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Review

"Role of MicroRNA-224 in The Field of Diabetes.: A comprehensive review"

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ABSTRACT

Small molecules, which are called microRNAs (miRNAs) control the expression of genes negatively by reducing the levels of their targeted mRNAs they target. Recent research has demonstrated that miRNAs have a variety of functions in diabetes mellitus. The two main kinds of diabetes are type 1 diabetes (T1DM) and type 2 diabetes (T2DM). T2DM is brought on by dysfunctional islet β-cells in response to insulin resistance, whereas T1DM is characterized by a decrease in insulin release from the pancreatic β-cells may be due to auto-immune diseases and lead to absolute deficiency in insulin production. The miRNAs that regulate insulin synthesis and release are discussed in this review. This study covers the role of miRNAs as possible biomarkers for T2DM, as well as the prospective applications of miRNA-224 in diabetes.

Keywords: T2DM; T1DM ; miRNA-224.

1. Introduction

The frequency of diabetes mellitus has drastically expanded over the past few decades, and by 2040, it is expected to impact 629 million people globally (1). In developing nations, type 2 diabetes mellitus (T2DM) accounts for 90–95% of all cases that have been recorded, while auto-immune variants currently account for 5–10% of all instances (2, 3).

T2DM is a chronic metabolic disturbance syndrome caused by the combined actions of genetic and environmental factors (4). The majority of people with T2DM experience severe consequences from chronic hyperglycemia, such as nephropathy, neuropathy, retinopathy, and accelerated cardiovascular disease (CVD) development (5).

The etiology of T2DM involves a number of several genes. Most of these genes relate to insulin action, glucose metabolism, pancreatic beta-cell function, or other metabolic issues that raise the risk of T2DM (6). Disruption of these molecules by pathogenic (causative) and non-pathogenic (DNA polymorphism) mutations is responsible for the risk of T2DM (7).

The most common risk factor for the development of T2DM is obesity, which is also a potential risk factor for CVDs, hypertension, stroke, metabolic syndrome, hypercholesterolemia, renal diseases, and cancer (8).

More to the point, miRNAs (mirs) have become effective glucose homeostasis regulators (8). About 19–25 nucleotide non-coding RNA molecules known as miRNA have been linked to a variety of disorders. One of two mechanisms—suppression of mRNA translation or mRNA degradation—had the potential to play a part in the regulation of posttranscriptional gene expression by miRNA. The 3' untranslated region of the target mRNAs for more than 60% of the protein-coding genes has miRNA target sites, which can inhibit the synthesis of proteins or cause mRNA destruction (9).

It has been demonstrated that miRNAs are essential for insulin secretion, pancreatic development, and insulin resistance (10). Future diagnostics and biomarkers for disease development and treatment responses are also being studied using miRNAs, in addition to being possible therapeutic targets. They have been identified in the serum, plasma, and whole blood of both human and animal models of diabetes. They exhibit differential expression in the tissues affected by diabetes as compared to unaffected healthy tissues (11). Furthermore, it has been discovered that miRNAs are indicators for both micro- and macrovascular issues, with the

potential to be clinically beneficial in the treatment of diabetes (12). A new miRNA in the realm of diabetes is called miRNA-224 (13). To date, miRNA-224 has been shown to express abnormally in a variety of malignancies, including colorectal, hepatocellular, and prostatic cancer. It has been demonstrated to target TGF-signaling via the SMAD-4 pathway and is acknowledged to enhance invasion, cell proliferation, and migration (14).

2. Diabetes mellitus

2.1. Definition of Diabetes mellitus

Diabetes mellitus is a collection of metabolic illnesses caused by inadequate insulin production, improper insulin action, or both that frequently manifest as bouts of hyperglycemia and glucose intolerance (15). Such changes result from abnormalities in the regulatory mechanisms that control the storage and mobilization of metabolic fuels, such as the catabolism and anabolism of carbs, lipids, and proteins, which are caused by suboptimal insulin production, insulin action, or both (16).

2.2. Classification of diabetes mellitus

Classification of diabetes mellitus (DM) is based on its etiology and clinical presentation. As such, there are four types of DM (17).

2.2.1. Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune condition that causes the loss of insulin-producing beta cells in the pancreas, typically resulting in a complete lack of insulin. This kind was once known as juvenile-onset or insulin-dependent diabetes mellitus, and it is now known as autoimmune diabetes (18). T1DM is usually characterized by the presence of anti-glutamic acid decarboxylase, islet cell, or insulin antibodies, which identify the autoimmune processes that lead to β-cell destruction (19).

There are approximately 34.2 million individuals worldwide who have T1DM, a number that has tripled in the past ten years. T1DM is commonly referred to as "juvenile diabetes" because an estimated 500,000 of these patients are under the age of 15 (20). T1DM accounts for only a minority of the total burden of diabetes in a population although it is the major type of

diabetes in younger age groups (21). All type 1 diabetic patients will require lifelong insulin therapy to maintain normoglycemia (22).

2.2.2. Type 2 diabetes mellitus

T2DM is a complex condition brought on by a mix of environmental variables such as obesity, overeating, lack of exercise, stress, and aging, as well as genetic factors associated with decreased insulin production, insulin resistance, and these factors (23). Adult-onset diabetes or non-insulin-dependent diabetes mellitus were earlier names for this condition. The susceptibility genes that predispose to T2DM have not been identified in the majority of individuals, and T2DM is not an autoimmune condition. This might be a result of the variability of the genes that cause T2DM susceptibility.

T2DM is the predominant form of diabetes and accounts for at least 90% of all cases of DM (24). Traditionally, T2DM is common in individuals over the age of 40 (25). T2DM is usually controlled through dietary therapy, exercise, and hypoglycemic agents (26). Due to insufficient insulin demand and impaired peripheral glucose utilization, the characteristic phenotype of hyperglycemia in type 2 diabetes (T2DM) develops, which in turn causes a number of metabolic complications, including diabetic retinopathy, nephropathy, cardiovascular and cerebrovascular disorders, and neuropathy (27).

2.2.3. Gestational diabetes mellitus

With an onset or initial recognition during pregnancy, gestational diabetes is a form of carbohydrate intolerance that causes hyperglycemia of varying severity. The diagnosis is typically made in the middle or final trimester. Older women, those with a history of glucose intolerance, and women from specific high-risk ethnic groups are among those at high risk for gestational diabetes. For the fetus to grow and develop properly, the high blood glucose level that is transmitted from the placenta to the fetus must be under control. After delivery, this kind of diabetes typically goes away on its own. Pregnancy-related diabetes raises the likelihood that a mother will later acquire T2DM (28).

The prevalence of T2DM among women of reproductive age has increased due to the continuous obesity and diabetes epidemic, and there is a rise in the number of pregnant women with undiagnosed T2DM in the first trimester (29).

Pregnancy-related diabetes is frequently a sign of underlying cell malfunction (30). This significantly raises the mother's risk of developing diabetes later in life, usually but not always T2DM (31). As effective prevention interventions, Pregnant women should undergo lifelong monitoring for prediabetes to enable diabetes risk reduction strategies and the earliest possible therapy for type 2 diabetes (32).

2.2.4. Other specific types of diabetes mellitus

This class includes monogenic diabetes syndromes such as neonatal diabetes and maturity-onset diabetes in the young. Neonatal or "congenital" diabetes is defined as diabetes that develops before the age of six months, and an underlying monogenic etiology may be identified in roughly 80–85% of cases. Neonatal diabetes is substantially less common beyond the age of six months, but autoimmune type 1 diabetes is quite uncommon before the age of six months. There are two types of neonatal diabetes: temporary and permanent (33). Diseases of the exocrine pancreas, such as pancreatitis or cystic fibrosis, can cause diabetes. Also, drug- or chemical-induced diabetes has been documented in instances such as treating acquired immune deficiency syndrome with glucocorticoids or after organ transplantation. The presence of diabetes after organ donation is referred to by a number of different names in the literature. One such term used to characterize people who acquire new-onset diabetes after transplant is "new-onset diabetes after transplantation" (NODAT). Patients with undetected pre-transplant diabetes and post-transplant hyperglycemia are excluded from NODAT if they resolve by the time of discharge. Another term, "post-transplantation diabetes mellitus" (PTDM) (34), explains the occurrence of diabetes following transplantation, regardless of when it first developed (35).

2.3. Epidemiology of diabetes mellitus

All around the world, the prevalence of diabetes is constantly rising, although it is most pronounced in middle-income nations. In comparison, 422 million adults were predicted to have diabetes in 2014. Since 1980, the prevalence of diabetes (age-standardized) in the adult population has increased nearly two-fold, from 4.7% to 8.5%. This sharp increase is mostly attributable to the rise in T2DM and the variables that contribute to it, such as overweight and obesity. Diabetes prevalence has increased more rapidly in low- and middle-income nations during the past ten years than in high-income countries, and it is anticipated to reach 592 million

in 2035 (36). In 2012, 1.5 million people died from diabetes (37). In the Middle East and North Africa region, more than 35.4 million people have diabetes, and by 2040, this number is expected to rise to 72.1 million (38). There were over 7.8 million cases of diabetes in Egypt in 2015 (39). With about 400 million adult cases worldwide, the frequency of T2DM has reached epidemic proportions after an abrupt rise over the past three decades (40). One explanation put out to explain this rise is a potential epidemiologic shift away from communicable diseases as the main cause of premature death. But it's also possible that this is related to a shift towards less healthful eating patterns and physical inactivity (41). It is a major contributor to death and disability (42). In most countries, even though females have lower mortality rates than males, they experience poorer health. Diabetes tends to affect males more than females since more males are diagnosed with T2DM. In addition, males are diagnosed at lower body mass index (BMI) levels than females (43).

Morbidity, mortality, and the availability of medical resources are all significantly impacted by Egypt's rapidly expanding type 2 diabetes mellitus health issue. Around 15.6% of Egyptians aged 20 to 79 have T2DM, and the disease accounts for 86478 annual deaths (44). According to the International Diabetes Federation, Egypt ranks ninth globally in terms of the proportion of T2DM patients. Over the past 20 years, T2DM prevalence in Egypt has practically tripled. This sudden increase could be related to other risk factors specific to Egypt or to an increasing pattern of typical T2DM risk factors such as obesity, inactivity, and altered eating habits. These include a rise in the prevalence of chronic hepatitis C and greater exposure to environmental risk factors like pesticides (45).

2.4. Diagnosis of diabetes mellitus

Diabetes mellitus is diagnosed when hemoglobin A1c (HbA1c) is 6.5%, fasting plasma glucose (FPG) is ≥ 126 mg/dl, 2-hour postprandial plasma glucose is ≥ 200 mg/dl during an oral glucose tolerance test, or random plasma glucose is ≥ 200 mg/dl plus symptoms of diabetes (46).

2.5. Pathogenesis of T2DM

Type 2 diabetes mellitus involves at least two primary pathogenic mechanisms (47):

(1) a progressive decline in pancreatic islet cell function resulting in reduced insulin secretion and inadequate suppression of glucagon secretion.

(2) peripheral insulin resistance resulting in a decrease in the metabolic responses to insulin.

Normal insulin resistance and glucose tolerance mark the beginning of T2DM's natural course. Insulin resistance is characterized by insulin's diminished capacity to carry out its physiologic tasks in the liver, muscles, and lipids. Skeletal muscle exhibits insulin resistance, which is particularly significant because, under physiologically normal settings, this tissue is in charge of more than 80% of the body's insulin-mediated glucose disposal (48). Reduced glucose absorption into peripheral locations, i.e., lipids and muscles, is one of the effects of insulin resistance at the tissue level. This causes hyperglycemia when coupled with increased hepatic glucose production. Additionally, hyperglycemia does not suppress the rise in glucagon during fasting. In the early stages of the insulin-resistant state, the pancreas can overcome insulin resistance by producing more insulin, a condition known as hyperinsulinemia. However with time, decreased glucose tolerance develops, and finally, the pancreatic cells are unable to support increased insulin secretion, which results in the onset of T2DM (49).

The release of incretins, specifically glucagon-like peptide 1 and glucagon inhibitory peptide, from the gut is one of the main processes that regulate insulin secretion after food consumption. These molecules are responsible for the absorption of around 50% of the immediately released insulin following a meal; however, their insulin trophic effects gradually diminish as T2DM progresses. This decline can be attributed to a synergistic interaction between decreased incretin secretion, elevated circulating levels of dipeptidyl peptidase 4, the enzyme that breaks down incretin, and resistance to incretin signaling in pancreatic cells. In fact, incretins work by binding to specific G protein-coupled receptors and activating the adenylyl cyclase pathway to increase the amount of insulin produced in response to glucose (50).

Numerous factors have been linked to the pathophysiology of T2DM. Cellular, molecular, and biochemical abnormalities include reduced insulin intracellular signaling, diminished insulin-stimulated glucose uptake, diminished hexokinase II expression and activity, diminished glycogen synthase activity, diminished pyruvate dehydrogenase activity, and diminished mitochondrial function. T2DM's etiology has also been linked to low-grade inflammation, lipotoxicity, and glucotoxicity (51).

The pathophysiology of T2DM varies by tissue or organ. The contribution of each tissue or organ to T2DM is summarized in (Figure 1). The theories on T2DM include a defect in insulin-mediated glucose uptake in skeletal muscle, a disruption of the secretory function of adipocytes, a dysfunction of pancreatic β-cells, impaired sensing and response to hyperglycemia in the central nervous system (CNS), an excessive accumulation of lipids, and impaired fatty acid oxidation due to obesity, physical inactivity, and genetic predisposition (52).

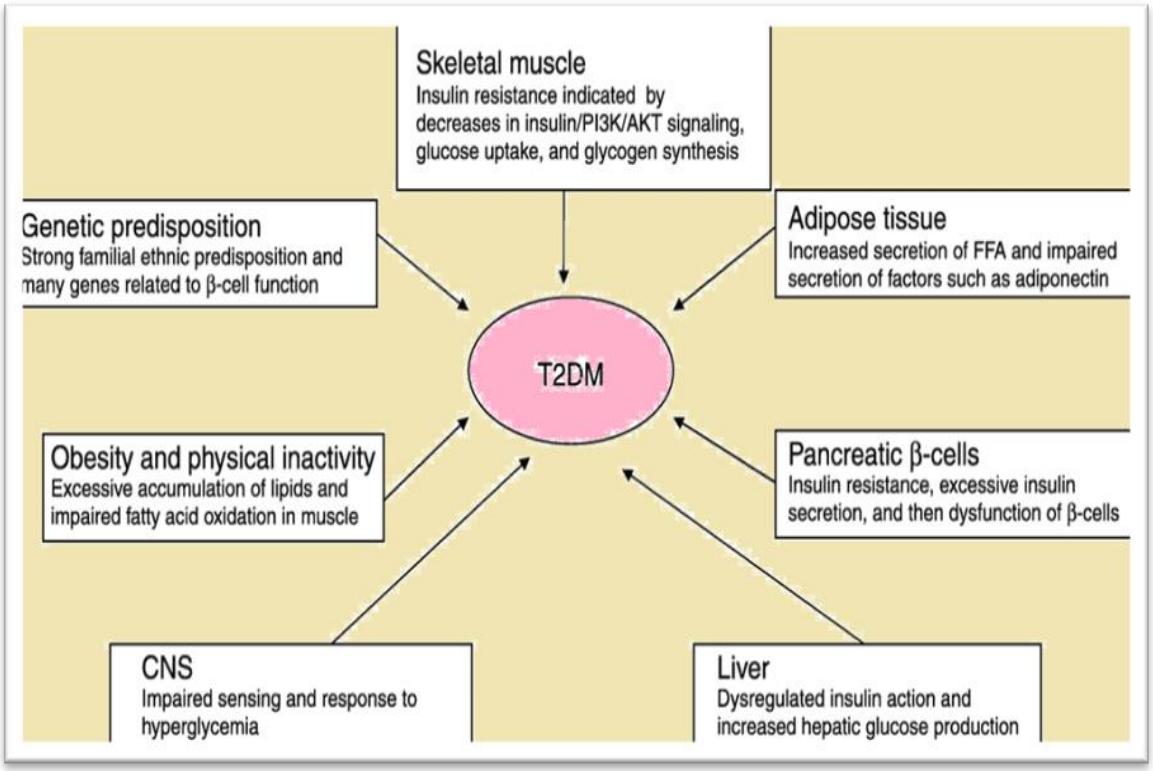


Figure 1. The pathophysiology of T2DM varies with tissues or organs (53). PI3K: Phosphatidylinositol-3-kinase; AKT: Protein kinase B; FFA: Free fatty acid.

2.6. Diabetic complications

The complications are related to the disease of blood vessels which is classified into microvascular and macrovascular complications.

2.6.1. Microvascular complications

These involve complications of a small blood vessel disease of the eye, kidney, and nerves (54).

2.6.1.1. Diabetic retinopathy

Diabetic retinopathy (DR) is a well-recognized complication occurring both in T1DM and T2DM, and it has been shown that nearly all T1DM and 75% of T2DM will develop DR after 15 years of diabetes (55).

The two main types of diabetic retinopathy are background (non-proliferative) and proliferative. Small hemorrhages in the intermediate layers of the retina are among the characteristics of retinopathy, also known as nonproliferative DR. They are frequently referred to as "dot hemorrhages" because of how they typically manifest clinically. Lipid deposition, which often happens at the edges of hemorrhages, is what leads to hard exudates. Small vascular dilations called microaneurysms develop in the retina, frequently as the first retinopathy sign (56). When a retinal exam is performed, they are clinically visible as red dots. Microvascular leakage may cause retinal edema, which is a sign that the blood-retinal barrier has been compromised. Retinal regions have a greyish appearance. Retinal edema may require treatment because it is occasionally accompanied by a decline in vision (57).

A vitreous hemorrhage may result from proliferative retinopathy, which is characterized by the growth of new blood vessels on the retina's surface. Cotton wool spots, which are white spots on the retina, may indicate proliferative retinopathy is about to develop. Blindness may result from vitreous hemorrhage and traction retinal detachment if proliferation persists. Without treatment, vision loss could happen. Close monitoring for the presence or advancement of retinopathy in diabetic patients is essential because laser photocoagulation can frequently stop proliferative retinopathy from progressing to blindness (58). Also, an excess of glucose activates the polyol pathway, which causes the accumulation of sorbitol in the lens and is accompanied by cataracts (59).

2.6.1.2. Diabetic neuropathy

The most prevalent cause of neuropathy in the world is diabetes mellitus. There are two types of neuropathies: symmetrical and asymmetrical (focal or multifocal). Symmetrical neuropathies are predominantly sensory and autonomic, whereas asymmetrical neuropathies can

also affect specific cranial or peripheral nerves. Distal symmetrical polyneuropathy is the most common type of diabetic neuropathy and accounts for 75% of diabetic neuropathies. Diabetic foot occurs often due to a combination of sensory neuropathy (numbness or insensitivity) and vascular damage, which increases rates of skin ulcers (diabetic foot ulcers), infection, and, in serious cases, necrosis and gangrene. Diabetic neuropathy also causes erectile dysfunction by affecting the nerve, which is important for penile erection. Diabetic neuropathy may also cause nausea, diarrhea, weight loss, and other gastroparesis by affecting the nerves of the stomach and intestine (60).

2.6.1.3. Diabetic nephropathy

Kidney disease brought on by diabetes is known as diabetic nephropathy. About one-third of dialysis patients experience renal failure, which is caused by nephropathy, the most common cause of chronic renal failure in the world. Microalbuminuria is one of the early indicators of this illness and denotes an enhanced risk of both cardiovascular events and the development of nephropathy. Patients with T2DM should undergo a test for the presence of microalbumin at the time of diagnosis. The following three techniques can be used to screen for microalbuminuria:

(1) A random spot collection is used to measure the albumin-to-creatinine ratio (ACR).
(2) A 24-hour urine collection containing creatinine that enables the determination of creatinine clearance at the same time.
(3) Timed (overnight or for four hours) collection (61).

2.6.2. Macrovascular complications

These encompass disorders of large blood vessels that ultimately lead to early myocardial infarctions, ischemic events, stroke, and premature death (62).

One of the main risk factors for CVD as a result of diabetes mellitus is dyslipidemia. High plasma triglyceride concentrations in conjunction with low circulating HDL cholesterol levels are the defining characteristics of dyslipidemia in people with T2DM. This characteristic is infrequently observed in T1DM patients until renal illness appears, in contrast to T2DM patients. These alterations in the lipid profile that are linked to T2DM are typically explained by an increase in free fatty acid (FFA) flow, which is a result of insulin resistance (63).

Hyperglycemia promotes the reaction of glucose with components of the arterial wall to form AGEs. These products cross-link with collagen, thereby increasing arterial stiffness (64).

2.7. Major risk factors of T2DM

Environmental factors, including physical inactivity, obesity, overeating, lack of exercise, stress, and aging, have also been widely described in the pathogenesis of T2DM (23). The etiology of T2DM is also influenced by genetic variables, and more recently, the role of epigenetic factors in the pathogenesis of this complicated disease has been suggested. These factors include DNA methylation, histone changes, and miRNAs. In most cases, the condition is complex, including a variety of genetic and environmental variables to differing degrees (65).

3. miRNAs

Our knowledge of the biological control of genetic material has substantially increased during the past twenty years. More than 90% of the genome's genes are not involved in making proteins, compared to 2% of protein-coding genes. The genes that produce proteins are first translated into RNA and then transcribed into proteins. The genes for non-coding proteins are translated into non-coding RNA but not into proteins (66). Non-coding RNA is crucial for the regulation of genomic activity. Non-coding RNAs called miRNAs have been found to play a key role in a number of disorders (67). miRNA was discovered in 1993 in the nematode Caenorhabditis elegans. It is a member of the endogenous small non-coding RNA family. It is composed of 22 nucleotides (68). By one of two mechanisms—the first being the inhibition of mRNA translation and the second being mRNA degradation—they are involved in the posttranscriptional regulation of gene expression. The 3' UTR of the target mRNAs (UTR) of more than 60% of the human protein-coding genes has miRNA target sites. These genes are therefore controlled by miRNAs (69). According to the miRNA database, there are more than 1000 miRNAs identified in the human genome (70). Those miRNAs regulate 1–5% of human gene expression, thus making miRNAs one of the largest classes of genomic regulators (71).

The nomenclature of a miRNA contains the prefix mir," followed by a dash and a number. In the prefix "mir", uncapitalized (r) means immature miRNA, while capitalized (R) means mature miRNA. The number indicates the order of naming. For example, miRNA-16 was named and discovered before miRNA-132. miRNAs with only one or two nucleotides are

annotated with an additional lowercase letter, e.g., miRNA-123a is closely related to miRNA-123b. Pre-miRNA that give the same mature miRNA but are located in different sites in the genome need to add a new dash and number for the prefix, e.g., pre-miRNAs miRNA-194-1 and miRNA-194-2 give the same mature miRNA (miRNA-194) but are located in different genome regions. An additional three letters are added to the prefix to identify the species. For example, Homo sapien-miRNA-123 is a human miRNA (hsa-miRNA-123), and Ovis aries-miRNA-123 is a sheep miRNA (oarmiRNA-123) (72).

3.1. Biogenesis of miRNAs

miRNAs are divided into two kinds based on where the miRNAs were produced:

(1) Transcripts from miRNA genes interspersed between protein-coding genes are used to make intergenic miRNAs.

(2) Transcripts of sequences found within the protein-coding genes are used to make intragenic miRNAs. Introns or exons contain intragenic miRNAs (73).

About 50% of the known human miRNAs are found in clusters and are transcribed by RNA polymerase II as lengthy primary transcripts that are mono- or polycistronic, commonly known as primary miRNA or pri-miRNA. These transcripts range in length from roughly 200 nucleotides to several kilobases and are folded into hairpin structures with poorly base-paired stems (74). Each cluster typically has two to three genes, with the largest cluster having seven genes. Clustered miRNAs may be functionally linked by focusing on a single gene or a group of related genes that are involved in the same metabolic pathway (75).

Nuclear RNase III endonuclease (Drosha) and its cofactor DiGeorge syndrome critical region gene 8 (DGCR8) cleave pri-miRNAs into pre-miRNA, which is a 70–100 nucleotide long hairpin with a monophosphate at the 5′ end and a 2-nucleotide overhang with a hydroxyl group at the 3′ end (76). The pre-miRNAs are transported into the cytoplasm by exportin-5 and its RAN-GTP cofactor. The pre-miRNAs are further digested by Dicer, a separate RNase III endonuclease, and its companion protein TRBP (transactivator RNA binding protein), to yield the final miRNA/miRNA* duplex, which is made up of 20–22 nucleotides. Dicer cuts both strands about two helical turns away from the pre-miRNA's stem-loop (77).

The miRNA "guide" strand, which is now known as The RISC (RNA-induced silencing complex) is then chosen for integration because it has a 5′ terminus and is less energetically

stable, whereas the miRNA* "passenger" strand is released, and destroyed. A helicase unwinds the duplex as shown in (Figure 2). Occasionally, the pre-miRNA hairpin produces mature miRNAs from both of its arms (78). Pre-miRNAs and mature miRNAs are two types of miRNAs that can leave cells and circulate outside of them, where they may interact with cells in the same tissue or in different tissues (79).

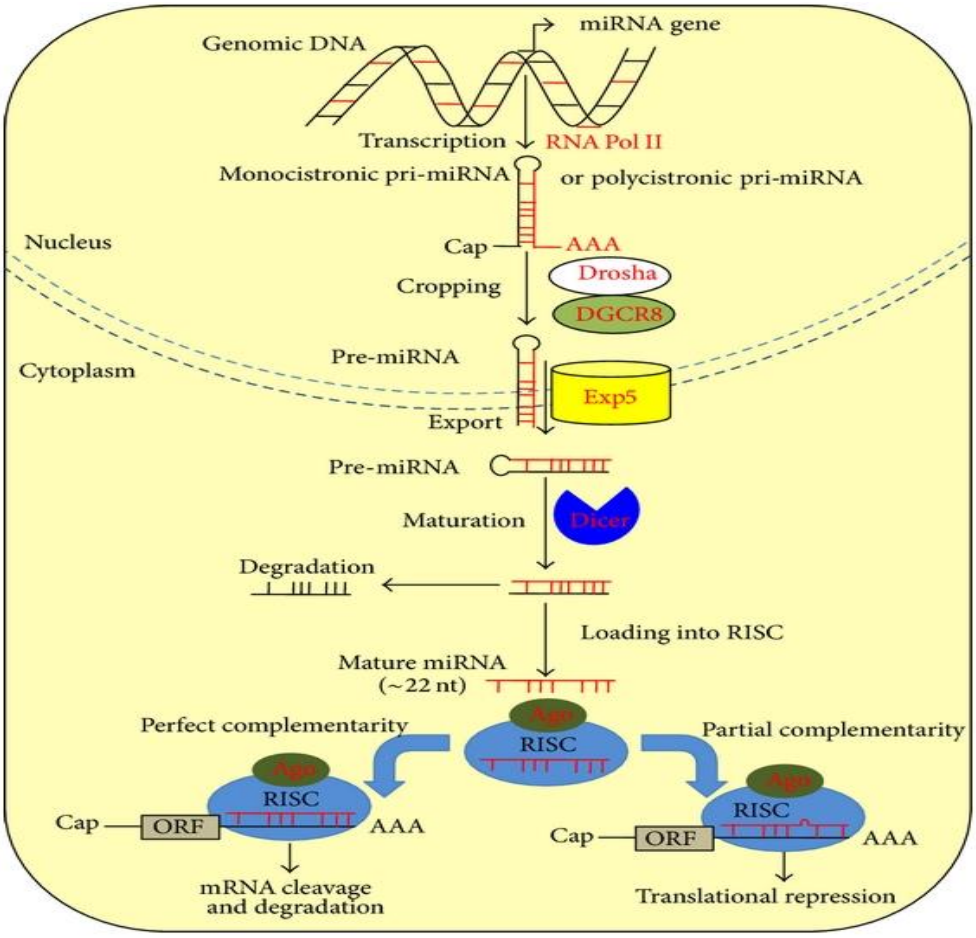


Figure 2. miRNA biogenesis and mode of action (80).

3.2. Mode of action *of miRNAs*

The target mRNA's 3'-UTR (also known as "templates") is where the miRNA molecule directs the RISC complex. The translation is suppressed by the interaction between the miRNA/RISC and its target mRNA. MiRNAs are post-transcriptional regulators as a result. The ‘seed region' of a miRNA, which is nucleotides 2 to 8 from the miRNA's 5' end and is crucial for

miRNA binding, attaches to its target site on a specific mRNA by Watson-Crick complementarity. The control mechanism of miRNA is based on the degree of complementarity between mRNA and miRNA. The interference regulation process is caused by the precise alignment of the miRNA with its target mRNA. The target mRNA is cleaved during this procedure by the RISC complex. Individual miRNAs can work together to jointly influence a single mRNA target in this way.

Variable inhibition of the target's translation is caused by an imperfect match between the miRNA and the mRNA. As a result, a single miRNA can regulate a large number of target mRNAs without fully matching them. The primary method through which miRNAs function in mammals is this one. The targeted genes are then shown to be expressed at the protein level without a decrease in mRNA levels. Instead, repressed mRNAs are sent to specific intracellular organelles known as P-bodies for storage, where miRNAs and associated proteins are co-localized (81). The partially complementary miRNA can accelerate deadenylation and cause mRNA degradation.

Several research have demonstrated that translational repression occurs prior to translation beginning, while other studies imply that repression happens after translation initiation (82).

3.3. Role of miRNAs

The study of miRNAs has advanced quickly since their discovery. miRNAs may be able to control roughly 60% of the protein-coding genes in humans, according to some research (83). miRNAs have been shown to have a crucial role in both the etiology of serious disorders like cancer and normal physiological balance (84).

3.3.1. Physiological role

miRNAs play a role in a number of physiological and developmental processes. These biological processes include DNA methylation, cell cycle, DNA repair, differentiation, apoptosis, and embryonic development (85). After being released into the extracellular fluids, miRNAs can operate in cell-to-cell communication within the same tissue or between the same and different cell types in distant tissues through endocytosis-like internalization or receptor-ligand interactions (86). miRNAs can act as tumor suppressors or oncogenes and are negative regulators of gene expression, according to growing data (87). Additionally, miRNAs and the

immune system have been linked in several studies. Innate and adaptive immunity depend on miRNAs , inflammatory diseases, and defense against both RNA and DNA viruses (88).

3.3.2. Pathological role

Alterations of miRNA are involved in the initiation and progression of many diseases. This alternation may be due to expression disorders, gene mutations, or natural genetic variants that may cause a single nucleotide mismatch between miRNA and its target site. This mismatch can change the efficacy and thermodynamics of miRNA. miRNAs are involved in disease pathogenesis, including autoimmune diseases, microbial infections, skin diseases, neurological diseases, psychiatric diseases, cancer, cardiovascular diseases, and asthma (89). miRNAs and human diseases

miRNA shortages or excesses have been associated with a range of clinically significant disorders, including autoimmune disease and myocardial infarction. The goal is that in the near future, miRNAs will have huge potential for the detection and treatment of many diseases as a result of outstanding discoveries and quick progress in the field (90).

miRNAs have a critical regulatory role in many human diseases, such as:

(1) Cancer: It is now well documented that up-regulation or down-regulation of miRNAs occurs in various human cancers. When tumor-suppressor genes are downregulated by overexpressed miRNAs, they can act as oncogenes and/or regulators of biological processes, including cell differentiation or apoptosis. Numerous cancer forms, including ovarian, breast, colon, hematological, endometrioid adenocarcinoma, esophageal, gastrointestinal, lung, bladder, and thyroid tumors, have been linked to specific miRNA expression profiles (91).

(2) Cardiovascular diseases: Deregulation of developmental processes and cardiac disorders like heart failure and cardiac hypertrophy have been linked to miRNA expression levels (92).

(3) Inflammatory diseases: The role of miRNAs in vascular inflammation, leukocyte activation, and their infiltration into the vascular wall has been described in numerous investigations (90).

(4) Autoimmune diseases: Patients with rheumatoid arthritis and systemic lupus erythematosus have been found to have aberrant miRNA expression (90).

(5) Skin diseases: According to reports, miRNAs play a part in the formation of hair follicles, andthe development of autoimmune and chronic inflammatory skin diseases (92).

(6) Neurodevelopmental diseases: Compared to other organs, the brains of humans and other mammals have an overexpression of miRNAs. They are believed to have a crucial role in the molecular etiology of neurodevelopmental disorders such Down syndrome, Alzheimer's disease, and schizophrenia (92).

(7) Liver diseases: Hepatic illnesses, including polycystic liver disease and viral hepatitis, involve abnormal miRNA expression. Additionally, miRNAs can have an impact on Non-alcoholic fatty liver disease (NAFLD) via a number of different pathways, including fibrosis, insulin resistance, lipid metabolism, and metabolic syndrome. Recently, it was shown that NAFLD-related altered miRNA expression was present in animal and human liver samples (93).

3.3.3. Clinical value of miRNA

Since 2008, numerous studies have demonstrated the feasibility of discovering miRNAs in body fluids like serum, plasma, urine, saliva, tears, amniotic, and placental fluids, increasing the potential uses for this unique class of diagnostic biomarkers (94). Most studies examining the use of miRNAs as biomarkers in fluids or diseased tissues have focused on cancer. Given the abundance of cell-free miRNAs originating from the primary tumor seen in the plasma of cancer patients, numerous lines of evidence point to circulating miRNAs as a prospective source of prognostic and/or diagnostic cancer biomarkers (95). Given that RNA molecules are unstable in the bloodstream, the discovery of miRNAs in serum came as a complete surprise. Studies have shown that because miRNAs are protected from endogenous RNase activity by membrane vesicles such as exosomes or microparticles, they demonstrate great stability in the serum and plasma. These microvesicles express tissue-specific markers and are resilient to extreme environments. Given their remarkable stability and ability to endure repeated freezing and thawing cycles, the miRNAs found in blood circulation are promising biomarkers for human diseases (96). The discovery that miRNAs are involved in human diseases may pave the way for the creation of a cutting-edge treatment approach. Therapeutic tests that target miRNA in vivo have recently been carried out (97).

3.3.4. Future biomarkers for the detection of diabetes based on miRNA signatures

T2DM is considered a multifactorial, chronic, and complex metabolic disease in which family medical history, age, lifestyle, diet, genetics, and environmental factors play a role. T2DM usually develops gradually; the early stages of the disease may be asymptomatic and undetected for several years. Initial symptoms commonly include polydipsia, polyuria, polyphagia, and eventually weight loss. This chronic disease triggers a series of complications with a high degree of morbidity and mortality, resulting in a significant number of medical consultations, hospitalizations, disabilities, and deaths. Examples of these multisystemic complications include microvascular events, such as retinopathy, nephropathy, and neuropathy, and macrovascular events, including ischemic heart disease, stroke, and peripheral vascular disease (98). A significant fraction of T2DM patients often present advanced complications that can be difficult to manage and costly to treat. In this context, the high incidence of T2DM presents a heavy burden on worldwide public health systems. Screening strategies have a positive impact on the quality of life and reduction of health costs since they allow early diagnosis, lowering the prevalence of underdiagnosis, thus reducing the generation of complications, which in the long run decreases the pressure on health systems (99). Therefore, developing strategies focused on prevention, diagnosis, control, and treatment will be a priority in the next few years. This review focuses on the recent development of new biomarkers and methods that represent cost-effective alternatives for screening and early diagnosis of T2DM and could be widely implemented in apparently healthy people.

According to the "Standards of Medical Care in Diabetes" published by the American Diabetes Association (ADA) and the World Health Organization (WHO) guidelines, diabetes may be diagnosed based on the concentration of plasma glucose—either fasting plasma glucose (FPG) or two-hour plasma glucose during a 75 g oral glucose tolerance test (OGTT)—or based on glycated hemoglobin A1c (HbA1c) concentration. The classification and diagnosis of diabetes historically relied solely on plasma glucose concentration and patient symptomatology until HbA1c emerged as a useful glycemic biomarker. Currently, there are several challenges in the management of T2DM that need to be addressed. On the technical side, there is a need for novel, more comprehensive strategies for optimal screening, early diagnosis, and adequate management of T2DM. Approaches combining the use of resources for risk assessment, such as the Finnish Diabetes Risk Score (FINDRISC) (100). along with more effective biomarkers for screening and

progression of T2DM, have a higher probability of success in managing the global diabetes epidemic. It will also positively impact the prevention of complications caused by hyperglycemic episodes in individuals diagnosed with diabetes and prediabetes by reducing the underdiagnosis and undertreatment of diabetes.

3.3.5. Novel Biomarkers

Metabolomics is especially useful in identifying biomarkers of T2DM because of the metabolic basis of its etiology and the fact that its development is strongly related to lifestyle and environmental factors. Several studies have been carried out to evaluate novel biomarkers in conventional fluids such as blood and urine. Some examples of these studies are presented in the sections "Clinically Validated Biomarkers" and "Novel Biomarkers." Few novel biomarkers have shown significant advantages over those already established and validated, such as FPG, OGTT, and HbA1c. However, it is expected that more extensive studies will lead to new resources for the management of the T2DM epidemic. For examples of some metabolites used for diagnosis of T2DM:

Biomarkers	Finding	References
Amino acids	The ability of amino acid levels, including BCAAs (isoleucine, leucine, valine) and aromatic amino acids (tyrosine and phenylalanine), to predict prediabetes risk was evaluated. Levels of aspartic acid, asparagine, and histidine significantly predicted the incidence of prediabetes, with the increased risk differing between African Americans and European Americans. The evidence observed in prediabetes suggests that changes in the amino acid profile occur in the transition from normoglycemia to the development of T2DM.	(101)

Biomarkers	Finding	References
α-HB α-hydroxybutyrate	The α-HB was the biomarker with the best performance to identify individuals with insulin resistance. This behavior was consistent in both screening and targeted assays. α-HB predictive potential can be explained both by its metabolic relevance and that its synthesis is stimulated by the elevation of the NADH/NAD+ ratio due to increased lipid oxidation.	(102)
Adiponectin and leptin	The relationship between plasma leptin levels and biomarkers associated with energy and hormone metabolism was explored. The untargeted metabolomics analysis showed that 64 metabolite features were associated with fasting leptin levels. The profile of metabolites associated with leptin levels varied by gender—the leptin level was approximately three times higher in women. A positive correlation was found between leptin and adiponectin, and a negative correlation with caloric intake, serum triglyceride levels, and VLDL. The evidence supports the role of leptin as a mediator of energy and hormone metabolism.	(103)

Also, we need to investigate other noninvasive biomarkers, such as miRNAs, The recent advent of genome-wide association studies (GWAS) has led to the identification of several T2DM-related genes; their application as biomarkers, such as in the individual prediction of disease risk, still needs further study, while a better understanding of gene expression regulatory mechanisms during the development of T2DM will have potential applications in prevention, early diagnosis, and treatment. Interestingly, miRNAs are present in human peripheral blood at consistent and reproducible levels. Unlike messenger RNA (mRNA), miRNAs are remarkably stable and are even resistant to RNase activity, which makes peripheral miRNAs potentially novel sources of non-invasive biomarkers of cancer and other diseases. We are particularly interested in the feasibility of the clinical application of circulating miRNAs in T2DM (98).

3.3.6. Examples of miRNAs used for diagnosis of T2DM

In recent years, miRNA has remained one of the most encouraging and fruitful fields in biological research. Presently (December 2009), about seven hundred human miRNAs have been described in miRbase (http://microrna.sanger.ac.uk), and computational analyses predict that up to 1,000 miRNAs exist in the genome (104). It is believed that since one miRNA family might bind to as many as 200 target genes, they may be able to regulate up to 30% of our gene set (105). So, it could be an advantage of miRNA that profiling the relatively small number of master regulators may be superior to profiling the large number of their downstream target genes.

Recent progress in GWAS has revived the initial optimism and accelerated the discovery of diabetes susceptibility genes. The phenotypes of these susceptibility genes exist not only in T2DM patients but also in prediabetic individuals, and some even exist in "healthy" individuals who are more susceptible to T2DM, so they can only serve as risk factors and are not able to be applied to the diagnosis of T2DM. As the key gene expression regulators, we explored if circulating miRNAs present different expression patterns among T2DM-susceptible individuals, pre-diabetic individuals, and T2DM patients.

3.3.7. miRNAs in diabetes

Powerful regulators of glucose homeostasis have been identified as miRNA It has been demonstrated that miRNA is essential for insulin secretion and pancreatic development as well as insulin resistance (106). miRNAs are being studied as future diagnostics and indicators for sickness onset and therapy responses, in addition to being possible therapeutic targets. They have been found in both human and animal models of diabetes in serum, plasma, and whole blood. Compared to unaffected healthy tissues, they show a distinct expression in tissues affected by diabetes (107). Diabetes patients' serum has been found to include various miRNA profiles, including those from both pediatric and adult cohorts (108). Additionally, miRNA has been identified as a biomarker for both microvascular and macrovascular problems, with the potential to be clinically advantageous in the treatment of diabetes.

Nearly all research on the utilization of human volunteers' serum to study the role of circulating miRNA in diabetes However, current interest has focused on miRNA detection in several more accessible bodily fluids (109). The most prevalent monogenic form of diabetes is

Hepatocyte nuclear factor 1 (HNF1A), a pancreatic transcription factor, that is responsible for HNF1A-maturity-onset diabetes of the young (HNF1A-MODY). Recently, it was shown that INS-1 cells, a cellular model of HNF1A-MODY, exhibit induced inhibition of endogenous HNF1A function, elevated levels of miRNA-224 and miRNA-103, two particular miRNA (110).

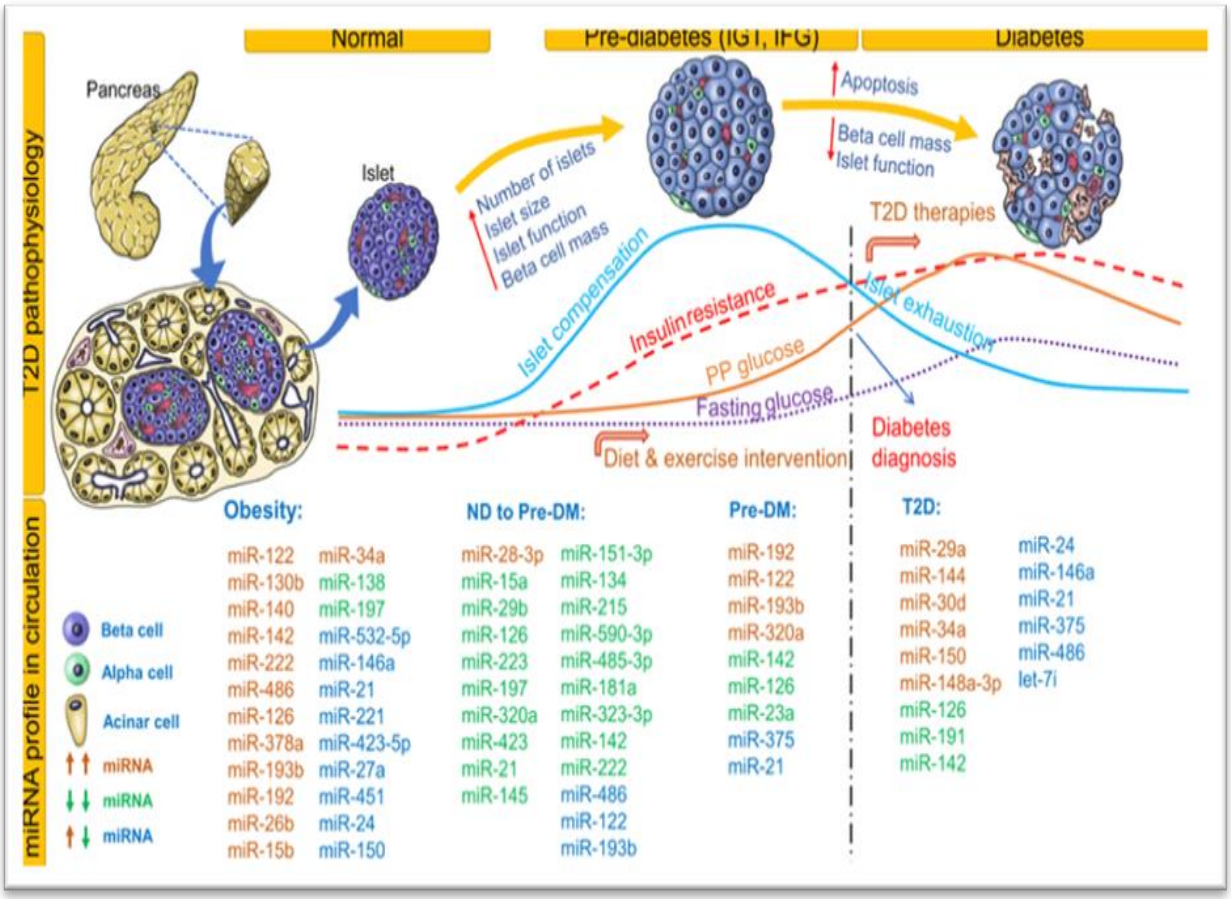
Cells can release miRNA, which can then collect in microvesicles, either free or attached to the Ago2 protein in extracellular fluids. It's interesting to note that HNF1A is expressed not only in the pancreas but also in the liver, digestive system, and particularly the kidney (111).

For examples of miRNAs involved in the pathogenesis of T2DM:

(1) miR-9 can diminish the expression of the transcription factor Onecut-2 and then, by increasing the level of Granuphilin/Slp4, a Rab GTPase effector associated with -cell secretory granules, exert a negative control on insulin release (112).

(2) Overexpression or inhibition of mir-124a2 leads to a decrease or increase in insulin mRNA levels, respectively, and its downstream targets are also critical for regulated insulin release (10).

(3) miR-375 is abundant in pancreatic islets and β-cells; its overexpression or inhibition represses or enhances insulin secretion. Knockdown of myotrophin (Mtpn), which is one of the target genes of miR-375, also leads to decreased insulin secretion (113). As



shown in (Figure 3), there are many miRNAs included in thepathogenesis of T2DM.

Figure 3. miRNAs involved in diabetes (114).

3.4. miRNA-224

1.1. miRNA-224 gene is located along the arm of chromosome X (q28). (Figure 4) shows the secondary structure and sequence conservation of miRNA-224 (115).

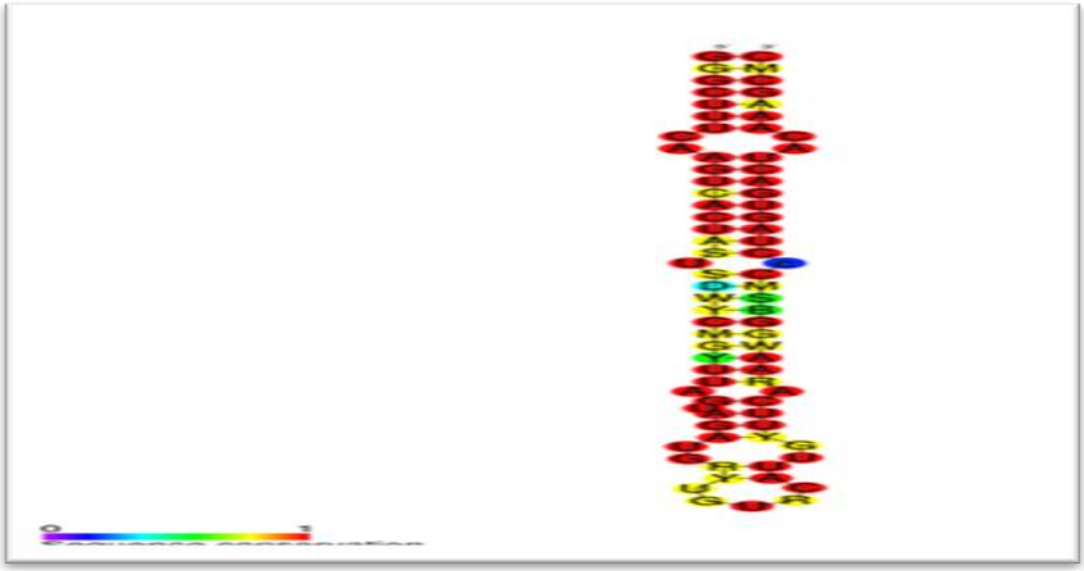


Figure 4. miRNA-224 secondary structure and sequence conservation. https://www.ncbi.nlm.nih.gov/gene/407009**.**

3.4.1. miRNA-224 in cancer

Several biological processes, including metastasis, invasion, tumor differentiation, apoptosis, and tumor growth, are known to be significantly regulated by miRNAs. They have been identified in a number of human disorders, such as cancer, where the neoplastic cells may exhibit upregulation or downregulation compared to their healthy counterparts (116). When miRNA-224 binds to the 3'UTR sections of several genes, It encourages the development and growth of tumors by being implicated in cell apoptosis, proliferation, migration, invasion, and autophagy (117).

miRNA-224 was discovered to be much more prevalent in cervical cancer, colon cancer, pancreatic ductal adenocarcinoma, and breast cancer (117). Researchers have examined the

function of miRNA-224 and its expression in HCC tissues in the development of the disease. They found that miRNA-224 is one of the most frequently overexpressed miRNAs, influencing critical cellular mechanisms in the development of HCC (118). This molecule was found to be a master controller of cell cycle execution in one study, and its exaggeration sped up cell proliferation (119).

Previous studies reported that by focusing on the apoptosis inhibitor API-5 and encouraging cell proliferation, it prevented tumor cell apoptosis. Some people claimed that miRNA-224 acted as an oncomiR in HCC by activating the AKT signalling pathway and promoting the migration and proliferation of malignant hepatocytes (120), while others associated the overexpression of miRNA-224 with the induction of inflammatory pathways by lipopolysaccharide, lymphotoxin, and tumor necrosis factor, which encouraged cell invasion and migration in HCC.

3.4.2. miRNA-224 and its functional association with the aromatase in ovarian granulosa cells

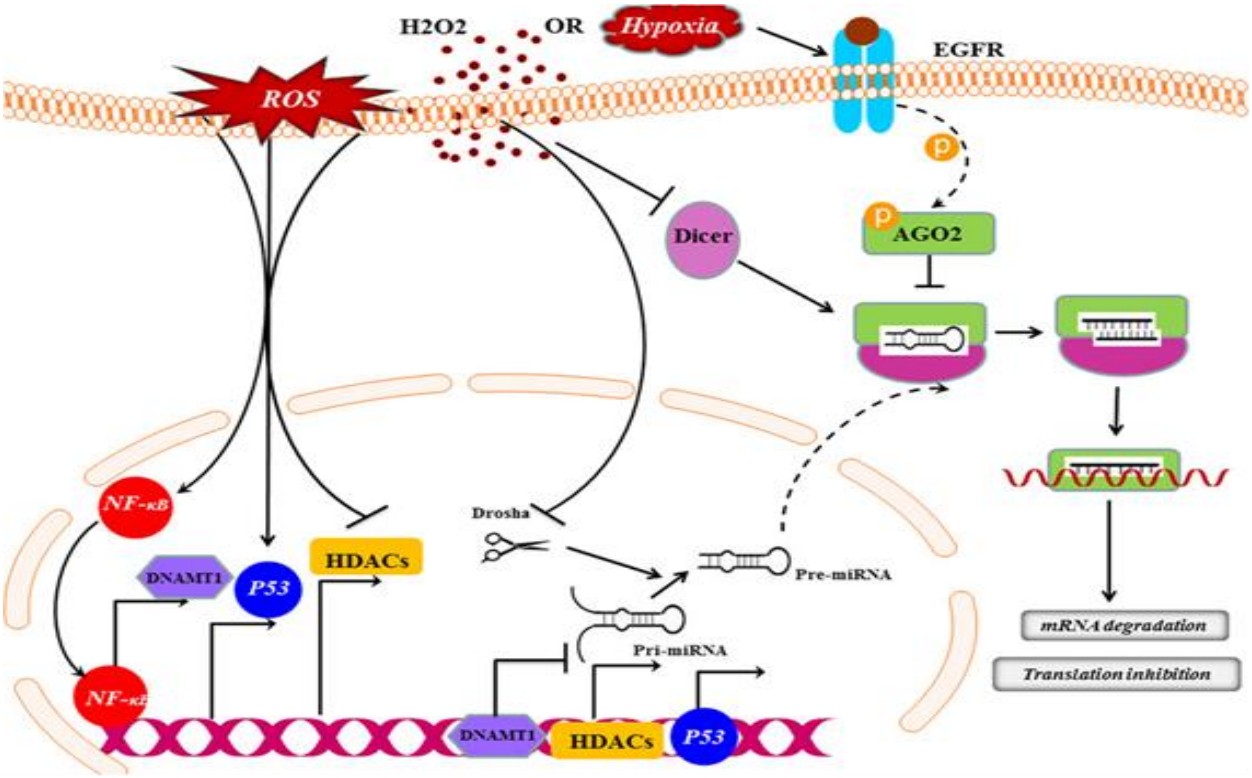
miR-224	**Reported to target smad-4 in the TGF-β1/Smads pathway**	**Both miR-244 and TGF-β1 promote estradiol synthesis in granulosa cells by increasing the expression of CYP19A1 mRNA levels**

miR, microRNA; TGF-β1, transforming growth factor-β1(121).

3.4.3. Role of miRNA-224 in oxidative stress.

Oxidative stress refers to elevated levels of intracellular reactive oxygen species (ROS). ROS homeostasis functions as a signaling pathway for normal cell survival and appropriate cell signaling. Chronic inflammation induced by imbalanced levels of ROS contributes to many diseases and different types of cancer. ROS can alter the expression of oncogenes and tumor suppressor genes through epigenetic modifications, transcription factors, and non-coding RNAs. MicroRNAs (miRNAs) are small non-coding RNAs that play a key role in most biological pathways. Each miRNA regulates hundreds of target genes by inhibiting protein translation and/or promoting messenger RNA degradation. In normal conditions, miRNAs play a physiological role in cell proliferation, differentiation, and apoptosis. However, different factors that can dysregulate cell signalling, and cellular homeostasis can also affect miRNA expression.

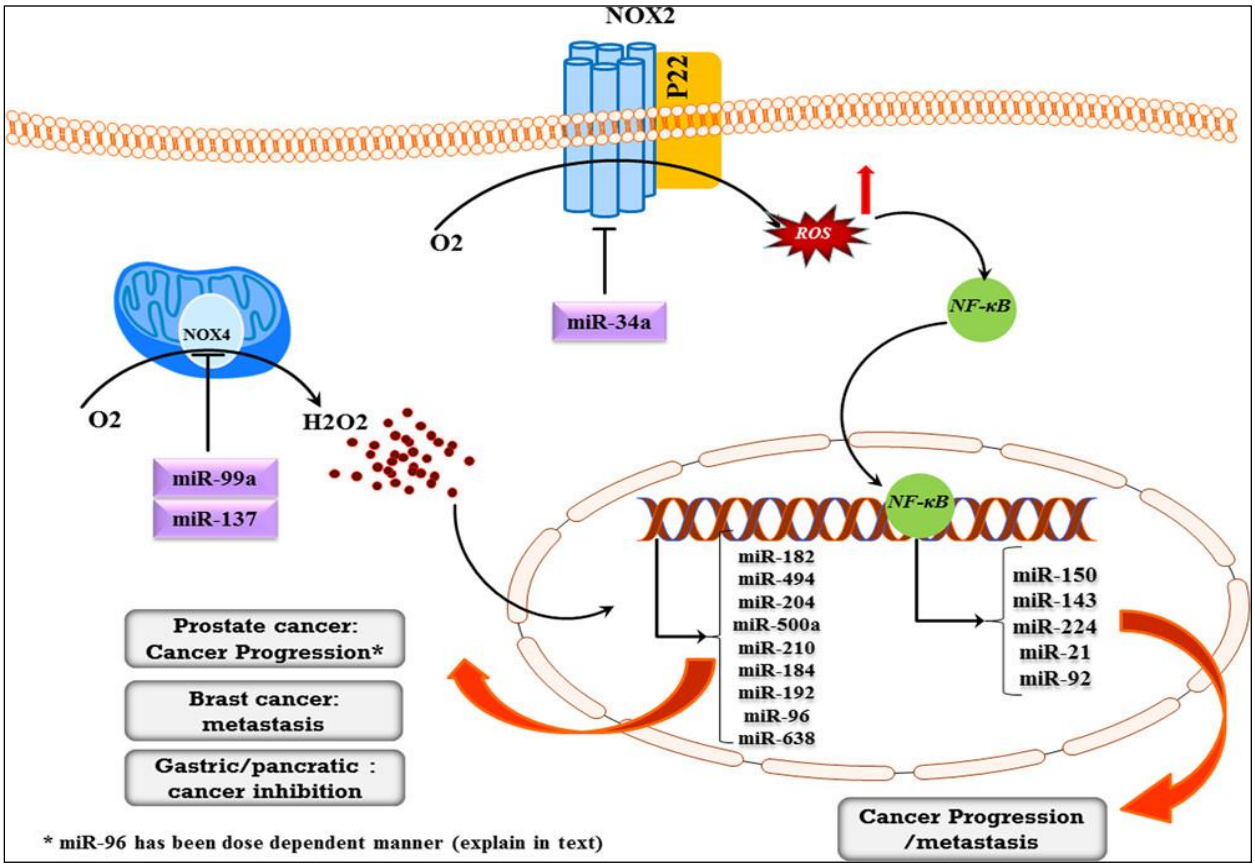
The alteration of miRNA expression can work against disturbing factors or mediate their effects. Oxidative stress is one of these factors. Considering the complex interplay between ROS level and miRNA regulation and both of these with cancer development, we review the role of miRNAs in cancer, focusing on their function in oxidative stress. NF-kB (nuclear factor-kappa B) also upregulates miR-224 in hepatocellular carcinoma (122). ROS production often occurs in inflammatory conditions and can activate NF-kB. The NF-kB pathway plays an important role in inflammation and oxidative stress responses and can regulate the expression of several miRNAs, one of which is miR-224 in hepatocellular carcinoma. The expression of miR-224 is increased under the influence of NF-kB and causes metastasis and invasion in hepatocellular carcinoma by targeting the fibronectin type III domain-containing 3B. Increased expression of miR-224 is



associated with metastasis and cell invasion. This carcinogenesis occurs due to ROS-mediated DNA damage and oxidative stress as well as the induction of telomerase activity, as shown in (Figure 6).

Figure 5. Illustration ROS/H_2O_2 or hypoxia effect in miRNA processing and expression. H_2O_2 inhibits Drosha and Dicer expression and ROS as direct and indirect causes up/downregulating some miRNA. ROS can affect the expression of miRNAs through epigenetic

modifications such as methylation or acetylation by DNAMT1 and HDACs. EGFR, epidermal growth factor receptor; HDACs, histone deacetylases; miRNA, microRNA; mRNA, messenger



RNA; NF-κB, nuclear factor-κB; ROS, reactive oxygen species (123).

Figure 6. Oxidative stress affects direct/indirect miRNAs upregulation. ROS upregulates NF-κB and leads to expressional induction in NF-κB target genes, such as some miRNAs. The H_2O_2-mediated miRNAs upregulation results in cancer progression or inhibition dependent on cancer microenvironment. miRNAs, microRNAs; NF-κB, nuclear factor-κB; ROS, reactive oxygen species (123).

Increase miR-224 upregulation in NF-KB which, by the way, plays an important role in the pathogenesis of T2DM and its related complications. NF-kB is important for the expression of GLUT2, which contributes to glucose-stimulated insulin secretion by B-cells. Inhibition of this transcription factor, therefore, may have deleterious effects leading to the development of insulin resistance and type 2 diabetes. Although there is a wealth of evidence surrounding the

role of NF-KB in insulin regulation, its specific contribution to the pathogenesis of human type 2 diabetes remains to be examined. Hyperglycemia-induced activation of NF-KB in vascular smooth muscle cells Prolonged hyperglycemia is believed to be one of the major causes of vascular complications associated with diabetes (124). NF-KB is a pleiotropic oxidant-sensitive transcription factor, and hyperglycemia-induced oxidative stress may play a key role in the pathogenesis of diabetic vascular disease. Prolonged hyperglycemia can also lead to the formation of advanced glycation end products (AGEs), which act through specific receptors on vascular cells, leading to oxidant stress and cellular dysfunction in the pathology of atherosclerosis and diabetic complications, Diabetic cardiomyopathy is the leading cause of death in diabetic patients is characterized by both systolic and diastolic dysfunction resulting from reduced contractility, prolonged relaxation, and decreased compliance. The development of diabetic cardiomyopathy is multifactorial. Putative mechanisms include metabolic disturbances, small vessel disease, autonomic dysfunction, insulin resistance, and myocardial fibrosis. Diabetes induces oxidative imbalance, this interaction involves the activation of transcription factors, such as NF-KB, which is a major target of ROS. Activation of NF-KB-dependent genes triggers several pathways via the production of proinflammatory cytokines, which are mainly involved in heart damage. TNF-alpha, in turn, activates NF-KB and induces the RAGE gene, thus amplifying its detrimental effects on the diabetic heart. Furthermore, NF-kB activation triggers a signaling cascade that ultimately leads to a switch in cardiac myosin heavy chain (MHC) gene expression from the alpha-MHC isoform to the alpha-MHC isoform (125).

Diabetic retinopathy is the leading cause of blindness and visual impairment in adults in developed countries. Retinal NF-kB is activated early in diabetes and remains activated for up to 14 months. In diabetes, the accumulation of AGE and its receptor, RAGE, is increased in the retinal microvasculature. In the late stages of retinopathy, AGEs are irreversibly formed and accumulate within retinal capillary cells. It is postulated that more ROS are generated via the AGE pathway, leading to the activation of NF-kB and causing further damage to the cells. Diabetic retinopathy shares similarities with chronic inflammatory disease, and inflammation may play a central role in the development and progression of diabetic retinopathy. Activation of NF-kB modulates the expression of several proinflammatory factors, including cytokines, tumor necrosis factor, and inducible nitric oxide synthase. This expression, in turn, can result in increased free radical production. The levels of cytokines, including interleukins IL-1B, IL-6,

and IL-8, are increased in the vitreous fluid of patients with proliferative diabetic retinopathy (126). So this proved that upregulating of miR-224 affects the formation of diabetes and complications by upregulation of NF-KB.

3.4.4. miRNA-224 in vitiligo pathogenesis as an inflammatory cytokines production modulator.

Vitiligo, a depigmenting skin disease, is characterized by the selective loss of melanocytes, which in turn leads to pigment dilution in the affected areas of the skin. The theories have been shown to combine biochemical, immunological, and environmental events, in a genetic milieu. miRNAs may play a potential role in multiple physiological and developmental processes in humans. Several studies demonstrated that miRNAs act as regulatory factors and are also involved in various aspects of the pathological process of vitiligo, like the growth, differentiation, and apoptosis of melanocytes (127). Cellular and humoral immunity are shown to be of etiological importance in vitiligo pathogenesis. It has been noticed that some miRNAs are implicated in the immune response. These miRNAs regulate a wide range of biological processes and have a potential value in skin T lymphocytes in the mechanism of immune disturbance in vitiligo (128). It is most widely accepted that melanocyte destruction in vitiligo is caused by autoimmunity and oxidative-stress-mediated toxicity. There is research that shows that many cutaneous pigmentation disorders can benefit from miRNA-based therapeutic applications, including vitiligo. miRNA-224-3p was identified to be related to a decline in the expression of tumor-necrosis factor-a, keratinocyte-derived chemokines, and macrophage inflammatory protein-2, suggesting an important role for miRNA-224-3p in the regulation of inflammatory cytokine production (129). Vitiligo melanocytes are more sensitive to accumulated reactive oxygen species due to their intrinsic antioxidant defects. This imbalance between pro-oxidants and antioxidants can disrupt the homeostasis of melanocytic cells, causing the accumulation of multiple oxidized and damaged proteins or organelles, leading to the destruction of melanocytes. The hypersensitivity caused by oxidative stress plays an important role in the degeneration of melanocytic cells. Multiple studies found that the miRNA expression profile might be regulated by the oxidative-stress process and can also mediate the pathogenic effects of reactive oxygen species in vitiligo. This suggests that miRNAs may contribute to vitiligo pathogenesis by

regulating the oxidative-stress-related gene expression in the cutaneous melanocytes. MiRNAs modulate cellular proliferation, differentiation, and apoptosis, including melanocyte and immune-cell development and function. miRNAs are shown to be unusually well preserved in serum or plasma derived from immune cells and other tissues and serve as promising biomarkers for different diseases (130). Wang *et al.* found that the expression of miRNA-224-3p was significantly increased in the peripheral blood mononuclear cells of patients with nonsegmental vitiligo. Their analysis was associated with responses to the biological functions of the immune modulator thymosin α1, which has an important role in the mechanism of immune imbalance in vitiligo, so miRNAs could act as potential biomarkers to distinguish nonsegmental vitiligo from healthy volunteers (131). Therapeutic applications based on miRNAs may be beneficial for many cutaneous pigmentation disorders, like vitiligo. The therapeutic application of miRNAs includes two strategies. One strategy is suggested to inhibit pathogenic miRNAs through the use of miRNA antagonists, such as anti-miRNAs, locked-nucleic acids, or antagomiRs. These miRNA antagonists are oligonucleotides with sequences complementary to the endogenous miRNA. The second strategy, miRNA replacement, includes the reintroduction of a gene-suppressor miRNA mimics to modulate gene expression, often resulting in a loss-of-function effect. This approach represents a second-generation RNAi-based therapy (132).

3.4.5. miRNA-224 up-regulation with thyroid cancer

Upregulation of miR-224-5p and downregulation of EGR2 expression were detected in PTC (papillary thyroid carcinoma) tissues and cells. Upregulation of miR-224-5p was found to be associated with TNM stage and lymph node metastasis. Meanwhile, it also predicted a poor prognosis for PTC patients. Functionally, upregulation of miR-224- 5p promoted cell metastasis and EMT (epithelial-mesenchymal transition) in PTC. In addition, miR-224-5p was detected to directly target EGR2. EGR2 expression was negatively correlated with EGR2 expression in PTC. Of note, overexpression of EGR2 attenuated the carcinogenic effects of miR-224-5p in PTC. miR-224-5p promotes cell migration, invasion, and EMT in PTC by targeting EGR2 (133).

3.4.6. miRNA-224 in diabetes

A new miRNA in the study of diabetes is miRNA-224. miRNA-224 has so far been demonstrated in a variety of malignancies, including colorectal, hepatocellular, prostatic, and hepatocellular, to be aberrantly expressed. It has been demonstrated to target TGF- signaling via

the Smad 4 pathway and is known to enhance cell migration, proliferation, and invasion. There is a previous study that proved that miRNA-224 is readily detectable in the urine of individuals with Diabetes Mellitus and is a potential indicator of beta-cell demise (134).

In the previous study, they reported, for the first time, the detection of circulating diabetes-associated miR-224 and miR-103 in the urine of patients, notably in the absence of renal pathology. This was also the first study to detect miRNA in the urine of a large cohort of HNF1A-MODY mutation carriers. HNF1A-MODY is a monogenic form of diabetes and therefore an ideal model for the study of the beta-cell. HNF1A-MODY is primarily a disorder resulting in beta-cell dysfunction, with mutation carriers lacking features of the metabolic syndrome such as insulin resistance, as opposed to the multifactorial etiology synonymous with T2DM. They decided to study miR-224 and miR-103 in particular based on preliminary work performed in our laboratory, whereby the endogenous expression of HNF1A in INS-1 cell lines resulted in the elevated expression of both miR-224 and miR-103 (111).

Of interest, they found that miR-224 is highly expressed in the urine of patients with T1DM and HNF1A-MODY mutation carriers when compared to both the T2DM and the normal control cohorts. miR-224 is a novel miRNA in the field of diabetes. To date, it has been shown that miR-224 is aberrantly expressed in a wide variety of malignancies, including prostate, hepatocellular, renal cell, and colorectal. It is known to promote cell migration, proliferation, and invasion and has been shown to target TGF-β signaling via the Smad 4 pathway. The elevated expression of miR-224 in both HNF1A-MODY mutation carriers and T1DM patients is not surprising given that both share certain traits. Beta-cell failure and ultimately a reduction in beta-cell mass are noted in both HNF1A-MODY mutation carriers and T1DM. In contrast to T2DM, which is a *relatively* insulin-deficient state with the pathology attributed largely to insulin resistance, it is a defect in insulin secretion that is the principal pathology of both HNF1A-MODY mutation carriers and T1DM. This defect predates the clinical manifestation of diabetes, as demonstrated by research in the carriers of HNF1A-MODY who were normoglycemic and likewise in islet antibody-positive relatives of those with T1DM. They hypothesized that the altered miR-224 expression profile may be a contributor to the beta-cell dysfunction noted in both HNF1A-MODY mutation carriers and T1DM. They speculated that miR-224, as a marker of beta-cell demise, has the potential to be an important clinical development given the current

inability to perform pancreatic biopsies in humans and the lack of beta-cell imaging techniques. There are currently no available biomarkers of beta-cell demise.

miR-224 was significantly elevated in the urine of HNF1A-MODY mutation carriers when compared to the T2DM cohort studied. They, therefore, proposed that the clinical utility of miR-224 may be further expanded as an additional screening tool to decipher who should be screened for HNF1A-MODY genetic testing. This is of clinical relevance, given that it is not feasible to perform genetic testing on all potential mutation carriers, and the greatest clinical challenge is in the discrimination of potential HNF1A-MODY mutation carriers from lean patients with T2DM (135).

Given the present limitations on human pancreatic biopsies and the absence of beta-cell imaging methods, miRNA-224's potential as a marker of beta-cell death could be a significant clinical development. There are no recognized biomarkers for beta-cell death at the moment. So, we decided to measure the concentration of miRNA-224 in T2DM Egyptian patients and try to find its association with diabetic complications, and we found that miRNA-224 was upregulated in T2DM Egyptian patients by a 2-fold change and could be used in medical settings as a potential technique for anticipating the onset of T2DM. It may be used as a novel marker for the detection of diabetic complications (134).

Also, previous studies have found that the functional involvement of miRNA-224-5p in adipogenesis and adipocyte metabolic activity is stage-dependent. miRNA-224-5p is present at low levels in the early stages of adipogenesis in 3T3-L1 cells and rises to higher levels in the later stages. Higher levels of miRNA-224-5p can stop adipogenesis in its early stages by targeting EGR2. Consequently, miRNA-224-5p can control fatty acid metabolism during terminal differentiation by targeting Acyl-CoA synthetase long-chain family member 4 (ACSL4), leading to an increase in free fatty acids, which will increase the risk of T2DM.

3.4.7. miR-224 and other diseases related to diabetes

3.4.7.1. Post-transcriptional Regulation of PCSK9 by miR-224 and its role in cardiovascular disease:

Since the discovery of proprotein convertase subtilisin kexin 9 (PCSK9), a gene involved in LDL metabolism regulation and cardiovascular diseases (CVD), many therapeutic strategies

have been introduced for the direct targeting of PCSK9. The main goal of these strategies has been to reduce PCSK9 protein levels either by the application of antibodies or by inhibiting their production. The role of miR-224 in lipid metabolism has been investigated in several studies, and as this data also demonstrated, it could be considered a major regulator in lipid metabolic pathways. Peng et al. reported that miR-224 is a negative regulator of adipocyte differentiation. This miRNA also regulates fatty acid metabolism by directly targeting Acyl-CoA synthetase long-chain family member 4 (ACSL4), which is an essential enzyme in fatty acid metabolism (136). This study revealed that miR-224 could negatively regulate PCSK9 expression by directly targeting its transcript. Also, it is in accordance with former studies that introduced miR-224 as a regulator of lipid metabolism and of PCSK9 in particular (136). Overall, the study demonstrated that miR-224 could play important roles in lipid and cholesterol metabolism and participate in the development of disease conditions such as hypercholesterolemia and CVD, by targeting PCSK9, which has a critical role in LDLR degradation and cellular LDL uptake. Due to the importance of LDLR and PCSK9 regulation, miRNA could be considered novel biomarkers and therapeutic targets in hypercholesterolemia, obesity, and cardiovascular diseases. However, metabolic disorders are multifactorial and caused by complex deregulation in biochemical and cellular signaling pathways (136).

3.4.7.2. miR-224 and pancreatic cancer:

miR-224 is overexpressed in various cancers and promotes cancer progression. In a previous study, a novel mechanism underlying the role of miR-224 was elucidated, namely that miR-224 promotes pancreatic ductal adenocarcinoma (PDAC) cell growth through miR-224- (thioredoxin-interacting protein (TXNIP-HIF1) TXNIP-HIF1α signalling. A previous report showed that E2F1 modulates the expression of miR-224 by targeting TXNIP, which can drive cancer metastasis in melanoma (137). However, the mechanism through which TXNIP inhibits cancer progression was not addressed clearly. In previous studies, they have also shown that miR-224 targets TXNIP and suppresses its expression in pancreatic cancer, which may promote PDAC growth. Furthermore, the suppression of TXNIP by miR-224 leads to the nuclear translocation of HIF1α, which is an important stimulator of cancer progression. The loss of TXNIP has been shown to correlate with the advanced stages of breast, gastric, colorectal, and bladder cancers.

However, TXNIP genetic alterations, such as deletions, translocations, or somatic mutations, have not been found in these cancers (138).

3.4.7.3. miR-224 and coronary heart disease

While a potential role for miR-224 in regulating vascular disease has not been defined, this miRNA has been studied in association with multiple cancer cell types and other cellular systems, and these data provide some insight into upstream pathways that might affect *TCF21* expression and thus CHD risk. Most significant among these are NFκB, WNT, and TGF, all of which have been linked to atherosclerotic signaling pathways. NFκB is a well-characterized transcription factor and mediator of cellular activation by inflammatory cytokines and chemokines, and in the context of hepatocellular carcinoma, miR-224 was shown to be upregulated by tumor necrosis-α (TNF-α) and miR-224 regulation is linked to hepatocellular migration and invasion. TGF-β stimulation of miR-224 expression has been characterized in ovarian granulosa cells where it has been implicated in cellular proliferation and estradiol release in this cell type. Further, miR-224 has been shown to be upregulated by the WNT signaling pathway in medulloblastoma, where it was linked to inhibition of proliferation, increased radiation sensitivity, and reduced anchorage-independent growth of tumor cells. Each of these pathways has been linked to atherosclerotic processes in the diseased blood vessel wall and could have a role in the *TCF21*-mediated risk for CHD (139).

4. Conclusion

A novel non-invasive diagnostic biomarker called miRNA-224 may be used to identify people who are genetically predisposed to developing diabetes and may improve the clinical course of the disease. One such approach would be to use the circulating miRNAs for predicting the beginning of T2DM in clinical situations.

Conflict of Interest

The Authors declare no conflict of interest.

5. References

1. Gregory GA, Robinson TI, Linklater SE, Wang F, Colagiuri S, de Beaufort C, et al. Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. 2022;10(10):741-60.
2. Mizukami H, Kudoh K. Diversity of pathophysiology in type 2 diabetes shown by islet pathology. Journal of Diabetes Investigation. 2022;13(1):6-13.
3. Perween S, Abidi M, Faizy AFJIJoBM. Biophysical changes in methylglyoxal modified fibrinogen and its role in the immunopathology of type 2 diabetes mellitus. 2022;202:199-214.
4. Ning C, Jiao Y, Wang J, Li W, Zhou J, Lee Y-C, et al. Recent advances in the managements of type 2 diabetes mellitus and natural hypoglycemic substances. 2022;11(5):1121-33.
5. Suárez R, Chapela SP, Álvarez-Córdova L, Bautista-Valarezo E, Sarmiento-Andrade Y, Verde L, et al. Epigenetics in Obesity and Diabetes Mellitus: New Insights. 2023;15(4):811.
6. Mageed SSA, Doghish AS, Ismail A, El-Husseiny AA, Fawzi SF, Mahmoud AM, et al. The role of miRNAs in insulin resistance and diabetic macrovascular complications–A review. 2023:123189.
7. Yang W, Zhang T, Song X, Dong G, Xu L, Jiang FJC. SNP-target genes interaction perturbing the cancer risk in the post-GWAS. 2022;14(22):5636.
8. Zhang C, Liu S, Yang M. Hepatocellular carcinoma and obesity, type 2 diabetes mellitus, cardiovascular disease: Causing factors, molecular links, and treatment options. Frontiers in Endocrinology. 2021;12:808526.
9. Sebastian-delaCruz M, Gonzalez-Moro I, Olazagoitia-Garmendia A, Castellanos-Rubio A, Santin I. The role of lncRNAs in gene expression regulation through mRNA stabilization. Non-coding RNA. 2021;7(1):3.
10. Kaur P, Kotru S, Singh S, Behera BS, Munshi A. Role of miRNAs in the pathogenesis of T2DM, insulin secretion, insulin resistance, and β cell dysfunction: the story so far. Journal of physiology and biochemistry. 2020;76:485-502.
11. Deiuliis J. MicroRNAs as regulators of metabolic disease: pathophysiologic significance and emerging role as biomarkers and therapeutics. International journal of obesity. 2016;40(1):88-101.
12. Bielska A, Niemira M, Kretowski A. Recent highlights of research on miRNAs as early potential biomarkers for cardiovascular complications of type 2 diabetes mellitus. International Journal of Molecular Sciences. 2021;22(6):3153.
13. Roy SG. Regulation of autophagy by miRNAs in human diseases. The Nucleus. 2021;64(3):317-29.
14. Matsuoka T, Yashiro M. The Role of the Transforming Growth Factor-β Signaling Pathway in Gastrointestinal Cancers. Biomolecules. 2023;13(10):1551.
15. Rivas AM, Nugent K. Hyperglycemia, insulin, and insulin resistance in sepsis. The American Journal of the Medical Sciences. 2021;361(3):297-302.
16. McKnight CL, Griffard JH, Bhat SG, Bollig RW, Lawson CM, Long CA, et al. Introduction to metabolism. Surgical Metabolism: The Metabolic Care of the Surgical Patient. 2020:3-16.
17. Association AD. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2021. Diabetes Care. 2020;44(Supplement_1):S15-S33.
18. Biswas S. A review on the progress of stem cell therapy as a treatment for Diabetes mellitus: Brac University; 2022.

19. Ochocińska A, Wysocka-Mincewicz M, Cukrowska B. Autoantibodies against islet cell antigens: Current diagnostic possibilities. Journal of Laboratory Diagnostics. 2022;58(3):114-9.
20. de Jesús Ríos-Ríos W, Sosa-Luis SA, Torres-Aguilar H. Current advances in using tolerogenic dendritic cells as a therapeutic alternative in the treatment of type 1 diabetes. World journal of diabetes. 2021;12(5):603.
21. Demeterco-Berggren C, Ebekozien O, Rompicherla S, Jacobsen L, Accacha S, Gallagher MP, et al. Age and hospitalization risk in people with type 1 diabetes and COVID-19: data from the T1D Exchange Surveillance study. The Journal of Clinical Endocrinology & Metabolism. 2022;107(2):410-8.
22. Moberg LJUjoms. The role of the innate immunity in islet transplantation. 2005;110(1):17-56.
23. Janssen JA. Hyperinsulinemia and its pivotal role in aging, obesity, type 2 diabetes, cardiovascular disease and cancer. International Journal of Molecular Sciences. 2021;22(15):7797.
24. Atre S, Deshmukh S, Kulkarni M. Prevalence of type 2 diabetes mellitus (T2DM) in India: A systematic review (1994–2018). Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2020;14(5):897-906.
25. Bellary S, Kyrou I, Brown JE, Bailey CJ. Type 2 diabetes mellitus in older adults: clinical considerations and management. Nature Reviews Endocrinology. 2021;17(9):534-48.
26. Blahova J, Martiniakova M, Babikova M, Kovacova V, Mondockova V, Omelka R. Pharmaceutical drugs and natural therapeutic products for the treatment of type 2 diabetes mellitus. Pharmaceuticals. 2021;14(8):806.
27. Lima JE, Moreira NC, Sakamoto-Hojo ET. Mechanisms underlying the pathophysiology of type 2 diabetes: From risk factors to oxidative stress, metabolic dysfunction, and hyperglycemia. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2022;874:503437.
28. McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm PJNrDp. Gestational diabetes mellitus. 2019;5(1):47.
29. Jovanovič L, Liang Y, Weng W, Hamilton M, Chen L, Wintfeld N. Trends in the incidence of diabetes, its clinical sequelae, and associated costs in pregnancy. Diabetes/metabolism research and reviews. 2015;31(7):707-16.
30. Kasture V, Sahay A, Joshi S. Cell death mechanisms and their roles in pregnancy related disorders. Advances in protein chemistry and structural biology. 2021;126:195-225.
31. Rughani A, Friedman JE, Tryggestad JB. Type 2 diabetes in youth: the role of early life exposures. Current diabetes reports. 2020;20:1-11.
32. Serbis A, Giapros V, Kotanidou EP, Galli-Tsinopoulou A, Siomou E. Diagnosis, treatment and prevention of type 2 diabetes mellitus in children and adolescents. World Journal of Diabetes. 2021;12(4):344.
33. Edress HAG. Evaluation of Fibrinogen Level among Patients with Diabetes Mellitus Type2: Sudan University of Science & Technology; 2017.
34. Chaitou AR, Valmiki S, Valmiki M, Zahid M, Aid MA, Fawzy P, et al. New-Onset Diabetes Mellitus (NODM) After Liver Transplantation (LT): The Ultimate Non-diabetogenic Immunosuppressive Therapy. Cureus. 2022;14(3).
35. Rysz J, Franczyk B, Radek M, Ciałkowska-Rysz A, Gluba-Brzózka A. Diabetes and cardiovascular risk in renal transplant patients. International journal of molecular sciences. 2021;22(7):3422.

36. Chinnasamy A. Screening for Type 2 Diabetes Mellitus initiated through the dental setting: a cost-effectiveness analysis: The University of Melbourne; 2019.
37. Tao Z, Shi A, Zhao J. Epidemiological perspectives of diabetes. Cell biochemistry and biophysics. 2015;73:181-5.
38. Shera AS, Basit A, Fawwad A. Middle East and North Africa region guidelines for the management of type 2 diabetes. Journal of Diabetology. 2019;10(3):134-9.
39. Khalil SHA, Megallaa MH, Rohoma KH, Ismael H, AbouSeif M, Kharboush I, et al. Prevalence of type 2 diabetes mellitus in a sample of the adult population of Alexandria, Egypt. Diabetes research and clinical practice. 2018;144:63-73.
40. Madede T, Damasceno A, Lunet N, Augusto O, Silva-Matos C, Beran D, et al. Changes in prevalence and the cascade of care for type 2 diabetes over ten years (2005-2015): results of two nationally representative surveys in Mozambique. BMC Public Health. 2022;22(1):2174.
41. Cole J. The epidemiological transition. Planetary Health: Human Health in an Era of Global Environmental Change. 2019:58.
42. Yefet E, Jeda E, Tzur A, Nachum Z. Markers for undiagnosed type 2 diabetes mellitus during pregnancy—A population-based retrospective cohort study. Journal of Diabetes. 2020;12(3):205-14.
43. Lee S-W, Han K, Kwon H-SJOI. Weight change and the risk of hip fractures in patients with type 2 diabetes: a nationwide cohort study. 2022;33(8):1755-67.
44. A Mohamed R, Mohammed Abd-Elghan S, Hussein Mohammad Abd Elrhman S, Nabeh Taref N. Effect of Applying Health Belief Model on Type 2 Diabetic Patients' Knowledge, Self-Care Practices and Health Beliefs. Egyptian Journal of Health Care. 2022;13(2):67-81.
45. Melaram R. Environmental risk factors implicated in liver disease: a mini-review. Frontiers in Public Health. 2021;9:683719.
46. Habeeb AAS. Assessment of Triglyceride/HDL-c Ratio and Triglyceride/glucose Index as Markers for Glycemic Control in Patient with Type2 Diabetes Mellitus in Khartoum State: Sudan University of Science & Technology; 2020.
47. Spellman CW. Pathophysiology of type 2 diabetes: targeting islet cell dysfunction. Journal of Osteopathic Medicine. 2010;110(s32):2-7.
48. Reddel HK, Bacharier LB, Bateman ED, Brightling CE, Brusselle GG, Buhl R, et al. Global Initiative for Asthma Strategy 2021: executive summary and rationale for key changes. Respirology. 2022;27(1):14-35.
49. Al-Suhaimi EA, Aljfary MA, Khan FA. Endocrine Pancreas and Glucose Metabolism. Emerging Concepts in Endocrine Structure and Functions: Springer; 2022. p. 247-85.
50. Holst JJ. Treatment of type 2 diabetes and obesity on the basis of the incretin system: the 2021 Banting medal for scientific achievement award lecture. Diabetes. 2021;70(11):2468-75.
51. Mengeste AM, Rustan AC, Lund J. Skeletal muscle energy metabolism in obesity. Obesity. 2021;29(10):1582-95.
52. Čolak E, Pap D. The role of oxidative stress in the development of obesity and obesity-related metabolic disorders. Journal of Medical Biochemistry. 2021;40(1):1.
53. Lin Y, Sun Z. Current views on type 2 diabetes. The Journal of endocrinology. 2010;204(1):1.
54. Chen C, Liu D. Establishment of Zebrafish Models for Diabetes Mellitus and Its Microvascular Complications. Journal of Vascular Research. 2022:1-9.
55. Forrester JV, Kuffova L, Delibegovic M. The role of inflammation in diabetic retinopathy. Frontiers in immunology. 2020;11:583687.

56. Bhardwaj C, Jain S, Sood M. Hierarchical severity grade classification of non-proliferative diabetic retinopathy. Journal of Ambient Intelligence and Humanized Computing. 2021;12(2):2649-70.
57. Pradas M, Rodriguez-Merchante MP, Estébanez N, Sarraf D, Freund KB, Fawzi A, et al. Navigating the white dot syndromes with optical coherence tomography (OCT) and OCT angiography (OCT-A). 2022;30(3):664-74.
58. Mansour SE, Browning DJ, Wong K, Flynn Jr HW, Bhavsar AR. The evolving treatment of diabetic retinopathy. Clinical Ophthalmology. 2020:653-78.
59. Mrugacz M, Pony-Uram M, Bryl A, Zorena K. Current Approach to the Pathogenesis of Diabetic Cataracts. International Journal of Molecular Sciences. 2023;24(7):6317.
60. Shao M-M, Xiang H-J, Lu H, Yin P-H, Li G-W, Wang Y-M, et al. Candidate metabolite markers of peripheral neuropathy in Chinese patients with type 2 diabetes. American Journal of Translational Research. 2022;14(8):5420.
61. Sawaf H, Thomas G, Taliercio JJ, Nakhoul G, Vachharajani TJ, Mehdi AJJoCM. Therapeutic advances in diabetic nephropathy. 2022;11(2):378.
62. Amran MS, Bahar NB, Akash S. Physiology and Pathology of the Cardiovascular System. Cardiovascular Diseases: IntechOpen; 2022.
63. Shetty SS, Kumari S. Fatty acids and their role in type-2 diabetes. Experimental and therapeutic medicine. 2021;22(1):1-6.
64. van der Bruggen MM, Spronck B, Delhaas T, Reesink KD, Schalkwijk CG. The putative role of methylglyoxal in arterial stiffening: A review. Heart, Lung and Circulation. 2021;30(11):1681-93.
65. Overstreet RM. Parasitic diseases of fishes and their relationship with toxicants and other environmental factors. Pathobiology of marine and estuarine organisms: CRC press; 2021. p. 111-56.
66. Tonti-Filippini J, Nevill PG, Dixon K, Small I. What can we do with 1000 plastid genomes? : Wiley Online Library; 2017. p. 808-18.
67. Bhatti GK, Khullar N, Sidhu IS, Navik US, Reddy AP, Reddy PH, et al. Emerging role of non-coding RNA in health and disease. Metabolic Brain Disease. 2021;36:1119-34.
68. Britton C, Laing R, Devaney E. Small RNAs in parasitic nematodes–forms and functions. Parasitology. 2020;147(8):855-64.
69. Barreda-Manso MA, Nieto-Díaz M, Soto A, Muñoz-Galdeano T, Reigada D, Maza RM. In silico and in vitro analyses validate human MicroRNAs targeting the SARS-CoV-2 3′-UTR. International Journal of Molecular Sciences. 2021;22(11):6094.
70. Yu T, Xu N, Haque N, Gao C, Huang W, Huang Z. Popular computational tools used for miRNA prediction and their future development prospects. Interdisciplinary Sciences: Computational Life Sciences. 2020;12:395-413.
71. Ali Syeda Z, Langden SSS, Munkhzul C, Lee M, Song SJ. Regulatory mechanism of MicroRNA expression in cancer. International journal of molecular sciences. 2020;21(5):1723.
72. Ghalehnoei H, Bagheri A, Fakhar M, Mishan MA. Circulatory microRNAs: promising non-invasive prognostic and diagnostic biomarkers for parasitic infections. European Journal of Clinical Microbiology & Infectious Diseases. 2020;39:395-402.
73. Morselli M, Dieci G. Epigenetic regulation of human non-coding RNA gene transcription. Biochemical Society Transactions. 2022;50(2):723-36.
74. Stankov K, Stankov S, Katanic J. Genetic and epigenetic drug targets in myelodysplastic syndromes. Current Pharmaceutical Design. 2017;23(1):135-69.

75. Pathak GA, Singh K, Wendt FR, Fleming TW, Overstreet C, Koller D, et al. Genetically regulated multi-omics study for symptom clusters of posttraumatic stress disorder highlights pleiotropy with hematologic and cardio-metabolic traits. Molecular Psychiatry. 2022;27(3):1394-404.
76. Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SHJJocp. An overview of microRNAs: biology, functions, therapeutics, and analysis methods. 2019;234(5):5451-65.
77. Vishnoi A, Rani S. MiRNA biogenesis and regulation of diseases: an overview. MicroRNA Profiling. 2017:1-10.
78. Playfoot CJ, Sheppard S, Planet E, Trono D. Transposable elements contribute to the spatiotemporal microRNA landscape in human brain development. bioRxiv. 2022.
79. Van Vleet TR, Mahalingaiah PK. MiRNA as Biomarkers of Mitochondrial Toxicity. Mitochondrial Dysfunction Caused by Drugs and Environmental Toxicants. 2018:347-72.
80. Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, Ramirez-Acuna JM, Perez-Romero BA, Guerrero-Rodriguez JF, et al. The roles of matrix metalloproteinases and their inhibitors in human diseases. 2020;21(24):9739.
81. Deogharia M, Gurha P. The "guiding" principles of noncoding RNA function. Wiley Interdisciplinary Reviews: RNA. 2021:e1704.
82. Das S, Vera M, Gandin V, Singer RH, Tutucci E. Intracellular mRNA transport and localized translation. Nature Reviews Molecular Cell Biology. 2021;22(7):483-504.
83. Margaritis K, Margioula-Siarkou G, Giza S, Kotanidou EP, Tsinopoulou VR, Christoforidis A, et al. Micro-RNA implications in type-1 diabetes mellitus: a review of literature. International Journal of Molecular Sciences. 2021;22(22):12165.
84. Yates AG, Pink RC, Erdbrügger U, Siljander PRM, Dellar ER, Pantazi P, et al. In sickness and in health: The functional role of extracellular vesicles in physiology and pathology in vivo: Part I: Health and Normal Physiology. Journal of extracellular vesicles. 2022;11(1):e12151.
85. Majidinia M, Mir SM, Mirza-Aghazadeh-Attari M, Asghari R, Kafil HS, Safa A, et al. MicroRNAs, DNA damage response and ageing. Biogerontology. 2020;21:275-91.
86. Wang Y-T, Tsai P-C, Liao Y-C, Hsu C-Y, Juo S-HH. Circulating microRNAs have a sex-specific association with metabolic syndrome. Journal of biomedical science. 2013;20:1-10.
87. Abdalla F, Singh B, Bhat HK. MicroRNAs and gene regulation in breast cancer. Journal of biochemical and molecular toxicology. 2020;34(11):e22567.
88. Chandan K, Gupta M, Sarwat M. Role of host and pathogen-derived microRNAs in immune regulation during infectious and inflammatory diseases. Frontiers in immunology. 2020;10:3081.
89. Murdaca G, Greco M, Negrini S, Casciaro M, Gangemi S. The role of skin and gut microbiome and epigenetic modifications in skin-autoimmune disorders. Current Molecular Medicine. 2021;21(4):283-90.
90. Ardekani AM, Naeini MMJAjomb. The role of microRNAs in human diseases. 2010;2(4):161.
91. Khan AQ, Ahmed EI, Elareer NR, Junejo K, Steinhoff M, Uddin S. Role of miRNA-regulated cancer stem cells in the pathogenesis of human malignancies. Cells. 2019;8(8):840.
92. LaCasse E, Mahoney D, Cheung H, Plenchette S, Baird S, Korneluk RJO. IAP-targeted therapies for cancer. 2008;27(48):6252-75.

93. Matboli M, Gadallah SH, Rashed WM, Hasanin AH, Essawy N, Ghanem HM, et al. mRNA-miRNA-lncRNA regulatory network in nonalcoholic fatty liver disease. International journal of molecular sciences. 2021;22(13):6770.
94. Weber JA, Baxter DH, Zhang S, Huang DY, How Huang K, Jen Lee M, et al. The microRNA spectrum in 12 body fluids. Clinical chemistry. 2010;56(11):1733-41.
95. Condrat CE, Thompson DC, Barbu MG, Bugnar OL, Boboc A, Cretoiu D, et al. miRNAs as biomarkers in disease: latest findings regarding their role in diagnosis and prognosis. Cells. 2020;9(2):276.
96. Lan H, Lu H, Wang X, Jin H. MicroRNAs as potential biomarkers in cancer: opportunities and challenges. BioMed research international. 2015;2015.
97. Hanna J, Hossain GS, Kocerha J. The potential for microRNA therapeutics and clinical research. Frontiers in genetics. 2019;10:478.
98. Angelescu MA, Andronic O, Dima SO, Popescu I, Meivar-Levy I, Ferber S, et al. miRNAs as biomarkers in diabetes: moving towards Precision Medicine. International Journal of Molecular Sciences. 2022;23(21):12843.
99. Alzaid A, Ladrón de Guevara P, Beillat M, Lehner Martin V, Atanasov P. Burden of disease and costs associated with type 2 diabetes in emerging and established markets: systematic review analyses. Expert Review of Pharmacoeconomics & Outcomes Research. 2021;21(4):785-98.
100. Sert UY, Ozgu-Erdinc AS. Gestational diabetes mellitus screening and diagnosis. Diabetes: from Research to Clinical Practice: Volume 4. 2021:231-55.
101. Owei I, Umekwe N, Stentz F, Wan J, Dagogo-Jack SJM. Amino acid signature predictive of incident prediabetes: a case-control study nested within the longitudinal pathobiology of prediabetes in a biracial cohort. 2019;98:76-83.
102. Gall WE, Beebe K, Lawton KA, Adam K-P, Mitchell MW, Nakhle PJ, et al. α-Hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population. 2010;5(5):e10883.
103. Kumar AA, Satheesh G, Vijayakumar G, Chandran M, Prabhu PR, Simon L, et al. Plasma leptin level mirrors metabolome alterations in young adults. 2020;16:1-13.
104. Kong L, Zhu J, Han W, Jiang X, Xu M, Zhao Y, et al. Significance of serum microRNAs in pre-diabetes and newly diagnosed type 2 diabetes: a clinical study. Acta diabetologica. 2011;48:61-9.
105. Dexheimer PJ, Cochella L. MicroRNAs: from mechanism to organism. Frontiers in cell and developmental biology. 2020;8:409.
106. Agbu P, Carthew RW. MicroRNA-mediated regulation of glucose and lipid metabolism. Nature reviews Molecular cell biology. 2021;22(6):425-38.
107. Alexandru N, Procopciuc A, Vîlcu A, Comarița IK, Bădilă E, Georgescu A. Extracellular vesicles—Incorporated microRNA signature as biomarker and diagnosis of prediabetes state and its complications. Reviews in Endocrine and Metabolic Disorders. 2022;23(3):309-32.
108. Cui X, You L, Zhu L, Wang X, Zhou Y, Li Y, et al. Change in circulating microRNA profile of obese children indicates future risk of adult diabetes. Metabolism. 2018;78:95-105.
109. Zen K, Zhang CY. Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. Medicinal research reviews. 2012;32(2):326-48.
110. Miyachi Y, Miyazawa T, Ogawa Y. HNF1A mutations and beta cell dysfunction in diabetes. International journal of molecular sciences. 2022;23(6):3222.

111. Bacon S, Engelbrecht B, Schmid J, Pfeiffer S, Gallagher R, McCarthy A, et al. MicroRNA-224 is readily detectable in urine of individuals with diabetes mellitus and is a potential indicator of beta-cell demise. Genes. 2015;6(2):399-416.
112. Pöstyéni E, Kovács-Valasek A, Urbán P, Czuni L, Sétáló Jr G, Fekete C, et al. Analysis of mir-9 expression pattern in rat retina during postnatal development. International journal of molecular sciences. 2021;22(5):2577.
113. Frias S. miR-375 IS NECESSARY FOR THE GROWTH OF PANCREATIC BETA-CELL: California State University, Chico; 2020.
114. He X, Kuang G, Wu Y, Ou CJC, medicine t. Emerging roles of exosomal miRNAs in diabetes mellitus. 2021;11(6):e468.
115. Mika-Gospodorz B. Development and application of bioinformatics tools for analysis of dual RNA-seq experiments: Universität Würzburg; 2022.
116. Daniel M, Tollefsbol TO. Epigenetic linkage of aging, cancer and nutrition. Journal of Experimental Biology. 2015;218(1):59-70.
117. Nomair A, Issa N, Madkour M, Shamseya M. The clinical significance of serum miRNA-224 expression in hepatocellular carcinoma. Clinical and Experimental Hepatology. 2020;6(1):20-7.
118. Li Y, Di C, Li W, Cai W, Tan X, Xu L, et al. Oncomirs miRNA-221/222 and tumor suppressors miRNA-199a/195 are crucial miRNAs in liver cancer: a systematic analysis. Digestive diseases and sciences. 2016;61:2315-27.
119. Han S-K, Herrmann A, Yang J, Iwasaki R, Sakamoto T, Desvoyes B, et al. Deceleration of the cell cycle underpins a switch from proliferative to terminal divisions in plant stomatal lineage. Developmental Cell. 2022;57(5):569-82. e6.
120. El-Mahdy HA, Sallam A-AM, Ismail A, Elkhawaga SY, Elrebehy MA, Doghish AS. miRNAs inspirations in hepatocellular carcinoma: detrimental and favorable aspects of key performers. Pathology-Research and Practice. 2022;233:153886.
121. Yao G, Yin M, Lian J, Tian H, Liu L, Li X, et al. MicroRNA-224 is involved in transforming growth factor-β-mediated mouse granulosa cell proliferation and granulosa cell function by targeting Smad4. 2010;24(3):540-51.
122. Doghish AS, Elballal MS, Elazazy O, Elesawy AE, Elrebehy MA, Shahin RK, et al. The role of miRNAs in liver diseases: Potential therapeutic and clinical applications. Pathology-Research and Practice. 2023:154375.
123. Ebrahimi SO, Reiisi S, Shareef SJJocp. miRNAs, oxidative stress, and cancer: A comprehensive and updated review. 2020;235(11):8812-25.
124. Li C-l, Liu X-h, Qiao Y, Ning L-n, Li W-j, Sun Y-s, et al. Allicin alleviates inflammation of diabetic macroangiopathy via the Nrf2 and NF-kB pathway. European Journal of Pharmacology. 2020;876:173052.
125. De Munck J. The effect of quercetin on in vitro cell models for endothelial dysfunction: Ghent University; 2020.
126. López-Contreras AK, Martínez-Ruiz MG, Olvera-Montaño C, Robles-Rivera RR, Arévalo-Simental DE, Castellanos-González JA, et al. Importance of the use of oxidative stress biomarkers and inflammatory profile in aqueous and vitreous humor in diabetic retinopathy. Antioxidants. 2020;9(9):891.
127. Singhvi G, Manchanda P, Rapalli VK, Dubey SK, Gupta G, Dua K. MicroRNAs as biological regulators in skin disorders. Biomedicine & Pharmacotherapy. 2018;108:996-1004.

128. Abdallah HY, Faisal S, Tawfik NZ, Soliman NH, Kishk RM, Ellawindy A. Expression Signature of Immune-Related MicroRNAs in Autoimmune Skin Disease: Psoriasis and Vitiligo Insights. Molecular Diagnosis & Therapy. 2023;27(3):405-23.
129. Rashed LA, Ibrahim HA. Expression of MicroRNA-224-3p in vitiligo. Journal of Pakistan Association of Dermatologists. 2021;31(4):672-6.
130. Correia CN, Nalpas NC, McLoughlin KE, Browne JA, Gordon SV, MacHugh DE, et al. Circulating microRNAs as potential biomarkers of infectious disease. Frontiers in immunology. 2017;8:118.
131. Abdallah HY, Abdelhamid NR, Mohammed EA, AbdElWahab NY, Tawfik NZ, Gomaa AH, et al. Investigating melanogenesis-related microRNAs as disease biomarkers in vitiligo. Scientific Reports. 2022;12(1):13526.
132. Li L. The role of MicroRNAs in vitiligo: Regulators and therapeutic targets. Annals of Dermatology. 2020;32(6):441.
133. Zang C-S, Huang H-T, Qiu J, Sun J, Ge R-F, Jiang L-W. MiR-224-5p targets EGR2 to promote the development of papillary thyroid carcinoma. European Review for Medical & Pharmacological Sciences. 2020;24(9).
134. Mohamed AA, Abo–Elmatty DM, Esmail OE, Salim HSM, Abd El Salam SM, El-Ansary AR, et al. MICRORNA-224 UP-REGULATION: A RISK FOR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS EGYPTIAN PATIENTS. Pharmacophore. 2022;13(6).
135. Bacon S, Engelbrecht B, Schmid J, Pfeiffer S, Gallagher R, McCarthy A, et al. MicroRNA-224 is readily detectable in urine of individuals with diabetes mellitus and is a potential indicator of beta-cell demise. 2015;6(2):399-416.
136. Naeli P, Mirzadeh Azad F, Malakootian M, Seidah NG, Mowla SJ. Post-transcriptional regulation of PCSK9 by miR-191, miR-222, and miR-224. Frontiers in genetics. 2017;8:189.
137. Zhu G, Zhou L, Liu H, Shan Y, Zhang X. MicroRNA-224 promotes pancreatic cancer cell proliferation and migration by targeting the TXNIP-mediated HIF1α pathway. Cellular Physiology and Biochemistry. 2018;48(4):1735-46.
138. Zhou J, Yu Q, Chng W-J. TXNIP (VDUP-1, TBP-2): a major redox regulator commonly suppressed in cancer by epigenetic mechanisms. The international journal of biochemistry & cell biology. 2011;43(12):1668-73.
139. Godoy PM, Bhakta NR, Barczak AJ, Cakmak H, Fisher S, MacKenzie TC, et al. Large differences in small RNA composition between human biofluids. Cell reports. 2018;25(5):1346-58.