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MINIREVIEW PAPER

THE NEW ERA OF NON-CODING RNAs: THE STATE OF ART AND FUTURE PERSPECTIVES IN ADVANCED MOLECULAR THERAPIES

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Abstract
Nowadays, thanks to deep sequencing technologies, it was discovered that the transcription of Eukaryotic genomes produces a huge variety of non-coding RNAs (ncRNAs). During last years, the idea of ncRNAs as "evolutionary junk" was substituted with a new hypothesis involving ncRNAs in several molecular mechanisms, from protein translation to the copying of DNA during cell replication. Several classes of small (20–30 nucleotides) and long (>200 nucleotides) non-coding RNAs have been validated as key regulators of gene expression in many processes ranging from embryonic development to innate immunity. In this review, we analyze the current scenario of the molecular mechanisms underlying the biogenesis and function of microRNAs (miRNAs), small interfering RNAs (siRNAs), Piwi – interacting RNAs (piRNAs), long non–coding RNAs (lncRNAs) and circular RNAs (circRNAs). Furthermore, we briefly exposed the relevance of small and long non-coding RNAs to human physio-pathology and their potential to be exploited as therapeutic agents.

Keywords: Non–coding RNAs, sncRNAs, miRNA, lncRNAs, circRNAs.

Introduction

Until last 10 years, the scientific community claimed that RNAs transcribed from human genome coded only for proteins. Nowadays, thanks to deep sequencing technologies and advances in bioinformatics, it was discovered that the transcription of Eukaryotic genomes produces a huge variety of RNA species, many of them without coding potential, referred to as non-coding RNAs (ncRNAs) (1). During past years, ncRNAs were considered as "evolutionary junk", but increasing evidence suggests a huge impact on several molecular mechanisms, from protein translation to the copying of DNA during cell replication (2). Moreover, the amount of ncRNAs in an organism correlates with its complexity, suggesting a relevant role on development and organization of higher structured animals (3). Specific biological functions of ncRNAs depend on their structure and length, which permit us to classify them in small non-coding RNAs (sncRNAs) and long non-coding RNAs (lncRNAs) (3). Aim of this review is to discuss the molecular mechanisms of non-coding RNAs, their involvement in gene expression regulation and in the onset and progression of diseases.

Small NON-CODING RNAs (sncRNAs)

Small ncRNAs act as guides for recognition of target RNAs, playing a key role in sequence – specific gene expression regulation (4). Their lengths vary between 20 and 30 nucleotides (nt) and result conserved throughout a wide range of organism (5). Small ncRNAs can be clusterized into three principal categories, based on their length, biogenesis, and target proteins: microRNAs (miRNAs), small interfering RNAs (siRNAs) and Piwi-interacting RNAs (piRNAs).

microRNAs

miRNA genes could be transcribed as polycistronic primary transcripts, from individual transcription units or could be embedded in the in-

tronic or exonic regions of host transcripts (6). Singular miRNAs can have hundreds of targets, while an individual target transcript may be regulated by several different miRNAs (7). miRNA biogenesis could begin in the nucleus from two different processes, generating precursor miRNAs (pre-miRNAs): 1) microprocessor complex (Drosha and DGCR8) RNase III enzyme processing of primary miRNA (pri-miRNAs) transcripts; 2) spliceosome processing of precursor mRNA (pre-mRNA) transcripts, followed by Lariat Debranching Enzyme (DBR1) processing of the resulting introns (7). Then pre – miRNAs exit from the nucleus using the karyopherin Exportin 5 and RAN – GTP and will be processed by the RNase III enzyme Dicer into the cytoplasm, producing ~25 nt mature miRNAs (8). Next step foresees the incorporation into the RNA – induced silencing complex (RISC), constituting the miRISC active complex (9). In this way, miRNAs bind the 3′ untranslated regions (3′-UTR) of target mRNAs and cleave them with the catalytic activity of Argonaute (AGO) family proteins, down – regulating target transcript expression by enhancing mRNA degradation, repressing protein translation, and/or sequestering target mRNAs to specific cellular compartments such as P bodies and stress granules (10). Curiously, a second biogenesis pathway is available for a subset of miRNAs, realized by the help of small nucleolar RNAs (snoRNAs), transfer RNAs (tRNAs) and group II introns via non-canonical mechanisms (11). Additionally, many evidences suggest that miRNA binding sites may also be present in the coding region and possibly the 5′ UTR of mRNAs (12). More than 60% of cellular mRNAs are thought to be regulated by miRNAs, and mutations or alterations involving them could determine the onset of many pathologies. For example, Fragile X syndrome (FXS) related proteins FXR1P and FXR2P form complexes with miRNA – sized nucleotides in control subject cells but not in FXS patients (13). Various miRNA pathway components resulted differentially expressed in multiple neoplasias (14), like non-small cell lung cancer (NSCLC) (15), and may be considered as potential biomarkers for those diseases (16). Moreover, many studies evidenced miRNAs involvement in psychiatric pathologies like schizophrenia (17), and other neurodegenerative diseases like Alzheimer's (18).

Small interfering RNAs

siRNAs are derived from long double-stranded precursor RNAs (dsRNAs) (19). Depending on the source of dsRNA precursor, siRNAs can be classified into exogenous and endogenous siRNAs (exo- and endo-siRNAs, respectively). Exo-siRNAs, generally ~21 nt long, derived from exogenous long dsRNAs from viruses, transgenes or exogenously supplied dsRNAs, and are involved in RNA silencing phenomena such as RNAi (20). Dicer cleaves dsRNA into siRNA duplexes, then loaded onto the RISC with an Argonaute protein and the guide strand of the siRNA duplexes as the core components, similar to that of miRISC, to direct the gene silencing (21). In animals, the exo-siRNAs regulate post-transcriptional modifications and antiviral defense (22). Thanks to their strong ability to silence target gene expression, exo-siRNAs are used to knock – down gene in order to identify gene functions in all eukaryotes, and are modified to silence gene expression that could determine etiopathogenesis of several human diseases (23). Endogenously produced-siRNAs (endo-siRNAs) are widely diffused in eukaryotes (24). The first mammalian endo-siRNA was characterized against one of the retrotransposons, L1, a long interspersed nuclear element (LINEs). Bidirectional transcription from the L1 loci is thought to yield dsRNAs that are processed into siRNAs by DCR-1 (25). Endo-siRNAs originate from long dsRNAs derived from transposon transcripts, from structured loci where the transcripts can fold into long stem hairpins or from hybridization between genic and pseudogenic transcripts (26). Although the exact biological role of most animal endo-siRNAs is not totally clear today, several studies showed that they could act silencing transposons (27). Such function is proved for a novel type of endogenous 26 nt RNA in C. elegans, RNA that acts as primary small RNA leading to the production of the transposon silencing and centromere function-related 22-nt RNAs (28).

Piwi-interacting RNAs (piRNAs)

One of the most uncharted small RNAs populations is represented by piwi – interacting RNAs (piRNAs). They are the longest known (24–29 nt) small ncRNAs and are found mainly in the animal germline cells (29). piRNAs show 3' 2′-O-methyl modification sites and are processed from single-stranded precursor transcripts expressed from intergenic regions, called piRNA clusters, via a Dicer-independent mechanism (30). The most of transposons in the piRNA cluster are bidirectionally transcribed (31). piRNA biogenesis could follow two distinct pathways: primary processing

takes place in both germline and somatic cells while secondary one, thanks to a feed-forward loop called the ping-pong amplification cycle, occurs only in germline cells (32). Moreover, piRNAs form specific RISCs, called piRISCAs, with PIWI subfamily proteins (PIWI proteins). Although PIWI proteins constitute the core of the piRNA biogenesis pathway, additional proteins, including RNA helicases, Tudor domain-containing proteins, and nucleases, also play a key role in the piRNA pathway (33). This scenario is confirmed by loss of function mutations in these genes, which often results in serious defects in gametogenesis and piRNA production (34). In details, several piRNA pathway components localize to nuage/chromatoid bodies, unique germline-specific structures found at the perinuclear region of germ cells (35). The most interestingly role established for piRNAs regards the ability to harbor a huge number of and different types of transposons, regulating their activity within the genome (36). Such skill results essential for protect physiological gametogenesis and reproduction, because it is widely known that transposition of transposons has a high risk of damaging the genome intracellularly (37). To highlight the fundamental role of piRNAs in safeguarding genomic integrity, mutations in genes encoding several piRNA pathway components impair fertility in animals (38). Curiously, piRNAs can be transmitted vertically through maternal inheritance, thereby providing an important defense against retrotransposons (39). A possible mechanism of piRNAs transposon regulation was proposed in analogy with immune systems "self" and "non – self" recognition, trying to select and regulate the "non – self" genes (29).

Long NON-CODING RNAs (lncRNAs)

Long non – coding RNAs (lncRNAs) are typically long more than 200 nt and show several features of typical protein-coding transcripts, such as a poly(A) tail, 5′ cap and introns (40). Several lncRNAs are located within intergenic sequences, but the most is transcribed as complex, interlaced networks of overlapping sense and antisense transcripts that often include protein-coding genes (3). LncRNAs have been involved in the regulation of various biological processes. lncRNAs could regulate transcription acting as cofactors for transcription factors, targeting transcriptional activators or repressors, regulating the association and activity of transcription factor coregulators or complexing with components of the transcriptional machinery (41). One example is given by regulation of the Cyclin D1 (CCND1) (42). lncRNAs may also regulate several post-transcriptional RNA processing aspects, such as pre-mRNA splicing (43), cytoplasmic transport (44), translation (45), and degradation, frequently involving base pairing between lncRNAs and the target mRNAs (46). One example is the lncRNA MALAT-1, which interacts and regulate the phosphorylation of various splicing (47). Recent studies have been highlighted the involvement of lncRNAs in mRNA translation control, especially in its (48). Recently, many lncRNAs have been implicated in epigenetic regulation of gene expression, as mammalian Hox genes (49). The expression of these lncRNAs is temporally and spatially regulated throughout human development, and characterize chromatin domains of differential histone methylation and accessibility by RNA polymerase (50). Among the most interesting lncRNA tasks, the contribute to cell cycle regulation and apoptosis plays a major role, as evidenced by the lncRNA Gas5 (growth arrest specific 5), which adapt the glucocorticoid activity in response to nutrient starvation, inducing cells to apoptosis (51). Moreover, very curiously, many imprinted genes are clustered on the chromosome and are often associated with lncRNAs, like the lncRNAs Kcnqot1 and Air (52). The expression of lncRNAs has been shown to be altered in numerous diseases such as various types of cancers (53), coronary and neurodegenerative diseases (54). In details, about cancer involvement, colorectal carcinoma (55), chronic lymphocytic leukemia (56), and hepatocellular carcinoma (57) show aberrant expression profiles for conserved lncRNAs, like the previously cited MALAT-1, compared to normal cells.

Circular RNAs (circRNAs)

Circular RNAs (circRNAs) were recently discovered as a particular novel type of endogenous non-coding RNA, and represent a new research hotspot in the field of RNA (58). Unlike linear RNAs that are terminated with 5′ caps and 3′ tails, circRNAs create covalently closed loop structures without 5′–3′ polarities and polyadenylated tails (59). Circular RNAs can originate from the direct ligation of 5′ and 3′ends of linear RNAs (CircRNAs), as intermediates in RNA processing reactions, or by "backsplicing," (CiRNAs) wherein a downstream 5′ splice site (splice donor) is joined to an upstream 3′ splice site (splice acceptor) (60). Circular RNAs show unique properties including

the ability to rearrange the order of genomic information, the potential for rolling circle amplification of RNA, protection from exonucleases, and constraints on RNA folding (61). Recent studies about Circular RNAs functions highlight their role as templates for viroid and viral replication, as intermediates in RNA processing reactions, as regulators of transcription in cis, as snoRNAs, and as miRNA sponges (62). In details, it was shown that some ciRNAs could enhance the transcription of the genes from which they are produced. CircRNAs, instead, can modulate miRNA activity, probably sequestering specific miRNA complexes and potentially by releasing them again when the circRNA is cleaved (63). Moreover, circRNAs production could regulate alternative splicing and "sponging" other factors, such as RNA-binding proteins or RNPs (64). Finally, it was shown that circRNAs could be used to bind and store components, to sort and deliver factors to particular subcellular locations, or as scaffolds for the assembly of other complexes or reactions (65). Recent studies have suggested that circRNAs may play important roles in the initiation and development of disease could potentially become new biomarkers for these processes. Among them, we remember prion disease (66), myotonic dystrophy (67), Parkinson disease (68), stress handling (69), brain development (70), and cellular proliferation (71). Additionally, circRNAs have also been described as a class of aging biomarkers (72).

Future perspective

Non – coding RNAs represent a new frontier towards medicine could focus and, even if the clinical utility of RNAi is not been established, a huge number of existing ongoing clinical trials could indicate the potential success in treatment of several metabolic diseases, cancer, liver fibrosis, viral infection, and inherited neurodegenerative diseases like Huntington disease, subsets of Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis (73). Thus, in a couple of years it will be possible that non-coding RNAs will serve as one of the most relevant target of personalized medicine, improving patient's quality of life and compliance.

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