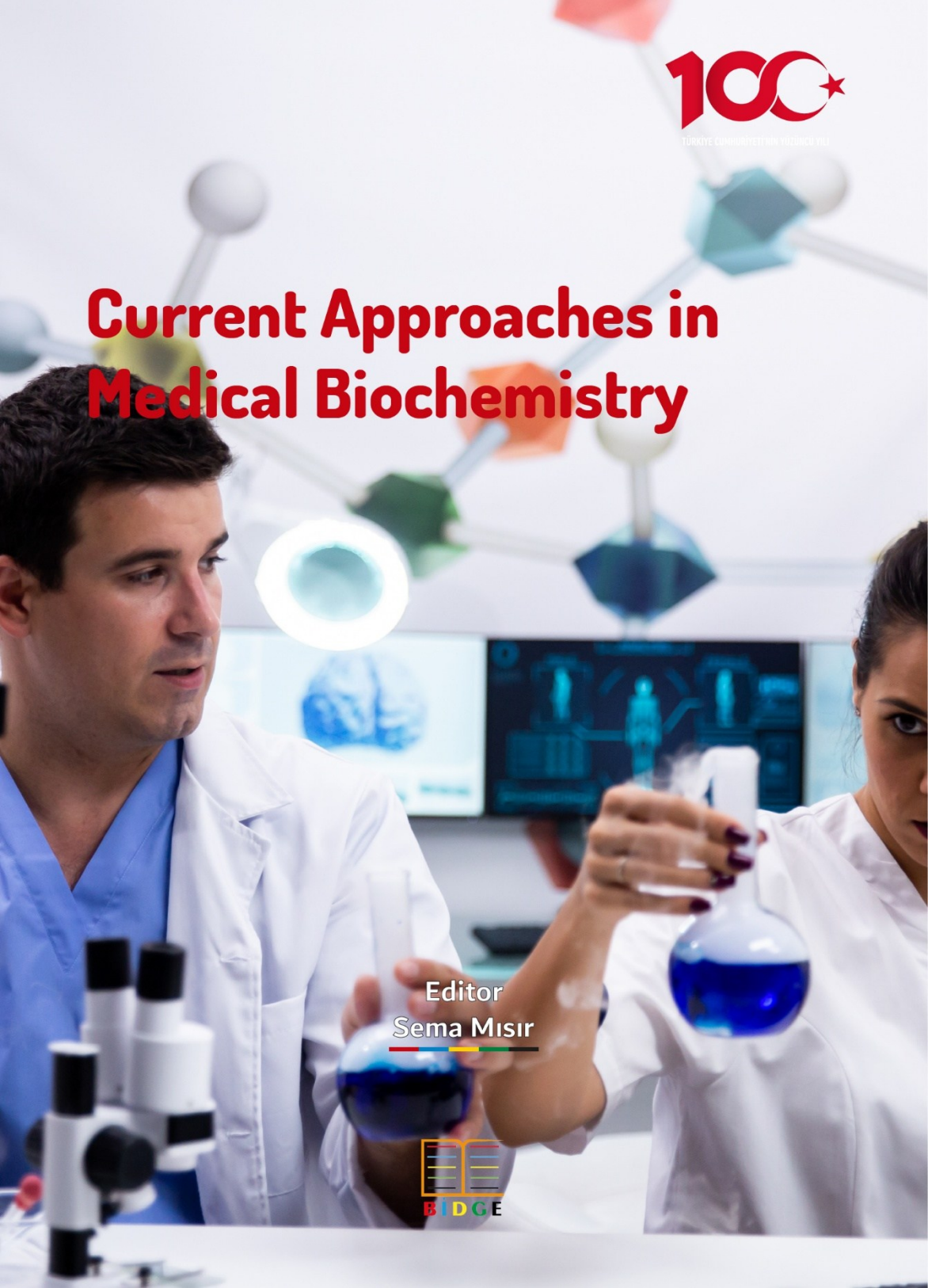
Current Approaches in Medical Biochemistry



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Content

CHAPTER I

miRNAs as a Biomarker Potential in Lung Cancer

Sema MISIR[1]

Lung cancer

Cancer is a pathological condition that arises due to uncontrolled cell growth and/or reduced apoptosis, promoting tumor formation and metastasis (Guz et al., 2014, Kuno et al., 2012). Lung cancer is the type of cancer with the highest mortality rate worldwide, and its rapid progression, combined with the inability to detect it in early stages, leads to reduced patient survival (Nielsen & Fredberg, 2022,Wheless et al., 2013). In accordance with data from the World Health Organization, lung cancer is the leading cause of cancer-related deaths worldwide (Guz et al., 2014,Takamizawa et al., 2004) and the most common cancer diagnosed in men and women (Lobera et al., 2023; Misir, Hepokur, et al., 2020). Lung cancer is histologically classified into two types: non-small cell lung

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cancer (NSCLC) and small cell lung carcinoma (SCLC). NSCLC accounts for over 80% of lung cancer cases and is further divided into subtypes, including adenocarcinoma (ADC), squamous cell carcinoma (SCC), and large cell carcinoma (LCC) (Raczkowska et al., 2023). The development of lung cancer is complex and multi-factorial, influenced by factors such as genetics, exposure to environmental carcinogens, and lifestyle (Mbemi et al., 2020, Wu et al., 2019). Environmental factors such as smoking, air pollution, radiation, and exposure to heavy metals play a significant role. Among these, smoking is the most critical risk factor, with lung cancer incidence being higher in the male population. In individuals who do not smoke, the development of lung cancer is attributed to genetic predisposition, exposure to harmful gases, and air pollution (De Sousa Monteiro et al., 2014). Additionally, factors such as family history, specific mutations, gender, age, race, and ethnicity can influence a person's susceptibility to the disease (Sweef et al., 2023). The treatment of lung cancer involves methods such as surgery, chemotherapy (drug therapy), and radiotherapy (radiation therapy), depending on the type and stage of cancer. The goal of cancer treatment is to eliminate the disease if possible, or if not, to control its progression, increase survival, and enhance the patient's quality of life (Lahiri et al., 2023). Detecting lung cancer early, maximizing treatment effectiveness, and identifying new biomarkers that can help predict tumor responses are crucial. Current studies focus on liquid biopsy, a non-invasive and easily accessible method based on simple blood collection. Despite extensive clinical research, advanced diagnostic techniques, and improved supportive care, the majority of lung cancer patients (60-80%) are diagnosed at an advanced stage. Low survival rates and a lack of therapeutic choices result from this. Consequently, reliable biomarkers that can aid in early identification, predict patient outcomes, and track treatment responses are required (Suri et al., 2024).

Biomarkers in Lung cancer

Identifying biomarkers is considered one of the most critical areas of personalized medicine in clinical oncology. Different types

of biomarkers are identified based on their predictive values in clinical and practical settings. Prognostic biomarkers indicate the likely progression of the disease, while pharmacological biomarkers demonstrate the effectiveness of a treatment. Predictive biomarkers estimate a patient's prognosis for a selected treatment. For a biomarker to be ideal, it should be helpful for diagnosis, prognosis, and/or response to treatment, meeting specific criteria. First, the biomarker should be quickly and repeatedly accessible in biological material and measurable through a non-invasive method (Behulová et al., 2023). Additionally, these molecules should possess high sensitivity, specificity, and positive predictive value (Lobera et al., 2023).

These molecules can be used for early diagnosis, predicting patient prognosis, monitoring tumor progression, and assessing responses to lung cancer treatment. The overall 5-year survival rate for lung cancer is no more than 15% (Zhao et al., 2016). Early lung cancer diagnosis in its initial stages is essential for improving treatment efficacy and patient survival rates. Therefore, it is crucial to identify easily detectable biomarkers that determine the presence of lung tumors or reveal disease progression in cancer patients (Guz et al., 2014, Kosaka et al., 2010).

Due to various advancements in molecular genetics, it has become possible to identify different types of molecules that can be used as biomarkers for cancer, in addition to genomic mutations (Lobera et al., 2023, Maharjan et al., 2020). Among the molecules that can be used as biomarkers are circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), tumor-educated platelets (TEPs), exosomes-extracellular vesicles measuring 40-100 nm and microRNAs (miRNAs) (Lobera et al., 2023).

miRNA

Gene expression is largely controlled by miRNAs, which are small, non-coding RNA molecules that are around 22 nucleotides in length (H. Yang et al., 2023). miRNAs are involved in several biological processes, such as apoptosis, differentiation, growth, and

development. In order to control gene expression at the post-transcriptional stage, they bind to complementary regions in target mRNAs (Suri et al., 2024). Human tissue-specific and highly conserved miRNAs (Suri et al., 2024) are detectable in nearly every bodily fluid, including plasma, serum, urine, saliva, and tears (Chen et al., 2008). Changes in regular physiological processes may be reflected in abnormal blood miRNA expression levels (Sredni et al., 2011). Many studies have reported that aberrant levels of miRNA expression are linked to various diseases, such as cancer, cardiovascular, inflammatory, and gynecological conditions (Misir et al., 2020, Misir et al., 2021, Small & Olson, 2011, H. Yang et al., 2008).

Biogenesis and functions of miRNAs

miRNA biogenesis is several steps that spans from transcription in the nucleus to the formation of mature miRNA in the cytoplasm (Conrad et al., 2014). miRNA precursors, known as pri-miRNAs, are transcribed from genomic DNA by RNA polymerase II. These pri-miRNAs are 500-3000 bases long, with a stem-loop structure and 5' cap and 3' poly-A tail (Wu et al., 2019). In the nucleus, pri-miRNAs are processed by Drosha, an endonuclease of the RNAse III enzyme family, and its cofactor DGCR8 (DiGeorge Syndrome Critical Region 8). This processing cleaves the pri-miRNAs at specific sites, forming pre-miRNAs approximately 70 nucleotides in length (Conrad et al., 2014). The pre-miRNAs are then transported from the nucleus to the cytoplasm by exportin 5, a dsRNA-binding protein that operates in a RanGTP-dependent manner (Lund et al., 2004). In the cytoplasm, the pre-miRNA hairpin is further cleaved by Dicer, another RNAse III enzyme, converting pre-miRNAs into ~22-nucleotide miRNA:miRNA duplexes. Typically, one strand of the miRNA duplex is incorporated into a multi-protein complex known as the RNA-induced silencing complex (RISC). The RISC/miRNA complex then binds to target mRNAs based on base-pairing properties, inhibiting protein translation of the corresponding gene and/or degradation of the mRNA (Wu et al., 2019). It is known that a single miRNA can

control the expression of multiple mRNAs, and multiple miRNAs can target each mRNA. Therefore, miRNAs involve multiple biological processes, including their role in genomic and epigenomic interactions (Nana-Sinkam & Geraci, 2006, Wu et al., 2019).

Since their discovery, many miRNAs have been identified, and studies continue to explore their functions and potential applications in research and clinical settings (Ho et al., 2022).

The Roles of miRNA in Lung Cancer

miRNAs are reported to play roles in cell proliferation, invasion, angiogenesis, and metastasis in lung cancer (Wu et al., 2019). Tumor suppressor and oncogenic miRNAs are the two main categories of cancer-related miRNAs (Otmani & Lewalle, 2021). In lung cancer treatment, it is essential first to understand the roles of oncogenic or tumor suppressor miRNAs and how they affect the development and progression of lung cancer (Frydrychowicz et al., 2023, Wani et al., 2022). In a particular cell type, a miRNA gene may be regarded as a tumor suppressor if its main target is an oncogene. On the other hand, a miRNA gene is considered an oncogene if its target is a tumor suppressor gene present in various cell types (Otmani & Lewalle, 2021). In anticancer therapy, overexpression of tumor suppressor miRNAs and suppression of oncogenic miRNAs may be important (Lima et al., 2011).

Because of its synergistic action with tumor suppressor p53, the miR-34 family (miR-34a, miR-34b, and miR-34c) is regarded as a tumor suppressor miRNA in many cancer types (L. Zhang et al., 2019). miR-34 prevents uncontrolled cell proliferation and promotes apoptosis by targeting Cyclin D1, CDK4/6, E2F family members, and anti-apoptotic proteins like BCL2 (Rokavec et al., 2014). The Let-7 family is highly expressed in normal lung tissue, but its expression has been reported to decrease in lung cancer cells (Y. Wang et al., 2022). Let-7a reduces the development of lung cancer by inhibiting Ras and c-Myc expression (He et al., 2010). Similarly, let-7b-3p has been demonstrated to directly target the BRF2-mediated MAPK/ERK pathway, thereby inhibiting the growth and

metastasis of lung cancer cells (Li et al., 2021). miR-486 has been reported to function as a tumor suppressor in lung cancer, particularly in the development of NSCLC, by targeting relevant signaling components such as insulin-like growth factor 1 (IGF1) and the IGF1 receptor (IGF1R), both in vitro and in vivo (Peng et al., 2013). The overexpression of miR-200c-3p and miR-485-5p has been reported to target RRM2 in cisplatin-resistant NSCLC tissues and cells, suppressing drug resistance and malignant behaviors (Liu et al., 2022).

Unlike tumor suppressor miRNAs, oncogenic miRNAs often exhibit functions that promote uncontrolled growth and/or anti-apoptotic effects in cancer types (Suri et al., 2024). Among the most studied oncogenic miRNAs in lung cancer, miR-21 is frequently reported to have increased expression in various lung cancer types (Iqbal et al., 2019). miR-21 contributes to tumor development by targeting PTEN (Zhou et al., 2019) and PDCD4 (Matsuhashi et al., 2019), leading to the downregulation of these suppressor molecules (Suri et al., 2024). miR-486-5p regulates tumor formation and progression by stabilizing metastasis and facilitating intercellular communication via exosomes (B. Yang et al., 2024, F. Yang et al., 2017). Overexpression of miR-25 is associated with increased cell proliferation and invasion. Other miRNAs, including miR-126, miR-100, and miR-145, have an reverse interaction with the VEGF family and are expressed at higher levels in lung cancer (Iqbal et al., 2019, Lobera et al., 2023). Overexpression of miR-224 in NSCLC CAFs has been reported to induce NSCLC tumor progression by regulating the SIRT3-AMPK-mTOR-HIF-1α signaling pathway (J. Zhang et al., 2021).

miRNAs as a biomarker in Lung cancer

Early lung cancer diagnosis improves treatment efficacy and patient survival rates. Thus, it is necessary to have easily detectable biomarkers in order to diagnose lung tumors or track the progression of cancer patients' illnesses (Kosaka et al., 2010). Among lung cancer biomarkers, miRNAs are the most promising due to their

prognostic and diagnostic potential, stability, and tissue-specific nature, making them vital in the fight against lung cancer (Suri et al., 2024). The possibility of employing miRNAs as biomarkers is further increased by the association found between various miRNA profiles in bodily fluids and tumor growth, metastasis, and patient clinical prognosis (Guz et al., 2014). miRNAs are also associated with therapeutic response and patient survival, making these molecules important as therapeutic biomarkers (Guz et al., 2014, Lobera et al., 2023).

miRNAs as Diagnostic Biomarkers

One of the significant challenges in the classification of lung cancer is its late diagnosis, which significantly limits available treatment options (Lobera et al., 2023). Many studies have examined at the diagnostic use of miRNAs in lung cancer to date, and early detection of the disease may result in higher survival rates (Gao et al., 2011). The primary miRNAs used as diagnostic biomarkers in lung cancer are shown in Table 1. miR-21 has been reported to have potential clinical value for lung cancer diagnosis and could serve as a therapeutic target (W. Wang et al., 2022). In a study conducted on plasma and tissue samples from patients with early-stage NSCLC, the AUC values of miR-486 and miR-150 were reported as 0.926 (sensitivity: 0.909; specificity: 0.818) and 0.752 (sensitivity: 0.818; specificity: 0.818), respectively. According to these findings, miR-486 and miR-150 may be useful biomarkers for NSCLC early detection (W. Li et al., 2015). In another study, the downregulation of miR-30a-3p, miR-30a-5p, miR-30c-2-3p, miR-30d-5p, and miR-27a-5p was identified as promising biomarkers for the early detection of lung cancer. The corresponding AUC values for the diagnostic accuracy of miR-27a-5p, miR-30a-3p, miR-30a-5p, miR-30c-2-3p, and miR-30d-5p were reported as 0.637, 0.758, 0.772, 0.734, and 0.776, respectively (Y. Zhang et al., 2017). In a study by Ma and colleagues, serum miR-125b was identified as a potential novel biomarker for NSCLC patients, and high miR-125b expression was found to be an independent prognostic factor for survival (Yuxia et al., 2012). A study on the serum of lung cancer patients reported

that the expression levels of miR-21, miR-210, and miR-155 were increased, suggesting their potential as non-invasive biomarkers for the early diagnosis of lung cancer (Lianidou et al., 2015). Hennessey and colleagues demonstrated that serum miRNAs miR-15b and miR-27b could potentially be sensitive biomarkers for the early detection of NSCLC (Hennessey et al., 2012). A study analyzing sputum samples from lung cancer patients found that miR-21 expression was increased compared to non-cancer individuals. This elevated miR-21 expression demonstrated diagnostic value for lung cancer with 69.9% sensitivity and 100% specificity (Xie et al., 2010). Similarly, Xing and colleagues identified miR-205, miR-210, and miR-708 in the sputum of lung cancer patients as diagnostic biomarkers with 73% sensitivity and 96% specificity (Xing et al., 2010). Furthermore, miRNAs can be utilized to differentiate between different kinds of lung neoplasms. For instance, miR-205 has been shown to differentiate between SCC and NSCC (Aharonov et al., 2009). According to Barshack et al., initial lung cancers are linked to high expression of miR-182, whereas metastatic tumors are linked to high expression of miR-126 (Barshack et al., 2010). In another study, miR-944 was suggested to play a role in the early squamous differentiation of lung tumors and serve as an accurate marker for distinguishing NSCLC subtypes (Powrózek et al., 2018).

Table 1. Diagnostic miRNA biomarkers in lung cancer

miRNA	**Expression**	**Sample**	**References**
miR-21	Upregulated	Tissue, A549 Cells	Wang et al., 2022
miR-486 and miR-150	Upregulated	Blood Sample	W. Li et al., 2015
miR-30a-3p, miR-30a-5p, miR-30c-2-3p, miR-30d-5p, miR-27a-5p	Downregulated	Tissue	Zhang et al., 2017
miR-125b	Upregulated	Blood Sample	Yuxia et al., 2012
miR-21, miR-210, miR-155	Upregulated	Blood Sample	Lianidou et al., 2015

miR-15b, miR-27b	Upregulated	Blood Sample	Hennessey et al., 2012
mir-21	Upregulated	Sputum	Xie et al., 2010
miR-205, miR-210, miR-708	Downregulated	Sputum	Xing et al., 2010
miR-205	-	Tissue	Aharonov et al., 2009
miR-182, miR-126	-	Tissue	Barshack et al., 2010
miR-944	Upregulated	Tissue	Powrózek et al., 2018

miRNAs as Prognostic Biomarkers

Despite significant advancements in diagnosis, classification, and treatment efficacy, lung cancer is often detected at advanced stages, leading to poor prognosis and low overall survival rates (Wu et al., 2019). In addition to the importance of early detection for the prevention and treatment of lung cancer, understanding the prognosis of this type of neoplasm is also crucial. Studies have shown that different miRNA profiles can serve as biomarkers for tumor progression, the presence of metastasis, and the clinical prognosis of patients (Table 2). These studies have also demonstrated how altered miRNA expression is associated with low or high survival outcomes (Guz et al., 2014, Lobera et al., 2023).

Table 2. Prognostic miRNA biomarkers in lung cancer

miRNA	**Expression**	**Sample**	**References**
miR-200c	Downregulated	Tissue, A549 Cell	Si et al., 2017
Let 7	Downregulated	Tissue	Takamizawa et al., 2004
miR-21 and miR-155	Upregulated	Tissue, Blood Sample	Yang et al., 2013
miR-125b	Upregulated	Blood Sample	Yuxia et al., 2012
miR-128b	Downregulated	Tissue	Weiss et al., 2008
miR-146-5p	Downregulated	Blood Sample	Yuwen et al., 2017

miR-23b-3p,miR-10b-5p and miR-21-5p	Upregulated	Blood Sample	Q. Liu et al., 2017
miR-16	Downregulated	Tissue	Navarro et al., 2011
miR-21	Upregulated	Tissue	Markou et al., 2008
miR-34a	Downregulated	Tissue	Wiggins et al., 2010
miR-186	Downregulated	Tissue	Cai et al., 2013

For instance, miR-200c, a member of the miR-200 family, is frequently downregulated in lung cancer, contributing to tumor progression and metastasis. In lung cancer, low expression levels of miR-200c have been linked to a poor prognosis (Mutlu et al., 2016). Another study revealed that let-7 expression is linked to shorter survival in lung cancer patients (Takamizawa et al., 2004). Yang and colleagues indicated that specific miRNAs, such as miR-21 and miR-155, could predict recurrence and poor survival in NSCLC (M. Yang et al., 2013). In another study, serum miR-125b levels correlated with clinical stages, and high miR-125b expression was identified as an independent prognostic factor for survival (Yuxia et al., 2012). miR-128b was reported to be prevalent in NSCLC tumor tissues and associated with clinical response and improved survival following gefitinib treatment (Weiss et al., 2008). Decreased levels of miR-146-5p in serum exosomes have been associated with poor survival and cisplatin resistance in NSCLC patients (Yuwen DL et al., 2017). Additionally, plasma exosomal miR-23b-3p, miR-10b-5p, and miR-21-5p have been shown to correlate with poor overall survival in NSCLC patients and represent promising non-invasive prognostic biomarkers for NSCLC (Q. Liu et al., 2017). Moreover, miR-16 has been suggested as a prognostic marker for disease-free survival (DFS) and overall survival (OS) in NSCLC (Navarro et al., 2011). Similarly, Markou et al. reported that overexpression of miR-21 is a separate negative predictor of overall survival in patients with non-small cell lung cancer (Markou et al., 2008). In contrast, low levels of miR-34a expression in cancer tissue were associated with a high likelihood of recurrence (Wiggins et al., 2010), whereas low

expression level of miR-186 in NSCLC tissues was linked to poor patient survival (Cai et al., 2013).

miRNAs as Therapeutic Biomarkers

Platinum-derived therapies (cisplatin-DDP, carboplatin) form the basis of chemotherapy treatment in patients with lung cancer (Lobera et al., 2023). Drug resistance is one of the most significant causes of therapy failure (Rossi & Di Maio, 2016, Sève & Dumontet, 2005). Many studies show that miRNAs also play important roles in chemotherapy-induced resistance and may help predict response to treatment (Lobera et al., 2023). Due to the complex molecular structure of lung tumors, it is crucial to identify therapeutic biomarkers and gaps associated with treatment and adverse therapeutic outcomes (Du et al., 2018, Lobera et al., 2023). miRNAs as potential therapeutic biomarkers in lung cancer are shown in Table 3.

Galluzzi et al. showed that miR-630 inhibits apoptosis by targeting p53-related pro-apoptotic pathways that are triggered by carboplatin and cisplatin (Galluzzi et al., 2010). Another study revealed that increased expression of miR-146a plays a significant role in the development of acquired cisplatin resistance in NSCLC cells by reducing cyclin J expression (Shi et al., 2017). According to Li et al., cisplatin-resistant A549/CDDP cells have downregulated miR-326, and its overexpression reverses chemotherapy resistance through the specificity protein 1 (SP1) pathway (J. Li et al., 2016). It has been proposed that miRNA-128-b can regulate EGFR expression in NSCLC cells, potentially contributing to the development of new strategies for the treatment of lung cancer patients (L. Li & Wang, 2019). Furthermore, by inhibiting Reck and triggering Notch1 signaling, miR-221/222 has been found to be a crucial regulator in NSCLC, indicating that it may be a viable therapeutic target for the treatment of NSCLC (Guo et al., 2021). According to another study, miR-34a is markedly downregulated in NSCLC cell lines and tissues, and it targets EGFR to prevent NSCLC tumor growth and metastasis (Li et al., 2017). In A549/DDP

cells, miR-29b-3p has been demonstrated to reverse cisplatin resistance by upregulating PTEN and BAX expression and suppressing COL1A1 gene expression (Jia & Wang, 2020). Additionally, studies have demonstrated that the tumor-suppressive miR-451 enhances DDP sensitivity in lung cancer cells by regulating MCL-1 expression (Cheng et al., 2016), miR-335 modulates chemoradiotherapy resistance in SCLCs by targeting PARP-1 (Luo et al., 2017), and miR-135b suppresses chemotherapy resistance in NSCLC cells by targeting FZD1 (Su et al., 2016).

Table 3. miRNAs as potential therapeutic biomarkers in lung cancer

miRNA	**Expression**	**Sample**	**References**
miR-630	Upregulated	A549 Cells	Galluzzi et al., 2010
miR-146a	Upregulated	A549 Cells	Shi et al., 2017
miR-326	Downregulated	A549 Cells	J. Li et al., 2016
miR-128-b	Downregulated	Tissue	Li & Wang, 2019
miR-221/222	Upregulated	A549 Cells	Guo et al., 2021
miR-34a	Downregulted	Tissue, Cells	Li et al., 2017
miR-29b-3p	Downregulated	Tissue, Cells	Jia & Wang, 2020
miR-451	Downregulated	A549 Cells	Cheng et al., 2016
miR-335	Upregulated	SCLC H69AR H446DDP	Luo et al., 2017
miR-135b	Downregulated	A549 Cells	Su et al., 2016

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CHAPTER II

Irisin in Cancer

Diler US ALTAY[2]

Introduction

Transcription factors (TFs) are essential for regulating gene expression, orchestrating cellular functions by binding to specific DNA sequences. However, their activity is significantly enhanced by the involvement of coactivators and corepressors. These accessory molecules do not directly interact with DNA but instead modulate transcription factor activity, ensuring precise control over gene expression. Among the wide array of coactivators, the PGC-1 (peroxisome proliferator-activated receptor-gamma coactivator-1) family stands out due to its versatile role in cellular energy metabolism and mitochondrial function. This family comprises three members: PGC-1α, PGC-1β, and PGC-1-related coactivator (PRC),

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all of which play critical roles in fine-tuning transcriptional responses to various metabolic cues (Liu & Lin, 2011).

PGC-1α: A Key Metabolic Regulator

Peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), the most studied member of the PGC-1 family, is a master regulator of energy metabolism. It is primarily expressed in tissues with high energy demands, such as skeletal muscle, the heart, and brown adipose tissue. Its activity is particularly enhanced in response to physiological stimuli, including exercise, fasting, and exposure to cold. Exercise-induced energy stress is a potent activator of PGC-1α, leading to its increased expression in skeletal muscle.The upregulation of PGC-1α triggers a cascade of metabolic adaptations, such as mitochondrial biogenesis, which increases the capacity for oxidative phosphorylation and ATP production. This process is critical for maintaining cellular energy homeostasis during periods of heightened demand. Furthermore, elevated levels of PGC-1α provide a protective mechanism against muscle damage caused by exercise-induced stress (Boström & et al., 2018).

Gene Expression and Biological Functions

PGC-1α influences the expression of numerous genes that are pivotal for muscle function and adaptation. Among these are interleukin 15 (IL-15), a cytokine involved in muscle hypertrophy and immune regulation, and vascular endothelial growth factor (VEGF), which promotes angiogenesis and improves oxygen delivery to tissues. PGC-1α also regulates leucine-rich glycoprotein 1 (LRG1) and **tiss**ue inhibitor of matrix metalloproteinase 4 (TIMP4), both of which contribute to maintaining muscle structure and function.

Notably, PGC-1α enhances the production of fibronectin type III domain-containing protein 5 (FNDC5), a membrane protein that serves as the precursor to irisin. Irisin is a hormone-like peptide secreted into the bloodstream, where it exerts systemic effects, including the promotion of browning in white adipose tissue (Us Altay et al., 2016). This browning process enhances thermogenesis and energy expenditure, linking PGC-1α to broader metabolic benefits beyond skeletal muscle.

The Multifaceted Role of PGC-1α

PGC-1α's influence extends beyond energy metabolism and muscle physiology. It plays a role in anti-inflammatory responses, antioxidant defense, and the regulation of autophagy, further highlighting its importance in cellular health. Its activation during exercise underscores its potential therapeutic relevance for metabolic disorders such as diabetes, obesity, and cardiovascular diseases. In summary, transcription factors require the assistance of coactivators like PGC-1α to effectively regulate gene expression. PGC-1α’s multifaceted role in energy metabolism, mitochondrial function, and systemic adaptation to exercise underscores its significance in both health and disease. The intricate regulation of muscle-related genes by PGC-1α exemplifies its pivotal position as a molecular integrator of metabolic signals and cellular responses.
This expanded text provides a more detailed overview of PGC-1α and its functions, highlighting its biological significance and therapeutic potential.

FNDC5 and Its Role in Metabolism and Exercise

Fibronectin type III domain-containing protein 5 (FNDC5), first identified independently by two research groups in 2002, is a protein of significant interest due to its role in energy metabolism and

exercise-induced adaptations. FNDC5 is also known by alternative names, such as fibronectin type III domain-containing protein 2 (FRCP2) and peroxisomal protein (PeP), highlighting its multifaceted functions in different cellular contexts (Teufel et al., 2013).

Structure of FNDC5

The FNDC5 protein is composed of 209 amino acids, organized into several functional regions. These include:

- A 29-amino acid signal peptide essential for directing the protein to its proper cellular destination.
- A 94-amino acid fibronectin type III domain, which is a hallmark of proteins involved in cellular adhesion and signaling.
- A 28-amino acid region of unknown function that may play a role in the protein's structural stability or interaction with other molecules.
- A 19-amino acid transmembrane domain, characteristic of type I membrane proteins, anchoring the protein to cellular membranes.
- A 39-amino acid C-terminal region, which likely contributes to the protein's regulatory functions or interactions (Erickson, 2013).

FNDC5 Cleavage and Irisin Production

FNDC5 undergoes a crucial post-translational modification at its N-terminal domain through proteolytic cleavage by an as-yet unidentified enzyme. This process generates irisin, a biologically active peptide consisting of 112 amino acids. Irisin is then secreted into the bloodstream, where it serves as a messenger molecule linking muscle activity to systemic metabolic changes.The expression of FNDC5 varies significantly across tissues, with some

organs displaying higher levels of expression. For example, skeletal muscles, where FNDC5 expression is most prominent, serve as the primary site for irisin production, especially in response to exercise. Other tissues, such as the liver, adipose tissue, and brain, also express FNDC5, albeit at varying levels, suggesting a wide-ranging influence on physiological processes (Huh et al., 2012).

Exercise-Induced Secretion of Irisin

Studies have demonstrated that irisin is secreted into circulation following exercise. Huh et al. (2012) showed that muscle contraction stimulates FNDC5 expression and subsequent irisin release, leading to notable metabolic adaptations. These adaptations include changes in subcutaneous adipose tissue, such as: Increased expression of uncoupling protein-1 (UCP1), a marker of thermogenic activity.

- Activation of thermogenesis, which facilitates energy expenditure and heat production.
- The browning of white adipose tissue (WAT), a process wherein white fat cells acquire characteristics of brown fat, including enhanced mitochondrial content and thermogenic capacity.

Evolutionary Conservation of Irisin

Human and mouse irisin share an identical amino acid sequence, indicating a high level of evolutionary conservation. This remarkable similarity suggests that irisin's function is preserved across species. Boström et al. (2012) proposed that this conservation underpins its critical role in energy regulation and adaptive responses to physical activity.

Endurance Exercise and FNDC5 Expression

Boström et al. conducted a study involving muscle biopsies and plasma samples collected before and after a 10-week endurance exercise program. They observed:

- A significant increase in FNDC5 gene expression in both muscle and beige adipose tissue.
- Elevated levels of circulating irisin, correlating with exercise-induced metabolic benefits.

The study revealed findings in humans that mirrored observations in mouse models, reinforcing the idea that irisin mediates some of the beneficial effects of exercise. These benefits may include enhanced glucose uptake, improved insulin sensitivity, and reduced inflammation, which collectively contribute to the prevention and management of metabolic disorders such as diabetes, obesity, and cardiovascular diseases.

Therapeutic Potential of FNDC5 and Irisin

Given its role in promoting systemic metabolic health, irisin is being explored for its potential as a therapeutic agent. By mimicking the positive effects of physical activity, irisin-based treatments could offer novel approaches for combating conditions like obesity and type 2 diabetes. Furthermore, its ability to induce the browning of white fat highlights its potential for addressing energy imbalance and weight management in clinical settings. FNDC5 and its cleavage product irisin represent a fascinating intersection of exercise biology and metabolic regulation. From its structural complexity to its far-reaching effects on energy homeostasis, FNDC5 exemplifies the intricate mechanisms underlying the benefits of physical activity. As research continues, the therapeutic applications of irisin may pave the way for innovative treatments targeting a wide range of metabolic and degenerative diseases.

Irisin Receptor: A Key Discovery

The identification of αVβ5 integrin as the receptor for irisin represents a pivotal advancement in understanding irisin's molecular mechanisms and physiological roles. This breakthrough, first reported by Kim et al. (2018), has opened new avenues for exploring how irisin mediates its diverse effects across different tissues. Further insights into irisin's receptor-binding process were provided by Mu et al. (2023), who demonstrated that extracellular heat shock protein 90a (HSP90a) acts as a cofactor in facilitating irisin's binding to αVβ5 integrin. This cofactor is thought to enhance the receptor's affinity for irisin, thereby amplifying its signaling potential. Irisin's binding to the αVβ5 receptor has been observed not only in skeletal muscle cells but also in intestinal epithelial cells, as shown in both in vitro and in vivo studies. This discovery suggests that irisin may play a role in regulating gastrointestinal physiology. For instance, its interaction with these cells could influence processes such as nutrient absorption, gut motility, and barrier function.The interaction of irisin with αVβ5 integrin is not limited to the gastrointestinal tract (Pinkas & et al., 2024). By engaging this receptor, irisin may exert beneficial effects on a variety of tissues, including:

- Adipose tissue: Enhancing the browning of white adipose tissue and promoting thermogenesis.
- Skeletal muscle: Supporting mitochondrial function and reducing exercise-induced muscle damage.
- Liver and cardiovascular tissues: Improving lipid metabolism and reducing inflammation.

The identification of αVβ5 integrin as an irisin receptor underscores the peptide's far-reaching impact on systemic health. Further research is needed to elucidate the full spectrum of signaling.

Methods for Detecting Irisin in Serum or Plasma Samples

Detecting irisin in human serum or plasma samples is a topic of ongoing research due to challenges in ensuring accuracy and reliability. Here is an overview of the primary methods used, along with their advantages and limitations.

1. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is the most commonly employed method for detecting irisin in biological samples. The technique involves the use of specific antibodies to capture and quantify irisin. However, its application in irisin detection has raised certain concerns:

- Cross-Reactivity: Antibodies used in ELISA kits may bind to other proteins in serum or plasma, leading to false-positive results. This highlights the importance of selecting kits with high specificity.
- Variability Among Kits: Commercial ELISA kits can detect a wide range of irisin concentrations, from picograms to micrograms per milliliter, but the results may vary depending on the manufacturer (Albrecht et al., 2020; Zhong et al., 2021).
- Validation: Some kits, such as those from Aviscera and Phoenix Pharmaceuticals, have been validated using Western blotting. These validations demonstrate their ability to accurately measure irisin under physiological conditions, especially when the protein is spiked into samples (Polyzos et al., 2015).

To improve consistency and reliability, it is crucial to:

- Use ELISA kits validated through Western blotting or mass spectrometry.
- Avoid comparing results obtained from different manufacturers within a single study.

2. Mass Spectrometry

Mass spectrometry (MS) is regarded as the gold standard for irisin detection due to its high precision and accuracy. Unlike ELISA, MS can provide a detailed quantitative analysis of irisin without the risk of antibody cross-reactivity.

- Key Studies: Jedrychowski et al. (2015) used mass spectrometry to perform a quantitative analysis of human irisin in plasma, representing a significant advancement in detection methods.
- Limitations: Despite its accuracy, mass spectrometry is not widely adopted due to its complexity, cost, and need for specialized equipment (Albrecht et al., 2020).

3. Western Blotting

Western blotting is often used to validate ELISA results and confirm the presence of irisin in serum or plasma samples.

- It involves separating proteins by gel electrophoresis, transferring them to a membrane, and detecting irisin with specific antibodies.
- While highly reliable for qualitative confirmation, Western blotting is less suitable for routine quantitative analysis.

Recommendations for Accurate Detection

- Consistency in Methods: Use the same ELISA kit throughout a study to avoid variability in measurements.
- Kit Validation: Select kits that have been validated with methods such as Western blotting or mass spectrometry.

- Complementary Approaches: Where feasible, use mass spectrometry alongside ELISA to ensure accuracy.
- Trends vs. Absolute Values: ELISA is suitable for identifying trends in irisin concentration changes but may not provide precise quantitative measurements.

By combining rigorous validation, careful kit selection, and complementary techniques, researchers can achieve more reliable and accurate detection of irisin in serum or plasma samples.

Irisin and Its Potential Role in Cancer

Physical exercise is a protective factor against cancer, reducing adverse toxicities, the likelihood of relapse or death following the initiation of antineoplastic treatments, and improving quality of life in individuals with oncological diagnoses, although the mechanisms underlying these beneficial effects remain unclear.

Irisin, a thermogenic protein secreted in response to physical activity, has attracted attention for its potential role in cancer prevention and progression. Epidemiological studies have shown that physical activity can significantly lower the risk of developing various types of cancer and can inhibit tumor progression, providing substantial benefits in cancer treatment (Moore et al., 2016).

These benefits are largely attributed to the anti-inflammatory effects of physical exercise, which may be partially mediated by irisin, a key player in the thermogenic response. Observational studies report that physical activity and metformin use are associated with a 38% and 34% relative risk reduction in cancer-specific mortality, respectively (Schmid &Leitzmann,2014; Gandini et al.,2014). However, the biological mechanisms regulating the relationship between engaging in physical activity, using metformin, and clinical outcomes among cancer survivors are not yet fully understood (Ballard-Barbash et al.,2012; Morales & Morris .,2015).Metformin alters myokine

concentrations, such as irisin, by increasing adenosine monophosphate-activated protein kinase (AMPK) activity in skeletal muscle, sensitizing insulin-resistant skeletal muscle, and promoting muscle protein synthesis (Li et al.,2015; Li et al.,2015).Collectively, these data suggest that exercise and metformin may have independent effects on myokine concentrations and, when combined, could produce synergistic effects consistent with improved cancer prognosis. Physical exercise is a protective factor against cancer, reducing adverse toxicities, the likelihood of relapse or death following the initiation of antineoplastic treatments, and improving quality of life in individuals with oncological diagnoses, although the mechanisms underlying these beneficial effects remain unclear.

Inflammation and Cancer

Chronic inflammation is a known factor in cancer development and progression. Inflammatory molecules and biomarkers such as NFκB, hypoxia-inducible factor-1α, STAT3, and pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6, IL-23) are implicated in tumor growth, survival, and metastasis. Given that irisin is known for its anti-inflammatory properties, it is thought to have the potential to influence cancer development and progression by modulating these inflammatory pathways. However, despite numerous studies indicating an association between irisin and cancer, its precise role in tumor biology remains somewhat unclear.

Clinical Studies on Irisin and Cancer

Several clinical studies have explored the relationship between irisin levels and different types of cancer, with varying results: Provatopoulou et al. (2015) found that elevated levels of irisin were linked to a reduced risk of developing breast cancer in women. This suggests that irisin may play a protective role in breast cancer

prevention. However, a contrasting study by Zhang et al. (2018) showed that women with primary breast cancer had higher irisin levels compared to those with spinal metastases. This finding suggests that irisin may not consistently act as a protective factor and its role might vary depending on the stage or type of cancer. Elevated levels of irisin have been observed in patients with renal cell cancer, with levels significantly higher than those found in healthy controls (Us Altay et al., 2018). This may point to a potential involvement of irisin in renal cell carcinoma, although the exact mechanism remains to be elucidated. In stark contrast, bladder cancer patients exhibited significantly lower serum irisin levels (Esawy et al., 2020). This suggests that irisin might have a tumor-suppressive role in certain cancers, potentially serving as a biomarker for bladder cancer diagnosis or prognosis. In prostate cancer, irisin levels were notably decreased, suggesting a possible inverse relationship between irisin and cancer progression. This could indicate that irisin might be a potential biomarker in prostate cancer, especially when used alongside free and total prostate-specific antigen (PSA) measurements (Aslan et al., 2020). Uğur et al. (2019) found that irisin expression was significantly elevated in oncocytic variants of thyroid cancer when compared to other thyroid carcinomas. This finding points to the possibility that irisin might play a specific role in certain subtypes of thyroid cancer, potentially influencing tumor growth or acting as a prognostic indicator.

Laboratory Studies on Irisin and Cancer Cells

Several in vitro studies have demonstrated that irisin has anti-cancer effects by suppressing the proliferation, migration, and viability of various cancer cell lines. These studies have provided valuable insights into how irisin might be used as a potential therapeutic agent in cancer: Gannon et al. (2015) showed that irisin suppressed the

proliferation and migration of malignant breast cancer cells, such as MCF-7 and MDA-MB231, by activating caspase activity and inducing apoptosis. Interestingly, irisin did not affect normal breast epithelial cells like MCF-10A, indicating that its anti-cancer effects might be selective for malignant cells. Similarly, Liu et al. (2018) reported that irisin inhibited the migration, invasion, and growth of pancreatic cancer cell lines (MIA PaCa-2 and Panc 03.27) in vitro. These effects were linked to the activation of the AMP-activated protein kinase (AMPKα) pathway and the inhibition of the mechanistic target of rapamycin (mTOR) signaling pathway. This suggests that irisin may exert its anti-cancer effects by disrupting key metabolic and survival pathways in cancer cells. Osteosarcoma is a primary malignant bone tumor commonly found in children and young adults. Recent studies have indicated that irisin can influence osteosarcoma cell behavior, particularly through its effects on epithelial-mesenchymal transition (EMT), a crucial process in cancer metastasis. According to Kong et al. (2017), irisin inhibits the IL-6-induced EMT pathway in osteosarcoma cell lines, such as U2OS and MG-63. By doing so, irisin suppresses the activation of the STAT3/Snail signaling pathway, which is involved in cancer progression and metastasis. This suggests that irisin might have potential therapeutic benefits in osteosarcoma by reducing metastasis and inhibiting the invasive properties of tumor cells. Lung cancer, one of the leading causes of cancer-related deaths worldwide, has also been shown to be affected by irisin. Research by Shao et al. (2017) demonstrated that irisin exerts a protective effect against lung cancer cells by inhibiting Snail expression. Snail is a transcription factor that plays a significant role in EMT, a process that facilitates cancer cell migration and invasion. Irisin's ability to inhibit Snail expression occurs through the PI3K/Akt/Snail signaling pathway, which is known to regulate cancer cell survival and

metastasis. This suggests that irisin may inhibit the progression of lung cancer by targeting key pathways involved in cancer cell migration and invasion. Prostate cancer, one of the most common cancers in men, has been another focus of research regarding irisin's potential therapeutic effects. Tekin et al. (2015) reported that irisin exerted cytotoxic effects on DU-145 and PC3 prostate cancer cell lines, suggesting that irisin could be a potential agent for prostate cancer treatment. However, the exact mechanisms of action remain unclear, as irisin's effects on prostate cancer cells may involve multiple signaling pathways, including those regulating apoptosis and cell cycle progression. In contrast to its potential anti-cancer effects in certain cancer types, irisin may promote the growth and invasiveness of cancer cells in some contexts. In hepatocellular carcinoma (HCC) cell lines, irisin has been shown to enhance cell proliferation, migration, and invasion by activating the Akt/PI3K pathway (Shi et al., 2017). The PI3K/Akt pathway is a crucial regulator of cell survival, growth, and metastasis, and its activation by irisin may suggest that irisin could facilitate the progression of HCC. These findings highlight the complex and context-dependent role of irisin in cancer biology. Pancreatic cancer, known for its aggressive nature and poor prognosis, has also been studied in relation to irisin. Liu et al. (2019) found that irisin enhanced apoptosis in pancreatic cancer cells, such as MIA PaCa-2 and BxPC-3, in response to chemotherapy drugs like doxorubicin. This effect was mediated through the inhibition of the PI3K/NF-κB pathway, a critical signaling pathway involved in cancer cell survival and inflammation. By promoting apoptosis and sensitizing pancreatic cancer cells to chemotherapy, irisin may play a potential role in enhancing the efficacy of cancer treatments, particularly in combination with other therapeutic agents. Irisin's potential role in gastrointestinal (GI) cancers has also been explored. Aydın et al.

(2016) found that irisin expression was elevated in most gastrointestinal system (GIS) cancers, except in liver tissues. The absence of increased irisin levels in liver cancers remains unclear, but it is thought to be related to the liver's role in gluconeogenesis and its metabolic functions. This raises an interesting question about the tissue-specific effects of irisin and its potential as a biomarker for GI cancers. Other studies have demonstrated similar effects of irisin on the motility, proliferation, and viability of various other cancer cell lines. For instance, irisin has been shown to impact cancer cell behavior by modulating both cell signaling pathways and mitochondrial function, two essential processes in cancer cell growth and survival. Beyond these specific cancer types, research by Moon et al. (2014) revealed that irisin demonstrated anticancer activity on several other cancer cell lines, including those from lung, pancreatic, osteosarcoma, prostate, and breast cancers. However, irisin did not exhibit anticancer effects on certain cell lines, including those from human and mouse colon, esophageal, thyroid, and endometrial cancers. Specifically, irisin had no significant impact on the proliferation and adhesion properties of these cell lines, suggesting that its effects may be restricted to specific types of cancers or cellular contexts.

Animal Studies on Irisin and Cancer Cells

Us Altay et al. (2016) observed an increase in FNDC5 expression (the precursor of irisin) in both white and brown adipose tissues in a mouse model of gastric cancer. This suggests that FNDC5, and consequently irisin, might contribute to the cachexia (muscle wasting) associated with cancer progression. However, the elevated expression of FNDC5 alone does not fully explain the relationship between irisin and gastric cancer, and more research is needed to clarify this connection.

Anti-Inflammatory Effects of Irisin in Cancer

Inflammation plays a crucial role in cancer initiation, progression, and metastasis. Irisin has been shown to exert anti-inflammatory effects by lowering the levels of inflammatory cytokines such as TNF-α and IL-6, both of which are implicated in tumor growth and metastasis. These effects are primarily mediated through the inhibition of the NF-κB pathway, a key regulator of inflammation and immune responses. By reducing inflammation, irisin may not only help prevent cancer but also potentially slow down the progression of established tumors (Kong et al., 2017; Mantovani et al., 2008).

Conclusion

The relationship between irisin and cancer is complex and still under active investigation. While several studies suggest that irisin may play a protective or therapeutic role in some cancers, its effects appear to be cancer-type specific. Irisin's ability to modulate inflammatory pathways, influence cell signaling (such as AMPK and mTOR), and induce apoptosis in malignant cells positions it as a potential target for cancer therapy. However, further research is needed to fully understand its mechanisms of action, therapeutic potential, and how it may be integrated into cancer prevention and treatment strategies. Given its emerging role in cancer biology, irisin could potentially be used as a biomarker for cancer diagnosis, prognosis, or as a therapeutic agent in the future. The role of irisin in cancer is multifaceted, with both anti-cancer and pro-cancer effects depending on the cancer type and context. In some cancers, such as osteosarcoma, lung cancer, and pancreatic cancer, irisin appears to have protective or therapeutic potential by inhibiting cancer cell

proliferation, migration, and invasion. In contrast, in hepatocellular carcinoma, irisin may promote cancer cell growth and invasiveness through the activation of key signaling pathways. While irisin's anti-inflammatory properties contribute to its potential as an anti-cancer agent, its role in cancer biology remains complex and requires further investigation. Ultimately, understanding the tissue-specific effects of irisin and its interactions with different signaling pathways will be crucial for harnessing its potential as a therapeutic target in cancer treatment.

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CHAPTER III

Examination of the Antioxidant Properties of Melatonin Hormone

Elif Ebru ALKAN[3]

Introduction

Melatonin is a hormone long known to be produced by the pineal gland. It mainly has immune modulatory, circadian and seasonal rhythm regulating, and sleep-inducing effects. Melatonin receptors are widespread throughout the body. Despite its long-known presence, the biosynthesis and chemical structure of melatonin, the primary hormone secreted by the pineal gland, were only identified by Lerner in 1958. Melatonin is found at its highest concentration during the night and is responsible for circadian regulation and sleep control. It shows a circadian rhythm in the blood, with nighttime levels being significantly higher than daytime levels. Chemically,

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melatonin is described as N-acetyl-5-methoxytryptamine and is primarily secreted by the pineal gland and retina. It is a lipophilic molecule. Additionally, melatonin is synthesized and secreted by the ovaries, bone marrow cells, bile, and gastrointestinal system (Topal and Korkmaz, 2009). The pineal gland was first described in the 3rd century BC by Herophilus (Erlich and Apuzzo, 1985). Gallen likened the pineal gland to the top of a pine tree and used the Latin term “conarium” to describe it. The name "pineal" is derived from the Latin word "pinea," meaning pine cone.

Melatonin secretion is regulated by a biological clock located in the suprachiasmatic nucleus of the hypothalamus. The suprachiasmatic nucleus is synchronized with the daily light/dark cycle and set to a 24-hour period. This synchronization begins with light signals transmitted to the suprachiasmatic nucleus via retinal pathways. The biological clock in the suprachiasmatic nucleus regulates the rhythmic secretion of melatonin by sending circadian signals to the pineal gland through multiple synaptic neuronal pathways.

The secretion of melatonin is directly related to the light sensitivity of pinealocyte cells. This sensitivity disappears with the inhibition of light, triggering melatonin secretion by pinealocytes in the dark. On average, 30 mg of melatonin is synthesized throughout the night.

Antioxidants are substances in the human body and foods that halt or neutralize the adverse effects of free radicals. The oxidative damage caused by free radicals and active oxygen can be prevented through antioxidant supplementation and an antioxidant-rich diet. Melatonin is considered a very potent free radical scavenger. It can neutralize both reactive oxygen species and reactive nitrogen species. It is known to capture highly toxic hydroxyl radicals,

peroxynitrite anions, and peroxyl radicals. Melatonin also inhibits the pro-oxidative enzyme nitric oxide synthase in some locations. Additionally, it is known to stimulate mRNA levels for the superoxide dismutase enzyme and activate glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase.

Structure and Synthesis of Melatonin

Melatonin was first discovered by Lerner in 1958. He named the hormone, derived from the pituitary gland and known for lightening skin color, "melatonin," combining the Latin words "melas" (black) and "tosos" (work) (Hilton, 2002, Beyer et al., 1998). The primary organs for melatonin secretion are the pineal gland and the retina (Wiechmann, 1986). The pineal gland, named after its resemblance to a pine cone, is located in the midline of the brain, behind the third ventricle, weighing 100-150 mg and measuring 5-10 x 3-5 mm, making it the smallest endocrine gland in the human body. The pineal gland, lacking a blood-brain barrier, is also the most vascular tissue after the kidneys, with a blood flow of 4 ml/min/g (Sen and Chakraborty, 2011, Hilton, 2002, Claustrat et al., 2005). Pinealocytes in the gland not only synthesize melatonin but also biological amines like serotonin, norepinephrine, histamine, dopamine, and peptides like luteinizing hormone-releasing hormone (LHRH), thyroid-stimulating hormone (TRH), somatostatin, and arginine vasopressin (Beyer et al., 1998).

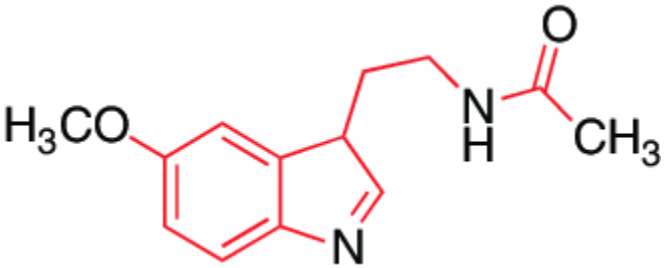

Figure 1: The chemical structure of melatonin (N-acetyl-5-methoxytryptamine)

The similarity between the pathways used by pinealocytes in the pineal gland and retinal photoreceptors to convert serotonin to melatonin supports the idea that the pineal gland evolved from a photoreceptive organ (Lynch, Wurtman, Moskowitz, Archer, & Ho, 1975; Wiechmann, 1986). Melatonin, also known as N-acetyl-5-methoxytryptamine, is secreted by the pineal gland and increases its secretion in the dark, serving as a potent antioxidant, aiding in cell regeneration, strengthening the immune system, and regulating sleep rhythm and body temperature (14). The circadian rhythm of melatonin secretion becomes irregular with age. Disruption of melatonin's circadian rhythm is also significant in certain clinical disorders, such as mood disorders.

The Antioxidant Property of Melatonin

The antioxidant property of melatonin was first proposed in the literature by Ianas and colleagues in 1991 (Ianas, 1991) and has since been supported by various in vitro and in vivo studies. Antioxidants exert their effects by scavenging free radicals, converting them into less reactive molecules, reducing their activity through interactions, binding to them and breaking the reaction chain, or through repair mechanisms. Melatonin's antioxidant property is attributed to the

pyrrole ring in its structure. Melatonin prevents oxidative stress caused by certain toxins leading to oxidative tissue damage. It does not require a binding site or receptor for this free radical scavenging property (Reiter, 1996). In vitro studies have demonstrated that melatonin neutralizes free radicals such as hydroxyl radicals (•OH), hydrogen peroxide (H2O2), and singlet oxygen (↑O2•-), and inhibits lipid peroxidation. Melatonin stimulates a range of antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, and catalase. It has been shown that melatonin increases intracellular glutathione levels by stimulating γ-glutamylcysteine synthetase, an enzyme that limits its synthesis, and inhibits peroxidative enzymes like nitric oxide synthase and lipoxygenase. There is evidence that melatonin stabilizes microsomal membranes, helping them resist oxidative damage (Karbownik, 2001). Additionally, melatonin enhances the efficiency of the electron transport chain, reducing electron leakage and free radical formation (Reiter, 2001). The antioxidant and immune-supporting properties of melatonin are also related to cancer processes. Since melatonin is soluble in both water and lipid phases, it can readily permeate all intracellular components, effectively protecting cell membranes, organelles, and the nucleus from free radical damage. Melatonin differs from classical antioxidants (vitamin E, vitamin C, β-carotene, etc.) in various ways. It does not enter redox cycling or radical-producing reactions. Classical antioxidants convert into pro-oxidant substances after exhibiting their effects. It is suggested that melatonin supports the antioxidant system by reducing free radical formation through inhibiting certain pro-oxidant enzymes (Pandi-Perumal et al., 2008). Melatonin protects not only proteins and lipids but also both nuclear DNA and mitochondrial DNA. Consequently, it has been found that melatonin stimulates antioxidant enzymes, inhibits lipid peroxidation, and

protects brain tissue from oxygen-induced free radicals. In summary, melatonin provides extensive protection by functioning as a direct free radical scavenger and an indirect antioxidant.

Melatonin is an indole derivative compound known to exhibit antioxidant, anti-inflammatory, and anti-tumor activity, working as a free radical scavenger against carcinogenic agents and is easily released into the bloodstream from the pineal gland. The emergence of the effects of free radicals on cancer, cardiovascular, and neurodegenerative diseases, and aging has brought about the use of antioxidants, with research on this topic gaining importance. Compared to many other antioxidants, the potent radical-scavenging effect and the ability to enhance antioxidant enzyme activities of melatonin have increased research efforts by scientists, especially concerning its use in preventive treatments. Consequently, studies on the clinical application of melatonin are on the rise. Results from studies comparing various antioxidants have proven that melatonin is one of the strongest antioxidants.

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CHAPTER IV

A Natural Approach to Diabetes Treatment: Verbascoside as an SGLT-2 Inhibitor

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1. Introduction

Diabetes Mellitus, commonly known as diabetes, is a chronic metabolic disorder characterized by hyperglycemia due to insufficient insulin production by the pancreas or the body's inability to use insulin effectively. Despite advancements in diabetes treatment, there is currently no cure, and management is limited to alleviating symptoms. This has raised concerns, and in recent years, the incorporation of Sodium-Glucose Co-Transporter-2 (SGLT-2) inhibitors into therapeutic regimens has not only contributed to glucose management but also addressed critical complications

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associated with diabetes, making them a vital component of modern diabetes care. SGLT-2, a central mechanism in glucose regulation, plays a significant role in renal glucose reabsorption, making it a critical target for innovative diabetes therapies like SGLT-2 inhibitors. In addition, in the recent year, the use of natural compounds in the treatment and management of diabetes has garnered significant interest. The current study investigates the inhibitory effect of verbascoside, a natural compound found in olive plants, on SGLT-2 using *in silico* methods. SGLT-2 inhibitors have emerged as an innovative treatment option with a unique mechanism of action and additional health benefits. These inhibitors function by preventing glucose reabsorption in the kidneys, thereby increasing glucose excretion through urine. The implications of this study could be significant in the field of diabetes research, as the development of effective inhibitors for specific targets is crucial for successful treatment.

2. General Information

2.1. Definition of Diabetes Mellitus

Diabetes Mellitus is a prevalent chronic condition marked by hyperglycemia, arising from an absolute or relative deficiency or ineffectiveness of insulin—a hormone produced by the beta cells of the pancreas (American Diabetes Association, 2014). Diabetes manifests as elevated blood glucose levels. To convert glucose into energy, it needs to be transported from the bloodstream into body cells, a process facilitated by the insulin hormone. In individuals with diabetes, the lack or ineffectiveness of insulin results in significantly elevated blood glucose levels (hyperglycemia), a key clinical marker of the disease. This condition damages various tissues throughout the body and creates a pathway for the emergence

of life-threatening health complications (Magliano, Boyko & Atlas, 2021).

2.2. Diabetes Epidemiology

Diabetes represents one of the most significant global health challenges of the 21st century, with many countries still underestimating its profound social and economic impacts. This lack of awareness inevitably contributes to the increasing prevalence of the condition and poses a significant barrier to developing effective treatment strategies. Since the year 2000, the International Diabetes Federation (IDF) has extensively studied diabetes, detailing how it affects and will continue to affect every country, age group, and economy worldwide through its comprehensive research efforts. According to IDF data, the treatment of diabetes and its associated complications, which constitutes a significant portion of total expenditures, continues to drive global healthcare costs upward by 12%. Alongside increased urbanization, lifestyle changes, and the anticipated population growth, a substantial rise in healthcare expenses is projected in the future. As shown in Table 1, the data presented in the IDF's Seventh Diabetes Atlas indicates that approximately 536 million people had diabetes in 2021, and this number is expected to rise to around 783 million by 2045. In 2021, one in 8 people was living with diabetes, whereas in 2045, it is estimated that one in 10 people will be affected. Additionally, it is reported that one in two adults with diabetes worldwide remains undiagnosed. The number of men with diabetes globally is higher than the number of women, and it is also estimated that there are more individuals with diabetes living in urban areas compared to rural regions (IDF, 2021).

Table 1: IDF's global estimates for 2021 and 2045 years

Global Overview	2021	2045
Population		
Total world population	7.9 billion	9.5 billion
Adult population (20-79 years)	5.1 billion	6.4 billion
Diabetes (20-79 years)		
Global diabetes prevalence	10.5%	12.2%
Number of individuals with diabetes	536.6 million	783.2 million
Deaths due to diabetes	6.7 million	-
Diabetes-related healthcare expenditures (20-79 years)		
Total healthcare expenditures (2021, USD)	966 billion	1,054 billion
Gestational hyperglycemia (20-49 years)		
Live birth ratio	16.7%	-
Number of live birth	21.1 million	-
Impaired glucose tolerance (IGT) (20-79 years)		
Global IGT prevalence	10.6%	11.4%
Number of individuals with IGT	541.0 million	730.3 million
Type 1 Diabetes (0-14 years)		
Number of Children with type 1 diabetes	1.2 million	-
Number of newly diagnosed cases of type 1 diabetes annually	184.100	-

Source: IDF Diabetes Atlas-10th Edition, 2021

Diabetes is a significant metabolic disorder caused by an absolute or relative deficiency of endogenous insulin or the peripheral ineffectiveness of insulin. As a chronic condition requiring ongoing medical care, DM is associated with high mortality and morbidity rates globally, impacting both developed and developing countries. Beyond its medical implications, DM exerts substantial social and economic burdens, negatively affecting the lives of thousands. It remains one of the leading causes of death worldwide (Magliano, Boyko & Atlas, 2021).

2.3. Oxidative Stress in Diabetes

In diabetes, uncontrolled hyperglycemia can trigger the excessive production of free radicals, highly reactive molecules capable of interacting with various cellular components due to irregularities in their structures. When these free radicals are not effectively neutralized, they accumulate in the body, leading to a state known as oxidative stress (King & Loeken, 2004).

Diabetes is commonly associated with a chronic state of oxidative stress, which, over time, can contribute to the development of severe and disabling health complications such as retinopathy, nephropathy, neuropathy, and atherosclerosis (Caturano, 2023). Additionally, it is well established that antioxidant enzyme activities are reduced in individuals with diabetes (Jemai, El Feki, & Sayadi, 2009), the glutathione redox status changes (Lutchmansingh et al., 2018), and the hepatic enzymes increase (Ahn et. Al., 2014) in diabetic individuals. Moreover, oxidative stress has an increasing effect on lipid peroxidation (Davi, Falco, & Patrono, 2005), which increases significantly in diabetic patients due to uncontrolled hyperglycemia.

2.4. Oral Antidiabetic Drugs in Diabetes Management

Oral antidiabetic drugs (OADs) are medications taken orally to manage blood sugar levels in individuals with type 2 diabetes. These drugs work through various mechanisms, such as increasing insulin production, improving insulin sensitivity, or reducing the absorption or production of glucose. Unlike insulin injections, OADs are non-invasive and are often prescribed as the first line of treatment for type 2 diabetes. OADs include biguanides, with metformin being the primary drug in this group. Metformin reduces glucose production in the liver and improves the body's sensitivity to insulin, making it more effective in lowering blood sugar levels.

Sulfonylureas stimulate the pancreas to release more insulin, while DPP-4 inhibitors enhance incretin hormones, which increase insulin secretion and reduce glucagon production. Thiazolidinediones increase insulin sensitivity in fat and muscle tissues, helping the body utilize glucose more effectively. Alpha-glucosidase inhibitors delay the digestion and absorption of carbohydrates in the intestines, controlling post-meal blood sugar spikes. Lastly, meglitinides stimulate the pancreas to release insulin rapidly, but only for a short duration (Piragine et. al., 2023).

2.5. SGLT2 Inhibitors in Diabetes Management

SGLT-2 inhibitors have gained significant attention due to their unique mechanism of action and additional health benefits. Among them, ertugliflozin canagliflozin, dapagliflozin, and empagliflozin are FDA-approved medications designed to help manage blood sugar levels in adults with type 2 diabetes mellitus (T2DM). These drugs are intended to be used alongside a healthy diet and regular exercise for optimal glucose control. These inhibitors work by promoting glucose excretion through the urine, preventing its reabsorption in the kidneys. This not only helps to lower blood sugar levels effectively but also provides cardiovascular and renal protective benefits, which are especially critical for individuals with diabetes who are at higher risk for heart and kidney diseases. The ability of SGLT-2 inhibitors to reduce body weight and blood pressure further enhances their importance as part of diabetes management (Baghel et. al., 2024).

Oral antidiabetic drugs are prescribed for individuals with type 2 diabetes who cannot control their blood sugar levels through diet, exercise, or lifestyle changes alone. Depending on the patient's needs, they may be used as monotherapy or in combination with other antidiabetic drugs or insulin therapy. However, these drugs are

not effective for type 1 diabetes, which requires insulin replacement due to the body's inability to produce insulin. The inclusion of SGLT-2 inhibitors in the treatment regimen not only aids in glucose management but also addresses critical complications associated with diabetes, making them a vital component in modern diabetes care.

Despite extensive clinical and experimental research by scientists on the treatment and prevention of diabetes, a definitive cure for the disease remains elusive. While advancements in diabetes treatment have been made, current approaches primarily focus on symptom management rather than a complete cure. This ongoing challenge has heightened concerns and drawn significant attention to the use of natural antioxidants in mitigating oxidative damage associated with diabetes. Consequently, many individuals with diabetes have increasingly sought natural alternative therapies as potential remedies.

2.6. Natural Antioxidants

Throughout history, the olive plant (*Olea europaea* L.) has been extensively utilized for its diverse applications, particularly in promoting health and well-being. Recognized as a staple in traditional medicine, the olive plant is rich in various bioactive compounds, including luteolin, verbascoside, oleuropein, tyrosol, hydroxytyrosol, diosmetin, and rutin (Filardo et al., 2024). Among these, verbascoside, also referred to as acteoside, is a phenylethanoid compound characterized by hydroxyphenylethyl and cinnamic acid moieties connected to a β-glucopyranose via a glycosidic bond (Andary et al., 1982; Alipieva et al., 2014). The structure of verbascoside was shown in Figure 1.

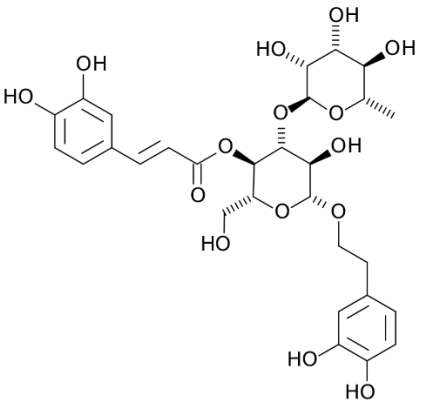

Figure 1: Structure of verbascoside

This compound demonstrates diverse biological activities, such as antioxidant, antimicrobial, anti-inflammatory, and antimutagenic effects (Rice et. al., 1996; Miyazawa et. al., 2003; Pereira et. al., 2007; Vertuani et. al., 2011; Carrillo-Ocampo et. al., 2013). The current study investigates the inhibitory effect of verbascoside, a natural compound found in olive plants, on SGLT-2 using *in silico* methods.

2.7. Molecular Docking Studies

Molecular docking serves as an essential technique for analyzing interactions between ligands and receptors. This method involves evaluating a ligand's potential binding interactions with a receptor by positioning one or more ligands within the high-resolution structure of the target receptor. Docking algorithms can predict the geometries of protein-ligand complexes, ligand binding affinities, and the pose of the ligand within the protein.

In this study, the Schrödinger Molecular Modeling Suite's Maestro 12.5 program was used for *in silico* studies. The software employs two key scoring functions: Emodel and GlideScore. While

the EmodelScore determines the optimal pose of a ligand, the GlideScore ranks ligands based on their binding affinities. For this study, the crystal structure of SGLT2 was imported into Maestro 12.5 using the PDB code 2XQ2 from the RCSB Protein Data Bank. Protein preparation was conducted via Schrödinger's Protein Preparation Wizard. To define the active site, Maestro's Receptor Grid Generation tool was utilized. The compound structure was created using Maestro 12.5's 2D structure software and subsequently imported into the program. Clinical inhibitors of SGLT2, including dapagliflozin, canagliflozin, and empagliflozin, were sourced from PubChem. Optimization of the compounds was carried out using Maestro's LigPrep software. Docking of all compounds into the target enzyme was performed using Glide/XP. Conformers with the lowest binding free energy were analyzed, and their binding modes and interaction diagrams were generated with Maestro 12.5.

3. Results

Our findings indicate that verbascoside exhibits the strongest inhibitory effect on SGLT2 among the tested compounds. Docking results revealed that verbascoside achieved a superior docking score and lower free binding energy compared to the positive control compounds, which may explain its high inhibitory activity. The binding affinity of verbascoside for SGLT2 was notably high, with a free binding energy of −8.824 kcal/mol, outperforming the other compounds. Detailed data are provided in Table 2.

Table 2: Binding Energies and Docking Scores of Compounds for SGLT2

Protein	Compounds	Docking Score	XP GScore	Glide Emodel
SGLT2 (PDB: 2XQ2)	Verbascoside	-8.824	-8.824	-52,985
	Dapagliflozin[a]	-7,875	-7,875	-42,543
	Canagliflozin[a]	-7,875	-7,875	-42,543
	Empagliflozin[a]	-7,956	-7,956	-48,940

[a] Clinically used SGLT2 inhibitors

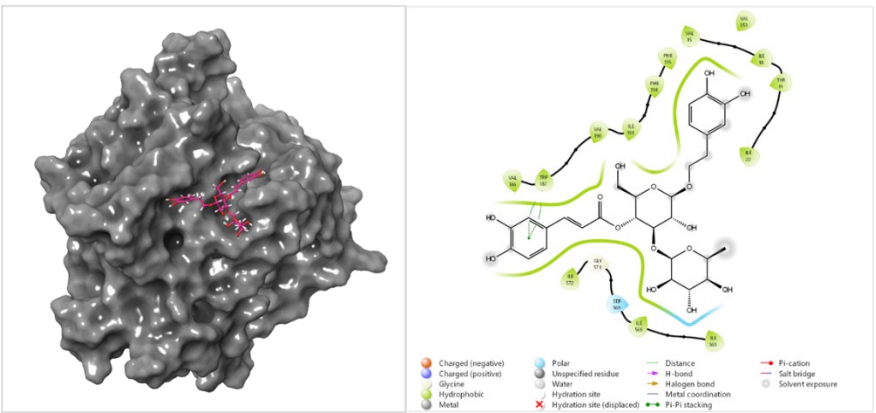

Figure 2: Three-dimensional (3D) docking poses (left) and two-dimensional (2D) ligand interactions (right) of Verbascoside with SGLT2 protein (Verbascoside is depicted in pink balls, and stick modelling)

Verbascoside demonstrated dual π-π stacking interactions with the TRP187 amino acid. Additionally, the compound exhibited hydrophobic interactions with several amino acids, including VAL15, ILE18, TYR19, ILE22, VAL186, TRP187, VAL190, ILE191, PHE194, PHE195, VAL353, ILE565, ILE572, and ILE568.

Furthermore, it engaged with a polar residue within the active site, as illustrated in Figure 3.

In conclusion, among the tested compounds, preliminary findings indicate that verbascoside could be more effective than some existing commercially available SGLT-2 inhibitors, offering a natural and potentially superior alternative. verbascoside was identified as the most effective inhibitor. Verbascoside, a phenolic glycoside found in olive leaves, could be a promising candidate for a new generation of SGLT-2 inhibitors. Its unique chemical structure has shown potential in inhibiting SGLT-2 transporters. This mechanism not only enhances glucose excretion but may also provide additional health benefits. The current evidence suggests that this compound may play a positive role in diabetes management. We anticipate that these findings could aid in the development of new SGLT2 inhibitors and pave the way for innovative treatments targeting a range of global health conditions. Nonetheless, additional studies are required to fully elucidate the impact of verbascoside on diabetes.

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BÖLÜM V

The Key Role of MicroRNAs in Cardiovascular Disease

Serap ÖZER YAMAN[1]

The Key Role of MicroRNAs in Cardiovascular Disease

Introduction

Cardiovascular illnesses rank among the leading causes of mortality in both developing and industrialized nations globally. Despite advancements in primary prevention, the incidence of cardiovascular illnesses has continued to rise in recent years. Therefore, it is essential to thoroughly examine the molecular pathogenesis of cardiovascular illnesses and identify new biomarkers for their early and accurate prevention and detection (Benjamin et al., 2018). Cardiovascular diseases (CVD) rank among the leading causes of global mortality, affecting both developing and developed nations. Notwithstanding advancements in primary prevention, the

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prevalence of cardiovascular disease has persisted in rising in recent years. Consequently, it is essential to thoroughly examine the pathogenesis of cardiovascular disease and to identify new biomarkers for the early and successful prevention and diagnosis of these conditions (Zhou et al., 2018, Kramna et al., 2023)).

Biogenesis, stability, and functions of miRNA

MikroRNAs (miRNA, miR) consist of non-coding RNA sequences that aggregate to create around 22 to 24 nucleotides. Their primary functions encompass the modulation of gene expression, regulation of tissue growth, and maintenance of homeostasis (Small & Olson, 2011; Condorelli & Dimmeler, 2008). miRNAs modulate gene expression by affecting both the source cell and intercellular communication considerably. MiRNAs are initially generated from pre-miRNA, which is synthesized by the RNA polymerase II enzyme on a DNA template. A sequence of enzymes alters the initial configuration of miRNA and aids in its transport to the cytoplasm. The mature miRNA ultimately incorporates into the RNA-induced silencing complex (RISC) alongside several proteins, such as Dicer, Argonaute 2, and transformation reply RNA-binding protein (Pratt & MacRae 2009). MicroRNAs bind to the 3' untranslated regions of targeting messenger RNAs through an eight-nucleotide seed sequence. Partial homology between mature miRNA and the target mRNA results in transcriptional repression, while perfect complementarity leads to mRNA degradation (Liu, Li, & Cairns, 2014).

Approximately 80% of genes in the human genome are transcribed; however, only 1% to 2% are translated into proteins, leading to a significant amount of noncoding RNA (ncRNA) transcripts. Non-coding RNAs include short nuclear and nucleolar RNAs, PIWI-interacting RNAs, Y-RNAs, microRNAs (miRNAs), and long non-

coding RNAs. Non-coding RNAs (ncRNAs) play a vital function in gene expression regulation and epigenetic applications. They may also constitute one of the most critical etiological variables in the onset of cardiovascular disease. At now, miRNAs are the most thoroughly investigated and delineated non-coding RNAs in the literature (Bartel, 2004). MicroRNAs (miRNAs) are endogenous, which retained, single-stranded noncoding RNAs ranging from 21 to 25 nucleotides in length.

While supplementary pairing beyond the seed sequence may improve target recognition, only a limited number of microRNAs seem to depend on these relationships (Broughton et al., 2016). MicroRNA response elements, which act as target sites for microRNAs on mRNA, are predominantly situated in the 3' untranslated region (3-UTR) and, to a lesser degree, in the 5' untranslated region (5-UTR) or coding regions (Grimson et al., 2007). Unlike lncRNAs or circRNAs, which possess various mechanisms of action, miRNAs demonstrate two distinct functions. Their principal role is to facilitate degradation, but their secondary role is to commence translating silence of target miRNAs (Bartel, 2018). The miRNA folder is augmented by non-genetic modifications known as isomiRs, which result from various miRNA processing, nucleotide splicing, or editing (Gebert & MacRae, 2019). A multitude of cardiovascular isomiRs has been found and recognized at different stages of illness. Significant distinctions and target sites have been recorded for the R isomers of miR-487b-3p (van der Kwast et al., 2017). The synthesis of a microRNA is linked to its enzymatic destruction at the end of its life cycle. Studies demonstrate that the half-life of the majority of miRNAs significantly exceeds that of mRNAs, with considerable variance affected by the miRNA strand and sequence, cell type, and processing variables (Marzi et al., 2016). Furthermore, the targets of

miRNAs have been discerned. Notwithstanding the recognized molecular attributes of targeted miRNA degradation (TDMD) and its shown in vivo significance, pinpointing the mRNAs implicated in TDMD continues to be a formidable task (Bitetti et al., 2018; Shi et al., 2020).

MicroRNAs are essential for embryonic development, physiological control, and cardiovascular system function (Kalayinia et al., 2021). Changes in the expression patterns of miRNAs are observed in several cardiovascular conditions, including acute myocardial infarction (AMI), heart failure, and cardiac hypertrophy. Moreover, hypertension, obesity, hyperlipidemia, and diabetes correlate with elevated levels of miRNA (Quiat & Olson, 2013; Cheng et al., 2023). Various experimental models of atherosclerosis have been evaluated, highlighting the significance of miRNA functioning. Both in vitro and in vivo investigations of atherogenesis have revealed a substantial function for different miRNAs. Particular processes, such as inflammation, endothelial cell activation, angiogenesis, vascular smooth muscle cell proliferation and migration, and neointima formation, have been associated with increased miRNA expression (Quiat & Olson, 2013). The active and selective production of miRNA in living cells has been extensively described, underscoring its crucial function in cell-tissue communication. The transport of secreted miRNAs is facilitated by extracellular vesicles linked to particular molecular constituents, including Argonaute 2, nucleophosmin, and lipoproteins. MicroRNAs (miRNAs) may be increased in extracellular macrovesicles and later transported to recipient cells, where they alter gene expression (Cheng et al., 2023). These data suggest that, in contrast to mRNA degradation, miRNAs exhibit stability during circulation, hence providing optimum protection against endogenous ribonuclease activity. The results substantiate the concept that circulating miRNAs could serve as

possible biomarkers for identifying different types of acute coronary syndrome (ACS) (Vickers & Remaley, 2012; Boon & Vickers, 2013).

The functions of miRNAs in the cardiovascular system

Numerous studies indicate a substantial elevation in miR-21-5p expression within impaired human heart tissue. The correlation between miR-21-5p and fibrosis indicates that its expression is elevated in renal and pulmonary diseases, where the degradation of fibrotic tissue is a prevalent characteristic. In animal studies, miR-21 inhibitors have demonstrated efficacy in mitigating heart fibrosis and neointimal formation. The general deficiency of miR21-5p is not well acknowledged; nonetheless, the long-lasting impacts of its inhibitors have been proven by the genetic ablation of miR-21 in nonmyocyte cells, underscoring its critical role in these cells. Cardiac macrophages and fibroblasts demonstrate markedly increased levels of miR-21-5p. The absence of miR-21-5p in murine macrophages led to resistance against transverse aortic constriction, characterized by altered structural and functional characteristics, and correlated with diminished inflammatory responses. Moreover, the administration of anti-miR-21 to pigs post-ischemia-reperfusion demonstrated enhanced cardiac function and decreased inflammation (Thum et al., 2008a).

The findings indicate that miR-21-5p has a significant pro-fibrotic and pro-inflammatory function in the myocardium. A phase II trial is now assessing the efficacy of locked nucleic acid (LNA)-anti-miR-21 for the treatment of fibrotic kidney disease. The miR-29 family comprises four closely related variants thought to affect collagen and other matrix proteins. Consequently, it has been recognized as a viable target for anti-fibrotic treatments. A seminal study by von Roji et al. shown that miR-29 mimics suppress collagen

synthesis, leading to enhanced cardiac function (Van Rooij et al., 2008). The study demonstrated the ability of miR-29 mimics to enhance cardiovascular health. The notion has been reiterated in other articles, leading to the development of a contemporary miR-29 mimic (MRG-201), which has demonstrated efficacy in the treatment of idiopathic pulmonary fibrosis. The theory highlights the reduction of collagen expression; however, anti-miR-29b enhances the stability of the arterial wall, which experiences structural alterations resulting in abdominal aortic aneurysms in murine models (Van Rooij et al., 2008; Boon et al., 2011). Sassi et al. found that blocking miR-29, rather than upregulating it, effectively reduced cardiac fibrosis (Sassi et al., 2017). Despite the unexpected nature of this conclusion, which astonished experts, the authors clarified the underlying mechanism. The miR-29 variants are mostly expressed in cardiac myocytes, with elevated levels observed in cardiac fibroblasts just during prolonged culture. The principal pathophysiological mechanism by which miR-29 induces fibrosis in fibroblasts involves the activation of the Wnt pathway, resulting in cellular hypertrophy and paracrine signaling. Inhibiting miR-29 represents a viable therapeutic approach in the myocardium, whereas increasing its levels may reduce fibrotic pathways in fibroblasts in patients with dermatological disorders. The elevated levels of miR-92a-3p in endothelial cells and its breakdown in animal models of cardiac and vascular tissue injury were analyzed in two separate studies (Bonauer et al., 2009; Hinkel et al., 2013). An efficient LNA anti-miR targeting miR-92a has demonstrated the ability to enhance angiogenesis and tissue repair in these models (Bonauer et al., 2009), a finding corroborated by studies utilizing a porcine ischemia-reperfusion model (Hinkel et al., 2013). The data were modified for therapeutic application based on findings from a pharmacological study with anti-miR-92a (MRI-110). The cohort comprised healthy

adults who were administered a single drug via intravenous injection. The trial group comprised healthy adults who were administered a single drug by intravenous injection (Abplanalp et al., 2020).

Patients with myocardial inflammation and relevant animal models have shown an elevation of miR-155-5p (Heymans et al., 2020). Research on bone marrow transplantation in mice indicated that the proinflammatory role of miR-155 is associated with macrophages exhibiting elevated NF-κB expression. Conversely, elevated levels of miR146a-3p attenuate this impact (Mann et al., 2017). The inhibition of miR-155 reduces cardiac inflammation in mice; however, additional research is necessary to confirm initial findings that macrophage-specific miR-155 deficiency hinders arteriogenesis following vascular injury (Heymans et al., 2020). Cobomarsen, an anti-miR targeting miR-155, has advanced to a phase I trial for cutaneous T-cell lymphoma (Anastasiadou et al., 2021). A phase II trial was discontinued because to speculative dedication to cardiovascular research. Although some miRNA-targeted therapies, such as miravirsen, RG-101, cobomarsen, and AZD4076, have been discontinued, their influence on the cardiovascular domain seems to be less significant than initially expected. Data from both preclinical and clinical studies employing inhibiting oligonucleotides, even those that have been discontinued, offer significant insights for the design and application of miRNA-targeted therapies in cardiovascular medicine. This pertains to miR-17-5p, miR-21-5p, miR-29b-3p, and miR-92a-3p, which have been thoroughly investigated in both laboratory and clinical settings. This is especially pertinent for the miR-132-3p inhibitor (CDR132L), used for the management of heart failure. CDR132L, now designated for phase II studies, may represent a significant advancement in

microRNA-targeted therapy for cardiovascular diseases (Batkai et al., 2021; Hinkel et al., 2021; Täubel et al., 2021).

miR-132-3p has progressed from preclinical investigations to clinical trials, demonstrating significant enhancements in safety, effectiveness, and benefits. Research indicates that genetic disruption of the miR-132/-212 cluster or the administration of an antagomir targeting miR-132-3p can obstruct TAC-induced maladaptive cardiac remodeling. The findings necessitated the assessment of miR-132-3p inhibition in mouse models of BP-induced heart failure (Heymans et al., 2021). In a separate investigation utilizing a porcine heart failure model, researchers found that miR-132 remains in cardiac tissue for a prolonged duration and exhibits a positive safety profile. The authors confirmed the suppression of miR-132 targets. In pig models of produced myocardial infarction, the administration of anti-miR-132 enhanced cardiac function following myocardial damage. In a comparable porcine model, analogous effects were reported in alleviating chronic pressure overload. A pilot human study on increasing doses in heart failure patients shown great tolerability and preliminary indications of therapeutic benefit (Täubel et al., 2021).

miRNA and cardiac hypertrophy

Cardiac remodeling is crucial for the heart's adaptive responses to stresses. Extended duration may lead to pathological hypertrophy, fibrosis, myocardial infarction, arrhythmias, tissue necrosis, cardiomyopathies, and heart failure. Cardiac remodeling includes cardiomyocyte death, hypertrophy, interstitial fibrosis, and regeneration. Prior research has demonstrated the participation and functions of several miRNAs in the normal and pathological progression of heart hypertrophy. Studies demonstrate that miRNAs have a role in the modulation of cardiac hypertrophy in both healthy

and pathological contexts. Moreover, miRNAs have been linked to cardiac fibrosis. Moreover, miR-221, miR-590, miR-33b, miR-320, and miR-34a may contribute to cardiac cell regeneration (Thum et al., 2008b).

miRNA and vascular pathology

Endothelial cells and vascular smooth muscle cells are essential for the preservation of vascular cell viability, integrity, and functionality in the human body. Modified miRNA expression correlates with numerous cardiovascular disorders, including atherosclerosis, vascular inflammation, diabetes with vascular consequences, and both coronary and peripheral artery illnesses. Elevated expression levels of miR-200, miR-34a, miR-217, and miR-146a have been recorded in relation to endothelial cell senescence. This syndrome is characterized by unregulated apoptosis, pronounced inflammation, and diminished production and secretion of endothelial nitric oxide, leading to endothelial dysfunction, atherosclerosis, and related problems (Arunachalam et al., 2015). Impaired endothelial cell function affects angiogenesis, perhaps leading to increased inflammation. Elevated inflammation ultimately leads to vascular disease, ischemic heart episodes, and atherosclerosis. miRNAs have distinct expression in vascular cells and tissues linked to angiogenesis. Vascular inflammation is defined by the activation and infiltration of leukocytes, as well as the synthesis and release of inflammatory cytokines, growth factors, and adhesion molecules. This mechanism may lead to changes in the expression and activities of several miRNAs in endothelial cells (Arunachalam et al., 2015; Thum et al., 2008b).

miRNA and hypertension

Hypertension is a significant risk associated with ischemic heart disease, heart failure, stroke, and peripheral artery disease. Arterial stiffness, aging, inflammation, the renin-angiotensin-aldosterone system, and endothelial dysfunction are associated with the pathophysiology of hypertension. Recent research indicates that the altered expression of some miRNAs, including miR-132, miR-155, miR-212, and miR-143/145, modulates blood pressure in humans through the renin-angiotensin-aldosterone system. Furthermore, miR-145 has been recognized as a modulator of vascular deterioration induced by hypertension in prior research (Maegdefessel, 2014). Studies have demonstrated a connection between single nucleotide polymorphisms in miRNA binding sites and essential hypertension. Furthermore, single nucleotide polymorphisms situated in miRNA binding sites frequently result in alterations in blood pressure levels (Bátkai & Thum, 2012). Certain miRNAs, including miR-130a, miR-210, miR-150, miR-191, miR-23b, miR-1246, and miR-451, have been identified in human blood or plasma as significantly expressed biomarkers for the early and accurate diagnosis of hypertension (Arunachalam et al., 2015). Additionally, miRNAs have been identified as promising noninvasive indicators for the early and precise detection of hypertension-related stroke. A recent study identified miR-30a, let-7b, and miR-126 as potential biomarkers for hypertension-associated ischemic stroke (Maegdefessel, 2014).

miRNA and pulmonary hypertension

Pulmonary arterial hypertension is a primary contributor to global mortality. The ailment is categorized into multiple subtypes, notably idiopathic pulmonary arterial hypertension, hereditary pulmonary arterial hypertension, and pulmonary arterial hypertension linked to

other conditions. While genetic, environmental, and epigenetic factors are considered etiopathological contributors to this syndrome, no specific factors have been identified. Inflammation and endothelial cell proliferation in the pulmonary artery are essential processes that necessitate further investigation (Zhou, Chen, & Raj, 2015).

Marked compression, contractions, thereby and migration of pulmonary vascular smooth muscle cells, together with fibroblast activation, emigration, and proliferation, are acknowledged pathogenetic mechanisms of pulmonary arterial hypertension. Previous studies have investigated and recorded the potential pathogenetic role of miRNAs. miR-21, miR-204, miR-17-92, miR-145, miR-124, and miR-210 have been identified as miRNAs exhibiting instability in the growth, immigration, and contract of pulmonary artery smooth muscle cells (Zhou, Chen, & Raj, 2015). Furthermore, miR-503, miR-27a, miR-424, miR-17-92, and miR-21 were recognized as expressed in endothelial cells, promoting proliferation and conferring resistance to apoptosis. Recent study indicates that miRNAs may function as new biomarkers for the early identification and treatment of pulmonary arterial hypertension. Rhodes et al. demonstrated a significant correlation between reduced expression levels of miR-150 and poor prognosis in patients with pulmonary arterial hypertension (Rhodes et al., 2013). Furthermore, Schlosser and colleagues established a positive correlation between miR-26a and right ventricular systolic pressure, right ventricular hypertrophy, and exercise capacity, as measured by the six-minute walking distance. MicroRNAs may be crucial to the precise and timely diagnosis, risk evaluation, and therapy of pulmonary arterial hypertension. These compounds may serve as novel therapeutic agents, functioning as agonists and antagonists in human programs, owing to their capacity to influence numerous genes within a

genome, hence enhancing their therapeutic value (Schlosser et al., 2013).

miRNA and acute myocardial infarction

Acute myocardial infarction is caused by atherosclerosis and is linked to considerable mortality and morbidity. The rupture of susceptible atherosclerotic plaques, acute coronary artery blockage due to thrombus development, and heightened myocardial demand are essential pathophysiological factors contributing to acute myocardial infarction. Cardiac remodeling, characterized by the dilatation of the coronary arteries and the deterioration of ventricular walls caused by significant necrosis and fibrosis following acute myocardial infarction, can result in systolic heart failure. Timely and precise diagnosis, together with appropriate treatment, is essential to prevent complications associated with acute myocardial infarction, particularly mortality. In recent years, numerous biomarkers have been utilized to assess global mortality and morbidity rates. Recent studies have examined the role of miRNAs as novel indicators in acute myocardial infarction. The expression of many miRNAs was shown to be elevated, while miR-197, miR-106, and miR-223 were downregulated in the serum/plasma of individuals with acute myocardial infarction. These miRNAs have been identified as distinctive biomarkers for predicting significant cardiovascular events in previous studies (Arunachalam et al., 2015). Potential mechanisms by which miRNAs may serve as biomarkers for acute myocardial infarction encompass the spread of circulating miRNAs to recipient target cells to modulate protein translation, alongside specific release pathways or passive release of miRNAs into the bloodstream subsequent to cellular degeneration and demise. Cyclical variations in miRNA expression linked to myocardial

survival, growth, fibrosis, and remodeling may influence ventricular activity and cardiac contractility (Zile et al., 2011).

miRNA and restenosis

Vascular remodeling and restenosis are pathophysiological consequences of atherosclerosis. The utilization of drug-eluting coronary stents in percutaneous coronary intervention may reduce the occurrence of restenosis; nonetheless, the complication rate persists at a high level. Severe neointimal proliferation and hyperplasia, vascular remodeling, enhanced proliferation and migration of vascular smooth muscle cells, and persistent inflammation subsequent to coronary stent implantation are the principal factors leading to restenosis. Restenosis may cause recurrent ischemia and angina, require repeat revascularization, and lead to acute coronary syndrome. A prior study indicated that circulating miR-143 levels can predict in-stent restenosis in coronary or peripheral artery disease. Additionally, miRNAs have been recognized as critical regulators in the development of vascular smooth muscle cells, as evidenced by a previous study. Moreover, miR-133 has been recognized as a dysregulated miRNA in relation to restenosis. This investigation established that elevated transcoronary miR-133 levels are a dependable marker for the need for target lesion revascularization in instances with in-stent restenosis. Moreover, miRNAs have been recognized as dysregulated in restenosis (Indolfi et al., 2019).

miRNA and heart failure

Heart failure is a clinical disorder characterized by the heart's inability to supply adequate blood, oxygen, and nutrients to fulfill the body's metabolic demands. Heart failure is classified into three subtypes: systolic, diastolic, and mid-range ejection fraction. Heart

failure is one of the primary causes of global mortality. Prevalent etiological factors of heart failure encompass coronary artery disease, hypertension, valvular heart disease, arrhythmias, myocarditis, and cardiomyopathies, which may result from viral infections and specific cardiotoxic agents or substances (Xiao et al., 2017). Recent studies demonstrate a substantial independent function of circulating miRNAs in the diagnosis and prognosis of heart failure (Watson et al., 2015). The results of these research provide significant evidence concerning the essential role of miRNAs in the disease's genesis and progression. Moreover, prior research has established a correlation between miRNAs and clinical, imaging, and laboratory outcomes (Xiao et al., 2017; Watson et al., 2015). The diagnostic efficacy of NT-pro-BNP for heart failure has enhanced through its integration with miRNAs by previous research. Furthermore, the concentrations of circulating miRNA may be altered by specific medications. Left ventricular assist devices may affect miRNA levels (miR-499, miR-208a and b, miR-133, and miR-1) in either a muscle-specific or general manner (Akat et al., 2014). Adverse remodeling after cardiac resynchronization therapy correlates with changes in miRNA levels, characterizing "responders". The study identified increased concentrations of miR-30e, miR-92a, miR-26b, miR-145, and miR-29a in the responder group compared to non-responders. Baseline levels of miR-30d and miR-1306 correlated with detrimental left ventricular remodeling in end-stage chronic heart failure patients classified as “responders.” Their prognostic relevance was apparent after one year (Bernardo et al., 2015).

Potential for therapy

Therapeutic modulation of miRNAs influences serum lipoproteins in clinical trials. A clinical study utilizing anti-sense suppression of

miR-122-5p offers significant data. This phase 2 clinical trial examines the safety and efficacy of miR-122-5p reduction for Hepatitis C, noting a dose-dependent reduction in serum total cholesterol levels (Janssen et al., 2013). The mechanisms remain only partially understood; however, studies employing animal models have pinpointed 3-hydroxy-3-methylglutaryl-CoA reductase, an essential enzyme in cholesterol biosynthesis, and microsomal triglyceride transfer protein, which aids in the transfer of triglycerides to ApoB throughout very low-density lipoprotein assembly, as significant targets. The therapeutic modulation of additional miRNAs to enhance lipid and glucose metabolism has been evaluated in animal models. The inhibition of miR-148a-3p with antisense nucleotides led to decreased LDL cholesterol and increased HDL cholesterol in serum, indicating the reactivation of hepatic LDL receptors and ABCA1 (Esau et al., 2006; Tsai et al., 2012, Goedeke et al., 2015,). Antagomir-induced suppression of miR-103-3p led to reduced plasma glucose concentrations and heightened insulin resistance in the hepatic and adipose tissues of obese murine models (Trajkovski et al., 2011). The data indicate that miR-103-3p and let-7 may serve as potential therapeutic targets in diabetes.

Ultimately, miRNAs have undergone experimental evaluation as medications in hypertension and associated organ damage. The systemic administration of antisense nucleotides targeting miR-208a-3p decreased cardiac stress and related abnormal hypertrophy in hypertensive rats (Dickinson et al., 2013). The administration of miR-29b-3p following Angiotensin II-induced hypertension improved cardiac function in mice and mitigated histological indicators of hypertensive cardiopathy (Zhang et al., 2014). The study illustrates the therapeutic potential of miRNAs in hypertension and its associated effects. Furthermore, miRNAs may enhance

clinical outcomes. Conversely, off-target effects and immune system activation provide significant challenges to the practical application of miRNA-based therapeutics (103). The primary advantages of miRNAs include their lasting influence on target tissues or organ systems, regulation ease, capacity to modify many pathways, and substantial impacts at lower dosages. Conversely, the challenges associated with these therapeutic systems encompass off-target effects, possible toxicities from delivery agents or miRNAs, problems in discerning tissue-specific pharmacodynamic impacts, and the stringent delivery mechanisms required for tissue targeting.

miRNAs and Future Perspectives

Circulating miRNAs serve as possible biomarkers for diagnosing and prognosticating cardiovascular disease (CVD). miRNAs display specificity for certain cells or tissues, maintain stability in serum or plasma, exhibit tolerance to degradative factors such as freeze-thaw cycles and blood enzymes, and possess rapid release kinetics. They function as potential surrogate indicators for early and accurate disease diagnosis, as well as for assessing medium- or long-term prognosis.

Furthermore, the use of miRNAs with traditional biomarkers may improve risk categorization and long-term prognostic accuracy. miRNA-based therapeutics have the potential to improve cardiovascular disease treatment by incorporating innovative platforms and computational tools, as MicroRNAs (miRNAs) play a crucial role in various cardiovascular risk factors and are essential to the pathophysiology of atherosclerosis, cardiac remodeling, and myocardial infarction (MI). These compounds exhibit considerable promise as biomarkers and therapeutic targets in diverse illnesses; yet, further investigation and clinical validation are required. MicroRNAs could significantly augment current biomarkers,

enhancing diagnostic accuracy and furthering the objective of individualized therapy. Additional validation of the most promising miRNA candidates in clinical environments and varied patient populations is crucial for their therapeutic applicability. The utilization of miRNA targeting as a therapeutic strategy is still in the preliminary phases of development. Preclinical investigations yield encouraging outcomes; nonetheless, most have been conducted solely in mouse models. Additional examination of this therapeutic method in a more applicable context is necessary to enable further clinical trials evaluating the efficacy and safety of these treatments.

In conclusion, miRNAs are substantially involved in numerous cardiovascular diseases, and initial research indicates their potential future use as biomarkers or therapeutic targets alongside traditional analytical approaches. Despite being in the experimental phase, the extensive application of miRNA shows significant potential for the diagnosis, prognosis, and treatment of several cardiovascular diseases. The prospective therapeutic application may also enhance the subsequent creation of stents. The adoption of this method, either autonomously or in conjunction with current biomarkers, may occur imminently, especially in instances of diagnostic ambiguity. Further investigation is required to validate its suitability in standard clinical settings, highlighting repeatability and sensitivity.

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