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miRNA and other non-coding RNAs as promising diagnostic markers

Dorota Trzybulska[1], Eleni Vergadi[2] and Christos Tsatsanis[1,2]

[1] *Molecular Reproductive Medicine, Department of Translational Medicine, Lund University, Malmö, Sweden*

[2] *Department of Clinical Chemistry, School of Medicine, University of Crete, Heraklion, Greece*

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Corresponding author:
Christos Tsatsanis
Medical School
University of Crete
Voutes, 70013 Heraklion, Crete
Greece
Phone: +30 2810394833
E-mail: tsatsani@uoc.gr

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ABSTRACT

Since the discovery of non-coding RNAs (ncRNAs) a new area has emerged in the field of biomarkers. NcRNAs are RNA molecules of different sizes that are transcribed as independent genes or as part of protein coding genes and are not translated, therefore they do not produce proteins. They have been classified according to their size and function and include microRNAs (miRNAs), piwiRNAs (piRNAs), snoRNAs and long non-coding RNAs (lncRNAs). These non-coding RNAs are present in different cell compartments participating in multiple cell functions, but they have also been identified in biological fluids, also known as cell-free or circulating ncRNAs, where they can be detected in exosomes, bound on lipoproteins as well as free circulating molecules. The role of circulating ncRNAs is still under investigation but are believed to be paracrine or endocrine messengers to systematically deliver signals between cells and tissues. Detecting ncRNAs in biological fluids has opened a new field in Clinical Chemistry utilizing them as biomarkers of diseases or prognostic markers for different pathological conditions. Herein, the different types of ncRNAs and their potential in the field of diagnostics are outlined.

INTRODUCTION

Protein-coding genes have been studied thoroughly during the last decades, even though they represent only 1.5% of the genome, which can increase to 2% if untranslated regions (UTRs) are included. The remaining genome, the non-protein coding region has remained a 'black box' until recently when characterization of non-coding RNAs (ncRNAs) has emerged, due to the development of novel nucleotide sequencing technologies. The importance of the ncRNAs has become increasingly apparent and our knowledge on the significance and contribution of ncRNAs in development and disease pathogenesis is expanding rapidly (1). NcRNAs can also explain phenotypic diversity between species given the fact that i.e. protein-coding genes are very similar among mammals while their ncRNAs do not exhibit the same level of similarity. The functional role of a class of ncRNAs, this of microRNAs (miRNAs), in cell physiology and human disease has been widely studied and acknowledged. In nearly all diseases miRNA expression pattern has been shown to differ both in tissues and extracellularly, contributing to disease pathogenesis. However, miRNAs are only the tip of the iceberg since additional ncRNAs are emerging as contributors of tissue homeostasis and regulators of cell function and fate. Hence, PIWI-interacting RNAs (piRNAs), transcribed small nucleolar RNAs (snoRNAs), ultraconserved regions (T-UCRs), large intergenic non-coding RNAs (lincRNAs) and the heterogeneous group of long non-coding RNAs (lncRNAs), are also key contributors to tissue homeostasis and disease pathogenesis. Among those, a significant number has been detected in biological fluids, allowing their use as circulating biomarkers.

Emergence of novel technologies has allowed to characterize sequences of ncRNAs in different healthy and diseased tissues as well as biological fluids from healthy and diseased individuals. The most prominent and widely studied ncRNAs are miRNAs which are already utilized as disease biomarkers.

miRNAs AS BIOMARKERS OF HUMAN DISEASE

After the discovery of miRNAs and their first association with human disease sixteen years ago (2), their contribution in human disease has been established. miRNAs are ncRNAs 19-24 nucleotides(nt) long, and control gene expression by targeting the 3'UTR of mRNAs leading them to degradation or inhibiting translation. Alternatively, miRNAs stabilize mRNA molecules and lead to more efficient translation, thus positively affecting gene expression (3). Changes in intracellular miRNA expression have been causally associated with multiple diseases including cancer (4), neurodegenerative diseases (5), cardiovascular diseases (6) and more. Similar as well as distinct changes in miRNA expression pattern has been observed in the serum of patients, introducing detection of serum miRNAs as biomarkers of human diseases (7). MiRNAs are present in the serum in different configurations; they are transported inside exosomes, bound on lipoproteins such as LDL and HDL, and also bound on proteins such as Argonaut 2 (Ago2) (8).

All configurations allow miRNAs to be uptaken by distant tissues and cells, altering gene expression in the target cells. Initial studies were focusing in functionally relevant miRNAs but utilization of serum-specific miRNA arrays has led to the identification of additional diagnostic and prognostic miRNAs. Recent work from our group has shown that miRNA levels in the serum are potential markers linking different diseases. For example, changes in miR-155-5p, miR-200a-3p, miR122-5p and miR-200c-3p are common determinants of male subfertility and metabolic disease, suggesting potential

common causal events and mechanisms (9, 10). MiRNAs in the circulation can transmit information similar to that of cytokines, chemokines and hormones but with more specificity in targeting gene expression in the recipient cell. In the context of inflammation, expression of miRNAs such as miR-155-5p and let-7 family in the serum may modulate expression of their target genes such as SOCS1 or TLR4, thus limiting detrimental effects of inflammation or augmenting anti-pathogen responses from distant cells and organs (11-14).

piRNAs AS BIOMARKERS

PiRNAs are ncRNAs slightly larger than miRNAs having size of 24–30 nt and are characterized by their ability to bind to the PIWI subfamily of Argonaut family proteins which are involved in maintaining genome integrity in germline cells, primarily in those of the male (15, 16). PiRNAs are transcribed from genomic regions that contain transposable elements and other repetitive elements and is believed that their function is to suppress those. PiRNA/PIWI complexes bind on transposable elements and inhibit their mobilization either by cleavage of transposable element transcripts by PIWI proteins using a mechanism based on recognition of homology between piRNAs and transposable elements or through heterochromatin-mediated silencing of transposable element transcription. PiRNAs, even though they are not abundant, they utilize the 'ping-pong' amplification cycle based on PIWI proteins (such as PIWILI1, PIWIL1 and PIWIL4) resulting in generation of antisense molecules that repress transcripts. PIWI proteins are also involved in DNA methylation and piRNAs mediate epigenetic changes (17). PiRNAs are also expressed in additional cell types to germ cells including endothelial cells but their function remains to be elucidated (18). Recent reports have shown that piRNAs are present in biological fluids, primarily in the seminal fluid and their expression has been associated to fertility (19). Whether soluble piRNAs are signals to mediate information imprinting between cells is not known. Profiling of soluble piRNAs can provide functionally significant and, more importantly, cell-source specific biomarkers for diseases.

snoRNAs AS POTENTIAL BIOMARKERS

SnoRNAs is another family of ncRNAs of intermediate size ranging from 60 to 300 nucleotides. They are components of small nucleolar ribonucleoproteins (snoRNPs), which are complexes controlling sequence-specific 2' *O*-methylation and pseudouridylation of ribosomal RNA (rRNA). RRNAs are post-transcriptionally modified in the nucleolus facilitating rRNA folding and stability. SnoRNA sequences are responsible for targeting the assembled snoRNPs to a specific target (20). Recently snoRNAs have been detected in biological fluids and have been suggested to be useful as biomarkers (21, 22). Whether they possess functional significance in biofluids remains to be determined.

lncRNAs AS POTENTIAL BIOMARKERS

LncRNAs include a broad and heterogeneous family of non-coding RNAs defined by their size being over 200 nt long. LncRNAs are involved in a diverse array of biological processes. This family is the largest family of ncRNAs including the largest portion of the mammalian non-coding transcriptome. The main function of lncRNAs is regulation of gene expression. Thus, lncRNAs mediate epigenetic modifications of DNA by recruiting chromatin remodeling complexes to target genes and therefore controlling their temporal and spatial expression (23). Among the known functions of lncRNAs, one involves regulating chromatin accessibility through histone modification enzymes and RNA polymerases. Physiological processes such as X chromosome inactivation occurs by the X-inactivation

specific transcript *(XIST)* lncRNA which recruits the polycomb complex to silence the X chromosome from which it is transcribed. *TSIX,* another lncRNA, is transcribed from the opposite strand to *XIST* and regulates *XIST* levels during X-chromosome inactivation (24). In addition, multiple lncRNAs are expressed by imprinted loci, where they contribute in genetic imprinting (25). A distinct family of lncRNAs are lincRNAs, which are transcribed from intergenic regions. LincRNAs control transcription, such as the p53 regulated lincRNA, lincRNA p21, which is located near the p21 gene and suppresses transcription when p53 is activated upon DNA damage (26). LincRNAs do not only regulate expression of neighboring genes but also act on distant ones. Another family of lncRNAs is this including lncRNAs transcribed from ultraconserved regions (UCRs). UCRs are conserved between vertebrates and are thought to date from a very early period in evolution. Some UCRs overlap with coding exons, although it is believed that more than half of the UCRs do not encode proteins. The UCRs that are transcribed are termed T-UTRs (27). The length of UCRs ranges from 200 to 80 nucleotides while T-UCRs have an unspliced length of up to 2kb. Their function remains unknown and the expression pattern has not been determined in disease conditions to allow them to serve as biomarkers.

Table 1 ncRNAs, their function and use as biomarkers

Family of ncRNAs	Size (nucleotides)	Potential function	Used as biomarker
miRNAs	19–24	Regulation of mRNA expression	Yes, widely
piRNAs	26–31	Repression of transposons and DNA methylation in germ cells	Yes, limited
tiRNAs	17–18	Regulation of transcription	no
snoRNAs	60–300	Regulation of rRNAs	Yes, limited
PASRs	22–200	Unknown	no
TSSa-RNAs	20–90	Transcriptional regulation	no
PROMPTs	<200	Transcriptional regulation	no
lincRNAs	>200	Chromatin regulation	Yes, limited
T-UCRs	200-780	Regulation of miRNAs	no
Other lncRNAs	>200	Transcriptional and epigenetic regulation of gene expression	Yes, limited

OTHER ncRNAs AND THEIR POTENTIAL AS BIOMARKERS

Different ncRNAs have been shown to associate with transcriptional initiation sites such as the promoter-associated small RNAs (PASRs), promoter upstream transcripts (PROMPTs), the Transcription Start Site-associated RNAs (TSSRNAs) and transcription initiation RNAs (tiRNAs) (28,29). The biological role of these ncRNAs is not well characterized but it is believed that they also regulate transcription. Another family of ncRNAs is this of telomeric repeat-containing RNAs (TERRAs), which are transcribed from telomeres (30). TERRAs regulate telomerase function and secure maintenance of heterochromatin integrity (31).

CONCLUSIONS

Since the initial characterization of non-coding RNAs and their identification as determinants on human disease pathogenesis, a new area in the field of biomarkers has emerged. Thus, our knowledge on the differential expression of different families of ncRNAs as well as their contribution in tissue homeostasis will open a new field of biomarkers that could be measured both in tissues and in biological fluids, and support disease diagnosis and prediction. A bottleneck at the moment in the field is the availability of automated rapid detection methods of ncRNAs and the identification of the most important ones that will serve as biomarkers. A list of different types of ncRNAs, their size, function and whether are currently used as biomarkers is presented in Table 1.

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