



The role of MicroRNAs as fine-tuners in the onset of puberty: a comprehensive review

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MicroRNA (miRNA) are small, noncoding RNA molecules that play pivotal roles in gene expression, various biological processes, and development of disease. MiRNAs exhibit distinct expression patterns depending on time points and tissues, indicating their relevance to the development, differentiation, and somatic growth of organisms. MiRNAs are also involved in puberty onset and fertility. Although puberty is a universal stage in the life cycles of most organisms, the precise mechanisms initiating this process remain elusive. Genetic, hormonal, nutritional, environmental, and epigenetic factors are presumed contributors. The intricate regulation of puberty during growth also suggests that miRNAs are involved. This study aims to provide insight into the understanding of miRNAs roles in the initiation of puberty by reviewing the existing research.

Keywords: MicroRNA, Precocious puberty, Puberty

Highlights

- This study explores the impact of miRNAs on the onset of central precocious puberty (CPP), with a particular focus on the roles of various miRNAs, including the Lin28/let-7 system, miR-200, miR-29, miR-30, miR-505-3p, and others.
- The research highlights how miRNAs act as key regulators in different pathways that control the initiation of puberty in the hypothalamus.
- The expression patterns of miRNAs indicate their potential as biomarkers for CPP, and targeting these miRNAs may contribute to the development of new diagnostic methods.

Introduction

Puberty is a pivotal milestone in both sexual and somatic maturation, marking a significant phase in an individual's life cycle.¹⁾ Unsurprisingly, the orchestration of puberty involves a sophisticated network of regulatory signals from central and peripheral sources, ensuring its timely occurrence and appropriate response to endogenous variables and environmental cues.¹⁻⁶⁾ Puberty is intricately linked to preceding developmental events, such as brain sexual differentiation,⁷⁾ which represents the process leading to reproductive competence. Recent advances in systems biology have gradually unveiled sets of genes and proteins that have the potential to globally regulate the timing of puberty.²⁾ Puberty emerges as a result of the harmonious and hierarchical activation/inactivation of excitatory and inhibitory networks rather than being a consequence of a single trigger. The regulation of these networks requires precise and multifaceted control mechanisms, which are not yet fully elucidated.

MicroRNAs (miRNA) are short noncoding RNAs that bind to the 3'-untranslated regions (UTR) of target genes. They play a crucial role in modulating gene expression by either targeting mRNAs for deregulation or causing translational repression.⁸⁾ MiRNA biogenesis (Fig. 1) is a complex and tightly regulated process that results in the production of the small RNA molecules. MiRNAs play a crucial role in posttranscriptional gene regulation by binding

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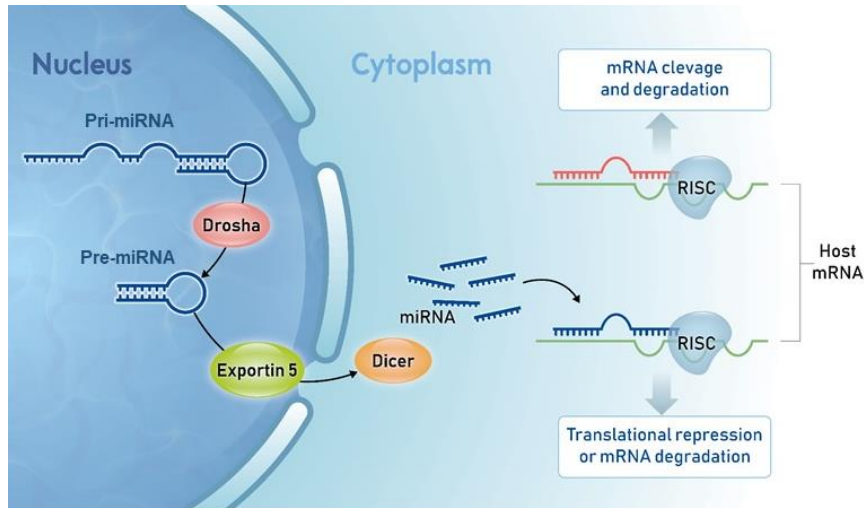


Fig. 1. Biogenesis of microRNA. miRNA, microRNA; mRNA, messenger RNA; RISC, RNA-inducing silencing complex.

to specific messenger RNA (mRNA) molecules, influencing their stability and translation. The process of miRNA biogenesis begins in the nucleus, where miRNA genes are transcribed by RNA polymerase II to generate primary miRNA transcripts (pri-miRNAs). Pri-miRNAs can be transcribed as independent genes or can be part of introns within protein-coding genes. The pri-miRNA transcript typically forms hairpin structures with stem and loop regions. The microprocessor complex, which consists of the RNA III enzyme Drosha and its cofactor DiGeorge syndrome critical region gene 8, recognizes and cleaves the stem-loop structure in the nucleus, generating precursor miRNAs (pre-miRNAs). Pre-miRNAs are short hairpin structures with a characteristic stem-loop. Exportin-5, a nuclear transport receptor, transports pre-miRNAs from the nucleus to the cytoplasm. In the cytoplasm, the enzyme Dicer further processes pre-miRNAs by recognizing their structure and cleaving it into a short double-stranded RNA molecule, which is typically approximately 22 nucleotides in length. One strand of the short RNA duplex, known as the guide strand, is selected to be incorporated into the RNA-inducing silencing complex (RISC). The RISC complex is a key player in the miRNA-mediated gene silencing mechanism. The guide strand of the miRNA within the RISC complex binds to the 3' UTR of target mRNAs through partial sequence complementarity. This binding can lead to mRNA degradation or translational repression depending on the degree of complementarity. If the miRNA is perfectly complementary to its target mRNA, it can induce mRNA degradation. If there is imperfect complementarity, it usually results in translational repression without mRNA degradation. The entire process of miRNA biogenesis is highly regulated; its dysregulation can have significant impacts on cellular processes and contribute to various diseases. MiRNA play essential roles in the regulation of gene expression and are involved in various biological processes, including development, cell differentiation, and response to environmental stimuli.

With finely regulated expression and the ability to modulate

numerous target genes, miRNAs emerge as promising candidates for regulating complex biological processes such as puberty. Notably abundant in the brain, miRNAs function as 'master regulators' or 'fine-tuners' of gene expression, contributing to regulatory roles spanning from neuronal development to various functions.⁹⁾ Despite suggestive evidence from Genome-Wide Association Studies (GWAS) and functional genomic studies, the direct involvement of miRNA regulatory pathways, particularly the *Lin28/let-7* system, in orchestrating the central developmental events leading to pubertal activation remains unexplored. More than 210 miRNAs have been detected in the arcuate (ARC) and paraventricular (PVN) nuclei of the rat hypothalamus,¹⁰⁾ where the machinery modulating puberty onset is located; however, only a small portion of them appears to regulate puberty onset in mammals based on direct evidence. Experimenting with the regulation processes of puberty using actual mammalian hypothalamic tissue is challenging. As an alternative, GT1-7 cells are widely employed in numerous studies. These cells, derived from mouse hypothalamic neurons, are immortalized GnRH neurons that offer an ideal model system for investigating the regulation of *Gnrh1* expression. While the majority of studies predominantly relies on animal experiments or experiments using GT1-7 cells, in this review, we aim to discuss the expression patterns of miRNA and their potential role in regulating the onset of puberty.

Lin28/let-7 miR system

GWAS have suggested the potential involvement of the *Lin28/let-7* axis in the control of puberty. These investigations have unveiled a plausible link between variations in or around the *LIN28b* locus and alterations in crucial developmental processes in humans. *Lin28b*, in conjunction with several targets of *let-7*, is associated with variations in human height.¹¹⁾ *LIN28b* is involved in the regulation of growth and the age of menarche

in humans.¹²⁻¹⁴⁾

Lin28, which was initially discovered in the nematode *Caenorhabditis elegans* (*C. elegans*), exhibits distinct patterns of expression specific to the particular tissue and developmental stage. Mutations in *Lin28* lead to either accelerated or delayed maturational events.¹⁵⁾ *Lin28* paralogs exhibit a high degree of conservation across evolutionary timelines, suggesting similar regulatory functions. In mammals, 2 *Lin28*-related genes called *Lin28* and *Lin28b* have been identified. *Lin28* and *Lin28b* are RNA-binding proteins that share a common function in suppressing the maturation of miRNAs belonging to the *let-7* family. While *Lin28* is broadly expressed during embryonic development, its expression is confined to specific tissues in adulthood.¹⁶⁾ The *let-7* miRNAs are known for their remarkable conservation, being widely and abundantly expressed across numerous species.¹⁷⁾ Members of the *let-7* family, along with *Lin28* counterparts, play a pivotal role in regulating essential developmental events in various species, including mammals.

In recent studies, the dynamic interplay of *Lin28/Lin28b*, *c-Myc*, and *let-7* has emerged as a key regulator orchestrating diverse cellular pathways.¹⁸⁾ The *Lin28* proteins have been demonstrated to bind to the terminal loops of precursors of the *let-7* family of miRNAs; in doing so, the *Lin28* proteins inhibit the formation of mature miRNAs.¹⁹⁾ Redundant *Lin28/Lin28b* represses the synthesis of mature *let-7* miRNAs, which can suppress *Lin28* levels. Additionally, one of the upstream regulators in the *c-Myc/Lin28/let-7* axis, *mir-145*, inhibits *c-Myc* expression at the posttranscriptional level.²⁰⁾ Moreover, *Lin28/Lin28b* downregulates *c-Myc* by suppressing mature *let-7* synthesis; *c-Myc*, in turn, transcriptionally activates the expression of both *Lin28* and *Lin28b*.¹⁸⁾ Bioinformatics analyses have suggested that miR-132 and miR-9 are potential miRNA that could repress *Lin28*.

In both male and female rats, the mRNAs of *Lin28* and *Lin28b*, as well as *c-Myc*, exhibit prominent expression in the hypothalamus during the neonatal period. However, this expression significantly decreases during the transition from infancy to juvenility and reaches minimal levels around or before puberty. Notably, experiments involving *Lin28* overexpression in mice have validated the presumed function of *Lin28* members in the regulation of postnatal growth and puberty. The outcome of *Lin28* overexpression in mice includes delayed puberty and increased body size, providing further evidence of the involvement of *Lin28* members in these developmental processes.²¹⁾

Previous studies have demonstrated that miR-145 suppresses *c-Myc* expression, leading to a reduction in *Lin28/Lin28b* transcription and consequently allowing the maturation of the *let-7* family of miRNAs to proceed without inhibition.^{20,22)} In adult rats, miRNA of the *let-7* family are notably more highly expressed in the hypothalamic arcuate and paraventricular nuclei.¹⁰⁾ In the nematode *C. elegans*, *Lin28* exhibits high levels at the beginning of the first larval stage and gradually decreases until the late larval stage; in contrast, *let-7* levels progressively increase to reach their maximum level in the adult stage.²³⁾ The reciprocal equilibrium between the level and timing of

mature *let-7* miRNA expression and *Lin28* plays a crucial role in defining key aspects of *C. elegans* development.

The *Lin28/let-7* miR system has been implicated in the maturation of the postnatal hypothalamus and the induction of puberty, particularly in a mouse model of hypogonadotropic hypogonadism.^{24,25)} Sangiao-Alvarellos et al.²⁴⁾ reported that developmental changes in hypothalamic *Lin28/let-7* expression may be involved in the mechanisms facilitating or leading to puberty onset. This study analyzed the expression profiles of *c-Myc*, *Lin28*, and *Lin28b* mRNAs, as well as *let-7a*, *let-7b*, *mir-145*, *mir-132*, and *mir-9* miRNAs in the hypothalamus of male and female rats. The finding revealed that *Lin28/Lin28b* and *c-Myc* mRNAs were abundant in the hypothalamus of both male and female rats during the neonatal period, with values declining significantly during the infant-to-juvenile transition. The expression levels of *c-Myc* mRNA in the rat hypothalamus mirrored those of *Lin28b* in both male and female rats. In contrast, *let-7a*, *let-7b*, *mir-132*, and *mir-145* miRNA levels were minimal during the neonatal period and progressively increased during postnatal maturation in both male and female rats. The authors suggested that *Lin28b* and its associated miRNA complex play a permissive role in the initiation of puberty. However, they emphasized that further functional studies and human studies are required for validation.

Obesity and high-fat intake have been demonstrated to induce premature activation of the hypothalamic-pituitary-gonadal (HPG) axis and central precocious puberty. In a study by Chen et al.,²⁶⁾ mice fed a high-fat diet showed increased expression of *p53* in the hypothalamus compared to that in the control group. The timing of vaginal opening, a marker of puberty onset, was also earlier in the high-fat diet group. In contrast, inhibiting *p53* expression led to a relative delay in vaginal opening. This finding suggests that a high-fat diet accelerates the onset of puberty through the upregulation of *p53* expression in the hypothalamus. The study proposed that *p53* might act as a crucial mediator linking metabolic changes to pubertal regulation. Moreover, the overexpression of *p53* was suggested to potentially expedite the activation of the HPG axis through the *c-Myc/Lin28/let-7* system. This implicates *p53* as a key player in the intricate interplay between metabolic alterations and the regulation of puberty.

MiR-200/429 and miRNA-155

The miR-200 family has been identified as a key player in the intricate regulation of neurogenesis, the migration of Gonadotropin hormone-releasing hormone (GnRH) neurons, and the process of sexual maturation.²⁷⁾ In a study by Messina et al.²⁸⁾ in 2016, it was reported that microRNAs, specifically miRNA-200/429 and miRNA-155, are critical components of a complex developmental switch that controls *Gnrh* promoter activity. The researchers generated mice in which *Dicer*, an RNase-III endonuclease essential for miRNA biogenesis, was selectively inactivated in GnRH neurons. Despite exhibiting normal body weights by adulthood, both male and female

mutant mice showed signs of hypogonadotropic hypogonadism and infertility. Markedly reduced serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were observed in both female and male adult mice. Notably, the pituitary function appeared intact, as LH levels increased after systemic GnRH injection; therefore, the mice had hypogonadotropic hypogonadism due to GnRH deficiency. Immunoreactivity for GnRH was completely absent in the hypothalamus of adult mutant mice. While some of the mutant female mice experienced vaginal opening during postnatal development, they never reached puberty despite normal somatic growth. Examination of the impact of Dicer inactivation on the migration of mouse GnRH neurons revealed that selective inactivation of *Dicer* expression in GnRH neurons did not result in migratory defects during embryogenesis. However, in mutant mice, hypothalamic GnRH immunoreactivity started to disappear during the infantile period, and this accelerated during the juvenile period. The deficiency of GnRH in mutant mice was attributed not to a developmental absence of GnRH neurons, but rather to an acquired postnatal deficit.

Among the differentially expressed miRNAs at different postnatal ages (P7 vs. P12), the miRNA-200 family and miR-429 emerged as the most highly enriched miRNAs in infantile GnRH neurons. These miRNAs are known to silence the transcriptional repressor *Zeb1* in pituitary gonadotropes, where it regulates LH expression. Both human and mouse *Gnrh* promoters were found to contain a putative *Zeb1* binding site. Through a series of experiments, the researchers hypothesized that increased *Zeb1* expression in the absence of miRNA biosynthesis could mediate the downregulation of the GnRH activators. To test this hypothesis, they administered a target-site blocker (TSB), which was selectively designed to silence the activity of target miRNA, directly into the brain of mice. TSB-200 selectively inhibits the activity of miR-200. This inhibition leads to a significant increase in the *Zeb1* mRNA level within GnRH neurons, while concurrently reducing transcripts of the *Gnrh* promoter activator and *Gnrh*. This miR-200-*Zeb1* network actively participates in the switch in *Gnrh* transcriptional control in the infantile mouse hypothalamus. MicroRNA-155 was also found to regulate the expression of *Cebpb*, *Gnrh*, and *Zeb1* mRNA in infantile GnRH neurons. TSB-155 upregulates *Cebpb* and *Zeb1* transcripts while indirectly suppressing *Gnrh* gene transcription. Both miR-200 and miR-155 facilitate the expression of *Gnrh1* by inhibiting the repressors of the *Gnrh1* promoter, specifically *Zeb1* and *Cebpb*, respectively. Remarkably, when TSB-200 and/or TSB-155 were injected into the brains of female wild-type mice during the infant–juvenile critical period, TSB-injected mice exhibited a precocious onset of puberty compared to control mice. The authors suggested that selectively blocking GnRH production and then releasing the block prematurely can trigger precocious puberty, possibly due to a rebound effect.

1. MiR-29 family

The miR-29 family is notably enriched in the central nervous system, particularly in mature neurons.²⁹⁾ Numerous studies have highlighted the regulatory role of miR-29s in puberty onset and reproduction. In the porcine developing hypothalamus, miR-29a-3p showed differential expression.³⁰⁾ In mice, the expression level of miR-29 family members in GnRH neurons was higher on postnatal day (PND) 12 than it was on PND 7; the abundance of these miR-29 family members was greater in GnRH neurons than in non-GnRH neurons.²⁸⁾ Takeda and Tanabe³¹⁾ reported that mice with brain-targeted miR-29 knockdown were developmentally indistinguishable from wild-type mice. However, mutant males exhibited subfertility, while mutant females showed hyperfertility. Similarly, turquoise killifish with neuron-specific miR-29 knockdown displayed reduced fertility and posthatch survival.³²⁾ GT1-7 cells, an immortalized clonal line of differentiated GnRH neurons derived from mouse hypothalamic neurons, were utilized as an ideal model system to study the regulation of *Gnrh1* expression.^{33,34)} Li et al.³⁵⁾ found that the absence of miR-29s resulted in elevated expression of *Gnrh1* and its target *Tbx21*, which promotes puberty onset. In GT1-7 cells, miR-29a, miR-29b, and miR-29c all have binding sites on the 3'UTR of the *Tbx21* gene. In this study, the transcription factor *Tbx21* was demonstrated to directly and indirectly promote *Gnrh1* expression. *TBX21* and GnRH were co-expressed in the hypothalamus, exhibiting a concurrent increasing trend during the onset of mouse puberty. When mice were subjected to stereotactic brain infusions of an miR-29a inhibitor, they exhibited a precocious onset of puberty compared with the control. The repression of miR-29a, mediated by the overexpression of *Tbx21*, promotes the onset of puberty and reproductive processes in mice. These findings suggest that miR-29 family members play a crucial role in regulating *Gnrh1* expression and, consequently, the onset of puberty.

2. MiRNA-375

MiR-375 is prominently expressed in the mouse pituitary gland and exerts a negative regulatory effect on pro-opiomelanocortin and related hormone secretion.³⁶⁾ According to Li et al.,³⁷⁾ miR-375 enhances *Gnrh* expression in GT1-7 cell by suppressing the levels of *Sp1* and *Tle4*. *Tle4* is known to repress the transcriptional activity of the *Gnrh* gene. *Sp1*, in contrast, negatively regulates *Gnrh* expression by directly binding to the *Gnrh* promoter to repress *Gnrh* transcription. *Sp1* also attaches to the promoters of *Cebpb*, *Msx1*, and *Tle4*, which act as repressors for the *Gnrh* promoter. This indirect mechanism affects *Gnrh* expression. The enhanced expression of miR-375 results in the reduced transcription activities of *Sp1*, *Cebpb*, *Msx1*, and *Tle4*. By doing so, miR-375 releases their repressive actions on *Gnrh* transcription, ultimately leading to an elevation in *Gnrh* expression.

3. *MiR-505-3p*

MiR-505-3p is commonly recognized as a biomarker of various diseases, including Parkinson disease and inflammatory bowel disease. Prenatal stress has been shown to lead to the downregulation of miR-505-3p in newborn rat brains. Target of miR-505-3p is the alternative splicing factor/splicing factor2 (ASF/SF2 of SRSF1),³⁸⁾ which acts as a regulator of the mammalian target of rapamycin (mTOR) pathway. Activation of the mTOR signaling pathway has been associated with an accelerated onset of vaginal opening in female rats.^{39,40)} In a study by Zhou et al.,⁴¹⁾ the role of miR-505-3p in the onset of puberty was investigated. The researchers found that miR-505-3p expression, which is most abundant in the female hypothalamus, reaches its peak during the neonatal period and gradually decreases thereafter. Overexpression of miR-505-3p resulted in delayed puberty, growth retardation, and reduced fertility. Conversely, female mice lacking miR-505-3p exhibited an increased growth rate, earlier vaginal opening, and larger litter sizes compared to those of wild-type mice. Furthermore, the serum levels of LH and FSH, as well as the expressions of *Srsf1*, *Kiss1*, and *Gnrh* in the hypothalamus, were significantly increased in knockout mice. *Srsf1* was identified as a target gene of the *miR-505-3p* gene in the GT1-7 cell line, primarily binding to ribosomal protein. Knocking down *Srsf1* expression in the GT1-7 cells inhibited the expression of the puberty-related genes *Kiss1* and *Gnrh*. The study concluded that miR-505-3p acts as a repressor of puberty onset in female mice by regulating the expression of *Srsf1*.

4. *MiR-199-3p*

Kisspeptin neurons are integral components of the hypothalamic GnRH pulse generators that play a crucial role in triggering the central mechanism that controls the onset of puberty.⁴²⁾ Li et al.⁴³⁾ investigated the regulatory mechanism influencing the onset of puberty through miRNA associated with *Kiss1*. The researchers observed that overexpression of miR-199-3p inhibited the expression of *Kiss1*, resulting in delayed puberty onset in mice. Furthermore, inhibition of the p38 mitogen-activated protein kinase (MAPK) pathway was found to delay puberty onset and decrease serum LH levels in both female and male mice. MiR-199-3p was shown to effectively repress the activity of the p38 MAPK pathway. Reduced p38 MAPK activity, in turn, inhibited the transcription of the *Kiss1* gene by blocking the activation of its transcription factors, c-FOS and cAMP response element-binding protein (CREB). The study proposed that sustained downregulation of miR-199-3p gradually ceases inhibition of the p38 MAPK pathway. This regulatory process, in turn, modulates the expression of *Kiss1* and plays a role in the precise timing of puberty onset in mice.

5. *MiR-664-2*

N-Methyl-D-aspartate (NMDA) signaling has been associated with the activation of GnRH neurons and linked to early menarche.⁴⁴⁾ NMDA receptors (NMDARs) are heteromeric receptors composed of various combinations of its 7 identified subunits⁴⁵⁾; they are present in different regions of the hypothalamus.⁴⁶⁾ Agonists and antagonists of NMDAR1 can influence the secretion of GnRH.⁴⁷⁾ Coexpression of NMDAR1 and GnRH within the same cellular environment has been noted in previous studies, highlighting the role of upregulated NMDAR1 expression in the increased release of GnRH at the initiation of puberty.⁴⁸⁾

In a study by Ju et al.,⁴⁹⁾ the expression of hypothalamic NMDARs in rats was assessed to elucidate the role of hypothalamic NMDARs and the associated miRNA in the pathogenesis of precocious puberty. In normal female rats, the average timing of vaginal opening typically occurred around PND 35. Concurrently, GnRH, LH, FSH, and estradiol serum levels reached their peak on PND 35, coinciding with the peak in hypothalamic NMDAR1 mRNA and protein levels. The researchers identified that miR-664-2 targets NMDAR1 in rat hypothalamic neurons, and the inhibition of miR-664-2 significantly increased NMDAR1 protein expression. Reduction in hypothalamic miR-664-2 levels was proposed to contribute to the pathogenesis of precocious puberty by enhancing NMDAR1 signaling. This study sheds light on the potential involvement of miR-664-2 and NMDAR1 in the regulation of puberty onset.

6. *MiR-30 family*

Recent evidence points to *Mkrn3*, a maternally imprinted gene encoding the *makorin RING-finger protein-3* (*MKRN3*), as a crucial inhibitory element in the gene regulatory network governing puberty.⁵⁰⁾ Loss-of-function mutations in *MKRN3* have been identified as the most common genetic cause of central precocious puberty. Additionally, a prepubertal decrease in *MKRN3* serum levels has been observed in both boys and girls.^{51,52)} The diminished hypothalamic expression of *Mkrn3* mRNA and protein before puberty onset in mice suggest its potential role as a repressive factor in the central control of puberty. The 3' UTR of the *MKRN3* transcript has been identified as a crucial miRNA-mediated regulatory element, which is highly conserved in both mice and humans.^{50,53)}

Heras et al.⁵⁴⁾ investigated the potential role of miRNAs in regulating *Mkrn3* activity and their functional contribution to puberty timing. The miR-30 family, identified through 4 miRNA-target prediction tools, was considered a strong candidate for regulating the *Mkrn3* gene. Specifically, miR-30b was selected for analysis. The expression profiles of miR-30b and *Mkrn3* in the hypothalamus of mice during postnatal maturation were examined. *Mkrn3* exhibited peak expression during the neonatal-infantile period, significantly decreased in expression during the transition to juvenile, reached minimal

levels during the peripubertal period, and maintained low levels in adulthood. In contrast, the miR-30b levels were lowest during the neonatal period, gradually increased during postnatal maturation, and peaked during the peripubertal period in both female and male rats. Hypothalamic Mkrn3 protein expression significantly declined during the transition from juvenile to pubertal stages in female rats. LH serum levels, serving as a surrogate marker for GnRH neuron activity, exhibited patterns inversely related to hypothalamic miR-30b expression in both female and male rats. The lowest LH levels were observed during the infantile period, followed by a progressive increase toward the peripubertal stage, and a peak during adulthood. It was confirmed that miR-30b targets the 3' UTR of *Mkrn3* and exerts a repressive signal on Mkrn3 expression in vitro. To prevent miR-30 from binding to its seed regions at the 3' UTR of *Mkrn3*, TSB-miR-30 was intracerebroventricularly injected. When TSB-miR-30 treatment was administered during the juvenile period, a delayed onset of puberty was observed. Additionally, the concentration of Mkrn3 in the hypothalamus increased in mice with delayed puberty after the administration of TSB-miR-30b. This study highlights the contrasting expression profile of Mkrn3 and miR-30b in the central control of puberty.

Mørup et al.⁵⁵⁾ conducted a study measuring the serum levels of miR-30b-5p, miR-200b-3p, and miR-155-5p in 46 boys from the longitudinal part of the Copenhagen puberty cohort study. They observed a significant increase in the circulating levels of miR-30b-5p when comparing pre- and peripubertal samples with postpubertal levels. In prepubertal boys, the circulating levels of miR-30b were low, while the MKRN3 levels were high. However, as puberty progressed, the levels of miR-30b increased, with a larger fraction becoming biologically active; concurrently, the MKRN3 level decreased. The researchers concluded that circulating levels of miR-30b-5p increase during the pubertal transition in boys. They suggested that miR-30b-5p may play a role in the activation of the HPG axis in the onset of puberty. This finding supports the idea that miRNAs, specifically miR-30b-5p, may contribute to the regulatory processes involved in the activation of the reproductive axis during the pubertal transition in boys.

Sexual dimorphism of miRNA expression

Sex chromosome-linked genes play a significant role in the sex dimorphism of gene expression, but they can only explain a limited number of sexually dimorphic genes. In fact, numerous sexually dimorphic genes are located on autosomes, which constitute 95% of the annotated human genome. The differential expression of autosomal genes between males and females is influenced by epigenetic controls and regulated by sex hormones.^{56,57)} While sex differences in pituitary function and associated disease are well recognized, the underlying gene regulatory networks that contribute to these differences remain unclear. The pituitary gland exhibits sex differences in its regulation of physiological function, including stress response, somatic growth, reproduction, and pubertal timing. Hormone

production and various physiological processes controlled by the pituitary gland are sex-biased.

Hou et al.⁵⁸⁾ conducted a study on the pituitary gene and miRNA profile in mice during postnatal development, encompassing the pubertal transition. They found that miRNA that was differentially expressed in the pituitary glands of male and female mice contributed to the sex difference observed in postnatal pituitary development and function.

In addition, miRNA expression has been extensively studied in the testes and ovaries of various species, including the human testis.⁵⁹⁻⁶³⁾ Differentially expressed miRNAs in these reproductive organs indicate the crucial role of miRNAs in guiding the development and function of gonadal tissues. Moreover, sex hormones such as estrogen and androgen stimulate and regulate miRNA expression in various diseases. Sex differences in miRNA expression are not limited to gonadal tissues; they have also been observed in diverse somatic tissues such as the liver, lung, and brain.

In a study by Ziats and Rennert,⁶⁴⁾ differentially expressed miRNAs in normal human donor brains were examined from infancy through adolescence. Notably, 40 miRNAs exhibited a significant sex-biased expression pattern in the prefrontal cortex, particularly during adolescence. These sex-biased miRNA-targeted genes are associated with Wnt and transforming growth factor-beta pathways. Additionally, besides inherent sex differences in miRNA expression, males and females exhibit sex-specific miRNA expression in response to environmental stimuli and/or pathological changes.⁶⁵⁾ It is plausible that miRNAs are expressed differentially between males and females across various diseases, leading to sex differences in disease susceptibility and severity.

Potential biomarkers for measuring puberty onset

MiRNA that exhibit distinct expression patterns based on developmental stage have the potential to be utilized as biomarkers for the onset of puberty. Han et al.⁶⁶⁾ suggested that circulating miRNAs are potential biomarkers for measuring puberty onset in chicken. They proposed a 7 miRNA panel (including miR-29c, miR-375, miR-215, miR-217, miR-19b, miR-133a, and let-7a) that displays a substantial potential to serve as a novel biomarker for evaluating puberty onset in chickens. Mørup et al.⁵⁵⁾ observed an increase in circulating levels of miR-30b-5p in boys as puberty progressed, suggesting the potential utility of circulating miRNA as a biological indicator within the context of reproductive maturation.

Conclusions

In conclusion, miRNAs play a crucial role as delicate regulators in the onset of puberty (Table 1). Changes in the expression patterns of miRNAs in the hypothalamus can influence the timing of puberty onset and may contribute to the development of precocious or delayed puberty.

Table 1. MicroRNA (miRNA) related to pubertal onset

miRNA	Target gene	Potential relation to pubertal onset
<i>Lin28/let-7</i> system	-	Permissive role <i>Lin28</i> gradually decreased <i>Let7</i> gradually increased
<i>Mir-200</i>	<i>Zeb1</i>	Promote <i>Gnrh1</i> expression
<i>Mir-155</i>	<i>Cebpb</i>	
<i>Mir-29</i>	<i>Tbx21</i>	Suppress <i>Gnrh1</i> expression
<i>Mir-375</i>	<i>Tle4</i> and <i>Sp1</i>	Promotes <i>Gnrh</i> expression
<i>Mir-505-3p</i>	<i>Srsf1</i>	Repressor of pubertal onset
<i>Mir-199-3p</i>	p38 MAPK pathway	Inhibits <i>Kiss1</i> expression
<i>Mir-664-2</i>	NMDAR1	Reduced expression of miR-664-2 may contribute to precocious puberty
<i>Mir-30b</i>	<i>Mkrn3</i>	Repress <i>Mkrn3</i> and contribute to the activation of the HPG axis

HPG, hypothalamic-pituitary-gonadal.

There are several limitations to the clinical application of miRNA research. First, the lack of a consensus regarding methodologies for quantifying miRNA poses a significant obstacle to the broader utilization of transcripts. Additionally, there is a lack of reproducibility across various technologies used for biomarker discovery. Most studies have been conducted in animals, with limited research in humans, especially in children. Furthermore, the majority of studies are based on case-control designs. The scarcity of large-scale cohort studies means that result may vary between studies. Despite several limitations, the identification and characterization of the miRNA profile during puberty are expected to provide valuable insights into the enigmatic processes of puberty. MiRNA research holds the potential to clarify the underlying processes of puberty and could help to predict the onset of puberty and treat associated disorders.

Notes

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