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Research Article

Bioinformatic Investigation of Mirnas Involved in the Regulation of β-Catenin and Cyclin D1 Expression.

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ABSTRACT:

Hepatocellular Carcinoma (HCC) is one of the most prevalent malignant worldwide. Recent finding have demonstrated that microRNAs (miRNAs) can use as therapeutic and diagnostic biomarker in many types of diseases, especially in cancer. Considerable improvement in bioinformatical algorithms and computer modeling systems has led to increase in the quality and quantity of the software and databases provide to predict genes targeting miRNAs. We use Clinical Bioinformatics, the new science that translate bioinformatics to clinical informatics and medical application. Extensive studies have shown Wnt/β-catenin signaling pathway play essential and critical role in growth, development and differentiation of cell and was impaired in HCC and many types of cancer. β-cateninis a multifunctional protein that regulates transcription factors and important genes involved in tumorigenesis. Another important gene in this pathway is CyclinD1, a proto-oncogene and an important regulator of cell cycle, which is located downstream of β-catenin. Blocking the function of β-catenin and CyclinD1 causes apoptosis and decline in tumorigenesis of various cancers, especially in HCC. The purpose of this study is to bioinformatically predict miRNAs that involved in the regulation of the expression of CyclinD1 and β-catenin. miRNA prediction databases, miRWalk, DIANA, Target Scan, miRanda, mirZ, PicTar, miRPath, microCosm and Qiagen were recruited to find microRNAs targetingCyclin D1(CCND1) and β-catenin(CTNNB1). Then the number of databases confirming miRNA:3'UTR attachment, a table of miRNA prediction was prepared.

This bioinformatical approach showed that, with the highest score, miR-214 and miR-20 more probably connect to 3'UTRs of CTNNB1 and CCND1.It seems that miR-20 andmiR-214 can regulate expression of CCND1 and CTNNB1, subsequently play an important role in prevention of development and progression of a variety of malignant tumors.

Keywords: Bioinformatics; miRNA; CyclinD1; β-catenin; Hepatocellular Carcinoma

[I] NTRODUCTION

Hepatocellular carcinoma (HCC) is the dominant form of primary liver cancer (85-90%) which is one of the most prevalent malignancies and the third cause of cancer deaths [1, 2]. In spite of the advances in cancer treatments, current therapies for HCC have some limitations [3]. Therefore, more comprehensive and detailed understanding of the molecular processes and metastasis

mechanisms of HCC is necessary to develop new therapies [4]. In some cases, disruptions in the regulation of genes that encode proteins of signaling pathways such as Wnt/β-catenin have been observed [5-8]. Wnt/β-catenin signaling pathway plays an important part in growth, development and differentiation of cells and is one of the major signaling pathways affected in HCC. It has also been determined that β-catenin expression is upregulated in more than 62% of HCC cases [7-13]. β-catenin is a critical mediator of Wnt signaling pathway transported form cytoplasm to the nucleus of cells and activates T-cell factor, a transcription factor [2, 10]. Recently, some genes that have been proved to play a role in tumorigenesis are reported to be targets of β-catenin, one of which is Cyclin D1 (CCND1). Increased expression of CCND1 in various cancers has been reported, and it is confirmed that inhibiting CCND1 expression in cancerous cells results in declined tumorigenesis and increased apoptosis and drug sensitivity [14, 15]. A principal cause of HCC is epigenetic alterations [10], a state of hereditary gene expression alteration accompanied with no mutations in DNA sequence. Epigenetic mechanisms include deoxycytosine methylation, histone modification and microRNAs (miRNAs) [1]. miRNAs are small non-coding RNAs, 19-25 nucleotide long, that post-transcriptionally regulate gene expression.miRNAs bind a complementary/semi-complementary region within 3'UTR of one or more mRNAs and repress or inhibit their expression [16-18]. So far, 35,828 miRNAs in 223 different species have been recorded [19]. Human genome encodes for nearly 1,200 miRNAs that target and regulate 60% of human genes [20]. miRNAs play a pivotal role in scores of biological processes such as differentiation, cell division, apoptosis, cell migration and invasion [18].

At the present time, by exploiting methods such as northern blot, microarray, in-situ hybridization, next generation sequencing, bead-based flowcytometry and qRT-PCT, researchers are able to identify miRNAs and investigate their roles in various diseases [21-24]. Among these methods, microarray and qRT-PCR are the most widely used ones, and since microarray is capable of determining expression profile status of mRNAs, it is the standard method to study genes regulated by miRNAs [25-28]. Nevertheless, it is costly, time-consuming, and it needs an experienced technician all of which make it difficult to be used broadly. Furthermore, noticeable advances in bioinformatics have led to the appearance of "clinical bioinformatics". By establishing a relationship between bioinformatic tools and clinical data, it greatly aids researchers in using bioinformatics in clinical settings. By means of clinical bioinformatics researchers will be able to wisely decide between bioinformatic tools to analyze microarray data, to optimize and improve disease-specific biomarkers and to screen and detect therapeutic targets. In addition, bioinformatic algorithms and in silico modeling systems that are able to predict miRNA targets have recently been improved to a great extent [29]. Therefore, using bioinformatics to discover miRNA targets is will be relatively inexpensive and less time-consuming.There are several web-based applications for the detection and prediction of miRNA targets all of which are based on complementarities of miRNA seed region with mRNA and thermodynamic stability of resulting duplexes [30-32]. Nonetheless, each application has its own unique algorithm.The goal of the present study is to bioinformatically predict miRNAs that target and regulate CCND1 and β-catenin mRNAs post-transcriptionally.

[II] MATERIALS AND METHODS

Nucleotide sequence of CCND1 (Cyclin D1, Ensembel accession #:ENSG00000110092) and CTNNB1 (β-catenin, Ensembel accession #:ENSG00000168036) was retrieved from NCBI. In order to find miRNAs targeting CCND1 and CTNNB1 mRNAs, we utilized the most comprehensive bioinformatic databases such as miRanda, TargetScan, DIANA, MicroCosm, mirPath and PicTar that provide only

bioinformatics predictions and miRZ, miRWalk and Qiagen that provide confirmed laboratory results in conjunction with bioinformatic predictions. Then a scoring table was prepared for candidate miRNAs, based on stability of miRNA:mRNA duplexand the number of repeats of target region in 3'UTR of the mRNAs. Finally, the miRNAs with the highest score were selected for functional analyses.

2.1. Bioinformatic prediction of miRNA

We used TargetScan which is a well-known web-based miRNA target prediction application used for vertebrates, especially human, and categorizes miRNAs in three groups: highly conserved, conserved and poorly conserved. This algorithm evaluates binding of miRNA seed region to target mRNA based on the thermodynamic stability of 7- and 8-mers and scores miRNAs from 0 to 1according to probability of conserved targeting [31, 33, 34].We also used miRanda, an online database that provides expression profile of miRNAs in addition to predicting targets of miRNAs [31, 35, 36]. Contrary to PicTar and microCosm, which predict targets only bioinformatically. microCosm is a web-based application at the EMBL-EBI that contains computationally predicted miRNA targets. PicTar also identifies targets of miRNAs and provides details about miRNA targets in vertebrates, Drosophila, nematodes and human. Contrary to PicTar and microCosm, which predict targets only bioinformatically [31, 37], miRWalk, along with bioinformatic predictions, provides users with confirmed laboratory results related to genes, pathways, organs, transcription factors and diseases. miRWalk uses data from 8 databases publishing information about miRNAs and their targets to offer predictions accurately and precisely about pathways and diseases [31, 38]. In addition, DIANA-tools applications were used. These applications analyze deep sequencing data, regulatory factors and miRNA targets to evaluate probable roles of miRNAs in diseases. The major goal of DIANA web server is to develop algorithms, databases and tools for interpretation of genomic information through systematic analysis.[31, 39-41] Finally, we benefited from miRZ and Qiagen to evaluate accuracy and precision of the results. Qiagen gives a collection of miRNAs playing a role in cancers such as breast cancer and their targets.

Algorithm	Website	Organisms	Input	Features	References
TargetScan	www.targetscan.org	mammals, fly, worm	miRNA name, miRNA family, gene name	seed match, conservation	Lewis et al., 2003[34] Friedman et al., 2009[33]
PicTar	pictar.mdc-berlin.de	vertebrate, fly, worm, mouse	microRNA name, gene name	seed match, pairing stability	Krek et al., 2005[37]
miRWalk	www.umm.uni-heidelberg.de/apps/zmf/mirwalk	human, rat, mouse	genes, pathways, diseases, organs, cell lines, OMIM disorders, and proteins	Watson–Crick complementarity Poisson distribution	Dweep, H., et al,2011[38]
miRanda	www.microrna.org	human, rat, mouse, fly, worm	miRNA name, miRNA sequence gene name	seed match, conservationfree energy	John et al., 2004 [35] Enright and John et al, 2003[36].
DIANA-microT	www.microrna.gr/microT-CDS	human, mouse, fly, worm	miRNA name, gene name, Ensembl ID	seed match, conservation, free energy, site accessibility, target-site abundance	Paraskevopoulouet al., 2013[39]

Table 1: Bioinformatic algorithms used for miRNA: mRNA predictions

[III] RESULTS

The result obtained from each algorithm was analyzed separately. Different algorithms were used to eliminate false positive results. Then, based on parameters such as 1) miRNA-mRNA 3'UTR attachment score from each individual algorithm 2) the number of algorithms that confirmed miRNA binding to the 3'UTR of target mRNAs (CTNNB1 and CCND1) and 3) practical results confirming miRNA binding to the target mRNAs, miRNAs were selected and a scoring table was prepared. Finally, it was concluded that miR-214 and miR-20 respectively target and regulate CTNNB1 and CCND1 mRNAs with the highest probability.

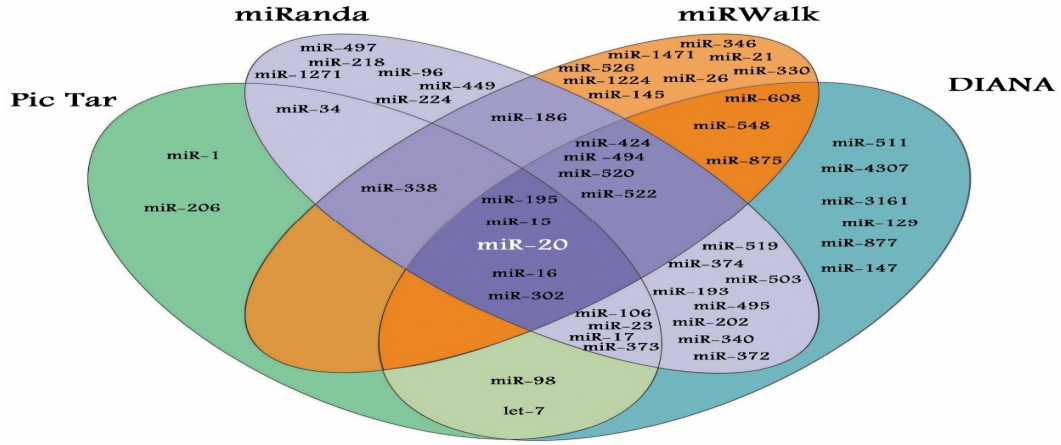

Fig: 1. miRNAs predicted for 3'UTR ofCCND1 mRNA by different algorithms

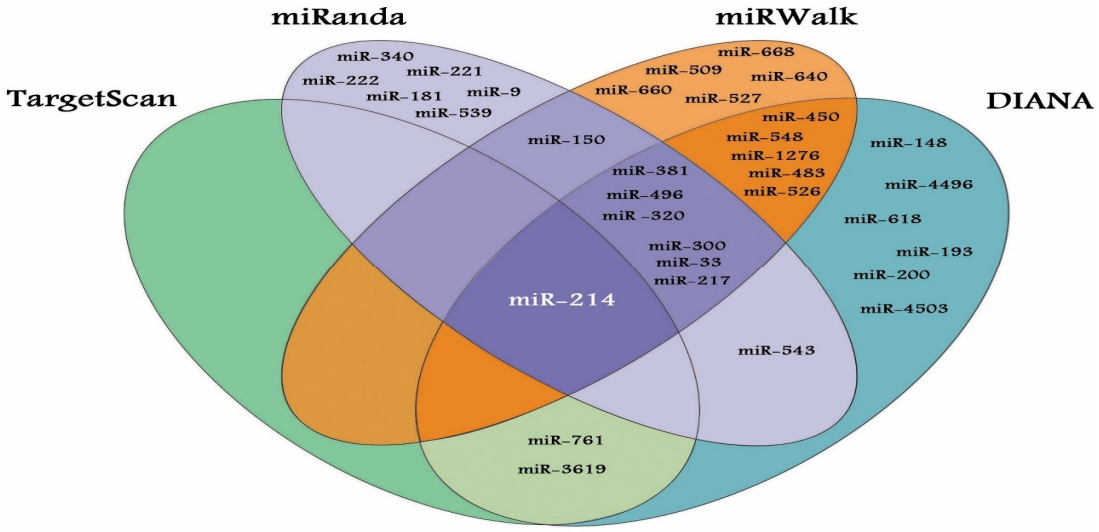

Fig: 2. miRNAs predicted for 3'UTR of CTNNB1 mRNA by different algorithms

	Target scan	Pic Tar	MiRWalk	miRanda	DIANA	MicroCosm	miRZ	Qiagen	Total Score
Mir-20 Target CD1	0	1	1	1	1	0	1	1	6
Mir-214 Target β-catenin	1	0	1	1	1	0	1	0	5

0	Not predicted
1	Predicted

Table2. Scoring table for selected miRNAs and respective targets in the applications

It should be mentioned that after selection of miR-214 and miR-20, which both predicts miRNA targets and compares their expression profile in various cell lines miRZ database, was used to evaluate the selected miRNAs. It also confirmed that these miRNAs target CTNNB1 and CCND1. To further assure that the results were reliable, we repeated the process in reverse order in that by using different bioinformatic algorithms, we sought genes that were targets of the miRNAs. Results showed that miR-214 and miR-20 are capable of targeting CCND1 and CTNNB1 mRNAs.

DIANA TOOLS-mirPath

This web-server was used to determine the role of miRNAs in signaling pathways. It uses the information from DIANA-microT-CDS and DIANA-TarBase v6.0, which respectively give bioinformatically predicted and practical data. By combining DIANA-micriT-CS and –TarBase data, mirPath predicts miRNAs targets and pathways in which they are involved [40]. The results showed that miR-20 and miR-214 participate in the regulation of CCND1 and CTNNB1 mRNAs which are parts of Wnt signaling pathway. In addition, it is noteworthy that Wnt pathway is one of the most important signaling pathways afflicted in HCC.

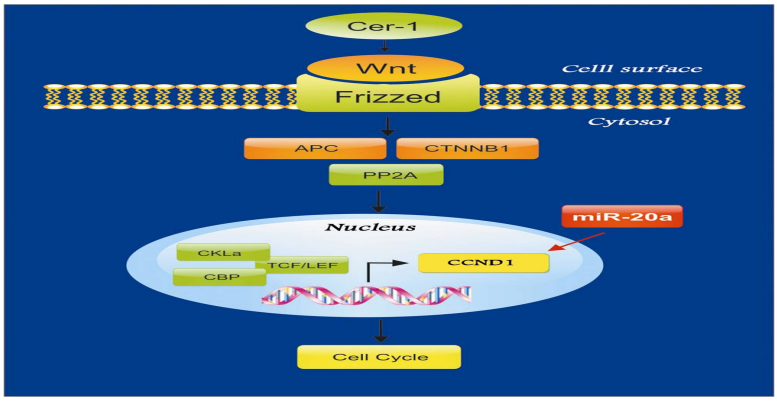

Fig: 3. Yellow-colored boxes indicate genes targeted by miR-20 in signaling pathway.

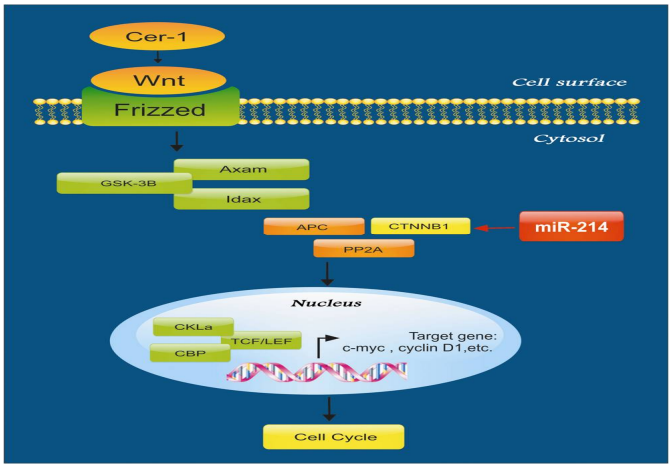

Fig: 4. Yellow-colored boxes indicate genes targeted by miR-214 in signaling pathway.

[V] CONCLUSION

HCC is the sixth prevalent cancer in the world and the third cause of cancer death which results in nearly 600000- 1000000 death annually [4]. In spite of advances made in cancer treatment, current therapeutic methods for HCC malignancies, metastasis and recurrence are not fully effective. Surgery, chemotherapy and radiotherapy are regularly used to reduce the size of HCC tumor. Nevertheless, after these treatments, tumor growth and recurrence are usually observed [42]. Generally, HCC is diagnosed in the late stages of the disease, i.e. when traditional therapies have the least efficacy. Therefore, it is critical to thoroughly understand the molecular mechanisms of HCC to develop new therapies and therapeutics [3, 4]. miRNAs are one of the regulatory elements of gene expression recently considered to be used as therapeutic agents in cancer treatment. Numerous scientists have conducted research on miRNAs' potential in the treatment of cancers among which miR-34 (MRX34®) is currently being used in phase I clinical trial of liver cancer [43-45]. Studies also indicate that miR-21 and miR-221 upregulation, which are observed in various cancers such as liver cancer, results in the onset and progression of malignancies. Therefore, anti-miR-21 and anti-miR-221 are being used in liver cancer treatment in preclinical phases [46, 47]. Identification of miRNAs that play a key role in regulation of gene expression and signaling and metabolic pathways is a positive step in exploitation of miRNAs in cancer treatment [48]. There are a number of studies which have utilized bioinformatic approaches to choose appropriate miRNAs involved in cancers [49]. There are a lot of databases that give useful information about miRNAs and their targets. One of such databases is miRBase that provides users with comprehensive information about miRNAs like the nucleotide sequence of the mature miRNA, stem-loop structure and other characteristics [50]. In the present study, we used several databases and web-based bioinformatic applications that evaluate the binding of miRNA and mRNA. Nevertheless, each application uses different parameters for evaluation. miRNAs that had the highest scores in the mentioned databases most probably regulate the target mRNAs [31]. There are different methods to analyze miRNAs expression profile and the downstream genes that are affected and regulated by them [51, 52]. Since microarray is a suitable and reliable method for gene expression (mRNA) analysis, researchers tend to use it for miRNA expression analysis [52]. In general, microarray is based on two approaches: 1) the total number of target genes should be adequate, e.g. more than 10000 and 2) the expression level of most of the genes should be constant. Since the number of miRNAs used in miRNA arrays is less than 2000, and most of the miRNAs are not expressed constantly and continuously, microarray for miRNAs is not a perfectly suitable method [53]. It is noteworthy that since miRNAs are very short, amplifying them during miRNA array is difficult to achieve. Furthermore, miRNA arrays results are not quantitative [27]. Considering the obstacles mentioned about miRNA arrays, and since it is costly, time-consuming, and it needs an experienced technician, using this method in research centers especially in developing countries is limited. Therefore, it is possible to use bioinformatic databases and applications as an alternate method. Clinical bioinformatics is an emerging interdisciplinary field created by combining bioinformatics, clinical data, mathematics, information technology and Omics. Clinical bioinformatics has substantial clinical applications in various areas such as biomarker discovery and development, omics (metabolomics, transcriptomics, proteomics and genomics), signaling pathways, drug discovery, etc.

At the moment, there is a lot of information of human omics in databases. In addition, tremendous improvement of medical and laboratory instruments has led to enhanced results. Integration of these data and exploitation of these tools will result in more reliable information. Therefore, better understanding of the relationship

between bioinformatics and clinical data is the key measure to develop novel therapies and therapeutics [29].

CONCLUSION

Based on the bioinformatic analysis performed by using various databases and web-based applications, miR-214 and miR-20 target 3'UTR of CTNNB1 and CCND1 mRNA, respectively, and regulate their expression. Nonetheless, functional studies are in process to confirm these in silico analyses and to ensure the results

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