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Network based multifactorial modelling microRNA-target interaction.

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ABSTRACT

Competing endogenous RNA (ceRNA) regulations and crosstalk between various types of non-coding RNA in human is remarkable in means of miRNA regulation. Many studies have pointed out that an alteration in miRNA:target interaction can result in unexpected changes due to indirect and complex interactions. In this paper, we defined a new network-based model that handles miRNA:ceRNA interactions with expression values. Our model is able to handle miRNA interaction factors such as seed type, binding energy, if provided. Our approach is able to reveal that a perturbation in an element of network affects whole competing elements differently and cooperative efficiencies of miRNAs on common targets could be calculated. Our findings emphasized importance of miRNA:target ratios being crucial, as reported by previous studies. We have showed that the competing elements which have the same or close expression values may not be affected equally from the perturbation because of repression functionality depended on interaction factors of miRNA target pairs. We applied the model to real sample consisting of breast cancer gene and miRNA expression dataset and experimental miRNA:target interaction dataset all generated via high throughput sequencing methods. A gene over-expressed in tumor tissue, namely SERPINE2, is used as perturbing element. We have observed that change in expression level of single gene in miRNA:target network is sufficient to perturb regulations in whole network, due to unforeseen and unpredicted regulation which are only visible when considered in network context. Therefore, this model helps unveiling the crosstalk between elements in miRNA:target network where abundance of target and sponge effect are taken into account. The model is scalable and can be plugged in with emerging miRNA effectors such as circRNAs. The model is available as R package ceRNAnetsim **https://github.com/selcenari/ceRNAnetsim**.

INTRODUCTION

MicroRNAs (miRNAs) are a family of short non-coding RNAs which are key regulator of gene expression through various post-transcriptional mechanisms. Although the mechanisms by which miRNA represses are not fully understood, miRNAs predominantly repress their targets. Repressive activities of miRNAs vary depending on many factors that are significant to miRNA:target interactions. These factors include miRNA:target binding energy, binding location in target sequence, base pairing types between miRNA and target, abundance of miRNAs and targets (1). Binding energies of miRNA:target complexes vary based on nucleotide context and determine folding stability of complex (2). It has been demonstrated that the binding energy between miRNA and target indicates stability or affinity of complex (3) and does not directly determine repressive activity of miRNA (2). Early studies have argued that 2-8 nt sequence, seed, located in miRNA 5'end bind to specific sequence located in 3'UTR of its target (4, 5). In recent studies, it has been shown that miRNAs can interact with targets via sequences located in regions such as 5'UTR or CDS (3, 6, 7). These studies also showed that binding location either indicate functionality of miRNA:target interaction or affect abundance of targets. It has been shown that miRNAs exhibit repressive activity via, 6-8 nt long sequence that is perfectly complementary with targets, seed region at the 5' end of miRNAs (1, 8). On the other hand, some researchers have reported that seed sequence of miRNA can have mismatches or bulged/wobble nucleotides (9) and may locate in region other than 5'end of miRNAs (3, 10). On top of all these factors, abundance of miRNAs and targets and miRNA:target ratio in cells predominantly affect efficiency of miRNA:target interaction (11, 12, 13).

As it is possible for miRNAs to suppress multiple targets, an individual mRNA molecule can also be targeted by multiple miRNAs. In that case, the targeted mRNAs exhibit competitor behavior, that is hypothesized as competing endogenous RNAs (ceRNAs) (14, 15), against their miRNAs. Briefly, Ala et al. have explained the ceRNA hypothesis as disturbance of the other target when one of the targets on a steady-state system that included one miRNA and two target was perturbed with expression change (14). Regarding interaction between miRNAs and their target in a cell, explaining and predicting results of an individual perturbation is difficult due to complexity of interactions. Various computational and experimental studies have tackled the problem of unraveling ceRNA:miRNA interactions.

It has been observed that when abundance of one of the targets of miR-122 was increased, the other targets'

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expression also slightly increased as a result of decreasing repressive activity of miR-122 on them (13). Bosson et al. have developed a mathematical model for changes on total target pool concentration after grouping targets according to affinity and demonstrated that miRNA activity correlated with affinity between miRNA and target (12). Cooperative efficiency of miRNAs as well as competitor behaviors of targets were also studied and it has been demonstrated to be crucial for regulating available mRNA levels of targets (16). MiRNA:target interactions have been modeled as stoichiometric and catalytic mechanisms and Figliuzzi et al. have recommended handling models in network context (17). The model that can explain miRNA target interaction through topological features has been applied at bipartite network by Nitzan et al. (18). Robinson and Henderson applied the model that handles miRNA:target direct and indirect interactions via common targeting miRNA of genes and target of miRNAs, at bipartite network. It has been demonstrated that all miRNAs and targets in the network can interact with each other through common miRNAs and genes, without interaction between the same type of nodes (19).Associated genes that are targets of the same miRNAs have been found with help of correlation of gene expression changes in recent algorithm (20). List et al. have specified that their approach can be useful for ceRNA studies and published their approach as an R package.

MATERIALS AND METHODS

Construction of miRNA:target network

miRNA and target pairs per line should be provided as edge list to construct the network. At each line minimum required information is expression levels of miRNA and the target. If available, additional data about factors effecting binding or efficiency of miRNA can be provided as separate columns (see Table S1 in Supplementary Tables). After construction of the network, amount of miRNA per target is calculated and kept as edge data. Simply, a target will sequester miRNA proportional to its ratio amount among other targets. If additional criteria effecting the binding of miRNA to its target is provided, distribution of miRNA will be calculated accordingly (available at Table S6 in Supplementary Tables). Target can be mRNA or any other ceRNA (circRNA, ncRNA, etc.) thus, throughout the manuscript terms target, gene and ceRNA are used interchangeably.

Triggering perturbation and subsequent calculations

Initially, the network is assumed in steady-state (Figure 1A and Figure S2) condition and needs least one trigger for initiating calculations. The trigger can be a change in expression level of one or more genes (Figure 1B and Figure S3). After a trigger, the network undergoes iterative cycle of calculations at each of which distribution of miRNA in local neighborhood is recalculated (Figure 1C and Figure S4A). Based on new miRNA distribution, expression level of each node (i.e. ceRNA) is updated. This update results in change of expression value of common target (G4) in system. In this case, the common target acts as trigger for the other group (the targets interacted with M2 miRNA) in the network. Due to common targeted elements, the change in one neighborhood spreads to other neighborhoods (Figure 1D and Figure S4B), consequently have potential to affect whole network due to "ripple effect" (see Section 2 in Supplementary Materials and Methods).

During calculations, following assumptions were adopted; 1) Transcription and degradation rates of miRNAs are steady and equal. 2) All available miRNAs are recycled as in miRNA:ceRNA binding, target is degraded and miRNA is unaffected. 3) ceRNA targets also have stable transcription and degradation rates and these rates are equal.

The repression efficiency of a miRNA on the individual target (Eff_{gi}) is calculated according to equation (1); where miRNA expression (C_m) in local neighborhood is distributed among targets using individual gene expression levels (C_{gi}). For the genes targeted by multiple miRNAs, cooperative activity of miRNAs on a target gene, R, is calculated by summing repression activity of each miRNA Equation (2).

$$Eff_{gi} = C_m \times C_{gi} / \sum_{1}^{i} C_{gi} \tag{1}$$

$$R_{gi} = Eff_{i1} + Eff_{i2} \ldots \tag{2}$$

Multifactorial calculations in miRNA:target network

Interactions between miRNAs and their targets can be affected from various factors. So, our model integrates multiple factors when calculating overall miRNA activity. We classified factors into two categories. Binding factors determine interaction between miRNA and target and they alter amount of miRNA sequestered to target. Efficiency factors dictate degradation efficiency of miRNA on its target and define amount of target to be degraded. In other words, binding factors exert their influence before or during binding, efficiency factors exert their influence after binding. In the literature, binding free energy (2, 3) and seed type (21) in miRNA:target interactions are described as factors effecting binding affinity. Also, binding region (e.g, 5'UTR, 3'UTR, CDS) was shown to drastically affect miRNA degradation efficiency (3, 6). Since binding or degradation efficiency from literature have various and broad ranges, both binding and efficiency factors are normalized to their maximum values and scaled to [0,1] interval. The normalized values of factors are used to determine binding activity and miRNA efficiency on targets (Figure 2). Binding affinities (activity, Eff) of miRNAs on each individual gene are calculated as shown in equation (3); C_m, miRNA expression in the group; C_{gi}, individual gene expression; gi, individual gene (Figure 2C).

$$Eff_{gi} = C_m \times E'_{gi} \times STE'_{gi} \times C_{gi} / (\sum_{1}^{i} E'_{gi} \times STE'_{gi} \times C_{gi}) \tag{3}$$

$$Eff_{gi} = Eff_{gi} \times RE'_{gi} \tag{4}$$

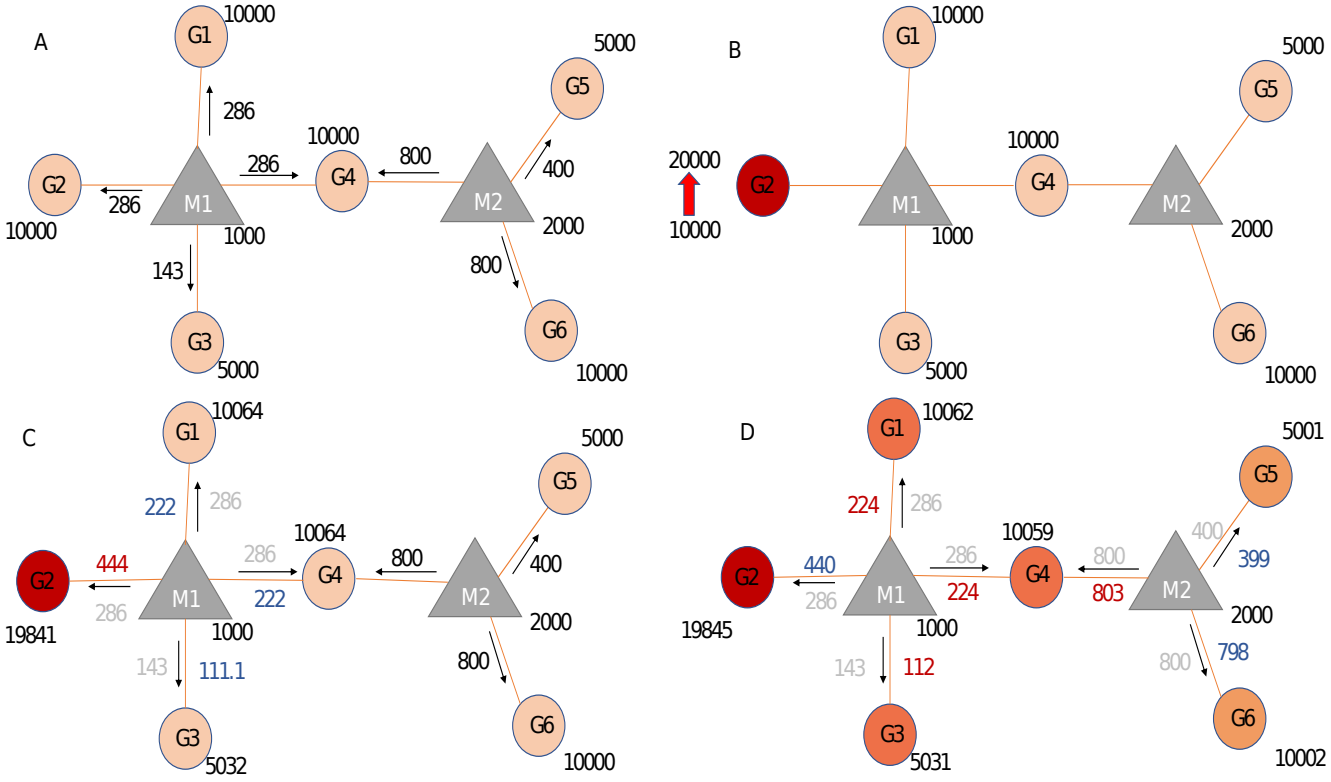

Figure 1. Schematic presentation of mechanism of network based model. (**A**) In steady state, miRNAs (M: triangles) repress targets (G: circles) according to proportion of target expression level. (**B**) Two fold increase in transcript level of Gene2 (G2) acts as a trigger (shown in red). (**C**) Distribution of miRNA1 (M1) changes. (**D**)) The change at expression of common target affects changes of proportional distribution of miRNA2 (M2). Expression values are rounded to integers for simplicity. Gray values on edges indicate initial distribution, red values indicate increase and blue values indicate decrease in distribution. Shades of circles indicate different levels of increase.

Not all miRNA:target binding events result in degradation of target. The degradation of target by bound miRNA depends on efficiency factors such as binding region. Exact repression efficiency of miRNA is calculated according to equation (4) (Figure 2D); RE'_{gi}, normalized values of region efficiency coefficient between miRNA and gene. The cooperative repression activity of miRNAs to their common targets is figured out as shown in Figure 2E.

Breast cancer patient dataset

We have applied our model in a real dataset for which experimental measurements of various factors were available. Expression levels of miRNA and genes in tumor and normal tissue of single patient are retrieved from TCGA Research Network https://www.cancer.gov/tcga. High-throughput experimental datasets which are provided miRNA:gene target pairs with interaction factors (3, 7) (Detailed codes are available at Section 6 in Supplementary Materials and Methods). We have combined miRNA and gene expression datasets via miRNA:target gene dataset retrieved from published datasets (3, 7) (Section 6 in Supplementary Materials and Methods and Table S4 in Supplementary Tables). SERPINE2, one of the most effective nodes, has been used as trigger in ceRNA network simulation in breast cancer patient network. Relevant steps and codes are available at Supplementary Materials and Methods.

RESULTS

Network with miRNA:ceRNA expression level

We have developed a network-based approach to assess effects of expression level changes in competitive ceRNA regulation. The basic mode of miRNA repression activity has been based on miRNA and target abundance in various researches (11, 13). Our approach can effortlessly calculate effects of expression changes when abundance levels of miRNAs and targets is only available factor. In sample network given in Figure (1), after an increase in expression level of a gene (G2), expression values of other genes also changed due to redistribution of miRNA among its targets. Previous studies have shown that if a gene abundance increases in ceRNA system, expression levels of genes targeted by shared miRNA are also affected (22, 23, 24). It was observed that expression levels of primary neighborhoods which do not interact with

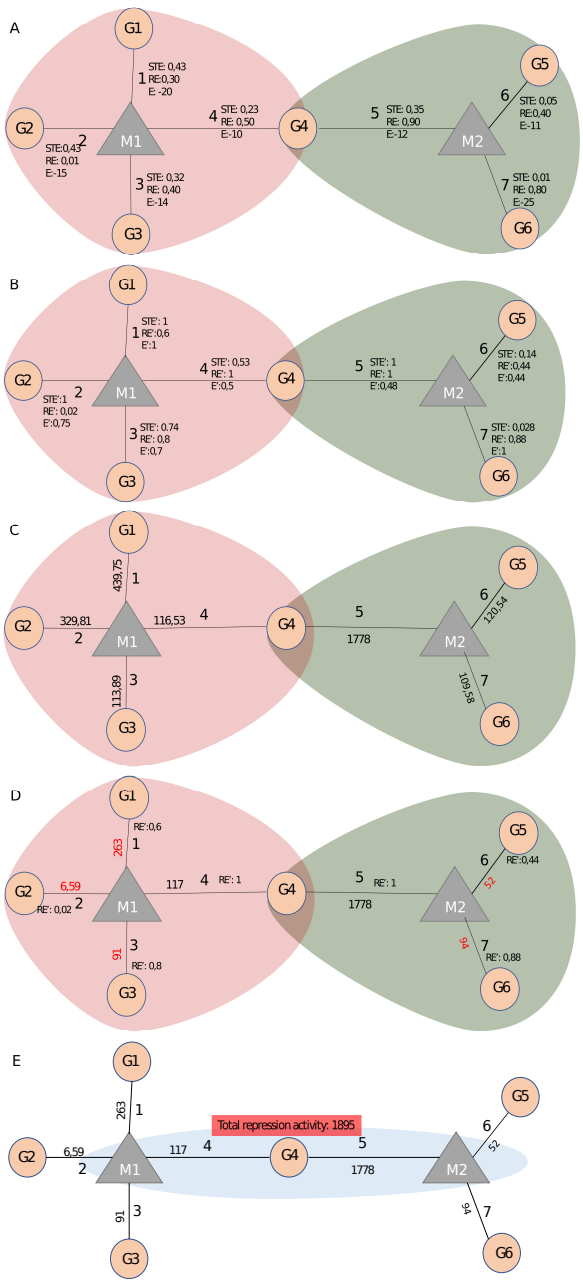

Figure 2. Calculations to determine of miRNA binding and repression efficiency. G, Gene; M, miRNA; STE, seed type effect; RE, Region Effect; E, Energy; STE', normalized values of seed type efficiency coefficient; RE', normalized values of region efficiency coefficient; E', normalized values of energy coefficient. Numbering on edges match the pair order in Table 1

another miRNA of the trigger gene change in relation to their expression.

Genes targeted by multiple miRNAs act as a trigger for adjacent local neighborhood of targeting miRNAs, causing changes in expression levels of genes outside the local neighborhood of original trigger gene. Therefore, primary expression level change in gene (G2) causes changes in other group of genes (G5 and G6) even though original trigger gene (G2) and genes in other group are not targeted by common miRNA. In addition, as shown by ceRNA hypothesis model of Ala et al., after the increase of gene expression level of G2, the miRNA that is found in the same group (M1) tended to be less repressive on its remaining targets (G1, G3 and G4). It's important to note that the changes in gene expression levels will have more pronounced effect if miRNA:target ratio is high, i.e., more miRNA available per target, which was reported in previous findings (11, 12, 13).

ceRNA:Target networks based on interaction factors

Earlier studies reported that miRNA regulatory interactions are affected by different parameters. For example, Xu et al. have investigated the importance of seed pairing type between miRNAs and their targets and target site location by using proteomics dataset (25). They have proposed that the features of binding between miRNA and target can be critical for miRNA efficiency. In addition, binding energy between miRNA and targets is a significant determinant for miRNA efficiency and it has been reported that strength of miRNA:target interactions is depended on binding energy of complexes (26). Similarly, another study revealed that affinity is correlated with seed pairing of miRNA:target pairs and suggested that affinity is correlated with number of canonical seed base pairing (12). Therefore, we have integrated aforementioned interaction parameters that could be useful for predicting miRNA repression activity more accurately. The sample dataset used in Figure 1 is recalculated with additional factors Table 1 in effect with same trigger (Detailed codes can be found at Section 3 in Supplementary Materials and Methods), two fold increase in Gene2 (Figure 3B, and Figure S6). When the factors were taken into account in the system, miRNA efficiencies varied as shown in Figure 3A (Figure S5). Although the miRNA:target expression ratios in steady-state were same in comparison with the sample dataset without factors, efficiency of binding and repression have changed. On the other hand, changes in expression levels of G4 (Detailed codes are available in Figure S8 and Section 4 in Supplementary Materials and Methods) and G1 differ although they have same initial conditions. This is due to fact that G4 is targeted by two miRNAs, M1 and M2 (Figure 3D) and changed interaction factors.

When the model run based on expression values of miRNAs and ceRNAs (genes), proportional distribution of miRNA on targets is determined in accordance with equations (1) (2). In that case, the system is observed as shown in Figure 1A at steady-state. However, if interaction factors are taken into account in the approach, specified interaction factors (i.e, binding factors such as seed type and energy) affect distribution of miRNA on targets as calculated in equation (3) (shown in Figure 2C). After distributed miRNA efficiency was re-calculated with degradation factor(s) (i.e region effect) according to equation (4), steady-state condition of network is obtained (shown in Figure 2D and Figure 3A). For instance, proportional distribution of G1:M1 interaction in Figure 1A is calculated lower than the same pair (G1:M1) in the other approach that includes interaction factors (Figure 2C). But, a decrease is observed in repression efficiency of M1 miRNA on G1 in interaction factor based network approach (Figure 2D and 3A).

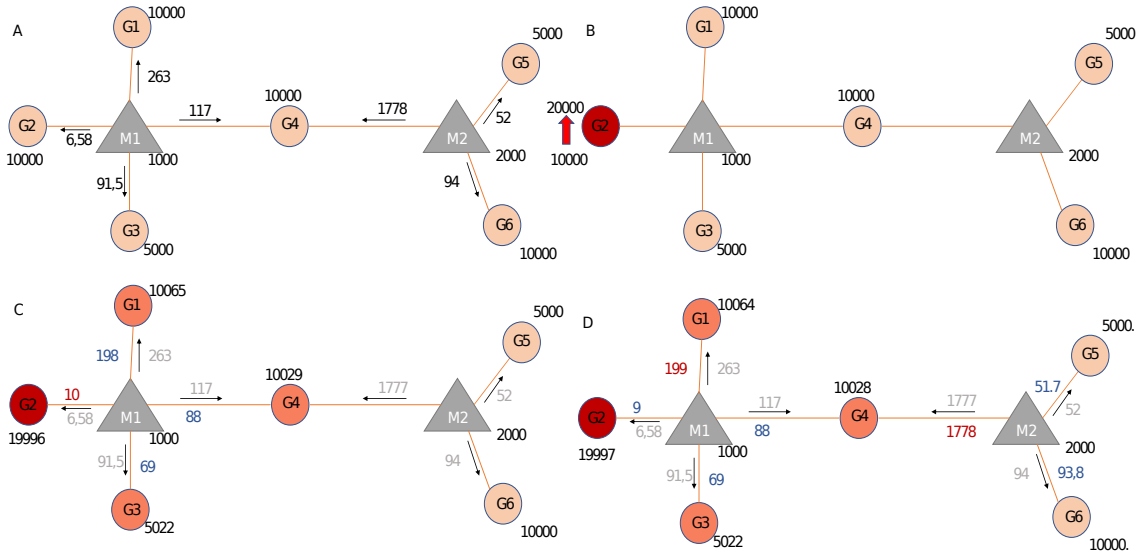

Figure 3. Target regulations with interaction parameters. (**A**) In the steady-state the repression activity of miRNAs on the targets after binding and repression efficiency. (**B**) The changes the repression activities after increasing of G2 expression. (**C**) Perturbation of primary neighborhoods of M1 miRNA (M1 miRNA group). (**D**) Regulation of gene expression of other gene group via triggering target (common target between M1 and M2)

Table 1. Expression values of elements and interaction factors of miRNA:target interactions

competing	miRNA	Competing expression	miRNA expression	seed type	region	energy
Gene1	Mir1	10000.00	1000.00	0.43	0.30	-20.00
Gene2	Mir1	10000.00	1000.00	0.43	0.01	-15.00
Gene3	Mir1	5000.00	1000.00	0.32	0.40	-14.00
Gene4	Mir1	10000.00	1000.00	0.23	0.50	-10.00
Gene4	Mir2	10000.00	2000.00	0.35	0.90	-12.00
Gene5	Mir2	5000.00	2000.00	0.05	0.40	-11.00
Gene6	Mir2	10000.00	2000.00	0.01	0.80	-25.00

It is considered that entire miRNAs in the system affect targets according to target:total target ratio Figure 1A, when interaction factors were not taken into account. However, in presence of interaction factors in miRNA:target interaction network (shown in Table 1), miRNAs are distributed according to target:total target ratio and affinity factors in first like shown in Figure 2C. After affinity mediated proportional distribution of miRNA expression, degradation factor is considered to specify count of repressive miRNA in pairs because entire bound miRNA target pairs might not be resulted with degradation (It is figured out shown in Figure 2D). For this reason, miRNA1 (M1) has more weak repressive effect (shown as edge variables) on target Gene2 Figure 3A in comparison with miRNA1 (M1):Gene2 (G2) interaction in Figure 1A. On the other hand, it is possible that taking into account of these interaction factors could cause increasing miRNA repression activity like in miRNA2:Gene4 interaction Figure 3A. When expression of Gene2 (G2) increased, expression values of all genes also changed differentially because of contribution of efficiency factors (Figure 3 B,C,D and Figure S6-7). We have considered that in our approach energy and seed type of pairs is significant for binding and targeted region is important for repression.

In regulation of miRNA target sample system (Figure 3 and Figure S5-7), miRNA (M1) repressive efficiency on the primary triggering gene (G2) is low in steady-state. So it has been observed that the regulatory activities of miRNA on the targets are weak after the increase of miRNA target G2. When model is triggered with two fold increase in expression level of common target (G4), more prominent changes were observed in gene expression levels in network compared to changes caused by G2 increase. Furthermore, change in expression level of target gene that has strong miRNA repression efficiency resulted in evident perturbation in network. On the other hand, it was observed that Gene2 was weakly affected from change of Gene4 expression because of its weak interaction factors. Since perturbation efficiency of each gene is different, we have developed a function which screens each gene in the network for their perturbation efficiencies. When we applied the method on the minimal dataset (i.e. Gene4 is trigger element), Gene4 has been found to be the most efficient element in terms of number of perturbed elements and miRNA2 (M2) has been found to be causing the highest mean in expression changes (see Section 5 in Supplementary Materials and Methods).

Simulation of large and realistic network

Apart from tools predicting miRNA:target pairs, there are databases that provide experimentally validated miRNA:target interaction datasets. The reliability in these datasets range from weak (e.g, western blot) to strong (.g, CLiP, CLASH). High-throughput methods ensure handling of AGO (Argonaute Protein) interacted miRNAs (CLiP) and their targets as well (CLEAR-CLiP, CLASH). We constructed a large ceRNA network utilizing high-throughput datasets containing chimeric reading of miRNA:target pairs (3, 7) which expose exact complementary seed regions.

Although these experimental sources include exact binding sites, they do not imply that each miRNA binding event is functional (27). Seed type, energy, binding region affects functionality of miRNA drastically. In order to construct realistic network, we incorporated such factors in the network. Energy values in miRNA:target pairs represented by high-throughput studies (3, 7), seed type (1, 8, 28) and binding region effect (3, 6) data were collected and integrated into network.

The network was finalized by integrating gene and miRNA expression levels from TCGA determined by RNA-Seq. Our model can successfully simulate perturbations in such large network despite complex behaviors and struggle to reach steady-state (see details at Section 6 in Supplementary Materials and Methods). Simulations show that change in expression level of single gene has potential to affect whole network, perturbing almost all nodes. These observations are in accordance with competing endogenous RNA hypothesis where genes targeted with many common miRNAs subsequently transmit perturbation to neighboring groups.

We have analysed the realistic network in context of perturbation efficiencies of nodes. This process was run with 3 fold upregulation and 10 iteration in conditions where elements that have less than five percent change are not taken into account (Codes are found at Section 6.5 in Supplementary Materials and Methods). With this approach, SERPINE2 has been found as one of the most effective gene nodes in network. We have observed that gaining steady-state in realistic network takes a lot of time because of complex interactions, as expected, and SERPINE2 exhibit highest efficiency at iteration 31 with given conditions (see Section 6.6 in Supplementary Materials and Methods and Figure S9 in Supplementary Figures). We ran the model with two fold of iteration which is observed as most effective. We have observed that the results of simulation with SERPINE2 gene, a serine protease inhibitor, one of the overexpressed genes of breast cancer (29, 30) is not convenient with tumor tissue expression values, when we compared the tumor gene expression dataset retrieved from TCGA and simulation results obtained via our approach (see Section 6.6 in Supplementary Materials and Methods). Simulation results were not expected to be consistent with tumor tissue expression values because large number of abnormal regulations were observed in these tissues. So, it might not said that a single gene is responsible for entire regulations in cancer cells. Besides, the all other factors such as up/down regulation of miRNAs or other genes were ignored. Therefore, it would be more useful to test our approach with an ideal dataset that includes entire informations of gene expression values before and after the regulation of a known gene at existing tissue, and miRNA expression values of this tissue at initial conditions.

DISCUSSION

Network based approaches for analyzing miRNA:target interactions have been developed in earlier studies. An initial attempt demonstrated the ceRNA crosstalk in a network-like minimal interaction structure with concentrations of ceRNA and miRNAs (17). Next, a network based kinetic model integrating miRNA and target rates of transcription, degradation, binding and unbinding was developed (18) using high throughput experimental dataset about miR-92a depletion (3). It was demonstrated that distant ceRNAs can interact with each other via indirect links, and the interactions are affected depending on distance between ceRNAs or topological features of network (18). More recently, an approach to detect ceRNA interaction by using the miRNA expression, gene expression and common miRNAs between gene targets was developed (20) which was effective in analyzing genes through miRNAs. Based on the observations that a miRNA can exhibit strong functionality to a target but may not against an other, the authors have concluded that existing miRNA based approach may not be suitable for understanding regulations of ceRNA interactions. Additionally, miRmapper package (31) utilizes an adjacency matrix to associate miRNAs using differentially expressed genes and identifies significant miRNAs in network.

In our approach, we have not taken into account transcription, degradation or binding rates of elements in network. Because, although it is known as the miRNAs are highly stable, the transcription and degradation rates of miRNAs change depending on cellular conditions (32). However, due to lack of datasets including degradation and transcription rates of miRNAs at specific cellular conditions, such factors are neglected in our model. Additionally, other regulation parameters such as gene-gene interactions and transcription factors are ignored but the network structure is flexible and can integrate additional regulation elements as they become available.

CONCLUSION

The main advantage of ceRNAnetsim package is providing various functions which can be run in accordance with user data. For example, the functions can work without interaction factors or with a simple interaction factor that is specified by user. Furthermore, it can also be operated according to a different parameter(s) provided by the user.In this study, we have preferred to run model, shown in *Multifactorial calculations in miRNA:target network* section.

In the future, with developments in experimental techniques about miRNAs:target interaction dynamics, more accurate results can be obtained from our model. Also, our model can integrate emerging miRNA effectors such as circRNAs. Consequently, our package may lead to better understanding and predictions of abnormal regulations and pathways underlying diseases or conditions.

Conflict of interest statement. None declared.

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