

## Chapter 2

# miRNA Biology in Pathological Processes

**Abstract** miRNAs are small (~22 nucleotides) noncoding RNAs, highly conserved in both plants and animals, which mainly function in posttranscriptional regulation through directly degrading target mRNAs or inhibiting the translation. In other words, the final roles of miRNAs are mediated by the regulation of their target genes, which are involved in a series of important pathophysiological events, such as embryonic development, metabolism, cell proliferation and differentiation, tumorigenesis, immune defense, etc. According to their roles as a disease driving force or accompanying feature, miRNAs could be used as molecular therapeutic targets or potential diagnostic/prognostic biomarkers, respectively. Here, we review the latest discoveries of miRNAs alteration involved in common human diseases and discuss their roles in diseases initiation and progression. On the basis of the increasing knowledge on miRNAs, it could be inferred that we might be able to precisely modulate the tissue or cell-specific miRNA levels and this would lead to a new revolution in medical treatment in the future.

**Keywords** Small noncoding RNAs • miRNAs biogenesis • Pathological processes • Inherited disease • Cancer initiation and progression • Cardiovascular disease • Diabetes mellitus

miRNA genes, located in intergenic regions as independent units or in introns of host genes, are generously transcribed by RNA polymerase II (Pol II). The resulting transcripts, named primary miRNAs (pri-miRNAs), are cleaved by “Microprocessor complex,” which is mainly formed by the DiGeorge syndrome critical region 8 (DGCR8) and the enzyme Drosha. The corresponding products, often termed as precursor miRNAs (pre-miRNAs), are then exported out of the nucleus by the nucleocytoplasmic shuttler Exportin-5. In the cytoplasm, pre-miRNAs are further cleaved by the RNase III enzyme Dicer to form an imperfect miRNA: miRNA\* duplex, which are about 22 nucleotides in length. Although

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either strand of the duplex might be functional miRNA, it must be incorporated into the RNA-induced silencing complex (RISC) to fulfill its function.

Due to the common features shared by miRNA genes and traditional DNA genes, it could be inferred that the regulating miRNAs might be regulated as well. Indeed, many miRNA genes could be transcriptionally regulated or posttranscriptionally edited, just like traditional genes. This expands the diversity and scope of miRNA function beyond that implicated from just the genome. Therefore, miRNA genes could be expressed in a time and tissue/cell-specific way under fine-tunings. It presents a new regulation layer of gene expression, which ensures that the body could make a rapid and accurate response to various external stimuli. Given that the expression of each individual miRNA is under strict regulation, the altered expression of certain miRNAs plays an important role in human diseases. Specifically, the expression changes could be induced by gene abnormalities, transcriptional or posttranscriptional alteration. Due to the limited space of this book, we mainly discuss the relationship of miRNAs with common diseases, such as inherited diseases, cancer, vascular diseases, diabetes, and so on.

## 2.1 miRNAs in Inherited Diseases

The first miRNA, named Lin-4, was discovered by Ambros in *Caenorhabditis elegans* in 1993 [1]. He found that Lin-4 is essential for the normal temporal control of diverse postembryonic developmental events in *C. elegans*. Specifically, adult Lin-4 loss-of function mutants lack many adult structures and they cannot lay eggs because of a failure to develop a vulva, so the eggs accumulate within their bodies [2]. Similarly, it could be inferred that some miRNA mutations might be involved in human inherited diseases due to the dysregulation of their targets. Until now, many hereditary diseases have been found to be caused by germline abnormalities in miRNA or miRNA-related genes [3].

### 2.1.1 Hereditary Deafness Caused by Mutations of miRNA-96 Gene

Growing evidence has demonstrated that both the mutations in certain miRNA genes and the duplication or deletion of miRNA genes could cause human inherited diseases. Although rare, several human single-gene disorders, which are associated closely with point mutations in miRNA genes have been reported till date. These mutations alter the subsequent processing and target recognition of the miRNAs. The first study of a Mendelian disorder that is associated with mutations in an miRNA gene is reported by Mencía et al. [4]. They found that point mutations in the seed region of miR-96, an miRNA expressed in hair cells of the inner ear, are responsible for nonsyndromic progressive hearing loss. According

to the literature, miRNA-96 is a member of the miRNA-183 family (miRNA-183, miRNA-96, and miRNA-182), which is expressed abundantly in specific types of sensory cells and is required for the physiological development of sensory hair cells [5–7]. The identified mutations are located in the seed region of miRNA-96, which indicate that the mutations result in either the loss of regulation of genes targeted by wild-type miRNA-96 or acquired repression of genes targeted by the mutant miRNA-96, or both. Interestingly, a recent study has found a novel mutation in the seed region of miRNA-96\* in an Italian family with autosomal dominant hearing loss [8]. These studies have shown that point mutations in the miRNA-96 gene might play pivotal roles in severe hereditary deafness through affecting the physiological development of sensory hair cells.

### ***2.1.2 Familial Keratoconus with Cataract Due to Mutations of the miRNA-184 Gene***

The second example of a human disease that is closely related to mutations in the seed region of an miRNA gene is familial keratoconus with cataract. Hughes and colleagues identified a heterozygous C-to-T transition within the seed region of miRNA-184 by deep sequencing of a linkage region known to contain the mutation [9]. The same point mutation was reported subsequently by another group using bidirectional sequencing [10]. The report suggested that the single-base-pair substitution in the seed region of miRNA-184 is responsible for the corresponding disease phenotype. Recently, two novel heterozygous substitution mutations in miRNA-184 were identified in two patients with isolated keratoconus: miRNA-184(+8C>A) and miRNA-184(+3A>G) [11]. Computational modeling predicted that these mutations would be involved in pathogenesis by altering the miRNA-184 stem-loop stability and secondary structure.

### ***2.1.3 Congenital Malformations Induced by Deletion of the miRNA-17~92 Cluster***

In addition to miRNA gene mutations, the duplication, deletion, or inversion of miRNA genes could cause also human inherited diseases. The gene regions that are altered in individuals with chromosomal abnormalities sometimes harbor both protein-coding genes and miRNA genes [12, 13]. Although previous studies mainly focused on the roles of protein-coding gene changes in diseases, the aberrant expression of miRNAs might cause some of the diseases. Here, one example of disease with chromosomal abnormalities that is associated with the dysregulation of miRNAs is introduced as the representative. In 2011, de Pontual and colleagues reported the identification of germline hemizygous deletions of miRNA17HG; encoding the miRNA-17~92 polycistronic miRNA cluster,

in individuals with microcephaly, short stature, and digital abnormalities [14]. Through the establishment of a mouse model, they demonstrated that insufficiency of miRNA-17~92 is responsible for these developmental abnormalities. It is the first example of an miRNA gene responsible for a syndromic developmental defect in humans.

#### ***2.1.4 Other Inherited Diseases Closely Related to miRNA Alterations***

Besides, many other miRNAs have been reported to be associated with inherited diseases, such as Down's Syndrome, Duchenne muscular dystrophy, methylmalonic acidemia, familial non-medullary thyroid cancer, and others [15–18]. Among these, although the microRNA genes are normal, their expression levels could change significantly during pathogenesis and progression.

Interestingly, the various mutations of miRNA processing enzymes or cofactors, including Drosha, DGCR8, Exportin-5, Dicer, TRBP, and AGO2, result in the reduced efficiency of miRNA processing, which could also lead to human diseases. For example, haploinsufficiency of the DGCR8 gene, induced by hemizygous microdeletions of the 22q11.2 locus, could contribute to the behavioral and cognitive deficits observed in DiGeorge syndrome [19]. Given that mutations in miRNA genes could induce human inherited diseases, it can be inferred that the variants with different miRNA-binding sites in the 3'UTRs of target mRNAs are also associated with diseases. In this regard, Simon and colleagues have found that a mutation in the miRNA-433 binding site of the HDAC6 gene is associated with X-linked dominant chondrodysplasia [20].

## **2.2 miRNAs in Cancer**

Both miRNAs and cancer are very hot and ever-changing fields of biomedical research. Growing evidence has shown that miRNAs play pivotal roles in the whole process of cancer development, including tumor initiation, progression, metastasis, and even the final outcome. Generously, miRNAs repress the expression of target genes through reducing the mRNA stability or inhibiting the following translation. It is estimated that about two-thirds of all protein-coding genes could be targeted and then regulated by miRNAs. These targets include genes that mediate different stages of tumorigenesis, such as inflammation, cell cycle regulation, stress response, differentiation, apoptosis, invasion, metastasis, and so on. On the other hand, it is not unexpected that miRNAs are involved in various aspects of cancer through the regulation of oncogenes and tumor suppressor genes. In this regard, cancer-related miRNAs could be divided into two kinds depending on their functions, namely the onco-miRNAs which inhibit tumor suppressor genes

and tumor suppressor miRNAs which inhibit oncogenes. Therefore, the mutation or dysregulation of miRNAs could play important roles in tumor initiation and development.

### ***2.2.1 Genetic Changes of miRNAs in Cancer***

Single nucleotide polymorphisms (SNPs) have been identified in many protein-coding genes and some of these variants have been found to be associated with cancer risk [21, 22]. It is not unexpected that the SNPs could be closely related to cancer risk or even prognosis. One of the most recent examples of miRNAs SNPs in cancer is the miRNA-196a2 SNP rs11614913. Du and colleagues found that the CC genotype was associated with the significantly decreased expression of miRNA-196a-5p in some renal cell cancer (RCC) tissues. Moreover, luciferase reporter assays revealed that this SNP could potentially affect the binding efficiency of miRNA-196a-3p to its targets. It showed that this SNP may contribute to the genetic susceptibility and prognosis for RCC, which may act as a biomarker for RCC occurrence and prognosis [23]. Similarly, other SNPs within miRNA genes could be associated with cancer risk as well. For instance, a single SNP in the miRNA-499 gene is closely related to the risk of various cancers including breast cancer, squamous cell carcinoma of the head and neck, and hepatocellular carcinoma [24–27]. However, further study is needed to elucidate the detailed mechanisms.

In addition, point mutations could also be associated with cancer risk. Using miRNA-16-1 as an example, in 2005, a germline point mutation in the miRNA-16-1-miRNA-15a primary precursor, which caused low levels of miRNA expression *in vitro* and *in vivo*, was identified in patients with chronic lymphocytic leukemia (CLL) [28]. This suggested that the mutations in miRNA transcripts are common and may have functional importance. Aside from SNPs and point mutations, the most common chromosomal abnormality identified in CLL is deletion of 13q14.3, which could cause both hemizygous and homozygous loss [29]. This was also found in other tumors, including multiple myeloma, diffuse large B-cell lymphoma (DLBCL), and prostate cancer, which suggests that this region harbors tumor suppressor genes [30–32]. Combining with mouse models, Klein and colleagues found that miRNA-15a/16-1-deletion could accelerate the proliferation of both human and mouse B cells by modulating the expression of genes controlling cell-cycle progression [33].

Interestingly, SNPs within miRNA binding sites could be a novel genre of cancer biomarkers as well. Given that miRNA regulation is dependent on sequence complementarity between the target mRNA and miRNA seed region, it can be inferred that even single nucleotide alterations have significant effects. In recent years, many examples of such functional SNPs within the miRNA binding site have been identified as cancer biomarkers [34]. For instance, Dzikiewicz and colleagues found that variant alleles of TLX1\_rs2742038 and ETV6\_rs1573613 were

associated with increased risk of childhood ALL, while PML\_rs9479 was associated with decreased ALL risk. Using luciferase reporter assays, it was revealed that SNPs within an microRNA-binding site could modulate leukemia risk by interfering with the miRNA-mediated regulation [35].

### ***2.2.2 Alterations of miRNAs Expression in Cancer***

As reported by many studies, miRNAs play more and more important roles in the whole process of tumor development, such as cancer initiation, progression, metastasis, and so on. Here, we will take some typical examples to illustrate the specific mechanisms involved in various steps of cancer development: For the first step, miRNAs expression might be associated with carcinogenesis. Through qPCR detection, Wang and colleagues found that miRNA-185 expression decreased in human breast cancer tissues compared with healthy tissue controls. In addition, upregulation of miRNA-185 could inhibit breast cancer cell proliferation and invasion and vice versa. Using bioinformatics techniques and a dual luciferase reporter system, they found that miRNA-185 was shown to bind to the 3'-untranslated region (UTR) of vascular endothelial growth factor a (Vegfa), which was found to be high in human breast cancer tissues [36]. Coincidentally, Zhang and colleagues revealed that miRNA-125b is abundantly expressed in both human and mouse, particularly at early stages of malignant progression to squamous cell carcinoma (SCC). Through further molecular and genetic analysis of miRNA-125b targets, they uncovered new insights underlying miRNA-125b's oncogenic function. On the one hand, miRNA-125b directly represses stress-responsive MAP kinase genes and associated signaling. On the other hand, it indirectly prolongs activated (phosphorylated) EGFR signaling [37]. These findings suggested that miRNAs could be associated with cancer initiation by targeting key molecules of cell proliferation and differentiation pathways.

For the second step, accumulating evidence has indicated that miRNAs act as critical regulators in tumor progression. Recently, Wang and colleagues reported that miRNA-199a-3p was significantly upregulated in gastric cancer (GC) cell lines and tissues. Functional studies demonstrated that miRNA-199a-3p dramatically increased cell proliferation and suppressed cell apoptosis both in vitro and in vivo by targeting the transcriptional regulator zinc fingers and homeoboxes 1 (ZHX1) [38]. Similarly, Zhang and colleagues reported that miRNA-214 was overexpressed in nasopharyngeal carcinoma (NPC) cell lines and tissues. Silencing of miRNA-214 by LNA-antimiRNA-214 in NPC cells resulted in promoting apoptosis and suppressing cell proliferation in vitro, while it suppressed tumor growth in nude mice in vivo. In addition, Bim was identified as a direct target of miRNA-214 by luciferase reporter assay [39]. This suggested that miRNAs could be associated with cancer progression by regulating key molecules of cell proliferation and apoptosis pathways.

For the third step, angiogenesis is a fundamental characteristic of cancer and is necessary in its multi-step progression. Although evidence for arsenite-induced lung cancer in humans is strong, the molecular mechanisms by which arsenite causes cancer remain to be established in practice. During a recent investigation, Zhao and colleagues evaluated the mechanism for arsenite-induced angiogenesis. They found that the knockdown of miRNA-21 could prevent tumors, which were formed from human bronchial epithelial (HBE) cells transformed by arsenite, from developing new blood vessels. Furthermore, downregulation of miRNA-21 in human umbilical vein endothelial cells (HUVEC) might inhibit the arsenite-induced increases of VEGF levels, which promotes angiogenesis. Thus, it is concluded that miRNA-21 could mediate tumor angiogenesis induced by arsenite [40]. Additionally, Kumar and colleagues demonstrated that ectopic expression of miRNA-34a in head and neck squamous cell carcinoma (HNSCC) cell lines significantly inhibited tumor cell proliferation, colony formation, and migration. Through an SCID mouse xenograft model, they found that ectopic expression of miRNA-34a also significantly inhibited tumor growth and tumor angiogenesis, which is mediated by blocking VEGF production as well as directly inhibiting endothelial cell functions [41]. This suggested that miRNAs could be associated with cancer initiation and progression by targeting key molecules in angiogenesis.

Finally metastasis, which can be regulated by miRNAs, causes most cancer deaths. Comparing the expression of miRNAs in metastatic and nonmetastatic primary mouse sarcomas, Sachdeva and colleagues found that miRNA-182 was markedly overexpressed in some tumors that metastasized to the lungs. By utilizing genetically engineered mice, they discovered that deletion of miRNA-182 substantially decreased, while overexpression of miRNA-182 considerably increased the rate of lung metastasis. Moreover, overexpression of miRNA-182 increased circulating tumor cells (CTCs), while deletion of miRNA-182 decreased CTCs, suggesting that miRNA-182 regulates invasion of cancer cells into the circulation. They identified four miRNA-182 targets that inhibit either the migration of tumor cells or the degradation of the extracellular matrix [42]. In addition, Wang and colleagues demonstrated that miRNA-133a expression negatively correlates with cell invasiveness in both transformed normal bronchial epithelial cells and lung cancer cell lines. miRNA-133a can inhibit cell invasiveness and cell growth through suppressing the expressions of three oncogenic receptors, including insulin-like growth factor 1 receptor (IGF-1R), TGF-beta receptor type-1 (TGFBR1), and epidermal growth factor receptor (EGFR) [43]. These results demonstrate that a single miRNA can regulate metastasis by coordinated regulation of multiple genes.

## 2.3 miRNAs in Cardiovascular Development and Disease

Cardiovascular disease is the leading cause of morbidity and mortality in developed countries and its incidence has increased gradually in developing countries. The implications of miRNAs in the pathological mechanism of cardiovascular



disease have widely been recognized, and research on their relationship has now become one of the most rapidly evolving fields. Many studies have demonstrated that miRNAs are abnormally expressed in the cardiovascular system under some pathological conditions. Using in vitro and in vivo models, both gain- and loss-of-function studies have revealed various roles for specific miRNAs in cardiovascular development, and physiological processes. Here, we review the latest studies that show the association of miRNAs with different aspects of cardiovascular disease.

### ***2.3.1 miRNAs in Cardiovascular Development***

Previous studies indicated that miRNAs play pivotal roles in proper cardiac development. However, their specific temporal and spatial functions during organogenesis are largely unknown. The results of inhibition of miRNA expression have been tested by deletion of Dicer, which is the essential RNase for miRNA biosynthesis. Interestingly, using Cre recombinase under control of cardiac regulatory DNA sequences to achieve cardiac deletion of Dicer could result in lethality at different developmental stages; which depends on the temporal expression pattern of the Cre transgene [44, 45]. Similarly, using a tamoxifen-inducible Cre recombinase to delete Dicer in the adult heart could cause heart failure and death [46]. Although these reports verify the importance of miRNAs in heart development and function, it is still unclear which miRNAs play decisive roles in the process.

miRNA-1, which is highly conserved from fruit flies to humans, was the first miRNA to be implicated in heart development. Interestingly, miRNA-1 and miRNA-133 are generated from a common bicistronic transcript in vertebrates, whereas these miRNAs are transcribed separately in invertebrates. Research on embryonic stem (ES) cells has revealed roles for miRNA-1 and miRNA-133 in the specification of mesodermal cell fates. However, miRNA-1 and miRNA-133 have opposite effects on the differentiation of muscle lineages: miRNA-1 promotes differentiation of ES cells toward a cardiac fate, whereas miRNA-133 inhibits the process. It has been suggested that miRNA-1 exerts its effects by targeting the Notch ligand Delta-like (Dll-1) [47]. Both miRNA-1 and miRNA-133a could regulate the fundamental aspects of cardiac growth and development. Furthermore, miRNA-1 could control cardiac rhythm and remodeling through modulating numerous ion channels involved in cardiac conduction.

Additionally, many other miRNAs have been found to be involved in cardiac development. For example, miRNA-138 is specifically expressed in the ventricular chamber of the zebrafish heart and plays pivotal roles in the control of cardiac patterning. Some research has shown that miRNA-138 plays similar roles in patterning of the mammalian four-chambered heart because it is conserved from zebrafish to human [48]. This will be interesting to investigate in future studies. In addition, although miRNA-143/145 has different sequences, they are transcribed as a bicistronic unit. These miRNAs are cardiovascular-specific miRNAs and play key roles in modulating vascular smooth muscle cell (VSMC) phenotypes between



differentiated, proliferative, or migratory states in response to vascular injury or growth factor signaling [49]. Specifically, both miRNA-143 and 145 could target various genes involved in the regulation of SRF activity and actin dynamics to promote differentiation, repress proliferation of VSMCs, and modulate cytoskeletal assembly and dynamics [50, 51].

Despite current progress in the roles of miRNAs in cardiovascular development, our understanding of the specific mechanism is far from complete and numerous conceptual and experimental questions remain to be solved in future studies. Till now, only a small part of the miRNAs expressed in the cardiovascular system has been functionally determined by study. Identification and verification of additional miRNAs and further analysis of the functions of their targets might provide innovative insights into mechanisms of cardiovascular development, function, and dysfunction [52].

### ***2.3.2 miRNAs in Cardiovascular Diseases***

Expression profiles of miRNAs have been identified and verified in a variety of cardiovascular disorders, such as hypertrophy, heart failure, ischemic cardiomyopathy, myocardial infarction, and so on. According to the multifactorial nature of cardiovascular disease, it could be inferred that miRNAs might orchestrate many aspects of disease progression, from regulating metabolic risk factors (e.g., cholesterol and hormones) to controlling the response to acute cardiovascular events (e.g., inflammation and hypoxia) [53].

At the very beginning, miRNAs regulate lipid metabolism, lipoprotein clearance, and the pro- or anti-atherogenic effects in multiple organs. As we know, low-density lipoprotein (LDL) delivers cholesterol and phospholipids from the liver to tissues in need, whereas high-density lipoprotein (HDL) carries redundant lipids away from peripheral tissues back to the liver for excretion. Both high levels of LDL cholesterol and low levels of HDL cholesterol are independent risk factors for the development of atherosclerosis and its downstream disorders. Based on knockdown experiments using antisense technology, Najafi-Shoushtari and colleagues found that miRNA-33 acts in concert with the SREBP host genes to control cholesterol homeostasis and suggested that miRNA-33 may represent a therapeutic target for ameliorating cardiometabolic diseases [54]. Work by Ramírez and colleagues suggest that miRNA-144 regulates cholesterol metabolism via suppressing ABCA1 expression and modulation of miRNAs may represent a potential therapeutic intervention for treating dyslipidemia and atherosclerotic vascular disease [55]. Unlike that of HDL, the miRNA-mediated regulation of LDL cholesterol has been less documented. miRNA-122, which is the most plentiful miRNA expressed in liver, controls both LDL and HDL cholesterol levels, mainly by indirect modulation of cholesterol biosynthesis [56].

Coronary atherosclerosis is one of the most common cardiovascular diseases. Till now, various studies have identified many risk factors for it, including

hypertension, dyslipidemia, overweight/obesity, high blood sugar/diabetes, unhealthy lifestyle, smoking, unreasonable diet, excessive drinking, and so on. Interestingly, cardiovascular inflammation or injury is common in early molecular events of coronary atherosclerosis. Inflammation in the vessel wall is regulated by multiple miRNAs, including miRNA-126, miRNA-143/145, miRNA-155, miRNA-342-5p, and so on [53]. For example, miRNA-126 in endothelial cells (ECs) directly targets the 3'untranslated region of VCAM-1 mRNA to repress its expression, and inhibition of miRNA-126 could upregulate VCAM-1 expression and leukocyte adherence to ECs [57]. In addition, EC apoptotic bodies shed microvesicles containing miRNA-126, which represses RGS16 (regulator of G protein signaling 16) in arteries, inhibits the expression of inflammatory chemokine CXCL12 (chemokine (C-X-C motif) ligand 12), and then decreases the recruitment of inflammatory cells. This further reduces inflammation and overall stabilizes the atherosclerotic plaque [58]. miRNAs also play pivotal roles in cardiac fibrosis, hypertrophy, and remodeling and repair post-ischemic injury or myocardial infarction (MI). Although miRNA-21 is the most well studied miRNA in the post-MI environment, its role in cardiac remodeling remains controversial. Thum and colleagues found that blocking of miRNA-21 inhibits ERK-MAPK kinase pathways in cardiac fibroblasts through miRNA-21's specific target, *Spry1*, resulting in the attenuation of interstitial fibrosis and cardiac hypertrophy and dysfunction [59]. In contrast, another group used locked nucleic acid modified anti-miRNA oligonucleotides to inhibit miRNA-21 and verified that no difference was found in pathological cardiac remodeling in response to cardiac stress, which was further confirmed in an miRNA-21-knockout model [60].

In summary, the understanding that miRNAs play pivotal roles in modulation of inherited and acquired diseases of a cardiovascular system provides a new perspective on these disorders and has shown innovative cellular mechanisms of disease and potential new therapeutic targets. Therefore, the results that abnormal miRNA expression patterns contribute at various levels to the pathogenesis of cardiovascular disease has brought about considerable optimism for their use as therapeutic targets.

## 2.4 miRNAs in Diabetes Mellitus

According to the high prevalence and associated complications, diabetes mellitus (DM) is a major socioeconomic health problem worldwide. Diabetes is currently the most common metabolic disorder and its prevalence is rapidly increasing in both developed and developing countries. Specifically, diabetes affected 285 million adults in 2010, and it is expected to ascend to 7.7 % or 439 million adults by 2030 [61]. Diabetes is a complex metabolic disorder with an etiology that includes genetic, environmental, and lifestyle factors that lead to several different defects of glucose homeostasis. Depending on the distinct pathogenesis, diabetes could be classified into two forms: type I and type II. Between these, type I mostly occurs

in children and adolescents. This type is associated with absolute insulin deficiency due to a destruction of pancreatic  $\beta$  cells, often subsequent to autoimmune  $\beta$ -cell destruction. Type II, often seen in older people, is caused by peripheral insulin resistance and  $\beta$ -cell dysfunction. It can clearly be seen that the absolute deficiency or relative lack of insulin is the root cause of diabetes. Till now, many studies suggest that miRNAs regulate multiple biological processes, including the pathogenesis of diabetes.

### ***2.4.1 miRNAs Involved in $\beta$ Cell Development and Function***

Pancreatic  $\beta$  cells, located in the islet of Langerhans, secrete insulin in response to increased blood glucose concentration, thereby maintaining glucose homeostasis within the body. These cells play key roles in the pathogenesis and progression of diabetes. Obviously, a variety of genes in them are distinctly regulated in response to alterations in blood glucose. Many researchers examined the contribution of miRNAs to this process.

In order to assess the overall contribution of miRNAs to pancreatic development, Dicer1, an essential enzyme for miRNA processing, was conditionally deleted from an embryonic developing mouse pancreas. The results showed that the expression of a unique profile of miRNAs is required during pancreas development and is necessary for  $\beta$  cell formation [62]. A recent study by Wang and colleagues shows that Dicer is essential for maintenance of acinar cell identity. Specifically, acinar cells lacking Dicer showed increased plasticity (as evidenced by loss of polarity), initiation of epithelial-to-mesenchymal transition (EMT), and acinar-to-ductal metaplasia (ADM) [63]. Poy and colleagues cloned and identified a novel, evolutionarily conserved and islet-specific miRNA (miRNA-375) and found that it is a regulator of insulin secretion and may thereby constitute a novel pharmacological target for treatment of diabetes [64]. miRNA-375, which is highly expressed in pancreatic  $\beta$  cells, is one of the most widely studied miRNAs involved in  $\beta$  cell functions. Soon after this discovery, the same group found that miRNA-375 is essential for normal glucose homeostasis, alpha- and beta-cell turnover, and adaptive beta-cell expansion in response to increasing insulin demand in insulin resistance [65]. In addition, increasing evidence has suggested that many other miRNAs are involved in insulin secretion, such as miRNA-124a, miRNA-9, miRNA-96, and so on [66].

### ***2.4.2 miRNA Alterations During Pre-diabetic Metabolism***

As discussed in the part “miRNAs in cardiovascular diseases,” miRNAs play pivotal roles in glucose and lipid metabolism, which is very important in the pathogenesis of both cardiovascular diseases and diabetes. On one hand, several studies

have revealed the role of miRNAs in glucose-induced vascular dysfunction. For example, Caporali and colleagues found that the expression of miRNA-503 was shown to be increased in human umbilical vein endothelial cells (HUVECs) and human microvascular endothelial cells (HMVECs) in culture conditions mimicking diabetes mellitus (high D-glucose) and ischemia-associated starvation (low growth factors) [67]. On the other hand, under diabetes conditions the vascular smooth muscle cell (VSMC) changes its phenotype from a contractile to a synthetic and proliferative state, which is an early event in the pathogenesis of atherosclerosis and is also accompanied by increased inflammation. Interestingly, Villeneuve and coworkers demonstrated a novel upstream role for miRNA-125b in the epigenetic regulation of inflammatory genes in VSMC of db/db mice through downregulation of Suv39h1 [68].

#### ***2.4.3 miRNAs in the Manifestation of Long-Term Complications***

Due to consistently high blood glucose levels, diabetics have an elevated risk of developing a number of serious health problems, which could affect the heart and blood vessels, eyes, kidneys, nerves, and teeth. Additionally, these patients also have a higher risk of suffering from infectious diseases. In almost all developed countries, diabetes is a leading cause of cardiovascular disease, blindness, kidney failure, and lower limb amputation. According to statistics by the WHO, diabetes could lead to the largest number of complications in the world. Until now, it is widely reported that miRNAs play important roles in these processes.

Diabetic cardiomyopathy is one of the diabetes-induced organ complications. Using a microarray analysis of myocardial tissue, Shen et al. [69] identified changes in expression in 19 miRNAs, of which 16 miRNAs were further validated by qPCR. Specifically, overexpression of miRNA-373 decreased the cell size, and also reduced the level of its target gene MEF2C; this allows edp38 to regulate miRNA-373 expression. Another important complication of diabetes is represented by diabetic nephropathy (DN), which is the major cause of end-stage renal disease in developed countries. Krupa [70] found that loss of miRNA-192 expression associates with increased fibrosis and decreased estimated glomerular filtration rate (eGFR) in diabetic nephropathy in vivo, perhaps by enhancing TGF-beta-mediated downregulation of E-cadherin in proximal tubular cells (PTCs). Furthermore, diabetic neuropathy is a major debilitating complication of diabetes, which leads to high psychological strain. Recently, combining a mouse model of diabetic peripheral neuropathy with cultured dorsal root ganglion (DRG) neurons, Wang and colleagues showed that hyperglycemia downregulated miRNA-146a expression and elevated interleukin-1 receptor-activated kinase (IRAK1) and tumor necrosis factor receptor-associated factor 6 (TRAF6) levels in DRG neurons. Their data provide the first evidence showing that miRNA-146a plays an important role in mediating DRG neuron apoptosis under hyperglycemic conditions [71].

## 2.5 Conclusions

It is well known that one miRNA could target up to hundreds of mRNAs and one mRNA could be regulated by multiple miRNAs at the same time. Therefore, various miRNAs collaboratively work together to form a comprehensive regulatory net, which provides a new layer of gene regulation, mainly at the transcriptional level. Unexpectedly, it could be inferred that miRNAs participate in nearly all of the physiological and pathological processes of the body, including embryonic and tissue development, stem cell differentiation, apoptosis, inflammation, and so on. Up to now, miRNAs are widely reported to be involved in many kinds of diseases. Apart from the above-mentioned diseases, many other diseases are closely related to aberrantly expressed miRNAs, such as infertility, infectious diseases, autoimmune diseases, neurodegenerative diseases, and so on. Therefore, modulation of miRNA levels by administration of specific miRNA mimics or antisense oligonucleotides has recently come into focus as an attractive and promising alternative therapeutic option to halt or attenuate disease progression. These findings give us hope that one day we might be able to precisely modulate the tissue or cell-specific miRNA levels by targeting technology and this would lead to a new revolution in medical treatment.

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