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miRNA–miRNA crosstalk: from genomics to phenomics

Juan Xu, Tingting Shao, Na Ding, Yongsheng Li and Xia Li

Corresponding authors: Xia Li, College of Bioinformatics Science and Technology, Harbin Medical University, Harbin 150081, China. E-mail: lixia@hrbmu.edu.cn; Yongsheng Li, College of Bioinformatics Science and Technology, Harbin Medical University, Harbin 150081, China. E-mail: liyongsheng@ems.hrbmu.edu.cn

Abstract

The discovery of microRNA (miRNA)–miRNA crosstalk has greatly improved our understanding of complex gene regulatory networks in normal and disease-specific physiological conditions. Numerous approaches have been proposed for modeling miRNA–miRNA networks based on genomic sequences, miRNA–mRNA regulation, functional information and phenomics alone, or by integrating heterogeneous data. In addition, it is expected that miRNA–miRNA crosstalk can be reprogrammed in different tissues or specific diseases. Thus, transcriptome data have also been integrated to construct context-specific miRNA–miRNA networks. In this review, we summarize the state-of-the-art miRNA–miRNA network modeling methods, which range from genomics to phenomics, where we focus on the need to integrate heterogeneous types of omics data. Finally, we suggest future directions for studies of crosstalk of noncoding RNAs. This comprehensive summarization and discussion elucidated in this work provide constructive insights into miRNA–miRNA crosstalk.

Key words: *genomics*; *global and context specific*; *miRNA–miRNA crosstalk*; *miRNA–target interaction*; *phenomics*

Introduction

A major surprise since the completion of the human genome is that a significant fraction of the human genome is transcribed as noncoding RNAs (ncRNA), primarily the well-characterized microRNAs (miRNAs) [1–3]. MiRNAs are a kind of single-stranded small RNA molecules comprising approximately 22 nucleotides. Most of the functions of miRNAs are unknown, but the characterized examples of miRNAs demonstrate their widespread participation in biological functions [4, 5]. Increasing evidence suggests that miRNAs play key roles in the life cycle of the cell, where their dysfunction can lead to various diseases, including cancers [6].miRNAs are recognized most for their roles as regulators of specific target RNA molecules by forming miRNA-induced silencing complexes, thereby resulting in RNA degradation or hindering of mRNA translation into functional proteins. Bioinformatics analyses estimate that miRNAs regulate >60% of the protein-coding genes [7–9]. In general, one miRNA can target more than one gene, thereby indicating the function complexity of miRNAs, and one gene can also be regulated by more than one miRNA, which indicates cooperative control. At present, miRNA cooperation is widely accepted and many cooperative pairs of miRNAs have been detected. For example, in *Caenorhabditis elegans*, both lin-4 and let-7 were first discovered as key regulators of lin-41, where they control the developmental timing of early larval developmental transitions [10]. Genetic evidence also suggests that the lin-28 gene is cooperatively regulated by the lin-4 miRNA and another unidentified miRNA [11]. After analyzing the human miRNA target interactions predicted using the miRanda program, Bino *et al.* found that regulation of one mRNA by one miRNA is rare, which the early examples (lin-4 and let-7) seem to support [12]. They also confirmed the existence of a human analog of the let-7/miR-125 relationship predicted in *C. elegans*. In addition, the distribution of the predicted targets reflects more complicated combinations, in terms of both target multiplicity (more than one target per miRNA) and signal integration (more than one miRNA per target gene). Studies have increasingly shown that some miRNAs can participate in the same biological pathways either as effectors or regulators [12–14]. For example, Krek *et al.* showed that for the known targets of miR-375, a combination of miR-124 and let-7b can lead to synergistic target inhibition in mammals [13]. Similarly, the expression of miR-16, miR-34a and

Juan Xu is an associate professor in the College of Bioinformatics Science and Technology at Harbin Medical University, China.
Tingting Shao is an instructor in the College of Bioinformatics Science and Technology at Harbin Medical University, China.
Na Ding is a MS student in the College of Bioinformatics Science and Technology at Harbin Medical University, China.
Yongsheng Li is an associate professor in the College of Bioinformatics Science and Technology at Harbin Medical University, China.
Xia Li is a professor in the College of Bioinformatics Science and Technology at Harbin Medical University, China.
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miR-106b can alter the cell cycle. Combining these miRNAs leads to stronger cell cycle arrest compared with each of the miRNAs alone [10]. In addition, miRNA target analysis has shown that both miR-17-5p and miR-17-3p repress TIMP3 expression and induce prostate tumor growth and invasion [15]. The synergistic effects of miR-21 and miR-1 were also functionally validated in terms of their significant influences on myocardial apoptosis, cardiac hypertrophy and fibrosis [16]. Wang *et al.* demonstrated that entinostat can specifically induce the expression of three miRNAs (miR-125a, miR-125b and miR-205), which act together to downregulate erbB2/erbB3 in breast cancer cells [17, 18]. Another study showed that six miRNAs significantly inhibited cell proliferation in a cooperative manner [19]. Moreover, recent results obtained using cross-linking and immunoprecipitation technologies confirmed the combinatorial nature of miRNA regulation. Therefore, studying the cooperativity of miRNAs can greatly improve our understanding of the contribution of co-regulating miRNAs toward the complex interplay between miRNAs.

In addition to identifying miRNAs that are expressed dynamically during development or dysregulated in complex diseases, a growing number of studies have suggested an intriguing hypothesis, where complex diseases are affected by several miRNAs rather than a single miRNA. Studies of miRNAs in cancers have shown that some miRNAs that are up-regulated in tumors can act as oncogenes (oncomiRs), whereas miRNAs that are down-regulated in cancer can act as tumor suppressors [20–22]. Interestingly, Wu *et al.* found that 28 miRNAs from different miRNA clusters or families could substantially inhibit p21Cip1/Waf1 expression, thereby signaling a new era of miRNA research by focusing on networks more than the individual connections between miRNAs and strongly predicted targets [23]. According to Peter [24], the study by Wu *et al.* represents the first defined example where multiple miRNAs target the same gene. Another example is the regulation of the tumor suppressor FUS1 in cancer cells [25], which depends on the presence of at least three miRNAs (miR-93, miR-98, miR-197 and additional unidentified miRNAs). Adam *et al.* also identified three miRNAs, i.e. miR-21, miR-23a and miR-27a, which act as cooperative repressors of three well-known suppressor genes [26], where the level of inhibition was greater than that of inhibition by miR-21 alone. Moreover, high levels of the three miRNAs combined were associated with shorter survival times after surgical resection. In 2015, Chen *et al.* discovered that miR-142-3p, miR-494-3p and miR-BART20-5p regulate a molecular circuit involving T-bet, PTEN, AKT and RICTOR, which is involved in the pathogenesis of nasal natural killer cell lymphoma. Moreover, antagomirs to miR-BART20-5p or miR-494-3p, miR-142-3p mimics or AKT inhibitors may be useful in therapy [27]. These findings indicate that miRNAs act together to promote tumor progression, and thus therapeutic strategies might require the inhibition of several miRNAs.

The cooperative integration of signals for target genes is a key feature of miRNA regulation. miRNA–miRNA crosstalk is common in all species, and studying the potential functional effects of this type of regulation is an interesting and challenging area of miRNA research. However, the application of experimental methods to infer miRNA cooperation must address many bottlenecks, such as lengthy experimental periods, the requirement for large amounts of equipment and a high number of miRNA combinations. By June 2016, investigators had discovered and documented 2813 miRNA entries in the miRBase database, with 3 955 078 potential miRNA combinations. The crosstalk among the miRNAs still needs to be tested, but studying the modular regulation of miRNAs and investigating their combined effects are important steps for elucidating the functions of miRNAs at a system-wide level. The use of computational methods to supplement experimental approaches can dramatically reduce the number of candidate miRNA combinations. Thus, in this review, we discuss the computational methods that have been proposed for inferring miRNA crosstalk in genomic sequence-based prediction, or sequencing-based analyses of miRNA–target interactions, as well as describing recent efforts to integrate several data sources for inferring miRNA crosstalk. Moreover, we present methods that have been introduced for identifying context-specific miRNA functional pairs, such as developmental stage-specific and disease-specific methods. Finally, we will discuss the advantages and disadvantages of the proposed approaches, as well as highlighting remaining challenges and future improvements, and research directions for ncRNA crosstalk detection.

Global computational methods for studying crosstalk among miRNAs: from genomics to phenomics

A number of computational methods have been proposed for identifying the crosstalk among miRNAs, which range from those based on genomic sequence to phenomics. In this section, we discuss several representative global computation methods (Table 1). We consider that global crosstalk among miRNAs represents generalized miRNA relationships without reference to any particular condition(s).

miRNA crosstalk based on genomic similarity

miRNAs regulate mRNAs to facilitate cleavage or translational repression via the complementary binding of a 'seed sequence' where they typically target in the 3′ un-translated region (UTR) of mRNAs. In addition, different miRNA sequences from the same genomic region can bind to each other in a complementary manner (Figure 1A) to affect the transcription of each other. This interesting phenomenon is common in human miRNAs. Guo *et al.* showed that miRNA–miRNA complementary matching pairs can be detected in different species, and some miRNAs pairs are conserved across species [28]. In addition, Xu *et al.* demonstrated that functional synergetic miRNA pairs exhibit high seed sequence similarity [29]. Thus, we may obtain a global view of miRNA crosstalk by analysis of the similarity of seed sequences among miRNAs.

Moreover, mammalian genomes are known to be organized in an intensive manner into a higher-order conformation inside the micron-sized nuclear space. Chromosome Conformation Capture (3C) and similar techniques have demonstrated that chromatin interactions must have roles in the mechanisms of transcriptional regulation and coordination. In addition, our knowledge of the role of higher-order chromatin structures during the transcription of miRNA is evolving rapidly (Figure 1B). Chen *et al.* investigated the effects of three-dimensional (3D) architecture of chromatin on the transcriptional regulation of miRNAs [30]. They also demonstrated the existence of spatial miRNA–miRNA chromatin interacting networks by assembling miRNA pairs that interact with each other at the chromatin level, and showed that groups of spatially coordinated miRNAs frequently come from the same family and they are involved with the same disease category. Recently, Ma *et al.* described a method for comprehensively mapping global chromatin contacts called DNase Hi-C. They applied targeted DNase

Table 1. The commonly used methods to construct the miRNA–miRNA network based on genomics to phenomics

Name	Omics data	Hypothesis	Software	PubMed ID
Guo *et al.*	Genomics (sequence)	miRNA:miRNA duplex with complete complementary structure	No	23031806
Chen *et al.*	Genomics (chromatin interaction)	miRNA interaction at chromatin level	No	24357409
miRWalk2.0	Regulatory omics (predicted or validated miRNA regulations)	Shared target genes	Yes	26226356
DIANA miRPath v.2.0	Regulatory and functional omics (predicted or validated miRNA regulations and KEGG pathway)	Co-regulated similar target genes	Yes	22649059
mirBridge	Genomics and regulatory omics (miRNA regulations and conservation signature)	Co-regulated a list of genes	No	20385095
GeneSet2miRNA	Regulatory omics (predicted miRNA regulations)	Co-regulated a list of genes	Yes	19420064
miRror2.0	Regulatory omics (predicted miRNA regulations)	Co-regulated a list of genes	Yes	22904063
Shalgi *et al.*	Regulatory omics (predicted miRNA regulations)	Function similarity measured by proportion of common target genes	No	17630826
Yu *et al.*	Regulatory and functional omics (miRNA regulations and GO annotation)	GO semantic similarities of target genes	No	–
C2Analyzer	Regulatory and functional omics (miRNA regulations and GO annotation)	Co-functionally enriched in GO terms	Yes	24862384
Mal *et al.*	Regulatory and functional omics (miRNA regulations and GO annotation)	Co-functionally enriched in GO terms	No	26066638
Yoon *et al.*	Regulatory omics (miRNA regulations)	Co-regulated functional modules	No	16204133
miRFunSim	Regulatory and functional omics (miRNA regulations and PPI)	Targeting propensity and proteins connectivity in PPI	No	23874989
Xu *et al.*	Regulatory and functional omics (miRNA regulations and PPI)	Site accessibility and the topology of target gene functional network	No	24149053
Meng *et al.*	Regulatory and functional omics (miRNA regulations and PPI)	Function similarity of target genes	No	26538106
Xu *et al.*	Regulatory and functional omics (miRNA regulations, GO annotation and PPI)	Co-regulated functional modules	No	20929877
Zhu *et al.*	Regulatory and functional omics (miRNA regulations and PPI)	Co-regulation and functional association of target genes	No	23691029
Wang *et al.*	Phenomics (miRNA–phenotype association)	miRNA FS by measuring the similarity of their associated disease DAG	Yes	20439255

Hi-C to characterize the 3D organization of 998 ncRNA promoters in two human cell lines and, showed that the expression of ncRNAs is tightly controlled by complex mechanisms involving both super-enhancers and the Polycomb repressive complex [31]. To store the chromatin interaction data obtained by comprehensive literature curation, the 4DGenome database was proposed to store both low- and high-throughput assays, including 3C, 4C-Seq, 5C, Hi-C, ChIA-PET and Capture-C data sets [32]. These chromatin data sets provide valuable resources for investigating the crosstalk among ncRNAs, including miRNAs, at the chromatin level.

miRNA crosstalk based on regulatory omics

miRNAs function though their regulated target genes and many miRNA target identification methods have been proposed, which were reviewed by Fan *et al.* [33]. A simple method for detecting cooperation between miRNAs is identifying miRNA pairs that co-regulate at least one target. In particular, some studies have attempted to identify miRNA pairs that co-occur in a large set of shared targets compared with the number expected by chance (Figure 1C). A common statistical test for this purpose is the cumulative hypergeometric statistic. miRWalk2.0 is a comprehensive archive of miRNA–target interactions, which also provides a framework for obtaining miRNA pairs that significantly co-regulate genes [34]. In addition, DIANA miRPath v.2.0 provides two approaches (union or intersection of targets) for investigating combinations of miRNAs [35]. These methods mainly use the hypergeometric test to investigate the significance of co-regulating target genes. However, given that the length of 3′ UTRs can vary, the assumption of the hypergeometric test may no longer hold. Some targets with long 3′ UTRs are likely to contain more binding sites for miRNAs, so a *P*-value calculated based on the hypergeometric test may overestimate the co-regulation rate. Thus, Shalgi *et al.* proposed an alternative, randomization-based test for identifying co-regulating miRNA pairs [35]. In this model, they first calculate the 'meet/min' score for each pair of miRNAs, before using an edge-swapping algorithm to generate the randomized miRNA-gene regulations. For each randomized miRNA-gene regulation, the score is computed repeatedly for all the pairs of miRNAs. The *P*-value for a pair of miRNAs is defined as the fraction of the randomized conditions where the score for that pair is greater than or equal to the original score.

In addition, specific statistical methods have been proposed for identifying miRNA pairs that co-regulate a list of genes. Tsang *et al.* introduced mirBridge [36] to consider the crosstalk among miRNAs based on gene sets to further elucidate the functions of miRNAs. Specifically, mirBridge first computes a score by combining the results of three statistical tests to evaluate different aspects of likely functional site enrichment for a given gene set. The miRNAs grouped into the same families are then assumed to function together by regulating common targets. GeneSet2miRNA [37] and miRror2.0 [38] are similar tools

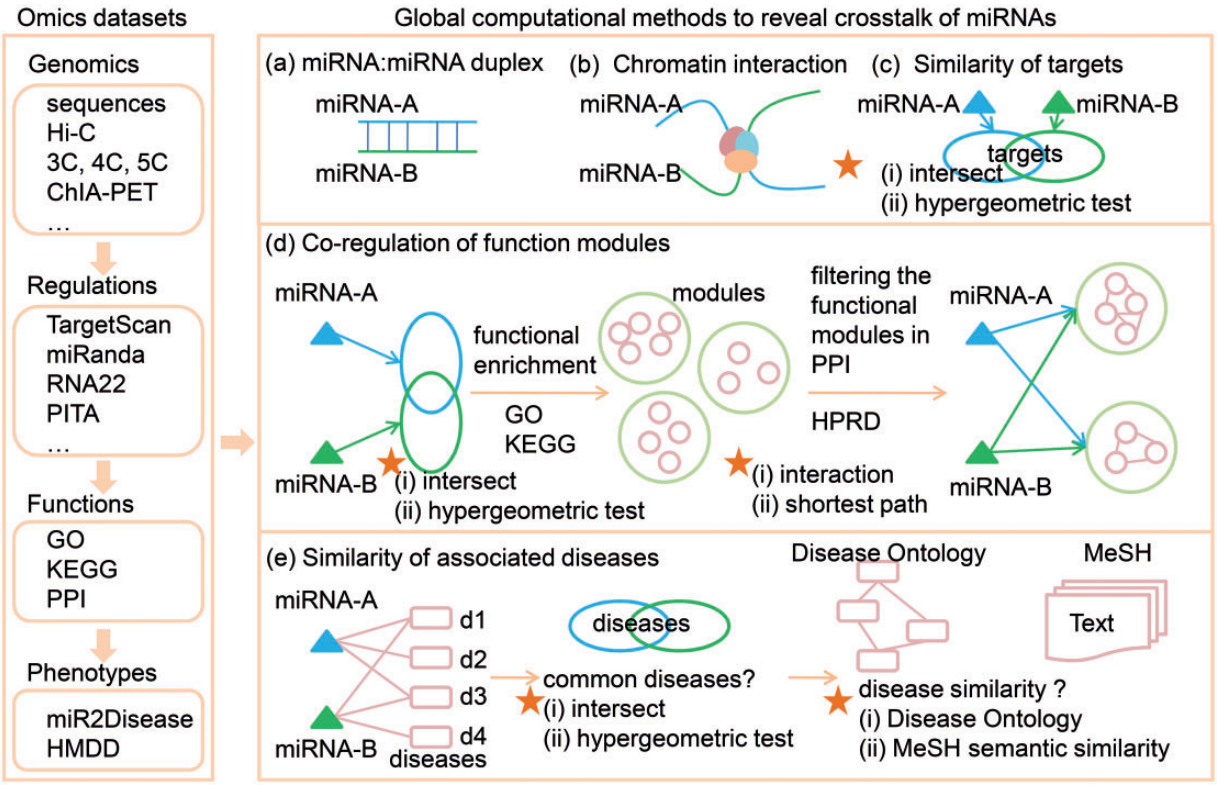

Figure 1. Identify the crosstalk among miRNAs from genomics to phenomics. (**A**) The methods based on genomic sequence information; (**B**) the methods based on chromatin interaction; (**C**) the methods based on the miRNA-gene regulations; (**D**) the methods based on co-regulation of function modules; (**E**) the methods based on similarity of associated diseases (phenomic similarity).

for exploring the links between miRNAs and a gene set. GeneSet2miRNA takes a list of target genes as an input and performs the hypergeometric test to examine whether there is a regulatory association between the miRNAs and the gene set. This tool may evaluate the cooperation among up to four miRNAs. miRror estimates the likelihood that the combinatorial effects of miRNAs can explain the observed data. These gene-set-based tools have identified crosstalks among miRNAs, but their performance depends greatly on the gene-sets used, which limits their generalization.

miRNA crosstalk based on functional omics

In terms of the co-regulation of targets, another common assumption is that the genes regulated by miRNA groups should behave in a similar functional manner. Thus, many methods have been proposed for identifying miRNA pairs with similar functions. However, the functions of most miRNAs are unknown. To better understand the crosstalk among miRNAs, it is necessary to measure their functional similarity (FS). Many successful methods have been proposed for determining the FS of coding genes [39–41], but some of them are limited to infer the FS of miRNAs. The simplest method reported by Shalgi *et al.* defines the miRNA function similarity as the proportion of common targets [42], where they used the Jaccard similarity between two target gene lists for two miRNAs to measure the crosstalk among miRNAs. However, the functions of the targets are not considered in this method. In addition, Yu *et al.* proposed a second method for inferring the FS of miRNAs by using the semantic similarities according to Gene Ontology (GO) for their target genes (Figure 1D). The FS can also be represented as the likelihood of the co-regulated genes sharing the same GO or Kyoto Encyclopedia of Genes and Genomes (KEGG) categories, which is usually identified by the hypergeometric test. Mal *et al.* constructed an miRNA–miRNA functional synergistic network in rice by considering the co-targeting genes in the context of GO annotations [43], where they defined them as synergistic if at least one GO category is significantly co-regulated by a pair of miRNAs. The Cotarget–Co-function Analyzer [44] is a Perl-based, versatile and user-friendly web tool for determining whether given pairs of miRNAs are co-functionally enriched.

Owing to the absence of functional databases such as GO for annotating some coding genes, gene functional networks are also used to infer the FS of miRNAs. The first study in this area was performed by Yoon *et al.* [44], who introduced the concept of miRNA regulatory modules to define the coordinated activity of miRNAs with their targets. They demonstrated the performance of the proposed method on only one of the modules using enriched GO annotations. In addition, protein–protein interactions (PPIs) involve the FS between proteins. It has been shown that interacting proteins are regulated by similar miRNA types [45] and clustered miRNAs also jointly regulate proteins that are close in the network, where the number of co-regulations between proteins is negatively correlated with their distance in the network [46]. Subsequent studies proposed PPI rules for defining the FS of miRNAs. Thus, Sun *et al.* proposed a novel graph theoretic property-based computational framework and method [47] called miRFunSim, for quantifying the associations between miRNAs based on the targeting propensity of miRNAs and the connectivity of protein in the integrated PPI network. They found that the FS scores of miRNAs in the same family or cluster were significantly higher compared with other miRNAs, which is consistent with existing knowledge. Moreover, Xu *et al.* proposed a new method for measuring the FS of miRNAs by considering

both the site accessibility and the interactive context of target genes in functional gene networks [48]. They applied this method to soybean and investigated the crosstalk among miRNAs in soybean. In these studies, GO and PPI were used mainly to obtain functional information for investigating the crosstalk among miRNAs. Recently, PPI network with semantic similarity weights generated using GO terms was used to calculate the FS of miRNAs [49], where the experimental results showed that the proposed method was more effective and reliable than previous methods. Moreover, Xu *et al.* [50] constructed an miRNA–miRNA functional synergistic network via co-regulating functional modules, which were usually defined as groups of interacting genes with more edges inside the module compared with the other genes in the network (Figure 1D). The functional modules were defined based on three features: common targets of corresponding miRNA pairs, enriched in the same GO category and close proximity in the PPI network. These studies indicate that GO and PPI functional annotations can contribute jointly to synergistic miRNA identification. Thus, Zhu *et al.* proposed an integrated parameter synergy score by combining the miRNA-mediated gene co-regulation and functional association between targets into a single parameter [16], where they demonstrated that this synergy score can accurately identify the GO-defined miRNA synergy.

miRNA crosstalk based on phenomics

It has been reported that genes or miRNAs with similar functions are often implicated in similar diseases, and vice versa. Thus, a commonly used method is to identify miRNA crosstalk based on their associated diseases (Figure 1E). However, few disease miRNAs are known because the limited number of miRNA functional annotations and diseases are not independent. According to previous research, similar to GO, the relationships between different diseases can be represented in a directed acyclic graph (DAG) structure, which allows the inference of miRNA FS based on their associated diseases. Wang *et al.* described a method for inferring the pairwise FS of human miRNAs based on the structures of their disease relationships [51], where the results showed that the calculated miRNA FS was strongly associated with the prior miRNA functional relationship. Importantly, the proposed method can also be used to predict novel miRNA biomarkers and to infer novel potential functions or associated diseases for miRNAs.

Context-specific computational methods for determining crosstalk among miRNAs: transcriptome integrated methods

The global methods mentioned in the section above focus on miRNA–miRNA networks at a global level. However, a major limitation of these approaches is that we might expect miRNA crosstalk to be reprogrammed in different biological contexts. To address this limitation, methods have been proposed for modeling context-specific miRNA–miRNA networks (Table 2). These context-specific miRNA–miRNA networks are mainly constructed in the same manner as the global methods except described above but by considering two main features (Figure 2): (A) differentially expressed (DE) miRNAs or genes; and (B) context-specific miRNA–mRNA regulation. Next, we introduce some representative methods for constructing the miRNA–miRNA networks for specific diseases.

By analyzing the publicly available microarray data set of miRNA expression, Chaulk *et al.* investigated the miRNA–miRNA co-expression and showed that the co-expression groups of miRNAs have similar biological activities [52]. In addition, by integrating the miRNA and mRNA expression profiles of colorectal cancers, Yin *et al.* first identified the DE miRNAs and genes [53], before extracting the regulation of DE miRNAs relative to the DE genes in the miRNA target gene database (TargetScan). The synergistic relationships between DE miRNAs were identified based on two restrictions: their target genes overlapped in a nonrandom manner and the overlapping targets were significantly enriched in pathways. In addition, Hua *et al.* proposed the construction of a coronary artery disease (CAD)-related miRNA–miRNA synergistic network by combining miRNA expression data with genome-wide single nucleotide polymorphism (SNP) genotyping [54]. This process involves three main steps. First, the DE miRNAs are identified using CAD-related miRNA express profiling data and the miRNASNP tool is then used to extract DE miRNAs and 3′-UTR SNP pairs. Finally, logistic regression is used to detect the significant interactions among 3′-UTR target SNPs of DE miRNAs. The miRNA–miRNA pairs are identified based on the corresponding SNP pairs. In another study, Xiao *et al.* identified the functional synergistic relationships among DE miRNAs in ischemic stroke [55], where miRNAs that can commonly regulate at least one target gene were used to construct an miRNA–miRNA network and these miRNA pairs were then filtered based on the co-regulation of functional modules. By integrating miRNA and mRNA expression profiles, Alshalalfa *et al.* investigated mRNA-mediated miRNA–miRNA interactions based on conditional mutual information [56].

In addition to focusing on DE miRNAs and genes, increasing evidence suggested that miRNA–mRNA regulation is context specific. Many studies have combined the computational target predictions obtained at the sequence level and the inverse expression relationships between miRNAs and mRNAs in the context of specific diseases to construct functional miRNA–mRNA regulatory networks (Figure 2B), before identifying the crosstalk among miRNAs using the global methods mentioned above. Na *et al.* created miRNA association network using miRNAs that share target genes based on the sequence binding and co-expression patterns of miRNA–target pairs [57], where they then applied this method to circadian rhythm, actinomycinD treatment, prostate cancer and radiation treatment. In another study, Hua *et al.* analyzed miRNA and mRNA dual expression profiling data obtained from the same breast cancer subtypes samples and identified the dysregulated miRNA–mRNA pairs. They then identified the miRNA–miRNA network for each subtype by co-targeting at least one gene. They found that luminal A and basal-like subtype-specific networks exhibited changes in the hubs that connected the most miRNAs [58]. In addition, Li *et al.* proposed a novel model called Mirsynery to integrate miRNA/mRNA expression profiles, target site information and PPIs [59]. After applying this method to ovarian, breast and thyroid cancer, they proposed several prognostically promising cancer-specific miRNA regulatory modules as biomarkers. Yang and Ying *et al.* also constructed miRNA–miRNA networks in the context of breast and ovarian cancer by integrating GO annotations [60, 61]. Recently, Xiao *et al.* proposed a multistep method for identifying dysfunctional miRNA–mRNA regulatory modules in a specific disease by considering that an miRNA cooperatively regulates a group of targets involved with a specific function [62]. This method first identifies the DE miRNAs and genes, and the inverse regulations among miRNAs and genes. The cooperative functional units, in each of which a pair of miRNAs cooperative repressed function-enriched and highly

Table 2. The methods integrated transcriptome to construct miRNA–miRNA networks

Name	Omics data	Hypothesis	Context	PubMed ID
Chaulk *et al.*	miRNA expression	Co-expression	Multiple context	26563430
Yin *et al.*	miRNA, mRNA expression profiles; miRNA-mRNA regulations	DE genes and miRNAs	Colorectal cancer	23246904
Hua *et al.*	miRNA expression profiles; SNP	DE miRNAs; associated with common SNP	Coronary artery disease	25641175
Xiao *et al.*	miRNA expression profiles; miRNA-mRNA regulations; GO annotation	DE miRNAs	Ischemic stroke	25108467
Alshalalfa *et al.*	miRNA, mRNA expression profiles; miRNA-mRNA regulations	Conditional mutual information	Prostate cancer	23193399
Na *et al.*	miRNA, mRNA expression profiles; miRNA-mRNA regulations	Context-specific inverse co-expression	Circadian rhythm; ActinomycinD; Prostate cancer; Radiation	24552551
Hua *et al.*	miRNA, mRNA expression profiles; miRNA-mRNA regulations	Context-specific co-expression of miRNA and mRNA	Breast cancer subtype	23619378
Mirsynergy	miRNA, mRNA expression profiles; miRNA-mRNA regulations; gene–gene interactions	Context-specific inverse co-expression	Ovarian cancer; Breast cancer; Thyroid cancer	24894504
Yang *et al.*	miRNA, mRNA expression profiles; miRNA-mRNA regulations; GO annotation	Context-specific expression co-functionality	Breast cancer	25680412
Ying *et al.*	miRNA expression profiles; miRNA-mRNA regulations; GO annotation	DE miRNAs; Co-functionality	Ovarian cancer	24444095
Xiao *et al.*	miRNA, mRNA expression profiles; miRNA-mRNA regulations; GO annotation and PPI	DE miRNAs, mRNAs; coregulated functional modules	Glioblastoma	23516263
Zhang *et al.*	miRNA expression profiles; miRNA-phenotype relations	Coexpressed miRNAs	Small-cell Lung Cancer	23464461
Song *et al.*	miRNA expression profiles; miRNA-mRNA regulations; GO annotation and PPI	Coexpressed miRNAs; co-regulated functional modules	Lung cancer	26026830
Meng *et al.*	miRNA, mRNA expression profiles; miRNA-mRNA regulations and PPI	Context-specific inverse co-expression proximity in PPI	33 cancer types	26690544

interconnected targets, were identified. They applied this method to glioblastoma (GBM) and identified GBM-associated miRNA regulatory modules at the population, subtype and individual levels. Furthermore, some studies have considered the co-expression of miRNA pairs to filter the identified miRNA–miRNA pairs. For example, Zhang *et al.* analyzed the crosstalk among miRNAs in small cell lung cancer based on the co-expression and co-regulation of miRNA pairs [63]. In addition, Song *et al.* identified lung cancer miRNA–miRNA co-regulation networks using a progressive data refinement approach, where the co-expression miRNA pairs are identified first before filtering them based on the co-regulation of functional modules [64]. According to a literature survey and database validation, many of their results regarding lung cancer are biologically meaningful. All of these studies have demonstrated the importance of miRNA synergism in cancer, but most focused mainly on a specific type of cancer, thus a pattern analysis across diverse cancer types is needed. Recently, Meng *et al.* constructed cancer-specific miRNA–miRNA synergistic network for 33 human cancer types, which were stored in the CancerNet database [65]. This is a useful resource for assessing the roles of protein and miRNAs, as well as their interactions across cancers.

Conclusions and future directions

Elucidating the crosstalk among multiple miRNAs is important for understanding the complex mechanisms posttranscriptional regulations. Complex diseases, including cancers, are affected by several miRNAs rather than a single miRNA. Thus, it is important to identify miRNA synergisms and to further determine the functions of miRNAs at a system-wide level. Previous biological theories indicate that researchers can use computational methods to infer the crosstalk among miRNAs. In this review, we summarized the computational methods that are available for constructing the global and context-specific miRNA–miRNA networks, which are useful for experimental biologists and bioinformatics specialists. For experimental biologists, we summarized the relative strengths and limitations of these commonly used methods, as well as providing suggestions for selecting a suitable method. In addition, this summary provides important insights for bioinformatics specialists, who can develop more accurate and efficient methods for identifying miRNA crosstalk. A key issue when investigating an miRNA–miRNA network is computing the similarities of the two miRNAs. Thus, we described the commonly used approaches for determining similarity measures using data ranging from genomics to phenomics. In general, the crosstalk among miRNAs can be determined by considering the sequence, chromatin interaction, co-regulation of target genes, semantic similarity of the targets, co-regulation of functional modules and co-association of diseases. However, most of these approaches are based on miRNA-gene regulation, but miRNA-gene assignments are not reliable because they are usually identified by miRNA-target prediction tools, which may contain incorrect data. Hence, researchers should aim to obtain more comprehensive miRNA–miRNA networks by using highly related biological information, such as GO and PPI. These methods for integrating predicted targets and functional information

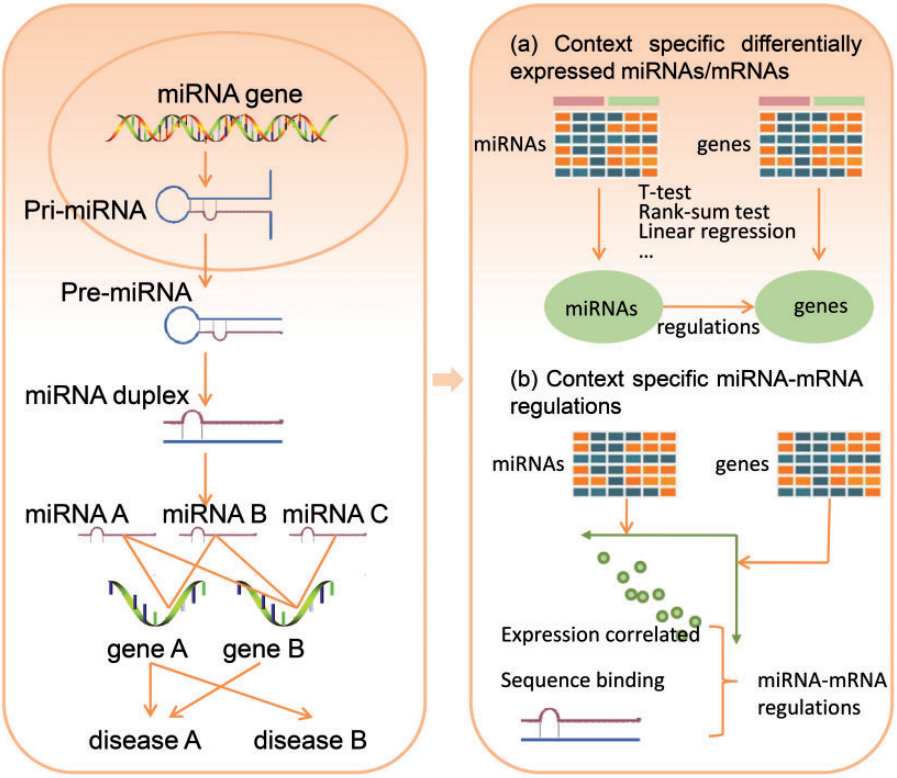

Figure 2. Transcriptome integrated approaches for identifying the context-specific crosstalk among miRNAs. (**A**) The methods based on context-specific DE miRNAs and/or mRNAs; (**B**) the methods based on context-specific miRNA-mRNA regulations.

could identify synergistic miRNA pairs and as well as elucidating their underlying functions. In addition, the crosslinking-immunoprecipitation and high-throughput sequencing data have been used to annotate the functional targeting sites of miRNAs, but little effort has been made to use these data as a filter to improve the specificity of currently available prediction methods for miRNA–miRNA crosstalk.

Given that miRNA crosstalk is often reprogrammed in different tissues, or different biological stages even within the same tissues [66], then context-specific miRNA–miRNA networks may provide a better representation. To identify context-specific networks, the methods proposed for *de novo* network reconstruction in recent studies only operate on miRNAs or genes that are DE. However, these approaches might not capture miRNA subnetworks that have been filtered out by expression. Other methods are based on context-specific miRNA-gene regulation, but these methods may be limited by the need for the paired miRNA/mRNA expression profiles of the same patients. With the development of genomic data for human diseases, e.g. cancer-related data from The Cancer Genome Atlas project, so the construction of cancer-specific miRNA–miRNA networks may be particularly useful for inferring cancer-specific miRNA biomarkers.

Furthermore, long noncoding RNAs (lncRNAs) are RNA molecules comprising >200 nucleotides, which are not translated into proteins. lncRNAs comprise a major but still poorly characterized component of the human transcriptome. Most of the functions of lncRNAs are unknown, but the existing examples of characterized lncRNAs demonstrate their widespread participation in biological functions [67]. Increasing evidence suggests that lncRNAs play key roles in the life cycle of the cell and that their dysfunction can lead to various diseases, including cancer. Evidence also indicates that miRNA can also engage in crosstalk with lncRNA, but the identification of miRNA–lncRNA interactions is mainly blocked by the conservation of lncRNAs. At present, there are a limited number of miRNA-lncRNA prediction methods, but several examples suggest that lncRNAs can also contribute cooperatively to tumorigenesis by directly modulating oncogenesis or tumor suppressor pathways. For example, some lncRNAs form part of the large pool of genes coordinated by the P53 transcriptional factor, but they are also required to fine-tune the p53 response and to fully complete its tumor suppressor program [68]. In addition, it has been reported that combined lncRNA signatures may facilitate more accurate predictions of patient survival than individual lncRNAs [69]. However, compared with miRNAs, the identification of crosstalk among lncRNAs is a more challenging task because the targets of lncRNAs are mainly unknown. Recently, Li *et al.* extended a previous approach for identifying significant functional synergistic lncRNA pairs based on the functional modules that they jointly regulate [70]. They applied this approach to three cancers, and cancer-specific lncRNA–lncRNA networks were constructed before identifying prognostic biomarkers using the modules in these networks. This proof-of-principle study indicates that synergistic lncRNA pairs can be identified by the integrative analysis of genome-wide expression data sets and functional information. In addition, this study also demonstrated that previous approaches used for identifying miRNA crosstalk can be extended to lncRNAs.

Recent network-based methods for prioritizing disease-related miRNAs have proved useful in cancer research [71, 72]. Integrating miRNA–miRNA network data to identify the associations between miRNAs and diseases can help researchers to obtain better performance. In this review, we described

representative methods in the field of miRNA–miRNA relationship network modeling, but it was not possible to include all of the available methods. Moreover, as numerous computational methods are available, it is difficult to decide the best method because they are often complementary to each other. We consider that the use of each method depends on the type of data set or the confidence of the miRNA–miRNA relationships that need to be identified. There is a lack of large-scale power comparisons among these methods, but the users can select a particular method by understanding the relative strengths and limitations of these commonly used methods. Single data set-based methods (such as those based on genomic sequences, chromatin similarity or miRNA–mRNA regulation) are simple to realized. However, these methods may yield many combinations of miRNA pairs with a higher false positive rate. Moreover, the increased volume of high-throughput data means that integrative methods (such as methods based on co-regulating functional modules) may be good candidates. Because these methods can dramatically benefit from each integrated data set and reduce the number of false positives. In addition, these methods can identify miRNA crosstalk as well as revealing their underlying functional patterns. Given that the miRNA–miRNA crosstalk may be reprogrammed in different contexts, integrated methods based on transcriptome data may provide a better representation of miRNA crosstalk in a specific context. However, these methods may be limited by the requirement for paired miRNA/mRNA expression profiles. In general, all of the methods described in the present review have demonstrated the importance of miRNA synergism as well as indicating that the integration of functional information with context-specific genomic data sets can yield more accurate inferences. With the increase in omics data set of complex diseases, it is envisioned that these approaches may be applied to more biological systems in the future.

Key Points

- Functional miRNA–miRNA crosstalk helps to improve our understanding of complex regulatory networks in normal and disease-specific physiological condition.
- Numerous approaches have been proposed for modeling miRNA crosstalk, which can range from genomics to phenomics.
- We reviewed the representative state-of-the-art miRNA–miRNA network inference methods, with an emphasis on the integration of multiple omics data sets.
- Numerous computational methods are available but it is difficult to decide which method is best because they are often complementary to each other.

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References

1. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;**116**:281–97.
2. Ambros V. The functions of animal microRNAs. *Nature* 2004;**431**:350–5.
3. Sun K, Lai EC. Adult-specific functions of animal microRNAs. *Nat Rev Genet* 2013;**14**:535–48.
4. Kloosterman WP, Plasterk RH. The diverse functions of microRNAs in animal development and disease. *Dev Cell* 2006;**11**:441–50.
5. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009;**136**:215–33.
6. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. *Trends Mol Med* 2014;**20**:460–9.
7. Cummins JM, Velculescu VE. Implications of micro-RNA profiling for cancer diagnosis. *Oncogene* 2006;**25**:6220–7.
8. Bentwich I, Avniel A, Karov Y, *et al*. Identification of hundreds of conserved and nonconserved human microRNAs. *Nat Genet* 2005;**37**:766–70.
9. Migliore C, Giordano S. MiRNAs as new master players. *Cell Cycle* 2009;**8**:2185–6.
10. Vella MC, Choi EY, Lin SY, *et al*. The C. elegans microRNA let-7 binds to imperfect let-7 complementary sites from the lin-41 3'UTR. *Genes Dev* 2004;**18**:132–7.
11. Seggerson K, Tang L, Moss EG. Two genetic circuits repress the Caenorhabditis elegans heterochronic gene lin-28 after translation initiation. *Dev Biol* 2002;**243**:215–25.
12. John B, Enright AJ, Aravin A, *et al*. Human MicroRNA targets. *PLoS Biol* 2004;**2**:e363.
13. Krek A, Grun D, Poy MN, *et al*. Combinatorial microRNA target predictions. *Nat Genet* 2005;**37**:495–500.
14. Ivanovska I, Cleary MA. Combinatorial microRNAs: working together to make a difference. *Cell Cycle* 2008;**7**:3137–42.
15. Yang X, Du WW, Li H, *et al*. Both mature miR-17-5p and passenger strand miR-17-3p target TIMP3 and induce prostate tumor growth and invasion. *Nucleic Acids Res* 2013;**41**:9688–704.
16. Zhu W, Zhao Y, Xu Y, *et al*. Dissection of protein interactomics highlights microRNA synergy. *PLoS One* 2013;**8**:e63342.
17. Wang S, Huang J, Lyu H, *et al*. Functional cooperation of miR-125a, miR-125b, and miR-205 in entinostat-induced downregulation of erbB2/erbB3 and apoptosis in breast cancer cells. *Cell Death Dis* 2013;**4**:e556.
18. Wahdan-Alaswad R, Liu B. "Sister" miRNAs in cancers. *Cell Cycle* 2013;**12**:3703–4.
19. Hrdlickova R, Nehyba J, Bargmann W, *et al*. Multiple tumor suppressor microRNAs regulate telomerase and TCF7, an important transcriptional regulator of the Wnt pathway. *PLoS One* 2014;**9**:e86990.
20. Calin GA, Ferracin M, Cimmino A, *et al*. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med* 2005;**353**:1793–801.
21. He L, Thomson JM, Hemann MT, *et al*. A microRNA polycistron as a potential human oncogene. *Nature* 2005;**435**:828–33.
22. Lee YS, Dutta A. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. *Genes Dev* 2007;**21**:1025–30.
23. Wu S, Huang S, Ding J, *et al*. Multiple microRNAs modulate p21Cip1/Waf1 expression by directly targeting its 3' untranslated region. *Oncogene* 2010;**29**:2302–8.
24. Peter ME. Targeting of mRNAs by multiple miRNAs: the next step. *Oncogene* 2010;**29**:2161–4.
25. Du L, Schageman JJ, Subauste MC, *et al*. miR-93, miR-98, and miR-197 regulate expression of tumor suppressor gene FUS1. *Mol Cancer Res* 2009;**7**:1234–43.

26. Frampton AE, Castellano L, Colombo T, *et al*. MicroRNAs cooperatively inhibit a network of tumor suppressor genes to promote pancreatic tumor growth and progression. *Gastroenterology* 2014;**146**:268–77.e218.
27. Chen HH, Huang WT, Yang LW, *et al*. The PTEN-AKT-mTOR/RICTOR pathway in nasal natural killer cell lymphoma is activated by miR-494-3p via PTEN but inhibited by miR-142-3p via RICTOR. *Am J Pathol* 2015;**185**:1487–99.
28. Guo L, Sun B, Wu Q, *et al*. miRNA-miRNA interaction implicates for potential mutual regulatory pattern. *Gene* 2012;**511**:187–94.
29. Xu J, Li Y, Li X, *et al*. Dissection of the potential characteristic of miRNA-miRNA functional synergistic regulations. *Mol Biosyst* 2013;**9**:217–24.
30. Chen D, Fu LY, Zhang Z, *et al*. Dissecting the chromatin interactome of microRNA genes. *Nucleic Acids Res* 2014;**42**:3028–43.
31. Ma W, Ay F, Lee C, *et al*. Fine-scale chromatin interaction maps reveal the cis-regulatory landscape of human lincRNA genes. *Nat Methods* 2015;**12**:71–8.
32. Teng L, He B, Wang J, *et al*. 4DGenome: a comprehensive database of chromatin interactions. *Bioinformatics* 2015;**31**:2560–4.
33. Fan X, Kurgan L. Comprehensive overview and assessment of computational prediction of microRNA targets in animals. *Brief Bioinform* 2015;**16**:780–94.
34. Dweep H, Gretz N. miRWalk2.0: a comprehensive atlas of microRNA-target interactions. *Nat Methods* 2015;**12**:697.
35. Vlachos IS, Kostoulas N, Vergoulis T, *et al*. DIANA miRPath v.2.0: investigating the combinatorial effect of microRNAs in pathways. *Nucleic Acids Res* 2012;**40**:W498–504.
36. Tsang JS, Ebert MS, van Oudenaarden A. Genome-wide dissection of microRNA functions and cotargeting networks using gene set signatures. *Mol Cell* 2010;**38**:140–53.
37. Antonov AV, Dietmann S, Wong P, *et al*. GeneSet2miRNA: finding the signature of cooperative miRNA activities in the gene lists. *Nucleic Acids Res* 2009;**37**:W323–8.
38. Balaga O, Friedman Y, Linial M. Toward a combinatorial nature of microRNA regulation in human cells. *Nucleic Acids Res* 2012;**40**:9404–16.
39. Pesquita C, Faria D, Bastos H, *et al*. Metrics for GO based protein semantic similarity: a systematic evaluation. *BMC Bioinformatics* 2008;**9**(Suppl 5):S4.
40. Pesquita C, Faria D, Falcao AO, *et al*. Semantic similarity in biomedical ontologies. *PLoS Comput Biol* 2009;**5**:e1000443.
41. Harispe S, Ranwez S, Janaqi S, *et al*. The semantic measures library and toolkit: fast computation of semantic similarity and relatedness using biomedical ontologies. *Bioinformatics* 2014;**30**:740–2.
42. Shalgi R, Lieber D, Oren M, *et al*. Global and local architecture of the mammalian microRNA-transcription factor regulatory network. *PLoS Comput Biol* 2007;**3**:e131.
43. Mal C, Deb A, Aftabuddin M, *et al*. A network analysis of miRNA mediated gene regulation of rice: crosstalk among biological processes. *Mol Biosyst* 2015;**11**:2273–80.
44. Aftabuddin M, Mal C, Deb A, *et al*. C2Analyzer: co-target-co-function analyzer. *Genomics Proteomics Bioinformatics* 2014;**12**:133–6.
45. Liang H, Li WH. MicroRNA regulation of human protein–protein interaction network, *RNA* 2007;**13**:1402–8.
46. Yuan X, Liu C, Yang P, *et al*. Clustered microRNAs' coordination in regulating protein-protein interaction network. *BMC Syst Biol* 2009;**3**:65.
47. Sun J, Zhou M, Yang H, *et al*. Inferring potential microRNA-microRNA associations based on targeting propensity and connectivity in the context of protein interaction network. *PLoS One* 2013;**8**:e69719.
48. Xu Y, Guo M, Liu X, *et al*. Inferring the soybean (Glycine max) microRNA functional network based on target gene network. *Bioinformatics* 2014;**30**:94–103.
49. Meng J, Liu D, Luan Y. Inferring plant microRNA functional similarity using a weighted protein-protein interaction network. *BMC Bioinformatics* 2015;**16**:360.
50. Xu J, Li CX, Li YS, *et al*. MiRNA-miRNA synergistic network: construction via co-regulating functional modules and disease miRNA topological features. *Nucleic Acids Res* 2011;**39**:825–36.
51. Wang D, Wang J, Lu M, *et al*. Inferring the human microRNA functional similarity and functional network based on microRNA-associated diseases. *Bioinformatics* 2010;**26**:1644–50.
52. Chaulk SG, Ebhardt HA, Fahlman RP. Correlations of microRNA:microRNA expression patterns reveal insights into microRNA clusters and global microRNA expression patterns. *Mol Biosyst* 2016;**12**:110–9.
53. Zhao X, Song H, Zuo Z, *et al*. Identification of miRNA-miRNA synergistic relationships in colorectal cancer. *Int J Biol Macromol* 2013;**55**:98–103.
54. Hua L, Xia H, Zhou P, *et al*. Combination of microRNA expression profiling with genome-wide SNP genotyping to construct a coronary artery disease-related miRNA-miRNA synergistic network. *Biosci Trends* 2014;**8**:297–307.
55. Xiao S, Ma Y, Zhu H, *et al*. miRNA functional synergistic network analysis of mice with ischemic stroke. *Neurol Sci* 2015;**36**:143–8.
56. Alshalalfa M. MicroRNA Response Elements-Mediated miRNA-miRNA Interactions in Prostate Cancer. *Adv Bioinformatics* 2012;**2012**:839837.
57. Na YJ, Kim JH. Understanding cooperativity of microRNAs via microRNA association networks. *BMC Genomics* 2013;**14**(Suppl 5):S17.
58. Hua L, Zhou P, Li L, *et al*. Prioritizing breast cancer subtype related miRNAs using miRNA-mRNA dysregulated relationships extracted from their dual expression profiling. *J Theor Biol* 2013;**331**:1–11.
59. Li Y, Liang C, Wong KC, *et al*. Mirsynergy: detecting synergistic miRNA regulatory modules by overlapping neighbourhood expansion. *Bioinformatics* 2014;**30**:2627–35.
60. Yang Y, Xing Y, Liang C, *et al*. Crucial microRNAs and genes of human primary breast cancer explored by microRNA-mRNA integrated analysis. *Tumour Biol* 2015;**36**:5571–9.
61. Ying H, Lyu J, Ying T, *et al*. Risk miRNA screening of ovarian cancer based on miRNA functional synergistic network. *J Ovarian Res* 2014;**7**:9.
62. Xiao Y, Ping Y, Fan H, *et al*. Identifying dysfunctional miRNA-mRNA regulatory modules by inverse activation, cofunction, and high interconnection of target genes: a case study of glioblastoma. *Neuro Oncol* 2013;**15**:818–28.
63. Zhang TF, Cheng KW, Shi WY, *et al*. MiRNA synergistic network construction and enrichment analysis for common target genes in small-cell lung cancer. *Asian Pac J Cancer Prev* 2012;**13**:6375–8.
64. Song R, Catchpoole DR, Kennedy PJ, *et al*. Identification of lung cancer miRNA-miRNA co-regulation networks through a progressive data refining approach. *J Theor Biol* 2015;**380**:271–9.
65. Meng X, Wang J, Yuan C, *et al*. CancerNet: a database for decoding multilevel molecular interactions across diverse cancer types. *Oncogenesis* 2015;**4**:e177.

66. Li Y, Xu J, Chen H, *et al*. Comprehensive analysis of the functional microRNA-mRNA regulatory network identifies miRNA signatures associated with glioma malignant progression. *Nucleic Acids Res* 2013;**41**:e203.
67. Ma H, Hao Y, Dong X, *et al*. Molecular mechanisms and function prediction of long noncoding RNA. *ScientificWorldJournal* 2012;**2012**:541786.
68. Grossi E, Sanchez Y, Huarte M. Expanding the p53 regulatory network: LncRNAs take up the challenge. *Biochim Biophys Acta* 2016;**1859**:200–8.
69. Li J, Chen Z, Tian L, *et al*. LncRNA profile study reveals a three-lncRNA signature associated with the survival of patients with oesophageal squamous cell carcinoma. *Gut* 2014;**63**:1700–10.
70. Li Y, Chen J, Zhang J, *et al*. Construction and analysis of lncRNA-lncRNA synergistic networks to reveal clinically relevant lncRNAs in cancer. *Oncotarget* 2015;**6**:25003–16.
71. Zeng X, Zhang X, Zou Q. Integrative approaches for predicting microRNA function and prioritizing disease-related microRNA using biological interaction networks. *Brief Bioinform* 2016; **17**:193–203.
72. Xu J, Li CX, Lv JY, *et al*. Prioritizing candidate disease miRNAs by topological features in the miRNA target-dysregulated network: case study of prostate cancer. *Mol Cancer Ther* 2011;**10**:1857–66.