TFmiR: a web server for constructing and analyzing disease-specific transcription factor and miRNA co-regulatory networks

Mohamed Hamed, Christian Spaniol, Maryam Nazarieh and Volkhard Helms*

Center for Bioinformatics, Saarland University, 66041 Saarbrucken, Germany

Received February 27, 2015; Revised April 16, 2015; Accepted April 18, 2015

ABSTRACT

TFmiR is a freely available web server for deep and integrative analysis of combinatorial regulatory interactions between transcription factors, microRNAs and target genes that are involved in disease pathogenesis. Since the inner workings of cells rely on the correct functioning of an enormously complex system of activating and repressing interactions that can be perturbed in many ways, TFmiR helps to better elucidate cellular mechanisms at the molecular level from a network perspective. The provided topological and functional analyses promote TFmiR as a reliable systems biology tool for researchers across the life science communities. TFmiR web server is accessible through the following URL: http://service.bioinformatik.uni-saarland.de/tfmir.

INTRODUCTION

Among many genetic regulators, transcription factors (TFs) and microRNAs (miRNAs) are the essential key players for regulating gene expression (1). Together they play important roles in regulating virtually all cellular processes such as differentiation, proliferation, survival and apoptosis (2). Also genetic disorders and complex diseases including cancers and other network motifs to regulate cellular transcription in a connective manner (4,5). Therefore, utilizing the combined regulatory information on TFs and miRNAs as well as their target genes could shed light on key driver genes and miRNAs in human diseases and, in turn, suggests novel therapeutic strategies in disease treatment (5,6).

Several databases have been developed in order to facilitate research on transcriptional and post-transcriptional interaction types between TFs, miRNAs and target genes. For instance, TransFac (7), OregAnno (8) and MsigDB (9) provide compilations of TFs regulating genes (TF → gene). TransmiR (10) provides information on which TFs regulate miRNAs (TF → miRNA). mirTarBase (11), TarBase (12) and miRecords (13) collect target genes of miRNAs (miRNA → genes) in different organisms. Although still little is known about miRNA-mediated miRNA regulations, recent studies reported plausible evidences that miRNAs may regulate the expression of other miRNAs as well as their target genes (5,14). Thus, miRNA → miRNA interactions were computationally predicted and made available in the PmmR database (15).

Despite the general availability of such databases, generalized repositories integrating different kinds of molecular interactions and enabling to analyze their contributions to diseases are still missing. To this end, we present TFmiR, a web server that allows for integrative and comprehensive analysis of interactions between a set of deregulated TFs/genes and a set of deregulated miRNAs within the relevant pathways of a certain disease. The tool unravels the disease-specific co-regulatory network between TFs and miRNAs and performs over representation analysis (ORA) for the involved TFs/genes and miRNAs. Our web server also detects FFLs consisting of miRNAs, TFs and co-targeted genes (TF–miRNA co-regulatory motifs) and statistically assesses the functional homogeneity between the co-regulated targets. Furthermore, TFmiR utilizes seven different methods for identifying key network players that could possibly drive oncogenic processes of diseases and thus could act as potential drug targets. Especially when combined with experimental validation, these putative key players as well as the novel TF–miRNA co-regulatory motifs could promote novel insights to develop new therapeutic approaches for human diseases.

MATERIALS AND METHODS

Description

TFmiR integrates genome-wide transcriptional and post-transcriptional regulatory interactions to elucidate human diseases. For a specified disease and based on user-supplied lists of deregulated genes/TFs and miRNAs, TFmiR investigates four different types of interactions, TF → gene, miRNA → TF, TF → miRNA and miRNA → gene.
**TF → miRNA, miRNA → miRNA and miRNA → gene.** It also unravels the circuitry between miRNAs, TFs and target genes with respect to specified diseases. For each interaction type, TFmiR utilizes information provided by established and curated regulatory databases of both predicted and experimentally validated interactions (Figure 1) whereby all duplicate interactions were removed. For TF → miRNA interactions, we also integrated manually curated regulatory relationships from (∼5000) published papers (16). From the predicted miRNA → miRNA interactions in the PmmR database (15), we considered only the best hits having score <0.2, which was computed as the normalized path length between the two involved miRNAs. The incorporated predicted miRNA → gene interactions were retrieved from starBase (17) by selecting only those predictions confirmed by three out of five prediction algorithms (targetScan (18), picTar (19), RNA22 (20), PITA (21) and miRanda (22)). Supplementary Table S1 lists the included databases and the number of regulations available for each interaction type. In total, TFmiR currently integrates information on almost 10 000 genes, 1856 miRNAs, ∼3000 diseases including subtypes and more than 111 000 interactions.

**TFmiR user input scenarios**

TFmiR can be called through two scenarios. If the user submits only a set of deregulated genes. In this case, TFmiR identifies the set of miRNAs whose target genes as well as regulator TFs are significantly enriched within the input deregulated genes using the hypergeometric distribution function followed by the Benjamini–Hochberg (BH) adjustment with a cutoff value of 0.001. Sample input files of the deregulated genes and miRNAs are provided in Supplementary Figures S1 and S2. The user can optionally set the P-value cutoff (default is 0.05) for ORA on the resulting network nodes (genes/miRNAs). Finally, the user can control the evidence level (experimentally validated, predicted or both) for the constructed regulatory interactions that will be used in the subsequent network analysis.

**Functionality of TFmiR**

TFmiR pools all four interactions types based on the input deregulated genes and miRNAs and accordingly generates the entire combinatorial regulatory network. If a disease was selected, TFmiR uses data retrieved from the human miRNA disease database (HMDD) (23) as well as DisGeNET (a database for gene-disease association) (24) as sources for disease-associated miRNAs and genes, respectively. Interactions whose target nodes or regulator nodes are known to be associated with the disease compose the putative disease-specific network. TFmiR then offers three levels of downstream analysis: (i) the regulatory subnetwork of the four interaction types, (ii) the combined network of all interaction types and (iii) the disease-specific network (if disease was selected).
For each interaction type subnetwork of regulator $\rightarrow$ target links, we display the total number of targets and regulators in the corresponding interaction databases, a Venn diagram depicting the overlap between the input deregulated targets (miRNAs/gene) and the targets of the input deregulated regulators (genes/miRNAs) available from the database. The significance of overlap is computed using the hypergeometric distribution test as an indicator how representative the provided deregulated inputs are for the known cellular targets of the considered regulators. This measure suggests whether the follow-up downstream analysis is likely to yield meaningful functional enrichments or disease associations. To avoid the effect of false-positives in the regulator $\rightarrow$ target databases and to account for a different number of targets for the input deregulated regulators, a randomization test is conducted ($n=1000$). Furthermore, TFmiR carries out ORA for both gene analyses and miRNA sets comprising the interaction subnetwork.

For gene set analysis, TFmiR employs DAVID (25) to check for enrichment of GO terms (BP subcategory), KEGG pathways and OMIM diseases as well as for clustering the genes based on their functional similarities. For miRNA set analysis, we used the miRNA-functional association data and miRNA-disease association data from HMDD to statistically relate the functional and disease terms to the miRNA set.

For levels 2 and 3, TFmiR calculates for each network basic topological features, relevance to the disease-associated genes/miRNAs by testing the overlap significance with the network nodes, degree distribution plot, ORA analyses for both gene and miRNA nodes, network key nodes, and detects 3-node motifs. To measure the strength of correlation between the potential disease-specific network, the input disease and the input deregulated genes and miRNAs, we compute a coverage ratio (CR) between the nodes of the disease-specific network and the nodes of the entire combined network.

$$C_R = \frac{N_d}{N_t}$$

Here, $N_d$ represents the number of disease-specific network nodes and $N_t$ represents the total number of nodes in the entire network. We also calculate the CR ratio between the edges of the two networks. All resulting networks are visualized using the interactive Cytoscape-web viewer (26).

Identification of network key nodes

We defined the key nodes as the top 10% highest centrality nodes of the TFs, miRNAs and genes in the disease-specific and whole network. TFmiR uses degree centrality, closeness centrality, betweenness centrality and eigenvector centrality as well as the common and union sets of the key nodes identified by these four measures. We also determine the minimal set of dominating nodes that regulate the entire network. This can be modeled as the following optimization problem: Let graph $G(V,E)$ be a connected graph, $n = |V|$, $adj$ is the adjacency matrix of $G$, and $adj(i,j) = 0$, $X$ is a binary array of size $n$, such that $X(i) = 1$ if node $i$ was marked as a key node, and 0 otherwise. Then the objective function is:

$$\min \sum_{i=1}^{n} X(i)$$

subject to $\forall i \sum_{j} adj(i,j) X(j) >= 1$.

The last constraint guarantees that every node in the network must have at least one key node in its neighborhood. To solve such an optimization problem, we apply the algorithm presented by Rai et al. 2009 (27) to search for the minimum dominating node set.

Identification of TF–miRNA co-regulatory motifs

FFLs are interconnection patterns that recur in many different parts of a network and form key functional modules (5,28). They have been demonstrated as one of the most important motif patterns in transcriptional regulation networks (28) that govern many aspects of normal cell functions and diseases (29,30). Here, TFmiR identifies four types of 3-nodes motifs (three FFLs and one co-regulation motif) consisting of a TF, a miRNA and their co-targeted gene and defines them as TF–miRNA co-regulatory motifs (Figure 2). (i) The Coregulation-FFL includes only TF regulation of a target gene as well as miRNA repression of that target gene. (ii) The TF-FFL includes TF regulation of the expression of both a miRNA and a target gene and it also includes miRNA repression of that target gene. (iii) The miRNA-FFL includes miRNA repression of both a TF and a target gene, as well as TF regulation of this target gene. (iv) The Composite-FFL describes TF regulation of both a miRNA and a target gene as well as miRNA suppression of that TF and that target gene.

Identifying significant TF–miRNA co-occuring pairs. We identified statistically significant TF and miRNA pairs that
cooperatively regulate the same target gene using the hyper-

\[ P-value = 1 - \sum_{i=0}^{x} \binom{i}{k} \binom{M-k}{N-i} \binom{M}{N} \]

where \( k \) is the number of target genes of a certain miRNA, \( N \) is the number of genes regulated by a certain TF, \( x \) is the number of common target genes between these TF and

\[ N_{h} = \frac{N_{s}}{N} \]

\[ Zscore = \frac{N_{o} - N_{m}}{\sigma} \]

Here \( N_{o} \) is the number of motifs observed in the real network, whereas \( N_{m} \) and \( \sigma \) are the mean and standard deviation of the motif occurrence in 100 random networks, respectively.

Functional homogeneity

In order to evaluate the biological evidence of the identified TF–miRNA co-regulatory motifs and better understand their functional roles, TFmiR allows the user to analyze the GO semantic similarity for all pairs of genes targeted by the same TF and miRNA pair or for all pairs of genes regulated by the TF or the miRNAs of that TF–miRNA pair (see Supplementary Figure S3). The GoSem-

\[ \text{Sim R package (32) is used to compute the semantic simi-larity scores according to the Gene Ontology (GO) annotations. Statistical significance is determined by randomly selecting the same number of genes (co-targeted genes or co-regulated genes) from all Entrez genes with GO annotations, and computing their similarity scores. The permutation procedure is repeated 1000 times. Then, we carry out a Kolmogorov–Smirnov test to check whether the functional similarity scores of all gene pairs from the FFL motif are significantly higher than that of randomly selected pairs.} \]

RESULTS

Case study

TFmiR was applied to several data sets related to complex diseases such as cancer, Alzheimer and diabetes. In a recent study on breast cancer (6), we identified 1262 deregulated genes and 121 deregulated miRNAs using gene and

\[ (\text{Supplementary Table S4). Additionally, TFmiR identified 22 key network players (10 genes and 12 miRNAs}) \]

\[ \text{based on the union set of four centrality measures described above (Supplementary Table S2). Interestingly, some of the identified key genes such as BRCA2, ESR1, AKT1 and TP53 were previously implicated and significantly mutated in breast cancer samples (33). More importantly, the protein products of the genes ESR1, TP53, TGF\text{\textbar}1, AKT1 and BRCA2 are binding targets for anti-breast cancer drugs (6) (Supplementary Table S4).} \]

Next, we examined the TF–miRNA co-regulatory motifs that were significantly enriched in the entire interaction network. We identified 53 FFL motifs (three composite-FFLs, two TF-FFLs, six miRNA-FFLs and forty-two coreg-FFLs). An interesting motif involving the TF SPI1, the miRNA hsa-mir-155 and the target gene FLL1 reveals how FFL motifs may help to better understanding the pathogenicity of breast cancer (Supplementary Figure
S4 from the tool). Recent studies reported that the oncoprobe SPI1 is involved in tumor progression and metastasis (34,35). However, the co-regulation of the oncogene FLI1 (36) by both SPI1 and the oncomiR hsa-mir-155 was not reported before. As the co-regulated genes of SPI1 and hsa-mir-155 have significantly more similar cellular functions than randomly selected genes (Supplementary Figure S5), this FLFL motif provides novel insights into SPI1-miRNA networks alteration in breast cancer and suggests a cooperative functional role between SPI1 and potential miRNA partners.

DISCUSSION

Compared to the web services of related databases and tools such as TransmiR (10), ChiPBaSE (37), CircuitsDB (38), starBase (17), miR2Disease (39) and cGRNB (40), our TFmiR web server has several distinctive features: (i) TFmiR performs integrative analysis of molecular interactions between a set of deregulated genes and a set of deregulated miRNAs within or without the pathogenic pathways of a certain disease. In contrast, the above mentioned web tools only search the regulatory interactions of a single gene or a single miRNA. (ii) TFmiR performs a rich network analysis, TF–miRNA co-regulatory motif detection, network visualization, statistical significance of the extracted interactions, and ORA analysis for each interaction type, the combined interaction network, and the disease network. Such an integrated analysis is not provided by other web tools. (iii) TFmiR allows the user to retrieve either experimentally validated or predicted interactions or both. Such an option is not available using the other tools. In a somehow similar fashion, DisTMGneT (41) was developed for obtaining cancer-specific network based on user-selected sets of deregulated genes and miRNAs. However, it lacks the downstream analysis, the varieties of user input parameters, and it is limited to a predefined set of miRNAs and genes as well as cancer disease. Also miTRail (42) performs ORA and Gene Set Enrichment analyses of interactions of genes and miRNAs based on expression profiles. However, it explores only miRNA → gene interactions.

CONCLUSION

We developed TFmiR as a comprehensive web server for integrative analysis of the molecular interactions between TFs/genes and miRNAs and their interwoven critical roles in the pathology of human diseases. TFmiR provides an extended downstream analysis, a variety of user parameters, user input scenarios and incorporates information from various well-established regulatory databases. TFmiR is based on user-provided sets of deregulated genes and/or miRNAs regardless of the data producing technologies of either microarray experiments, next generation sequencing or PCR. We showed that unlike the traditional separate analysis of gene expression profiles (43,44) or the aberration of miRNA expression in cancer tissues (45,46), this integrated molecular analysis of deregulated miRNAs and genes using TFmiR was able to uncover literature confirmed core regulators as well as important new aspects of the TF/gene-miRNA interactomes, their co-regulation mechanisms, and the underlying pathogenesis of human breast cancer. The novel hub nodes of TFs/miRNAs could be further experimentally investigated as new potential drug targets. TFmiR was also able to characterize important TF miRNA co-regulatory motifs whose co-regulated genes form cooperative functional modules in breast oncogenesis processes.

OUTLOOK AND PERSPECTIVE

TFmiR is planned to be integrated with other useful ORA tools such as KeyPathwayMiner (47), GiGA (48), HotNet (49) and jActiveModules (50) to allow the user to benefit their advances within TFmiR. We also intend to allow for submitting multi case expression data and times series data as well as the currently supported case/control data. Finally, expanding the TFmiR to elucidate the regulatory mechanisms of cellular processes (ex. stem cell differentiation) in addition to diseases would sort TFmiR of great interest for wide range of researchers and most of life science community.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

We thank Dr Michael Hutter for valuable comments.

FUNDING

DAAD (to M.H.), SFB 1027 funded by DFG (to M.H., M.N.) and Graduate school of computer science, Saarbrücken, Germany (M.N., M.H.). Funding for open access charge: Publication charges will be covered from SFB 1027 funded by DFG.

Conflict of interest statement. None declared.

REFERENCES

1. Hobert,O. (2008) Gene regulation by transcription factors and microRNAs. Science, 319, 1785–1786.
2. Esquela-Kerscher,A. and Slack,F.J. (2006) Oncomirs - microRNAs with a role in cancer. Nat. Rev. Cancer, 6, 259–269.
3. Hanahan,D. and Weinberg,R.A. (2011) Hallmarks of cancer: the next generation. Cell, 144, 646–674.
4. Poos,K., Smida,J., Nathrath,M., Maugg,D., Baumhoer,D. and Korsching,E. (2013) MicroRNA and transcription factor co-regulatory networks affect osteosarcoma cell proliferation. PLoS Comput. Biol., 9, e1003210.
5. Yan,Z., Shah,P.K., Amin,S.B., Samur,M.K., Huang,N., Wang,X., Misra,V., Ji,H., Gabuzda,D. and Li,C. (2012) Integrative analysis of gene and miRNA expression profiles with transcription factor miRNA feed-forward loops identifies regulators in human cancers. Nucleic Acids Res., 40, e135.
6. Hamed,M., Spaniol,C., Zapp,A. and Helms,V. (2015) Integrative network based approach identifies key genetic elements in breast invasive carcinoma. BMC Genomics, 16(Suppl. 5), S2.
7. Matys,V., Fricke,E., Gellers,R., Gössling,E., Haubrock,M., Hehl,R., Hornischer,K., Karas,D., Keil-A.E., Kel-Margoulis,O.V. et al. (2003) TRANSFAC: transcriptional regulation, from patterns to profiles. Nucleic Acids Res., 31, 374–378.
8. Griffith,O.L., Montgomery,S.B., Bernier,B., Chu,B., Kasaian,K., Aerts,S., Mahony,S., Sleumer,M.C., Blenky,M., Haeussler,M. et al. (2008) ORAregAnno: an open-access community-driven resource for regulatory annotation. Nucleic Acids Res., 36, D107–D113.
9. Liberzon, A., Subramanian, A., Pinchback, R., Thorvaldsdottir, H., Tamayo, P. and Mesirov, J. P. (2011) Molecular signatures database (MSigDB). *Biotechniques*, 47, 1739–1740.

10. Wang, J., Lu, M., Qiu, C. and Cui, Q. (2010) TransmiR: a transcription factor-microRNA regulation database. *Nucleic Acids Res.*, 38, D119–D122.

11. He, Q. (2008) MicroRNA database. *FEBS Lett.*, 582, 1057–1061.

12. Setu, A. H. and Hutz, G. A. (2004) TarBase: a comprehensive database of experimentally supported animal microRNA targets. *RNA*, 10, 192–197.

13. Xiao, A. F., Zuo, Z., Cai, G., Kang, S., Gao, X. and Li, T. (2009) MicroRecords: an integrated resource for microRNA-target interactions. *Nucleic Acids Res.*, 37, D105–D110.

14. Matkovich, S., Hu, Y. and Dorn, G. (2013) Regulation of cardiac microRNAs by cardiac microRNAs. *Circ. Res.*, 113, 62–71.

15. Sengupta, D. and Bandyopadhyay, S. (2011) Participation of microRNAs in human interactions: extraction of microRNA-microRNA regulations. *Mol. BioSyst.*, 7, 1966–1973.

16. Qiu, C., Wang, J., Yao, P., Wang, E. and Cui, Q. (2010) MicroRNA evolution in a human transcription factor and microRNA regulatory network. *BMC Syst. Biol.*, 4, 90.

17. Yang, J.-H., Li, J.-H., Shao, P., Zhou, H., Chen, Y.-Q. and Qu, L.-H. (2011) starBase: a database for exploring microRNA mRNa interaction maps from Argonaute CLIP-Seq and Degradome-Seq data. *Nucleic Acids Res.*, 39, D202–D209.

18. Bartel, D. P. (2009) MicroRNAs: target recognition and regulatory functions. *Cell*, 136, 215–233.

19. Krek, A., Grün, D., Poy, M. N., Wolf, R., Rosenberg, L., Epstein, E. J., MacMenamin, P., da Piedade, I., Gunsalus, K. C., Stoffel, M. et al. (2005) Combinatorial microRNA target predictions. *Nat. Genet.*, 37, 495–500.

20. Miranda, K., Huynh, T., Tay, Y., Ang, Y. S., Tam, W. L., Thomson, A. M., Lim, B. and Rigoutsos, I. (2006) A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. *Cell*, 126, 1203–1217.

21. Kertesz, M., Iovino, N., Unnerstall, U., Gaul, U. and Segal, E. (2007) The role of site accessibility in microRNA target recognition. *Nat. Genet.*, 39, 1278–1284.

22. John, B., Enright, A. J., Aravin, A., Tuschl, T., Sander, C. and Marks, D. S. (2004) Human microRNA targets. *PLoS Biol.*, 2, e363.

23. Lu, M., Zhang, Q., Deng, M., Xiao, J., Guo, Y., Guo, W. and Cui, Q. (2008) An analysis of human microRNA and disease associations. *PLoS ONE*, 3, e3420.

24. Bauer-Mehren, A., Rautschka, M., Sanz, F. and Furlong, L. I. (2010) Prognostic values of microRNAs in human disease. *Nucleic Acids Res.*, 37, D98–D104.

25. Huang, D. W., Sherman, B. T. and Lempicki, R. A. (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.*, 4, 44–57.

26. Lopes, C. T., Franz, M., Kazi, F., Donaldson, S. L., Morris, Q. and Bader, G. D. (2010) Cytoscape web: an interactive web-based network browser. *Bioinformatics*, 26, 2347–2348.

27. Bai, W. M., Verma, S. and Tapaswi, S. (2000) A power aware minimum connected dominating set for wireless sensor networks. *J. Netw.*, 4, 511–519.

28. Shen-Orr, S. S., Milo, R., Mangan, S. and Alon, U. (2002) Network motifs in the transcriptional regulation network of Escherichia coli. *Nat. Genet.*, 31, 64–68.

29. He, L., He, X., Lin, L., De Stanchina, E., Xuan, Z., Liang, Y., Xue, W., Zender, L., Magnus, J. and Radzoun, D. (2007) A microRNA component of the p53 tumour suppressor network. *Nature*, 447, 1130–1134.

30. Li, X., Cassidy, J. J., Reinke, C. A., Fischboeck, S. and Cartewbh, R. W. (2009) A microRNA imparts robustness against environmental fluctuation during development. *Cell*, 137, 273–282.

31. Bennett, Y. and Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B (Methodological)*, 57, 289–300.