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Analysis of breast cancer specific mRNA - miRNA relationship

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Abstract

Cancer is a sophisticated disease in which the proliferation of cells which are unrestrained lead to the development of tumour. A detailed research into the mechanisms underlying the development of tumour, has paved a way towards the characterization of tumour suppressors like miRNAs (microRNAs). miRNAs are considered to regulate the expression of more than 30 percentage of the entire gene in the cells of mammals. The miRNAs with the largest change in the levels of expression are not necessarily the ones that are most relevant. However, miRNAs which are expressed differentially have greater importance in a biological context in relation to the progression of cancer than miRNAs that target and modulate just a few transcripts of mRNA (messenger RNA). The application of miRNAs in the breast cancer therapeutics is an area of interest. In this paper, a new technique for analyzing this nature of modulation by miRNA, has been developed, which determines the binding regions of miRNAs to the mRNAs using a normalised correlation method. 30 breast cancer specific mRNAs and the various miRNAs that target each of the mRNAs, are considered for the analysis. The new correlation method identifies the binding regions of all the miRNAs with respect to the ground truth.

Keywords: Binding region; Normalization; Normalised correlation; Seed region.

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1. Introduction

microRNAs (miRNA) are short double stranded 19–23 nucleotides long molecules antisense in nature, which are processed endogenously. The miRNAs are significant gene regulators that play a major role in several aspects of cellular functions including differentiation, cell cycle control, stemness, progression and development of cancer. The main function of miRNA involves regulating the expression of a gene by exhibiting nearly perfect base pairing with the mRNA it targets. In this manner, it inhibits the expression of mRNA at posttranscriptional level by either degrading the mRNA or repressing the translation process [1].

The seed region consists of a contiguous string of minimum 6 nucleotides. miRNA recognizes its target by the degree of complementarity between the motif of the special nucleotide (seed region, which is found within the mature miRNA) and some specific binding sites found along the mRNA sequence. Longer seeds which are 7 or 8 nucleotides long are considered to have greater efficacy as it is related to the repression of mRNA.

The miRNA guides the complex RISC and relates with the Argonaute protein to form silencing complexes in targeting the mRNA to induce translational repression and thus accelerates mRNA deadenylation [2-5].

In the deadenylation process, the targets of the miRNAs are channeled to the cellular 5’ to 3’ mRNA decay pathway. In this pathway, the deadenylase complex deadenylates the mRNAs followed by decapping. The regions where the microRNAs target a gene are seen to be mostly clustered together leading to a collective effect in repression [6-10].

Identifying the targets of microRNAs through

experiments is a critical step in obtaining prediction results that are reliable. The study on microRNA–mRNA interactions is by far incomplete due to the challenges involved in predicting the nature of interactions, computationally. Many tools with various algorithms have been developed for predicting the interactions. The outputs of commonly used software that predict the behavior of miRNA–mRNA interactions, fail to experimentally spot the confirmed microRNA-binding regions correctly.

The 3'UTR (Untranslated Region) is that region of mRNA that follows the termination codon of translation. It comprises of sequences that are capable of either degrading or stabilizing the mRNA transcript.

The methods used for target recognition are mainly based on pattern matching and offer a set of probable binding sites that are redundant. The algorithms available for predicting the targets of miRNA are based on the search for a sequence complementarily between the target and the miRNA. The results of these algorithms give a large number of miRNA targets making it less possible to experimentally validate all the targets [11-.14]. cDNA microarray has found application in the study of gene expression [13]. However, the accurate detection of the gene targets would also require proteomic data analysis since a miRNA can repress the production of a large number of proteins. Thus miRNA-target interaction is importantly influenced by the seed region [15]. The most important requirement for a proper repression is a perfect pairing of the seed [16-18].

In this study, the new correlation method is applied for obtaining the binding regions of several miRNAs for 30 breast cancer specific mRNAs. The results obtained for this method is same as the ground truth.

2. Materials and Methods

The interaction between a miRNA and an mRNA is affected by the strength of binding between the two at the seed region. Tests were done to evaluate the hypothesis by calculating the differential expressions of all the miRNAs that target the mRNA [19, 20]. The seed region/sequence plays a very significant role in analysing the extent of interaction between miRNA and mRNA towards cancer [21, 22].

The importance of methods based on signal processing, is attributed to their application in collecting, processing and interpreting the information present in both genomics and proteomics data. Digital signal processing and control has been widely used in many areas of science and engineering. The importance of genomic signal processing is rising as it has been recognized that the characterization of genomic and proteomic regulations require various disciplines related to signal processing. Bioinformatics and Genomic Signal Processing both apply computational methods to tackle various problems related to biology.

Several Digital Signal Processing techniques including STFT have found application in the search for genomic repeats using Fourier analysis. DFT was used for spectrum analysis of biological data where initially the DNA sequence was mapped into a numeric sequence and spectrum of finite-length windowed DNA numerical sequences was computed. The applications of digital filters also helped eliminate the background 1/f noise of the spectrum exhibited by nearly all DNA sequences. A new method was introduced based on a modified Gabor-wavelet transform (MGWT) for the identification of protein coding regions. This novel transform was tuned to analyze periodic signal components and presented the advantage of being independent of the window length.

Decomposition of genomic DNA sequences could be employed using discrete wavelets followed by thresholding algorithms that are data-dependent in order to remove the background noise. Following this, entropic segmentation method was applied to track boundaries between segments well-characterized genes. Before the wavelet decomposition, the sequences were digitized into numerical sequences on the basis of their contents.

Several DSP based algorithms have been applied to studying the characteristics of DNA and RNA sequences. However, the solutions provided by these algorithms include much background noise and the results obtained are computationally complex and less accurate. Additional techniques are needed to remove the noise.

When some of the miRNAs bind with mRNAs, degradation may occur, leading to cancer. Our purpose is to find out such binding regions and seed binding regions specific to breast cancer, without the presence of any background noise. Normalised Correlation method is used for this purpose.

2.1 Materials

The program codes for the normalised correlation method, was designed using the built-in utility of MATLAB. The lists of the miRNAs that target each mRNA are taken from the exiqon website [https://www.exiqon.com/miRSearch] and the sequences of the miRNA are obtained from the miRBase website [https://www.mirbase.org/]. The 3'UTR of the breast cancer specific mRNAs are obtained from the UCSC Genome browser website [https://www.genome.ucsc.edu/]. The breast cancer specific mRNAs BRCA1, BRCA2 and the miRNAs that target these two mRNAs were used for analysis, in this study. The ground truth is taken from the CNR-ISMAC, Institute for the Study of Macromolecules [http://www.bi.ismac.cnr.it/].

2.2 Experiment

When some of the miRNAs bind with mRNAs, degradation may occur, leading to cancer. Our purpose is to find out such binding regions and seed binding regions specific to breast cancer, without the presence of any background noise. Normalised Correlation method is used for this purpose.

2.2.1 Correlation Method for determining miRNA binding and seed binding regions

The various miRNAs and the breast cancer specific mRNAs whose regions of binding to the miRNAs are to be found, are selected. The calculation of maximum correlation between microRNA and mRNA initially requires finding the reversed compliment of microRNA [23, 24]. The normalised correlation between mRNA and miRNA was computed while laterally shifting miRNA throughout the mRNA sequence and computing the normalised correlation value each time it is shifted. The maximum strength from among the correlation values is noted.

There are 4 types of seed regions; 8-mer (bases 1–8), 7-mer-m8 having bases 2–8), 7-mer (7-mer-A1 having bases 1–7, and 6-mer (bases 2–7) [25]. As per the observations made by the authors, the seed regions are positioned at the first 8 bases of the miRNA [26] and hence the only requirement would be to do a one-to-one match for the short length of bases between the reverse complemented miRNA and the mRNA.

As per the normalised correlation method, the binding and the seed binding regions of various miRNAs are obtained for 30 breast cancer specific mRNAs. The results obtained for the breast cancer specific mRNAs, BRCA1, and BRCA2 are tabulated in Tables I and III respectively. BRCA1 is a gene that suppresses tumour and the mutations in these genes are found to be a major cause of breast cancer. However, this gene has a major role in the genome stability. With mutations in BRCA1, there is 80% risk in developing breast cancer. Investigations done on BRCA1 for various organisms have provided knowledge of the involvement of BRCA1 in breast cancer [27]. BRCA2 is also a tumor suppressor gene, and is found in all humans. The BRCA2 protein, also known by the name breast cancer type 2 susceptibility protein, is expressed in the breast cells where they together help in the repair of damaged DNA or destroy the cells if the DNA cannot be repaired [28].

2.2.2 Implementation of the Correlation Method

According to the normalised correlation method, the binding and seed binding regions are obtained for 30 breast cancer specific mRNAs of which the miRNA binding and seed binding regions of BRCA1 and BRCA2 are presented in this paper and tabulated in tables I, and III respectively.

3. Results

35 miRNAs binding to BRCA1, and 7 binding to BRCA2 were chosen for the study. Tables I and II show the details of the binding regions and the seed regions with respect to the correlation method, for BRCA1 and BRCA2 respectively.

Table I: Study on Binding region and Seed binding region details with respect to Correlation Methods for BRCA1

BRCA1	*From Ground Truth*	*From Ground Truth and Correlation Method*	*From Correlation Method*		
Sl No.	*miRNA Name and Length*	*Actual Binding Region of mRNA*	*Actual Seed Binding Region wrt mRNA, Sequence*	*miRNA Seed Region*	*Maximum Correlation value at the binding Region*
1	hsa-miR-1915-3p(20)	17-36	18-24,CCCTGGG	2-8	0.9788
2	hsa-miR-6764-5p(22)	68-89	68-75,CCCTGGGA	1-8	0.9874
3	hsa-miR-6847-3p (22)	207-228	208-214, CATGAGC	2-8	0.9844
4	hsa-miR-125a-3p (22)	233-254	234-240,TCACCTG	2-8	0.9895
5	hsa-miR-7158-5p(24)	271-294	272-278, ATTTGAGC	2-8	0.9764
6	hsa-miR-4267(82)	282-363	283-288,TGCTGA	2-7	0.9711
7	hsa-miR-4446-3p(22)	300-321	301 -307, CAGCCCT	2-8	0.9881
8	Has-miR-6512-3p (22)	339-360	339-346, GGCTGGAA	1-8	0.9882
9	hss-miR-6720-5p(23)	339-361	339-346, GGCTGGAA	1-8	0.9808
10	hsa-miR-6749-3p(21)	527-547	528-534, GGGAGGA	2-8	0.9792
11	hsa-miR-2113(91)	569-659	570-575, TTGAAA	2-7	0.9771
12	hsa-miR-6807-5p(22)	587-608	5S7-594, TGGCTCAC	1-8	0.9899
13	hsa-miR-6878-3p(21)	616-636	617-623, GAGGCCA	2-8	0.9800
14	hsa-miR-4435(22)	663-684	664-670, CTGGCCA	2-8	0.9905
15	hsa-miR-193b-5p(22)	678-699	679-685, AAACCCC	2-8	0.9950
16	hsa-miR-4284(81)	700-780	701-706, ACAGAA	2-7	0.9822
17	hsa-miR-6071(78)	701-773	702-707, CAGAAA	2-7	0.9752
18	hsa-miR-552-3p(21)	728-748	729-735, CACCTGT	2-8	0.9924
19	hsa-miR-6747-3p(21)	759-779	759-766, AGGCAGGA	1-8	0.9798
20	hsa-miR-504-3p(21)	819-839	820-826, GCACTCC	2-8	0.9925
21	hsa-miR-6760-3p(22)	819-840	820-826, GACAGTG	2-8	0.9723
22	hsa-miR-500b-3p(20)	829-848	830-836, CTGGGTG	2-8	0.9827
23	hsa-miR-6379-3p(21)	832-852	832-839, GGGTGACA	1-8	0.9803
24	hsa-miR-215-3p (23)	834-856	835-840,TGACAG	2-7	0.9842

25	hsa-miR-1304-3p(22)	838-859	838-845, CAGTGAGA	1-8	0.9791
26	hsa-miR-4447(91)	883-973	884-889, CTAGAA	2-7	0.9835
27	hsa-miR-671 -3p (21)	964-984	965-971, GAACCGG	2-8	0.9805
28	hsa-miR-1976(52)	1162-1213	1163-1168, TTGCTG	2-7	0.9795
29	hsa-miR-4267(82)	1163-1244	1164-1169, TGCTGA	2-7	0.9711
30	hsa-miR-629-5p(21)	1226-1246	1226-1233, TAAACCCA	1-8	0.9930
31	hsa-miR-328-5p(23)	1279-1301	1280-1286, GCCCCCC	2-8	0.9962
32	hsa-miR-6885-5p(25)	1279-1303	1280-1286, GCCCCCC	2-8	0.9836
33	hsa-miR-7155-5p(19)	1283-1301	1284-1289, CCCCAG	2-7	0.9887
34	hsa-miR-6499-3p (22)	1350-1371	1351 -1356, CACTGC	2-7	0.9897
35	hsa-miR-6516-5p (22)	1352-1373	1352-1358, ACTGCAAA	1-8	0.9921

Table II: Study on Binding region and Seed binding region details with respect to Correlation Methods for BRCA2

BRCA2	*From Ground Truth*	*From Ground Truth and Correlation method*	*From Correlation Method*		
Si No.	*miRNA Name and Length*	*Actual Binding Region of mRNA*	*Actual Seed Binding Region wrt mRNA, Sequence*	*miRNA Seed Region*	*Maximum Correlation value at the binding region*
1	hsa-miR-6073 (89)	240-328	241-246., ACATCT	2-7	0.9637
2	hsa-miR-1224-3p (21)	297-313	298-304, AGGTGGG	2-8	0.9943
3	hsa-miR-146a-5p (22)	573-594	574-580,. AGTIVTC	2-8	0.9606
4	hsa-miR-6744-5p (19)	850-868	850-857, GTCATCCA	1-8	0.9692
5	hsa-miR-513c-5p (22)	866-837	866-873, CTTGAGAA	1-8	0.9555
6	hsa-miR-514b-5p (22)	866-887	866-873, CTTGAGAA	1-8	0.9489
7	hsa-miR-4425 (84)	966-1049	967-972, AAAGCA	2-7	0.8612

Considering the correlation method, seeds of all the 35 miRNAs binding to BRCA1 (Table I), have exactly the same binding position as that in the ground truth. Columns 3 and 4 give the seed sequence and seed type with respect to the ground truth. Similarly, considering the correlation method, seeds of all the 7 miRNAs binding to BRCA2 (Table II) have the same binding position as that in the ground truth. Tables III and IV highlight the position and the length of the seed within the 3'UTR of breast cancer specific mRNAs, BRCA1 and BRCA2 respectively.

Table III. Seeds highlighted in the 3'UTR of BRCA1.

CTGCAGCCAGCCACAGGTACAGAGCCACAGGACCCCAAGAATGAGCTTACAAAGTGGCCTTCCAGGCCCTGGGAGCTCCTCTCACTCTCA
GTCCTCTACTGCCTGGCTACTAAATATTTTATGTACATCAGCCTGAAAAGGACTCTGGCTATGCAAGGGTCCCTTAAAGATTCTGCTGAAG
TCTCCCTGGAAATCTGCCATGAGCACAAAATTATGGTAATTTTCACCTGAGAAGATTTTAAAACCATTAAACGCCACCAATTGAGCAAGAT
GCTGATTCATTATTTATCAGCCCTATCTTCTATCAGGCTGTGTTGGCTTAGGGCTGGAAGCACAGAGTGGCTTGGCCTCAAGAGAATAGCT
GGTTTCCCTAAGTTACTCCTAAAACCCTGTGTCACAAAGGCAGAGAGTCAGACCCTCAATGGAAGGAGAGTGCTGGGATCGATTATGTGA
CTTAAAGTCAGAATAGTCCTGGGCAGTTCTCAAATGTTGGAGTGGAACATTGGGAGGAAATTCTGAGGCAGGTATTAGAAATGAAAAGGA
AACTTGAAACCTGGGCATGGTGGCTCACGCCTGTAATCCCAGCACTTGGGCATGAGCAGGCCAAGGTGGGCAGATCACTGGAGGTCAGGA
GTCGAAACCAGCTGGCCAACATGGTGAAACCCCATCTCTACTAAAAATACAGAAATTAGCCGGTCATGGTGGGAGCACCTGTAATCCCAG
CTACTCAGGTGGCTAGGCAGGAGATCACTCAGCCCGGGAGGTGGAGGTTGCAGTGAGCCAAGATCATACCACGCACTCCAGCCTGGGTGA
CAGTGAGACTGTGGCTCAAAAAAAAAAAAAAAAAAAGGAAAATGAAAGTAGAAGAGATTCTAGAAAGTCTGAGATATATTTGCTAGATT
CTAAAGAATGGTCTAAAACAGCAGAAGATTCAAGAACCGGTTCCAAAGACAGCTCTAATTCCTCATTAGTAATAAGTAAAATGTTTATTG
TGTAGCTCTGGTATATAATCCATTCCTCTTAAAATATAAGACCTCTGGCATGAATATTTCATATCTATAAAATGACAGATCCCACCAGGAA
GGAAGCTGTGCTTCTTGAGGTGATTTTTTCCTTGCTGCCCTGGCTGAAACCATACAGCTTCATAAATAATTTTGCTGCTAAGGAAGAAAAA
GTGTTTTAAAACCCATTATCCAGGACTGTTATAGCTGTTGGAAGGACTAGGTCTCCCTAGCCCCCCAGGTGCAAGGGCAGTGAAGACTTGA
TTGTACAAAATACGTTGTAAATGTTGTGCTGTTAACACTGCAAATAAACTGGTAGCAAACACTCCA

Table IV. Seeds highlighted in the 3'UTR of BRCA2.

GCATTTGCAAAGGCGACAATAAATTATTGACGCTTAACCTTCCAGTTATAAGACTGGAATATAATTCAAACCACACATTAGTACTTATGTT
GCACAATGAGAAAAGAAATTAGTTTCAAATTTACCTCAGCGTTTGTGTATCGGGCAAAAATCGTTTGCCCGATTCCGTATTGGTATACTTG
CTCAGTGCATATCTTAAAACTAAATGTAATTATTAACTAATCAAGAAAAACATCTTGGCTGAGCTCGGTGGCTCATGCCTGTAATCCCAAC
ACTTTGAGAAGCTGAGGTGGGAGGAGTGCTTGAGGCCAGGAGTTCAAGACCAGCCTGGGCAACATAGGGAGACCCCCATCTTACAAAGA
AAAAAAAAAGGGGAAAAGAAAATCTTAAATCTTGGATTGATCACTACAAGTATTATTTACAAGTGAAATAAACATACCATTTCTTAGATT
GTGTCATTAAATGGAATGAGGTCTCTTAGTACAGTTATTTTGATGCAGATAATCCTTTAGTTCTCTACTATTTAGGGGATTTTTTAGAGGTA
ACTCACTATGAAATAGTCTCTAATGCAAATATGTTGGTTCTGCTATAGTCCATCCTGTCAAAAGTCAGGATGAATATGAAGAGTGGTGTCC
TTGAGCAATCTCATCCTTAAGCAGCATGATTATAAGAAAAATAGAACCCTCAGTGTAACTCTAATCCTTTGTCATCCAGTGTGATCTCTTG
AGAATTAAATTACTTCAACTAAAAATCAAATACTTAAATCAGAAGATTCATAGTTAATTTATTTTTTCAACAAAATGCATCAAACTCAAAC
TGAGAAAATATCTGCTTTCASATTGGCACTGATCTGCCTGCTTATTTAGCGCTATCACAGGACCCAGAGCCTATGCCCTTTTAAACTTACCA
CAAAGCAGAAGATTAATTCAATTAAGATGATACTCTCATTGTTACGTCCTTTTTTTTGGAGATGGAGTCTTGCTTGTCGCCCATGCTGGAGT
GCAGTGGCATGATCCTGGCTCACTGCAGCCTCCACTCCCGGGTCACGTAATTCTCCCACCTCAAGCCTCCCTAGTAGCTGGGATTACAGGG
ACGCACCACCATGCCCAGCTAATTTGCATTTTAGTAGAGACTGGGTTTACCATGTTGGCCAAGCTGGTCTCAAACTCCTGATGTCAGGTGA
TCCATCTGCCTCAGCCTCCCAAAGTGCTGGGATTATAGGCGTGAGCCACTCCCGGCCAATATTTGTACTTCTTAGGTTAATAGAGAAAAGG
GATAAAACATTCTAACTGGGAGTTAATTGCATGGAGAAGGTCTTAAATCAGATGTTTTAATGCCTTAAATGTCTGTATAATATCATGTTTC
AAATCTAATTATAAATACGTTTAAAGCCAAGAATAAATCTTTAAAAAATT

The correlation method was also applied to EGFR, a breast cancer specific mRNA, to obtain similar results. The Epidermal Growth Factor Receptor (EGFR) is one among the first identified important targets of the antitumor agents. Approximately half the numbers of cases of triple negative breast cancer and also the inflammatory breast cancer have EGFR in the overexpressed state. Extensive research has indicated that EGFR targeted therapy might have promising roles in different types of breast cancer [29, 30].

4. Discussions

4.1 STFT Method versus Normalised Correlation Method

The authors attempted a comparison between STFT (Short-time Fourier transform) and correlation method to detect the binding regions of miRNAs to a specific mRNA considered. The following observations were made:

1) The binding regions obtained using STFT method was approximate and it deviated from the ground truth. However, the binding regions obtained using the correlation method were exact and close to the ground truth.
2) Since the STFT method involves much FFT calculations, the computation required for finding the binding region is more. In the case of correlation, the binding regions are obtained at the point of maximum correlation.
3) Determination of the seed region using the STFT method, involves finding the maximum value of normalised correlation using circular shift between miRNA and the obtained binding region. The application of the correlation method to finding the seed region involves just a one-to-one match for the short length of bases with respect to the miRNA and binding region of the mRNA.
4) Analysis has been tried on finding a relationship between the highest values of correlation strength and miRNAs having the highest seed length; miRNAs having 8-mer seed. This analysis was tried on the breast cancer specific miRNA, BRCA1. It was noted that the 7-mer m8 and 6-mer seeds have correlation strength values much lesser than the 8-mer and 7-mer-A1 seeds. The relationship noted needs to be validated on other breast cancer specific mRNAs.

4.2 Concentration of nucleotides and Correlation value

In table I, the concentration of the nucleotides a and c in a seed has effect on the maximum correlation value and may be also on the grade or severity of the cancer. This is under study.

5. Conclusions

Investigation on the roles of miRNA in cancer exhibits a promising and developing field of research in the war against cancer. Mutations in miRNAs lead to abnormalities thus preventing the cells from attaining the differentiated form in full manner. The new correlation method identified the binding regions of all the miRNAs specific to the 30 breast cancer specific mRNAs considered for the analysis, to obtain results close to the ground truth.

5.1 The correlation method for detecting the miRNA binding and seed binding regions for the breast cancer specific mRNAs, BRCA1, BRCA2 and EGFR *provided results exactly the same as that in ground truth.*

5.2 The correlation method provided results superior to the STFT method in which the results were just approximate and not accurate when compared to the ground truth.

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