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Review

Emerging fundamental roles for non-coding RNA species in toxicology

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Abstract

microRNAs (miRNAs) are a large family of small regulatory RNA molecules found in all multicellular organisms. Since their discovery in 2001, there has been impressive progress in miRNA research, and a great deal is now known about the biosynthesis of miRNAs and their regulatory role in translation. It is becoming increasingly clear that miRNAs have fundamental roles to play in cellular responses to xenobiotic stress, the development of pathophysiological changes and other toxicological phenomenon such as susceptibility and resistance. Furthermore, the expression of miRNAs, like many of the genes important in toxicology, can be regulated by xenobiotics and DNA methylation. In this article we review the present understanding of the miRNA field with particular reference to toxicology. We also give an insight into our current projects within this exciting area and highlight some of the new challenges that now face miRNA research.

Keywords: microRNA; Toxicology; mRNA translation; Non-coding RNA

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1. What are miRNAs?

microRNAs (miRNAs) are short (18–26 nucleotide) RNA molecules that play an important role in translational regulation. They are endogenously produced and their biosynthesis involves a number of processing steps (Fig. 1). Long primary miRNA (pri-miRNA) molecules are transcribed from non-coding polycistronic regions of the genome under the control of RNA polymerase II (Pol II) promoters (Zhou et al., 2007). These pri-miRNA transcripts can exceed 1 kb and may give rise to a number of different miRNAs (Du and Zamore, 2005). The nuclear ribonuclease III (RNase III) Drosha, together with its double-stranded RNA-binding domain (dsRBD) protein partner DGCR8, cleave the pri-miRNA to yield a 70–100 nt stem loop precursor miRNA (pre-miRNA) molecule (Du and Zamore, 2005; Kosik, 2006). The pre-miRNA is then exported from the nucleus into the cytosol by Exportin5, and is cleaved by a second RNase III Dicer and its dsRBD protein partner TRBP to produce a mature miRNA duplex (Du and Zamore, 2005; Kosik, 2006). The duplex unwinds and the guide miRNA strand is incorporated into the RNA-induced silencing complex (RISC), where it

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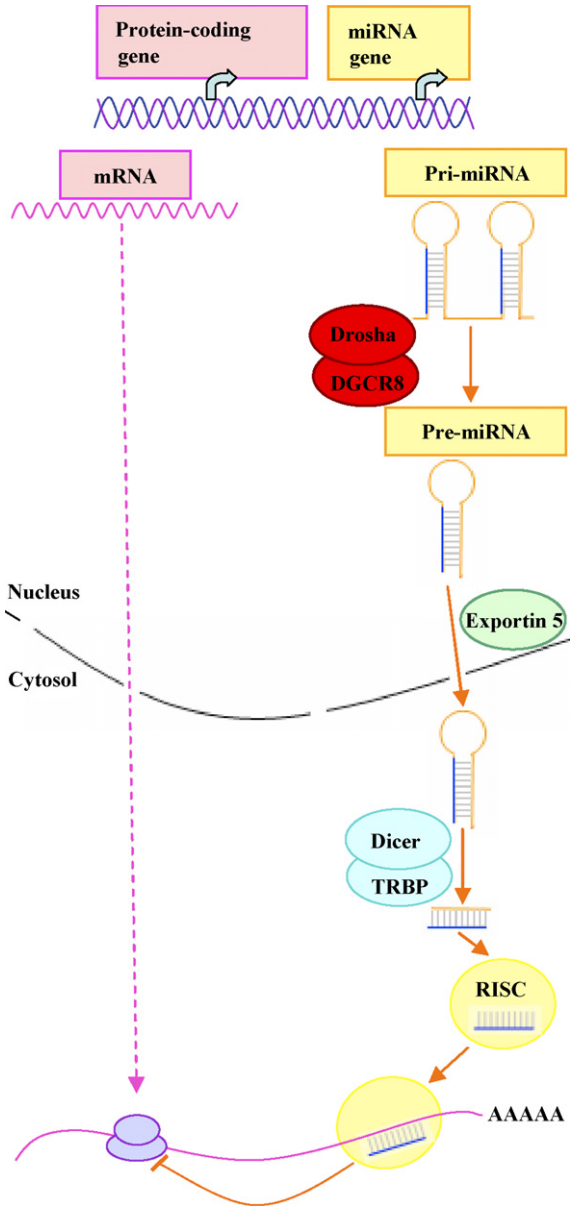

Fig. 1. miRNA biosysnthesis and function. miRNAs are transcribed as long pri-miRNA transcripts which undergo two processing steps to produce the mature miRNA duplex. The guide strand is incorporated into the RISC complex where it binds to, and inhibits the translation of, its target mRNA.

binds to the 3′UTR of, and silences, its target miRNA (Du and Zamore, 2005; Kosik, 2006). This silencing can result in degradation or repressed translation of the target mRNA, and probably depends on the degree of complementarity between the miRNA and its target (Kosik, 2006; Meister, 2007). The majority of miRNAs in animals exhibit only partial complementarity to their mRNA targets and so are thought to function predominantly by repressing target translation, most likely through the inhibition of translational initiation (Meister, 2007). The exact mechanism of miRNA-mediated translation repression, however, is beyond the scope of this article and is reviewed in Meister (2007) and Rana (2007).

2. microRNAs and toxicology

It is now clear that miRNAs have a fundamental role in normal development and in disease pathology, particularly cancer. The fact that miRNA transcription involves pol II promoters, which often contain toxicologically significant enhancer regions, infers that miRNAs are also likely to be of crucial importance in cellular responses to xenobiotics. Indeed, miRNA expression in *Drosophila* and *C. elegans* can be regulated by enhancers or hormones (Brennecke et al., 2003; Johnson et al., 2003; Sempere et al., 2003), and various chemotherapeutics and chemicals such as ethanol can control the expression of miRNAs in rodents and humans (Meng et al., 2006; Saito et al., 2006; Pogribny et al., 2007; Rossi et al., 2007; Sathyan et al., 2007). More recently, xenobiotic-mediated miRNA expression has been directly linked with downstream protein expression and cell proliferation in mice (Shah et al., 2007). The peroxisome proliferator-activated receptor alpha (PPARα) agonist Wy-14,643 down-regulates the expression of let-7C, which in turn reduces let-7C-mediated repression of *c-myc* translation. This increases *c-myc*-induced expression of the proto-oncogenic miR-17-92 cluster, resulting in hepatocyte proliferation (Shah et al., 2007).

There is also evidence for epigenetic modulation of miRNA expression and for miRNA-mediated epigenetic alterations. Both DNA methylation and histone modification can affect the expression of a number of miRNAs, and aberrant DNA hypermethylation of miRNAs is being increasingly identified in cancer cells (Saito et al., 2006; Brueckner et al., 2007; Lujambio and Esteller, 2007; Lujambio et al., 2007; Meng et al., 2007; Weber et al., 2007). Furthermore, miRNAs themselves appear to play a role in epigenetic modification. The miRNA-29 family reverses aberrant methylation in non-small-cell lung cancer through a direct reduction of DNA methyltransferase (DNMT) 3A and 3B mRNA levels, which leads to decreased expression of the DNMT proteins, reduced global DNA methylation, increased expression of tumour suppressor genes and inhibition of tumour cell growth (Fabbri et al., 2007). In addition, miRNA-140 directly represses histone deacetylase 4 expression (Tuddenham et al., 2006) and may thus regulate chromatin structure. The potential of miRNAs to influence epigenetic changes is supported by the fact that the closely related small interfering RNAs (siRNAs) are also involved in DNA methylation and histone modifications (Chuang and Jones, 2007).

Many miRNA sequences occur within the introns of protein coding genes (Chuang and Jones, 2007; Saetrom et al., 2007). Biosynthesis of such intronic miRNAs relies on pol II transcription and correct splicing of their host genes. This implies that intronic miRNAs are co-regulated with their host genes (Chuang and Jones, 2007) and can thus be under the control of similar enhancer and epigenetic regions as their host genes and non-intronic miRNAs. Furthermore, co-regulation with a miRNA may provide a simple mechanism for a gene to down-regulate other genes (Saetrom et al., 2007). It also remains possible that intronic miRNAs have their own promoters and the discovery of CpG islands within introns suggests that even intronic miRNAs with their own promoters could still be regulated by DNA methylation (Chuang and Jones, 2007).