Chapter

Functional Role of MicroRNAs in Embryogenesis

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Abstract

This book chapter will provide an overview of the functional role of microRNAs (miRNAs) in embryogenesis. A brief introduction to embryogenesis and emphasis on the importance of miRNAs in gene regulation will be provided. The biogenesis and mechanism of action of miRNAs will be discussed in detail with a focus on the importance of miRNA-mRNA interaction in gene regulation. The chapter will then delve into the role of miRNAs in early embryonic development, including their importance in the establishment of the three germ layers, cell proliferation, differentiation, and apoptosis during embryogenesis. The role of miRNAs in organogenesis and tissue differentiation, specifically the formation of specific organs such as the heart, lung, liver, and brain, will also be discussed. The chapter will conclude by examining the dysregulation of miRNAs in embryonic development and disease, including teratogenicity, developmental disorders, and developmental cancer. The chapter will summarize the functional roles of miRNAs in embryogenesis and will offer future perspectives and potential therapeutic applications of miRNAs in embryonic development and disease.

Keywords: miRNAs, embryogenesis, gene regulation, organogenesis, development

1. Introduction

Small noncoding RNAs, also called miRNAs, which have a length of about 22 nucleotides, control the expression of genes at the posttranscriptional stage by selecting mRNAs for cleavage or transcriptional repression [1]. The majority of embryonic alterations can be connected to the genome's activity [2]. According to mouse studies, genomic data within preimplantation embryos undergoes wave-like alterations related with maternal transcriptome disintegration and zygote genome activation (ZGA) [3]. Despite the fact that miRNA regulates transcription, it is still unclear how it affects the removal of the maternal genome. According to studies, mouse oocytes and early preimplantation embryos exhibit usually decreased miRNA function [4]. The timing of extensive maternal gene breakdown is consistent with the inhibition of miRNA activity occurring before the two-cell stage. The expression of miRNA is revived, and the repression is lifted after the two-cell stage [5]. miRNA is short RNA molecules that regulate the translation and transcription.

The formation of miRNA is done by folding of small hairpin structure by the transcription of RNA polymerase II translation repression, and mRNA degradation is brought on by the synthesis of miRNAs in the nucleus as primary (pri)-miRNA and pre-miRNA, mature miRNA duplexes, RNA-induced silencing complex (RISC) strand-mediated complex, and complementary mRNA sequence synthesis (in the cytoplasm). Before implantation, a fertilized egg undergoes continuous cleavage to create an implantable embryo in a process known as embryogenesis. There are several factors, which characterize embryonic development, including the breakdown of maternal stores, activation of minor and major embryonic genomes, breaking down of the embryo and developing the embryonic cavity, and proliferation of embryonic cells into the inner cell mass and the outer epithelial trophectoderm [6]. The importance of stage-specific miRNAs throughout the early stages of mammalian embryogenesis is recognized, as is the involvement of coding genes. The first indication of the significance of micro RNAs (miRNAs) in embryonic development was acquired after the ablation of miRNA-treating genes such as Dicer, which is a key player in the start of RNA silencing because it recognizes double-stranded RNAs (dsRNAs) and uses its RNase III-like double-stranded RNA-specific nuclease activity to cleave them into short RNAs and Argonaute RISC Catalytic Component 2 (AGO2), which is a gene that codes for proteins. AGO2 serves a variety of roles, including RNA interference, translation repression, and heterochromatinization, which are all significant in small RNA-guided gene silencing processes [7, 8]. This resulted in an embryonic stop at E6.5 or embryonic mortality at gastrulation. In order to control the quantity or concentration of proteins or enzymes, miRNAs can either degrade mRNA or prevent protein translation. This can either promote or impede embryonic growth. The importance of miRNA before implantation, embryo growth between maternal zygote change, compaction, and blastocyst development has been shown by specific functional investigations or miRNA profiling research. From the zygote and two-cell stage, there is significant breakdown of maternally derived miRNAs, and during mouse embryogenesis, a variety of miRNAs are produced entirely from scratch reduced amounts of intact and hatched blastocysts and elevated Let-7a expression up to the eight-cell stage. The activity of miR-205, miR-150, miR-96, miR-122, miR-146a, miR-145, miR-208, and 496 elevated after bovine minor or major embryonic genome activation. In the initial bovine blast, miR-135a, miR-218, miR-335, and miR-449b are increased [9–12].

These critical developmental processes, such as embryonic cell segregation, embryonic outgrowth growth, and later implantation, are thought to be regulated by miRNA. Throughout these critical stages of embryo development, miRNA dysregulation may have an impact on cell differentiation and subsequent embryo implantation. In this context, Dicer knockout testing revealed that miRNA may retain trophoblast stemness by lowering the activity of Cdkn1a (p21) and Cdkn1c (p57) genes, as well as epiblast pluripotency by avoiding apoptosis. Furthermore, previous research found miRNA in both first trimester and term trophoblast cells, but different miRNAs were expressed in each of these cells, demonstrating that miRNAs may show a part in different trophoblast cell characteristics. The restricted expression of miR-93 on the trophectoderm and upcoming embryonic endoderm, as well as enhanced expression of miR-106a in the inner cell mass, point to a potential involvement for these miRNAs in embryonic differentiation. Additionally, 526 miRNAs, including let-7b, miR-23a, miR-27a, miR-291a, miR-425, and miR-429, showed differential expression among embryos in blastocyst stage and those in embryonic outgrowth stage. Similarly, eight-cell stage embryos and trophoblast tissue have variable expression levels of certain miRNAs [12–16].

miRNAs regulate posttranscriptional gene expression as supported by the fact that around 30% of human genes can have their expression altered by miRNAs, which are distributed in tissue-specific patterns. miRNAs show a significant part in regulating gene expression. Understanding the molecular mechanisms that govern miRNAs is critical since they are frequently dysregulated in a number of human ailments, such as cancer and immunological disorders. RNA polymerase II generally generates major transcripts from miRNA-encoding genes, which are subsequently cut into short RNAs of roughly 21 nucleotides by RNase III endonucleases Drosha and Dicer. The RNA-induced silencing complex (RISC) functions as a posttranscriptional regulator by binding to the 3′-untranslated region (UTR) of mRNAs. It is essential for selecting the start codon and facilitating ribosome binding to the mRNA. Additionally, it significantly influences translational efficiency regulation and the development of the cellular proteome [17], encompassing all miRNAs. This seed-dependent miRNA binding inhibits the translation of its mRNA targets and/or promotes their splicing into nonfunctional forms [18–22]. Unexpectedly, current work provides proof of a target-mediated decay mechanism that regulates the concentration of certain miRNAs. Unexpected miRNA activities have also been found, together with a variety of noncanonical genes harboring miRNA. For instance, some miRNAs work in the nucleus to activate or silence target genes during transcription. These epigenetic alterations are attracted to particular genomic loci by RISC which participates in the breakdown of target mRNA, lowering the amount of transcript accessible for ribosome translation and directed there by miRNAs [23] as shown in **Figure 1**.

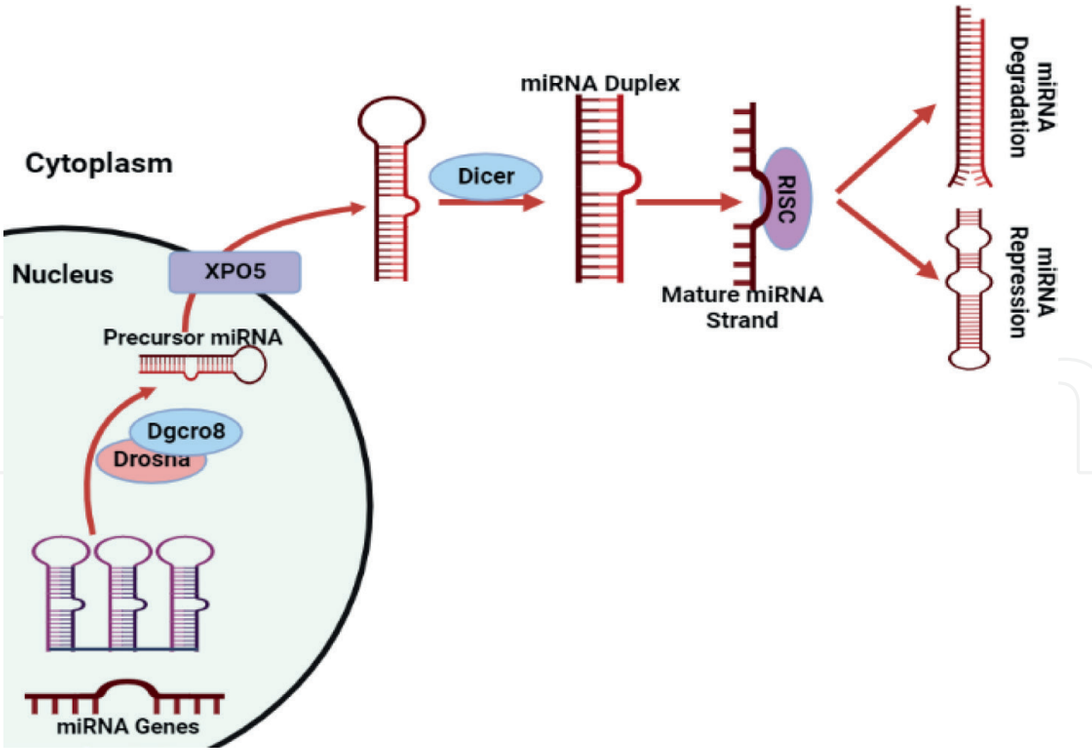

Figure 1.
miRNA synthesis, processing, completion, and control are depicted schematically. The noncoding region of the genome serves as the source for the miRNA transcription within the nucleus. The nuclear membrane protein XPO5 is responsible for the pre-export miRNA's cytoplasmic region from the nucleus. Primary-miRNA is converted into miRNA duplex by the endonuclease dicer in cytoplasm. The mature miRNA is created by when the miRNA duplex unwinds by a helicase. It is the RISC that contains the mature miRNA. Through translation inhibition or mRNA degradation, the miRNA-RISC complex regulates posttranslational gene expression by binding at the mature mRNA.

2. miRNAs biogenesis and mechanism of action

The process of creating miRNAs begins with DNA sequences known as miRNA genes, or with gene clusters that are only transcripted as miRNA molecules or collectively as polycistronic transcripts, correspondingly. A protein-coding gene's intron or non-translated region (UTR) may also be where miRNAs are found. The canonical and noncanonical versions of the two primary biogenesis routes were determined [24].

2.1 Canonical miRNA biogenesis and functions

By use of RNA polymerase II, miRNAs are produced as primary transcripts. Drosha and its cofactor DGCR8 recognize and cleave one or more stem loops that are present in pri-miRNAs. Exportin 5 is a Ran-GTP-dependent mechanism that exports the resultant pre-miRNA from the nucleus. A mature miRNA duplex with two strands that are each 21–22 nt long is produced in the cytoplasm by the processing of the pre-miRNA by Dicer and its cofactor TRBP. After that, the RNA-induced silencing complex (RISC) incorporates this miRNA duplex [25].

To initiate miRNA production, primary miRNA transcripts (pri-miRNAs) from the genome are processed post- or co-transcriptionally. Conventional pri-miRNAs, which are normally transcribed due to RNA polymerase II (POL II), have a 5′-cap, and include one, do not necessarily have a poly-A tail [26].

The majority of mature miRNA genes can express themselves singly or in groups and are found in functional gene coding or noncoding areas. Drosha and Dicer, two RNase III enzymes that catalyze two additional processing stages in the nucleus and cytoplasm, are responsible for the synthesis of miRNAs [27, 28]. In the nucleus, genomic DNA is translated into a long primary miRNA (pri-miRNA). Drosha then cleaves this miRNA to produce a precursor miRNA (pre-miRNA) with a hairpin structure and a length of around 70 nucleotides (nt). These hairpin RNAs are moved nuclear leakage enters the cytoplasm receptor exportin-5, where Dicer cleaves them into mature miRNAs of 19–23 nt active miRNAs can also be produced *via* noncanonical miRNA biogenesis mechanisms. Small nucleolar RNA (snoRNA) progenitors serve as the source of miRNAs. and mirtrons derived from pre-mRNA splicing are examples of these [28, 29].

The microprocessor complex is a protein complex, which involves in the initial steps of processing for microRNA (miRNA) and RNA interference (RNAi) in animal cells [30]. The complex, which converts main miRNA substrates to pre-miRNA in the cell nucleus, is made up mostly of the ribonuclease protein Drosha and the dimeric RNA-binding proteins DGCR8 (also called Pasha in nonhuman species) [31]. Microprocessor also comprises of two units of multiple proteins, transcribes primary miRNAs (pri-miRNAs) from their genes, and then processes them into precursor miRNAs (pre-miRNAs). Introns and noncoding RNA genes are both potential sources of pri-miRNA substrates. In the latter scenario, there is evidence that the spliceosome and microprocessor complex interact and that pri-miRNA processing takes place before splicing [32]. Its two components have been found to be both necessary and sufficient to mediate the formation of miRNAs from pri-miRNAs [30]. One of them is a massive multiprotein complex made up of multiple RNA-associated proteins, ribonucleoproteins, dsRNA-binding proteins, and the family of Ewing's sarcoma proteins. The other is Drosha, tiny multiprotein complex that contains the enzyme RNase III, as well as the DiGeorge syndrome critical region 8 RNA protein complex (DGCR8). The microcontroller identifies certain pri-miRNA patterns and splits it at the bottom

of the distinctive hairpin form, resulting in a pre-miRNA, there is a 3′–2–nt overhang and a 5′–monophosphate. Afterward, a complex of exportin5 (XPO5) and the nuclear protein guanosine-5'-triphosphate-ase, which is connected to RAS, was formed (Ran-GTPase). This complex exports pre-miRNAs from the nucleus to the cytoplasm. The RNase III endonuclease Dicer eliminates the terminal loop of pre-miRNA in the cytoplasm [33, 34]. miRNAs are currently loaded, entering the complex for RNA-induced silencing (RISC) as short RNA duplexes called miRNA duplexes [34, 35].

2.2 NonCanonical miRNAs

There are miRNAs known as noncanonical miRNAs that are produced using a distinct biogenesis mechanism. Recently, different miRNA biogenesis routes have been identified. Certain noncanonical pri-miRNAs, for example, are known as mirtrons and are stored in coding genes' introns [23]. Like typical introns, all mirtrons undergo initial processing by the nuclear splicing machinery before creating sturdy, stem-less hairpins than canonical pri-miRNAs. Only one annotated miRNA has been found to process independently of DICER so far. Pre-miR-451 miRNA maturation necessitates the AGO2 slicer activity because of its stem-loop shape is too shorter for DICER to cleave [23]. Pre-miR-451 processing by AGO2-mediated processes and eventual RISC loading are influenced by the length of the stem-loop structure, inappropriate base pairing in the stem, and low GC content in the distal stem, among other factors. Furthermore, miR-451 processing by AGO2 is dependent on the function of eukaryotic translation initiation factor 1A, another RISC component (EIF1A) [23].

It has been predicted that mirtron formation occurs during noncanonical miRNA biogenesis. RNA Pol II transcribes mirtrons as introns of protein-coding genes. These mirtrons are spliced by the spliceosome, debranched, and (if necessary) exosomal trimmed. Mirtrons are considered as canonical miRNAs after undergoing these preliminary processing stages, and Exp5 then transports them to the cytoplasm, where they are processed by Dicer and TRBP and then integrated into the RISC. Also, it has been highlighted the production of miRNAs independent of Dicer. RNA Pol II produces the Dicer-independent miRNA miR-451, which is then processed by Drosha/DGCR8 and exported from the nucleus by Exp5. Argonaute 2 (Ago 2) binds to miR-451; once it is inside the cytoplasm, where it is then cut into the functional, mature, single-stranded miRNA [25].

2.3 shRNA- snoRNA- and tRNA-derived miRNAs

The family of ncRNAs known as snoRNAs, which range in length from 60 to 300 nt, is typically found in cells' nucleolus, where they may alter tRNAs rRNAs and snRNAs. Through the H/ACA box on their rRNA precursors, snoRNAs can control the methylation and pseudouridylation of specific rRNA sites. According to a number of lines of evidence, certain snoRNAs can serve as the building blocks for noncanonical miRNAs. These noncanonical miRNAs are produced by use of a biological process, which resembles the synthesis of canonical miRNAs in that it involves the Dicer, Dgcr8, and AGO enzymes. Dicer and Dgcr8 are required for the stabilization and degradation of the purportedly derives from snoRNA miRNAs [36]. SnoRNA-derived miRNAs are 21 nt long, equivalent to miRNAs, and can link to AGO enzymes such as AGO1 and AGO4 to inhibit mRNA targeting [36, 37]. Contrarily, the production of two snoRNA-derived miRNAs called ACA45 and GlsR17 shows a dependence on Drosha/Dgcr8 rather than Dicer [36, 37]. The unusual precursor structure of these

snoRNA-derived miRNAs consists of two hairpins resembling pre-miRNA connected *via* a hinge. These distinct assemblies appear to give their capacity to silence mRNA transcripts posttranscriptionally, similar to miRNAs. ShRNA-derived miRNAs, such as snoRNA-derived miRNAs, are derived from non-miRtronic genomic areas and require Dicer but not Dgcr8 for synthesis. This subclass includes the isoleucine tRNA gene, shRNA-miR-320, miR-484, and miR-1980. They are all distinguished by distinctive physical characteristics, such as structures with hairpins that surround the conventional pre-miRNA hairpin but are invisible to Dgcr8. These shRNA-derived miRNAs have a complicated and unusual hairpin structure that is located at the 3′ end rather than the traditional 5′ end. Emerging information on shRNA-derived miRNA clusters suggests that a new biogenesis may represent additional crucial regulatory activities that have not yet been recognized [23, 36, 37].

tRNA-derived miRNAs are created through tRNA development, although they have activities comparable to miRNAs, such as RNA transcript suppression. However, compared to traditional miRNAs, their affinity for Ago proteins appears to be less., which is probably because of their susceptibility to posttranscriptional modifications. Although little is known about their function, tRNA-derived miRNAs may support cell growth and miRNA biogenesis [37–40].

Exportin 5 transports a pre-miRNA hairpin enters the cytoplasm and forms a miRNA duplex is formed following a Dicer's second cycle of endo nucleolytic cleavage [41]. Certain miRNAs are processed by Dicer a few organisms through interactions with supplementary RNA-binding proteins such as loquacious and TRBP. [42]. These RNA-binding proteins' posttranslational modifications can influence Dicer activity and the biogenesis of specific miRNAs [29]. Following duplex unwinding and strand selection, the mature miRNA is loaded into the Argonaute protein, which the miRNA-induced suppressing complex's core component (miRISC) [41]. Through sequence complementarity, the targeting mRNAs' 3'UTRs are bound by the miRISC-associated miRNA, which also attracts downstream effectors and typically causes translational suppression and mRNA degradation [43]. The seed sequence is the first 2–8 nucleotides from the mature miRNA's 5′ end. To pair with a target mRNA, the seed sequence must be nearly full, while exhibiting partial symmetry along the remainder of the miRNA and the target. MiRNAs that have the identical seed pattern are members with the same family of miRNAs. A plethora of computational and biological approaches have been developed to anticipate and confirm miRNA-mRNA target pairings [44].

Argonaute posttranslational changes can influence steady-state miRNA activity [45]. The orthologs of Argonaute in humans and *C. elegans*, AGO2 and ALG-1, have conserved phosphorylation sites that modulate mRNA target binding, according to mass spectrometry [46, 47]. To continue the overall a phosphorylation cycle, the effectiveness of miRNA-target associations in different cell lines controlled by the casein kinase CSNK1A1 and the phosphatase ANKRD52-PPP6A suggested, in which specific binding causes Quick phosphorylation and dephosphorylation of AGO2 [48]. Phosphorylation of the Argonautes AGO1 and AGO2 proteins was discovered to be essential for interaction with LIM domain-containing proteins in a separate study. To promote target silencing by certain miRNAs, these LIMD proteins establish a clamp between Argonaute and the adaptor protein GW182. In the absence of that phosphorylation event, AGO3, which has a phosphomimetic residue near the phosphorylation site of AGO1 and AGO2, is recruited to execute the identical silencing effect [49]. These findings imply that the Argonaute function may be situation sensitive. The production of a shortened version of AGO2, deficient in every functional area, has

been proposed as the cause in mouse oocytes of a general downregulation of miRNA activity. In the absence of mature miRNAs, the E3 ubiquitin ligase Iruka poly-ubiquitinates AGO1 at an unaltered lysine position, designating AGO1 for destruction [50].

3. miRNAs in early embryonic development

3.1 The role of miRNAs in preimplantation development

The free-living stage of mammalian development from the one-cell zygote to blastocyst adhesion or implantation into the uterine wall is known as preimplantation development. The first week of development is typically covered by it, and key moments such the change from parental to embryonic genomic activation (EGA), compaction, and cavitation or blastocyst formation are included [51]. The primary result of preimplantation development is the creation of an embryo that is implantation-competent for the start of pregnancy. To achieve this, the trophectoderm (TE), which develops into the blastocyst's outer layer and connects with the endometrium to start implantation, must first take shape. The embryonic element of the placenta is likewise derived from the TE. The initial stage of the embryo proper is the inner cell mass (ICM), a second cell type that is still pluripotent. These cells continue to be contained within the polar and mural TE of the blastocyst. Therefore, TE differentiation rather than embryogenesis is the focus of preimplantation development as placental mammals must first meet this criterion for implantation and placental growth before embryogenesis can occur [51].

The animal embryo moves between the oviduct and the uterus after fertilization. The embryo then implants, forming a link with maternal tissues as depicted in **Figure 2**. Embryonic development in mammals a diploid zygote is created when a sperm and an egg are united. The zygote proceeds through a sequence of cleavage divisions that increase the number of blastomere-containing cells while maintaining the embryo's total volume. Following cleavage, the eutherian embryo goes through compaction, in which the compaction of the embryo one another to form a tight ball of cells known as a morula [52].

miRNAs, which are around 22 nucleotides long, provide guidance molecules in the RNA silencing process. MiRNAs have a role in nearly every animal developmental and pathological process, targeting the majority of protein-coding transcripts [53]. A crucial step is embryo implantation. In the creation of a pregnancy in animals and happens over a "period of implantation" is a certain time frame [54]. Apposition, attachment, and invasion are the three stages of implantation [55]. The receptive endometrium, which is produced by widespread endothelial edema on the surface of the inner uterus, interlaces with the microvilli of the cytotrophoblast on the outer surface during apposition [56]. Furthermore, the receptive endometrium expresses heparin-binding growth factor similar to EGF (HB-EGF), and cells expressing Blastocysts are adhered to by transmembrane HB-EGF with ErbB4 on their cell surface [57]. Activated blastocysts increase the expression of HB-EGF, which causes its own expression of genes at the blastocyst apposition location in the endometrium *via* an auto-induction loop [58]. Lif regulates HB-EGF expression, which leads to a decrease in COX-2, a deficit of which results in implantation failure [59]. At the location of implantation, gene expression in the Wnt/−catenin pathway is also crucial. Wnt/−catenin signaling prior to attachment necessitates an active blastocyst and preimplantation. Transgenic mouse models of estrogen secretion [60].

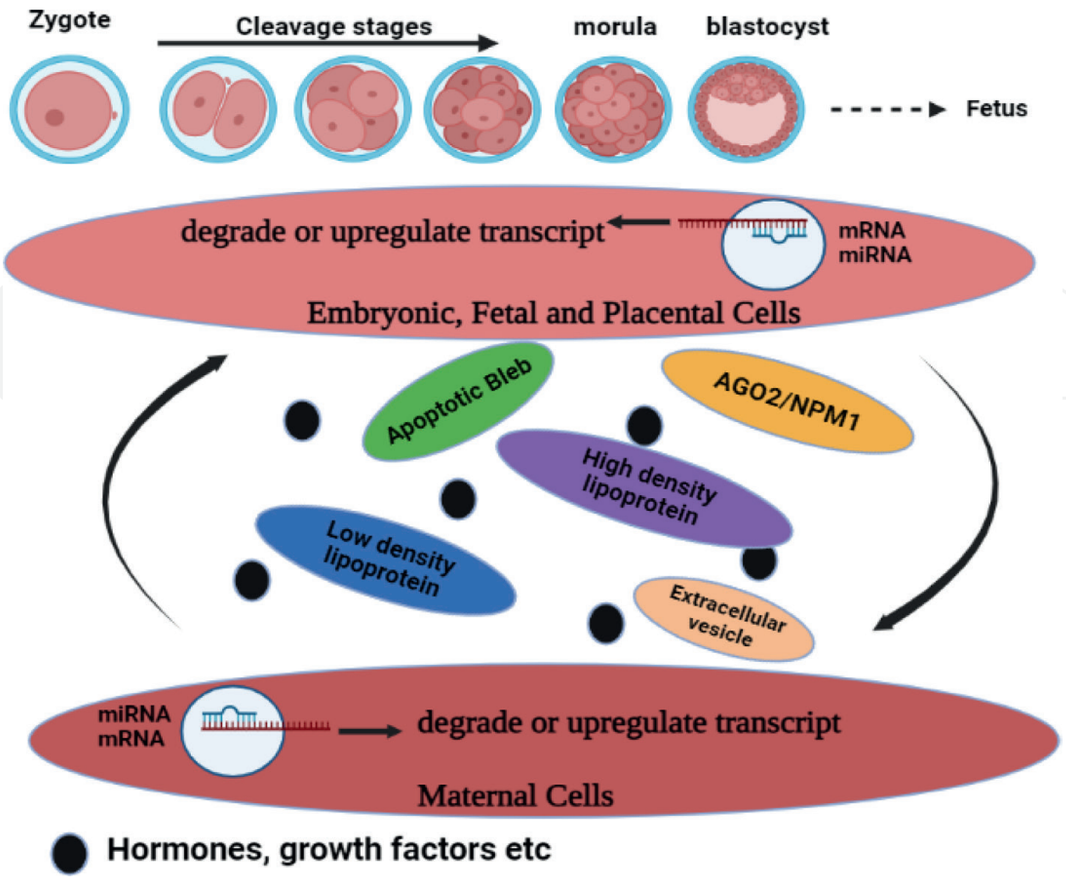

Figure 2.
miRNA transfer between the mother and fetus is depicted schematically. miRNAs may be transported in between fetal cells and the conceptus using a variety of carriers. Proteins such as extracellular vesicles, cytotoxic benign growths, and high-density and low-density lipoproteins, such as Argonaute 2 (AGO2) and Nucleophosmin 1 (NPM1), can all act as these vehicles. The miRNA pairs with a complementary target mRNA when it is delivered to the mother or conceptus and joins an RNA-induced silencing complex (RISC). The desire mRNA is subsequently dysregulated, increased, or degraded. Direct cell-cell contact can cause this crosstalk, or miRNAs can move around and be conveyed through fluids, such as uterine fluid.

During the preimplantation stage of development, the embryo secretes extracellular miRNAs are introduced. MiRNAs are secreted into biological fluids in the extracellular setting by a variety of cell types. These miRNAs generated from fluid have been demonstrated to circulate in the body. The appropriate packing of vesicles that contain miRNAs outside of cells, especially exosomes, is required for stable transport. At the forefront of studies into cell-to-cell communication are these vesicles, which transport RNA, DNA, and proteins. The presence of EVs in their miRNA content was found in uterine fluid. Their miRNA content was found in uterine fluid, which is expected to facilitate interaction between the mother and conceptus. Uterine fluid is one of the reproductive fluids where they have been discovered (**Table 1**) [61].

3.2 The importance of miRNAs in the establishment of the three germ layers

A large a group of noncoding RNAs known as miRNAs function as RNA silencing guide molecules and are approximately 22 nucleotides long. Animal processes, both developmental and degenerative virtually, universally involve miRNAs, which primarily target protein-coding transcripts [53]. The "window of implantation" refers to the constrained time period during which embryo implantation, an essential

miRNAs	Function	References
miR-143	Proliferating, migrating, and invading cells of human endometrial stroma	[62]
miR-193	In the uterus of mice, implantation rate is controlled.	[63]
miR-181	Control of the implantation rate in the mouse uterus	[64]
miR-29b	Development of a calf using somatic cell nuclear transfer (SCNT)	[65]
miR-126a-3p	Development utilizing nuclear somatic cell sojourning in bovine embryos (SCNT)	[66]
miR-200a	Counting the implantation spots in the rat endometrium	[67]

Table 1.
Role of miRNAs in the development and implantation of embryo.

step in the creation of pregnancy in mammals, takes place (WOI) [54]. Apposition, attachment, and invasion are the three stages of implantation [55]. The receptive endometrium, which is produced by widespread an inner uterine surface with stromal edema, interlaces with the microvilli of the cytotrophoblast on the outer surface during apposition [56]. Additionally, the receptive endometrium expresses heparin-binding growth hormone similar to EGF (HB-EGF), the ErbB4-expressing cells that participate in this adhesion to blastocysts [57], and the ErbB4-expressing cells that participate in this adhesion to blastocysts *via* an auto-induction loop [58]. Lif regulates HB-EGF expression, which leads to a decrease in COX-2, a deficit of which leads to unsuccessful implantation [59]. At the location of implantation, which results in the failure of the implant. Wnt/-catenin signaling prior to attachment necessitates an active blastocyst and preimplantation. In transgenic mouse models of estrogen secretion [60] various glycoproteins, ligands for carbohydrates, receptors, and integrins collaborate the uterine surface in order to secure the embryo during the attachment phase. Several integrins play a role in implantation in varying degrees. The embryo expresses 51, v3, v5, and v6 during attachment, and 111, 61, and 71 are later implicated in invasion [68].

The uterine surface in order to secure the embryo into biological fluids in the extracellular environment by a variety of cell types. These miRNAs generated from fluid have been demonstrated to circulate in the body. The appropriate packing of extracellular vesicles containing miRNAs (EVs), especially exosomes, is required for stable transport. The study of cell-to-cell communication is currently focused on these vesicles, which transport RNA, DNA, and proteins. EVs have been discovered in a number of reproductive fluids, such as uterine fluid, where it is believed that their miRNA content serves as a mechanism for mother and child chit-chatting conceptus [61].

3.3 The role of miRNAs in cell proliferation, differentiation, and apoptosis during embryogenesis

MiRNAs have a significant impact on a variety of cellular processes, such as differentiation and proliferation, that are regulated by intracellular signaling. Major examples of these signaling cascades are the nuclear factor kappa-light chain-enhancer of activated B cells (NFkB), mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinases (PI3K), notch, transforming growth factor (TGF), and hedgehog (Hh) pathways. MicroRNAs may regulate cell signaling by suppressing the

expression of their target genes, which produce molecules involved in these important signaling pathways [69, 70]. Additionally, a number of signaling mechanisms control miRNA expression. For instance, in rat vascular smooth muscle cells (VSMC), an active MAPK pathway increases miR-31 expression. In PC12 cells, a rat pheochromocytoma cell line of the adrenal medulla, the nerve growth factor (NGF) increases expression of miR221, miR-222, with NGF induction itself dependent upon activation of the extracellular signal-regulated kinase 1 and 2 (ERK1/2) pathways [71]. In signaling cascades, microRNAs may potentially establish regulatory feedback loops. In many physiological processes, there are several regulatory loops between miRNAs and their targets. In THP-1 cells, a cell line for human acute monocytic leukemia, a regulatory loop between miR-146 and NF-B has been identified [72]. Through mediating inter-pathway crosstalk, microRNAs may operate as a connecting agent between several signaling pathways [70, 73]. For instance, in the early stages of embryonic patterning, miR-15 and miR-16 link the Wnt and TGF- signaling pathways. To sum up, miRNAs play a role in controlling intracellular signaling pathways. Additionally, they create intricate links between various signaling pathways. Changes in intracellular pathways are linked to a number of diseases in humans.

Preimplantation embryos from mice and humans can produce the placenta, yolk sac, and the three germ layers of the fetus, respectively, through the actions of the epiblast (EPI), primitive endoderm (PrE), and trophectoderm (TE) [74]. Years of work have resulted ESCs, XEN (extraembryonic endoderm) stem cells, and trophoblast stem cells are three *in vitro* models for cell culturing reproduce the potentials of those three distinct lineages (TSCs) [75]. Shortly, after implantation, it is possible to identify two additional stem cell types: two stem cells: the primordial germ cell (PGC), the very first progenitor of the germ cell lineage, and the stem cells from epiblasts (EpiSC), which mimics the potential of the more primed epiblast [76]. It is worth noting that the expression patterns of the three preimplantation embryo lineages varied significantly between mice and humans [77].

MiRNAs can influence adult stem cell destiny in a variety of ways; a type of stem cell called epiblast (EpiSC), and hematopoietic stem cells (HSCs) hinders whereas miR-16, miR-103, and miR-107 expression decrease proliferation, and they promote differentiation [78]. MiRNAs have been shown to influence a variety of differentiation processes such as myogenesis, cardiogenesis, neurogenesis, and osteogenesis [79].

miRNAs linked with stem cell development are expressed is regulated at several levels, for instance, posttranscriptional, epigenetic, and/or transcriptional control; cluster of miR-362 and miR-367, for instance, is regulated by transcription factors (TFs) connected to the ESC, including Oct3/4, Sox2, and Rex1 [80], which promote the self-renewal or generation of pluripotent stem cells. Studies on the transcriptional control of miRNA production in ESCs have shown, which bicistronic or monocistronic miRNAs at intergenic loci are regulated by shared upstream transcription regulators, bicistronic or monocistronic in nature. Along with the bi-cistronic miRNAs mir-1 and mir-133, mir-206, mir-486, and mir-499, are induced by MyoD, Myogenin, and MYF5 during human muscle stem cell (hMSC) growth [81]. It is interesting to note that the co-regulated miRNAs mir-1 and mir-206 in various Chromosomes share a seed sequence with members of the same miRNA family that downregulates relevant targets [82].

Cells that would typically be harmful to the organism's survival are eliminated during apoptosis, a sort of active, and cell-autonomous caused cell death.

The so-called intrinsic pathway, which includes mitochondria and stressors, can start this death response. Alternatively, the extrinsic system, which involves a cognate ligand, might start it by activating death receptors on the cell membrane [83]. In 2003, the first papers on the regulatory functions of miRNAs in apoptosis were published. Through the inhibition of the effector caspase Dicer and the proapoptotic high temperature-induced Dauer synthesis family member (hid1) genes, respectively; it was demonstrated in these Drosophila research that miR-14 and bantam govern cell death. Since then, both intrinsic and extrinsic routes have been revealed to contain many miRNA families that regulate apoptosis. The discovered miRNAs have possibly pro- or antiapoptotic characteristics [84].

Extrinsic signaling pathways are occupied by a large number of miRNAs. miR-7 specifically sensitizes cells from hepatocellular cancer and glioblastoma multiforme to TRAIL-mediated apoptosis [85]. In human acute myelogenous leukemia cells to improve cell survival, miR-590 targets Fas ligand (FasL) and is regulated utilizing the STAT5 (Signal Transducer and Activator of Transcription) pathway [86]. MiR-21, a direct FasL target in pancreatic cancer, prevents cancer cells from dying from gemcitabine-induced apoptosis when it is expressed ectopically [87]. Fas expression in osteosarcoma cells is decreased by miR-20a, promoting tumor cell proliferation, and metastatic potential [88]. Additionally, miR-146a and miR-196b prevent the expression of Fas, and leukemia is brought on by ectopic miR-196b expression [89]. The expression of Fas is also inhibited by miR-146a and miR-196b, and leukemia is caused by ectopic miR-196b expression [89]. miR-25, which is associated with intrahepatic bile duct. It is more common for malignancy to directly target DR4 and resist TRAIL-mediated apoptosis [80]. There is an increase in malignancy, which has been demonstrated to directly target DR4 and shield against TRAIL-mediated apoptosis. Although miR-128a antagonists increase Fas-mediated apoptosis, ectopic miR-128a In Jurkat cells, expression raises Fas resistance by targeting FADD [90]. By inhibiting the expression of miRNA-186*, which targets the caspase-10 enzyme, which is identical to caspase-8, curcumin induces death in A549 cells [91]. MiR-708 improves the susceptibility a kidney cancer cells multiple apoptotic stimuli and adversely controls the expression of c-FLIP [19]. Three miRNAs, miR-378, miR-155, and miR-let-7a, target caspase-3 that can regulate the apoptosis of cancer cells [92]. Specifically targeting caspase-7, miR-106b is linked to the prostate cancer coming back [93]. For the purpose of controlling pancreatic cancer cells undergo apoptosis,miR-133 and miR-24a directly inhibit caspase-9 [94].

A variety of miRNAs are also involved in the regulation of the apoptotic regulator genes in the intrinsic route for apoptosis. It has been demonstrated that miR-365 specifically targets the protein adaptor Src homology 2-containing subtype 1and the Bcl-2-like protein 4 (Bax) (SHC1)in lung adenocarcinoma and lung cancer cell lines [95]. In gastric cancer cells, miR-125b expression is markedly downregulated. Overexpressing miR-125b, however, prevents stomach cancer cell lines from proliferating, migrating, and invading (HGC-27 and MGC-803) (95)miR-148a and miR-204 cause apoptosis *via* posttranscriptionally targeting Bcl-2 negatively [96]. The posttranscriptional level of miR-148a and miR-204 negatively targets Bcl-2 to cause apoptosis [97]. By triggering apoptosis, miR-608 greatly reduces the growth of chordoma cells by directly targeting Bcl-extra-large (Bcl-xL) [98]. Activator of apoptosis protein 1 (Apaf1)can be targeted by miR-23a/b and miR-27a/b, which controls the vulnerability of neurons to apoptosis (**Table 2**) [99].

miRNA	Function	References
miR-21-3p	Inhibits the cow granulosa cells undergo autophagy	[100]
miR-383	Prevents autophagy in granulosa cells of cattle	[101]
miR-183-96-182 cluster	Controls apoptosis in the granulosa cells of the porcine ovary	[102]
miR-1275	Involved in the synthesis of estradiol and death in porcine granulosa cells	[103]
miR-92a	Controls the apoptosis of granulosa cells in porcine ovary	[104]
miR-15a	Controls the granulosa cells in the human ovary produce steroid hormones and promote cell growth	[105]
miR-96	Controls the growth of cells and the synthesis of steroid hormones in human ovarian granulosa cells	[106]
miR-126-3p	Encouraging in porcine granulosa cells, cell growth, and averting cell death	[107]
miR-181a	Control oxidative stress-related acylation of the fork head box protein O1 (FOXO1) and mouse granulosa cell apoptosis	[108]
miR-23a. miR-27a	Granulosa cells in humans undergo apoptosis	[109]
miR-99a	Polycystic ovarian syndrome (PCOS) sufferers' granulosa cell growth and death are controlled.	[110]
miR-335-5p	In women with polycystic ovarian syndrome (PCOS), the regulation of granulosa cell growth and apoptosis is in place.	[111]
miR-125b	Controls the apoptosis of the yak ovary's granulosa cells	[112]
miR-143	Encourages human granulosa cell proliferation and estradiol synthesis that is produced by follicle-stimulating hormone (FSH)	[113]

Table 2.
miRNAs that are involved in autophagy, cell proliferation, and apoptosis.

4. miRNAs in organogenesis

4.1 The role of miRNAs in organogenesis and tissue differentiation

miRNAs may be crucial for differentiation and organogenesis in *D. rerio*, according to research on Dicer mutant animals. Maternal-zygotic Dicer mutants were discovered to have disruptions in several developmental processes, including neurogenesis, somatogenesis, and the development of the ear, eye, and heart [114]. It is not yet known if every one of these flaws results from a deficiency in miRNAs or from other Dicer-dependent mechanisms. There are currently no zebrafish strains with miRNA knockouts; however, rescue experiments on maternal-zygotic Dicer mutant animals show that the miR-430 family of miRNAs plays a crucial role in neurogenesis [114]. Defects in neurulation are severe in maternal-zygotic mutated Dicer embryos. These mutants have problems forming the neurocoele and neural tube, as well as the midbrain-hindbrain boundary and brain ventricles. Surprisingly, this mutant phenotype was partially reversed by miR-430, and a single miRNA was injected, which belongs to a broad kinship with it belongs to the miR-302 and miR-17 families of miRNAs . The midbrain-hindbrain border was saved after miR-430 injection but not after another unrelated miRNA. Additionally, the injection of, however, heart or ear development was not partially rescued by miR-430, only somatogenesis, retinal development, and gastrulation. These findings demonstrate

conclusively that the loss of miRNAs is the cause of at least some of the aberrant phenotypes seen during maternal-zygotic diverse mutant animals. Without a miRNA knockdown strain, a unique system is provided by the maternal-zygotic Dicer knockout to study the function of additional miRNAs in zebrafish.

M. musculus is comparable to *D. rerio* and comes with the same warnings. MiRNAs play a variety of mouse embryogenesis, according to Dicer1 mutant mice, which plays roles in differentiation and development [7]. Conditional-knockout strains in the mouse can be used to explore the necessity of both Dicer and miRNAs in various tissues at various developmental junctures, providing further insights. Conditional gene targeting led to the creation of ES cells with the Dicer mutation, which exhibit severe differentiation abnormalities *in vitro* [115]. MiRNAs appear to operate in cells, lineage commitment, not however in CD4/CD8; according one study found that examined the consequences early in T-cell development by genetically inactivating Dicer1 [116]. MiRNAs seem to function in various cellular processes and lineage commitment, but interestingly, they do not seem to play a role in CD4/CD8 development. A study investigating the effects of genetically inactivating Dicer1 during early T-cell development supports this observation [117].

This conditional-knockout research in mice has a special warning because it is frequently difficult to determine how effectively any and the Dicer preexisting When Dicer1 is deleted somatically, miRNA pools are depleted. In reality, miRNAs do seem to last a while [116]. One particular miRNA, MiR-181, has been directly associated to the maturation of B cells [118]. This miRNA is highly expressed in mouse B-lymphoid cells. Hematopoietic stem cells overexpress some genes and increases the proportion of B-lineage cells both *in vitro* and *in vivo* in adult mice during differentiation tests. Dicer1 was conditionally inactivated in particular areas of the mouse limb mesoderm, which resulted in, however, there are no mistakes in fundamental limb patterning or tissue-specific differentiation, only mutant embryos exhibit severe growth defects limbs [119]. This is an astonishing discovery that is similar to the Dicer1 victory over *D. rerio*. The Dicer1 victory over *D. rerio*. It is still unclear, though, whether lingering deciphering or miRNA pools may have covered up prior miRNAs' functions in the growth of limbs.

The Dicer1 knockdown in *D. rerio* has some similarities to this startling discovery. If any residual deciphering or miRNA pools may have covered up earlier miRNA the development of limbs, this is still unknown. [120]. Myocardin, Mef2, serum response factor (Srf), and Myod have all been found to operate as transcriptional controllers of *in vitro* miR-1 expression. Through investigation of the upstream-regulatory sequence of these two genes [121]. By employing a conditional Srf-knockout strain, it was discovered that Srf is necessary the expression of miR-1in a mouse heart that is still growing [116]. Developmental arrest occurred around heart failure caused by upregulation of miR-1 under the promoter of the -myosin heavy chain on embryonic day 13.5. ventricular cardiomyocyte proliferation anomalies and thin ventricle walls appeared in transgenic embryos. Mice with a mutated form of the miR-1 gene exhibited reduced levels of the transcription factor Hand2, even though there was no apparent alteration in Hand2 mRNA levels. This implies that Hand2, a potential miR-1 target in myocardial development, is affected [121].

5. The importance of miRNAs in the formation of specific organs

5.1 Heart

Each of the several stages of development involves a number of substances with inducing or suppressing capabilities [122].

Preliminary cardiac precursor cells have been found in the mouse and chick embryo's front portion of the primitive streak, according to tracing investigations using studies using cell/tissue explants and essential dyes [123]. The FGF family of molecules' action signaling molecules, which includes the FGFs FGF2, FGF4, and FGF8 have been postulated to indicator through FGFR1, controls the initial movement of these cells from the posterior to the anterior at the start of gastrulation [124, 125]. Similar to TGF-, bone-forming protein 2 (BMP2), a growth factor belonging to the TGF- superfamily, is first expressed toward the final phase of the primitive phase by the start of gastrulation and is associated with early cell migrations [126].

As a result of Mesp-1 and Mesp-2 being expressed in an FGF-8-dependent manner at embryonic day 6.5 (E6.5); Bilaterally, cardiac progenitor cells move to the front and side of the heart of the embryo, colonizing the lateral plate mesoderm splanchnic layer, and forming two early lateral cardiac fields [127]. Mesp-1 positive mesodermal lineage is induced by canonical Wnt/−catenin signaling in the neural ectoderm during early gastrulation [128]. These findings support significant roles for early Bmp2 and FGF signaling in establishing cardiogenic potential [129], limiting the position of the cardiac fields, and directing progenitor cell movement. By acting as a concentration gradient of Wnt/−catenin, the underneath anterior mesoderm can be induced into head tissue [130].

A day later (E7.5), the cardiac crescent, a horseshoe-shaped epithelial fold, is formed when these earliest cardiogenic stem cells travel cranially and congregate across the midline region of the embryo. Nkx2-5, a vertebrate homeodomain transcription factor and *D. melanogaster* tinman gene homolog, was first used to identify the lateral cardiac fields in mouse and chicken embryos [131, 132]. Nkx2-5 is the Nkx factor that is most abundantly expressed during early peripheral cardiac fields and the cardiac crescent undergoes cardiogenesis in mouse and chick embryos [133, 134]. But unlike the way Tinman works in flies, no Nkx gene controls cardiogenesis in a master manner [135]. The lateral portions of the cardiac crescent are subsequently drawn toward the midline by a dynamic process that causes the medial area of the crescent to expand and travel cranially; these two lateral cell populations eventually unite to form the heart tube (E8.0). The first heart areas can finally be anatomically and molecularly recognized when the heart tube is forming. Wnt/−catenin signaling, particularly Wnt-3a and Wnt-8, is required for the initiation and upkeep of Isl-1 expression during the development of the heart tube [136] and Fgf10 [137]. The development and stability of Isl-1 expression during the development of the heart tube depend on Wnt/−catenin signaling, particularly Wnt-3a and Wnt-8 [138].

At E9.5, the cardiac tube is bent to the right to adjust the heart's chambers (looping), and the caudal region of the cardiac tube is brought cranially to position the two sinus venosus horns dorsally to the outflow tract. Since the sinus venosus moved the right side of the heart by E10.5, the cardiac looping process is almost finished. The right atrium and common cardinal vein on the right are joined at this point, which is indicated by the emergence of two outgrowing structures known as the sinus valves. Additionally, at E10.5, the cardiac chambers have begun to septate [139, 140]. By E11.5, the heart's caudal region is at last dorsal to the arterial pole, and in oxygen-breathing vertebrate species, the heart chambers will then become septate is the last step in their specification. The outflow route is initially divided into an arterial and pulmonary arch by the creation of internal cushions. The left horn shrinks and changes into the coronary artery as the right sinus venosus horn is placed in close proximity to the right atrium. The interatrial and interventricular septa, respectively, divide the atria and ventricles [125, 141].

5.2 Lung

Similar divisions between the stages of lung development in mice and humans, including canalicular, terminal saccular, and pseudoglandular, and the final alveolar stage, are present in both species [142]. Total miRNA levels in the mouse embryo at the two-cell stage were found to be considerably lower than those in the one-cell zygote following fertilization [9]. A noteworthy difference between the two-cell stage and the four-cell stage embryos was the overall miRNA levels [9]. Inhibiting sections of cultured tissue from mouse lung E12 smoothened led to an increase in the distal epithelial size, as well as to disturb the usual branching pattern and mesenchymal integrity [143]. Inhibiting smoothened in cultured explanted E12 mouse lung caused distal epithelium to enlarge and disrupted the mesenchymal integrity and normal branching pattern. Mesenchymal progenitors' growth and differentiation in the lung development of mice were controlled by miR-142-3p after it was all said and done [144].

The amount in the forming mouse lung miR-17 was steady from E11.5 to E16.5; it was mostly present at the pseudoglandular stage, with greater epithelial levels around E12.5 [145]. MiR-17 levels in the developing mouse lung were steady (E11.5–E16.5), primarily when pseudoglandular with greater epithelial levels at E12.5 [145]. In the rat embryonic lung, miR-449a levels increased from E15.5 to E18.5, and miR-449a was upregulated in the human fetal lung at 18–20 weeks (canalicular stage) [146]. Levels of Mycn and Sox9 mRNA and SOX9 and Ki-67protein were all elevated at the distal end of the epithelium of E16.5 mouse lung culture when miR-449a was inhibited (end of pseudoglandular stage) [146].

The deletion of miR-26a-1 and miR-26a-2 in mice enhanced the creation of dilated lumens and aerated patches at the beginning of the neovascularization stage (E16.5 of lung development) [147]. The miR-26a mutant mice's results at the level of secularity (E18.5) likewise showed enlarged crystalline bodies and higher amounts of the proteins SP-A, SP-B, and SP-C. The findings also suggested that miR-26a is implicated in the pulmonary surfactant generation [147]. MiR-17-92 cluster and miRNAs in the Dlk1–Dio3 locus were upregulated in the E18.5 mouse lung after epithelial histone deacetylase 3 was lost [148]. Embryonic mouse lung epithelium overexpressing miR-17-92 indicated densely packed AT1 cells. The results of the study collectively demonstrated that histone deacetylase 3 controlled miR-17-92 levels, which, in turn, affected TGF- signaling and, ultimately, AT1 cell spreading and saccular formation [148]. The highest amount of miR-127 was found in the fetal rat lung at E21 [149]. Additionally, the size of the terminal and internal buds was increased by overexpressing miR-127 in fetal rat lung (E14) culture.

In research on alveolarization, bronchopulmonary dysplasia (BPD) mouse models have been used. After, the treatment of miR-876-3p mimics repaired alveolar architecture and reduced neutrophilic irritation in postnatal day 14 animals that had been subjected to hyperoxia [150]. After exposure to hyperoxia, levels of miR-30a-3p and -5p were significantly increased in the lungs of female mice on postnatal days 7 and 21 [151]. In total, significant reductions in miR-30a-3p and -5p levels were observed, whereas DLL4 mRNA and protein levels were elevated in human BPD lung samples [151].

5.3 Brain

A KO would be very difficult because of the 12 different members of the miRNA Let-7 family, which is strongly expressed in the brain. In addition, miRNAs are frequently transcribed in groups. Therefore, the manifestation of a different unrelated

miRNA in the same cluster may be affected if one miRNA gene is deleted or otherwise altered. Along with having overlapping targets, miRNA families also have the same seed sequence. It follows that other family members would probably make up for the loss an individual's family. Even with these challenges, reports of miRNA knockout (KO) mice have allowed for loss-of-function studies for particular During developing brain, miRNAs [151].

One of the most abundantly produced miRNAs in the mouse brain is miR-124, which is also highly conserved among mammals. When neural stem cells differentiate into neurons, miR-124 expression rises and is neural-specific [152, 153]. Reduced neurogenesis and a shift to gliogenesis are caused by neural stem cells permanently losing the ability to function as miR-124 [154]. MiR-124 is a neuronal destiny determinant, as determined by the forced neural differentiation that occurs when it is overexpressed in glioma cells, embryonic stem cells, HeLa cells, and neural progenitor cells [154–157]. Three distinct loci for the miRNAs mIR-124-1, mIR-124-2, and mIR-124-3 yield the same mature sequence and are used to transcribe miR-124. Retinal noncoding RNA gene 3 has an exon from which the miR-124-1 stem-loop is translated (Rncr3). The nuclear localization, consensus sequence, and high amounts of miR-124 expression in the brain are all due to this gene, which also serves as its main source. In order to study the function of miR-124-1 during brain development, Rncr3 was deleted to create miR-124-1 knockout (KO) mice. In this work, it was discovered indicated there was a decrease in miR-124 expression by 60–80% in KO mice. Around postnatal day 20, the majority (60%) of KO mice perished. In addition to a large loss of cone cells and cell apoptosis in the cortex and hippocampus, Rncr3 KO mice also had smaller brains. Axons in the dentate gyrus of animals lacking Rnc3 also showed aberrant outgrowth. The tail suspension assay results in KO mice clasping their front and back limbs further demonstrated severe neuronal injury. Additionally, mouse models that mimic neurodegenerative diseases show this neuronal impairment. In conclusion, the KO of Rncr3 suggests that miR124 plays a significant role in murine brain development [158].

6. Dysregulation of miRNAs in embryonic development and disease

miRNAs have a role in differentially implantation and non-implantation of embryos; proliferative endometrium within women who face failed implantation and are used as a biomarker for embryo implantation [159], blastocyst development, and morula to blastocyst transition [160]; in short, they are involved in gametogenesis, embryogenesis, and mammalian fertility [12]. The role of short noncoding RNAs in gene expression is found in fully developed oocytes [160], ovaries, testis, and follicular fluid. During gametogenesis, miRNA are involved in the division and differentiation of stem cell and cell proliferation [84, 161] proved by knockout of Dicer1 gene [162] and AGO2 [163]. miRNA is involved in follicular development [164] through the regulation of vitamins, methionine, lipids, and cysteine [165]. miRNA has its role in testis development [166] as it is evident by its presence in Sertoli cells [167] and proved by the Dicer and Drosha development [166]. miRNA-1285, miRNA-762, miRNA-638, miRNA-15a,miR-29b,miR-34b and miR-34c has its role in spermatogenesis [168]. miRNA are also found in body fluids such as serum, saliva, urine, and blood plasma in the form of exosome or apoptotic bodies with the playing role as lipid or protein carriers [169, 170].

6.1 The effects of miRNA dysregulation on embryonic development and disease

miRNA can has deregulatory functions by destroying the translation process of protein making from mRNA [171]. This dysregulation has negative effects on embryonic development through having impact on stem cell division, cell proliferation, and on early or late embryonic development [161]. These negative effects lead to deleterious diseases such as tumors and cancers at the embryonic stage [172]. A total of 156 mature miRNAs are involved in embryonic cancerous diseases out of which colon cancer is greater in number [173]. Most importantly, miR-290–295, miR-302–367, miR-17–92, and miR-106b–25 are the set of miRNAs that are the controlling centers of embryonic diseases. Recent researches has also shown the effect of miRNA on protein output [174]. miRNA-383 is involved in infertility of men, maturation arrest, hyperactive proliferation of germ cells, G1-phase arrest, suppression of proliferation, and induction of apoptosis, and this effect is increased by the downregulation of IRF1. As described earlier, Dicer1, along with miRNA, has significant role in spermatogenesis. The Dicer1 deletion in the testis leads to defective spermatogenesis and during proliferation arrested at early differentiation. Only miRNA-372 and 373 are seen in defective spermatogenesis. This all would happen during early spermatogenesis stages, which can result in germ cell death [175]. miRNA expression is seen during different embryonic stages in mammals and thus can act as a biomarker for the selection of embryos for successful *in vitro* pregnancy. MiRNA is involved in polycystic ovary syndrome [176]. Recently shown that miRNA-25 is involved in cardiomyocytes oxidative stress and apoptosis in human ovarian cancer [177, 178]. miRNA-181 is involved early embryonic developmental stages and regulates nucleoplasmin 2 protein [179]. miRNA-196a encode transcriptional factors through HOX genes necessary for embryonic development [180]. Polymorphism miRNA-196aCC found in aborted fetuses [123]. miRNA-302 is found in blastocyst in high quantity. It is linked with cell programming [181]. miRNA-370 regulated DNA methyltransferase 3a expression [182].

6.2 miRNAs in developmental cancer

Different types of these small noncoding miRNAs are involved in different types of cancers [172], such as miR-15a is involved in leukemia [172], miR-145 in colorectal cancer [183], miR-21 in breast cancer [184], and let-7 of miRNA in lung cancer [172]. These dysregulated miRNAs can be oncogenes or tumor suppressors [172]. miRNA 17–92 are highly expressed in cancerous tissues in humans [174]. Amplification overexpression and depletion of miRNA lead to cancer [185]. miRNA are seen in genome effected with cancer [169]. miRNA is highly stable during extreme conditions of temperature and environment [186, 187]; so, miRNA is used as a biomarker for cancer such as lung, prostate, and colorectal cancer [184, 187]. miRNA expression gives 60% surety of cancer by analyzing serum, while 100% specificity is based on the effectiveness of miRNA [9]. Mutations, deletions, amplifications, epigenetic silencing, and dysregulation of transcriptional factors in miRNA loci are various processes that contribute to changes in the miRNA genome leading to cancer. The dysregulated miRNA genes are managed by protein-coding oncogenes or tumor suppressor gene. The tumor suppressor miRNA genes are B cell leukemia, lymphoma 2, MYC, RAS, phosphatase and tensin homolog (PTEN), p27, p57, and tissue inhibitor of metalloproteinases 3 (TIMP3). The silencing of miRNA genes gives epigenetic alterations that result in the silencing of tumor-suppressing genes. For example, the

deletion of miRNA-29 causes DNA methyltransferase overexpression and results in tumor suppressor-silenced gene. The dysregulated miRNA can be used as biomarker for anticancer therapies [188].

Early B-cell lymphoma stages have greater levels of miRNA-21 expression; miRNA-200 and miRNA-17 are functionally active in cancer. The alternated expressions of miRNA in cancer were first described and studied by Chalin et al. [189]. miRNA-34a expression is reduced in breast, colon, and lung cancer [190]. The miRNA-331 expression is reduced in nasopharyngeal carcinoma. MiRNA-21, miRNA-155, miRNA-17b, and miRNA-106b has oncogenesis roles in hematological malignancies, B-cell lymphoma, breast, lung, and metastatic breast cancer, respectively [191]. Limited oxygen supply and hypoxia regulate the function of miRNA [192]. Altered miRNA genomic expressions in cancer arise from genomic variation. For instance, B-cell chronic lymphocytic leukemia has a high frequency of deletions at the chromosomal locus of the miR-15/miR-16 cluster (CLL) [193]. For instance, B-cell chronic lymphocytic leukemia frequently has genomic deletions at the miR-15/miR-16 cluster locus (CLL) [194].

Introduction of single nucleotide polymorphism (SNP) into miRNA sequence itself results in miRNA dysregulation both in epigenetic and at genetic level that leads to breast cancer [195]. A total of 50% of miRNA genes are involved in deletion, amplification, and translocations implicated tumorigenesis [196]. In fact, it is observed that 755 of miRNA genome is involved in breast cancer-affected copy number variations [196]. miRNA silencing *via* hypermethylation is observed in breast cancer with the discovery of miRNA-125b promotor in invasive breast cancer. It proves poor survival due to deletion of derepression of target gene ETSI. miRNA-335 gene is seen in breast cancer involving in suppressor of invasion, metastatic colonization, and tumor reinitiation [196]. The epigenetic discovery of miRNA discovered the new epigenetic-based cancer therapies. Mutations by XPO5 were seen during breast cancer, which is responsible for miRNA transportation to the cytoplasm. Both XOP5 and miRNA are responsible for breast cancer resulting in impaired RNA processing [197]. Genetic and epigenetic XOP5 is involved in breast cancer, while mutants are involved in the chances to get cancer [198]. While all this process, hypermethylation reduces the risk of cancer. Polymorphism, along with miRNA, is also responsible for breast cancer [199]. miRNAs are regulated by various signals released by progesterone and estrogen during breast cancer [200]. These signals dysregulate the miRNA.

7. Conclusion and prospects

MiRNAs control the physiological and developmental processes in a variety of animals, including embryos, by preventing the target genes' translation or degrading their activity. Endogenous tiny noncoding RNAs, which range in size from prokaryotes to eukaryotes, play a significant role in the transcriptional and post-transcriptional control of many genes. They control the complicated gene expression connected to the intricate process of embryogenesis throughout embryonic development. Further, study is, therefore, necessary to understand how miRNAs regulate gene expression at the posttranscriptional level, especially given that they are the most significant posttranscriptional regulators recently identified.

miRNA is playing its role in cell differentiation, proliferation, apoptosis, tumorigenesis, developmental timing, *in vitro* embryo development, and host-pathogen

interaction. miRNA is itself control by various mechanism to maintain basic functions such as SNP, miRNA editing, methylation, and circadian clock.

Proven by 24,000 peer-reviewed articles, miRNA has its role in cancer. miRNA is involved in breast cancer through non-targeting hyaluronic acid. The dysregulated miRNA is involved in the malignant phenotype of cancer cells. MiRNA has its role as a therapeutic agent. The combination of small anticancer drugs and miRNA is effective in the treatment of cancer. Reducing drug resistance, increasing apoptosis and autophagy, reducing tumor angiogenesis, and preventing the production of drug efflux are all ways to reverse the EMT are some of the useful advantages of miRNA therapies over the traditional convention ones. The use of targeted miRNA is also proved effective in therapies.

MiRNA is important in gametogenesis and spermatogenesis. During the preimplantation stage of development, the embryo releases miRNA in extracellular vesicle. miRNA is involved in oogenesis, spermatogenesis, and early embryogenesis. This also includes several proteins of miRNA, including Dicer, Drosha, and DiGeorge. Dicer1 has generalized functions in handling other short RNAs, particularly siRNAs; therefore, it is not exclusive to miRNAs. miRNA is also involved in abortion and infertility. It is seen that the expose of varies to external environment can bring changes in oogenesis in relation of miRNA. miRNA-regulated gene is important in normal spermatogenesis. The activity of miRNA changes in the testis with spermatogenesis development.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell. 2004;**116**(2):281-297

[2] Prather R, First N. A review of early mouse embryogenesis and its applications to domestic species. Journal of Animal Science. 1988;**66**(10):2626-2635

[3] Yang Y, Bai W, Zhang L, Yin G, Wang X, Wang J, et al. Determination of microRNAs in mouse preimplantation embryos by microarray. Developmental Dynamics: An Official Publication of the American Association of Anatomists. 2008;**237**(9):2315-2327

[4] Suh N, Baehner L, Moltzahn F, Melton C, Shenoy A, Chen J, et al. MicroRNA function is globally suppressed in mouse oocytes and early embryos. Current Biology. 2010;**20**(3):271-277

[5] Yang Q, Lin J, Liu M, Li R, Tian B, Zhang X, et al. Highly sensitive sequencing reveals dynamic modifications and activities of small RNAs in mouse oocytes and early embryos. Science Advances. 2016;**2**(6):e1501482

[6] Duranthon V, Watson AJ, Lonergan P. Preimplantation embryo programming: Transcription, epigenetics, and culture environment. Reproduction (Cambridge, England). 2008;**135**(2):141

[7] Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, et al. Dicer is essential for mouse development. Nature Genetics. 2003;**35**(3):215-217

[8] Alisch RS, Jin P, Epstein M, Caspary T, Warren ST. Argonaute2 is essential for mammalian gastrulation and proper mesoderm formation. PLoS Genetics. 2007;**3**(12):e227

[9] Tang F, Kaneda M, O'Carroll D, Hajkova P, Barton SC, Sun YA, et al. Maternal microRNAs are essential for mouse zygotic development. Genes & Development. 2007;**21**(6):644-648

[10] Cheong AW, Pang RT, Liu W-M, Kottawatta KSA, Lee K-F, Yeung WS. MicroRNA let-7a and dicer are important in the activation and implantation of delayed implanting mouse embryos. Human Reproduction. 2014;**29**(4):750-762

[11] Houbaviy HB, Murray MF, Sharp PA. Embryonic stem cell-specific MicroRNAs. Developmental Cell. 2003;**5**(2):351-358

[12] Salilew-Wondim D, Gebremedhn S, Hoelker M, Tholen E, Hailay T, Tesfaye D. The role of micrornas in mammalian fertility: From gametogenesis to embryo implantation. International Journal of Molecular Sciences. 2020;**21**(2):585

[13] Spruce T, Pernaute B, Di-Gregorio A, Cobb BS, Merkenschlager M, Manzanares M, et al. An early developmental role for miRNAs in the maintenance of extraembryonic stem cells in the mouse embryo. Developmental Cell. 2010;**19**(2):207-219

[14] Morales-Prieto D, Chaiwangyen W, Ospina-Prieto S, Schneider U, Herrmann J, Gruhn B, et al. MicroRNA expression profiles of trophoblastic cells. Placenta. 2012;**33**(9):725-734

[15] Foshay KM, Gallicano GI. miR-17 family miRNAs are expressed during early mammalian development and regulate stem cell differentiation.

Developmental Biology. 2009;**326**(2): 431-443

[16] Kim J, Lee J, Jun JH. Identification of differentially expressed microRNAs in outgrowth embryos compared with blastocysts and non-outgrowth embryos in mice. Reproduction, Fertility and Development. 2019;**31**(4):645-657

[17] Hinnebusch AG, Ivanov IP, Sonenberg N. Translational control by 5′-untranslated regions of eukaryotic mRNAs. Science. 2016;**352**(6292):1413-1416

[18] Date R. Bradyrhizobium effectiveness responses in Stylosanthes hamata and S. seabrana. Tropical Grasslands. 2010;**44**(3):141-157

[19] Ambros V. microRNAs: Tiny regulators with great potential. Cell. 2001;**107**(7):823-826

[20] Treiber T, Treiber N, Meister G. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. Nature Reviews Molecular cell biology. 2019;**20**(1):5-20

[21] Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? Nature Reviews Genetics. 2008;**9**(2):102-114

[22] Kura B, Szeiffova Bacova B, Kalocayova B, Sykora M, Slezak J. Oxidative stress-responsive microRNAs in heart injury. International Journal of Molecular Sciences. 2020;**21**(1):358

[23] Stavast CJ, Erkeland SJ. The non-canonical aspects of microRNAs: Many roads to gene regulation. Cell. 2019;**8**(11):1465

[24] Salim U, Kumar A, Kulshreshtha R, Vivekanandan P. Biogenesis, characterization, and functions of mirtrons. Wiley Interdisciplinary Reviews: RNA. 2022;**13**(1):e1680

[25] Olena A, Patton J. MiRNA biogenesis and function. In: Singh S, Rameshwar P, editors. MicroRNA in Development and in the Progression of Cancer. New York, NY: Springer; 2014. DOI: 10.1007/978-1-4899-8065-6_1

[26] Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, et al. MicroRNA genes are transcribed by RNA polymerase II. The EMBO Journal. 2004;**23**(20):4051-4060

[27] Michlewski G, Cáceres JF. Post-transcriptional control of miRNA biogenesis. RNA. 2019;**25**(1):1-16

[28] Liu J, Zhou F, Guan Y, Meng F, Zhao Z, Su Q, et al. The biogenesis of miRNAs and their role in the development of amyotrophic lateral sclerosis. Cell. 2022;**11**(3):572

[29] Ha M, Kim VN. Regulation of microRNA biogenesis. Nature reviews Molecular Cell Biology. 2014;**15**(8):509-524

[30] Nguyen DX, Bos PD, Massagué J. Metastasis: From dissemination to organ-specific colonization. Nature Reviews Cancer. 2009;**9**(4):274-284

[31] Siomi H, Siomi MC. Posttranscriptional regulation of microRNA biogenesis in animals. Molecular Cell. 2010;**38**(3):323-332

[32] Kataoka N, Fujita M, Ohno M. Functional association of the microprocessor complex with the spliceosome. Molecular and Cellular Biology. 2009;**29**(12):3243-3254

[33] Cullen BR. Transcription and processing of human microRNA

precursors. Molecular Cell. 2004;**16**(6):861-865

[34] Annese T, Tamma R, De Giorgis M, Ribatti D. microRNAs biogenesis, functions and role in tumor angiogenesis. Frontiers in Oncology. 2020;**10**:581007

[35] Noland CL, Doudna JA. Multiple sensors ensure guide strand selection in human RNAi pathways. RNA. 2013;**19**(5):639-648

[36] Ender C, Krek A, Friedländer MR, Beitzinger M, Weinmann L, Chen W, et al. A human snoRNA with microRNA-like functions. Molecular Cell. 2008;**32**(4):519-528

[37] Natarelli L, Weber C. A non-canonical link between non-coding RNAs and cardiovascular diseases. Biomedicine. 2022;**10**(2):445

[38] Cole C, Sobala A, Lu C, Thatcher SR, Bowman A, Brown JW, et al. Filtering of deep sequencing data reveals the existence of abundant dicer-dependent small RNAs derived from tRNAs. RNA. 2009;**15**(12):2147-2160

[39] Liu B, Cao J, Wang X, Guo C, Liu Y, Wang T. Deciphering the tRNA-derived small RNAs: Origin, development, and future. Cell Death & Disease. 2021;**13**(1):24

[40] Maute RL, Schneider C, Sumazin P, Holmes A, Califano A, Basso K, et al. tRNA-derived microRNA modulates proliferation and the DNA damage response and is down-regulated in B cell lymphoma. Proceedings of the National Academy of Sciences. 2013;**110**(4):1404-1409

[41] Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I, et al. Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control C. elegans developmental timing. Cell. 2001;**106**(1):23-34

[42] Fukunaga R, Han BW, Hung J-H, Xu J, Weng Z, Zamore PD. Dicer partner proteins tune the length of mature miRNAs in flies and mammals. Cell. 2012;**151**(3):533-546

[43] Jonas S, Izaurralde E. Towards a molecular understanding of microRNA-mediated gene silencing. Nature reviews Genetics. 2015;**16**(7):421-433

[44] Lee FC, Ule J. Advances in CLIP technologies for studies of protein-RNA interactions. Molecular Cell. 2018;**69**(3):354-369

[45] Gebert LF, MacRae IJ. Regulation of microRNA function in animals. Nature Reviews Molecular Cell Biology. 2019;**20**(1):21-37

[46] Golden RJ, Chen B, Li T, Braun J, Manjunath H, Chen X, et al. An Argonaute phosphorylation cycle promotes microRNA-mediated silencing. Nature. 2017;**542**(7640):197-202

[47] Quévillon Huberdeau M, Zeitler DM, Hauptmann J, Bruckmann A, Fressigné L, Danner J, et al. Phosphorylation of Argonaute proteins affects mRNA binding and is essential for micro RNA-guided gene silencing in vivo. The EMBO Journal. 2017;**36**(14):2088-2106

[48] Bridge KS, Shah KM, Li Y, Foxler DE, Wong SC, Miller DC, et al. Argonaute utilization for miRNA silencing is determined by phosphorylation-dependent recruitment of LIM-domain-containing proteins. Cell Reports. 2017;**20**(1):173-187

[49] Freimer JW, Krishnakumar R, Cook MS, Blelloch R. Expression of

alternative Ago2 isoform associated with loss of microRNA-driven translational repression in mouse oocytes. Current Biology. 2018;**28**(2):296-302.e3

[50] Kobayashi H, Shoji K, Kiyokawa K, Negishi L, Tomari Y. Iruka eliminates dysfunctional argonaute by selective ubiquitination of its empty state. Molecular Cell. 2019;**73**(1):119-29.e5

[51] Bell CE, Calder MD, Watson AJ. Genomic RNA profiling and the programme controlling preimplantation mammalian development. Molecular Human Reproduction. 2008;**14**(12):691-701

[52] Gerri C, Menchero S, Mahadevaiah SK, Turner JM, Niakan KK. Human embryogenesis: A comparative perspective. Annual Review of Cell and Developmental Biology. 2020;**36**:411-440

[53] Kaczmarek MM, Najmula J, Guzewska MM, Przygrodzka E. miRNAs in the peri-implantation period: Contribution to embryo–maternal communication in pigs. International Journal of Molecular Sciences. 2020;**21**(6):2229

[54] Liang J, Wang S, Wang Z. Role of microRNAs in embryo implantation. Reproductive Biology and Endocrinology. 2017;**15**:1-11

[55] Enders AC, Schlafke S, Hendrickx AG. Differentiation of the embryonic disc, amnion, and yolk sac in the rhesus monkey. American Journal of Anatomy. 1986;**177**(2):161-185

[56] Bentin-Ley U, Sjögren A, Nilsson L, Hamberger L, Larsen J, Horn T. Presence of uterine pinopodes at the embryo–endometrial interface during human implantation in vitro. Human Reproduction. 1999;**14**(2):515-520

[57] Chobotova K, Spyropoulou I, Carver J, Manek S, Heath JK, Gullick WJ, et al. Heparin-binding epidermal growth factor and its receptor ErbB4 mediate implantation of the human blastocyst. Mechanisms of Development. 2002;**119**(2):137-144

[58] Hamatani T, Carter MG, Sharov AA, Ko MS. Dynamics of global gene expression changes during mouse preimplantation development. Developmental Cell. 2004;**6**(1):117-131

[59] Song H, Lim H, Das SK, Paria BC, Dey SK. Dysregulation of EGF family of growth factors and COX-2 in the uterus during the preattachment and attachment reactions of the blastocyst with the luminal epithelium correlates with implantation failure in LIF-deficient mice. Molecular Endocrinology. 2000;**14**(8):1147-1161

[60] Mohamed OA, Jonnaert M, Labelle-Dumais C, Kuroda K, Clarke HJ, Dufort D. Uterine Wnt/β-catenin signaling is required for implantation. Proceedings of the National Academy of Sciences. 2005;**102**(24):8579-8584

[61] Gross N, Kropp J, Khatib H. MicroRNA signaling in embryo development. Biology. 2017;**6**(3):34

[62] Tian S, Su X, Qi L, Jin X-H, Hu Y, Wang C-L, et al. MiR-143 and rat embryo implantation. Biochimica et Biophysica Acta (BBA)-General Subjects. 2015;**1850**(4):708-721

[63] Li R, He J, Chen X, Ding Y, Wang Y, Long C, et al. Mmu-miR-193 is involved in embryo implantation in mouse uterus by regulating GRB7 gene expression. Reproductive Sciences. 2014;**21**:733-742

[64] Chu B, Zhong L, Dou S, Wang J, Li J, Wang M, et al. miRNA-181 regulates embryo implantation in mice through targeting leukemia inhibitory factor. Journal of Molecular Cell Biology. 2015;**7**(1):12-22

[65] Liang S, Nie Z-W, Guo J, Niu Y-J, Shin K-T, Ock SA, et al. Overexpression of microRNA-29b decreases expression of DNA methyltransferases and improves quality of the blastocysts derived from somatic cell nuclear transfer in cattle. Microscopy and Microanalysis. 2018;**24**(1):29-37

[66] Li Z, Jia J, Gou J, Tong A, Liu X, Zhao X, et al. Mmu-miR-126a-3p plays a role in murine embryo implantation by regulating Itga11. Reproductive Biomedicine Online. 2015;**31**(3):384-393

[67] Shen L-J, He J-L, Yang D-H, Ding Y-B, Chen X-M, Geng Y-Q, et al. Mmu-microRNA-200a overexpression leads to implantation defect by targeting phosphatase and tensin homolog in mouse uterus. Reproductive Sciences. 2013;**20**(12):1518-1528

[68] Bloor D, Metcalfe A, Rutherford A, Brison D, Kimber S. Expression of cell adhesion molecules during human preimplantation embryo development. MHR: Basic Science of Reproductive Medicine. 2002;**8**(3):237-245

[69] Ichimura A, Ruike Y, Terasawa K, Tsujimoto G. miRNAs and regulation of cell signaling. The FEBS Journal. 2011;**278**(10):1610-1618

[70] Inui M, Martello G, Piccolo S. MicroRNA control of signal transduction. Nature Reviews Molecular Cell Biology. 2010;**11**(4):252-263

[71] Terasawa K, Ichimura A, Sato F, Shimizu K, Tsujimoto G. Sustained activation of ERK1/2 by NGF induces microRNA-221 and 222 in PC12 cells. The FEBS Journal. 2009;**276**(12):3269-3276

[72] Taganov KD, Boldin MP, Chang K-J, Baltimore D. NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proceedings of the National Academy of Sciences. 2006;**103**(33):12481-12486

[73] Martello G, Zacchigna L, Inui M, Montagner M, Adorno M, Mamidi A, et al. MicroRNA control of nodal signalling. Nature. 2007;**449**(7159):183-188

[74] Rossant J, Tam PPL. Blastocyst lineage formation, early embryonic asymmetries and axis patterning in the mouse. Development. 2009;**136**(5): 701-713. DOI: 10.1242/dev.017178

[75] Okae H, Toh H, Sato T, Hiura H, Takahashi S, Shirane K, et al. Derivation of human trophoblast stem cells. Cell Stem Cell. 2018;**22**(1):50-63.e6

[76] Turnpenny L, Brickwood S, Spalluto CM, Piper K, Cameron IT, Wilson DI, et al. Derivation of human embryonic germ cells: An alternative source of pluripotent stem cells. Stem Cells. 2003;**21**(5):598-609

[77] Blakeley P, Fogarty NM, Del Valle I, Wamaitha SE, Hu TX, Elder K, et al. Defining the three cell lineages of the human blastocyst by single-cell RNA-seq. Development. 2015;**142**(18):3151-3165

[78] Georgantas RW III, Hildreth R, Morisot S, Alder J. Liu C-g, Heimfeld S, et al. CD34+ hematopoietic stem-progenitor cell microRNA expression and function: A circuit diagram of differentiation control. Proceedings of the National Academy of Sciences. 2007;**104**(8):2750-2755

[79] Gangaraju VK, Lin H. MicroRNAs: Key regulators of stem cells. Nature Reviews Molecular Cell Biology. 2009;**10**(2):116-125

[80] Barroso-delJesus A, Romero-López C, Lucena-Aguilar G, Melen GJ, Sanchez L, Ligero G, et al. Embryonic

stem cell-specific miR302-367 cluster: Human gene structure and functional characterization of its core promoter. Molecular and Cellular Biology. 2008;**28**(21):6609-6619

[81] Yeung F, Chung E, Guess MG, Bell ML, Leinwand LA. Myh7b/miR-499 gene expression is transcriptionally regulated by MRFs and Eos. Nucleic Acids Research. 2012;**40**(15):7303-7318

[82] Chen J-F, Tao Y, Li J, Deng Z, Yan Z, Xiao X, et al. microRNA-1 and microRNA-206 regulate skeletal muscle satellite cell proliferation and differentiation by repressing Pax7. Journal of Cell Biology. 2010;**190**(5):867-879

[83] Vecchione A, Croce CM. Apoptomirs: Small molecules have gained the license to kill. Endocrine-Related Cancer. 2010;**17**(1):F37

[84] Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM. Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in drosophila. Cell. 2003;**113**(1):25-36

[85] Zhang X, Zhang X, Hu S, Zheng M, Zhang J, Zhao J, et al. Identification of miRNA-7 by genome-wide analysis as a critical sensitizer for TRAIL-induced apoptosis in glioblastoma cells. Nucleic Acids Research. 2017;**45**(10):5930-5944

[86] Favreau AJ, Shaffiey F, Cross E, Sathyanarayana P. Mir-590 is a novel STAT5 regulated oncogenic miRNA and targets FasL in acute myeloid leukemia. Blood. 2013;**122**(21):3811

[87] Wang P, Zhuang L, Zhang J, Fan J, Luo J, Chen H, et al. The serum miR-21 level serves as a predictor for the chemosensitivity of advanced pancreatic cancer, and miR-21 expression confers chemoresistance by targeting FasL. Molecular Oncology. 2013;**7**(3):334-345

[88] Huang G, Nishimoto K, Zhou Z, Hughes D, Kleinerman ES. miR-20a encoded by the miR-17-92 cluster increases the metastatic potential of osteosarcoma cells by regulating Fas ExpressionmiR-20a regulates Fas and osteosarcoma metastatic potential. Cancer Research. 2012;**72**(4):908-916

[89] Huang X, Xiao S, Zhu X, Yu Y, Cao M, Zhang X, et al. miR-196b-5p-mediated downregulation of FAS promotes NSCLC progression by activating IL6-STAT3 signaling. Cell Death & Disease. 2020;**11**(9):785

[90] Yamada N, Noguchi S, Kumazaki M, Shinohara H, Miki K, Naoe T, et al. Epigenetic regulation of microRNA-128a expression contributes to the apoptosis-resistance of human T-cell leukaemia jurkat cells by modulating expression of fas-associated protein with death domain (FADD). Biochimica et Biophysica Acta (BBA)-molecular. Cell Research. 2014;**1843**(3):590-602

[91] Zhang J, Du Y, Wu C, Ren X, Ti X, Shi J, et al. Curcumin promotes apoptosis in human lung adenocarcinoma cells through miR-186* signaling pathway. Oncology Reports. 2010;**24**(5):1217-1223

[92] Czochor JR, Glazer PM. microRNAs in cancer cell response to ionizing radiation. Antioxidants & Redox Signaling. 2014;**21**(2):293-312

[93] Yin W, Chen J, Wang G, Zhang D. MicroRNA-106b functions as an oncogene and regulates tumor viability and metastasis by targeting LARP4B in prostate cancer. Molecular Medicine Reports. 2019;**20**(2):951-958

[94] Park JK, Doseff AI, Schmittgen TD. MicroRNAs targeting caspase-3 and-7 in

PANC-1 cells. International Journal of Molecular Sciences. 2018;**19**(4):1206

[95] Wang Y, Zhang S, Bao H, Mu S, Zhang B, Ma H, et al. MicroRNA-365 promotes lung carcinogenesis by downregulating the USP33/SLIT2/ROBO1 signalling pathway. Cancer Cell International. 2018;**18**(1):1-14

[96] Li Q, Ren P, Shi P, Chen Y, Xiang F, Zhang L, et al. MicroRNA-148a promotes apoptosis and suppresses growth of breast cancer cells by targeting B-cell lymphoma 2. Anti-Cancer Drugs. 2017;**28**(6):588-595

[97] Zhuo C, Jiang R, Lin X, Shao M. LncRNA H19 inhibits autophagy by epigenetically silencing of DIRAS3 in diabetic cardiomyopathy. Oncotarget. 2017;**8**(1):1429

[98] Zhang Y, Schiff D, Park D, Abounader R. MicroRNA-608 and microRNA-34a regulate chordoma malignancy by targeting EGFR, Bcl-xL and MET. PLoS One. 2014;**9**(3):e91546

[99] Chen Q, Xu J, Li L, Li H, Mao S, Zhang F, et al. MicroRNA-23a/b and microRNA-27a/b suppress Apaf-1 protein and alleviate hypoxia-induced neuronal apoptosis. Cell Death & Disease. 2014;**5**(3):e1132-e

[100] Ma L, Zheng Y, Tang X, Gao H, Liu N, Gao Y, et al. miR-21-3p inhibits autophagy of bovine granulosa cells by targeting VEGFA via PI3K/AKT signaling. Reproduction. 2019;**158**(5):441-452

[101] Li Z, Li W. miR-383 inhibits proliferation of granulosa cells by down-regulation of cell cycle-related proteins in mice. Xi bao yu fen zi Mian yi xue za zhi= Chinese journal of. Cellular and Molecular Immunology. 2019;**35**(6):518-525

[102] Gebremedhn S, Salilew-Wondim D, Hoelker M, Rings F, Neuhoff C, Tholen E, et al. MicroRNA-183-96-182 cluster regulates bovine granulosa cell proliferation and cell cycle transition by coordinately targeting FOXO1. Biology of Reproduction. 2016;**94(6):127**:1-11

[103] Liu J, Li X, Yao Y, Li Q, Pan Z, Li Q. miR-1275 controls granulosa cell apoptosis and estradiol synthesis by impairing LRH-1/CYP19A1 axis. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms. 2018;**1861**(3):246-257

[104] Liu J, Yao W, Yao Y, Du X, Zhou J, Ma B, et al. MiR-92a inhibits porcine ovarian granulosa cell apoptosis by targeting Smad7 gene. FEBS Letters. 2014;**588**(23):4497-4503

[105] Sirotkin V, A, Kisova G, Brenaut P, Ovcharenko D, Grossmann R, Mlyncek M. Involvement of microRNA Mir15a in control of human ovarian granulosa cell proliferation, apoptosis, steroidogenesis, and response to FSH. Microrna. 2014;**3**(1):29-36

[106] Mohammed BT, Sontakke SD, Ioannidis J, Duncan WC, Donadeu FX. The adequate corpus luteum: miR-96 promotes luteal cell survival and progesterone production. The Journal of Clinical Endocrinology & Metabolism. 2017;**102**(7):2188-2198

[107] Yuan X, Deng X, Zhou X, Zhang A, Xing Y, Zhang Z, et al. MiR-126-3p promotes the cell proliferation and inhibits the cell apoptosis by targeting TSC1 in the porcine granulosa cells. In Vitro Cellular & Developmental Biology-Animal. 2018;**54**:715-724

[108] Zhang M, Zhang Q, Hu Y, Xu L, Jiang Y, Zhang C, et al. miR-181a increases FoxO1 acetylation and promotes granulosa cell apoptosis via

SIRT1 downregulation. Cell Death & Disease. 2017;**8**(10):e3088-e

[109] Nie M, Yu S, Peng S, Fang Y, Wang H, Yang X. miR-23a and miR-27a promote human granulosa cell apoptosis by targeting SMAD5. Biology of Reproduction. 2015;**93**(4):98, 1-10

[110] Geng Y, Sui C, Xun Y, Lai Q, Jin L. MiRNA-99a can regulate proliferation and apoptosis of human granulosa cells via targeting IGF-1R in polycystic ovary syndrome. Journal of Assisted Reproduction and Genetics. 2019;**36**:211-221

[111] Yao L, Li M, Hu J, Wang W, Gao M. MiRNA-335-5p negatively regulates granulosa cell proliferation via SGK3 in PCOS. Reproduction. 2018;**156**(5):439-449

[112] Yao Y, Niu J, Sizhu S, Li B, Chen Y, Li R, et al. microRNA-125b regulates apoptosis by targeting bone morphogenetic protein receptor 1B in yak granulosa cells. DNA and Cell Biology. 2018;**37**(11):878-887

[113] Zhang L, Zhang X, Zhang X, Lu Y, Li L, Cui S. MiRNA-143 mediates the proliferative signaling pathway of FSH and regulates estradiol production. Journal of Endocrinology. 2023;**234**(1):1-14. DOI: 10.1530/JOE-16-0488

[114] Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, et al. MicroRNAs regulate brain morphogenesis in zebrafish. Science. 2005;**308**(5723):833-838

[115] Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, et al. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. Genes & Development. 2005;**19**(4):489-501

[116] Cobb BS, Nesterova TB, Thompson E, Hertweck A, O'Connor E, Godwin J, et al. T cell lineage choice and differentiation in the absence of the RNase III enzyme dicer. The Journal of Experimental Medicine. 2005;**201**(9):1367-1373

[117] Muljo SA, Ansel KM, Kanellopoulou C, Livingston DM, Rao A, Rajewsky K. Aberrant T cell differentiation in the absence of dicer. The Journal of Experimental Medicine. 2005;**202**(2):261-269

[118] Chen C-Z, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. Science. 2004;**303**(5654):83-86

[119] Harfe BD, McManus MT, Mansfield JH, Hornstein E, Tabin CJ. The RNaseIII enzyme dicer is required for morphogenesis but not patterning of the vertebrate limb. Proceedings of the National Academy of Sciences. 2005;**102**(31):10898-10903

[120] Lee RC, Ambros V. An extensive class of small RNAs in Caenorhabditis elegans. Science. 2001;**294**(5543):862-864

[121] Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Nature. 2005;**436**(7048):214-220

[122] Harvey RP. Nk-2homeobox genes and heart development. Developmental Biology. 1996;**178**(2):203-216

[123] Ahn DH, Rah H, Choi YK, Jeon YJ, Min KT, Kwack K, et al. Association of the miR-146aC> G, miR-149T> C, miR-196a2T> C, and miR-499A> G polymorphisms with gastric cancer risk and survival in the Korean

population. Molecular Carcinogenesis. 2013;**52**(S1):39-51

[124] Ciruna B, Rossant J. FGF signaling regulates mesoderm cell fate specification and morphogenetic movement at the primitive streak. Developmental Cell. 2001;**1**(1):37-49

[125] Harvey RP. Patterning the vertebrate heart. Nature Reviews Genetics. 2002;**3**(7):544-556

[126] Andrée B, Duprez D, Vorbusch B, Arnold H-H, Brand T. BMP-2 induces ectopic expression of cardiac lineage markers and interferes with somite formation in chicken embryos. Mechanisms of Development. 1998;**70**(1-2):119-131

[127] Kitajima S, Takagi A, Inoue T, Saga Y. MesP1 and MesP2 are essential for the development of cardiac mesoderm. Development. 2000;**127**(15):3215-3226

[128] Bondue A, Lapouge G, Paulissen C, Semeraro C, Iacovino M, Kyba M, et al. Mesp1 acts as a master regulator of multipotent cardiovascular progenitor specification. Cell Stem Cell. 2008;**3**(1):69-84

[129] Lough J, Barron M, Brogley M, Sugi Y, Bolender DL, Zhu X. Combined BMP-2 and FGF-4, but neither factor alone, induces cardiogenesis in non-precardiac embryonic mesoderm. Developmental Biology. 1996;**178**(1):198-202

[130] Brade T, Männer J, Kühl M. The role of Wnt signalling in cardiac development and tissue remodelling in the mature heart. Cardiovascular Research. 2006;**72**(2):198-209

[131] Bodmer R, Jan LY, Jan Y. A new homeobox-containing gene, msh-2, is transiently expressed early during mesoderm formation of drosophila. Development. 1990;**110**(3):661-669

[132] Kasahara H, Bartunkova S, Schinke M, Tanaka M, Izumo S. Cardiac and extracardiac expression of Csx/Nkx2. 5 homeodomain protein. Circulation Research. 1998;**82**(9):936-946

[133] Lyons I, Parsons LM, Hartley L, Li R, Andrews JE, Robb L, et al. Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2-5. Genes & Development. 1995;**9**(13):1654-1666

[134] Tanaka M, Chen Z, Bartunkova S, Yamasaki N, Izumo S. The cardiac homeobox gene Csx/Nkx2. 5 lies genetically upstream of multiple genes essential for heart development. Development. 1999;**126**(6):1269-1280

[135] Newman CS, Krieg PA. Tinman-related genes expressed during heart development in Xenopus. Developmental Genetics. 1998;**22**(3):230-238

[136] Cohen ED, Wang Z, Lepore JJ, Lu MM, Taketo MM, Epstein DJ, et al. Wnt/β-catenin signaling promotes expansion of Isl-1–positive cardiac progenitor cells through regulation of FGF signaling. The Journal of Clinical Investigation. 2007;**117**(7):1794-1804

[137] Lin L, Cui L, Zhou W, Dufort D, Zhang X, Cai C-L, et al. β-Catenin directly regulates Islet1 expression in cardiovascular progenitors and is required for multiple aspects of cardiogenesis. Proceedings of the National Academy of Sciences. 2007;**104**(22):9313-9318

[138] Tzahor E. Wnt/β-catenin signaling and cardiogenesis: Timing does matter. Developmental Cell. 2007;**13**(1):10-13

[139] Challice C, Virágh S. The architectural development of the early mammalian heart. Tissue and Cell. 1974;**6**(3):447-462

[140] Van Mierop L, Gessner IH. The morphologic development of the sinoatrial node in the mouse. The American Journal of Cardiology. 1970;**25**(2):204-212

[141] Buckingham M, Meilhac S, Zaffran S. Building the mammalian heart from two sources of myocardial cells. Nature Reviews Genetics. 2005;**6**(11):826-835

[142] Warburton D, El-Hashash A, Carraro G, Tiozzo C, Sala F, Rogers O, et al. Lung organogenesis. Current Topics in Developmental Biology. 2010;**90**:73-158

[143] Jiang Z, Cushing L, Ai X, Lü J. miR-326 is downstream of sonic hedgehog signaling and regulates the expression of Gli2 and smoothened. American Journal of Respiratory Cell and Molecular Biology. 2014;**51**(2):273-283

[144] Carraro G, Shrestha A, Rostkovius J, Contreras A, Chao C-M, El Agha E, et al. miR-142-3p balances proliferation and differentiation of mesenchymal cells during lung development. Development. 2014;**141**(6):1272-1281

[145] Carraro G, El-Hashash A, Guidolin D, Tiozzo C, Turcatel G, Young BM, et al. miR-17 family of microRNAs controls FGF10-mediated embryonic lung epithelial branching morphogenesis through MAPK14 and STAT3 regulation of E-cadherin distribution. Developmental Biology. 2009;**333**(2):238-250

[146] Sanford EL, Choy KW, Donahoe PK, Tracy AA, Hila R, Loscertales M, et al. MiR-449a affects epithelial proliferation during the pseudoglandular and canalicular phases of avian and mammal lung development. PLoS One. 2016;**11**(2):e0149425

[147] Sun YF, Kan Q, Yang Y, Zhang YH, Shen JX, Zhang C, et al. Knockout of microRNA-26a promotes lung development and pulmonary surfactant synthesis. Molecular Medicine Reports. 2018;**17**(4):5988-5995

[148] Wang Y, Frank DB, Morley MP, Zhou S, Wang X, Lu MM, et al. HDAC3-dependent epigenetic pathway controls lung alveolar epithelial cell remodeling and spreading via miR-17-92 and TGF-β signaling regulation. Developmental Cell. 2016;**36**(3):303-315

[149] Bhaskaran M, Wang Y, Zhang H, Weng T, Baviskar P, Guo Y, et al. MicroRNA-127 modulates fetal lung development. Physiological Genomics. 2009;**37**(3):268-278

[150] Lal CV, Olave N, Travers C, Rezonzew G, Dolma K, Simpson A, et al. Exosomal microRNA predicts and protects against severe bronchopulmonary dysplasia in extremely premature infants. JCI Insight. 2018;**3**(5)

[151] Zhang Y, Coarfa C, Dong X, Jiang W, Hayward-Piatkovskyi B, Gleghorn JP, et al. MicroRNA-30a as a candidate underlying sex-specific differences in neonatal hyperoxic lung injury: Implications for BPD. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2019;**316**(1):L144-LL56

[152] Åkerblom M, Sachdeva R, Quintino L, Wettergren EE, Chapman KZ, Manfre G, et al. Visualization and genetic modification of resident brain microglia using lentiviral vectors regulated by microRNA-9. Nature Communications. 2013;**4**(1):1770

[153] Cheng L-C, Pastrana E, Tavazoie M, Doetsch F. miR-124 regulates adult

neurogenesis in the subventricular zone stem cell niche. Nature Neuroscience. 2009;**12**(4):399-408

[154] Åkerblom M, Sachdeva R, Barde I, Verp S, Gentner B, Trono D, et al. MicroRNA-124 is a subventricular zone neuronal fate determinant. Journal of Neuroscience. 2012;**32**(26):8879-8889

[155] Krichevsky AM, Sonntag K-C, Isacson O, Kosik KS. Specific microRNAs modulate embryonic stem cell–derived neurogenesis. Stem Cells. 2006;**24**(4):857-864

[156] Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science. 2004;**303**(5654):95-98

[157] Xia H, Cheung WK, Ng SS, Jiang X, Jiang S, Sze J, et al. Loss of brain-enriched miR-124 microRNA enhances stem-like traits and invasiveness of glioma cells. Journal of Biological Chemistry. 2012;**287**(13):9962-9971

[158] Sanuki R, Onishi A, Koike C, Muramatsu R, Watanabe S, Muranishi Y, et al. miR-124a is required for hippocampal axogenesis and retinal cone survival through Lhx2 suppression. Nature Neuroscience. 2011;**14**(9):1125-1134

[159] Paul AB, Sadek ST, Mahesan AM. The role of microRNAs in human embryo implantation: A review. Journal of Assisted Reproduction and Genetics. 2019;**36**:179-187

[160] Paulson EE, Fishman EL, Ma J, Schultz RM, Ross PJ. Embryonic microRNAs are essential for bovine preimplantation embryo development. Proceedings of the National Academy of Sciences. 2022;**119**(45):e2212942119

[161] Hatfield S, Shcherbata H, Fischer K, Nakahara K, Carthew R, Ruohola-Baker H. Stem cell division is regulated by the microRNA pathway. Nature. 2005;**435**(7044):974-978

[162] Lei L, Jin S, Gonzalez G, Behringer RR, Woodruff TK. The regulatory role of dicer in folliculogenesis in mice. Molecular and Cellular Endocrinology. 2010;**315**(1-2):63-73

[163] Kaneda M, Tang F, O'Carroll D, Lao K, Surani MA. Essential role for Argonaute2 protein in mouse oogenesis. Epigenetics & Chromatin. 2009;**2**:1-8

[164] Gebremedhn S, Salilew-Wondim D, Ahmad I, Sahadevan S, Hossain MM, Hoelker M, et al. MicroRNA expression profile in bovine granulosa cells of preovulatory dominant and subordinate follicles during the late follicular phase of the estrous cycle. PLoS One. 2015;**10**(5):e0125912

[165] Salilew-Wondim D, Ahmad I, Gebremedhn S, Sahadevan S, Hossain MM, Rings F, et al. The expression pattern of microRNAs in granulosa cells of subordinate and dominant follicles during the early luteal phase of the bovine estrous cycle. PLoS One. 2014;**9**(9):e106795

[166] Yuan S, Schuster A, Tang C, Yu T, Ortogero N, Bao J, et al. Sperm-borne miRNAs and endo-siRNAs are important for fertilization and preimplantation embryonic development. Development. 2016;**143**(4):635-647

[167] Wainwright EN, Jorgensen JS, Kim Y, Truong V, Bagheri-Fam S, Davidson T, et al. SOX9 regulates microRNA miR-202-5p/3p expression during mouse testis differentiation. Biology of Reproduction. 2013;**89**(2):34, 1-12

[168] Gao H, Wen H, Cao C, Dong D, Yang C, Xie S, et al. Overexpression of microRNA-10a in germ cells causes male

infertility by targeting Rad51 in mouse and human. Frontiers in Physiology. 2019;**10**:765

[169] Wang Z, Yao H, Lin S, Zhu X, Shen Z, Lu G, et al. Transcriptional and epigenetic regulation of human microRNAs. Cancer Letters. 2013;**331**(1):1-10

[170] Rayner KJ, Hennessy EJ. Thematic review series: Functional regulation of lipid homeostasis by microRNA: Extracellular communication via microRNA: Lipid particles have a new message. Journal of Lipid Research. 2013;**54**(5):1174

[171] Narry KV, Nam J-W. Genomics of microRNA. TRENDS in Genetics. 2006;**22**(3):165-173

[172] Eder M, Scherr M. MicroRNA and lung cancer. New England Journal of Medicine. 2005;**352**(23):2446-2448

[173] Monzo M, Navarro A, Bandres E, Artells R, Moreno I, Gel B, et al. Overlapping expression of microRNAs in human embryonic colon and colorectal cancer. Cell Research. 2008;**18**(8):823-833

[174] Vidigal JA, Ventura A. Embryonic stem cell miRNAs and their roles in development and disease. In: Seminars in Cancer Biology. Vol. 22. Issue 5-6. Academic Press, Elsevier; 2012. pp. 428-436

[175] Lian J, Tian H, Liu L, Zhang X, Li W, Deng Y, et al. Downregulation of microRNA-383 is associated with male infertility and promotes testicular embryonal carcinoma cell proliferation by targeting IRF1. Cell Death & Disease. 2010;**1**(11):e94-e

[176] Grive KJ. Pathways coordinating oocyte attrition and abundance during mammalian ovarian reserve establishment. Molecular Reproduction and Development. 2020;**87**(8):843-856

[177] Varga ZV, Kupai K, Szűcs G, Gáspár R, Pálóczi J, Faragó N, et al. MicroRNA-25-dependent up-regulation of NADPH oxidase 4 (NOX4) mediates hypercholesterolemia-induced oxidative/nitrative stress and subsequent dysfunction in the heart. Journal of Molecular and Cellular Cardiology. 2013;**62**:111-121

[178] Zhang H, Zuo Z, Lu X, Wang L, Wang H, Zhu Z. MiR-25 regulates apoptosis by targeting Bim in human ovarian cancer. Oncology Reports. 2012;**27**(2):594-598

[179] Lingenfelter BM, Tripurani SK, Tejomurtula J, Smith GW, Yao J. Molecular cloning and expression of bovine nucleoplasmin 2 (NPM2): A maternal effect gene regulated by miR-181a. Reproductive Biology and Endocrinology. 2011;**9**:1-9

[180] Chen C, Zhang Y, Zhang L, Weakley SM, Yao Q. MicroRNA-196: Critical roles and clinical applications in development and cancer. Journal of Cellular and Molecular Medicine. 2011;**15**(1):14-23

[181] Lee MR, Prasain N, Chae H-D, Kim Y-J, Mantel C, Yoder MC, et al. Epigenetic regulation of NANOG by miR-302 cluster-MBD2 completes induced pluripotent stem cell reprogramming. Stem Cells. 2013;**31**(4):666-681

[182] Qi L, Hongjuan H, Ning G, Zhengbin H, Yanjiang X, Tiebo Z, et al. miR-370 is stage-specifically expressed during mouse embryonic development and regulates Dnmt3a. FEBS Letters. 2013;**587**(6):775-781

[183] Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N,

et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. Journal of the American Medical Association. 2008;**299**(4):425-436

[184] Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Research. 2005;**65**(14):6029-6033

[185] Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. Nature Reviews Cancer. 2015;**15**(6):321-333

[186] Chen K, Boettiger A, Moffitt J, Wang S, Zhuang X. Spatially resolved, highly multiplexed RNA profiling in single cells. Science. 2015;**348**(6233)

[187] Stupar RM. Into the wild: The soybean genome meets its undomesticated relative. Proceedings of the National Academy of Sciences. 2010;**107**(51):21947-21948

[188] Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nature Reviews Genetics. 2009;**10**(10):704-714

[189] Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proceedings of the National Academy of Sciences. 2002;**99**(24):15524-15529

[190] Xuefang Z, Ruinian Z, Liji J, Chun Z, Qiaolan Z, Jun J, et al. RETRACTED: miR-331-3p inhibits proliferation and promotes apoptosis of nasopharyngeal carcinoma cells by targeting elf4B-PI3K-AKT pathway. Technology in Cancer Research & Treatment. 2020;**19**:1533033819892251

[191] Medina PP, Nolde M, Slack FJ. OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma. Nature. 2010;**467**(7311):86-90

[192] Franovic A, Gunaratnam L, Smith K, Robert I, Patten D, Lee S. Translational up-regulation of the EGFR by tumor hypoxia provides a nonmutational explanation for its overexpression in human cancer. Proceedings of the National Academy of Sciences. 2007;**104**(32):13092-13097

[193] Allegra D, Bilan V, Garding A, Döhner H, Stilgenbauer S, Kuchenbauer F, et al. Defective DROSHA processing contributes to downregulation of MiR-15/−16 in chronic lymphocytic leukemia. Leukemia. 2014;**28**(1):98-107

[194] Zhao JL, Starczynowski DT. Role of microRNA-146a in normal and malignant hematopoietic stem cell function. Frontiers in Genetics. 2014;**5**:219

[195] Melo SA, Esteller M. Dysregulation of microRNAs in cancer: Playing with fire. FEBS Letters. 2011;**585**(13):2087-2099

[196] Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, et al. microRNAs exhibit high frequency genomic alterations in human cancer. Proceedings of the National Academy of Sciences. 2006;**103**(24):9136-9141

[197] Melo SA, Moutinho C, Ropero S, Calin GA, Rossi S, Spizzo R, et al. A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells. Cancer Cell. 2010;**18**(4):303-315

[198] Leaderer D, Hoffman AE, Zheng T, Fu A, Weidhaas J, Paranjape T, et al. Genetic and epigenetic association studies suggest a role of microRNA biogenesis gene exportin-5 (XPO5) in breast tumorigenesis. International

Journal of Molecular Epidemiology and Genetics. 2011;**2**(1):9

[199] Sung H, Jeon S, Lee K-M, Han S, Song M, Choi J-Y, et al. Common genetic polymorphisms of microRNA biogenesis pathway genes and breast cancer survival. BMC Cancer. 2012;**12**(1):1-12

[200] Yang Z, Wang L. Regulation of microRNA expression and function by nuclear receptor signaling. Cell & Bioscience. 2011;**1**(1):1-9