miReg: a resource for microRNA regulation

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Summary

MicroRNAs (miRNAs/miRs) are important cellular components that regulate gene expression at posttranscriptional level. Various upstream components regulate miR expression and any deregulation causes disease conditions. Therefore, understanding of miR regulatory network both at upstream and downstream level is crucial and a resource on this aspect will be helpful. Currently available miR databases are mostly related to downstream targets, sequences, or diseases. But as of now, no database is available that provides a complete picture of miR regulation in a specific condition.

Our miR regulation web resource (*miReg*) is a manually curated one that represents validated upstream regulators (transcription factor, drug, physical, and chemical) along with downstream targets, associated biological process, experimental condition or disease state, up or down regulation of the miR in that condition, and corresponding PubMed references in a graphical and user friendly manner, browseable through 5 browsing options. We have presented exact facts that have been described in the corresponding literature in relation to a given miR, whether it's a feed-back/feed-forward loop or inhibition/activation. Moreover we have given various links to integrate data and to get a complete picture on any miR listed. Current version (Version 1.0) of *miReg* contains 47 important human miRs with 295 relations using 190 absolute references. We have also provided an example on usefulness of *miReg* to establish signalling pathways involved in cardiomyopathy. We believe that *miReg* will be an essential miRNA knowledge base to research community, with its continuous upgrade and data enrichment.

This HTML based *miReg* can be accessed from: www.iioab-mireg.webs.com or www.iioab.webs.com/mireg.htm.

1 Background

MicroRNAs (miRNAs/miRs) are an endogenous pool of small (~22 nt) non-coding RNAs that post-transcriptionally inhibit mRNA expression by complementary base pairing at the 3′-untranslated regions (3′-UTRs) of target mRNAs. A perfect paring results in cleavage and an imperfect binding causes translation inhibition of the target mRNAs [1]. By virtue of this fine tuning of gene regulation, miRs regulate various cellular and biological processes (BP) for instance embryonic stem cells differentiation [2], heart development [3], apoptosis [4], cell proliferation [5], insulin secretion [6], immunity [7], and aging [8] etc. Deregulation of miRs have been implicated in various patho-physiological conditions such as cardiovascular [3], Alzheimer [9], Parkinson [10] diseases and various cancers [11]. Therefore, regulation of miRs has of prime importance in basic biomedical research as well as in disease diagnosis, prognosis, and therapy.

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It has been shown that a single miR can regulate ~200 mRNAs [12] and currently available evidences support that, similar to the transcription machinery, miR coding genes are transcribed by transcription factors (TFs). The latter ones act as upstream regulators, positively or negatively regulate transcription of miRs**,** which in turn fine tune the regulation of their targets mRNAs. These TFs are also fine tuned themselves by same miRs through feed-back loop regulation or can create a feed-forward loops that are curtail in regulating various biological and patho-physiological processes and to maintain proper physiology. Such feed-back loops have been identified for various miRs such as AP-1 and miR-21 [13], miR-200, ZEB1, and SIP1 [14], miR-34, SIRT1 and p53 [15] etc. Therefore TFs-miR interaction is a vital process behind any BP. Furthermore, miRs are also regulated by various physical and chemical stimuli. While miR-143 was found to be inhibited in response to Pourous polyethylene [16], miR-221 is upregulated in response to Tamoxifen [17], so is miR-29 in response to ionizing radiation [18], and so is the cancer-related miR-195 following RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) exposure [19]. Hence, identification of the upstream regulators of miRs is essential in understanding the basic mechanisms of miR-regulated fine tuning of gene expression in any given biological or patho-physiological condition, and in developing therapeutic strategies.

Although several databases and tools are available for miR targets (both validated and predicted), an extensive resource on upstream regulators of miRs is yet to be developed. This gap is somehow filled by TransmiR database [20] that has listed only upstream regulatory TFs. However other types of upstream regulators are also equally important as mentioned earlier. Therefore, we took the initiative to create the miR regulation web resource (*miReg*) to give comprehensive information of miRs in terms of all kinds of upstream regulators, specific feed-back and feed-forward loops, corresponding BPs and disease states etc. in a graphical and user friendly mode. We also tried to integrate various miRNA resources in a single platform providing hyperlinks in each miR page of the *miReg* so that user can have most of the information related to a miR of interest listed in this web resource. We believe *miReg* will be an indispensable knowledge base on miR regulation for the research community, with a continuous upgrade and data enrichment. The *miReg* is free only for academic research and can be accessed from: www.iioab-mireg.webs.com or www.iioab.webs.com/mireg.htm.

2 Methods

2.1 Data compilation

PubMed was scanned to select publications indicating relationships between miRs and their regulatory counterparts using various single or combination of keywords (e.g.: miR, regulator, feed-back, upstream etc.). 7000 references were retrieved and manually examined at title line level. Finally 400 references were selected for manual curation at abstract level. Experimentally validated relationships (hereafter denoted as reactions) of a miR with its upstream regulators (e.g.: TFs, proteins, drugs, chemicals, BPs etc.) and downstream targets (mainly mRNA) were manually captured in a graphical manner for each reference so that a single reaction can represent the fact reported in the corresponding literature. Similarly we also have captured the experimental system and the outcome of the reaction in term of BPs. The natural expression levels of miRs have also been curated for conditions described in the corresponding publication. Mostly mammalian (human, mouse, and rat) specific information had been captured in miReg, except a very few cases.

2.2 Data source description

HTML codes were used to design and represent data. *miReg* is a miR specific data source and each page has been allotted to a single miR. Each miR page contains reactions, corresponding BPs experimental systems, and references (PubMed ID/PMID) in tabular format. The miR of the page is hyperlinked to miRBase (a database of published miRNA sequences) [21] and each PMID is linked to the corresponding PubMed abstract.

In a reaction, each upstream regulator or down stream target (proteins/TFs/mRNAs) is hyperlinked to corresponding results of human specific "Search by Gene" module of miRNA path [22]. Therefore clicking on a protein/TF/mRNA of a reaction will directly lead to miRs that affect the protein/TF/mRNA and the pathways in which each miR is involved. All hyperlinks are human specific, although a different species may be mentioned in the corresponding literature (e.g.: Zebrafish). BPs are linked to AmiGO (gene ontology database) [23] and any disease condition of a reaction is hyperlinked to the corresponding disease search page result of miR2Disease database [24]. Hence, clicking on any disease link will give all experimentally validated and manually curated miRs associated with the particular disease. When an upstream regulator is a drug or chemical, they are hyperlinked to DrugBank [25] or PubChem (http://pubchem.ncbi.nlm.nih.gov), respectively. Below the reaction table, the summary of all reactions for the miR of interest is given. Here each protein/TF/gene is linked to a NCBI *Entrez* page. Below the summary, web browsing options are given for both experimentally validated and predicted targets for the miR using search results from miRecords [26] and miRWalk (http://www.ma.uni-heidelberg.de/apps/zmf/mirwalk/index.html). Below that, another link is provided for disease involvement of the miR using miR2Disease database link. At the bottom, the "BROWSE" option will take the reader to the *miReg* web browser (*Browse-miReg*) page.

The *miReg* web browser page will provide surfs specific to miRs, diseases, BPs, and upstream regulators (proteins/TFs, drugs). All web browsing options are hyperlinked to corresponding database pages as mentioned earlier and each miR in the "*Browse-miReg*" page is linked to the corresponding miR page of *miReg*. Each miR page also contains all important miR specific database links. Therefore, unlike other miRNA databases, *miReg* will provide a more detailed picture of any miR listed in this web recourse. A representative snapshot of *miReg* is depicted in Figure 1**.**

3 Utility

The *miReg* resource can be used for various miR-related researches. We employed *miReg* to establish a cardiac regulatory pathway. The cardiovascular domain benefited greatly from the advances in miRNA research. The identification of specific cardiac miRs as being important modulators of heart functions is increasing and the need for well organized databases for miRs with their upstream regulators is currently very strong. However it is not possible to provide a complete pathway for the cardiovascular system or a specific cardiovascular disease, because of the huge amount of information which is available concerning the validated and putatively involved miRs. Indeed, using *miReg* as starting point, a list of 7 validated and causal miRs implicated in the general development of cardiac hypertrophy for instance: miR-1, miR-21, miR-23a, miR-30, miR-133, miR-195 and miR-208 can be obtained. Each of the mentioned miRs has its own complex signaling pathway. Moreover they differently regulate the onset or development of cardiac hypertrophy. While miR-21, miR-23a, miR-195 and miR-208 would be pro-hypertrophic factors [3], miR-1, miR-30, and miR-133 would rather be considered as protective ([27], [28], [29]). Nevertheless such classification appears to be too simplistic in the light of dual effects exhibited by various

miRs, especially miR-1 which has been shown being pro-arrhythmic and pro-apoptotic ([30], [31]). In Figure 2, we concentrated on 2 particular "protective" miRs (miR-30 and miR-133) and on their roles in cardiac hypertrophy and associated consequences such as fibrosis, arrhythmias and apoptosis using *miReg*.

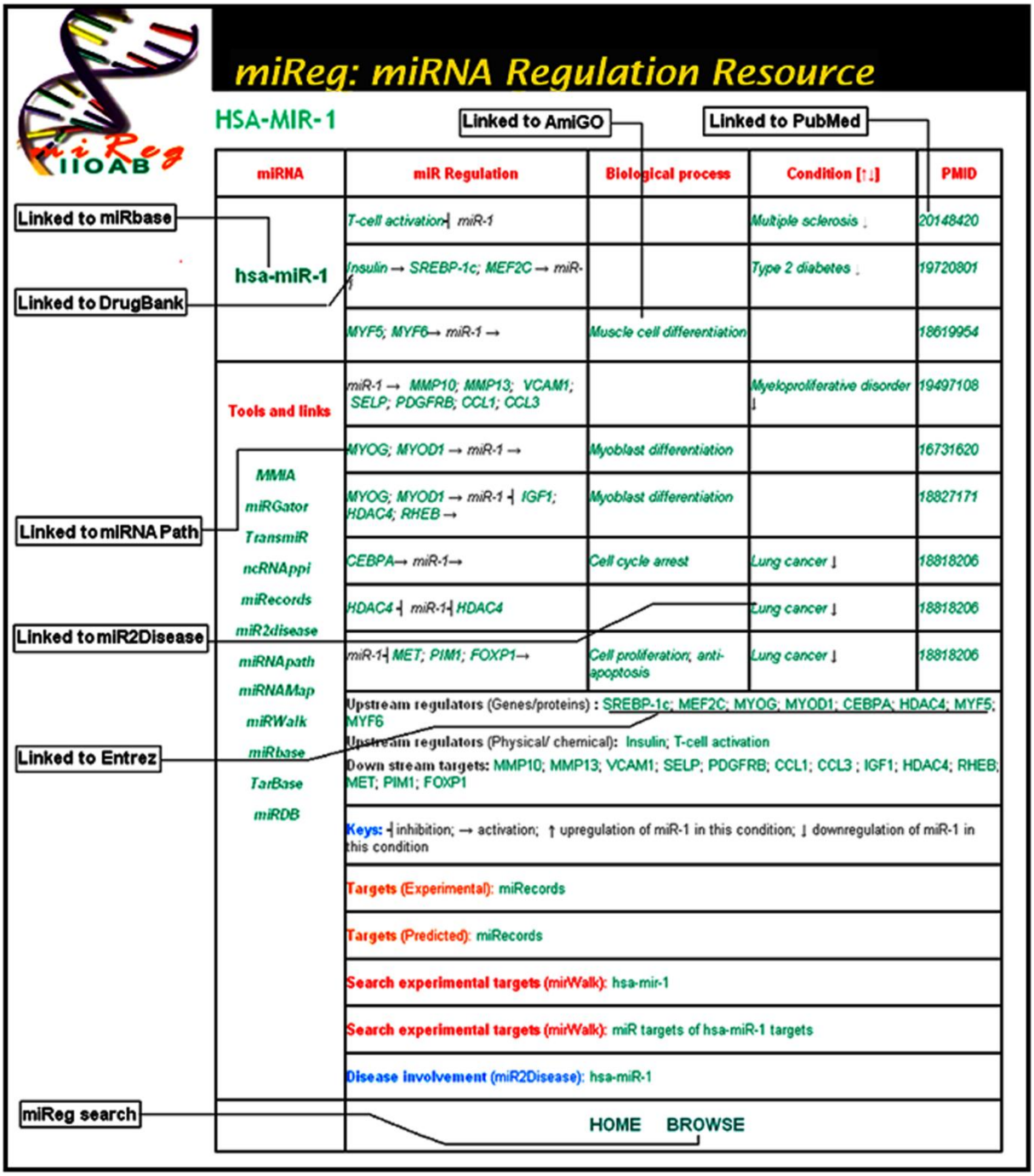

Figure 1: Snapshot of has-mir-1 page from *miReg*. The page shows miR-1 specific reactions, various upstream regulators of miR-1 and its targets, specific BPs, and experimental conditions along with corresponding PMID, reaction summery table, different web browsing options, and various external tools, links, and miRNA resources.

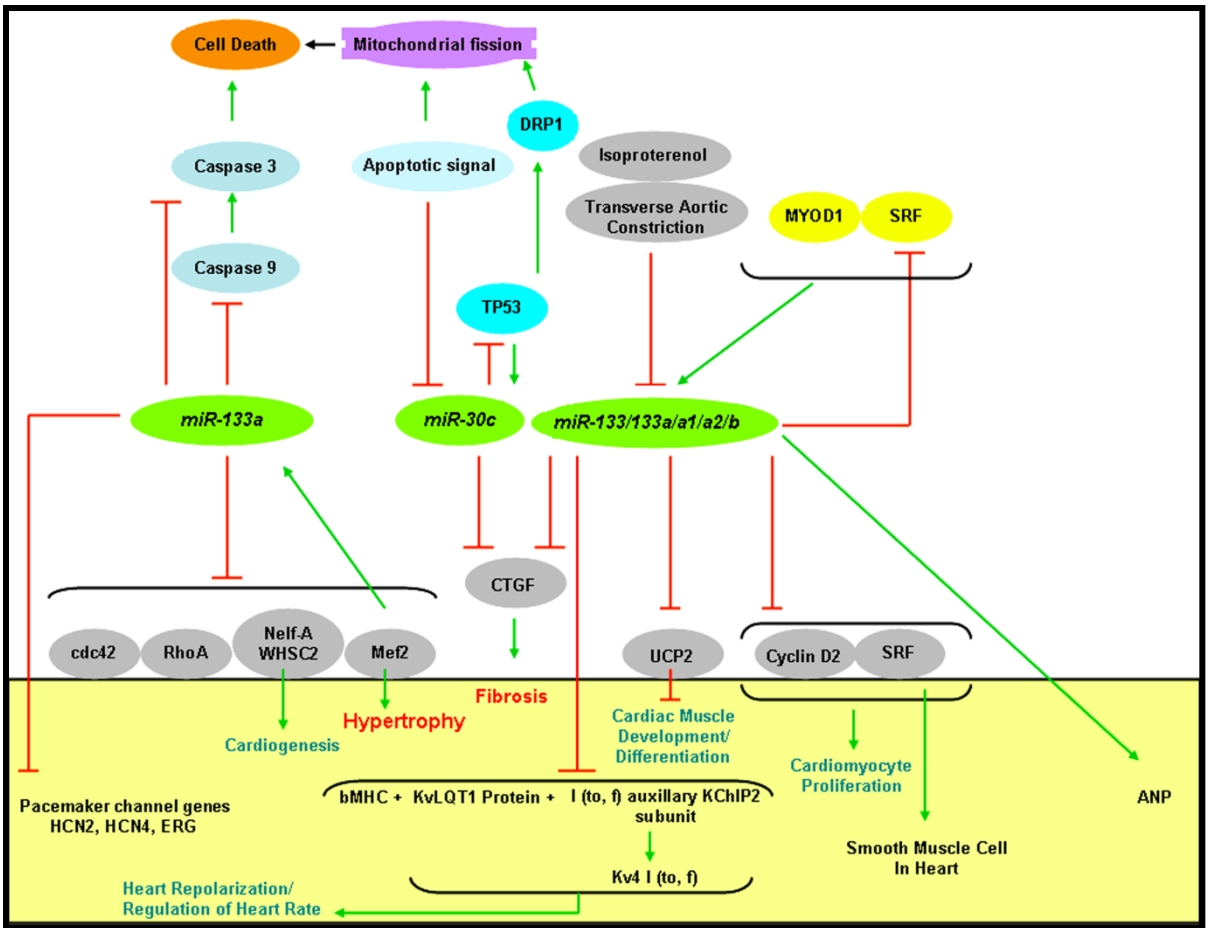

Figure 2: Proposed model of cardiomyopathy regulation by miR-30 and miR-133. This schematic representation depicts important signaling regulations between miR-30c/miR-133 and their upstream modulators and downstream targets, respectively. Abbreviations: DRP1: dynamin-related protein-1; TP53: tumor protein p53; CTGF: connective tissue growth factor; MYOD1: myogenic differentiation 1; SRF: serum response factor (c-fos serum response element-binding transcription factor); UCP2: uncoupling protein 2; KvLQT1: potassium voltage-gated channel, KQT-like subfamily, member 1; KChIP2: voltage-gated potassium Kv channel interacting protein 2; Kv4 I (to, f): Kv4-encoded fast transient outward (I_{to}) K^+ channels; HCN2: potassium hyperpolarization-activated cyclic nucleotide-gated channel 2; HCN4: potassium hyperpolarization-activated cyclic nucleotide-gated channel 4; ERG: ether-a-go-go-related K^+ channel gene; bMHC: beta myosin heavy chain; ANP: atrial natriuretic peptide; Mef2: myocyte enhancer factor-2; NelfA/WHSC2: negative elongation factor A/Wolf-Hirschhorn syndrome candidate 2; RhoA: Ras homolog gene family, member A, small GTPase protein; cdc42: cell division control protein 42. The symbols → and ⊥ indicate activation/upregulation and inhibition/downregulation, respectively. Please refer to *miReg* for corresponding references of published data.

4 Data collection statistics

This introductory version (Version 1.0) of *miReg* contains 47 important human miRs, 85 upstream validated regulators (proteins), 30 upstream validated regulators (drugs/ chemicals/physical or biological process), 165 experimentally validated targets, 38 disease information, 295 reactions, and 70 biological processes related miRs manually curated form 190 absolute PubMed references.

5 Future developments

In this release, we have given more emphasis on scientific information than on the technical aspects of the browser. This web resource is consistent and will be maintained continuously. Although at the current stage of development of *miReg*, a small number of miRs have been listed, we are working towards data enrichment and *miReg* data resource will constantly be updated with new published data added to the existing list of miRs and will incorporate most of the human miRs soon. We shall also include disease specific miR networks in the *miReg* in the near future. To create these regulatory pathways, we currently work with specialists in the domains of neurobiology, cardiovascular research, cancer and diabetes. Moreover, we shall shift from HTML based browser to Oracle/ SQL based *miReg* database in our next release with all technical demands of a database including the query system. Also, we plan to link *miReg* to ClinicalTrials.gov (http://clinicaltrials.gov/). Indeed, a search of the keywords "microRNA" or "miRNA" in ClinicalTrials.gov will give 47 results of miR-related clinical studies either completed or in progress.

6 Conclusion

Therefore, since miRNA research has implications in disease screening and therapies, we hope that *miReg* will provide a platform for scientists and clinicians, enabling the sharing and exchange of information on miRNA knowledge.

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References

[1] W. Filipowicz, S.N. Bhattacharyya, and N. Sonenberg, Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight?, Nat Rev Genet, vol. 9, pp 102-114, 2008.

[2] K.N. Ivey, A. Muth, J. Arnold, F.W. King, R.F. Yeh, J.E. Fish, E.C. Hsiao, R.J. Schwartz, B.R. Conklin, H.S. Bernstein, and D. Srivastava, MicroRNA regulation of cell lineages in mouse and human embryonic stem cells, Cell Stem Cell, vol. 6, pp. 219-229, 2008.

[3] M.V. Latronico, D. Catalucci, and G. Condorelli, Emerging role of microRNAs in cardiovascular biology, Circ Res, vol. 7, pp. 1225-1236, 2007.

[4] V. Tarasov, P. Jung, B. Verdoodt, D. Lodygin, A. Epanchintsev, A. Menssen, G. Meister, and H. Hermeking, Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest, Cell Cycle, vol. 6, pp. 1586-1593, 2007.

[5] J.F. Chen, E.M. Mandel, J.M. Thomson, Q. Wu, T.E. Callis, S.M. Hammond, F.L. Conlon, and D.Z. Wang, The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation, Nat Genet, vol. 38, pp. 228-233, 2006.

[6] M.N. Poy, L. Eliasson, J. Krutzfeldt, S. Kuwajima, X. Ma, P.E. Macdonald, S. Pfeffer, T. Tuschl, N. Rajewsky, P. Rorsman, and M. Stoffel, A pancreatic islet-specific microRNA regulates insulin secretion, Nature, vol. 11, pp. 226-230, 2004.

[7] A. Rodriguez, E. Vigorito, S. Clare, M.W. Warren, P. Couttet, D.R. Soond, S. van Dongen, R.J. Grocock, P.P. Das, E.A. Miska, D. Vetrie, K. Okkenhaug, A.J. Enright, G. Dougan, M. Turner, and A. Bradley, Requirement of bic/microRNA-155 for normal immune function, Science, vol. 316, pp. 608-611, 2007.

[8] O.C. Maes, J. An, H. Sarojini, and E. Wang, Murine microRNAs implicated in liver functions and aging process, Mech Ageing Dev, vol. 129, pp. 534-541, 2008.

[9] S.S. Hébert, K. Horré, L. Nicolaï, A.S. Papadopoulou, W. Mandemakers, A.N. Silahtaroglu, S. Kauppinen, A. Delacourte, and B. De Strooper, Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression, Proc Natl Acad Sci U S A, vol. 29, pp. 6415-6420, 2008.

[10] G. Wang, J.M. van der Walt, G. Mayhew, Y.J. Li, S. Züchner, W.K. Scott, E.R. Martin, and J.M. Vance, Variation in the miRNA-433 binding site of FGF20 confers risk for Parkinson disease by overexpression of alpha-synuclein, Am J Hum Genet, vol. 82, pp. 283-289, 2008.

[11] C. Blenkiron and E.A. Miska, miRNAs in cancer: approaches, aetiology, diagnostics and therapy, Hum Mol Genet, vol. 16, Spec No 1, pp. R106-R113, 2007.

[12] A. Esquela-Kerscher and F.J. Slack, Oncomirs - microRNAs with a role in cancer, Nat Rev Cancer, vol. 6, pp. 259-269, 2006.

[13] S. Fujita, T. Ito, T. Mizutani, S. Minoguchi, N. Yamamichi, K. Sakurai, and H. Iba, miR-21 Gene expression triggered by AP-1 is sustained through a double-negative feedback mechanism, J Mol Biol, vol. 378, pp. 492-504, 2008.

[14] C.P. Bracken and P.A. Gregory, ZEB1-SIP1 and the microRNA-200 family regulates epithelial-mesenchymal transition, Cancer Res, vol. 68, pp. 7846-7854, 2008.

[15] M. Yamakuchi and C.J. Lowenstein, MiR-34, SIRT1 and p53: the feedback loop, Cell Cycle, vol. 8, pp. 712-715, 2009.

[16] A. Palmieri, F. Pezzetti, G. Brunelli, M. Martinelli, L. Scapoli, M. Arlotti, E. Masiero, and F. Carinci, Medpor regulates osteoblast's microRNAs, Biomed Mater Eng, vol. 18, pp. 91-97, 2008.

[17] T.E. Miller, K. Ghoshal, B. Ramaswamy, S. Roy, J. Datta, C.L. Shapiro, S. Jacob, and S. Majumder, MicroRNA-221/222 confers tamoxifen resistance in breast cancer by targeting p27Kip1, J Biol Chem, vol. 283, pp. 29897-29903, 2008.

[18] J. Filkowski, Y. Ilnytskyy, J. Tamminga, I. Koturbash, A. Golubov, T. Bagnyukova, I.P. Pogribny, and O. Kovalchuk, Hypomethylation and genome instability in the germline of exposed parents and their progeny is associated with altered miRNA expression, Carcinogenesis, vol. 31, no. 6, pp. 1110-1115, 2010.

[19] B. Zhang and X. Pan, RDX induces aberrant expression of microRNAs in mouse brain and liver, Environ Health Perspect, vol. 117, pp. 231-240, 2009.

[20] J. Wang, M. Lu, C. Qiu, Q. Cui, TransmiR: a transcription factor-microRNA regulation database, Nucleic Acids Res, vol. 38, pp. D119-D122, 2010.

[21] S. Griffiths-Jones, R.J. Grocock, S. van Dongen, A. Bateman, A.J. Enright, miRBase: microRNA sequences, targets and gene nomenclature, Nucleic Acids Res, vol. 34, pp. D140-D144, 2006.

[22] A.O. Chiromatzo. T.Y. Oliveira, G. Pereira, A.Y. Costa, C.A. Montesco, D.E. Gras, F. Yosetake, J.B. Vilar, M. Cervato, P.R. Prado, R.G. Cardenas, R. Cerri, R.L. Borges, R.N. Lemos, S.M. Alvarenga, V.R. Perallis, D.G. Pinheiro, I.T. Silva, R.M. Brandão, M.A. Cunha, S. Giuliatti, W.A. Silva Jr, miRNApath: a database of miRNAs, target genes and metabolic pathways, Genet Mol Res, vol. 6, pp. 859-865, 2007.

[23] S. Carbon, A. Ireland, C.J. Mungall, S. Shu, B. Marshall, and S. Lewis; AmiGO Hub; Web Presence Working Group, AmiGO: online access to ontology and annotation data, Bioinformatics, vol. 25, pp. 288-289, 2009.

[24] Q. Jiang, Y. Wang, Y. Hao, L. Juan, M. Teng, X. Zhang, M. Li, G. Wang, and Y. Liu, miR2Disease: a manually curated database for microRNA deregulation in human disease, Nucleic Acids Res, vol. 37, pp. D98-D104, 2009.

[25] D.S. Wishart, C. Knox, A.C. Guo, D. Cheng, S. Shrivastava, D. Tzur, B. Gautam, and M. Hassanali, DrugBank: a knowledgebase for drugs, drug actions and drug targets, Nucleic Acids Res, vol. 36, pp. D901-D906, 2008.

[26] F. Xiao, Z. Zuo, G. Cai, S. Kang, X. Gao, and T. Li, miRecords: an integrated resource for microRNA-target interactions, Nucleic Acids Res, vol. 37, pp. D105-110, 2009.

[27] A. Carè, D. Catalucci, F. Felicetti, D. Bonci, A. Addario, P. Gallo, M.L. Bang, P. Segnalini, Y. Gu, N.D. Dalton, L. Elia, M.V. Latronico, M. Høydal, C. Autore, M.A. Russo, G.W. Dorn 2nd, O. Ellingsen, P. Ruiz-Lozano, K.L. Peterson, C.M. Croce, C. Peschle, G. Condorelli, MicroRNA-133 controls cardiac hypertrophy, Nat Med, vol. 13, no. 5, pp. 613-618, 2007.

[28] R.F. Duisters, A.J. Tijsen, B. Schroen, J.J. Leenders, V. Lentink, I. van der Made, V. Herias, R.E. van Leeuwen, M.W. Schellings, P. Barenbrug, J.G. Maessen, S. Heymans, Y.M. Pinto, and E.E. Creemers, miR-133 and miR-30 regulate connective tissue growth factor: implications for a role of microRNAs in myocardial matrix remodeling, Circ Res, vol. 104, pp. 170-178, 2009.

[29] S. Ikeda, A. He, S.W. Kong, J. Lu, R. Bejar, N. Bodyak, K.H. Lee, Q. Ma, P.M. Kang, T.R. Golub, and W.T. Pu, MicroRNA-1 negatively regulates expression of the hypertrophy-associated calmodulin and Mef2a genes, Mol Cell Biol, vol. 29, pp. 2193-2204, 2009.

[30] B. Yang, H. Lin, J. Xiao, Y. Lu, X. Luo, B. Li, Y. Zhang, C. Xu, Y. Bai, H. Wang, G. Chen, and Z. Wang, The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2, Nat Med, vol. 13, pp. 486-491, 2007.

[31] C. Xu, Y. Lu, Z. Pan, W. Chu, X. Luo, H. Lin, J. Xiao, H. Shan, Z. Wang, and B. Yang, The muscle-specific microRNAs miR-1 and miR-133 produce opposing effects on apoptosis by targeting HSP60, HSP70 and caspase-9 in cardiomyocytes, J Cell Sci, vol. 120, pp. 3045-3052, 2007.