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 (TOFIX: Hocam etkileşim faktörlerinden dolayı ekspresyonları yakın olsa bile baskılanma aktitesinin aynı sergilenmeyeceğini ifade etmeye çalıştım. Bu şekilde anlaşılıyor mu?). 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 *ABCC1*, 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 at https://github.com/selcenari/ceRNAnetsim. (TODO the link will change)

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 microRNA:target interactions. These factors include microRNA:target binding energy, binding location in target sequence, base pairing types between miRNA and target, abundance of miRNAs and targets (Grimson et al. 2007). Binding energies of miRNA:target complexes vary based on nucleotide context and determine folding stability of complex (Cao and Chen 2012). It has been demonstrated that the binding energy between miRNA and target indicates stability or affinity of complex (Helwak et al. 2013) and does not directly determine repressive activity of miRNA (Cao and Chen 2012). 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 (Bartel 2004; Lewis, Burge, and Bartel 2005). In recent studies, it has been shown that miRNAs can interact with targets via sequences located in regions such as 5'UTR or CDS (Hausser et al. 2013; Helwak et al. 2013; Moore et al. 2015). These studies also showed that binding location could indicate functionality of miRNA:target interaction or be effective on abundance of

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targets(TOFIX abundance etkisi derken, bazen azaltabilir, bazen arttırabilir, manasında mı?, evet hocam anlaşılmıyorsa değiştirebilirim). 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 (Bartel 2009; Grimson et al. 2007). On the other hand, some researchers have reported that seed sequence of miRNA can have mismatches or bulged/wobble nucleotides and may locate in region other than 5'end of miRNAs (Hafner et al. 2010; Helwak et al. 2013). On top of all these factors, abundance of miRNAs and targets and miRNA:target ratio in cells predominantly affect efficiency of miRNA:target interaction (Arvey et al. 2010; Bosson, Zamudio, and Sharp 2014; Denzler et al. 2014).

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) (Ala et al. 2013; Cesana and Daley 2013), 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 (Ala et al. 2013). 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 target expression also slightly increased as a result of decreasing repressive activity of miR-122 on them (Denzler et al. 2014). 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 (Bosson, Zamudio, and Sharp 2014). 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 (Denzler et al. 2016). MiRNA:target interactions have been modeled as stoichiometric and catalytic mechanisms and Figliuzzi et al. have recommended handling models in network context (Figliuzzi, Marinari, and De Martino 2013). The model that can explain miRNA target interaction through topological features has been applied at bipartite network by Nitzan et al. (Nitzan et al. 2014). Robinson and Henderson applied the model that handles miRNA:target direct and indirect interactions via common miRNA of gene and target of miRNA, 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 (Robinson and Henderson 2018).Associated genes that are targets of the same miRNAs have been found with help of correlation of gene expression changes in recent algorithm (Markus List 2017). List et al. have specified that their approach can be useful for ceRNA studies and published their approach as an R package.

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. 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. 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) condition and needs a trigger for initiating calculations. The trigger can be a change in expression level of one or more genes (Figure 1b). After a trigger, the network undergoes iterative cycle of calculations at each of which distribution of miRNA

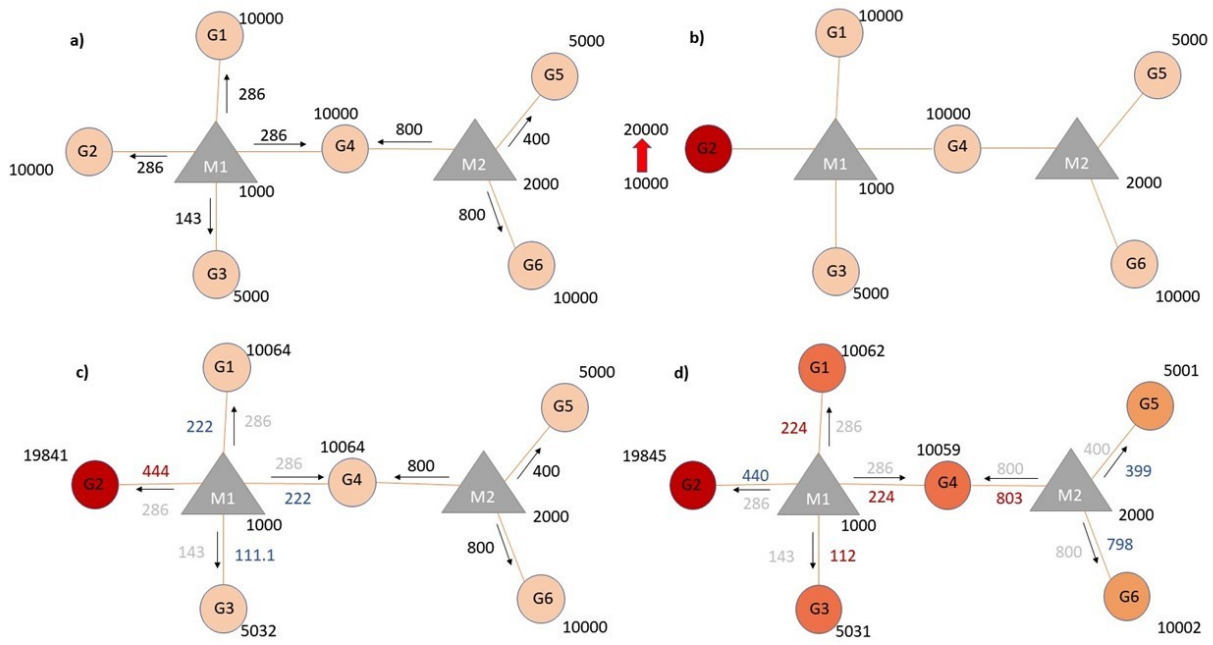

Figure 1: Schematic presentation of mechanism of network based model. a) In steady state, miRNAs (M: gray triangle) repress targets (G: circle coconut) according to proportion of their targets' expression. b) Two fold increase in transcript level of Gene2 (G2) acts as a trigger. c) Distribution of miRNA1 (M1) changes. d) The change at expression of common target effects changes of proportional distribution of miRNA2 (M2). Expression values are rounded to integers for simplicity

in local neighborhood is recalculated (Figure 1c). Based on new miRNA distribution, expression level of each node (i.e. ceRNA) is updated. Due to nodes which are targeted by multiple miRNAs, the change in one neighborhood spreads to other neighborhoods (Figure 1d), consequently have potential to effect whole network due to "ripple effect".

(#TODO caption needs more text, every color should be explained)

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}) (#TODO isn't C_g gene expressions in the group is equal to total of C_{gi} in group?). For the genes targeted by multiple miRNAs, cooperative activity of miRNAs, R, is calculated by summing repression activity of each miRNA (Equation (2)).

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

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

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.

Factors effecting binding determine interaction between miRNA and target and they alter amount of miRNA sequestered to target. Factors effecting efficiency determine proportion of target mRNA degraded by bound miRNA (#TODO fix this sentence). 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 (Cao and Chen 2012 ; Helwak et al. 2013) and seed type (Werfel et al. 2017) in miRNA:target interactions are described as factors effecting binding affinity. Efficiency factors determine how many of miRNA:target complexes will result in inhibition and binding region on target drastically effect miRNA degradation efficiency (Hausser et al. 2013; Helwak et al. 2013). Both binding and efficiency factors are normalized to their maximum values and scaled to [0,1] interval. The normalized values of factors take into account 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 (4); C_m, miRNA expression in the group; C_g, Gene expression; C_{gi}, individual gene expression; gi, individual gene; g, whole genes in a group (Figure 2c).

$$Eff_{gi} = C_m \times E'_{gi} \times STE'_{gi} \times C_{gi} / (\sum_{1}^{i} E'_{gi} \times STE'_{gi} \times C_{gi}) \tag{4}$$

$$Eff_{gi} = Eff_{gi} * RE'_{gi} \tag{5}$$

After miRNA binds to its target, but might not repress to bound target. The functionality of bound miRNA on target depends on efficiency factors like region that is binding sequence of miRNA on its target. Exact repression efficiency of miRNA is calculated according to equation (5) (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(#TODO cite). High-throughput experimental datasets which are provided miRNA:gene target pairs with interaction factors (Helwak et al. 2013; Moore et al. 2015). We have combined miRNA and gene expression datasets via miRNA:target gene dataset retrieved from ... (#TODO name of database and its citation). Detailed description of network construction and its code is available in Supplementary data (#TODO burada link nasıl olacak, word dosyası veya PDF dosyası ismi mi vermemiz mi gerekiyor, yoksa sadece Sup Data denilmesi yeterli mi? ilgili dosya: TCGA_E9-A1N5_article.Rmd). ABCC1 gene, over-expressed in tumor tissue, was selected as trigger for simulation of integrated dataset. After simulation of network, we have compared simulation results and tumor tissue expression levels.

(#TODO about formulas, 1) eq:3 F/Fmax can be removed, 2) in formulas eq:1 and eq:2, C_m or Eff_gi is not intuitive, is there consensus terms about effect or nth gene? 3) in formula eq:4, instead of, 4) in general, `*` sign should be replaced with `\times` and apostrophe should be replaced with `\prime`)

Results and Conclusions

We have developed a model approach and workflow for competitive ceRNA regulation in this study. The basic mode of miRNA repression activity has been based on miRNA and target abundance in various researches(Arvey et al. 2010; Denzler et al. 2014). For this reason, we performed first application as considering only abundance of miRNAs and targets (Figure 1). After the increasing in a gene expression value, expression values of other genes also changed slightly in comparison with alteration in perturbation started gene node. Different studies have shown that the other gene expression values also rise differently, if a gene abundance increases in ceRNA system (Lai, Wolkenhauer, and Vera 2016; Salmena et al. 2011; Tay, Rinn, and Pandolfi 2014). It was observed that primary neighborhoods of the trigger gene change with the same ratio, but cooperative efficiency of miRNAs on common target causes differently change in common target (Figure 1d). The common target in system has displayed like a trigger for the other group and induced

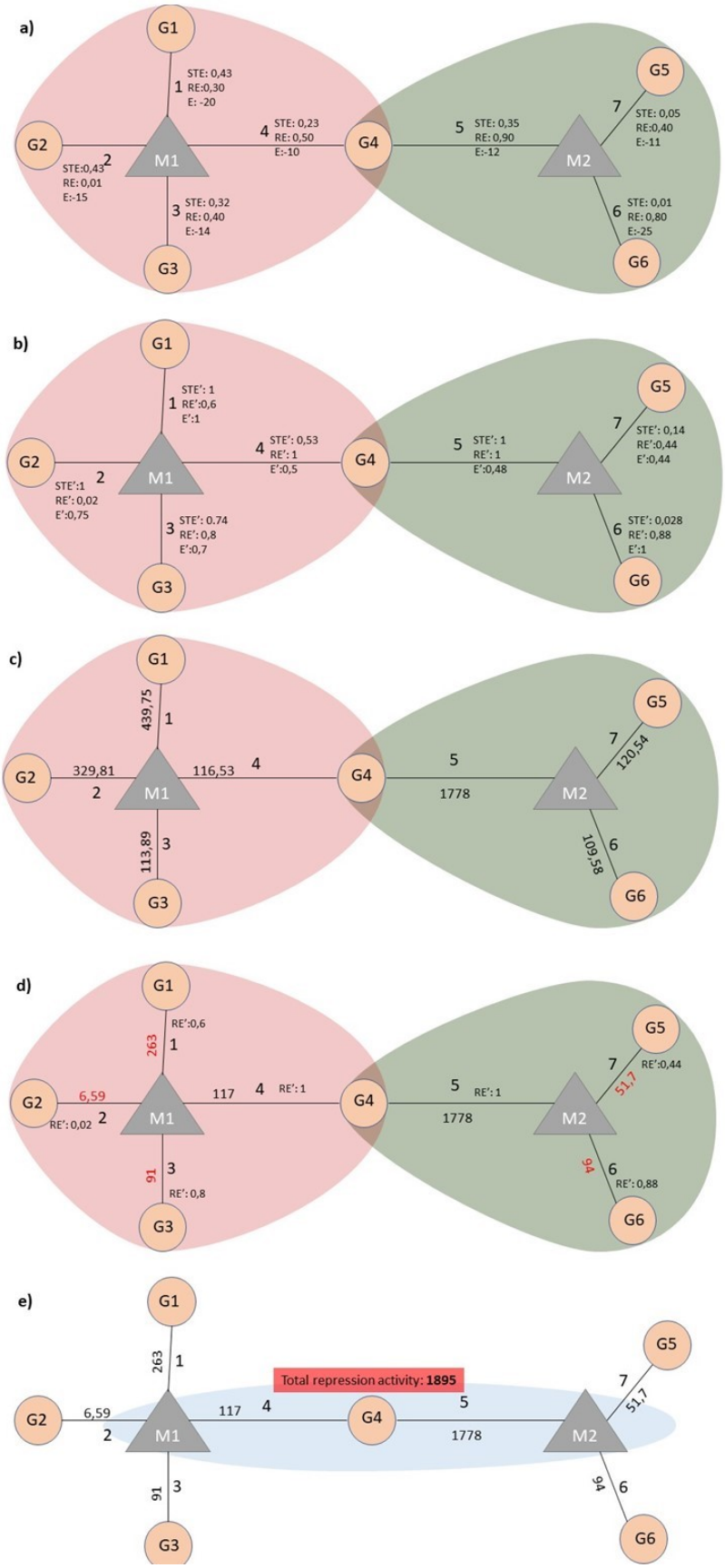

Figure 2: Calculations to determine of miRNA binding and repression efficiency. G, Gene; M, miRNA; STE, seed type effect; RE, Region Effect; E, Energy; STE', normalized values of seed type efficiency coefficient; RE', normalized values of region efficiency coefficient; E', normalized values of energy coefficient.

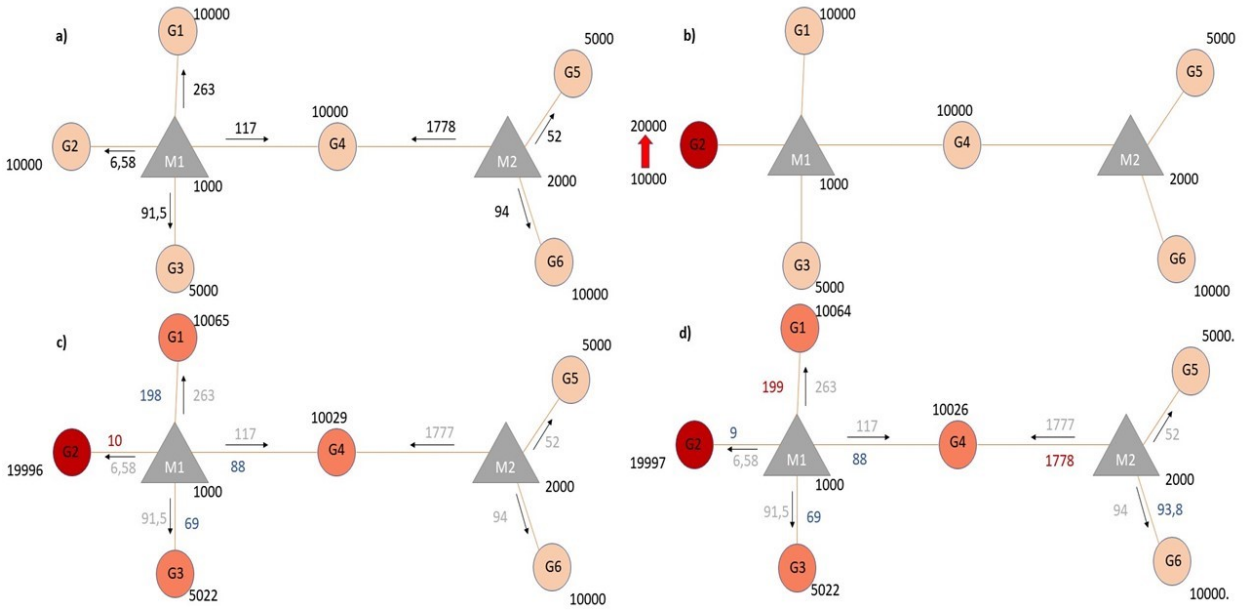

Figure 3: Target regulations with interaction parameters. a) In the steady-state the repression activity of miRNAs on the targets after binding and repression efficiency. b) The changes the repression activities after increasing of G2 expression. c) Perturbation of primary neighborhoods of M1 miRNA (M1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

changes of expression values of genes on the other group. Therefore, effect of primary change of the gene (G2) on genes of the other group was observed slightly (Figure 1d). In addition, like shown by ceRNA hypothesis model of Ala et al., after the increasing of a gene expression (G2), the miRNA that is found in the same group (M1) tended to be more repressive on this target. This has caused to decrease in increased expression value of primer triggering gene. When the expression of a gene (G2) was increased to two fold, we observed that the expression changes were more evident. So, we have considered that high miRNA:target ratio results in stronger miRNA repression activity. This results also are coherent with previous reports that were offered by Arvey et al. and Denzler et al.(Arvey et al. 2010; Bosson, Zamudio, and Sharp 2014; Denzler et al. 2014). When the simulation was run in sample dataset that includes lower target abundance, the first response of primary neighborhood of the same group was determined as similar with in system that have high target abundance. But regulations of ceRNAs were observed differences in the following steps of simulation. In overall state in the end of simulation, we have established that all regulations more prominent when compared with low miRNA:target ratio dataset. The model has appeared the importance of mRNA:target ratio in regulation of competing RNAs.

It has been reported in several studies that miRNA regulatory interactions also is affected 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 (Xu, Wang, and Liu 2014). They have proposed that the features of binding between miRNA and target can be critical efficiency of miRNA and can be used to find effective miRNA molecules. In addition, binding energy between miRNA and targets also could be determinant for miRNA efficiency. It has been reported by Breda et. al. that strength of miRNA:target interactions is depended on binding energy of complexes (Breda et al. 2015). As similar to these, Bosson et al. have associated affinity and seed pairing of miRNA target pairs and suggested that number of canonical base pairing is correlated with affinity. For these reasons, we have improved the model with optional interaction parameters that could be useful for understanding of miRNA repression activity. We have explained the model in the same sample dataset with using different factors and simulated via the same change (Figure 3). When the factors were taken into account in the system, miRNA efficiencies varied

as shown in Figure 3a. Although the miRNA target ratios in initial states were same in comparison with the sample dataset without factors, efficiency of binding and repression have changed. When expression of a gene (G2) increased, expression values of all genes also change differentially because of contribution of efficiency factors (Figure 3 b,c,d). We have considered that in our approach energy and seed type of pairs is significant for binding and targeted region is important for repression. But these factors can be handled optionally and differently.

In regulation of miRNA target sample system (Figure 3), 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 efficiencies on the targets are weak after the increase of miRNA target G2. When we ran to model with two fold increasing expression value of common target (G4), the changes of other gene expressions have observed more prominent. Furthermore, folding of expression of target gene that has strong miRNA repression efficiency also resulted in evident perturbation. On the other hand, It was observed that Gene2 was weakly affected from change of Gene4 expression because of its weak interaction factors.This also shows that the perturbation-initiation element in the system is important parameter. So, we also have developed an approach that is found perturbation efficiencies of elements in dataset. **(see details in Supplementary File(fig1_2app.Rmd)**. When we applied the method on the minimal dataset, while Gene4 is the most efficient element in terms of number of perturbed elements, it have observed that Mir2 is most potent element in the mean of expression change. In our approach, the elements behave differently based on type (competing or miRNA). When a competing element was changed, whole elements out of miRNAs are re-regulated. But, if a miRNA was perturbed, whole competing elements are re-regulated and miRNA preserves expression at after perturbation.

The defined miRNA' number is very low in comparison with defined protein coding genes' number. So, this results in that the miRNA amounts in the system must be high or targeted RNAs' amount must be low for effective miRNA activity. We have tried to simulate a real dataset which contains thousands of genes and hundreds of miRNAs. Firstly, we have observed the activities in the iterations and determined behaviors of the network after simulation. As expected, we have observed that the big dataset, compared to those containing a few genes and miRNAs, exhibited cumbersome behaviors and complexity to gain steady-state **(see details in Supplementary File(TCGA_E9-A1N5_article.Rmd)**. We realized that a small change in a system with a large change actually brought many arrangements. This can be explained with the competing groups. Because the genes are targeted with many common miRNAs, and this results in that the common targets exhibit competing behaviors in different groups. On the other hand, the changes of gene expressions were very different. While the change in some of genes were highly evident, some have changed slightly. So, we have compared two datasets; the tumor gene expression and simulation results. When we analyzed comparison of two datasets, we have observed that the results of simulation is not convenient with tumor tissue expression values. The simulation results were not expected to be concordant with tumor tissue expression values because of a single gene could not be responsible for gene regulation in all tissue. Besides, the all other factors such as up/down regulation of miRNAs or other genes were ignored. Of course, it would be more useful to test a dataset that includes gene expression values before and after the regulation of a gene and miRNA expression values at initial conditions. When we evaluated the suitability of approach, the approach allows different miRNA targets to be considered together and can work with different regulatory elements due to the network structure.

The network based approaches have been developed in previous studies. Figliuzzi et al. have tried to explain the ceRNA crosstalk in a network-like minimal interaction structure with concentrations of ceRNA and miRNAs. They have pointed out that the larger miRNA number can cause to evident crosstalk between ceRNAs. It has been thought that miRNAs and their targets interactions depend on rates of transcription, degradation, binding and unbinding in the network based kinetic model which has been developed by Nitzan et al. Nitzan and collaborators have used the high throughput experimental dataset (Helwak et al. 2013) for miRNA:target pair that occur high free energy and microarray dataset of a miR-92a depletion for expression values. They have been demonstrated that distant ceRNAs can interact with each other via indirect links, and the interactions change to depend on distance between ceRNAs, and topological features of network (Nitzan et al. 2014). List et al. have developed an approach to detect ceRNA interaction by using the miRNA expression, gene expression and common miRNAs between gene targets (Markus List 2017). Their approach could be useful to analysis interacted genes through miRNAs. When it has been thought that a

miRNA can exhibit strong functionality to a target but may not against an other, common miRNA based approach can be inconvenient to understand regulations of ceRNA interactions.

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 to depend on according to cellular conditions (Rüegger and Großhans 2012). According to our search, there is no dataset that was includes to degradation and transcription rates on specific cellular conditions for each miRNA. So, we preferred to accept that degradation and transcription rates of each miRNAs are equal to each other, instead of determining a constant value for all miRNAs in the system (The same applies to mRNA.). On the other hand other regulation parameters such as gene-gene interactions and transcription factors are ignored but the network structure can promote to integration of other regulation elements. In the future, with development in experimental techniques about features of miRNAs and their targets, more consistent and useful results can be obtained from our approach. Additionally, this can provide to contribution to prediction of abnormal regulations and pathways with the studies that will be developed.

(#TODO BRCA results go here) ABCC1 gene, *Multidrug resistance-associated protein 1*, is one of the most significant factor to develop resistance against chemotherapotic agents(Atalay, Demirkazik, and Gunduz 2008; Lu et al. 2015; Atalay et al. 2006)

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