Network based multifactorial modelling microRNA-target interaction.

Selcen Ari[*]
Alper Yilmaz[†]

11 April 2019

Abstract

Competing endogeneous RNA (ceRNA) regulations and crosstalk in human are remarkable issue in mean of regulations via miRNAs. Many studies have pointed out that an alteration in miRNA:target interaction can result in unexpected regulations because of indirect and complex interaction. In this paper, we defined a new network-based model that handles miRNA:ceRNA interactions with expression values and interaction factors optionally. We applied approach in sample dataset and defined dynamics of it. We have observed that a perturbation in an element of network affects whole competing elements differently and cooperative efficiencies of miRNAs on common targets could be shown. Importance of miRNA:target ratios was also observed consistently with previous reports. We have obtained that the competing elements which have the same or close expression values may not affected equal from the perturbation because of repression functionality of interactions. To apply model to the real sample that obtained combining breeast cancer gene and miRNA expression dataset via high throughput experimental miRNA:target dataset, we selected a gene, *ABCC1*, which over expressed in tumor tissue and utilized it as perturbating element. We have observed that one change can be sufficient to perturb regulations, and may not predicted all of regulations correctly, with this change. Therefore, this model which helps to understand crosstalking between elements could be tested and improved via results of well-constructed experimental dataset and takes to hope for future research.

Introduction

MicroRNAs (miRNAs) that are key regulator of gene expression through various post-transcriptional mechanisms are a family of short non-coding RNAs. Although repression mechanisms of miRNA are not fully understood, exact effect of miRNAs is regulation of direct target/targets negatively. 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'UTR 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 miRNAs can interact with targets via sequence that located in different region such as 5'UTR or CDS (Hausser et al. 2013; Helwak et al. 2013) **moore_mirnatarget_2015**. These studies also shown that binding location could indicate functionality of miRNA:target interaction or affect profile of target abundance. It has been shown that miRNAs exhibit repressive activity via, 6-8 nt long sequence that is prefectly complementary with targets, seed region at the 5'UTR 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 locates in different region of miRNAs (Hafner et al. 2010; Helwak et al. 2013). In addition to all of these, abundance of miRNAs and targets and miRNA:target ratio

[*]Yildiz Technical University Department of Bioengineering
[†]Yildiz Technical University Department of Bioengineering

in cells are determinant for 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 RNA molecule can also be targeted by various miRNAs. In that case, the targeted RNAs 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 disturbtion of the other target when a steady-state system that included one miRNA and two target, was perturbed with expression changes at one of the targets (Ala et al. 2013). When interaction between miRNAs and their target were dealed in the cell, explanation and prediction of results of perturbation is difficult due to varieties of interactions. Various computational and experimental studies have applied to be understood for 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 to affinity of targets. They have 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 also were studied and it has been demonstrated crucial for regulations at expression of targets (Denzler et al. 2016). MiRNA:target interactions have modelled as stoichiometric and catalytic mechanisms and Figliuzzi et al. have recommended to handling models in network structures (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 whole miRNAs and targets in network can interacted through common miRNAs and genes (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 study (Markus List 2017). List et al. have specified that their approach can be useful for ceRNA's studies and published these approach as a package that can use with R programming.

Methods

miRNA:target ratio theoretical action in network based ceRNA model

We developed a novel network based approach that can use to simulation of gene regulation after any perturbation at miRNA:target interactions. If a perturbation or change induces on graph, whole interacted nodes vary because of changes in miRNA:target ratios. The system was assumed as steady state that miRNAs affect according to the ratio of the individual target to all targets (Figure 1a, expression values shown as integer for comprehensible sampling). When expression of gene 2 (G2) increased to two fold (Figure 1b), distribution of miRNA (M1) expression changes (Figure 1c). Consequently, expression of common target (G4) of miRNA1 and miRNA2 also varies. The change at expression of common target triggers to changes of proportional distribution of miRNA2 (Figure 1d).

The model was developed by making some assumptions. Transcription rates and degradation rates of miRNAs were assumed as stationary and equal. Targets also have stabil transcription and degradation rates, at the same time they are equal. The factors that could be affect the miRNA:target interaction are disregarded in this approach. It is considered that miRNAs degradates or inhibits them via any mechanisms after binding to target while the miRNAs were used reversibly. In initial conditions, gene expression amounts in system have been considered after repression activity of miRNAs. If a change occurs in the system, variation on miRNA proportional distribution are reflected as change of repression activity.

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

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

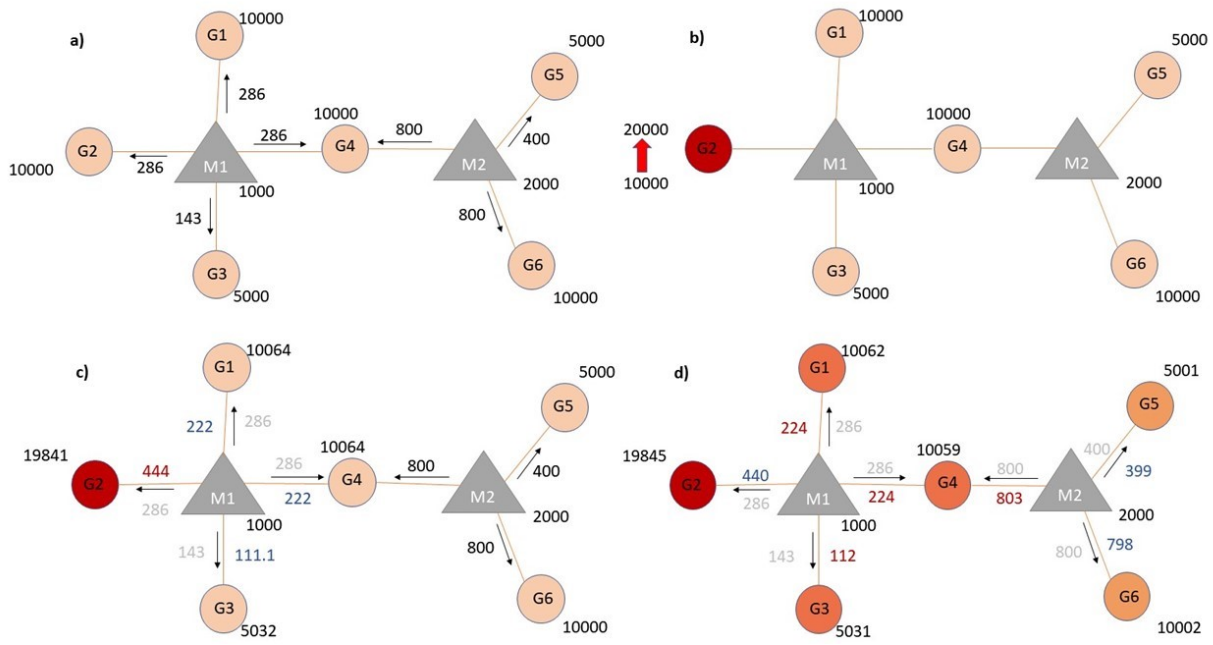

Figure 1: Shematic presentation of mechanism of network based model. a) In steady state, miRNAs (M: triangle grey) repress targets (G: circle coconut) according to proportion of their targets' expression. b-c-d) MiRNA expression distributions to targets vary when expression of one of the targets perturbed.

The efficiency of a miRNA on the individual target (Eff_{gi}) is calculated according to equation (1); C_m, miRNA expression in the group; C_{gi}, symbolize individual gene expression; C_g gene expressions in the group (Groups are determined depending on miRNAs. Targets that are interacted with the same miRNA find in a group.). In this approach, because a gene can be found in different groups, the cooperative activity of microRNAs also can be calculated. Total repression activity of different miRNAs on the common target, R, is calculated based on equation (2); sum of efficiency of different miRNAs on the target.

miRNA action in network based multifactorial ceRNA model

Interactions between miRNAs and their targets can be affected from different parameters. For this reason, we improved model with factors that can affect to miRNA activity. In our approach, the parameters are classified as binding and efficiency factors and all of these are evaluated in their groups. Binding factors determine interaction between miRNA and target. The efficiency factors decide functionality of 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 represented as binding affinity. Efficiency factors determine how many of microRNA:target complexes will result in inhibition. For example, binding region on the target shows whether the target can be inhibited (functionality)(Hausser et al. 2013; Helwak et al. 2013). Both of binding and efficiency factors are evaluated in the groups and normalized according to equation (3); each factor is normalized based on maximum variables that found in their groups.

$$F` = F/F_{max} \tag{3}$$

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

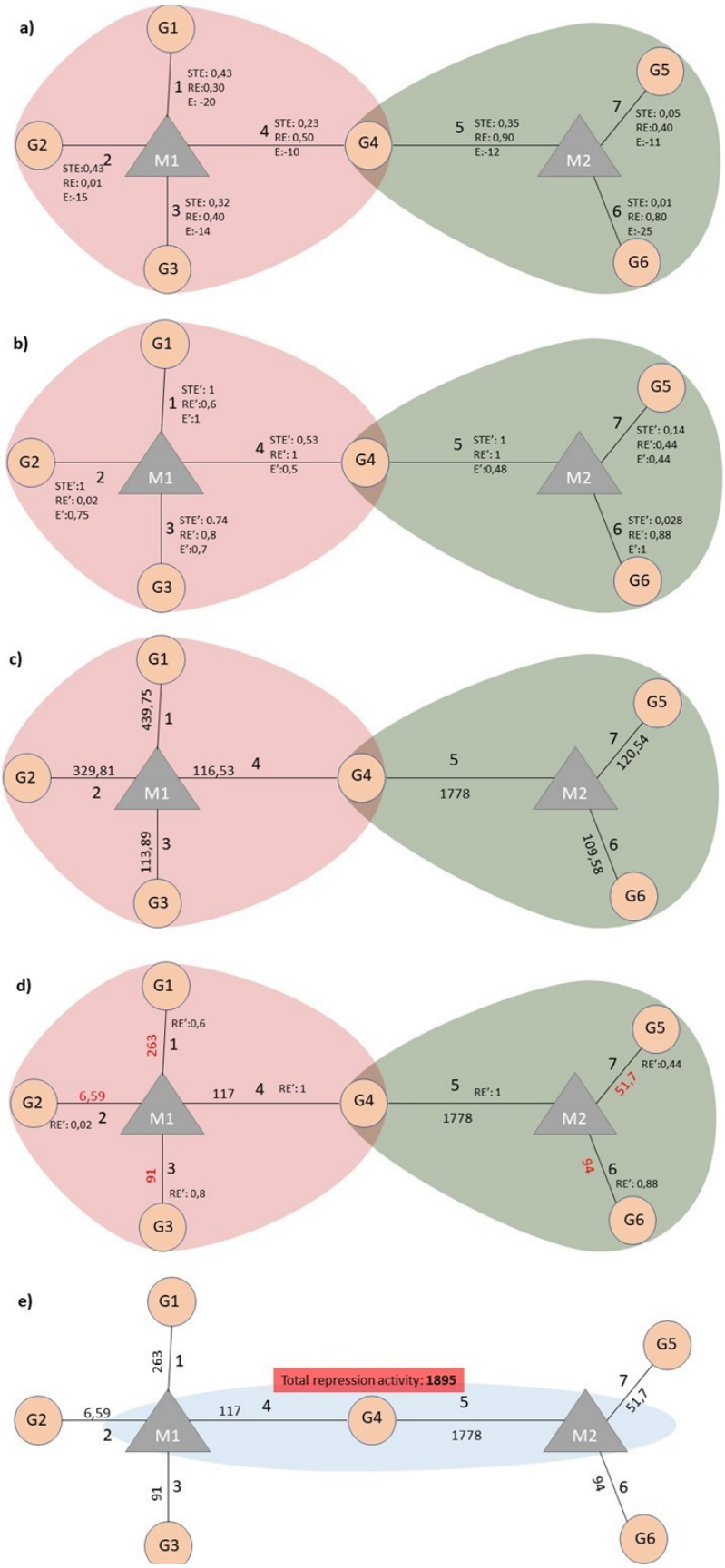

Figure 2: Calculations to determine of miRNA binding and repression efficiency. G, Gene; M, miRNA; STE, seed type effect; RE, Region Effect; E, Energy; STE', normalized values of seed type efficiency coefficient; RE', normalized values of region efficiency coefficient; E', normalized values of energy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

expression; gi, individual gene; g, whole genes in a group (Figure 2c).

$$Eff_{gi} = C_m * E`_{gi} * STE`_{gi} * C_{gi} / (\sum_{1}^{i} E`_{gi} * STE`_{gi} * C_{gi}) \quad (4)$$

$$Eff_{gi} = Eff_{gi} * RE`_{gi} \quad (5)$$

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.

Application of model in a breast cancer patient dataset

To determine suitability of method, we have tried to apply model in a real dataset. Firstly, we obtained the miRNA and gene expression datasets of the patient's tumor and normal tissue. We downloaded the high-throughtput experimental datasets which are provided miRNA:gene target pairs with interaction factors by **moore_mirnatarget_2015** and (Helwak et al. 2013). We have combined miRNA and gene expression datasets via miRNA:target gene dataset **(Whole steps can be found in Supplementary data(TCGA_E9-A1N5_article.Rmd)**). We selected a gene which is over expressed in tumor tissue with compared to normal tissue. It is pointed out that 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). So, we selected ABCC1 gene for simulation of integrated dataset. We have detected the iteration that is suitable for ABCC1 gene simulation. After simulation of system via ABCC1 gene with over expression, we have compared simulation results and tumor tissue expression levels.

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 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 neihgborhood of the same group was determined as similar with in system that have high target abudance. But regulations of ceRNAs were observed differences in the folowing steps of simulation. In overall state in the end of simulation, we have established that all regulations more prominent when compared with

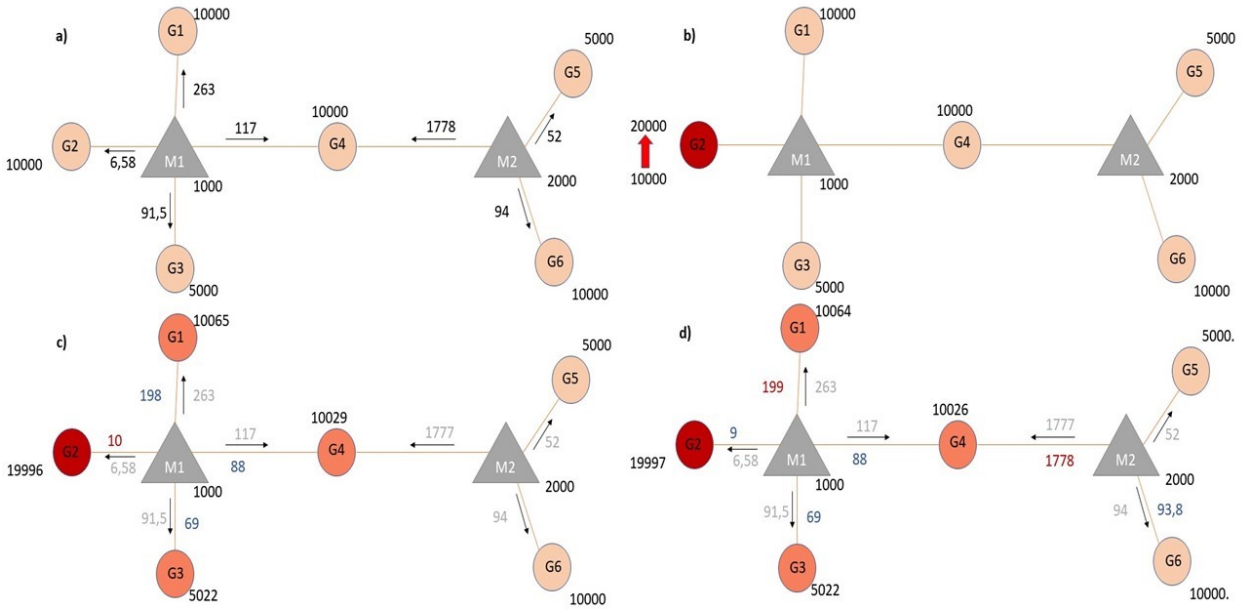

Figure 3: Target regulations with interaction parameters. a) In the steady-state the repression activity of miRNAs on the targets after binding and repression efficiency. b) The changes the repression activities after increasing of G2 expression. c) Perturbation of primary neighborhoods of M1 miRNA (M1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

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 proteomic 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 behaviours 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 behaviours 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 behaviours 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, somes have changed slightly. So, we have compared two datasets; the tumor gene expression and simulation results. When we analysed 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 throught 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.

References

Ala, U., F. A. Karreth, C. Bosia, A. Pagnani, R. Taulli, V. Leopold, Y. Tay, P. Provero, R. Zecchina, and P. P. Pandolfi. 2013. "Integrated Transcriptional and Competitive Endogenous RNA Networks Are Cross-Regulated in Permissive Molecular Environments." *Proceedings of the National Academy of Sciences* 110 (18): 7154–9. https://doi.org/10.1073/pnas.1222509110.

Arvey, Aaron, Erik Larsson, Chris Sander, Christina S Leslie, and Debora S Marks. 2010. "Target mRNA Abundance Dilutes microRNA and siRNA Activity." *Molecular Systems Biology* 6 (April). https://doi.org/10.1038/msb.2010.24.

Atalay, Can, Ismet Deliloglu Gurhan, Cigdem Irkkan, and Ufuk Gunduz. 2006. "Multidrug Resistance in Locally Advanced Breast Cancer." *Tumor Biology* 27 (6). Karger Publishers: 309–18.

Atalay, C, A Demirkazik, and U Gunduz. 2008. "Role of Abcb1 and Abcc1 Gene Induction on Survival in Locally Advanced Breast Cancer." *Journal of Chemotherapy* 20 (6). Taylor & Francis: 734–39.

Bartel, David P. 2004. "MicroRNAs." *Cell* 116 (2): 281–97. https://doi.org/10.1016/S0092-8674(04)00045-5.

Bartel, David P. 2009. "MicroRNAs: Target Recognition and Regulatory Functions." *Cell* 136 (2): 215–33. https://doi.org/10.1016/j.cell.2009.01.002.

Bosson, Andrew D., Jesse R. Zamudio, and Phillip A. Sharp. 2014. "Endogenous miRNA and Target Concentrations Determine Susceptibility to Potential ceRNA Competition." *Molecular Cell* 56 (3): 347–59. https://doi.org/10.1016/j.molcel.2014.09.018.

Breda, Jeremie, Andrzej J. Rzepiela, Rafal Gumienny, Erik van Nimwegen, and Mihaela Zavolan. 2015. "Quantifying the Strength of miRNA–Target Interactions." *Methods* 85 (September): 90–99. https://doi.org/10.1016/j.ymeth.2015.04.012.

Cao, Song, and Shi-Jie Chen. 2012. "Predicting Kissing Interactions in microRNA–Target Complex and Assessment of microRNA Activity." *Nucleic Acids Research* 40 (10): 4681–90. https://doi.org/10.1093/nar/gks052.

Cesana, Marcella, and George Q. Daley. 2013. "Deciphering the Rules of ceRNA Networks." *Proceedings of the National Academy of Sciences of the United States of America* 110 (18): 7112–3. https://doi.org/10.1073/pnas.1305322110.

Denzler, Rémy, Vikram Agarwal, Joanna Stefano, David P. Bartel, and Markus Stoffel. 2014. "Assessing the ceRNA Hypothesis with Quantitative Measurements of miRNA and Target Abundance." *Molecular Cell* 54 (5): 766–76. https://doi.org/10.1016/j.molcel.2014.03.045.

Denzler, Rémy, Sean E. McGeary, Alexandra C. Title, Vikram Agarwal, David P. Bartel, and Markus Stoffel. 2016. "Impact of MicroRNA Levels, Target-Site Complementarity, and Cooperativity on Competing Endogenous RNA-Regulated Gene Expression." *Molecular Cell* 64 (3): 565–79. https://doi.org/10.1016/j.molcel.2016.09.027.

Figliuzzi, Matteo, Enzo Marinari, and Andrea De Martino. 2013. "MicroRNAs as a Selective Channel of Communication Between Competing RNAs: A Steady-State Theory." *Biophysical Journal* 104 (5): 1203–13. https://doi.org/10.1016/j.bpj.2013.01.012.

Grimson, Andrew, Kyle Kai-How Farh, Wendy K. Johnston, Philip Garrett-Engele, Lee P. Lim, and David P. Bartel. 2007. "MicroRNA Targeting Specificity in Mammals: Determinants Beyond Seed Pairing." *Molecular Cell* 27 (1): 91–105. https://doi.org/10.1016/j.molcel.2007.06.017.

Hafner, Markus, Markus Landthaler, Lukas Burger, Mohsen Khorshid, Jean Hausser, Philipp Berninger, Andrea Rothballer, et al. 2010. "Transcriptome-Wide Identification of RNA-Binding Protein and MicroRNA Target Sites by PAR-CLIP." *Cell* 141 (1): 129–41. https://doi.org/10.1016/j.cell.2010.03.009.

Hausser, J., A. P. Syed, B. Bilen, and M. Zavolan. 2013. "Analysis of CDS-Located miRNA Target Sites Suggests That They Can Effectively Inhibit Translation." *Genome Research* 23 (4): 604–15. https:

//doi.org/10.1101/gr.139758.112.

Helwak, Aleksandra, Grzegorz Kudla, Tatiana Dudnakova, and David Tollervey. 2013. "Mapping the Human miRNA Interactome by CLASH Reveals Frequent Noncanonical Binding." *Cell* 153 (3): 654–65. https://doi.org/10.1016/j.cell.2013.03.043.

Lai, Xin, Olaf Wolkenhauer, and Julio Vera. 2016. "Understanding microRNA-Mediated Gene Regulatory Networks Through Mathematical Modelling." *Nucleic Acids Research* 44 (13): 6019–35. https://doi.org/10.1093/nar/gkw550.

Lewis, Benjamin P., Christopher B. Burge, and David P. Bartel. 2005. "Conserved Seed Pairing, Often Flanked by Adenosines, Indicates That Thousands of Human Genes Are MicroRNA Targets." *Cell* 120 (1): 15–20. https://doi.org/10.1016/j.cell.2004.12.035.

Lu, Lin, Fang Ju, Hui Zhao, and Xuezhen Ma. 2015. "MicroRNA-134 Modulates Resistance to Doxorubicin in Human Breast Cancer Cells by Downregulating Abcc1." *Biotechnology Letters* 37 (12). Springer: 2387–94.

Markus List, Marcel Schulz. 2017. "SPONGE." Bioconductor. https://doi.org/10.18129/B9.bioc.SPONGE.

Nitzan, Mor, Avital Steiman-Shimony, Yael Altuvia, Ofer Biham, and Hanah Margalit. 2014. "Interactions Between Distant ceRNAs in Regulatory Networks." *Biophysical Journal* 106 (10): 2254–66. https://doi.org/10.1016/j.bpj.2014.03.040.

Robinson, J. M., and W. A. Henderson. 2018. "Modelling the Structure of a ceRNA-Theoretical, Bipartite microRNA-mRNA Interaction Network Regulating Intestinal Epithelial Cellular Pathways Using R Programming." *BMC Research Notes* 11 (1): 19. https://doi.org/10.1186/s13104-018-3126-y.

Rüegger, Stefan, and Helge Großhans. 2012. "MicroRNA Turnover: When, How, and Why." *Trends in Biochemical Sciences* 37 (10): 436–46. https://doi.org/10.1016/j.tibs.2012.07.002.

Salmena, Leonardo, Laura Poliseno, Yvonne Tay, Lev Kats, and Pier Paolo Pandolfi. 2011. "A ceRNA Hypothesis: The Rosetta Stone of a Hidden RNA Language?" *Cell* 146 (3): 353–58. https://doi.org/10.1016/j.cell.2011.07.014.

Tay, Yvonne, John Rinn, and Pier Paolo Pandolfi. 2014. "The Multilayered Complexity of ceRNA Crosstalk and Competition." *Nature* 505 (7483): 344–52. https://doi.org/10.1038/nature12986.

Werfel, Stanislas, Simon Leierseder, Benjamin Ruprecht, Bernhard Kuster, and Stefan Engelhardt. 2017. "Preferential microRNA Targeting Revealed by in Vivo Competitive Binding and Differential Argonaute Immunoprecipitation." *Nucleic Acids Research* 45 (17): 10218–28. https://doi.org/10.1093/nar/gkx640.

Xu, Wenlong, Zixing Wang, and Yin Liu. 2014. "The Characterization of microRNA-Mediated Gene Regulation as Impacted by Both Target Site Location and Seed Match Type." Edited by Thomas Preiss. *PLoS ONE* 9 (9): e108260. https://doi.org/10.1371/journal.pone.0108260.