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The microrna 29 family and its regulation

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ARTICLE INFO

DOI:10.46223/HCMCOUJS.tech.en.8.1.332.2018

Received: February 6th, 2018

Revised: March 14th, 2018

Accepted: April 16th, 2018

Keywords:

growth factors, microRNAs, MiR-29 family, transcription factors

ABSTRACT

Since miRNAs have broad effects on tissue homeostasis and disease development, it is particularly interesting to work out how miRNAs themselves are being regulated. Such data could provide crucial information for further understanding the mechanism underlying disease development and for being able to manipulate these miRNAs therapeutically. Generally, the expression of miRNAs can be regulated transcriptionally, epigenetically, or controlled by different stimuli e.g., cytokines and growth factors. In review, just transcription factors, cytokines, and growth factors controlling the miR-29 family expression in human diseases were for the first time investigated.

1. Introduction

MicroRNAs are an abundant class of evolutionarily conserved, short (~22nt long), single-stranded RNA molecules that have recently emerged as important post-transcriptional regulators of gene expression by binding to specific sequences within a target mRNA (Ambros, 2004; Bartel, 2004). To date, 1424 miRNAs have been identified in human cells and each was predicted to regulate several target genes (Kozomara & Griffiths-Jones, 2011; Lim et al., 2005). Computational predictions indicate that more than 50% of all human protein-coding genes are potentially regulated by miRNAs (Friedman, Farh, Burge, & Bartel, 2009; Lewis, Burge, & Bartel, 2005). The abundance of mature miRNAs varies extensively from as few as ten to more than 80,000 copies in a single cell, which provides a high degree of flexibility in the regulation of gene expression (Chen et al., 2005; Suomi et al., 2008). The regulation exerted by miRNAs is reversible, as feedback/forward regulatory loops have been shown to exert modifying effects during translation (Inui, Martello, & Piccolo, 2010).

2. MicroRNA biogenesis

Despite the obvious differences between the biology of miRNAs and mRNAs, all available evidence suggests that these transcripts share common mechanisms of transcriptional regulation. Generally, the generation of a miRNA is a multi-step process that starts in the nucleus and finishes in the cytoplasm (Lee, Jeon, Lee, Kim, & Kim, 2002). Firstly, miRNAs are transcribed by the RNA polymerase II complex (Lee et al., 2004) and subsequently capped, polyadenylated, and spliced (X. Cai, Hagedorn, & Cullen, 2004). Transcription results in a primary miRNA transcript (prior-miRNA) that harbors a hairpin structure (Kim, 2005; Lee et al., 2002). Within the nucleus, the RNAse II-type molecule Drosha and its cofactor DGCR8

process the pri-miRNAs into 70- to 100-nt-long pre-miRNA structures (Han et al., 2004; Lee et al., 2003), which in turn are exported to the cytoplasm through the nuclear pores by Exportin-5 (Bohnsack, Czaplinski, & Gorlich, 2004; Lund, Güttinger, Calado, Dahlberg, & Kutay, 2004; Yi, Qin, Macara, & Cullen, 2003; Zeng & Cullen, 2004). Subsequently, the RNAse III-type protein Dicer generates a double-stranded short RNA in the cytoplasm that consists of the leading-strand miRNA and its complementary sequence (Chendrimada et al., 2005; Grishok et al., 2001; Hutvagner et al., 2001; Ketting et al., 2001). This duplex miRNA is unwound by a helicase into a single-stranded short RNA in the cytoplasm and the leading strand is incorporated into the argonaute protein (Ago 2)-containing ribonucleoprotein complex known as the miRNA-induced silencing complex, mRISC (Bossé & Simard, 2010; Hammond, Bernstein, Beach, & Hannon, 2000; Hutvagner & Simard, 2008). During this process, one strand of the miRNA duplex is selected as the guide miRNA and remains stably associated with mRISC, while the other strand, known as the passenger strand is rapidly removed and degraded (Martinez, Patkaniowska, Urlaub, Lührmann, & Tuschl, 2002). The selection of the appropriate strand is primarily determined by the strength of base pairing at the ends of the miRNA duplex. The strand with less-stable base pairing at its 5' end is usually destined to become the mature miRNA (Hutvagner, 2005; Khvorova, Reynolds, & Jayasena, 2003; Schwarz et al., 2003). However, some miRNA passenger strands are thought to negatively regulate gene expression. One hypothesis is that both strands could be used differently in response to extracellular or intracellular cues, to regulate a more diverse set of protein-encoding genes as needed, or strand selection could be tissue-specific (Ro, Park, Young, Sanders, & Yan, 2007). The mature miRNA guides the RISC complex to the 3'UTR of its target miRNA (Bartel, 2009; Lai, 2002). The seed sequence, comprising nucleotides 2-8 at 5'-end of the mature miRNA, is important for binding the miRNA to its target site in the mRNA (Lewis et al., 2005). Association of miRNA with its target results in mRNA cleavage (if sequence complementarity is high) or more usually in higher eukaryotes, blockade of translation (Zeng & Cullen, 2004) (see below).

In an alternative pathway for miRNA biogenesis, short hairpin introns termed mirtrons are spliced and debranched to generate pre-miRNA hairpin mimics (Berezikov, Chung, Willis, Cuppen, & Lai, 2007; Okamura, Hagen, Duan, Tyler, & Lai, 2007; Ruby, Jan, & Bartel, 2007; Sibley et al., 2012; Westholm & Lai, 2011). These are then cleaved by Dicer in the cytoplasm and incorporated into typical miRNA silencing complexes (Berezikov et al., 2007; Okamura et al., 2007; Ruby et al., 2007; Sibley et al., 2012; Westholm & Lai, 2011). The presence of mirtrons may be an evolutionary strategy to diversify miRNA-based gene silencing (Lau & MacRae, 2009).

3. MicroRNA 29 family genome

The miR-29 family is intergenic miRNAs encoded in two gene clusters e.g., one for the primary miR-29a/b1 on chr.7q32, and the other for the primary miR-29b2/c on chr.1q32.2 (Chang et al., 2008; Saini, Griffiths-Jones, & Enright, 2007). The miR-29b1 and miR-29a precursors are processed from the pri-miR-29a/b1 last intron (Genbank accession EU154353) whilst the miR-29b2 and miR-29c precursors are from the pri-miR-29b2/c last exon (Genbank accession EU154352 and EU154351) (Chang et al., 2008) (Figure 1).

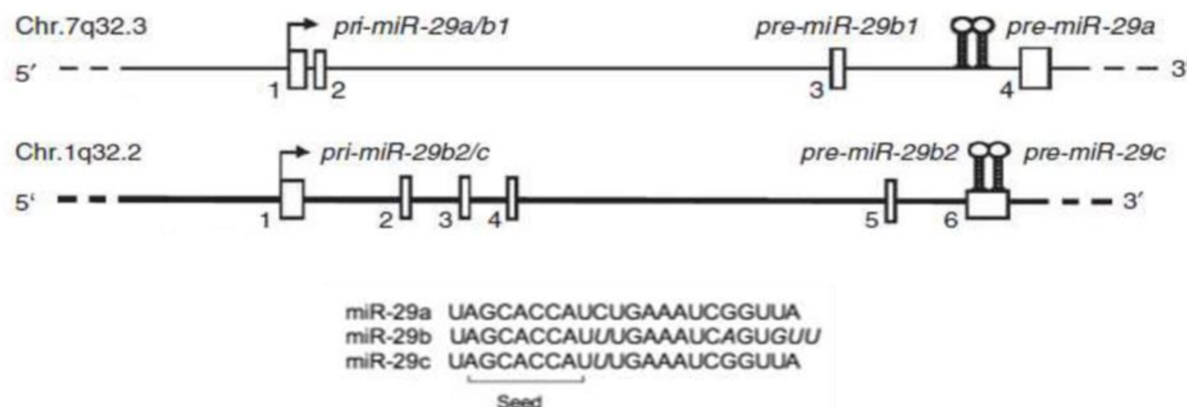

Figure 1. Genomic organization of the miR-29 family

The miR-29 family includes three members miR-29a, miR-29b and miR-29c. The primary pri-29a/b1 is located in chromosome 7 containing pre-29a and pre-29b1. The primary pri-29b2/c is located in chromosome 1 including pre-29b2 and pre-29c. The hairpins indicate the locations of the sequence encoding precursors of miR-29s. Pre-29a and pre-29c will process into mature miR-29a and miR-29c, respectively. Pre-29b1 and pre-29b2 will process into mature miR29b. The mature sequences of the miR-29 family members share identical seed regions. Nucleotides that differ among miR-29s are indicated in italics.

4. Transcriptional regulation of miR-29 expression

The miR-29 family precursors are all transcribed as polycistronic primary transcripts (Chang et al., 2008; Mott et al., 2010) upon which various transcriptional regulators e.g., NFκB (Liu et al., 2010; Mott et al., 2010), suppressors (Mott et al., 2010; Parpart et al., 2014), Sp1(Amodio et al., 2012; Liu et al., 2010), Gli (Mott et al., 2010), Yin-Yang-1, Smad3 (Qin et al., 2011), Ezh, H3K27, HDAC1, HDAC3), or inducers (Gli, SRF, Mef2, TCF/LEF, GATA3 (Chou et al., 2013), CEBPA (Eyholzer, Schmid, Wilkens, Mueller, & Pabst, 2010)), and signaling pathways e.g, Wnt, TGFβ, TLR/NFκB, TNFα/NFκB, hedgehog signalling have been reported to be directly and/or indirectly involved.

5. Wnt signalling

Both canonical and non-canonical Wnt signaling was reported to induce the miR-29 family level in different cellular contexts: Wnt3a rapidly induces miR-29 levels in osteoblastic cells (Kapinas, Kessler, & Delany, 2009; Kapinas, Kessler, Ricks, Gronowicz, & Delany, 2010) or in muscle progenitor cells (MPCs) (Hu et al., 2014), respectively, at least in part through the two putative TCF/LEF-binding sites in the miR-29a promoter (Kapinas et al., 2010); non-canonical Wnt signaling through Wnt7a/Frizzled 9 resulted in increased expression of only the mature miR-29b but not miR-29a or c or any miR-29b primary or precursor forms in non-small lung cancer cell lines H661 and H15 (Avasarala et al., 2013). In addition, ERK5 and PPARγ, key effectors of the Wnt7a/Frizzled 9 pathway, were also observed to be strong inducers of miR-29b expression (Avasarala et al., 2013).

6. TGFβ/Smad3 signalling

In contrast to Wnt signaling, TGFβ/Smad3 signaling was shown to negatively regulate miR-29 family expression in different cell lines e.g., human aortic adventitial different cell lines e.g., human aortic adventitial fibroblasts (Maegdefessel et al., 2012), renal fibrosis cells (Ramdas, McBride, Denby, & Baker, 2013; B. Wang et al., 2012), murine hepatic stellate cells (Roderburg et al., 2011), rat hepatic stellate cells (Kwiecinski et al., 2011), human skin fibroblasts (Maurer et al., 2010), human tenon's fibroblast (Li, Cui, Duan, Chen, & Fan, 2012), human lung fibroblast cell line (Cushing et al., 2011; Yang et al., 2013). The suppressive effect of TGFβ/Smad3 signaling on miR-29 expression was partly mediated through a Smad3 binding site in the highly conserved region around 22kb upstream of the miR-29b2/c promoter as showed by chromatin immunoprecipitation assay (Qin et al., 2011; Ramdas et al., 2013).

7. Toll-like receptor (TLR) and TNFα signallings

Similar to TGFβ, Toll-like receptor (TLR) signaling and TNFα signaling have been shown to mediate suppressive effects on miR-29 family expression. In man, treating human cholangiocarcinoma cells with TLR ligands e.g., TLR3 (Poly (I: C)), TLR4 (LPS), TLR5 (flagellin), TLR6 (MALP-2) showed a significant decrease in the miR-29 level beginning after 4 hours of LPS treatment but increasing to 24 hours (Mott et al., 2010); treating human stellate cells with LPS strongly decreased all miR-29 family expression after 1 hour (Roderburg et al., 2011); treating C2C12 myoblasts with TNFα substantially reduced miR-29b and miR-29c expression (H. Wang et al., 2008); stimulating the choroidal-retinal pigment epithelial cell line ARPE-19 with TNFα resulted in significant down regulation of all miR-29s; conversely, transfecting with a synthetic NFκB decoy, (NFκB inhibitor), rescued the downregulation of miR-29 by TNFα (J. Cai et al., 2014). The activation of NFκB through TLR signalling with its three binding sites in the miR-29a/b1 cluster promoter (-561, -110, and +134) was proven to be the mechanism for the suppression of miR-29a/b1 promoter function (Mott et al., 2010). In mice, miR-29a and miR-29b significantly decreased expression in murine natural killer (NK) cells stimulated with the TLR3 ligand (Poly (I:C)) or phorbol ester (PMA) as well as in splenocytes, NK and T cells of mice infected with *L. monocytogenes* or *Mycobacterium bovis* bacillus Calmette-Guérin (Ma et al., 2011). Consistent with the human miRNA, a region about 25 kb upstream of the murine promoter of miR-29a/b1 contains two NFκB binding sites. The second binding site is more conserved between humans and mouse and it has been shown to be key for suppression of the miR-29a/b1 promoter (Ma et al., 2011).

Importantly, other transcriptional factors, co-operating with NFκB to suppress or induce miR-29 family expression, have also been reported e.g., YY1, Sp1, Ezh, H3K27, HDAC1, HADC3, Mef2, SFR. Forced expression of YY1 in C2C12 lead to a 2-fold decrease of miR-29b and miR-29c levels; similarly, siRNA knockdown of YY1 significantly enhanced expression of miRNA expression. ChIP analysis showed that YY1 did not bind to the miR-29b2/c locus in cells in the absence of NFκB, suggesting that both pathways are necessary for silencing the miR-29b2/c locus. Amongst 4 putative binding sites of YY1 in highly a conserved region ~20kb upstream of miR-29b2/c, only one site is bound by YY1 on ChIP assay whereas all 4 sites produced a binding complex with EMSAs using nucleus extract from C2C12. Notably, Ezh, H3K27, HDAC1, whose expression is associated with repression of muscle-

specific genes, and recruited by YY1, was also detected by ChIP assay. In the line of these transcription factors, Mef2 and SFR, well-known for activating muscle genes, were also found to bind to the miR-29b2/c promoter. Again using luciferase reporter assay, a reporter containing a 4.5 kb fragment spanning YY1, Mef2, SFR binding sites was repressed by YY1 or loss of the YY1 binding site but stimulated with either YY1 knockdown or SRF or Mef2 (H. Wang et al., 2008). In addition, forced expression of Sp1 or NFκB (p65) reduced miR-29b expression; conversely, knockdown of Sp1 or NFκB (p65) by siRNAs resulted in induced miR-29b level (Liu et al., 2010). EMSA assay using probes spanning the -125/-75 miR-29b sequence yielded two major complexes, suggesting Sp1/NFκB acts as a repressive complex interacting with an element of the miR-29b enhancer (Liu et al., 2010). Interestingly, histone deacetylase (HDAC) 1 and 3 contribute to the repressor activity of Sp1/NFκB on miR-29b expression (Liu et al., 2010). Incubation of HDAC1/HDAC3 with the 32P-labelled probe from the miR-29a/b1 cluster region together with NFκB p50/p65 and Sp1 showed a delayed and more intense band; HDAC1/3 inhibitors increase miR-29b expression, supporting the interaction of HDAC1 and 3 and Sp1/NFκB with the miR-29b regulatory sequence (Liu et al., 2010).

8. Hedgehog signalling

Similar to other signaling mentioned previously, the hedgehog signaling pathway was also shown to repress miR-29 expression: cells treated with cyclopamine, an inhibitor of Smoothened (a hedgehog signaling component), or transfected with siRNA to knockdown Gli-3, the expression of miR-29b increased (Mott et al., 2010). Along with the transcription factors mentioned above, there are other transcriptional factors controlling miR-29 family expression. The serum alpha-fetoprotein (AFP), a membrane-secreted protein associated with poor patient outcome in hepatocellular carcinoma, was reported to inhibit miR-29a expression through facilitating c-MYC binding to the promoter of the pri-miR-29a/b. This conclusion was supported by the inability of AFP to decrease the miR-29a level in the absence of c-MYC protein; c-MYC was abundantly bound to the miR-29a/b1 promoter in the presence of AFP, but did not bind without AFP (Parpart et al., 2014); c-MYC promoter binding protein (MBP), originally described to bind to and repress c-MYC promoter function, up-regulated miR-29b expression by 6 fold in prostate cancer cells (Steele, Mott, & Ray, 2010). The hematopoietic master transcription factor, CCAAT/enhancer-binding protein-α (CEBPA), was also reported to activate the expression of miR-29a and miR-29b. Forced expression of CEBPA in acute myeloid leukaemic cells leads to two-fold induced expression of the primary miR-29a/b1 and the mature miR-29a and miR-29b whereas the expression of miR-29b2/c primary transcript remained stable. Using luciferase reporter assays, the sequence, having the conserved region spanning -682 bp upstream to +296 bp downstream of the miR-29a/b1 transcriptional start site and containing 6 potential CEBPA sites, was also strongly induced with CEBPA. Among these binding sites, the one located at +15 to +29 bp was identified to be responsible for CEBPA-mediated activation of the pri-miR-29a/b1 promoter on ChIP assay (Eyholzer et al., 2010). Another transcriptional factor, GATA3, specifying and maintaining luminal epithelial cell differentiation in the mammary gland, was also found to induce miR-29 expression directly by binding to three GATA3 sites in the miR-29a/b1 promoter. Interestingly, GATA3 can induce miR-29s expression by inhibiting the TGFβ and NFκB signaling pathways. Additionally, STAT1 (signal transducer and activator of transcription) a transcription factor induced by

interferon γ signalling, was reported to upregulate primary 29a/b1, the pre-29a, pre-29b1, and the mature miR-29a, miR-29b in melanoma cell and T cells (Schmitt, Margue, Behrmann, & Kreis, 2013).

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