RIC

RINCÓN DEL RESIDENTE

The microRNA biogenesis machinery: regulation by steroid hormones and alterations in cancer

Ramiro José González-Duarte*, Verna Cázares-Ordoñez*, Euclides Ávila-Chávez*

* Departamento de Biología de la Reproducción, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán.

ABSTRACT

MicroRNAs are a class of non-coding RNAs that regulate gene expression at the post-transcriptional level. The major proteins of the canonical microRNA biogenesis pathway in human are: Drosha, DGCR8, DDX5, DDX17, Exportin 5, Dicer and Argonaute 2. Recent studies suggest that gene expression of some canonical microRNA biogenesis components could be regulated by steroid hormones. Furthermore, various alterations in microRNA biogenesis have been associated with diseases like cancer. Due to the importance of microRNAs in cell physiology, the study of the factors that regulate or affect their biogenesis is critical.

Key words. microRNA biogenesis. Steroid hormones. Gene expression. Post-transcriptional regulation. Cancer.

La maquinaria de biogénesis de los microRNAs: regulación por hormonas esteroideas y alteraciones en el cáncer

RESUMEN

Los microRNAs son una clase de RNAs no codificantes que regulan la expresión génica en la etapa postranscripcional. Las principales proteínas de la vía canónica de biogénesis de los microRNAs en el humano son: Drosha, DGCR8, DDX5, DDX17, Exportina 5, Dicer y Argonauta 2. Estudios recientes sugieren que la expresión génica de algunos componentes de la biogénesis de los microRNAs podría ser blanco de las hormonas esteroideas. Por otro lado, diversas alteraciones en la biogénesis de los microRNAs han sido asociadas a padecimientos como el cáncer. Dada la importancia de los microRNAs en la fisiología celular, el estudio de los factores que regulan o afectan su biogénesis es fundamental.

Palabras clave. *Biogénesis de microRNAs. Hormonas esteroideas. Expresión génica. Regulación postranscripcional. Cáncer.*

INTRODUCTION

One of the most meaningful advances in modern cell biology has been the discovery of small non-coding RNAs (~20-30 nucleotides) as genome regulators in both plants and animals.[1] In general, the effects of those non-coding RNAs on gene expression are inhibitory; therefore, they are considered part of RNA silencing. To exert their regulatory functions, small non-coding RNAs associate with proteins to produce the inhibitory effect on gene expression. In other words, the RNA molecules act as factors of specificity that direct bound effector proteins to target nucleic acids. So far, until today, there are three major classes of small non-coding RNAs described in eukaryotes: short interfering RNAs (siRNAs), microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs).[2] The microRNAs, mainly, represent the best characterized class of small RNAs and growing evidence supports their relevance in cell physiology. The microRNAs were discovered through genetic studies in nematode worm *Caenorhabditis elegans* as endogenous regulators for developmental timing.[3,4] Later studies showed that plants and animals also require those RNAs as regulators in diverse physiological processes. In fact, it has been proposed that in humans, the microRNAs might regulate over half of the genome.[5] Regulation of microRNA bio-

genesis is an important issue but has not been extensively studied. However, recent findings suggest that a complex network of interacting factors is involved in the regulation process. This review focuses on the regulation of microRNA biogenesis by steroid hormones, and also show information about alterations on microRNA biogenesis involved in cancer development.

MicroRNA biogenesis

The canonical pathway of microRNA biogenesis begins in the nucleus with the transcription, usually by RNA polymerase II, of long primary microRNAs (~1,000 nucleotides) that need to be sequentially processed to generate mature microRNAs (Figure 1). At the end of the process, the RNA Induced Silencing Complex (RISC) associates with the mature microRNAs to repress the translation of RNA messengers into proteins.[6] It is important to note that the interaction between microRNAs and target messenger RNAs does not require total complementarity among the bases of both nucleic acids. Therefore one microRNA could regulate several messenger RNAs and, on the other hand, one messenger RNA can be regulated by different microRNAs. This implicates a high complexity on the study of gene expression regulation by microRNAs.[7]

Interestingly, characterization of the interaction between microRNAs and target messenger RNAs has provided the basis for the development of computational programs to predict associations between microRNAs and their potential targets.[8] However, experimental validation *(in vitro* and *in vivo)* is always necessary.

It is necessary to point out that microRNA biogenesis has alternative pathways. Several studies have shown that non-canonical pathways for microRNA biogenesis are conserved in different organisms. Although non-canonical microRNAs represent a small fraction of the total microRNAs, their discovery implicates that some microRNAs might be produced under specific developmental stages or altered cell conditions to achieve gene regulation.[9]

Regulation of microRNA biogenesis by steroid hormones

Steroid hormones regulate a wide diversity of fundamental processes in the organism. Recent studies

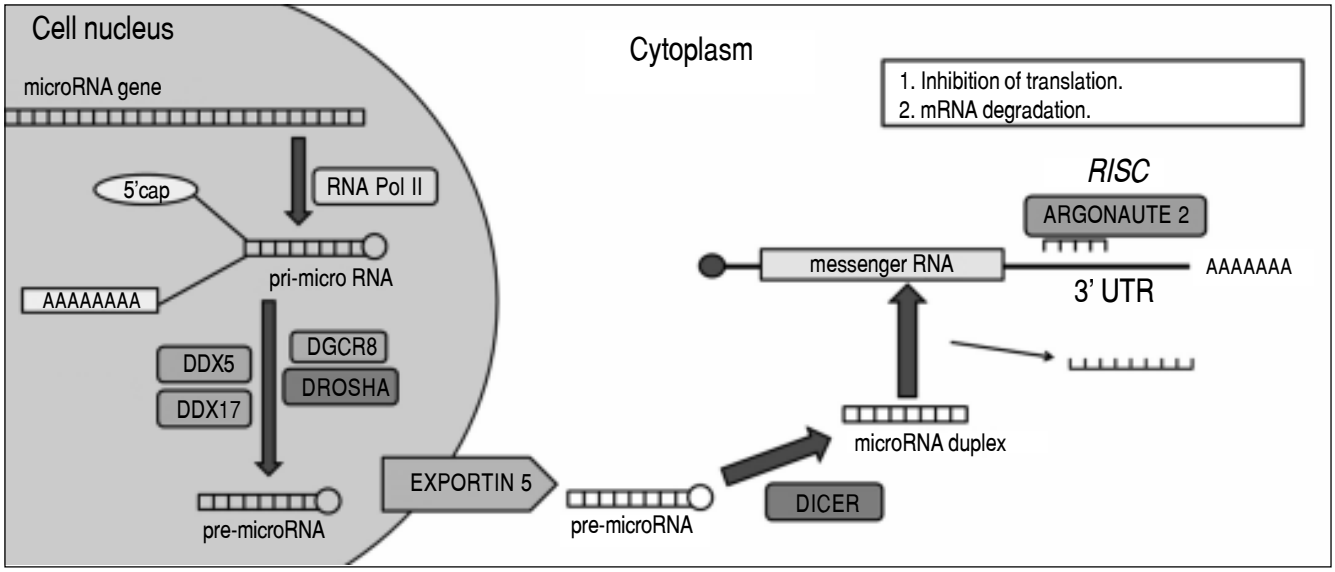

Figure 1. Canonical microRNA biogenesis pathway. Human genome encodes microRNA genes which are typically transcribed by RNA polymerase II to produce long, capped and polyadenylated, primary microRNA. The pri-microRNA stem-loop structure is processed by the microprocessor complex formed by: DGCR8, DROSHA and DDX5 and DDX17 RNA helicases. DROSHA enzyme crops the pri-microRNA into a hairpin-shaped precursor microRNA which is exported out of the nucleus by EXPORTIN 5. Once in the cytoplasm, DICER enzyme cleaves the loop from the pre-microRNA producing the mature microRNA (microRNA duplex). Only one strand of the microRNA duplex is loaded onto Argonaute 2 to form the RNA-induced silencing complex (RISC) to mediate gene silencing at the posttranscriptional level. microRNA binding sites in mRNAs generally lie in the 3' untranslated region (3' UTR). Partial complementarity between microRNA and target mRNA allows translational repression, without mRNA degradation. On the other hand, total complementarity allows mRNA degradation to produce the repressive effect.

Table 1. Effects of steroid hormones on canonical microRNA biogenesis pathway components.

Pathway component	Hormone	Tissue or cell line	Effect	Reference
Drosha	Estradiol and progesterone	Mouse uterus	No effect	13
DGCR8	Estradiol and progesterone	Mouse uterus	Messenger RNA increased (estradiol only)	13
DDX5	No reports have been published			
DDX17	No reports have been published			
Exportin 5	Estradiol and progesterone	Mouse uterus	Messenger RNA and protein increased	13
Dicer	Progesterone	Mouse uterus	Messenger RNA and protein increased	13
	Estradiol	MCF-7 cells	Messenger RNA increased	12
Argonaute 2	No reports have been published			

have demonstrated that hormonal effects also regulate microRNA expression. For example: estradiol stimulates or suppress microRNA expression in human breast cancer cells, endometrial cells, rat mammary gland and mouse uterus.[10] On the other hand, a study performed with calcitriol (the hormonal form of vitamin D_3) and testosterone, in human prostate cancer cells, evaluated both messenger RNAs expression profile and microRNAs expression profile showing that both hormones regulate messenger RNA degradation by microRNAs action. Those observations revealed feedback loops that result in global changes in messenger RNA and protein levels.[11]

As it was previously mentioned, in the canonical microRNA biogenesis pathway, many proteins participate coordinately from nucleus to cytoplasm. The genes that codify those proteins have been identified, but the factors that regulate their expression are little known. Although the regulation of microRNA biogenesis machinery components is in an early stage of study, some works suggest that hormonal action might have a major role. One of the first studies about this topic demonstrated that human Dicer gene expression is induced by estradiol. The study showed estradiol induced Dicer messenger RNA levels in MCF-7 human breast cancer cells. The authors described that estradiol modified the expression of several microRNAs and also induced the expression of Dicer enzyme, which is essential for the general mechanism of RNA silencing. These results indicate that estradiol may modulate microRNA biogenesis.[12]

Another important work explored the steroidal regulation of the microRNA biogenesis machinery components in vivo, using mouse uterus. In this study, estradiol and progesterone effects on Drosha, DGCR8, Exportin 5 and Dicer expression were evaluated. The authors described that estradiol and progesterone increased Exportin 5 messenger RNA expression, while only progesterone increased Dicer messenger RNA expression. They also observed the corresponding increment at protein level, in both genes.[13] To determine if the detected induction on Exportin 5 and Dicer expression were associated with microRNA processing, authors evaluated microRNA-451 expression, which they had previously described as an inducible microRNA by estradiol in uterine tissue.[14] They observed that microRNA-451 expression was increased by both hormones at the same treatment times where the induction of Exportin 5 and Dicer was detected. Therefore the authors suggest that their findings represent an additional mechanism by which steroid hormones may increase microRNAs processing.[13] The general findings from the mentioned studies are summarized in table 1.

Either DDX5 or DDX17 RNA helicases are also components of microRNA biogenesis, forming part of microprocessor complex.[15] It has been described that DDX5 is a multifunctional protein that interacts with other proteins as SMADs (the signal transducers of the TGF-β family of signaling pathways) and the tumor suppressor p53. In addition, DDX5 functions as a co-activator of several nuclear receptors as estrogen receptor α, androgen receptor and the vitamin D receptor.[16] Both DDX5 and DDX17 associate with estrogen receptor α upon estradiol stimulation.[17] That information indicates that both RNA helicases participate in the cell response upon hormonal stimulus and rise the question about if DDX5 and DDX17 could be target of hormones. At present, GRTH/DDX25 RNA helicase, which is essential for spermatogenesis, is the only RNA helicase known to be hormonally regulated.[18]

In general, the genomic effects exerted by hormones implicate the participation of specific nuclear receptors that regulate the transcription of target genes. The information presented in this brief re-

view shows that steroid hormones modify both protein coding genes and microRNA coding genes. Several works indicate that steroid hormones regulate microRNA expression through nuclear receptor mediated pathways. However, other possibility implicates that some of the microRNA biogenesis machinery components have hormonal response elements in specific genomic regions (as the gene promoter) and an induction in their expression may contribute to an induction in microRNA processing. It is clear that the precise mechanism by which steroid hormones regulate microRNA expression is not completely defined.

microRNA biogenesis alterations in cancer

A fundamental finding in cancer biology has been the observation of microRNA deregulation in human tumors compared with normal tissue.[19,20] Later studies demonstrated the accumulation of pri-microRNAs in cancer cells, which indicate an alteration in the microRNA biogenesis pathway.[21] The evidence that supports this observation was the abnormalities in the copy number of Dicer, Exportin 5 and Argonaute 2 genes described in breast and ovarian cancer, as well as in melanoma.[22,23] Furthermore, the reduction on microRNA biogenesis components expression has been associated with bad prognosis in hepatocellular carcinoma.[24] A key study in this context demonstrated that the depletion of Drosha, DGRC8 or Dicer drastically promoted tumor formation and invation.[25] As previously mentioned, Dicer ribonuclease is a key component of the microRNA biogenesis machinery. Interestingly, a low Dicer expression has been described in several tumors and has been related to bad prognosis in patients.[26-28] Moreover, the downregulation of Dicer expression has been associated with metastasis.[29]

DDX5 and DDX17 RNA helicases are multifunctional proteins, ubiquitously expressed in tissues, which participate in transcription, RNA processing and microRNA biogenesis. Studies performed in cell lines have linked both helicases with cell proliferation and differentiation, however the precise roles of DDX5 and DDX17 in those cellular processes remains unclear.[30] Altered expression and/or function of both RNA helicases has been related with cancer progression.

Indeed, some studies indicate that DDX5 and DDX17 induce cell proliferation and may be oncogenic; however there are other works that indicate the opposite, showing both DDX5 and DDX17 as tumor suppressors with inhibitory actions on cell proliferation. Even when those data appear to be contradictory, is important to point out that the precise function of both helicases may depend on tumor cell type and the expression of other proteins which interact with DDX5 and DDX17.[30,31] Reports that associate cancer progression with alterations on microRNA biogenesis machinery components represent valuable information to propose those genes, and their proteins, as potential cancer biomarkers and also as therapeutic targets.[32-34] Finally, as previously mentioned, some microRNA biogenesis pathways does not require canonical components as DGCR8, Exportin 5, Dicer or Argonaute 2 which adds a new level of complexity to cancer research.[35] Probably, future studies will reveal the role of those microRNAs in human cancer and contribute to understand the regulation of their biogenesis in cells.

CONCLUSIONS

The regulation of the microRNA biogenesis machinery is an emerging field of study that suggests a complex process in which many cellular factors participate. A recent body of experimental evidence shows that steroid hormones may increase microRNA maturation by stimulating the biosynthesis of some key components of microRNAs biogenesis machinery. A better understanding on how microRNA biogenesis is regulated will help to define its role in human diseases as cancer and also to design novel strategies to modify microRNAs expression for therapeutic purposes.

ACKNOWLEDGEMENTS

We want to thank Dr. Diego Arenas Aranda and Dr. Emilio Rojas del Castillo for critical review of the manuscript. We also thank Dra. Claudia Dolores Pérez Ortega for review the English version of the manuscript.

REFERENCES

1. Bartel DP. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell* 2004; 116: 281-97.
2. Carthew R, Sontheimer E. Origins and mechanisms of miRNAs and siRNAs. *Cell* 2009; 136: 642-55.
3. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993; 75: 843-54.
4. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, et al. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. *Nature* 2000; 403: 901-6.

5. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; 136: 215-33.
6. Finnegan EF, Pasquinelli AE. MicroRNA biogenesis: regulating the regulators. *Crit Rev Biochem Mol Biol* 2013; 48: 51-68.
7. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 2009; 19(1): 92-105.
8. Peterson SM, Thompson JA, Ufkin ML, Sathyanarayana P, Liaw L, Bates-Congdon C. Common features of microRNA target prediction tools. *Front Genet* 2014; 5: 1-10.
9. Xie M, Steitz JA. Versatile microRNA biogenesis in animals and their viruses. *RNA Biology* 2014; 11(6): 1-9.
10. Klinge CM. miRNAs and estrogen action. *Trends Endocrinol Metab* 2012; 23(5): 223-33.
11. Wang WL, Chatterjee N, Chittur SV, Welsh J, Tenniswood MP. Effects of 1α,25 dihydroxyvitamin D3 and testosterone on miRNA and mRNA expression in LNCaP cells. *Mol Cancer* 2011; 10: 58.
12. Bhat-Nakshatri P, Wang G, Collins NR, Thomson MJ, Geistlinger TR, Carroll JS, Brown M, et al. Estradiol-regulated microRNAs control estradiol response in breast cancer cells. *Nucleic Acids Res* 2009; 37(14): 4850-61.
13. Nothnick WB, Healy C, Hong X. Steroidal regulation of uterine miRNAs is associated with modulation of the miRNA biogenesis components Exportin-5 and Dicer1. *Endocr* 2010; 37: 265-73.
14. Nothnick WB. Regulation of uterine matrix metalloproteinase-9 and the role of microRNAs. *Semin Reprod Med* 2008; 26(6): 494-9.
15. Fukuda T, Yamagata K, Fujiyama S, Matsumoto T, Koshida I, Yoshimura K, Mihara M, et al. DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. *Nat Cell Biol* 2007; 9: 604-11.
16. Wagner M, Rid R, Maier CJ, Maier RH, Laimer M, Hintner H, Bauer JW, Onder K. DDX5 is a multifunctional co-activator of steroid hormone receptors. *Mol Cell Endocrinol* 2012; 361(1-2): 80-91.
17. Yamagata K, Fujiyama S, Ito S, Ueda T, Murata T, Naitou M, Takeyama K, et al. Maturation of microRNA is hormonally regulated by a nuclear receptor. *Mol Cell* 2009; 36(2): 340-7.
18. Tsai-Morris CH, Sheng Y, Gutti RK, Tang PZ, Dufau ML. Gonadotropin-regulated testicular RNA helicase (GRTH/DDX25): a multifunctional protein essential for spermatogenesis. *J Androl* 2010; 31(1): 45-52.
19. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, et al. MicroRNA expression profiles classify human cancers. *Nature* 2005; 435(7043): 834-8.
20. Jansson MD, Lund AH. MicroRNA and cancer. *Mol Oncol* 2012; 6(6): 590-610.
21. Thomson JM, Newman M, Parker JS, Morin-Kensicki EM, Wright T, Hammond SM. Extensive post-transcriptional regulation of microRNAs and its implications for cancer. *Genes Dev* 2006; 20(16): 2202-7.
22. Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, Liang S, et al. microRNAs exhibit high frequency genomic alterations in human cancer. *Proc Natl Acad Sci USA* 2006; 103(24): 9136-41.
23. Kim MS, Oh JE, Kim YR, Park SW, Kang MR, Kim SS, Ahn CH, et al. Somatic mutations and losses of expression of microRNA regulation-related genes AGO2 and TNRC6A in gastric and colorectal cancers. *J Pathol* 2010; 221(2): 139-46.
24. Kitagawa N, Ojima H, Shirakihara T, Shimizu H, Kokubu A, Urushidate T, Totoki Y, et al. Downregulation of the microRNA biogenesis components and its association with poor prognosis in hepatocellular carcinoma. *Cancer Sci* 2013; 104(5): 543-51.
25. Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nat Genet* 2007; 39(5): 673-7.
26. Merritt WM, Lin YG, Han LY, Kamat AA, Spannuth WA, Schmandt R, Urbauer D, et al. Dicer, Drosha, and outcomes in patients with ovarian cancer. *N Engl J Med* 2008; 359(25): 2641-50.
27. Guo X, Liao Q, Chen P, Li X, Xiong W, Ma J, Li X, et al. The microRNA-processing enzymes: Drosha and Dicer can predict prognosis of nasopharyngeal carcinoma. *J Cancer Res Clin Oncol* 2012; 138(1): 49-56.
28. He L, Wang HY, Zhang L, Huang L, Li JD, Xiong Y, Zhang MY, et al. Prognostic significance of low DICER expression regulated by miR-130a in cervical cancer. *Cell Death Dis* 2014; 1(5): e1205.
29. Martello G, Rosato A, Ferrari F, Manfrin A, Cordenonsi M, Dupont S, Enzo E, et al. A microRNA targeting Dicer for metastasis control. *Cell* 2010; 141(7): 1195-207.
30. Fuller-Pace F, Moore H. RNA helicases p68 and p72: multifunctional proteins with important implications for cancer development. *Future Oncology* 2011; 7(2): 239-51.
31. Paris O, Ferraro L, Grober OM, Ravo M, De Filippo MR, Giurato G, Nassa G, Tarallo R, et al. Direct regulation of microRNA biogenesis and expression by estrogen receptor beta in hormone-responsive breast cancer. *Oncogene* 2012; 31(38): 4196-206.
32. van Kouwenhove M, Kedde M, Agami R. MicroRNA regulation by RNA-binding proteins and its implications for cancer. *Nat Rev Cancer* 2011; 11(9): 644-56.
33. Díaz-García CV, Agudo-López A, Pérez C, López-Martín JA, Rodríguez-Peralto JL, de Castro J, Cortijo A, et al. DICER1, DROSHA and miRNAs in patients with non-small cell lung cancer: implications for outcomes and histologic classification. *Carcinogenesis* 2013; 34(5): 1031-8.
34. De Santa F, Iouse I, Del Rio A, Fazi F. microRNA Biogenesis Pathway as a Therapeutic Target for Human Disease and Cancer. *Curr Pharm Des* 2013; 19(4): 745-64.
35. Havens MA, Reich AA, Duelli DM, Hastings ML. Biogenesis of mammalian microRNAs by a non-canonical processing pathway. *Nucleic Acids Res* 2012; 40(10): 4626-40.

Reimpresos:

Ramiro José González-Duarte
Departamento de Biología de la Reproducción
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
Vasco de Quiroga, Núm. 15
14000, México, D.F.
Tel.: 52 (55) 5487-0900, Ext. 2418
Correo electrónico: ramirou2@yahoo.com.mx

Recibido el 09 de junio 2014.
Aceptado el 17 de septiembre 2014.