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General Article

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Micro RNA, A Review: Pharmacogenomic drug targets for complex diseases

Ritesh Bajaj*[1], Neelima Goel[1], Sushma Chaturvedi[1], Sandhya Bawa[2], Sushma Drabu[1]

Maharaja Surajmal Institute of Pharmacy, C-4 Janakpuri, New Delhi: 110058 [1]
Faculty of Pharmacy, Jamia Hamdard University [2]

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Abstract Micro RNAs (miRNAs) are non-coding RNAs that can regulate gene expression to target several mRNAs in a gene regulatory network. MiRNA related Single Nucleotide Polymorphisms (S.N.P.s) represent a newly identified type of genetic variability that can be of influence to the risk of certain human diseases and also affect how drugs can be activated and metabolized by patients. This will help in personalized medicines which are used for administrating the correct dosage of drug and drug efficacy. miRNA deregulated expression has been extensively described in a variety of diseases such as Cancer, Obesity , Diabetes, Schizophrenia and control and self renewal of stem cells. MiRNA can function as oncogenes and/or tumor suppressors. MiRNAs may act as key regulators of processes as diverse as early development, cell proliferation and cell death, apoptosis and fat metabolism and cell differentiation .miRNA expression have shown their role in brain development chronic lymphocytic leukemia, colonic adeno carcinoma, Burkiff's lymphoma and viral infection. These show their links with viral disease, neurodevelopment and cancer. It has been shown that they play a key role in melanoma metastasis. These may be differentially expressed in malignant cells compared to normal cells altering the regulation of expression of many important genes. MiRNA expression has been used for prognosis and early diagnosis of these complex diseases. The present paper focuses on the role of miRNAs in various complex diseases, which will help in improving the drug discovery process and personalized medicines.

Keywords: miRNAs, gene expression, Single Nucleotide Polymorphisms, personalized medicines, mRNA translation, complex diseases, drug discovery.

Biochemistry and basic mechanism

MicroRNAs (miRNAs) are non-coding small RNAs that regulate gene expression on the post-transcriptional level by inhibiting the translation of protein from mRNA hence degrading mRNA. MiRNAs are 18–25 nucleotides long firstly elucidated in the worm Caenorhabditis elegans as gene expression regulators of differentiation and development. Further experimentations confirmed existence of more than 700 miRNA as the class of non-coding genes which are predicted to regulate at least 30% of all the human protein-coding genes.[1-4]

The biochemical aspect of miRNAs suggests that they are initially transcribed as pri-miRNAs which can be processed into pre-miRNAs and subsequently into mature miRNAs. Mature miRNAs have the ability to affect the translational efficiency of various protein-coding genes at the same time. MiRNAs in posttranscriptional regulation of proteins with diverse roles have been implicated.

***for correspondence**
Ritesh Bajaj
Maharaja Surajmal Institute of Pharmacy,
C-4 Janakpuri, New Delhi,
E-mail: ritesh046@gmail.com

There is a 'seed' region of 8 nucleotide at the 5'-end of miRNAs that is essential to miRNA function. This region is important for binding to the mRNA targets and for mRNA target degradation.[5-7] SNPs (Single-Nucleotide Polymorphisms) can affect the way miRNAs regulate their targets, pointing to a function in drug metabolism and in phenotypic variation. For example, the presence of SNPs located in miRNA-binding sites of several genes and SNPs in the microRNA seed regions when evaluated by genome wide analyses identified twelve miRNA binding SNPs that display an aberrant allele frequency in human cancers and approximately 250 SNPs that create novel target sites for miRNAs in humans and may result in phenotypic differences. SNPs and other genetic variations will affect the expression of protein-coding and noncoding genes in the genome by changing the miRNA regulatory network.[8-12]
MiRNAs are of particular therapeutic relevance as one miRNA can down regulate multiple target proteins by interacting with different target mRNAs. As drug metabolism can involve a group of genes, it is considered that the determination of how patients react and metabolize drugs can be focused on master regulators of gene expression, such as miRNAs. In that regard, changes in the sequence of a miRNA and/or variations in the target region of a transcript that is regulated by a miRNA can have major effects in posttranscriptional regulation of proteins.[13-15]
The table 1 below is a critical review of the various diseases

and the kind of miRNA acting along with their reference.

Table 1: Critical review of the various diseases and the kind of miRNA actin

DISEASES	**IDENTIFIED miRNA**
CANCER AND TUMORIGENESIS MiRNA is opening up a whole new way of understanding carcinogenesis. MiRNAs in tumorigenesis have shown that the down regulation of these is a common occurrence in breast carcinomas and breast cancer line. MiRNA does not code for protein production. Instead, it causes the destruction of coding RNA directly, or garbles its translational activity so proper protein production cannot take place. Some of the breast cancer-specific miRNA we identified appear to act like tumor suppressors, and others appear to act like oncogenes, encouraging tumor growth. The role of miRNA in prognosis and diagnosis of cancer is attractive goal for researchers.	Micro RNA -143 identified as a tumor suppressor for bladder cancer.[21]
	MiR-372, miR-373 increase gene regulation of LAST2 in case of Testicular germ cell tumors.[22]
	29 miRNAs are significantly deregulated in breast cancer (some are identified as miR10b, miR-125b, miR-145, miR-21 and miR-155, miR -17-92 etc.)[23]
	MiR-143 and miR-145 constantly down-regulate in colorectal tumors.[24]
	MiR-15, miR-16 down regulate in Chronic lymphocytic leukemia targeting BCL2 gene.[25]
	MiR-21, miR-221 potentiates and miR-181 suppresses tumorigenesis in case of Brain Cancer.[26]
	MiR-221, miR-222, miR-146, miR-181 encourages expression of KIT gene in case of Papillary thyroid carcinoma.[27]
CARDIOVASCULAR DISORDERS • VASCULAR INFLAMMATION • HYPERTENSION • ISCHAEMIA • ATHEROSCLEROSIS • TUMOR ANGIOGENESIS • HEART ATTACK The miRNAs and their respective targets are new therapeutic strategies to treat vascular diseases such as atherosclerosis, to improve neovascularization after ischaemia, or to prevent tumor progression. miRNAs that are down regulated in the hypertrophic and failing heart are of interest as expression of a subset of these prevents hypertrophic cell growth .It is tempting to speculate that these miRNAs function as negative regulators of cell growth or as regulators of prosurvival pathways such that their down regulation predisposes the heart to pathological remodeling. The mRNA targets of the miRNAs that participate in cardiac remodeling are to be understood as the functions of their target mRNAs.	SNPs in miR-155 target sites down regulate the expression of the allele that has been associated with hypertension.[28]
	VASCULAR INFLAMMATION There is an association of the human angiotensin II type 1 receptor polymorphism and miR-155(miRNAs, e.g. MiR-21 and miR-126 are other examples).[29]
	MiR-221 and miR-222 inhibit endothelial cell migration, proliferation, and angiogenesis in vitro.[30]
	Some miRNAs are involved in tumor angiogenesis such as the miR-17-92 cluster and miR-378.[31]
	Induction of cardiac hypertrophy and heart failure by miR-195.[32]
	MiR-21 appears to function as a regulator of cardiac growth and fetal gene activation in cardiac hypertrophy.[33]
SCHIZOPHRENIA Altered miRNA profiles are seen in postmortem prefrontal cortex from schizophrenia patients. Post-transcriptional deregulation of gene expression, postmortem studies find altered levels of mRNA and proteins rather than a specific abnormal protein which connects it to the region of miRNA.	MiR-219 expressed in the brain is located in the susceptibility locus for schizophrenia and screening for mutations has identified a population polymorphism in the 5′- Upstream miR-219 gene region.[34]
	Two SNPs located in miR-206 and miR-198 sequences have been expressed by the respective miRNAs.[35]

ASTHMA Some genetic variants may only cause asthma when they are combined with specific environmental exposures. Single nucleotide polymorphisms may affect the miRNAs that are specifically identified.	An SNP has been identified in the 3'UTR of HLA-G that influences the targeting of three miRNAs (miR-148a, miR-148b and miR-152) to this gene. The allele-specific targeting of these miRNAs can account with asthma.[36]
VIRAL INFECTION Viral-encoded miRNAs have been in viruses transcribed from double-stranded DNA genome. T hey comes from three different viral families-herpes viruses, polyomaviruses, and retroviruses. Virus-encoded miRNAs have the polymerase that transcribes their location within the precursor transcript.	MiR-32 and miR-122 binds to the adenovirus and acts as an attractive drug target in its mechanism.[37]

Future prospects & application

- MiRNAs related to siRNA (small interfering RNA) which are studied and evaluated as a potential therapeutic strategy. Promising results have been obtained from studies on mammalian cell-culture systems and animal in vivo models in siRNA technology.[16]
- Antisense Oligonucleotides & Anti-mRNA Oligonucleotides (AMO) or antisense Oligonucleotide (ASO) techniques use the same rationale: a nucleic acid that is antisense to the miRNA, thus base pairs with the miRNA, resulting in impaired interaction between miRNAs and target mRNAs. ASOs targeting 86 human miRNAs were used to screen for potential miRNAs that might be involved in adipocyte differentiation.[17]
- Locked Nucleic Acids, (inaccessible RNAs), are a family of nucleic acid analogs containing one or more LNA nucleotide monomer with a bicyclic furanose unit locked in an RNA-mimicking sugar conformation. This conformational restriction results in an unprecedented hybridization affinity towards complementary single-stranded RNA molecules. LNA is thought to increase the functional half-life of miRNA *in vivo.*[18]
- Viral Vectors Expressing miRNA Genes, Lentiviral vectors (LVs), adenoviral vectors (AVs) or adeno-associated virus (AAV) vectors hold great promises for gene therapeutic applications and pharmaceutical target validation. LVs efficiently integrate into the genome of nondividing cells, such as pancreatic islets, hematopoietic stem cells, or terminally differentiated cells.[19]
- The regulatory and functional effect of the miRNAs can be studied individually or systematically using the whole set or a subset of miRNA expression constructs by transduction of precursor miRNAs into target cells either via transfection or viral infection which relates to regulation of gene expression.[20]

Conclusions

- Pharmacogenomic linked biochemical research has provided evidence that miRNAs may act as key regulators of processes as diverse as early development, oncogenic cell proliferation and cell death, apoptosis and fat metabolism and cell differentiation.
- MicroRNAs and other noncoding RNAs are new players in pharmacogenomics. Our current view of how genome variations such as SNPs can affect drug metabolism.
- Polymorphisms in one or a few protein-coding genes will affect drug resistance, efficacy and metabolism. MicroRNA variations, protein variations and noncoding RNA variations affecting the way drugs are metabolized are depicted.
- In this new integrated concept, all variations in the genome will have additive effects in pharmacokinetics and pharmacodynamics of drugs.
- MiRNA target gene identification and validation together with GeneCopoeia's miRNA target validation expression clones, miRNA expression constructs can be used to identify and verify their target genes.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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