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Short Communication

MicroRNA–mRNA interaction network using TSK-type recurrent neural fuzzy network

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ABSTRACT

MicroRNAs are short non-coding RNAs that can regulate gene expression during various crucial cell processes such as differentiation, proliferation and apoptosis. Changes in expression profiles of miRNA play an important role in the development of many cancers, including CRC. Therefore, the identification of cancer related miRNAs and their target genes are important for cancer biology research. In this paper, we applied TSK-type recurrent neural fuzzy network (TRNFN) to infer miRNA–mRNA association network from paired miRNA, mRNA expression profiles of CRC patients. We demonstrated that the method we proposed achieved good performance in recovering known experimentally verified miRNA–mRNA associations. Moreover, our approach proved successful in identifying 17 validated cancer miRNAs which are directly involved in the CRC related pathways. Targeting such miRNAs may help not only to prevent the recurrence of disease but also to control the growth of advanced metastatic tumors. Our regulatory modules provide valuable insights into the pathogenesis of cancer.

1. Introduction

MicroRNAs (MiRNAs) constitute a class of small non-protein-coding RNAs which regulates protein-coding genes at the post transcriptional and translational level (Carthew, 2006). There are at least 800 miRNAs within the human genome, each of which has a different function. MicroRNAs bind to partially complementary sites in the messenger RNA of other genes and inhibit the translation of these genes. They have been found to regulate a wide range of biological processes such as cell differentiation, proliferation, growth, mobility and apoptosis in diverse cancer-related biological processes (Lynam-Lenno et al., 2009; Schickel et al., 2008). Accumulating evidence suggests that altered miRNA expression correlates with the pathogenesis of cancers. The over-expression of several miRNAs results in tumor formation; however, some miRNAs are consistently downregulated in tumors and may have tumor-suppressive effects (Voorhoeve, 2010; Zhang et al., 2007). For example, microRNAs in the let-7 and miR-34 families may act as tumor suppressors by repressing certain oncogenes (Johnson et al., 2005; Tazawa et al., 2007) while miR-106b and miR-21 play roles in oncogenesis (Chang et al., 2005; Ivanovska et al., 2008). A recent study suggested that microRNAs can identify cancer tissue origin accurately (Rosenfeld et al., 2008). This is of great clinical importance because microRNAs may be used for tracing the tissue from which cancers of unknown primary origin arose. Thus the identification of miRNAs linked to cancer susceptibility is useful for cancer diagnosis, prognosis, treatment and drug target discovery (Volinia et al., 2006).

Recent experiments also show that miRNAs upregulate genes in one condition, but act as a negative regulator in another condition. For example, let7 and the synthetic microRNA miRcxcr4-likewise upregulate target mRNAs upon cell-cycle arrest; yet, they inhibit translation in proliferating cells (Vasudevan et al., 2007). The abundance and diversity of miRNA targets result in a large number of possible miRNA regulatory mechanisms. It is not feasible to test all the possibilities through biological experiments. Therefore, the development of various computational methods to recognize crucial regulatory functions of miRNA has been widely applied to cancer research as a powerful supplement to experimental methods.

Colorectal cancer is the third most commonly diagnosed cancer in the world and contributes to over 655,000 deaths per year. However, in almost all cases, early diagnosis can lead to complete cure. Uncontrolled growth of cells and loss of apoptosis function usually results in cancer formation. MicroRNAs have been found to regulate mechanisms such as cell growth and apoptosis (Cheng et al., 2005). Recognition of miRNAs that are differentially expressed in tumor and normal tissues may help to identify those miRNAs that are involved in pathogenesis of human cancers. Experimental methods such as microarray profiling and qRT-PCR have been used to monitor

Abbreviations: CRC, Colorectal Cancer; MiRNA, Micro RNA; miRO, Micro RNA Ontology Database; mRNA, Messenger RNA; QRT-PCR, Quantitative Reverse Transcriptase Polymerase Chain Reaction; TRNFN, Takagi Sugeno Kang-type Recurrent Neural Fuzzy Network.

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expression levels of miRNAs in various types of cancers. Microarray profiling is a powerful technique that can be used to systematically detect the differential expression of miRNAs in cancer and normal samples. By integrating miRNA target genes, cancer genes, miRNA and mRNA expression profile information, an interaction network can be developed to link miRNAs to cancer target genes.

Several computational methods have been proposed to study miRNA regulatory mechanisms through expression data. Huang et al. used Bayesian data analysis algorithm (Gene MIR++) (Huang et al., 2007) to identify miRNA targets by utilizing paired expression profiles of miRNA and mRNA. However the algorithm used in that paper is based on pairwise correlation method which may fail to undermine collinearities among the covariates (miRNAs). Partial least square (PLS) regression approach (Li et al., 2011) by Xiaohong et al. overcomes these issues and explores likely associations between miRNA and mRNA by taking advantage of the known inverse relationship between miRNAs and target mRNAs.

In the present paper we use Takagi Sugeno Kang type recurrent neural fuzzy network (TRNFN) (Vineetha et al., 2012) to model miRNA–mRNA interaction network from paired expression profiles of miRNA and mRNA for both colon tumors and normal tissues. This method combines the advantages of both neural network and fuzzy logic. Neural Fuzzy network has been previously used to simulate gene regulatory networks (Maraziotis et al., 2007; Vineetha et al., 2010a, 2012) but not for finding miRNA–mRNA interaction network yet. This hybridized model combines the features of connectionist and fuzzy logic approaches and infers information on gene interactions in the form of fuzzy rules and considers the dynamic aspects of gene regulation. Unlike other neural fuzzy model, there was no predefined structure and rules in TRNFN, all of them are constructed during online learning. The TRNFN includes memory elements in the form of feedback connections to store prior system states so that it can perform dynamic mapping of inputs and outputs. To achieve better performance TRNFN adopts a global feedback structure where the output of all rule nodes are fed back and summed, so each rule's firing strength depends not only on its previous value but also on others. Furthermore, the inclusion of TSK-type consequence can significantly reduce the rule number (Juang, 2002). TRNFN is characterized by small network size and fast learning speed.

In this study, using paired miRNA and mRNA microarray data previously collected from CRC cancer patients (Chia-Feng and Chin-Teng, 1991), we were able to capture a complex miRNA–mRNA association network. All these findings reveal the potential of microRNA profiling in cancer diagnosis.

2. Material and methods

The schematic diagram of the whole procedure is shown in Fig. 1. The TSK type recurrent neural fuzzy network was proposed by Chia-Feng Juang in 2002 (Juang, 2002). The model is constructed from a series of fuzzy if–then rules, with the consequence of each rule being of TSK-type fuzzy reasoning. The network precondition

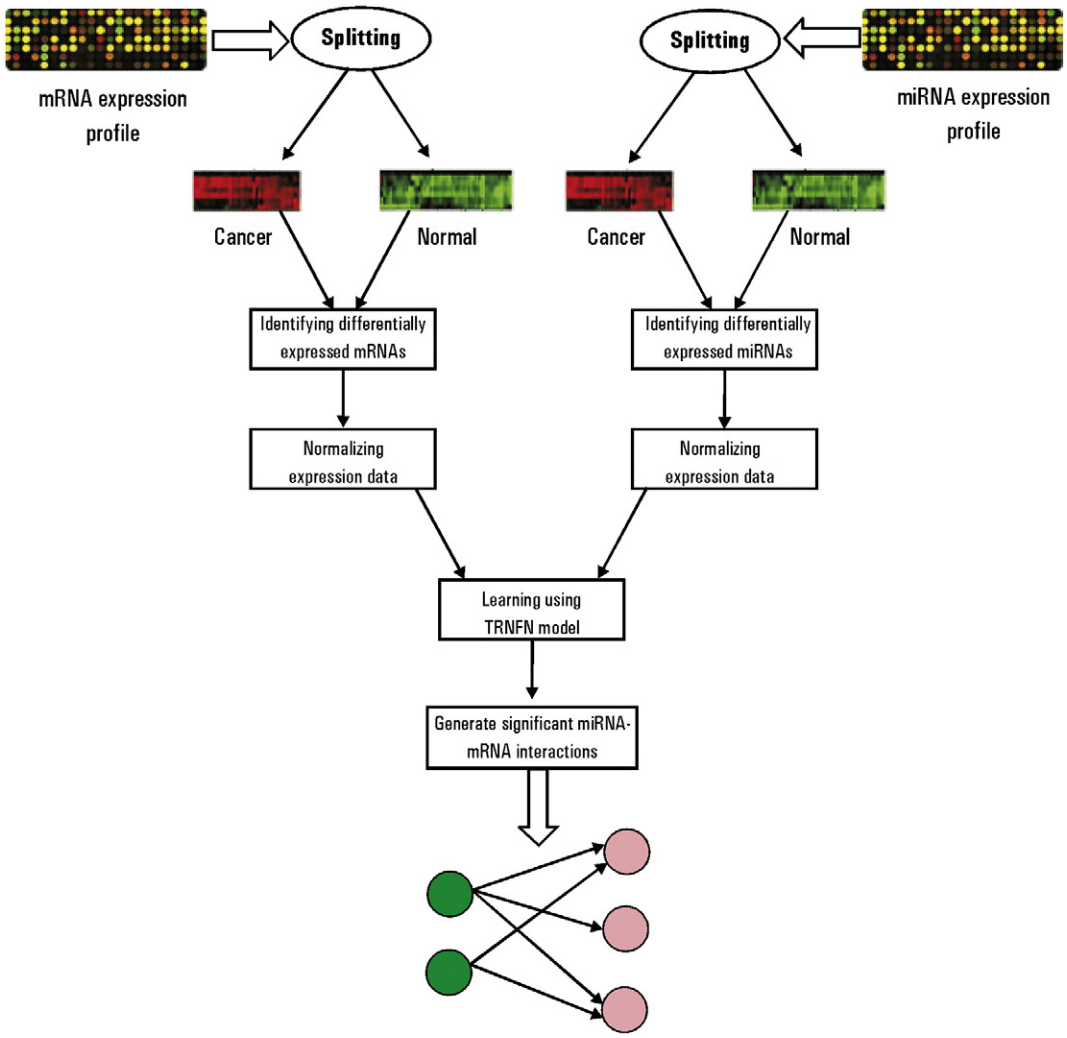

Fig. 1. Schematic diagram of the overall procedure for generating miRNA–mRNA interaction network.

part includes external variables and internal variables derived from fuzzy firing strengths, and the consequence is a linear combination of them plus a constant term. Each rule i has the following form:

$$R(i) = \text{if } x_1(t) \text{ is } A_{i1} \text{ and } x_2(t) \text{ is } A_{i2} \text{ and} \ldots x_n(t) \text{ is } A_{in} \text{ and } h_i(t) \text{ is } G \text{ then } y(t+1) = a_{i0} + \sum_j a_{ij} \cdot x_j(t) + a_{j\,n+1} h_i(t) \quad (1)$$

where A and G are fuzzy sets, h is the internal variable and a is the parameter for the inference output y. In TRNFN, there are two learning phases — structural and parameter learning. The structural learning phase is responsible for the construction of fuzzy if- then rules and the identification of feedback structure. The parameter learning phase is for tuning of free parameters of the network structure.

2.1. Architecture

The architecture of TRNFN is illustrated in Fig. 2. TRNFN is a six layered network, including a feedback layer that brings the temporal processing ability into a feed forward neural fuzzy network. Layer 1 acts as an input layer. Layer 2 is used to fuzzify the input value. Gauss membership function is employed in this layer. Nodes in layer 3 are called rule nodes and a new node will be created each time a new rule is generated during the structural learning phase. Nodes in this layer perform the precondition matching of the rule and compute the firing strength of each rule for the given input. Since Tasaki Sugeno output membership functions are linear, nodes in the layer 4 perform a linear summation of the external input variables and context node to evaluate the consequent. Layer 5 is called the feedback layer. The outputs of the feedback term nodes contain the firing history of the fuzzy rules. Layer 6 performs the defuzzification operation. Detailed description of the function and the equations used in each layer were reported in (Juang, 2002; Vineetha et al., 2012).

2.2. Learning process

TRNFN performs the structural as well as parameter learning process simultaneously during the training phase. The way the input space is partitioned determines the number of rules extracted from the training data. During the training phase, when a new rule is generated, it corresponds to the creation of a new cluster in the input space. The spatial firing strength of each rule represents the degree to which an input pattern belongs to the corresponding cluster. The spatial firing strength is computed using the following equation

$$F^i(x) = \prod_{k=1}^{n} O_k^{(2)} = \exp\left\{ -\sum_{j=1}^{n} \frac{\left(x_j - m_{ij}\right)^2}{\sigma_{ij}^2} \right\} \in [0,1] \quad (2)$$

where $O_k^{(2)}$ is the output of the k^{th} rule node in layer 2, x_j is the jth component of the input x, σ and m are the center and width of the Gauss membership function. The value of $F^i(x)$ determines whether a fuzzy rule should be generated or not. Since there were no rules initially, for the first incoming pattern $x(0)$, a new fuzzy rule is generated with center and width of Gauss membership function is assigned as

$$m_{1i} = x_i(0) \text{ and } \sigma_{1i} = \sigma_{init}, \text{for } i = 1.n \quad (3)$$

$$I = \arg \max_{i \le i \le r(t)} F^i(x) \quad (4)$$

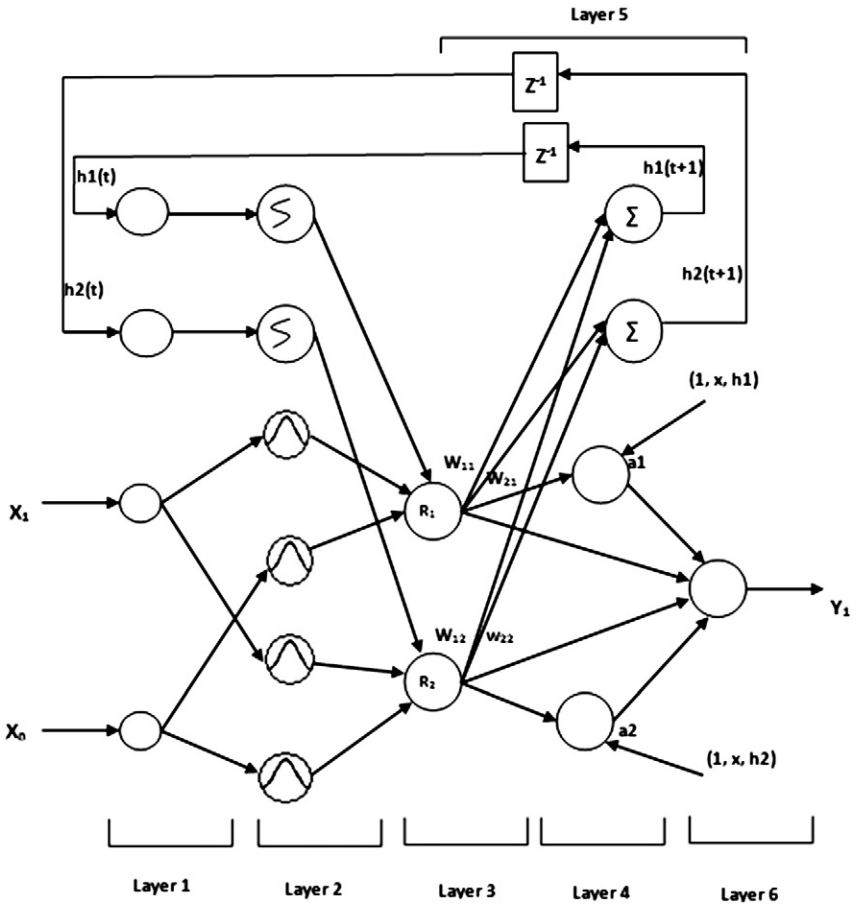

Fig. 2. TRNFN architecture.

where $r(t)$ is the number of rules at time t. The new rule is generated, if $F_I \leq F_{in}(t)$, where $F_{in}(t) \in (0, 1)$ is a pre-specified threshold that decays during learning process. The center and width of the new rule can be set according to the first nearest neighbor heuristic as

$$m(r(t)+1)i = x_i(t) \quad \text{and}$$
$$\sigma(r(t)+1)i = \beta \cdot \sum_{j=1}^{n} \frac{\left(x_j - m_{ij}\right)^2}{\sigma_{ij}^2} \tag{11}$$

where $\beta \geq 0$ decides the degree of overlap between the two clusters. The number of rules generated is determined by the parameters F_{in} and β.

After the generation of new rules the consequent nodes in layer 4 and context node in layer 5 are computed. The output, h, of the new context node in layer 4 is fed back as input in the precondition part of the newly generated rule. Thus, each rule has its own memory elements for memorizing the temporal firing strength history. For every incoming training data, the above process is repeated, new rules are generated one after another and a whole TRNFN is constructed finally. The free parameters such as m, σ, parameters in layer 4 and link weight w are tuned using the real time recurrent learning algorithm (Chia-Feng and Chin-Teng, 1991; Juang, 2002).

3. Results and discussion

We applied TRNFN to the paired miRNA and mRNA microarray data sets for both human colon tumors and normal tissues obtained from the cancer dataset provided by Broad Institute (http://www.broadinstitute.org/cgi-bin/cancer/datasets.cgi; Lu et al., 2005). The dataset originally consist of the expression profiles of 218 tumor samples representing 14 common human cancer classes out of which 10 are colon tumor tissue samples and 5 are normal colon tissue samples. We have taken 7 colon tumor tissue samples and 4 normal colon tissue samples which passed quality control criteria (Ramaswamy et al., 2001). Unpaired t-tests were used to identify a set of miRNAs which are differentially expressed in different conditions under the investigation. We selected 56 miRNAs whose p-value less than 0.05 for further analysis.

From our previous works, we identified a set of 27 genes, which are found to be highly regulated in CRC patients (Vineetha et al., 2010a, 2010b, 2011, 2012). In this work we chose to analyze the above 27 genes to model the miRNA–mRNA association network. We implemented 27 multi input–single output TRNFN model. Each model describes the state of output gene based on the expression value of the 56 miRNAs. Among the 11 available samples, 6 samples were used to train the network and the remaining 5 samples were used for testing the consistency. Training and test dataset contains samples from both healthy and unhealthy tissues. Structure of each

Table 1
Set of known relations predicted by TRNFN.

Target genes	miRNAs associated
EPAS1	hsa-miR-103, hsa-miR-107, hsa-miR-138, hsa-miR-150, hsa-miR-182, hsa-miR-30b
ANXA1	hsa-miR-221, hsa-miR-222
C18B11	hsa-let-7g,hsa-miR-136
ACP1	hsa-miR-141, hsa-miR-18, hsa-miR-98, mmu-miR-106a
HBE1	hsa-miR-218
LDHA	hsa-miR-15a,hsa-miR-15b,hsa-miR-16,hsa-miR-182,hsa-miR-30a, hsa-miR-30b,hsa-miR-30c, hsa-mir-30e,hsa-miR-33
MVP	hsa-miR-150
PCBP2	hsa-let-7a, hsa-let-7b, hsa-let-7c,hsa-miR-150,hsa-miR-15a, hsa-miR-195,hsa-miR-200a
PPP1CC	hsa-miR-21
PSMA3	hsa-let-7b,hsa-miR-135,hsa-miR-182,hsa-miR-210,hsa-miR-221, hsa-mir-32,mmu-miR-135b
RELN	hsa-miR-200c, hsa-miR-138,mmu-miR-200b
TIAF1	hsa-miR-150,hsa-miR-24,hsa-miR-30a,hsa-miR-30b,hsa-miR-30c, hsa-miR-30d
TRIP10	hsa-let-7d, hsa-miR-106b, hsa-miR-142-5p,hsa-miR-214, hsa-miR-195, mmu-miR-106a
UBE2D3	hsa-let-7b, hsa-miR-103,hsa-miR-107, hsa-miR-135,hsa-miR-138, hsa-miR-140, hsa-miR-144, hsa-miR-154,hsa-miR-185,hsa-miR-185, hsa-miR-203,hsa-miR-21, hsa-miR-9,mmu-miR-101b

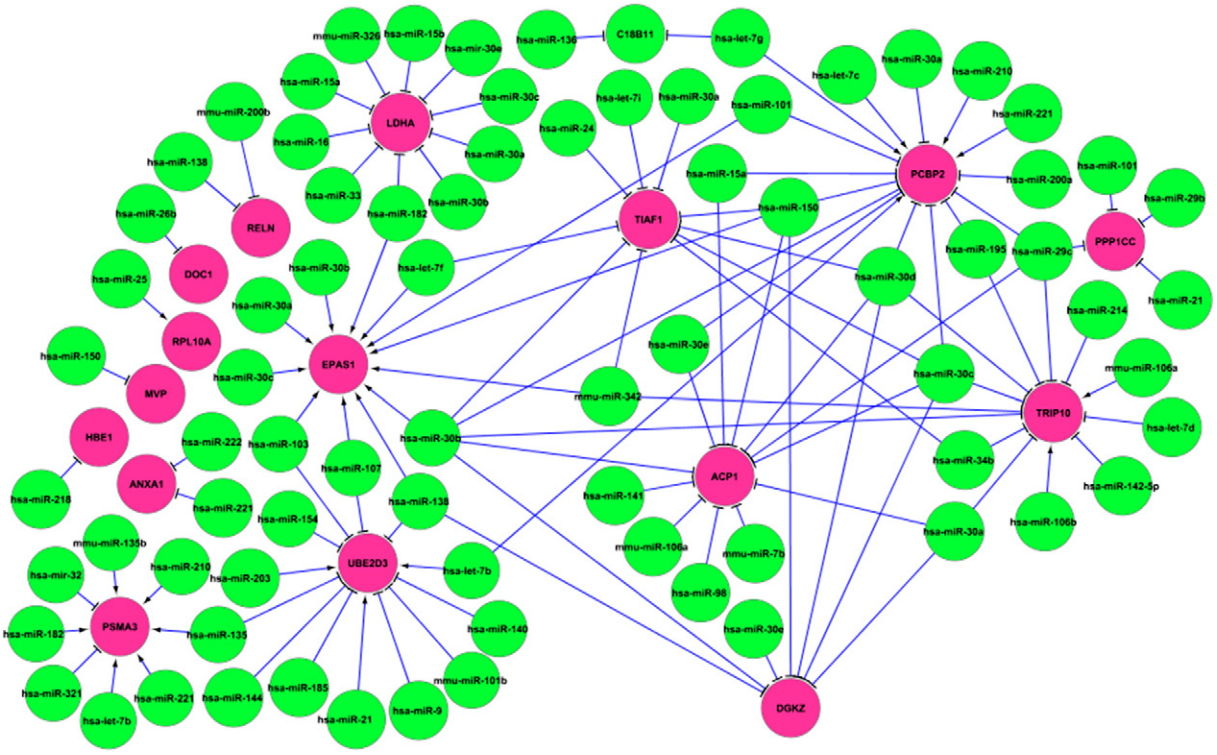

Fig. 3. miRNA–mRNA interaction network predicted by TRNFN.

Table 2
List of 17 miRNAs and target genes associated with colorectal cancer.

CRC related miRNAs	miRNA family	Target genes predicted by TRNFN
hsa-let-7b	Let-7b	PCBP2,UBE2D3,PSMA3
hsa-let-7 g	Let-7 g	PCBP2,C18B11
hsa-miR-106b	miR-106	TRIP10
hsa-miR-107	miR-107	EPAS1,UBE2D3
hsa-miR-140	miR-140	UBE2D3
hsa-miR-141	miR-141	ACP1
hsa-miR-15b	miR-15	LDHA
hsa-miR-182	miR-182	LDHA,EPAS1,PSMA3
hsa-miR-195	miR-195	PCBP2,TRIP10
hsa-miR-203	miR-203	UBE2D3
hsa-miR-21	miR-21	PPP1CC,UBE2D3
hsa-miR-221	miR-221	ANXA1,PCBP2,PSMA3
hsa-miR-25	miR-25	RPL10A
hsa-miR-29b	miR-29	PPP1CC
hsa-miR-30c	miR-30	LDHA,PCBP2,ACP1,TIAF1,TRIP10, DGKZ,EPAS1
hsa-mir-32	mir-32	PSMA3
hsa-miR-34b	miR-34	TIAF1,TRIP10

model is generated using the training dataset and the parameter tuning is done by the repeated learning. The fuzzy rule set derived from 27 TRNFN model was used to build a miRNA–mRNA interaction network. The inferred association network using TRNFN is shown in Fig. 3. There were two disjoint sets of nodes in this graph, miRNA (green circle) and mRNA genes (pink circle). A direct connection placed from a miRNA to an mRNA indicates that the mRNA was predicted to be the target of the miRNA. An edge → indicates activation of transcription, whereas, an edge ⊣ indicates repression of transcription. Cytoscape software (Shannon et al., 2003) has been used to draw the network. The resulting network had 76 nodes and 119 connections.

In order to better understand the biological processes linked to these miRNAs and their predicted target mRNAs in the context of colorectal cancer, we compared our results with the experimentally known miRNA–mRNA association available in literature. MicroRNA.org (Doron et al., 2008), is a widely used web resource for miRNA target prediction and expression profiles. In microRNA.org database, target predictions are based on a development of the miRanda algorithm (John et al., 2004) which incorporates current biological knowledge on target rules. The set relations predicted by TRNFN and are confirmed in mircoRNA.org database are listed in Table 1. For example, the down regulation of UBE2D3 by miR-107, miR-135 and miR-140 were predicted correctly by our algorithm and are confirmed in the literature. Further, by searching miR2Disease: a manually curated database for microRNA deregulation in human disease (Qinghua et al., 2009) we identified that 17 of our 56 miRNAs are known to be actively involved in the pathways associated with colorectal cancer. Table 2 lists each of those miRNAs and their target genes which have been previously reported to have associations with colorectal cancer.

It is interesting to investigate further the biological process and cancer related canonical pathways associated with these miRNAs which are associated with colon cancer. To obtain the biological process in which the above cancer related genes are involved, we used the miR-Ontology database (miRo) (Lagana et al., 2009).The results are presented in Table 3. A more detailed functional analysis has

Table 4
CRC related miRNAs and target genes involved in cancer related canonical pathways.

Cancer related canonical pathways	Target genes in respective pathways	The no. of CRC related miRNAs associated with the target genes
Hypoxia signaling pathway	EPAS1,LDHA,UBE2D3	8
Protein ubiquitination pathway	PSMA3, UBE2D3	8
Polyamine regulation in colon cancer	PSMA3	4
Pyruvate metabolism	LDHA	3
Insulin signaling pathway	TRIP10, PPP1CC, EPAS1	8
Renal cell carcinoma	EPAS1	3
Signaling by Wnt	PSMA3	4
Apoptosis	PSMA3, TIAF1	6
Cdc20:phospho-APC/C mediated degradation of cyclin A	PSMA3	4
Regulation of activated PAK-2p34 by proteasome mediated degradation	PSMA3	4
APC/C:Cdh1-mediated degradation of Skp2	PSMA3	4
VEGF signaling pathway	EPAS1	3

Table 3
CRC related miRNAs and their associated processes.

CRC related miRNA	Associated biological processes
hsa-miR-34b	Regulation of cyclin-dependent protein kinase activity, angiogenesis, ubiquitin-dependent protein catabolic process, regulation of transcription from RNA polymerase II promoter, regulation of apoptosis
hsa-miR-32	Beta-catenin binding, regulation of apoptosis, BMP signaling pathway, regulation of cell proliferation, ubiquitin-dependent protein catabolic process
hsa-miR-21	Cell proliferation; anti-apoptosis, ubiquitin–protein ligase activity
hsa-let-7g	Cell proliferation; anti-apoptosis, ubiquitin–protein ligase activity, anaphase-promoting complex(APC)-dependent proteasomal ubiquitin-dependent protein catabolic process, response to oxidative stress, regulation of transcription, DNA-dependent
hsa-miR-140	Cell cycle arrest, regulation of cell proliferation
hsa-let-7b	Cell proliferation, ubiquitin–protein ligase activity, anaphase-promoting complex(APC)-dependent proteasomal ubiquitin-dependent protein catabolic process, response to oxidative stress and regulation of apoptosis, hemopoiesis
hsa-miR-30c	Polyamine biosynthetic process, ubiquitin-dependent protein catabolic process, and regulation of apoptosis
hsa-miR-106b	Angiogenesis, regulation of cyclin-dependent protein kinase activity, regulation of cell proliferation, regulation of transcription from RNA polymerase II promoter
hsa-miR-107	Anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process, anti-apoptosis, angiogenesis, BMP signaling pathway, Wnt receptor signaling pathway
hsa-miR-15b	Anti-apoptosis, regulation of cell proliferation, anaphase-promoting complex(APC)-dependent proteasomal ubiquitin-dependent protein catabolic process
hsa-miR-182	BMP signaling pathway, apoptosis, angiogenesis, activation of MAPK activity, regulation of Wnt receptor signaling pathway through beta-catenin, protein ubiquitination during ubiquitin-dependent protein catabolic process,
hsa-miR-195	Wnt receptor signaling pathway, BMP signaling pathway, anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process, regulation of apoptosis
hsa-miR-141	Angiogenesis, regulation of transcription, DNA-dependent, hemopoiesis, regulation of cell proliferation
hsa-miR-203	Regulation of cell growth, activation of MAPKK activity, anti-apoptosis, BMP signaling pathway,
hsa-miR-221	Cytokine and chemokine mediated signaling pathway, regulation of Wnt receptor signaling pathway, regulation of Wnt receptor signaling pathway
hsa-miR-25	Angiogenesis, anti-apoptosis, BMP signaling pathway, regulation of cell proliferation, regulation of Wnt receptor signaling pathway, ubiquitin-dependent protein catabolic process
has-miR-29b	Regulation of cell proliferation, angiogenesis, BMP signaling pathway, regulation of transcription from RNA polymerase II promoter, regulation of apoptosis

been done to identify the cancer related canonical pathways in which these miRNAs and target genes are involved. The identified pathways are listed in Table 4.Overall, it is clear that the above CRC related miRNAs are involved in many biological processes and pathways by regulating the target genes predicted by our approach.

4. Conclusion

MicroRNAs are a class of non-coding RNAs that hybridize to mRNAs and regulate their activities at post transcriptional as well as translational level. Recently it has been reported that the miRNAs play an important role in the development of many cancers, including CRC. Therefore, identifying cancer related miRNAs and their target genes is a key step towards the diagnosis and treatment of cancer. In this paper, we applied TSK-type recurrent neural fuzzy network (TRNFN) to infer miRNA–mRNA association network from microarray gene expression data of CRC patients. Here, we are focusing on a small number of relevant genes, each of which can fairly classify colon tumor tissues from normal ones. Using TRNFN, we were able to identify miRNAs which are involved in the regulation of above cancerous genes. We demonstrated that the method we proposed achieved good performance in recovering known experimentally verified miRNA–mRNA associations. Moreover, we were able to identify 17 miRNAs which are directly involved in the CRC related pathways. Targeting such miRNAs may help not only to prevent the recurrence of disease but also to control the growth of advanced metastatic tumors. Our interaction network will provide valuable insights into cancer diagnostics, prognostics and therapy.

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