, sulfamethazine, sulfinpyrazone, tamoxifen, tetracycline, topotecan, troglitazone, valproic acid, vinblastine, vincristine, vitamin D and analogs, xenobiotics
PTGS2	let-7b, miR-16	Anti-inflammatory agents, BSI-201, coxibs, olaparib, aspirin, capecitabine, celecoxib, cetuximab, clomipramine, dexamethasone, diclofenac, gefitinib, glucocorticoids, HMG CoA reductase inhibitors, ibuprofen, interferon alfacon-1, nimesulide, omega-3 polyunsaturated fatty acids, oxaliplatin, prostaglandins, rofecoxib, tacrolimus, valdecoxib
TYMS	let-7b	Pyrimidine analogs, antimetabolites, antineoplastic agents, asparaginase, bevacizumab, capecitabine, carboplatin, cisplatin, cyanocobalamin, cytarabine, daunorubicin, dexamethasone, etoposide, fluorouracil, folic acid, gemcitabine, ibuprofen, irinotecan, L-glutamine, leucovorin, mercaptopurine, methotrexate, oxaliplatin, pemetrexed, prednisone, pyridoxine, raltitrexed, vincristine
VDR	let-7a, miR-125b	Alendronate, asparaginase, calcipotriol, calcitriol, calcium, clodronate, cyclophosphamide, cyclosporine, cytarabine, daunorubicin, dexamethasone, estrogens, etidronic acid, etoposide, leucovorin, mercaptopurine, methotrexate, prednisone, raloxifene, torcetrapib, tretinoin, vincristine, vitamin D and analogs

TYMS is therefore an important target for anticancer and immunosuppressant drugs such as methotrexate [44], and overexpression of TYMS has been linked to drug resistance [45]. Since TYMS is a verified target of let-7b, it is possible that let-7b (and possibly other miRNAs in the let-7 family) influences the effect of at least some of the many associated drugs. If this is true, let-7b mimics may have potential as adjuvant therapeutics to counter TYMS-related drug resistance. Over-expression of let-7b in, for instance, solid tumors may also indicate a high likelihood of treatment success for drugs targeting the folate cycle, such as methotrexate.

LIMITATIONS TO A ONE miRNA, ONE GENE, ONE DRUG MODEL

As apparent from above, the concept of one miRNA regulating one gene affecting response to one drug may not take the full scope of miRNA regulation into account. For instance, the same miRNA may target multiple genes that are relevant for the function of one particular drug [46, 47] as exemplified by the several miR-125b target genes in calcitriol resistance affecting both the drug's metabolism and its target protein level [26, 34]. In the context of Pharmaco-miR, the same gene–drug combination can, for instance, occur multiple times if the gene is predicted to be targeted by more than one miRNA. Also, multiple miRNAs are often deregulated in the same disease tissue, and drug resistance may therefore be the result of the combined actions of several miRNAs working on different genes. Such cases may be identified by using the 'overlapping associations' option in Pharmaco-miR. For instance, cancer cell line resistance to cisplatin has been associated with down-regulation of four different genes by seven different miRNAs [48–51]. Finally, miRNA deregulation may only initiate a signaling cascade which involves secondary effects that lead to deregulation of several genes. This is exemplified in a study by Giovannetti *et al*. [52], where in gemcitabine-resistant pancreatic cancer cells, miR-21 induction leads to an increase in metalloproteinase-2 and −9 (MMP-2 and MMP-9) and vascular endothelial growth factor (VEGF) expression levels as part of cell invasiveness. MMP-2, MMP-9 and VEGF are therefore indirect, downstream targets of miR-21 that play a role in gemcitabine resistance. Such cases where genes are deregulated due to secondary effects are not included in Pharmaco-miR, since the database only includes

direct targets, although Pharmaco-miR could be used to determine candidates for direct targets in gemcitabine resistance.

miRNAs AS DRUGS

Efforts are ongoing to develop miRNA-based drugs, either in the form of miRNA mimics, amplifying the impact of a miRNA, or miRNA inhibitors, essentially quenching the effect of a miRNA. miRNA drugs have the advantage that one miRNA may target and modify the expression of several genes with different roles in the same pathway. The most advanced miRNA drug to date is a miRNA inhibitor which targets miR-122 in liver to treat hepatitis C virus (HCV) [53]. One challenge as such drugs approach clinical use is testing for interactions between the novel miRNA drugs and traditional drugs already in the market. The link from miRNAs to drugs allows Pharmaco-miR to predict putative interactions between novel miRNA drugs and more traditional drugs, which can then be tested experimentally. miR-122 is for instance predicted to target estrogen receptor 1 (ESR1), whose gene product is essential for the important drug families of estrogens (e.g. estradiol) and anti-estrogens (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 anti-estrogens.

CONCLUSION

In recent years, an increasing number of papers describe a link between miRNAs and drug function through deregulation of pharmacogenomics relevant genes. These studies are mainly performed in cancer cell lines and mainly describe chemoresistance. Drug toxicity studies and studies on drug metabolizers are remarkable scarce. 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 relationship between genes and drug efficacy/toxicity. Pharmaco-miR is a web server designed to assist in identifying such interactions among miRNAs, target genes and associated drugs by integrating the leading sources on miRNA targeting and pharmacogenomics. The output typically consists of miRNA pharmacogenomic sets comprising a miRNA, a target gene and a drug annotated in the literature as being associated with the target gene. Accompanying Pharmaco-miR is the VerSe database, consisting of miRNA pharmacogenomic sets which have been experimentally verified. 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. We therefore believe that Pharmaco-miR will contribute significantly to the progress of miRNA pharmacogenomics both in short and long terms.

SUPPLEMENTARY DATA

Supplementary data are available online at http://bib.oxfordjournals.org/.

Key Points

- MicroRNAs (miRNAs) play a role in pharmacogenomics by regulating genes that are important for drug function.
- The web server Pharmaco-miR combines miRNA targeting data with gene–drug interactions and thereby helps identify sets of miRNAs, their target genes and drugs depending on these genes for their action. Thus, Pharmaco-miR predicts interactions between miRNA regulation and drug function.
- Pharmaco-miR can be an important tool when planning and interpreting miRNA pharmacogenomic experiments and building hypothesis on mechanisms of miRNA–drug interdependence.

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