Tabari Biomedical Student Research Journal

Original Article

Application of bioinformatic software and tools for prediction of miRNAs suppressing mTOR/S6K1 signaling pathway as new targets of cancer therapy

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mTOR/S6K1 signaling pathway is a center of cell growth and survival and dysregulation of this pathway is involved in the incidence of many cancers. MiRNAs are key regulators of gene expression at post-translational levels and their dysregulation is considered in many cancers. There are various methods for detection of miRNAs. Regarding extensive development of bioinformatics, these applications can be utilized for prediction of miRNAs and their target genes with acceptable accuracy. Therefore, the aim of this study was to apply bioinformatic software and programs, as a low cost method for prediction of miRNAs targeting mTOR/S6K1 signaling pathway. Prediction of miRNAs targeting mTOR and S6K1 genes was performed using bioinformatic software and programs named TargetScan, miRBase, miRanda, microCosm, microPIR, miRDB, miRSearch, miRWalk, DIANA-microT, PicTar, TarBase, miRTarBase, miRNAMap and MirZ bioinformatics tools Hsa-miR-100-5p, hsa-miR-99a-5p, hsa-miR-199a-3p, hsa-miR-99b-5p and hsa-miR-96-5p had the highest score and repeat among all databases to target 3′-UTR of mTOR and hsa-miR-557, hsa-miR-362-5p, hsa-miR-500a-5p, hsa-miR-223-3p and hsa-miR-200c-3p had the highest score to target 3′-UTR of S6K1. Bioinformatic approaches are accessible and low-cost techniques to find miRNAs targeting genes in different signaling pathways. Considering the role of mTOR/S6K1 signaling pathway in cell growth and survival and its dysregulation in different cancers, miRNAs that suppress this signaling pathway can be utilized as a potential effective therapeutic and diagnostic tool.

Keywords: mTOR, S6K1, miRNA, Bioinformatic

Introduction

The mTOR/S6K1 signaling pathway is important in many aspects of cell growth and survival whether in natural or pathologic conditions. Mechanistic Target of Rapamycin (mTOR) is a serine/threonine kinase located in short arm of chromosome number 1 (1p36) and is expressed in all mammalian cells [1]. In the interaction with several other proteins, mTOR forms multi-protein complexes, called mTORC1 and mTORC2, which are structurally and functionally different. One of the key downstream effectors of mTOR is S6K1, which initiates the translation of mRNA into a protein that is essential for cell growth and survival, cell development, and cellular metabolism [2]. One of the S6K1 substrates is S6 ribosomal protein and the programmed cell death protein 4 (PDCD4), a tumor suppressor. Totally, S6K1 activation results in increased protein synthesis by effects on various factors. Recent studies have also shown mTOR and S6K1 are involved in the biosynthesis of lipids and nucleotides. Changes and disturbances in the mTOR/S6K1 signaling pathway have been observed in a wide variety of cancers and studies declared that inhibition of this pathway by various factors can contribute to the treatment of various cancers [1,3,4].

Micro-RNAs (miRNAs) are a class of single-stranded RNAs with an average length of 18 to 22 nucleotides and are the key molecules for regulation of gene expression at post-translational levels. The functional mechanism of these molecules

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is binding to 3′ untranslated regions (3′-UTR) of the target gene's mRNA, which subsequently leads to suppression of the translation, or the complete degradation of mRNA [5–7]. MiRNAs are very important regulatory molecules in most biological processes. Over the past decades, many studies investigated the miRNAs expression changes and its correlation with a large number of disorders, especially cancers [8–10]. The results of these studies suggest that determination of various miRNAs expression profiles can be used as an appropriate tool for the diagnosis, prognosis and even treatment of many diseases and cancers [5].

There are different methods for quantitative measurement of miRNAs in tissues and investigation of their roles in different disorders. These techniques are in situ hybridization, microarrays, real-time PCR, next generation sequencing and Nanostring nCounter [11,12]. These methods are often expensive and are not usually available to everyone. One of the disadvantages of these methods is their inability to differentiate between pri-, pre- and mature miRNAs as well as their families [13]. For example: microarrays is known as the most popular diagnostic method for miRNAs and is a high power technology that help researchers to evaluate the expression of a large number of targets in only one experiment, but due to the costs, it is not commonly used, especially in developing countries and its sensitivity and specificity are limiting factors [13].

Today, due to the large amounts of biological information, the use of computers in biosciences has been developed. Bioinformatic is composed of biology and computer sciences and can provide a large amount of data over a short period of time. A large number of engineers and bioinformatic specialists are designing and developing new tools for better and faster management of data. Availability, high speed in performance and being cost beneficial are among the advantages of application of bioinformatic. Due to the possibility of errors during the use of bioinformatic tools, multiple programs and software can be simultaneously used to achieve more reliable results. Furthermore, the information of bioinformatic tools have acceptable accuracy.

Today, there is a wide range of information about miRNAs and their regulatory function on target genes and different pathways [12,13]. Considering the role of mTOR/S6K1 signaling pathway in many cancers, the purpose of this study was to identify and determine of miRNAs targeting mTOR and S6K1, as their inhibitors, by applying bioinformatic algorithms. The results of this study can take the first steps toward using these small RNA molecules for identification, pre-screening and even the treatment of the different cancers.

Materials and Methods

For prediction of miRNAs targeting 3′-UTR of mTOR and S6K1 mRNAs, the bioinformatic softwares named in table 1 were applied. All information obtained from mentined softwares were extracted and inserted in an excel file. The information of TargetScan was the basis of comparisons since the algorithm used in this software is the best one for prediction of miRNAs and their target genes [14]. MiRNAs with 8mer and 7mer-m8 seed matchs which had the highest levels of complementarity with the 3′-UTR of mTOR and S6K1 mRNA were selected. All acquired data were compared to TargetScan. Therefore, the number of repeats of miRNAs in other tools and databases were evaluated. Finally, the miRNAs with higher score and repitation in different databases were selected as the miRNA target gene.

Results

The TargetScan database is applied for prediction of miRNAs targetinf 3′-UTRs in mammals, including humans. In this software the status of binding of each miRNA to its target 3′-UTR is reported in three ways based on the type of hybrid they form. 1) Bindings with eight nucleotide protected seeds (8mer) which is the most complete type of connection, 2) bindings in the form of seven nucleotides (7mer) that is divided into two categories: 7mer-m8 and 7mer-1A and 3) bindings with six nucleotides are shown as 6mer. The algorithm used in this software is of the most powerful ones for prediction of miRNAs. The miRBase is also a searchable database of sequences and interpretations of miRNAs and is the most comprehensive reference in this regard. In this database, the information about the sequences of immature and mature miRNAs, with the symbol of mir and miR respectively is available. The results obtained from bioinformatic databases and tools mentioned in the materials and methods section demonstrated that miRNAs including hsa-miR-100-5p, hsa-miR-99a-5p, hsa-miR-199a-3p, hsa-miR-99b-5p and hsa-miR-96-5p have the highest score and repeat among all databases to target 3′-UTR of mTOR gene mRNA as well as hsa-miR-557, hsa-miR-362-5p, hsa-

miR-500a-5p, hsa-miR-223-3p and hsa-miR-200c-3p were the miRNAs which had the top scores in targeting the 3′-UTR of S6K1 mRNA.

Table 2 summarizes the results of bioinformatic prediction in databases. With this explanation that the number 1 represents prediction of miRNA at the desired database as the miRNA targeting the relevant mRNA and number 0shows failure to predict favarable miRNA.

Discussion

MiRNAs are one of the key regulators of gene expression at post-transcription levels and several studies have shown dysregulation in miRNAs involved in many diseases and cancers. In recent years, there was an increasing interest to miRNAs as the valuable diagnostic and prognostic molecules, even appropriate and effective potential molecular tools for targeted therapies [15,16]. The role of dysregulation in mTOR/S6K1 signaling pathway was approved in many types of cancers. Therefore, an interesting strategy against cancers maybe inhibition of mTOR signaling pathway by miRNAs targeting the key genes in this pathway [17]. This study aimed to identify the miRNAs targeting mTOR and S6K1 genes from mTOR signaling pathway. Results showed "miR-100, miR-99a, miR199a, miR-99b and miR-96" and also "miR-223, miR-557, miR-362, miR-500a and miR-200c" are potential inhibitors of mTOR and S6K1, respectively.

MiR-100 is a member of miR-99 family (miR-99a, miR-99b and miR-100). The potential role of miR-100 in diagnostic, prognostic and therapeutic strategies of cancers was investigated in different studies. Chu Qin et al. showed ectopic expression and imbalance in miR-100 is involved in tumor generation and progression in many cancers. These researchers used bioinformatic analysis in their study and the results indicated mTOR is directly targeted by miR-100 [18] that this finding is in agreement with ours. In another study, Wang et al. showed that miR-100 suppresses the mTOR signaling pathway in pulmonary hypertension induced by hypoxia [19]. In a study by Yang et al., miR-99a was introduced as a tumor suppressor miRNA in breast cancer. The results show that miR-99a presumably suppress the tumorigenicity and phenotype of breast cancer stem cells by targeting mTOR signaling pathway [20]. Since the mTOR has an oncogenic role, overexpression of this key regulator can lead to cancer; therefore, the miRNAs which target this gene, act as a tumor suppressor miRNA. In a research that was conducted by Wei et al., the results indicated that down-regulation of miR-99b can lead to mTOR overexpression. In this study, miR-99b introduced

Table 1. Software and bioinformatic databases

Rank	Software/Database	Web address
1	TargetScan	http://www.targetscan.org/vert_71/
2	miRBase	http://www.mirbase.org/
3	miRanda	http://www.microrna.org/microrna/home.do
4	microCosm	http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/
5	microPIR	http://www4a.biotec.or.th/micropir/
6	miRDB	http://mirdb.org/
7	miRSearch	https://www.exiqon.com/mirsearch/
8	miRWalk	http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/
9	DIANA-microT	http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index
10	PicTar	http://www.pictar.org/
11	TarBase	http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index
12	miRTarBase	http://mirtarbase.mbc.nctu.edu.tw/
13	miRNAMap	http://mirnamap.mbc.nctu.edu.tw/
14	MirZ	http://www.mirz.unibas.ch/

Table 2. The results of bioinformatic prediction of miRNAs targeting 3′-UTR of mTOR and S6K1 mRNAs

Gene	miRNA	TargetScan	miRanda	microCosm	microPIR	miRDB	miRSearch	miRWalk	DIANA-microT	PicTar	TarBase	miRTarBase	miRNAMap	MirZ	SUM
mTOR	hsa-miR-100-5p	1	1	1	1	1	1	1	1	1	1	1	0	1	12
	hsa-miR-99a-5p	1	1	1	1	1	1	1	1	1	1	1	0	1	12
	hsa-miR-199a-3p	1	1	1	0	1	1	1	1	1	1	1	0	1	11
	hsa-miR-99b-5p	1	1	1	1	1	1	1	0	1	1	1	0	1	11
	hsa-miR-96-5p	1	1	1	1	1	1	1	1	1	0	0	0	1	10
S6K1	hsa-miR-223-3p	1	1	0	1	1	1	1	1	1	0	0	1	1	10
	hsa-miR-557	1	1	0	1	1	1	1	1	0	0	0	1	1	9
	hsa-miR-362-5p	1	1	0	1	1	0	1	1	0	0	1	1	1	9
	hsa-miR-500a-5p	1	1	0	1	1	0	1	1	0	1	1	0	1	9
	hsa-miR-200c-3p	1	1	0	1	1	0	1	1	1	0	0	1	1	9

as a key mediator of mTOR [21]. Li and colleagues found that up-regulation of miR-199a can reduces the cell proliferation and increases the apoptosis rate. This phenomena is in favor of physiological conditions and against cancers. The results of this study showed that miR-199a mimics can target mTOR mRNA at 3′-untranslated region (3′-UTR) region and hence can reduce its expression. It should be noted the these researchers used TargetScan algorithm for miR-199a target gene identification [22]. In a study by Zhang et al. miR-233 introduced as a potential biomarker with the highest predictive accuracy for screening and early diagnostic of non-small cell lung cancer [23]. The results of a research conducted by Ni et al. on breast cancer showed that down-regulation of miR-362 inhibits the proliferation, differentiation, migration and invasion of cancerous cells. TargetScan algorithm was used in this study to identify of miR-362 target mRNAs, too [24]. In a large number of studies, the mutual effects of miR-96 and mTOR signaling pathway has been investigated, including the study of Sun et al. on interaction between miR-96 and mTOR. The results of this study approved the targeting of mTOR by miR-96 [25]. Also, the results of Oliveras-Ferraros et al. approved the tumor suppressor role of miR-96 [26]. Katayama et al. demonstrated that the expression of miR-557 in tumor tissues of hepatocellular carcinoma was decreased [27]. Therefore, regarding the ability to regulate gene expression, miRNAs play a key role in the incidence of cancers and tumor metastasis [28]. In general, one miRNA can target many genes and on the other side, one gene also affect by a lot of miRNAs. During cancer, dysregulation of these miRNAs and their target genes occurs. Today, the use of miRNAs in biological and medical sciences has become widespread and bioinformatic can de served as a rapid, economical and reliable tool in these fields.

References

1. Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR signaling in cancer. Front Oncol. 2014;4:64.
2. Ras PI. K and mTOR signalling controls tumour cell growth. Nature. 2006;441:424.
3. Hassan B, Akcakanat A, Holder AM, Meric-Bernstam F. Targeting the PI3-kinase/Akt/mTOR signaling pathway. Surg Oncol Clin. 2013;22(4):641–64.
4. Huang K, Fingar DC. Growing knowledge of the mTOR signaling network. In: Seminars in cell & developmental biology. Elsevier; 2014. p. 79–90.
5. Gulyaeva LF, Kushlinskiy NE. Regulatory mechanisms of microRNA expression. J Transl Med. 2016;14(1):143.
6 Hu Y-B, Li C-B, Song N, Zou Y, Chen S-D, Ren R-J, et al. Diagnostic value of microRNA for Alzheimer's disease: a systematic review and meta-analysis. Front Aging Neurosci. 2016;8:13.
7. O'Reilly S. MicroRNAs in fibrosis: opportunities and challenges. Arthritis Res Ther. 2016;18(1):11.
8. Hata A, Kashima R. Dysregulation of microRNA biogenesis machinery in cancer. Crit Rev Biochem Mol Biol. 2016;51(3):121–34.
9. Mohammadi-Yeganeh S, Mansouri A, Paryan M. Targeting of miR9/NOTCH1 interaction reduces metastatic behavior in triple-negative breast cancer. Chem Biol Drug Des. 2015;86(5):1185–91.
10. Katchy A, Williams C. Expression profiles of estrogen-regulated microRNAs in breast cancer cells. In: Estrogen Receptors. Springer; 2016. p. 373–93.
11. Moreno-Moya JM, Vilella F, Simón C. MicroRNA: key gene expression regulators. Fertil Steril. 2014;101(6):1516–23.
12. Abedi N, Mohammadi-Yeganeh S, Koochaki A, Karami F, Paryan M. miR-141 as potential suppressor of β-catenin in breast cancer. Tumor Biol. 2015;36(12):9895–901.
13. Ahsani Z, Mohammadi-Yeganeh S, Kia V, Karimkhanloo H, Zarghami N, Paryan M. WNT1 gene from WNT signaling pathway is a direct target of miR-122 in hepatocellular carcinoma. Appl Biochem Biotechnol. 2017;181(3):884–97.
14. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005;120(1):15–20.
15 Wang Q, Li C, Zhu Z, Teng Y, Che X, Wang Y, et al. miR-155-5p antagonizes the apoptotic effect of bufalin in triple-negative breast cancer cells. Anticancer Drugs. 2016;27(1):9–16.
16. Das SG, Romagnoli M, Mineva ND, Barillé-Nion S, Jézéquel P, Campone M, et al. miR-720 is a downstream target of an ADAM8-induced ERK signaling cascade that promotes the migratory and invasive phenotype of triple-negative breast cancer cells. Breast Cancer Res. 2016;18(1):40.
17. Mo W, Liu Q, Lin CC-J, Dai H, Peng Y, Liang Y, et al. mTOR inhibitors suppress homologous recombination repair and synergize with PARP inhibitors via regulating SUV39H1 in BRCA-proficient triple-negative breast cancer. Clin Cancer Res. 2016;22(7):1699–712.

18. Deng L, Chen J, Zhong XR, Luo T, Wang YP, Huang HF, et al. Correlation between activation of PI3K/AKT/mTOR pathway and prognosis of breast cancer in Chinese women. PLoS One. 2015;10(3):e0120511.
19. Wang A, Li X, Gong S, Li W, Hu C, Zhang Z, et al. miR-100 suppresses mTOR signaling in hypoxia-induced pulmonary hypertension in rats. Eur J Pharmacol. 2015;765:565–73.
20. Yang Z, Han Y, Cheng K, Zhang G, Wang X. miR-99a directly targets the mTOR signalling pathway in breast cancer side population cells. Cell Prolif. 2014;47(6):587–95.
21. Wei F, Liu Y, Guo Y, Xiang A, Wang G, Xue X, et al. miR-99b-targeted mTOR induction contributes to irradiation resistance in pancreatic cancer. Mol Cancer. 2013;12(1):81.
22. Li Q, Xia X, Ji J, Ma J, Tao L, Mo L, et al. MiR-199a-3p enhances cisplatin sensitivity of cholangiocarcinoma cells by inhibiting mTOR signaling pathway and expression of MDR1. Oncotarget. 2017;8(20):33621.
23. Zhang H, Mao F, Shen T, Luo Q, Ding Z, Qian L, et al. Plasma miR-145, miR-20a, miR-21 and miR-223 as novel biomarkers for screening early-stage non-small cell lung cancer. Oncol Lett. 2017;13(2):669–76.
24. Ni F, Gui Z, Guo Q, Hu Z, Wang X, Chen D, et al. Downregulation of miR-362-5p inhibits proliferation, migration and invasion of human breast cancer MCF7 cells. Oncol Lett. 2016;11(2):1155–60.
25. Sun X, Zhang C. MicroRNA-96 promotes myocardial hypertrophy by targeting mTOR. Int J Clin Exp Pathol. 2015;8(11):14500–6.
26. Oliveras-Ferraros C, Cufí S, Vazquez-Martin A, Torres-Garcia VZ, Barco S Del, Martin-Castillo B, et al. Micro(mi)RNA expression profile of breast cancer epithelial cells treated with the anti-diabetic drug metformin: Induction of the tumor suppressor miRNA let-7a and suppression of the TGFβ-induced oncomiR miRNA-181a. Cell Cycle. 2011;10(7):1144–51.
27. Katayama Y, Maeda M, Miyaguchi K, Nemoto S, Yasen M, Tanaka S, et al. Identification of pathogenesis-related microRNAs in hepatocellular carcinoma by expression profiling. Oncol Lett. 2012;4(4):817–23.
28 Mohammadi-Yeganeh S, Paryan M, Arefian E, Vasei M, Ghanbarian H, Mahdian R, et al. MicroRNA-340 inhibits the migration, invasion, and metastasis of breast cancer cells by targeting Wnt pathway. Tumor Biol. 2016;37(7):8993–9000.