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REGULATORY RELATIONSHIP OF MICRORNAS IN NEURODEGENERATIVE DISEASES:A CLADISTIC APPROACH

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ABSTRACT: **MicroRNAs (miRNAs) are small noncoding RNA gene products about 18- to 24-nt-long. Mainly they negatively regulate protein expression of specific mRNA by either translational inhibition or mRNAs degradation .Mature miRNAs are the results of successive processing of primary transcripts (pri-miRNAs)which are facilitated by two RNase III enzymes, Drosha and Dicer with a characteristic hairpin secondary structure. Reporting of the aberrant expressions of miRNAs have been done in respect to different human diseases mainly in concern with cancer viz lung cancer, brain cancer, chronic lymphocytic leukemia, neurodegenerative diseases etc.. It is well established fact that miRNAs defines the expression patterns of the tissue-specificity. Neurodegenerative disease states the physiological nature of the cell, expression of miR-29a, miR-29b-1 and miR-9 are found significantly down regulated in Alzheimer's disease. Biopsies of tumor from Parkinson's disease patients exposed association of miR-30b, miR-30c, and miR-26a. Phylogenetic analysis of miRNA of Alzheimer and Huntington diseases gives insight into evolutionary relationship and reveals regulation of Mir-22, Mir 29a, and mir-128-1 in both diseases. Mir-22 shows down-regulation in Parkinson disease and Alzheimer disease while mir-128-1 and mir-29a shows the difference in their regulation pattern .In Alzheimer disease mir-128-1 is up regulated while in Huntington disease it is down regulated. Mir-29a is up regulated in Huntington disease while in Alzheimer disease, it is down regulated. These findings illustrates the importance of miRNA research in Neurodegenerative diseases with reference to novel targets identification which can give a better lead in concern to protective or prophylective approaches.**

***Keywords*— miRNA; Parkinson disease; Alzheimer disease; Neurodegenerative diseases; phylogenetic analysis**

I. INTRODUCTION

MiRNAs are small (19-23nucleotide) molecules that regulate mRNAs through binding to their 3' UTR, mediated by the RNA induced silencing (RISC) complex. This binding event causes translational repression and mRNA destabilization [1]. The function of miRNAs in general appears to be as a fine-tuner of gene expression. The origin of small interfering RNAs appears to predate the emergence of eukaryotes. One of the first features observed for mature miRNAs was their high degree of similarity across taxa. So many miRNA families have identical mature sequences across a wide range of species, e.g. let-7[2]. This high-degree of similarity can hamper phylogenetic approaches to establish relationship. Functional constraints which surround the seed region (6-8nucleotide) of the miRNA represent an important fraction of their length, which is less amenable to mutational changes [3]. While many miRNAs are present in multiple species and are highly conserved, there are a growing number of miRNAs restricted to specific lineages[4]. Comparison of pre-miRNA sequences illustrates that they are highly conserved and more amenable to phylogenetic approaches than the mature sequences alone[5]. Experimental studies reveal relationship between miRNA and human diseases. The primary repository for miRNA sequence data is miRBase. In this context study has been done to highlight regulatory relationship between different miRNA involved in Neurodegenerative disease[6].

II. MIRNAS AND HUMAN DISEASES

A. miRNAs in neurodegenrative diseases

As discovery of human miRNAs increased, the research focus was gradually shifted towards functional characterization of miRNAs, particularly in the context of human diseases[7]. miRNA expression patterns are tissue-specific and in many cases define the physiological nature of the cell. The definitive evidence came from a report demonstrating that the gene expression profile of a non-neuron cell became more like that of a neuron when the neuron-specific miR-124 was artificially over-expressed within[8]. If the same premise holds true, certain miRNA expression patterns could be disease-specific and hold great prognostic value[9]. In fact, a more comprehensive miRNA profiling study demonstrated that distinct miRNA expression patterns were specific to various types of cancers and were able to reflect the developmental lineage and differentiation state of tumors[10].

More specifically, many miRNAs were found to play key roles in vital biological processes such as cell division and death, cellular metabolism, intracellular signaling, immunity and cell movement[11]. Therefore, aberrant miRNA expression should proportionately affect those critical processes, and as a result, lead to various pathological and occasionally malignant outcomes. Neurodegenerative diseases on molecular levels remain poorly understood, successful treatments are still unavailable[12]. With increasing investments from governments and pharmaceutical companies, biomedical research on neurodegenerative diseases has become proprietary[13]. Notably,

recent progresses from studies elucidating miRNA functions in Neurodegenerative diseases have shed new light on disease pathogenesis and may lead to novel treatment strategies [14].

B. miRNA in Parkinson disease

The miRNA profiling in peripheral blood mononuclear cells from Parkinson's disease patients revealed miR-30b, miR-30c, and miR-26a to be associated with the susceptibility of the disease and Deregulation of miR-133b expression may contribute to the pathogenesis of Parkinson's disease[15-18]. In Drosophila model for Parkinson's disease, pathogenic leucine-rich repeat kinase 2 (LRRK2) was shown to promote the expression of transcriptional factors E2F1 by down regulating expression of let-7 and miR-184 [18-20].

C. miRNA in Alzheimer's disease

Analysis of miRNA and mRNA expression in brain cortex from Alzheimer's disease and age-matched control subjects demonstrated strong correlations between the expression levels of mirnas and predicted mrna targets, implying functional relevance of microrna-mediated regulations in Alzheimer's disease pathogenesis [21-24]. More specifically, the expression of mir-29a, mir-29b-1 and mir-9 was significantly decreased in Alzheimer's disease patients, resulting in abnormally high expression of their target BACE1, a protein playing an important role in Alzheimer's disease pathogenesis[25-28]. These findings not only highlight the importance of mirna research in understanding Neurodegenerative diseases pathogenesis, but also provide a previously unrecognized venue for medical interventions [29-30].

D. miRNA in Huntington disease

Huntington disease is a rare neurodegenerative disorder of the central nervous system characterized by unwanted choreatic movements, behavioural and psychiatric disturbances and dementia[31-32]. Huntington disease is an autosomal dominant inherited disease caused by an elongated CAG repeat (36 repeats or more) on the short arm of chromosome 4p16.3 in the Huntington gene. The longer the CAG repeat, the earlier the onset of disease[33-35]. In cases of Juvenile Huntington's disease the repeat often exceeds 55 repeat blocks[36].

E. MiRBase

miRBase is the primary online database and repository for published microRNA sequences and annotation (http://www.mirbase.org/). MiRBase was established in 2002 (under the name the microRNA Registry) with the primary role of coordinating gene names of the then newly discovered miRNA gene class[37-40]. miRBase provides information about miRNA function, structure, regulation along with sequence and association with diseases[41].

III. Materials And Methods

miRNA Databases miRBase ((http://www.mirbase.org/) and mir2disease (http://www.mir2disease.org/) was used to retrieve high confidence miRNA sequence involved in Alzheimer's disease, Parkinson's disease and Huntington's disease [42]. Multiple sequence alignment tools Clustal omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) is used to study similarity and conserved region among miRNAs. PHYLIP (http://evolution.genetics.washington.edu/phylip.htm) DNApars, Drawgram, Seqboot, Consensus programs are used to create phylogenetic tree and analysis [43]

Fig. 1 Flowchart for phylogenetic tree construction between miRNAs associated with neurodegenerative diseases

Fig 1. describes the methodology for phylogenetic tree construction .Firstly miRNA ids associated with Alzheimer's disease, Parkinson's disease and Huntington's disease was retrieved from mir2disease database and sequence of these miRNAs is retrieved from miRBase database [44-45] .Three datasets were created

- Dataset 1: Alzheimer's disease and Huntington disease
- Dataset 2: Alzheimer's disease and Parkinson's disease
- Dataset 3:Parkinson's disease and Huntington disease

Multiple sequence alignment is performed using Clustal omega and phylip file is created. Phylip software packages are used to create phylogenetic tree and for phylogenetic tree validation.

IV. RESULT

A. Dataset 1:Alzheimer and Huntington disease

Phylogenetic tree was constructed for miRNAs involved in Alzheimer and Huntington disease using phylip program. The micro-RNA which are similar and are responsible for Alzheimer disease and Parkinson disease are Mir-22, Mir 29a, and mir-128-1. Fig.2 represents the phylogenetic tree and clades showing relationship of miRNA in both diseases.

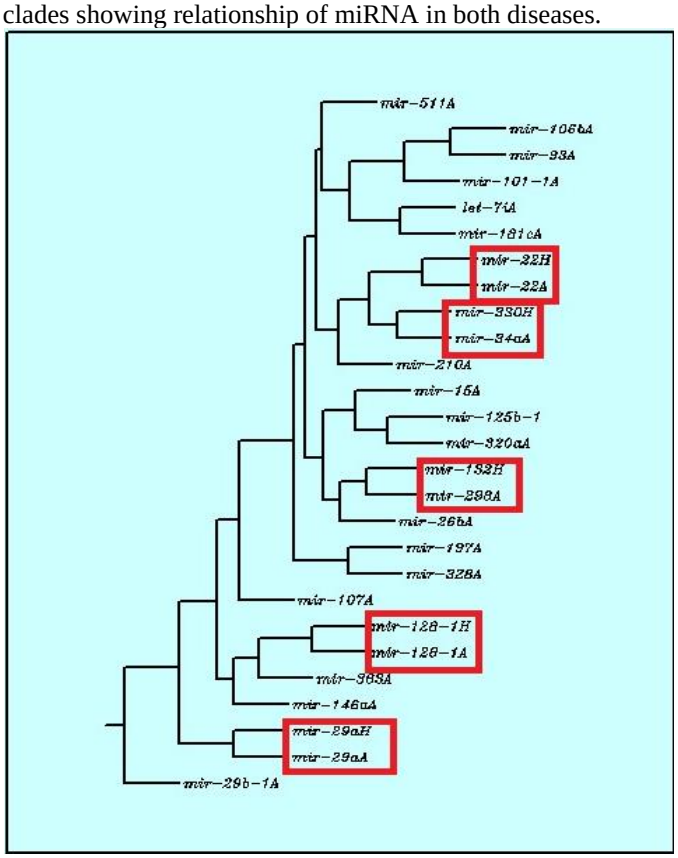

Fig. 2 Phylogenetic tree of miRNA's in Alzheimer's disease (A) and Huntington disease (H) , red box represents the clades ,miRNA showing close relationship and common in both diseases

Mir22 is expressed in both Alzheimer and Huntington disease having bootstrap score of 10 out of 10 replicates. Table 1 list the clades information of miRNA in both diseases and also their regulation pattern .Mir-330 is upregulated in Huntington disease and Mir-34a is downregulated in Alzheimer's disease, study shows differential expression of miRNA in both disease conditions.

Table 1 shows regulation pattern of miRNA in both diseases. Mir-132 and Mir-298 are downregulated in both diseases and are from common evolutionary origin. Mir-128 shows differential expression in both diseases it is downregulated in Huntington disease and upregulated in Alzheimer disease hence in these different diseases they exhibit different pathways. This shows regulatory relationship of miRNA with these diseases. Mir-29a is upregulated in Huntington disease and downregulated in Alzheimer disease.

This study can be used to classify the differential expression of miRNA in Alzheimer and Huntington disease.

Table 1 miRNA relationship and regulation pattern in Huntington and Alzheimer disease

S no.	miRNA	Gene family	Regulation	Bootstrap score
1.	Mir-22(H)	MIPF0000053	Down Regulated	10
	Mir-22(A)			
2.	Mir-330(H)	MIPF0000200	Up Regulated	5.17
	Mir-34a(A)	MIPF0000039	Down Regulated	
3.	Mir-132(H)	MIPF0000065	Down Regulated	5.00
	Mir-298(A)	MIPF0000206	Down Regulated	
4.	Mir-128-1(H)	MIPF0000065	Down Regulated	10
	Mir-128-1(A)		Up Regulated	
5.	Mir-29a(H)	MIPF0000009	Up Regulated	10
	Mir-29a(A)		Down Regulated	

B. Dataset 2:Alzheimer and Parkinson's disease

Mir-26a-1 and Mir-26b of same gene family are expressed in Alzheimer and Parkinson's disease and have same evolutionary relationship. Fig. 3 shows phylogenetic tree of miRNA between Alzheimer and Parkinson's disease. Two clades were identified that shows relationship of miRNA in both diseases. Clade information shows relationship between Mir-184 and Mir-320 that are expressed in Parkinson's and Alzheimer's disease and have same evolutionary relationship. Mir-184 and Mir-320 show similarity when the phylogenetic tree was constructed using DNApars but when the consensus

tree was generated then, these two miRNA did not show the evolutionary relationship and hence no bootstrap value is calculated. The MiRNA sharing the same sequence act differently because of their regulation pathway.

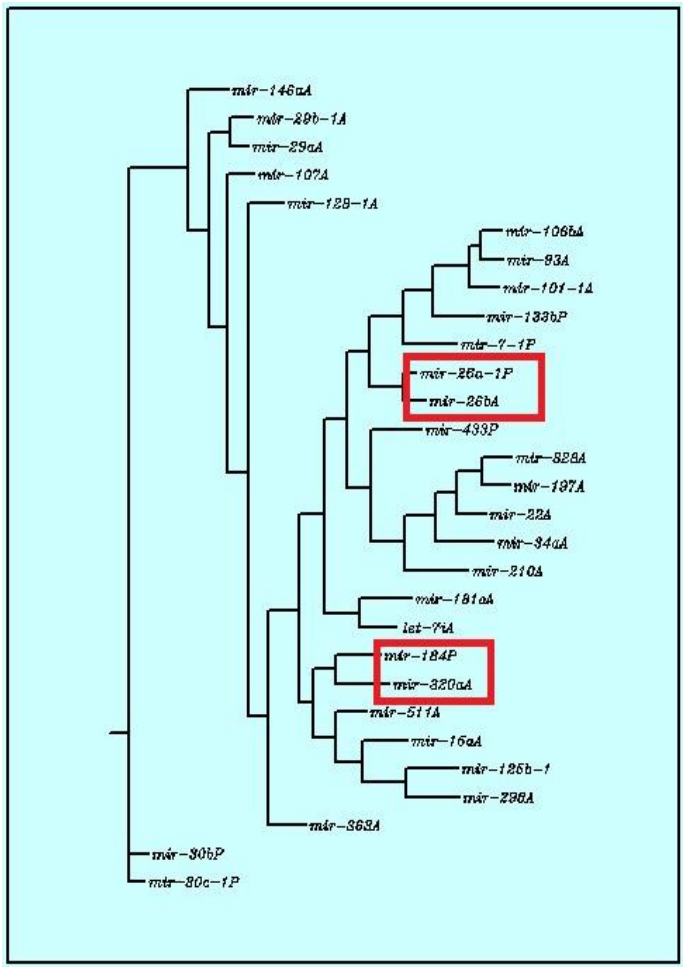

Fig. 4 Phylogenetic tree of miRNA's in Alzheimer's disease (A) and Parkinson's (P) , red box represents the clades ,miRNA showing close relationship and common in both diseases

Table 2 shows the regulation pattern of miRNA in Parkinson and Alzheimer disease .that shows Mir-26a-1and Mir-26b are downregulated in both diseases. Mir-320 is upregulated in Alzheimer disease. Mir-184 regulation pattern is unknown function of Mir-184 can be associated with function of Mir-320.

Table 2 miRNA relationship and regulation pattern in Parkinson and Alzheimer disease

S no.	miRNA	Gene family	Regulation pattern	Bootstrap score
1.	Mir-26a-1(P)	MIPF000	Down	10
	Mir-26b(A)	0043	regulated	
2.	Mir-184(P)	MIPF000 0059	N/A	-
	Mir-320(A)	MIPF000 0163	Up regulated	

C. Dataset 3: Parkinson's and Huntington disease

Mir-184is expressed in Parkinson disease and Mir-132 is expressed in Huntington disease and have common evolutionary relationship with a confidence score of 10 out of 10 replicates .Fig. 4 shows the phylogenetic tree of miRNA between Parkinson's and Huntington disease and Table 3 shows the regulation pattern of miRNA in both diseases

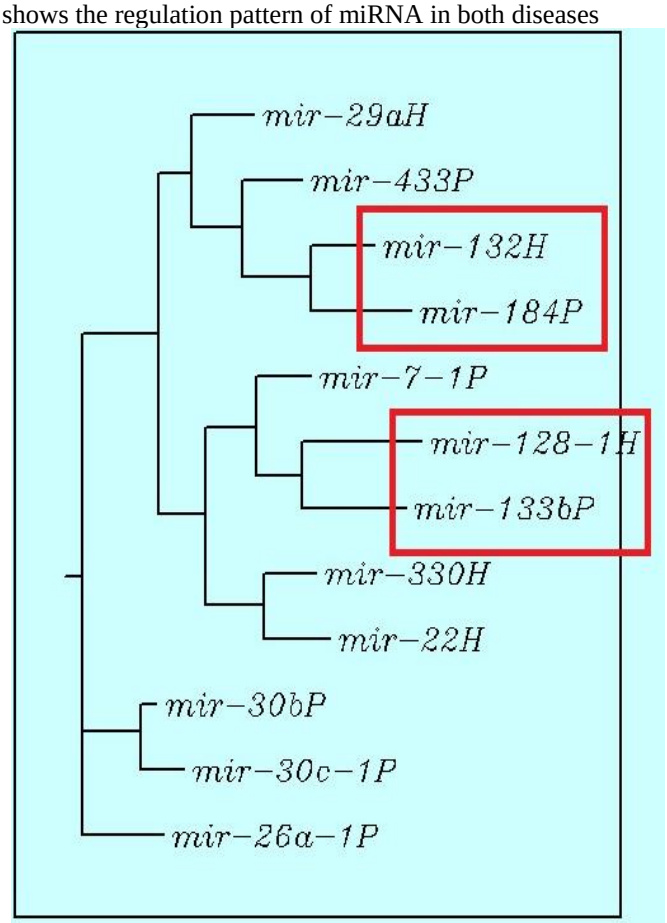

Fig. 5 Phylogenetic tree of miRNA's in Huntington disease (H) and Parkinson's (P), red box represents the clades, miRNA showing close relationship and common in both diseases

Table 3 represents the clade relationship of miRNA in Parkinson and Huntington disease. Study shows Mir-184 and Mir-132 are downregulated in both diseases.

Table 3 miRNA relationship and regulation pattern in Parkinson and Huntington disease

S no.	miRNA	Gene family	Regulation pattern	Bootstrap score
1.	Mir-184(P)	MIPF0000059	Down regulated	10
	Mir-132(H)	MIPF0000065		
2.	Mir-133(P)	MIPF0000029	Down regulated	6
	Mir-128-1(H)	MIPF0000048		

Mir-133and Mir-128-1 is downregulated in Parkinson and Huntington disease.

V. CONCLUSION AND DISCUSSION

miRNA can be used as drug target to silence genes that are upregulated in diseases [46-49]. This study signifies the role of miRNA in neurodegenerative diseases. As for example Mir-128-1 is Down Regulated in Huntington disease and Up Regulated in Alzheimer disease so Mir-128-1 can be used as potent drug target to regulate its expression and hence Alzheimer disease. Mir-29a can be used as drug target and hence regulation of Huntington disease. Also this study shows the evolutionary relationship between the miRNAs which are expressed in the neuro-degenerative diseases, Alzheimer, Huntington and Parkinson's disease. That can be used further to identify the gene as drug target that are involved in neuronal disease.

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