INTRODUCTION

Molecular biology’s central dogma explains life using 3 macromolecules: Deoxyribonucleic acid (DNA)—the genetic material of almost all living organisms, which is transcribed to Ribonucleic acid (RNA), which transmits genetic information from DNA to the cytoplasm to be translated to amino acids and forms protein which is a sequence of amino acids that forms the structural and functional basis of every cell.\[1\]

For decades, RNA was thought to play a very minor role in gene expression by converting genetic information from DNA into functional proteins upon receiving an appropriate signal. In the late 1960s, a subset of RNAs was found to control gene expression by stating which genes should turn on and which should turn off.\[2\] These non-coding RNAs, rightly named because they do not code for a protein, are of distinct classes distinguished based on their function and origin. These include microRNA (miRNA), small temporal RNA (stRNA), short interfering RNA (siRNA), short hairpin RNA (shRNA), small nuclear RNAs (snRNA), small nucleolar RNAs (snoRNA), transfer RNAs (tRNA) and ribosomal RNAs (rRNA).\[3\]

MiRNAs and siRNAs are currently among the most studied small non-coding RNAs. Following completion of the Human Genome Project, it was found that there are about 1000 genes in humans that encode miRNAs, which account for approximately 3% of the human genome.\[4–6\] MiRNAs are critical in determining cellular fate as they regulate development, maturation, differentiation and apoptosis of the cell, cell signaling, cellular interactions and homeostasis. Alternatively, they also assume central importance in our understanding of many pathologic conditions such as carcinogenesis.\[7,8\] Small non-coding RNAs are thus at the forefront of modern biology, heralding in an era of RNomics (the study of small non-messenger RNA) and challenging a central dogma proposed by Francis Crick more than half a century ago.

HISTORY

Living cells arose on Earth around 3.5 billion years ago when spontaneous reactions occurred between molecules of which RNA (Ribonucleic acid) molecules were the prime players. With time, protein catalysts accumulated, thereby resulting in the evolution of more complex and efficient cells and eventually the DNA double helix molecule, being more stable, replaced RNA in order to store the larger amounts of genetic information needed by these cells. The RNA molecule remained an intermediary, connecting the DNA, having the genetic function, with the proteins, having the catalytic function.\[9\]

Coding RNAs

Based on their function, RNA molecules can be broadly classified into coding RNAs and non-coding RNAs. Coding RNAs are molecules that code for a particular protein. They are key players in protein transcription and translation. The gene that codes for the protein of interest is unwound and transcribed into a single-stranded RNA molecule, the messenger RNA (mRNA), so called because it carries the genetic information from the nucleus into the cytoplasm, where it is translated into a sequence of amino acids forming a polypeptide chain. However, it remained unclear as to what made the genes to be transcribed into a particular protein and
how the process was turned on and off in each cell.\textsuperscript{[1]} Human

genome analysis has revealed that a very small portion of the
human genome is translated into functional proteins while the
majority (approximately 65\%) of the genome is transcribed
into RNAs, whose function is still not determined.\textsuperscript{[10]}

Non-coding RNAs

Non-coding RNAs, unlike mRNAs, do not encode protein
but control various levels of gene expression.\textsuperscript{[11,12]} Based
on their function, non-coding RNAs can be categorized
into housekeeping RNAs such as tRNAs, rRNAs, snRNAs
and snoRNAs and regulatory RNAs. Housekeeping RNAs
are usually constitutively expressed whereas regulatory
non-coding RNAs are produced only at certain stages of
cellular development and differentiation or in response to
external stimuli. Among the small regulatory RNAs, miRNAs
are the most phylogenetically conserved and function
post-transcriptionally to regulate physiological processes by
silencing the gene.\textsuperscript{[13-15]}

Gene regulation

Genetic regulation is essential to development and is the
process that controls the differentiation of a single totipotent
cell into a functional, complex multicellular organism. An
interspecies variation, or more simply, what makes us human,
is not only the difference in the genetic makeup but also
the differences in gene regulation.\textsuperscript{[16]} It is also the cause of
phenotypic variations among individuals of the same species
as well as the reason for disease processes when the regulation
is aberrant.

Both RNA and protein can be regulated to control the
amount of active gene product formed by epigenetic control,
chromatin remodeling through DNA modifications and
regulating the transcript. This can occur at the transcriptional
level, when the gene is transcribed to an RNA transcript; at
the translational level, when the gene encodes a protein; or the
post-transcription and post-translational level, after the gene
product is synthesized. Small non-coding regulatory RNAs
regulate post-transcriptional gene expression.\textsuperscript{[17]}

Gene regulation can result in up-regulation or down-regulation
of the gene product. Down-regulating the formation of active
gene products or "turning off" the gene is called gene
silencing. RNA interference (RNAi) is a method of sequence
silencing that differs significantly from a variety of other
mechanisms in its mechanism of action.\textsuperscript{[25,26]}

The first miRNA, lin-4 (abnormal cell L1Neage) was discovered
by Ambros and coworkers (1993) in \emph{C. elegans} as an
endogenous regulator of genes that regulate developmental
timing.\textsuperscript{[10]} The second miRNA, let-7 (LETal), was discovered
7 years later and found to function similar to lin-4. Eventually,
two categories of small RNAs were established that regulated
gene expression: miRNAs, which regulate endogenous genes
and siRNAs, which defend genome integrity in response to
foreign or invasive nucleic acids such as viruses, transgenes
and transposons.\textsuperscript{[27,28]}

Following the discovery of lin-4 and let-7, several hundreds of
miRNAs have been identified. Some miRNAs, such as let-7, are
highly conserved through evolution and are essential to many
biological processes, while the individuality of an organism
can be ascribed to lineage- and species-specific miRNAs.\textsuperscript{[29,30]}
There are currently 1872 precursor and 2578 mature human
miRNA sequences listed in the miRNA registry (Sanger
miRBase release 20; http://www.mirbase.org/). Almost 60\%
of mammalian miRNAs are predicted targets of a relatively
small number of miRNAs, suggesting that a given miRNA
can silence many target genes. This is thought to be because
miRNA does not require perfect sequence complementarity
with its target mRNA.\textsuperscript{[31]}

BIogenesis of miRNAs

miRNAs are produced through transcription of miRNA genes
in the nucleus known as miRNAs precursor genes (mir-gene).
The miRNA transcripts are then spliced and capped similar
to protein coding mRNA transcripts. These primary miRNAs
form a hairpin-shaped stem loop, prior to being processed
into pre-miRNAs. This processing is carried out by a
microprocessor complex that consists of Drosha (RNase
III endonuclease) and DiGeorge syndrome critical region
8 (DGCR8) or Pasha, which is an essential cofactor. This
miRNAs have been found to regulate almost all cellular functions including cell proliferation, growth, differentiation and apoptosis. They are thought to play a role in specifying tissue identity since they are involved in the process of differentiation into specific tissue. Thus, the expression of miRNA in a specific cell type can be a useful marker for identifying the particular cell type.[37]

Tooth development

Specific codes of miRNAs have been identified which regulate cell differentiation and are required for tooth patterning; size, shape and number determination.[38]
that were capable of differentiating aggressive from indolent chronic lymphocytic leukemia (CLL).[43]

Recent evidence indicates that miRNAs play an important role in p53 tumor suppressor pathways. He et al. found miR-34a, miR-34b and miR-34c to be closely linked to p53 status and oncogenic stress and DNA damage induced their expression.[44,45]

**Oral cancer**

miRNA profiling in head and neck squamous cell carcinomas (HNSCCs) revealed miR-451 to be a potential prognostic marker. miR-375 and miR-106b-25 cluster to be mediate the development and progression of HNSCC.[46] Kozaki et al. showed that miR-137 and miR-193a function as tumor suppressors and are silenced in oral carcinogenesis.[47] Wong et al. studied the expression patterns of miRNAs in squamous cell carcinoma of the tongue and found an over expression of miR-184 which was thought to have an oncogenic role by inducing proliferative and anti-apoptotic processes.[48,49]

Henson et al. found that the development and/or progression of oral squamous cell carcinoma are associated with the down-regulation of miR-100 and miR-125b and these miRNAs may be the reason for the low sensitivity to ionizing radiation.[50] Li et al. found that miR-21 was an independent prognostic indicator for tongue squamous cell carcinoma and played a role in its development by inhibiting apoptosis of cancer cells.[51]

Metastasis is a significant event in the progression of HNSCC and Liu et al. found miR-138 to act as a tumor suppressor which could be a potential target for therapy in patients with a risk of metastasis.[52]

Cervigne et al. found miRNA signatures that could potentially identify leukoplakias which are at a risk of malignant transformation. The expression of miR-21, miR-181b and miR-345 were found to be consistently increased and associated with increase in the severity of the lesion. Overexpression of these miRNAs was thought to play a major role in malignant transformation.[53]

**miRNAs and viruses**

RNA interference and gene silencing is an innate host cell mechanism to protect against viruses. Viruses, on the other hand, have evolved to bypass host interference by various mechanisms which include altering miRNA expression in the host cell in a way that promotes viral replication. Nef and rev are viral genes in the human immunodeficiency virus that suppress host silencing mechanisms.[54-56]

**miRNAs in autoimmune diseases**

miRNAs are found to regulate immune response, immune cell development and prevention of autoimmunity. A possible role has been suggested for miRNA in the development of autoimmune diseases such as Rheumatoid Arthritis (RA), Sjögren’s syndrome and Systemic Lupus Erythematosus (SLE). Distinctive miRNA expression patterns have been linked to salivary gland dysfunction in patients with Sjögren’s syndrome and these miRNAs can serve as potential biomarkers for the disease. Proteins such as Ago2, involved in the biogenesis of miRNAs, have been found to be targets of these autoantibodies.[57,58]

**Periodontal disease**

miRNAs have been shown to play a major role in regulating the immuno-inflammatory response. Xie et al. compared the miRNA profiles of inflamed and healthy gingival tissue and found 91 miRNAs up-regulated and 34 miRNAs down-regulated in the inflamed gingival tissue indicating a plausible relationship between periodontal inflammation and miRNAs. miRNAs may be involved in regulating toll-like receptors (TLRs) in periodontal inflammation.[59]

**THE CLINICAL POTENTIAL OF MIRNAS: DIAGNOSTIC, PROGNOSTIC AND THERAPEUTIC IMPLICATIONS**

The majority of miRNA are intracellular, but some miRNA exists in the extracellular compartment and are seen to be mediators of cell-cell communication. Extracellular miRNAs can be isolated from body fluids such as serum, plasma and saliva. They can act as potential biomarkers for the detection of various diseases.[60] Salivary miRNAs can be used clinically to detect oral cancer. Healthy saliva contains approximately 50 miRNAs. Two miRNAs in particular, miR-125a and miR-200a have been found exclusively in the saliva of oral cancer patients and are diagnostic markers of the disease.[61-63]

The presence of RNAases in body fluids precludes the existence of any intact RNA. Thus, it has been theorized and proven that miRNA exist extracellularly within small, cell-secreted vesicles called “exosomes.”[64] These vesicles can regulate intercellular communication and facilitate certain processes such as antigen presentation. Exosomes are present in body fluids that include plasma, blood, breast milk, saliva and urine and can have a potential role in immunotherapy and vaccination modalities and as a potential vector for gene therapy. Salivary exosomal miRNAs may be important not only as a diagnostic tool but can also provide information regarding the role of miRNAs in the pathophysiology of various salivary gland diseases.[64]

**QUANTIFICATION**

Microarrays and quantitative PCR (qPCR)-based methods are the major modalities used to profile miRNAs. Quantitative PCR methods are widely available, relatively inexpensive and allow for the measurements of minute quantities of
miRNAs. However, the primer design can influence the results. With microarray-based methods, it is difficult to detect different miRNAs at one time. Northern blotting, direct sequencing and ligation-based measurement can also be used.\[36]\[62\] In situ hybridization is a reliable method to localize and detect miRNAs in both frozen tissue and paraffin-embedded sections. To confirm the function of a specific miRNA, loss-of-function and gain-of-function approaches can be applied in vivo in mammals as well as in vitro in cultured cells.\[37]\[62\]

ACKNOWLEDGEMENTS

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