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Review Article

Transcriptional R loops and their significance in neurodegenerative disease and possible elimination by specific carrier mediated microRNA techniques; importance for clinical therapeutics

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ABSTRACT

The transcription process has been shown to stimulate both mutation and recombination in many living beings from bacteria to mammals, but the mechanisms underlying this phenomenon are still largely unknown. A particularly intriguing and physiologically relevant intermediate causing transcription-associated genetic instability is the R-loop, a co-transcriptionally formed structure between the nascent mRNA and the template DNA that displaces the non-template single-strand DNA. R-loop formation impairs DNA replication and that this is responsible for the deleterious effects of R loops on genome stability in humans and may be responsible for neurodegenerative diseases. Novel approaches using the using specific gene delivery systems for miRNAs or MicroRNAs can prevent or remove R Loop formation.

Keywords: R Loop, drug, miRNA, DNA, transcription

INTRODUCTION:

A transcriptional R loop is a structure in which a nascent transcript is hybridized with the template DNA strand, leaving the nontemplate strand unpaired. Such RNA, DNA hybrids have been detected in organisms from bacteria to humans under various physiological and/or pathological [1]. R-loops constitute a novel trigger for genomic instability and the accumulation of these structures may represent an underlying and contributing mechanism in autosomal recessive ataxias characterised by defective responses to DNA damage. Accumulating evidences based on recent studies have shown correlations between transcription deregulation, defective RNA processing, genome instability and neurodegeneration [2,3]. R-loops occur in proliferating cells due to the presence of unrepaired DNA lesion that can stall the transcription machinery. In addition, the extent of R-loop accumulation can exacerbate genomic instability, trigger apoptosis and may be indicative of the fertility status, cancer pathogenesis and neurodegenerative state. There is new data that provides fresh evidence for genome destabilization as a consequence of disrupted transcription in the presence of DNA double strand breaks arising during DNA replication or recombination.

R loops and drug mechanisms: There is potential for R loops as targets for pharmacotherapeutics of various disorders. R loops have recently been linked with the molecular mechanism of a cancer drug, topotecan, that reactivates the expression of the imprinted silenced gene *Ube3a [4]*. Angelman syndrome (AS) is an autism-related disorder that is caused by mutations or deletions of the maternal copy of the *Ube3a* gene [5,6]. Normally, the neurons express only the maternal copy of this gene and silence the paternal copy via the *Ube3a* antisense transcript. So, *Ube3a* mutations in the maternal copy result in a complete loss of the protein, a brain-specific ubiquitin E3 ligase. *Ube3a* antisense is located immediately downstream from the *Snord116* gene, mutations of which cause a second disorder, Prader-Willi syndrome. The anti-cancer drug topotecan was found to reactivate the paternal copy of *Ube3a* by reducing the antisense *Ube3a* transcript in neurons and therefore could be potentially used to treat AS [7]. Even though topotecan holds promise for AS treatment, it still remains unknown how it targets specifically *Ube3a* and no other genes within this locus. Importantly, topotecan is an inhibitor of topoisomerase, which, as mentioned above, relaxes negative supercoiling. It is now revealed that R-loop formation plays a role in the topotecan effect [8]. In essence, R loops form over the G-rich *Snord116* gene, which in turn causes nucleosome depletion and chromatin decondensation in the paternal allele.

Transcriptional R loops and neurodegenerative disorders:

Transcription of very long genes has also been shown to cause replication/transcription collisions and

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accumulation of R loops at the common fragile sites (CFSs) [9]. Some of these genes have been shown to be down-regulated in neurological diseases, such as Alzheimer's disease [10]. Additionally, defects in DNA repair proteins which as are connected to R loop and cause neurodegenerative syndromes [11]. Defective DNA repair in mature neuronal tissues has also been linked to aging and neurodegenerative disorders, such as Parkinson's disease and Alzheimer's disease. Senataxin has recently been suggested to act as a DNA repair protein by resolving R loops at human genes. Senataxin, is a 300-kDa protein that has putative RNA/DNA helicase activity and interacts with RNA polymerase II [12]. Given that mutations in senataxin cause specific neurodegenerative disorders [13], perhaps senataxin, despite being a ubiquitously expressed protein, has a special role in neuronal genes by controlling the transcription of some fragile sites present in long genes. Altogether, these studies reveal a complex and coordinated network in neurons between transcription, DNA repair, and R loops. Indeed, the general physiological relevance of R loops as transcriptional regulators seems more and more likely. R loops are often associated with neurodegenerative disease caused by abnormal expansion of repeated DNA sequences i.e so-called repeat expansion disorders [14,15]. In recent studies, R loops were shown to form over the promoter of the *fragile X mental retardation 1* (*Fmr1*) gene and coincide with its epigenetic silencing in fragile X syndrome [16]. A similar mechanism has also recently been shown to occur in another trinucleotide repeat expansion disease, Friedriech's ataxia [17].

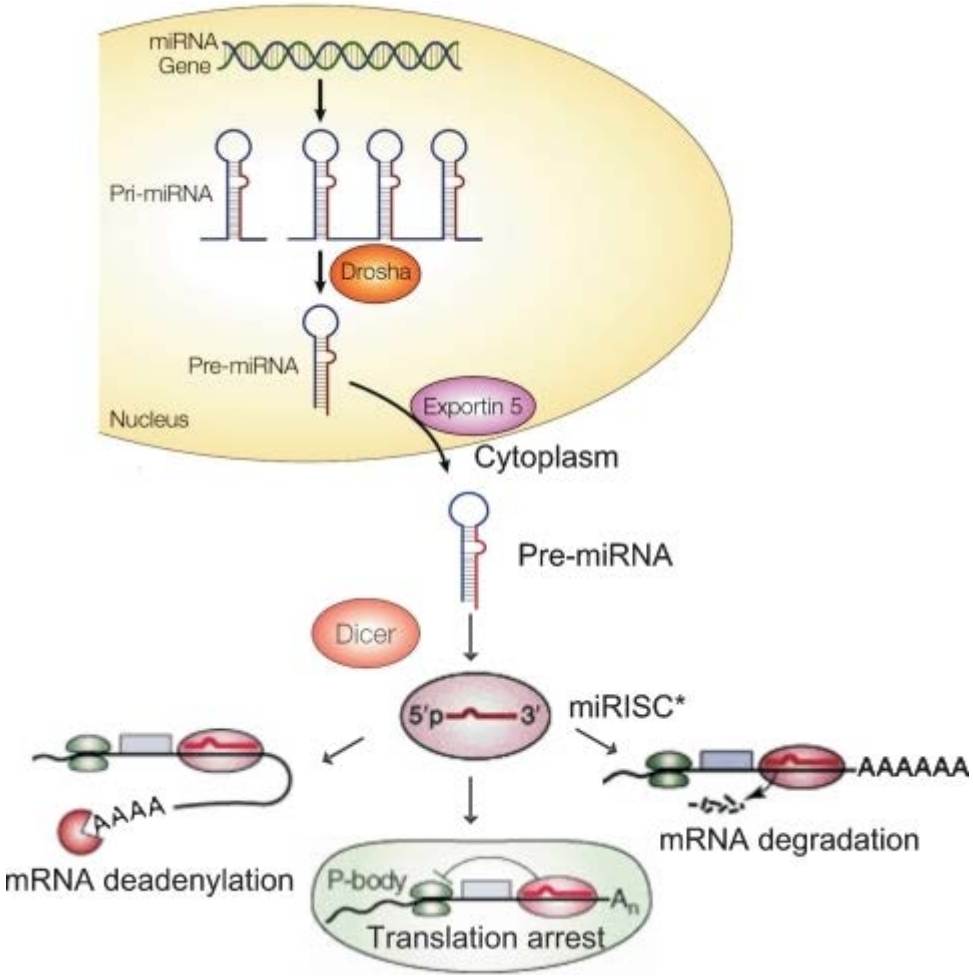

Figure 1: Courtesy: Pirkko Muhonen and Harry Holthofer, Epigenetic and microRNA-mediated regulation in diabetes. Nephrol Dial Transplant. 2009 Apr; 24(4): 1088–1096.

Targeting the R-loop formation using the microRNAs, a novel concept and hypothesis:

The discovery of the abundance of miRNAs in several multicellular species has raised multiple questions, including, perhaps most intriguingly, what these tiny noncoding RNAs may be doing for the cell function. Deep-sequencing technologies has greatly accelerated miRNA discovery and, till date, over 1500 mature miRNAs have

been identified in the human genome, many of which are highly conserved among species [18]. MiRNA gene regulatory networks are highly complex, as each specific miRNA can target up to several hundred distinct molecules of mRNA, and each mRNA can be targeted by many different miRNAs, which individually may have multiple target sites in the specific mRNA [19]. Although predictions are challenging and every algorithm uses a different concept, some general items can be found. (a) miRNA-mRNA interaction requires conserved Watson-Crick pairing to the 5' region of the miRNA centered on nucleotides 2–7, which is called the miRNA "seed" and markedly reduced the occurrence of false-positive predictions. (b) Conserved pairing to the seed region can be sufficient on its own for predicting conserved targets above the noise of false-positive predictions. (c) Highly conserved miRNAs have several conserved targets. These guidelines are not yet complete although other features have been discovered that might boost site predication efficacy in the future, including (d) positioning of the miRNA within the 3'UTR at least 15nt from the stop codon, (e) positioning away from the center of long UTRs, (f) AU-rich nucleotide composition near the site or other measures of site accessibility, and (g) proximity to sites for coexpressed miRNAs. Although the drawbacks due to the lack of knowledge of miRNA target selection, many researchers were successful in identification of true experimental validated targets, mostly *in vitro.* miRNAs have a high degree of sequence complementarity, then target mRNA degradation processes are facilitated through Ago protein slicer activity. The fact that messenger RNA (mRNAs) are also reduced with an abundance of miRNAs suggests that miRNAs are responsible for mRNA degradation processes [20]. Recent studies have suggested that not only the Ago-catalyzed mRNA degradation process is responsible for the mRNA degradation, but other mechanisms such as deadenylation, decapping, and exonucleolytic digestion of mRNA are also involved. mRNA degradation by miRNA requires Ago, GW182, and the cellular decapping and deadenylation machinery[21]. The exact procedure of target selection has yet to be determined by more comprehensive experimental studies. However, it has been shown that the number, type, and position of mismatches in the miRNA/mRNA duplex play a critical role in the selection of the degradation or translational repression mechanisms [22]. Because miRNAs are naturally occurring molecules, there are certain advantages in their application as therapeutic agents. Using specific gene delivery systems for e.g dendrimers and disialoganglioside targeting silica nano particles for miRNAs it is possible to cause the degradation of the R loop formation and alleviation and substantial cure of many a neurological based diseases which may have the R loop accumulation as a predisposing aetiopathological factor (Refer Fig.1). The specific miRNAs and gene delivery systems need to be ascertained and delineated.

CONCLUSION:

R loops are thought to play a role in neurodegenerative disorders even though strong evidence for this association has yet to be established. R loops could possibly be involved in regulation of transcription, and it is now the time to unravel their possible links with cancer and neurodegenerative disease. From the studies conducted in recent years, it is evident that R loops lie at the interphase of different fields: transcription, RNA processing, DNA damage, and chromatin. A specialized microRNA based technique to prevent or remove the R loops can be beneficial in clinical therapeutics. The pioneering groups of specialized pharmaceutical companies have initiated studies on creating viable therapeutic candidates with miRNA inhibitors and miRNA mimetics in diverse fields such as cancer, cardiovascular diseases, neurological disorders, and viral infections. MiRNAs are making their way in the pharmaceutical industry as therapeutic and diagnostic targets, and may hopefully target transcriptional R-Loops as part of the strategy.

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