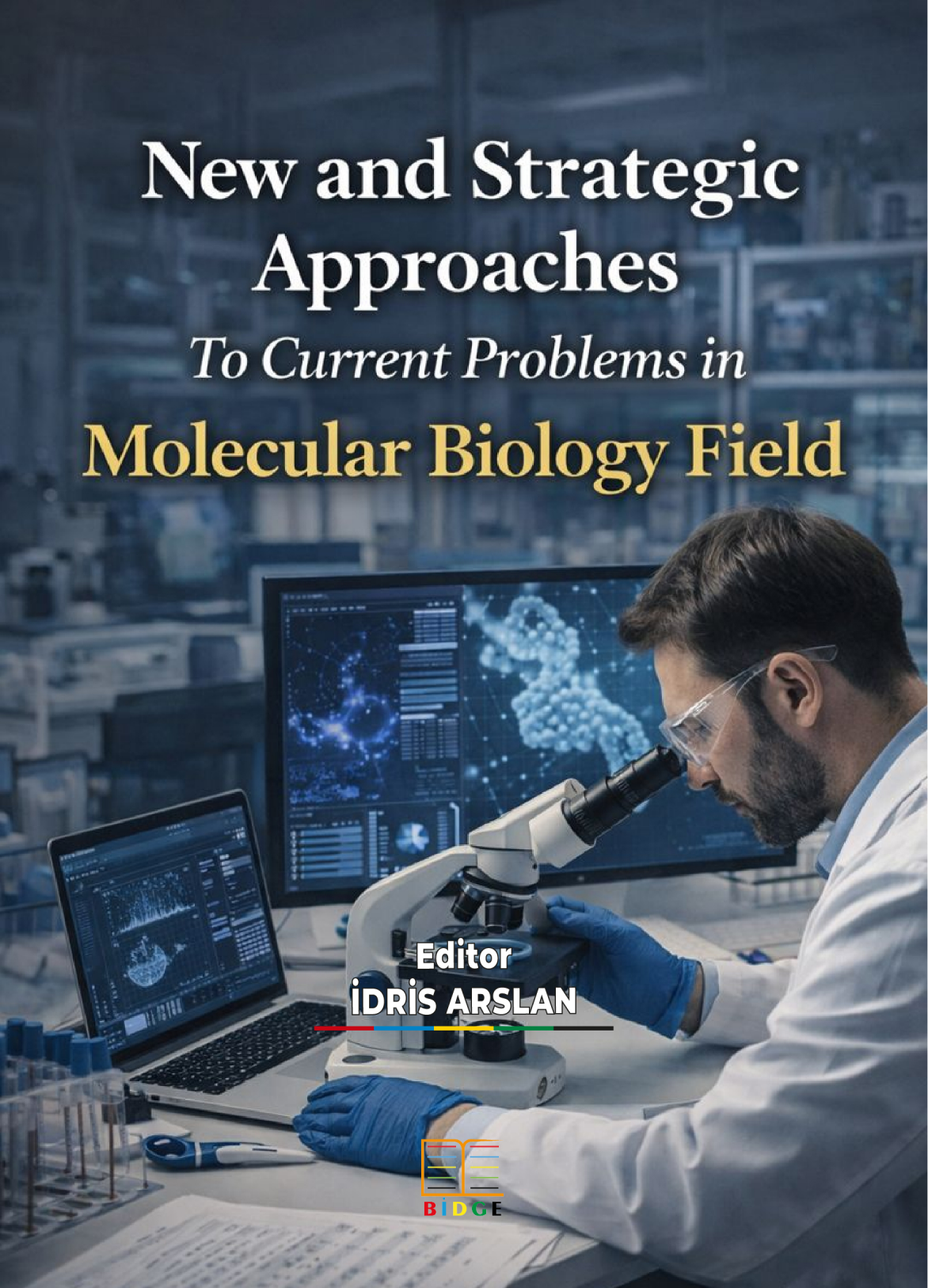
New and Strategic Approaches

To Current Problems in

Molecular Biology Field



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PREFACE

Molecular biology stands at the heart of modern life sciences, continually reshaped by rapid technological advances, expanding datasets, and increasingly complex biological questions. As classical approaches reach their limits, the field demands new perspectives—methods that are not only innovative, but also strategic in how they integrate theory, experimentation, and computation. *New and Strategic Approaches to Current Problems in the Molecular Biology Field* is conceived in response to this need, bringing together contemporary ideas aimed at addressing some of the most pressing challenges facing molecular biology today.

This book explores emerging concepts, tools, and interdisciplinary strategies that are redefining how molecular systems are studied and understood. Rather than focusing solely on established techniques, the chapters emphasize forward-looking approaches, including novel experimental designs, advanced analytical frameworks, and the convergence of molecular biology with fields such as bioinformatics, systems biology, and biotechnology.

Intended for researchers, educators, and advanced students, this book serves both as a reference and a source of inspiration. It encourages critical thinking about existing limitations while showcasing pathways for innovation and collaboration. Ultimately, *New and Strategic Approaches to Current Problems in the Molecular Biology Field* aims to contribute to the ongoing evolution of molecular biology by fostering ideas that will shape future research, applications, and scientific understanding.

Prof. Dr. İDRİS ARSLAN

ZONGULDAK BÜLENT ECEVİT ÜNİVERSİTESİ

İÇİNDEKİLER

BÖLÜM 1

Ferroptosis: Molecular Mechanisms and Roles in Human Diseases

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1. Introduction

1.1 Ferroptosis

Ferroptosis is a form of iron-dependent regulated cell death (RCD) characterized by the accumulation of lethal levels of lipid peroxides on cellular membranes (Dixon et al., 2012; Stockwell et al., 2017). This form of cell death develops independently of classical apoptotic pathways and is primarily shaped by disruption of cellular redox balance, iron metabolism, and lipid peroxidation. In recent years, ferroptosis has been considered a promising new

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therapeutic approach, particularly in oncology, due to its ability to target apoptosis-resistant tumor cells (Jiang et al., 2021).

A large proportion of chemotherapy, radiotherapy, and targeted agents commonly used in cancer treatment are based on inducing apoptosis in tumor cells. However, in many types of cancer, the suppression of apoptotic signaling pathways through genetic and epigenetic mechanisms leads to the development of treatment resistance. Ferroptosis, thanks to its unique mechanism that does not require caspase activation, offers an alternative cell death pathway in apoptosis-resistant cancer cells and thus gains strategic importance in oncology (Stockwell et al., 2017; Jiang et al., 2021).

1.2 History of Ferroptosis

The term ferroptosis was defined and introduced into the literature in 2012 by Brent R. Stockwell and colleagues (Dixon et al., 2012). However, the cellular and biochemical characteristics specific to ferroptosis were observed in different experimental contexts long before the emergence of this term.

Early studies in 1908 reported that iron could have toxic effects on cells (Stockwell et al., 2017). Pioneering cell culture studies conducted by Harry Eagle in the 1950s revealed that the deficiency of amino acids, particularly cysteine, could lead to cell death, and these findings later formed the basis for understanding the central role of glutathione metabolism in ferroptosis (Eagle, 1955). Studies conducted in 1989 showed that glutamate-mediated

cytotoxicity causes oxidative stress and cell death through inhibition of the system xc^- (SLC7A11/SLC3A2) amino acid antiporter, which facilitates cysteine uptake into the cell (Murphy et al., 1989). In 2001, a form of cell death induced by oxidative stress, particularly in neuronal cells, and differing morphologically and biochemically from apoptosis, was termed oxytosis (Tan et al., 2001). In the years that followed, during small molecule compound screenings conducted between 2003 and 2008, it was noticed that some compounds targeting oncogenic RAS mutations (e.g., erastin and RSL3) triggered a specific form of cell death (Yang & Stockwell, 2008). Finally, in 2012, this form of cell death was defined as a new, non-apoptotic, regulated form of cell death due to its iron dependence and unique morphological, biochemical, and genetic characteristics, and the term "ferroptosis" was introduced into the literature (Dixon et al., 2012).

2. Morphological Features of Ferroptosis

The morphological features of ferroptosis are characterized by distinctive ultrastructural changes that distinguish it from other forms of RCD, such as apoptosis, necroptosis, and pyroptosis. These morphological changes are concentrated predominantly at the mitochondrial level, rather than the nucleus-centered events seen in classical cell death pathways, reflecting the metabolic and redox-based nature of ferroptosis.

One of the most prominent morphological features observed in cells undergoing ferroptosis is mitochondrial shrinkage. In these cells, mitochondria are significantly reduced in size, exhibiting an irregular and dysmorphic appearance. This differs from the mitochondrial outer membrane permeabilization typically observed in apoptosis or the cellular swelling seen in necroptosis, and is considered one of the distinctive ultrastructural signatures of ferroptosis (Dixon et al., 2012; Stockwell et al., 2017). In addition, an increase in mitochondrial membrane density is noteworthy in ferroptotic cells. Electron microscopy studies clearly show that the inner and outer mitochondrial membranes acquire a denser and more condensed structure. This condensation is associated with the direct effects of lipid peroxidation and iron-dependent oxidative stress on mitochondrial membrane structures (Friedmann et al., 2017).

Another characteristic feature is the marked reduction or complete disappearance of mitochondrial cristae. Cristae, which are folds of the inner membrane of the mitochondria, are critically important for energy metabolism. The disruption of cristae structures in ferroptosis indicates that the metabolic functions of the mitochondria are severely affected and that cellular energy homeostasis is irreversibly disrupted (Gao et al., 2019). Despite these mitochondrial abnormalities, it has been reported that the nuclear membrane generally remains intact in cells undergoing ferroptosis, and apoptosis-specific nuclear changes such as chromatin condensation or DNA fragmentation are not observed.

However, in the later stages of the cell death process, necrosis-like morphological features such as disruption of plasma membrane integrity and cytoplasmic swelling (oncosis) may occur. This suggests that while ferroptosis progresses as a regulated process in its early stages, it can acquire necrotic features in the final stage due to the physical effects of membrane lipid peroxidation (Stockwell et al., 2017; Jiang et al., 2021).

These morphological changes observed in ferroptosis also reveal the complex role of mitochondria in the cell death process. Mitochondria are critical in the mechanism of ferroptosis because they are both a major source of oxidative stress and the center of cellular metabolism (Gaschler et al., 2018; Gao et al., 2019).

Consequently, mitochondrial shrinkage, characterized by increased membrane density and cristae loss, and the distinctive ultrastructural changes are key features that clearly distinguish ferroptosis morphologically and mechanically from other RCD pathways such as apoptosis, necroptosis, and pyroptosis.

3. Structural and Biochemical Basis of Ferroptosis

Ferroptosis is distinctly different, both mechanistically and morphologically, from other RCD pathways such as apoptosis, necroptosis, and pyroptosis (Stockwell et al., 2017).

Ferroptosis is a process dependent on the presence of intracellular iron. Free iron triggers the formation of highly reactive

oxygen species (ROS) via the Fenton reaction, which accelerates lipid peroxidation and increases cellular damage. Intracellular iron accumulation is a critical factor in the initiation and progression of ferroptosis (Dixon et al., 2012; Gao et al., 2019).

The main driving mechanism of ferroptosis is the lethal accumulation of oxidized phospholipids containing polyunsaturated fatty acids (PUFA) on cellular membrane phospholipids. These lipid peroxides disrupt membrane integrity, leading to irreversible damage to the cell (Stockwell et al., 2017; Jiang et al., 2021).

Ferroptosis occurs without the direct involvement of specific cell death-enhancing proteins such as caspases involved in apoptosis, MLKL proteins playing a critical role in necroptosis, or gasdermin proteins that form pores in pyroptosis (Dixon et al., 2012; Friedmann et al., 2017).

Morphological changes in ferroptotic cells are largely concentrated in the mitochondria. Mitochondrial shrinkage, increased mitochondrial membrane density, and reduced or absent mitochondrial cristae are defined as the distinctive ultrastructural features of ferroptosis. In contrast, the nuclear membrane is generally preserved, and apoptosis-specific nuclear fragmentation is not observed (Dixon et al., 2012; Gao et al., 2019).

While the structural and biochemical features of ferroptosis define the cellular context in which lipid peroxidation-driven cell death occurs, the execution and modulation of this process are

governed by complex molecular regulatory networks. Therefore, the following section focuses on the signaling pathways and molecular regulators that fine-tune ferroptotic sensitivity across different physiological and pathological conditions.

4. Molecular Mechanisms in Ferroptosis

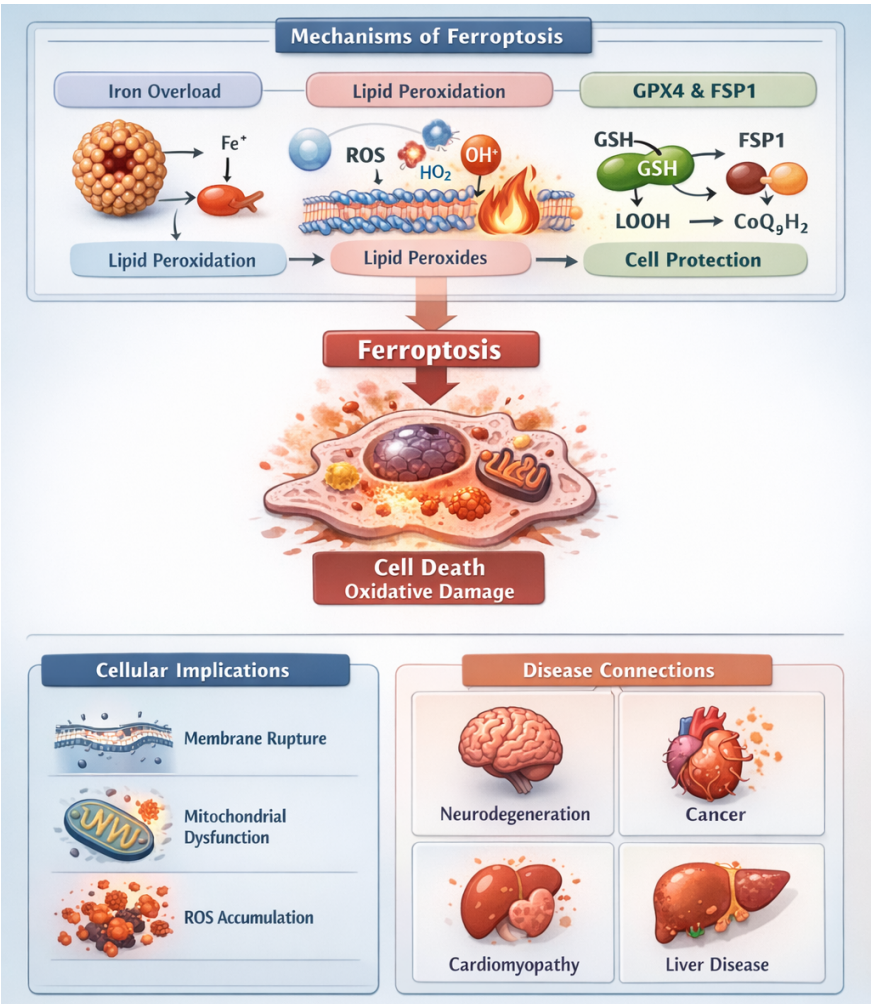

Figure 1: Mechanisms of ferroptosis

4.1 RAS Signaling

RAS signaling has emerged as an important modulator of ferroptotic sensitivity through its effects on cellular metabolism, redox homeostasis, and lipid peroxidation. Among RAS isoforms, KRAS has been most extensively investigated in the context of ferroptosis, particularly in cancer models, due to its high mutation frequency and strong association with metabolic reprogramming.

Oncogenic RAS, particularly KRAS, can affect ferroptosis susceptibility in two opposing ways: (i) by sensitizing cells to ferroptosis through enhanced lipid peroxidation, and (ii) by simultaneously facilitating escape from ferroptosis via the upregulation of antioxidant defense mechanisms. Initial observations revealed that RAS-selective small molecules, such as erastin, trigger an iron-dependent and caspase-independent form of cell death, later defined as ferroptosis (Dixon et al., 2012; Yang & Stockwell, 2008).

Mechanistically, RAS activation reprograms membrane lipid composition and cellular redox balance, thereby increasing susceptibility to peroxidation of PUFA-containing phospholipids. When GPX4/GSH-dependent antioxidant defenses are overwhelmed, this process leads to ferroptotic cell death characterized by the accumulation of PL-PUFA-OOH (Yang et al., 2014; Stockwell et al., 2017). In addition to GPX4-dependent protection, GPX4-independent defense systems, such as the FSP1-

CoQ10 axis, have been shown to suppress ferroptosis in certain RAS-driven tumors (Bersuker et al., 2019; Doll et al., 2019).

4.2 NRF2 Signaling (KEAP1-NRF2 Axis)

NRF2 (NFE2L2) is the main transcriptional regulator of the cell's oxidative stress response and initiates a strong "anti-ferroptotic" program against ferroptosis. In the basal state, KEAP1 directs NRF2 to the ubiquitin-proteasome pathway; Oxidative/metabolic stress or KEAP1 loss leads to the translocation of NRF2 to the nucleus, increasing antioxidant, iron-metabolism, and GSH biosynthesis genes (Stockwell et al., 2017; Jiang et al., 2021). NRF2 targets genes such as SLC7A11 (system xc^-), GCLC/GCLM (GSH synthesis), NQO1, HMOX1, and ferritin iron storage components, thereby (i) increasing the GSH pool, (ii) supporting the lipid hydroperoxide detoxification capacity of GPX4, and (iii) limiting iron-induced ROS production; ultimately, this raises the lipid peroxidation threshold and suppresses ferroptosis (Jiang et al., 2021; Stockwell et al., 2017). Therefore, resistance to ferroptosis induction is common in KEAP1 mutated/NRF2 hyperactive tumors, and treatment strategies aim to overcome this defense axis (Jiang et al., 2021).

4.3 mTOR Signaling

mTORC1 activates anabolic programs according to nutrient/energy status and affects ferroptosis susceptibility particularly through lipid biosynthesis and redox balance. mTORC1

activation reshapes fatty acid metabolism by supporting lipogenesis transcription factors (especially SREBP1); as a result, MUFA production may increase via desaturases such as SCD1/SCD5. Integration of MUFAs into the membrane reduces lipid peroxidation by partially replacing PUFA-PLs and contributes to ferroptosis resistance (Magtanong et al., 2019; Viswanathan et al., 2017). On the other hand, the interaction of the mTORC1 axis with cellular stress response and autophagy can indirectly alter the balance of iron pool and lipid peroxidation; therefore, mTOR signaling may suppress ferroptosis in some contexts while facilitating its development in others (Jiang et al., 2021; Stockwell et al., 2017).

4.4 Hypoxia Signaling (HIF-1α / HIF-2α)

Hypoxia modulates ferroptosis through two main mechanisms: (i) strengthening or weakening system xc^-/GSH/GPX4 defense, and (ii) reprogramming lipid metabolism and iron homeostasis. HIF-1α and HIF-2α alter lipid uptake/transport and fatty acid processing via different target gene sets depending on the cell type; this determines the membrane PUFA content and thus the risk of peroxidation (Jiang et al., 2021; Stockwell et al., 2017). For example, lipid reprogramming under hypoxia can contribute to therapy resistance in some tumors by suppressing ferroptosis, while in other contexts it can create a predisposition to ferroptosis by increasing iron-dependent oxidative processes (Jiang et al., 2021).

This bidirectional nature highlights that the hypoxia-ferroptosis relationship is “context-dependent.”

4.5 EMT (epithelial-mesenchymal transition)

EMT affects ferroptosis susceptibility alongside cellular reprogramming associated with invasion/metastasis. In states associated with EMT drivers such as ZEB1 and stem-like phenotype markers (e.g., CD44), lipid metabolism and antioxidant defenses are remodeled; this can alter ferroptosis thresholds (Viswanathan et al., 2017; Jiang et al., 2021). In particular, it has been shown that “treatment-resistant/metastatic” cell states may be dependent on a lipid peroxidase-dependent survival program, and these cells may present a sensitive therapeutic window for ferroptosis induction (Viswanathan et al., 2017). Furthermore, EMT-related glutaminolysis and TCA influx indirectly determine the tendency towards ferroptosis by affecting membrane lipid composition via NADPH/ROS balance (Stockwell et al., 2017; Jiang et al., 2021).

4.6 TP53 (p53) signaling

p53 is a “two-faced” regulator that can both suppress and enhance ferroptosis depending on the context. One of the best-defined ferroptosis connections of p53 is that it reduces cystine influx into the cell by suppressing SLC7A11 expression, thus decreasing GSH production and weakening GPX4 defense; this increases susceptibility to lipid peroxidation (Jiang et al., 2015; Stockwell et al., 2017). p53 can also exert effects toward ferroptosis

by modulating enzymatic pathways associated with lipid peroxidation (e.g., some lipoxygenase axes); However, conditions in which p53 delays/reduces ferroptosis by enhancing the antioxidant response through specific targets have also been reported (Stockwell et al., 2017; Jiang et al., 2021). Therefore, TP53 status is an important biomarker/contextual variable in ferroptosis-based therapies.

4.7 YAP/TAZ Signaling

The effectors of the hippo pathway, YAP/TAZ, establish transcriptional programs through cell density, mechanistic signals, and metabolic status and can significantly influence ferroptosis susceptibility. YAP/TAZ activity modulates membrane peroxidation susceptibility by altering the expression of genes associated with lipid metabolism, iron uptake/transport, and antioxidant defenses (Jiang et al., 2021). In some models, YAP/TAZ activity has been associated with a "pro-ferroptotic" program that increases ferroptosis susceptibility, and cell density-dependent ferroptosis susceptibility has been explained (Stockwell et al., 2017; Jiang et al., 2021).

4.8 Autophagy Pathways

Autophagy regulates ferroptosis, particularly through subtypes that alter the iron pool and lipid supply. One of the most critical mechanisms is ferritinophagy. NCOA4-mediated ferritin degradation increases the "labile iron pool" by releasing iron stored

in ferritin; this accelerates Fenton chemistry and lipid peroxidation, thus facilitating ferroptosis (Hou et al., 2016; Stockwell et al., 2017). Similarly, autophagic degradation of lipid droplets (lipophagy) or processes affecting mitochondrial quality can alter cellular ROS production and PUFA flux, thereby affecting the ferroptosis threshold (Stockwell et al., 2017; Jiang et al., 2021). Furthermore, influencing the stability of ferroptosis defense proteins through certain components of selective autophagy types (e.g., chaperone-mediated autophagy) can indirectly alter defense capacity (Jiang et al., 2021).

4.9 Energy metabolism pathways (AMPK, PPP, glutaminolysis)

Under energy stress, AMPK can readjust ferroptosis susceptibility by limiting lipid biosynthesis and the PUFA pool. The stress exerted by AMPK on ACC (acetyl-CoA carboxylase) can reduce de novo fatty acid synthesis and, in particular, the production of PUFA-PL, which is susceptible to peroxidation; This situation may be protective against ferroptosis in some contexts (Stockwell et al., 2017; Jiang et al., 2021). On the other hand, the pentose phosphate pathway (PPP) and NADPH production support anti-ferroptotic defense by providing reducing power for both the GPX4/GSH system and the FSP1-CoQ10 system (Bersuker et al., 2019; Doll et al., 2019). Glutaminolysis (via GLS/GLUD) can alter the lipid peroxidation threshold by affecting TCA influx and redox

homeostasis; therefore, energy-metabolism reprogramming is a key determinant of ferroptosis susceptibility.

5. Cellular Defense Mechanisms Against Ferroptosis

Cells have developed powerful antioxidant and lipid regulatory defense systems that work interconnectedly but partly independently to prevent the lethal accumulation of lipid peroxides. These defense networks aim to maintain a delicate balance between pro-oxidant processes that trigger ferroptosis and detoxification mechanisms that suppress lipid peroxidation (Stockwell et al., 2017; Jiang et al., 2021).

The GPX4-centered antioxidant defense system protects cells from ferroptosis by reducing membrane lipid hydroperoxides to non-toxic lipid alcohols in a glutathione-dependent manner, thereby preventing the propagation of lipid peroxidation (Figure 2).

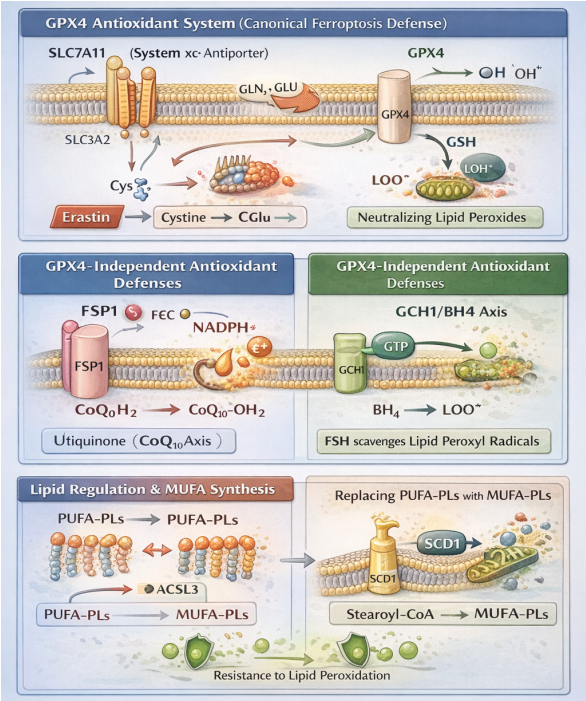

Figure 2: GPX4 antioxidant system

5.1 GPX4 Antioxidant System (Canonical Ferroptosis Defense)

The glutathione peroxidase 4 (GPX4) axis is considered the primary and canonical defense mechanism used by cells against ferroptosis (Yang et al., 2014).

5.1.1. SLC7A11 (System xc^-) Activity

The first and critical step in ferroptosis defense is the uptake of cystine (Cys_2) from the extracellular space into the cell via system xc^-, known as the cystine/glutamate antiporter. This antiporter

consists of the catalytic subunit SLC7A11 (xCT) and the structural subunit SLC3A2 (Dixon et al., 2012).

Intracellular cystine is rapidly reduced to cysteine (Cys_2) in the cytosol, and this amino acid acts as the rate-limiting precursor molecule in the synthesis of the cellular antioxidant glutathione (GSH). Suppression of system xc^- activity leads to decreased intracellular GSH levels and consequently to the accumulation of lipid peroxides. In this context, compounds such as erastin, sorafenib, and sulfasalazine inhibit cystine uptake by inhibiting SLC7A11 function and trigger ferroptosis via the extrinsic pathway (Yang et al., 2014; Louandre et al., 2013).

5.1.2. Glutathione Peroxidase 4 (GPX4) Activity

Glutathione serves as an essential cofactor for the GPX4 enzyme. GPX4 reduces toxic lipid hydroperoxides (PLOOH) formed on cellular membrane phospholipids to harmless lipid alcohols, thereby halting lipid peroxidation chain reactions (Yang et al., 2014). Direct inhibition of GPX4 leads to a strong induction of ferroptosis. Small molecules such as RSL3 directly inactivate GPX4, initiating ferroptosis independently of the presence of GSH. This mechanism is described as the intrinsic ferroptosis pathway (Yang et al., 2014; Stockwell et al., 2017).

5.2. GPX4-Independent Antioxidant Defense Systems

Recent studies have shown that cells are not only dependent on GPX4 for ferroptosis; in addition, they develop parallel and GPX4-independent radical scavenging systems (Bersuker et al., 2019; Doll et al., 2019).

5.2.1. FSP1 - Ubiquinone ($CoQH_2$) Axis

Ferroptosis Suppressor Protein 1 (FSP1, formerly AIFM2) is a plasma membrane-associated oxidoreductase and exerts its anti-ferroptotic effect through coenzyme Q10 (ubiquinone) metabolism (Doll et al., 2019).

FSP1 reduces ubiquinone using NADPH and forms ubiquinol ($CoQH_2$), a potent lipophilic antioxidant. Ubiquinol terminates lipid auto-oxidation chain reactions by directly scavenging lipid peroxyl radicals, thus providing a GPX4-independent defense against ferroptosis (Bersuker et al., 2019).

5.2.2. GCH1/BH4 Axis

Another GPX4-independent defense mechanism is the tetrahydrobiopterin (BH4) axis, synthesized by the GTP cyclohydrolase 1 (GCH1) enzyme. BH4, as a potent lipophilic antioxidant, suppresses lipid peroxidation and protects the cell against ferroptosis (Kraft et al., 2020).

5.3. Lipid Regulation Mechanisms and MUFA Synthesis

Cells can reprogram not only antioxidant defenses but also membrane lipid composition to protect against ferroptosis. Monounsaturated fatty acids (MUFAs), unlike polyunsaturated fatty acids (PUFAs), are more resistant to lipid peroxidation because they do not contain bis-allylic bonds. Therefore, the integration of MUFAs into membrane phospholipids provides a protective effect against ferroptosis (Magtanong et al., 2019).

In this process, increasing MUFA synthesis via stearoyl-CoA desaturase-1 (SCD1) and the incorporation of these fatty acids into membrane phospholipids by ACSL3 replace peroxidation-sensitive PUFA-PLs, thereby increasing ferroptosis resistance. This mechanism is described as a significant metabolic adaptation, particularly used by cancer cells to avoid ferroptosis (Magtanong et al., 2019; Viswanathan et al., 2017).

6. The Role of Ferroptosis in Diseases and Pathologies

Ferroptosis, as a form of iron-dependent and RCD, plays critical roles not only in cancer biology but also in the pathogenesis of ischemic tissue damage, neurodegenerative diseases, and various chronic pathologies (Figure 3). Located at the intersection of cellular metabolism, iron homeostasis, and lipid redox balance, ferroptosis appears as a protective target in some diseases and as a damage mechanism that needs to be prevented in others (Stockwell et al., 2017; Jiang et al., 2021).

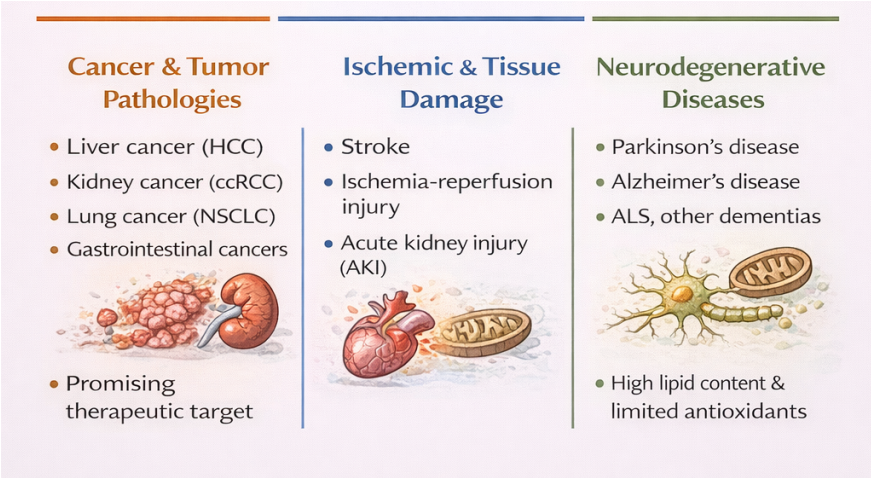

Figure 3: The role of ferroptosis in diseases and pathologies

6.1. Cancer and Tumor Pathologies

Ferroptosis functions as a natural tumor suppression mechanism under physiological conditions and offers a significant alternative pathway, particularly in eliminating malignant cells that have developed apoptosis evasion (Dixon et al., 2012; Stockwell et al., 2017). This feature has made ferroptosis a promising therapeutic target for tumors resistant to conventional chemotherapy and targeted therapies (Jiang et al., 2021).

Among hepatic cancers, hepatocellular carcinoma (HCC) is a model where ferroptosis-based approaches are being intensively investigated. HCC cells can show a significant sensitivity to ferroptosis due to high iron overload and metabolic stress, and this highlights the ferroptosis-related effects of agents such as sorafenib (Louandre et al., 2013; Llovet et al., 2021).

In kidney cancers, particularly in clear cell renal cell carcinoma (ccRCC) and chromophobe renal cell carcinoma subtypes, alterations in lipid metabolism and antioxidant defense mechanisms are important factors determining ferroptosis susceptibility (Miess et al., 2018).

In lung cancers, non-small cell lung cancer (NSCLC) subtypes, especially tumors with EGFR mutations or KEAP1 mutations, have been associated with impaired ferroptosis regulation. Alterations in the KEAP1-NRF2 axis may contribute to the development of resistance to ferroptosis (Singh et al., 2020).

Among gastrointestinal cancers, gastric, pancreatic, and colorectal cancers are among the tumor groups where ferroptosis-based therapeutic approaches may be potentially effective (Chen et al., 2021). In breast cancers, it has been reported that triple-negative breast cancer (TNBC) cells, despite being resistant to apoptosis, show a significant sensitivity to ferroptosis (Yu et al., 2017).

In addition, ferroptosis has been shown to be effective on tumor progression and treatment response in many malignancies such as melanoma, neuroblastoma, mesothelioma, prostate cancer, and acute myeloid leukemia (AML) (Stockwell et al., 2017; Jiang et al., 2021). Targeting ferroptosis, especially in cancer cells showing drug resistance and metastatic potential, offers a strategic approach for eliminating chemotherapy and radiotherapy-resistant cell populations (Viswanathan et al., 2017).

6.2. Ischemic and Tissue Damage

Unlike cancer, excessive or uncontrolled activation of ferroptosis plays a role in increasing cell loss in various ischemic and acute tissue damage situations. In this context, inhibition of ferroptosis is considered a tissue-protective strategy (Friedmann Angeli et al., 2014).

Ferroptosis has been identified as a central pathogenic mechanism, especially in ischemia/reperfusion (I/R) injuries. Increased iron-dependent lipid peroxidation with reoxygenation after ischemia triggers cell death. This process is closely associated with:

Acute kidney injury (AKI) and damage developing after kidney transplantation, ischemic heart disease and cardiomyopathy, brain ischemia and stroke, tissue damage developing after heart, kidney and liver transplantation (Linkermann et al., 2014; Fang et al., 2019).

Furthermore, ferroptosis also plays a role in pathologies associated with radiation damage. Ferroptosis-mediated lipid peroxidation has been reported to be effective in the development of acute lung injury and pulmonary fibrosis due to radiotherapy (Li et al., 2019).

6.3. Neurodegenerative Diseases

Ferroptosis stands out as an important cell death mechanism in the pathogenesis of neurodegenerative diseases due to its high lipid content and limited antioxidant capacity in the central nervous system (Do Van et al., 2016).

Neuronal loss due to lipid peroxidation in areas such as the brain's cortical regions and the hippocampus has been associated with ferroptosis. This mechanism has been linked to nerve cell death in specific neurodegenerative diseases such as Parkinson's disease and Pelizaeus-Merzbacher disease (Do Van et al., 2016; Stockwell et al., 2017).

Ferroptosis, a unique form of cell death situated at the intersection of cellular metabolism, iron balance, and lipid redox homeostasis, presents itself as a mechanism that needs to be therapeutically induced in some diseases and suppressed in others. This dual role has made ferroptosis a central focus of research in both cancer biology and degenerative and acute tissue damage pathologies (Stockwell et al., 2017; Jiang et al., 2021).

7. Conclusion

Ferroptosis represents a distinct form of regulated cell death driven by iron-dependent lipid peroxidation and shaped by complex interactions between metabolic, redox, and signaling pathways. A comprehensive understanding of the molecular determinants governing ferroptosis including lipid metabolism, iron homeostasis, antioxidant defense systems, and their integration with cellular

signaling networks is essential for elucidating its diverse roles across a broad spectrum of human diseases. Beyond cancer, accumulating evidence implicates ferroptosis in the pathogenesis of neurodegenerative disorders, ischemia–reperfusion injury, metabolic diseases, and inflammatory conditions. Accordingly, advancing our knowledge of ferroptotic regulatory mechanisms may facilitate the identification of disease-specific vulnerabilities as well as the development of context-dependent therapeutic and protective strategies aimed at modulating ferroptosis in both pathological and physiological settings.

Abbreviations

ACC: Acetyl-CoA carboxylase

ACSL3: Acyl-CoA synthetase long-chain family member 3

AKI: Acute kidney injury

AML: Acute myeloid leukemia

AMPK: AMP-activated protein kinase

BH4: Tetrahydrobiopterin

CD44: Cluster of differentiation 44

CoQ10: Coenzyme Q10 (ubiquinone)

CoQH2: Ubiquinol (reduced CoQ10)

COPD: Chronic obstructive pulmonary disease

EGFR: Epidermal growth factor receptor

EMT: Epithelial–mesenchymal transition

FSP1 (AIFM2): Ferroptosis suppressor protein 1

GCH1: GTP cyclohydrolase 1

GLS: Glutaminase

GLUD: Glutamate dehydrogenase

GPX4: Glutathione peroxidase 4

GSH: Glutathione

HCC: Hepatocellular carcinoma

HIF-1α / HIF-2α: Hypoxia-inducible factor-1α / -2α

I/R: Ischemia/reperfusion

KEAP1: Kelch-like ECH-associated protein 1

KRAS: Kirsten rat sarcoma viral oncogene homolog

MAPK/ERK: Mitogen-activated protein kinase / Extracellular signal-regulated kinase

MLKL: Mixed lineage kinase domain-like protein

mTORC1: Mechanistic target of rapamycin complex 1

MUFA: Monounsaturated fatty acid

NADPH: Nicotinamide adenine dinucleotide phosphate (reduced form)

NCOA4: Nuclear receptor coactivator 4

NFE2L2 (NRF2): Nuclear factor erythroid 2–related factor 2

NQO1: NAD(P)H quinone dehydrogenase 1

NSCLC: Non-small cell lung cancer

PPP: Pentose phosphate pathway

PUFA: Polyunsaturated fatty acid

PUFA-PL: PUFA-containing phospholipid

RCD: Regulated cell death

ROS: Reactive oxygen species

SCD1: Stearoyl-CoA desaturase 1

SLC3A2: Solute carrier family 3 member 2 (system xc^- subunit)

SLC7A11 (xCT): Solute carrier family 7 member 11 (system xc^- subunit)

SREBP1: Sterol regulatory element-binding protein 1

System xc^-: Cystine/glutamate antiporter

TCA: Tricarboxylic acid cycle

TNBC: Triple-negative breast cancer

TP53 (p53): Tumor protein p53

YAP/TAZ: Yes-associated protein / Transcriptional coactivator with PDZ-binding motif

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BÖLÜM 2

HEPATOCELLULAR CANCER and lncRNA

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INTRODUCTION

Hepatocellular Cancer

Hepatocellular carcinoma (HCC) represents approximately 90% of all primary liver malignancies and remains one of the most prevalent cancers globally. According to 2024 data from the World Health Organisation (WHO) and its affiliated cancer database (IARC-GLOBOCAN), HCC, the most common primary cancer of the liver, remains a global public health issue. HCC accounts for approximately 75-85 % of all primary liver cancers and ranks as the sixth most common cancer type and the third leading cause of

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cancer-related deaths worldwide. The incidence and mortality of HCC are particularly high in Asia and Sub-Saharan Africa, where chronic hepatitis B and C infections are the main risk factors; in contrast, alcohol consumption and metabolic dysfunction-associated fatty liver disease (MASLD) increase the burden of HCC in Western countries (Chen et al.,2025). There is a strong correlation between hepatitis B virus (HBV) carrier rates and the increased rate of HCC, and the majority of HBV-related HCC cases are seen in patients with cirrhosis. Long-term alcohol use leads to fatty liver disease, cirrhosis, hepatitis, oxidative stress, mitochondrial damage, endoplasmic reticulum stress, and consequently, liver damage. At the molecular level, excessive alcohol consumption can stimulate acetaldehyde toxicity, form adducts on proteins and DNA, produce excessive reactive oxygen species (ROS), and lead to changes in lipid metabolism (Feng & Zhao, 2024). The causes of HCC formation are shown in Figure 1. The global cancer burden is on the rise, and new cases of HCC are projected to increase significantly by 2050; this underscores the importance of preventive strategies such as early diagnosis, vaccination, and risk factor control.

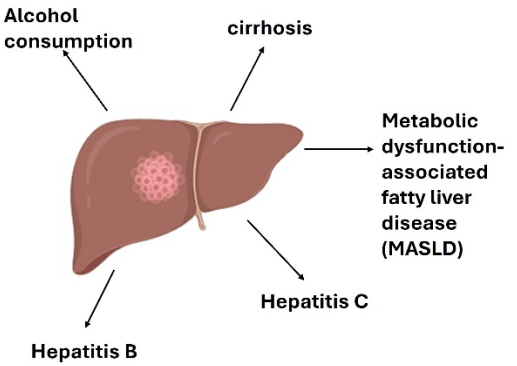

Figure 1: Causes of HCC formation

Molecular Heterogeneity of HCC

HCC is a highly heterogeneous malignancy characterized by genetic, epigenetic, transcriptomic, and tumor microenvironment–related diversity, which underlies the marked variability in clinical behavior, prognosis, and treatment response among patients (Villanueva, 2019; Llovet et al., 2021). Large-scale molecular profiling studies have demonstrated that HCC represents a dynamic spectrum of distinct molecular subtypes rather than a single disease entity (Villanueva, 2019).

At the genetic level, HCC heterogeneity is primarily driven by recurrent alterations in TP53, CTNNB1 (β-catenin), and TERT promoter regions. TP53 mutations are commonly associated with genomic instability and poor prognosis, whereas CTNNB1 mutations define a biologically distinct subgroup characterized by activation of Wnt/β-catenin signaling (Figure 2). TERT promoter mutations contribute to cellular immortalization via telomerase

activation, and the distribution of these alterations varies according to etiological factors such as HBV infection, alcohol exposure (Villanueva, 2019; Llovet et al., 2021).

Transcriptomic analyses have further classified HCC into proliferative and non-proliferative subtypes. The proliferative class is associated with activation of MYC and AKT/mTOR pathways, stem cell–like features, and aggressive clinical behavior, whereas the non-proliferative class displays more differentiated phenotypes and metabolic gene expression profiles with relatively better outcomes (Goossens, 2015). In addition, the tumor microenvironment, including chronic inflammation, fibrosis, hypoxia, and immune infiltration, critically shapes tumor biology and determines immunological subtypes with distinct responses to immunotherapy (Kurebayashi et al., 2022).

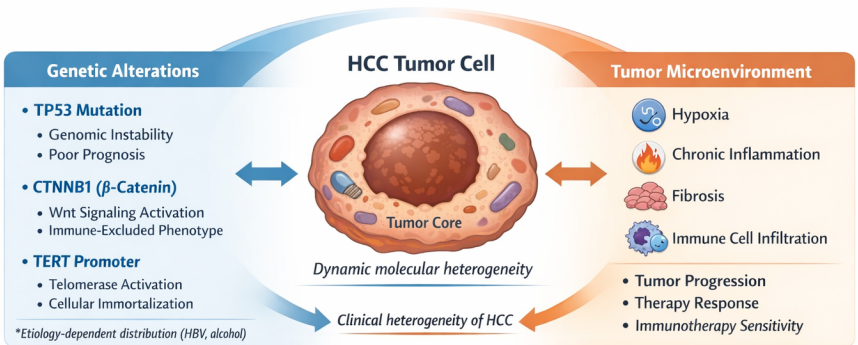

Figure 2: Genetic and Microenvironmental Determinants of Molecular Heterogeneity in Hepatocellular Carcinoma

Epigenetic alterations, such as aberrant DNA methylation, histone modifications, and chromatin remodeling, represent another major source of HCC heterogeneity and profoundly influence gene expression patterns, including those of long non-coding RNA (lncRNAs) (Romeo et al., 2023). Recent multi-omics studies emphasize that HCC heterogeneity arises from the integration of multiple molecular layers, highlighting lncRNAs as both products and regulators of this complexity through their context-specific expression and regulatory functions (Statello et al., 2021).

Classification of lncRNAs

lncRNAs are RNA transcripts longer than 200 nucleotides that do not encode proteins but play essential roles in the multilayered regulation of gene expression (Figure 3). Recent evidence demonstrates that lncRNAs are not passive transcriptional by-products in HCC; instead, they function as active, context-dependent regulators of tumor development, progression, and treatment response (Quinn and Chang, 2016; Statello et al., 2021).

lncRNAs are categorized according to their genomic localization and their positional relationship with neighboring protein-coding genes. Intergenic lncRNAs are transcribed from regions between protein-coding genes, whereas antisense lncRNAs originate from the opposite strand of protein-coding loci. Intronic lncRNAs arise from intronic regions of protein-coding genes, while divergent lncRNAs are transcribed in the reverse direction from

shared promoter regions with protein-coding genes. In addition, enhancer RNAs (eRNAs) are generated from active enhancer regions and function to increase the transcriptional activity of target genes (Ding et al.,2018).

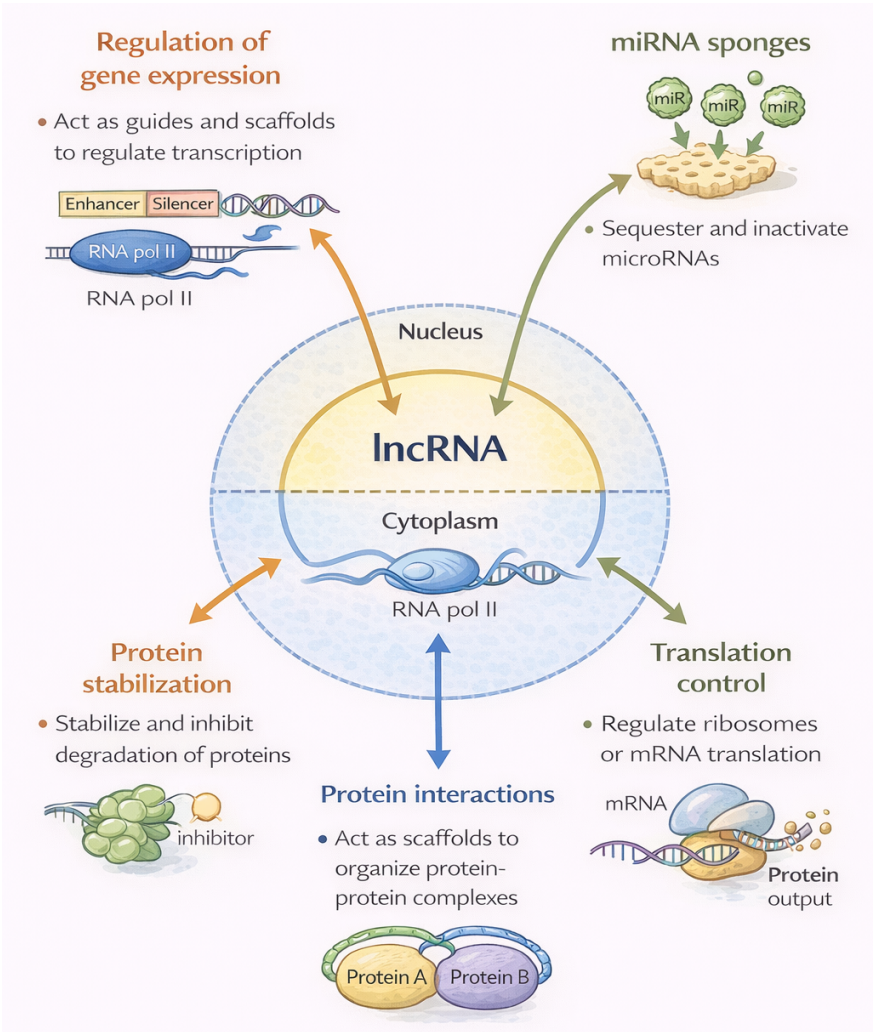

Figure 3: Multifunctional Roles of Long Non-Coding RNAs in Gene Regulation

Transcriptional Regulation

lncRNAs play essential roles in transcriptional gene regulation. Based on their genomic location, interaction partners, and mechanisms of action, lncRNAs are classified into distinct categories that help explain their cis- or trans-regulatory functions.

Intergenic lncRNAs (lincRNAs) reside in genomic regions between protein-coding genes and primarily function in trans by acting as scaffolding platforms for chromatin-modifying complexes. A prototypical example is HOTAIR, which recruits Polycomb Repressive Complex 2 (PRC2) and LSD1 to promote H3K27 trimethylation and transcriptional silencing, whereas MALAT1 contributes to nuclear organization and transcriptional elongation (Rinn & Chang, 2012; Quinn & Chang, 2016).

Antisense lncRNAs are typically exert cis-regulatory effects through transcriptional interference or local chromatin remodeling. ANRIL, located at the CDKN2A/B locus, exemplifies antisense-mediated epigenetic repression of cell cycle genes, while HIF1A-AS2 modulates HIF-1α signaling under hypoxic conditions (Pasmant et al., 2011; Liaoo et al., 2024).

Intronic lncRNAs arise from intronic regions of protein-coding genes and regulate parental gene expression by influencing

RNA polymerase II activity or chromatin accessibility (Ponting et al., 2009). Enhancer RNAs (eRNAs) are transcribed from active enhancers and facilitate transcription by strengthening enhancer–promoter interactions, particularly in hormone-responsive and inflammatory pathways (Kim et al., 2010; Natoli & Andrau, 2012).

Finally, divergent lncRNAs are transcribed bidirectionally from shared promoters and are characteristic of active transcriptional regions. LncRNA-p21, which regulates p53-dependent gene expression, represents a well-characterized example of this class (Dimitrova et al., 2014).

Post-Transcriptional Regulation

lncRNAs can direct processes such as mRNA stability, alternative splicing, translational efficiency, and RNA transport. In particular, it has been shown that nuclear localized lncRNAs modulate pre-mRNA processing by interacting with splicing factors; while cytoplasmic lncRNAs play roles in enhancing or suppressing mRNA stability (Quinn and Chang, 2016). These mechanisms are critical in the formation of gene expression patterns.

ceRNA (miRNA Sponge) Mechanism

The competing endogenous RNA (ceRNA) mechanism describes a mode of gene regulation in which different RNA species influence each other's expression by sharing common microRNA (miRNA) binding sites. In this model, lncRNAs act as miRNA sponges, reducing miRNA availability for target mRNAs and

thereby enhancing mRNA stability or translation (Salmena et al., 2011). The effectiveness of ceRNA interactions depends on the presence of miRNA response elements (MREs) and the relative abundance and binding affinities of the interacting RNAs, making ceRNA networks highly dynamic and context dependent (Tay et al., 2014).

Representative examples include H19, which sequesters let-7 family miRNAs to regulate genes involved in proliferation and differentiation, and PTENP1, a pseudogene-derived transcript that preserves PTEN expression by sponging PTEN-targeting miRNAs (Poliseno et al., 2010; Kallen et al., 2013). In cancer, lncRNAs such as MALAT1, TUG1, and XIST regulate pathways related to epithelial–mesenchymal transition, proliferation, and DNA damage responses through ceRNA mechanisms (Yildirim et al., 2013; Li et al., 2014; Wang et al., 2016). ceRNA regulation also contributes to stress adaptation, as lncRNAs including GAS5, MEG3, NEAT1, and LINC00152 modulate growth suppression, p53 signaling, and hypoxia-responsive pathways (Tao et al., 2015; Zhou et al., 2017; Choudhry et al., 2015; Yu et al., 2017).

Chromatin Modification

Many lncRNAs regulate gene expression by interacting with epigenetic regulatory proteins and shaping chromatin architecture in a locus-specific and context-dependent manner (Rinn & Chang, 2012; Statello et al., 2021). Acting as molecular guides, scaffolds, or

decoys, lncRNAs recruit chromatin-modifying complexes such as Polycomb Repressive Complex 2 (PRC2), lysine-specific demethylase 1 (LSD1), and DNA methyltransferases (DNMTs) to defined genomic regions, thereby enabling precise temporal and spatial control of epigenetic states (Kopp & Mendell, 2018; Rinn & Chang, 2020).

Several lncRNAs mediate transcriptional repression through PRC2-dependent mechanisms. HOTAIR recruits PRC2 and LSD1 to target loci, promoting H3K27 trimethylation and removal of activating H3K4 marks, resulting in stable transcriptional silencing (Rinn et al., 2007; Tsai et al., 2010). Similarly, XIST, the key regulator of X-chromosome inactivation, interacts with PRC2 and additional silencing factors to induce chromosome-wide heterochromatin formation (Clemson et al., 2009; Zhao et al., 2008). Beyond Polycomb-mediated repression, some lncRNAs regulate gene expression through DNA methylation–dependent mechanisms. For instance, KCNQ1OT1 recruits DNA methyl transferases DNMTs to neighboring genes, leading to promoter methylation and long-term silencing (Pandey et al., 2008), while ANRIL contributes to epigenetic repression at the CDKN2A/B locus by interacting with Polycomb group proteins (Pasmant et al., 2011).

Importantly, lncRNA-mediated epigenetic regulation is not restricted to gene silencing. Certain lncRNAs promote transcriptional activation by establishing a permissive chromatin

environment. HOTTIP, for example, interacts with the WDR5/MLL complex to enhance H3K4 trimethylation and activate HOXA gene expression. (Rinn and Chang, 2020; Statello et al., 2021).

Protein Interaction and Stability

In addition to interacting with DNA and RNA, lncRNAs exert important regulatory functions through direct interactions with proteins, thereby modulating protein activity, localization, complex formation, and stability. Through these interactions, lncRNAs act as molecular scaffolds, decoys, or adaptors, enabling fine-tuned regulation of cellular signaling networks (Kopp and Mendell, 2018).

Several lncRNAs function as scaffolds that facilitate the assembly of multi-protein structures. For example, HOTAIR coordinates the interaction of multiple chromatin-associated proteins, while NEAT1 serves as the structural backbone of paraspeckles by binding RNA-binding proteins such as NONO and PSPC1, thereby regulating their nuclear availability and function. These scaffold-based interactions allow lncRNAs to indirectly influence transcriptional and post-transcriptional responses, particularly under cellular stress conditions.

Beyond scaffolding, lncRNAs can regulate protein localization and activity by sequestering proteins within specific subcellular compartments. GAS5, for instance, binds the glucocorticoid receptor and prevents its nuclear translocation, leading to repression of glucocorticoid-responsive gene expression.

In addition, lncRNAs have been shown to control protein stability by modulating ubiquitination and proteasomal degradation pathways. Certain lncRNAs stabilize target proteins by interfering with E3 ubiquitin ligase–mediated degradation, whereas others promote protein turnover by facilitating ubiquitin-dependent proteolysis (Kopp and Mendell, 2018; Statello et al., 2021).

The Effect of Subcellular Localization

The biological functions of lncRNAs are strongly determined by their subcellular localization, which defines their interaction partners and regulatory mechanisms. Nuclear lncRNAs are mainly involved in chromatin organization, transcriptional regulation, and RNA processing, whereas cytoplasmic lncRNAs primarily regulate gene expression at the post-transcriptional level. This spatial compartmentalization enables lncRNAs to exert highly specific and context-dependent regulatory effects (Ulitsky and Bartel, 2013; Statello et al., 2021).

In the nucleus, lncRNAs often associate with chromatin and nuclear protein complexes, acting as guides or scaffolds for epigenetic regulators. Classic examples include XIST, which mediates chromosome-wide transcriptional silencing, and HOTAIR, which recruits Polycomb complexes to establish repressive chromatin states. Structural nuclear lncRNAs such as NEAT1 organize nuclear bodies, including paraspeckles, thereby regulating the availability of transcriptional and RNA-binding proteins. In

addition, some nuclear lncRNAs influence RNA maturation by interacting with splicing factors and modulating alternative splicing.

By contrast, cytoplasmic lncRNAs mainly control mRNA stability, translation, and signaling pathways. Many functions as competing endogenous RNAs, as exemplified by H19, MALAT1, and TUG1, while others directly interact with ribosomes or translation factors to modulate protein synthesis. Notably, certain lncRNAs exhibit dynamic localization, shuttling between the nucleus and cytoplasm in response to developmental cues or environmental stresses, which allows them to perform distinct regulatory functions depending on cellular context. Overall, subcellular localization represents a key determinant of lncRNA function and integrates spatial organization with gene expression control (Ulitsky and Bartel, 2013; Statello et al., 2021).

Roles of lncRNAs in Hepatocellular Carcinoma

In HCC, lncRNAs contribute to gene regulation at transcriptional, post-transcriptional, and epigenetic levels, thereby playing key roles in tumor initiation, progression, and therapeutic response. Nuclear lncRNAs regulate chromatin organization and transcriptional activity, frequently through interactions with epigenetic modifiers such as PRC2, whereas cytoplasmic lncRNAs commonly act through ceRNA mechanisms by modulating microRNA availability and downstream gene expression (Rinn and Chang, 2020; Kopp and Mendell, 2018). Disruption of these

regulatory networks has been closely linked to tumor growth, metastasis, metabolic reprogramming, and treatment resistance in HCC. Accumulating evidence indicates that lncRNAs can function either as oncogenes or tumor suppressors and respond dynamically to microenvironmental cues such as hypoxia, inflammation, and immune signaling. Moreover, lncRNAs influence not only tumor cell–intrinsic pathways but also tumor–microenvironment interactions, including immune modulation and therapy responsiveness, highlighting their potential as biomarkers and therapeutic targets (Jiang et al., 2020).

lncRNAs in HCC

HOTAIR (HOX Transcript Antisense Intergenic RNA)

Long non-coding RNA HOTAIR is a well-established oncogenic lncRNA that plays a pivotal role in HCC progression. Approximately 2158 nucleotides in length, HOTAIR is the first identified trans-acting lncRNA transcribed from the HOXC gene cluster on chromosome 12q13.13 (Wang et al., 2022). Its expression is significantly upregulated in HCC tissues, cell lines, and circulation, and high HOTAIR levels are consistently associated with aggressive tumor behavior and poor prognosis (Wu et al., 2017).

Clinically, elevated HOTAIR expression correlates with poor differentiation, metastasis, early recurrence, advanced TNM stage, portal vein tumor thrombosis, and reduced overall and recurrence-free survival. Circulating HOTAIR has therefore been proposed as a

potential non-invasive biomarker for HCC diagnosis and prognosis, including in HCV-associated HCC patients treated with direct-acting antivirals (El-Khazragy et al., 2020; El-Shendidi et al., 2022).

At the molecular level, HOTAIR functions as a modular scaffold in both the nucleus and cytoplasm. In the nucleus, its 5′ domain recruits Polycomb Repressive Complex 2 (PRC2), leading to EZH2-mediated H3K27 trimethylation and transcriptional silencing, while its 3′ domain interacts with the LSD1 complex to remove activating H3K4 methylation marks. Through EZH2, HOTAIR also promotes DNA methylation–dependent repression of tumor-suppressive miRNAs such as miR-122 (Cheng et al., 2018).

In the cytoplasm, HOTAIR acts as a competing endogenous RNA by sponging multiple miRNAs, thereby promoting oncogenic signaling. Functionally, HOTAIR enhances proliferation, cell cycle progression, epithelial–mesenchymal transition, and metastasis, while its silencing induces cell cycle arrest and apoptosis (Fu et al., 2015; Zhou et al., 2017). HOTAIR further supports metabolic reprogramming by upregulating GLUT1 and activating mTOR signaling, particularly under hypoxic conditions, where it facilitates glycolytic adaptation (Wei et al., 2017; Hu et al., 2020b). Additionally, HOTAIR contributes to tumor microenvironment remodeling by increasing CCL2 expression, promoting immune cell recruitment, and enhancing exosome biogenesis.

MALAT1 (Metastasis-Associated Lung Adenocarcinoma Transcript 1)

MALAT-1 is a lncRNA that functions as a transcriptional and post-transcriptional regulator and is considered a proto-oncogene in HCC. MALAT-1 has also been proposed as a potential cancer biomarker due to its role in promoting cell proliferation and migration. MALAT-1 expression is significantly higher in HCC tissues compared with paired tumor-free liver tissues (Liao et al., 2023; Lu et al., 2022).

In HepG2 and Hep3B, MALAT-1 has been shown to regulate gene expression by modulating transcriptional activity and alternative splicing. Mechanistically, MALAT-1 contributes to HCC development through activation of the Wnt/β-catenin signaling pathway and stimulates the oncogenic splicing factor SRSF1, thereby influencing downstream oncogenic targets including RAS and MAPK pathways (Ji et al., 2019; Liu et al., 2017). In addition, MALAT-1 regulates cancer-associated microRNAs such as miR-574 and miR-20b, further supporting tumor progression and invasiveness (Shin et al., 2017).

Functionally, MALAT-1 overexpression significantly enhances the migratory and invasive capacity of HCC cells, whereas its silencing suppresses colony formation, migration, and invasion, particularly in Huh7 cells (Youness et al., 2021). Beyond its tumor cell–intrinsic effects, MALAT-1 also contributes to immune evasion

in HCC. MALAT-1 has been shown to modulate immune checkpoint expression through epigenetic mechanisms, including the regulation of PD-L1 and CD155. Silencing MALAT-1 using siRNA reduces PD-L1/CD155 co-expression in tumor cells and enhances the cytotoxic activity of peripheral blood mononuclear cells (PBMCs), highlighting its role in shaping the immunosuppressive tumor microenvironment (Assal et al., 2020).

Recent studies further demonstrate an inverse correlation between MALAT-1 and the tumor-suppressive microRNA miR-423-5p in HCC. High miR-423-5p expression is associated with a less aggressive tumor phenotype and improved patient survival, whereas elevated MALAT-1 levels correlate with poor prognosis (Bocchetti et al., 2025). Collectively, these findings support MALAT-1 as a multifunctional oncogenic lncRNA that integrates transcriptional regulation, alternative splicing, ceRNA interactions, and immune modulation in HCC.

HULC (Highly Upregulated in Liver Cancer)

HULC has been identified as one of the most prominently overexpressed lncRNAs in gene library screenings for HCC (Panzitt et al., 2007). Located on chromosome 6p24.3 in the human genome, HULC is a transcript approximately 1.6 kb long, with two exons and no protein-coding capacity. HULC expression has been shown to be significantly increased in HCC tissues, and this increase has been

shown to be associated with an oncogenic role (Panzitt et al., 2007; Du et al., 2012).

Functional analyses have revealed that HULC suppression reduces proliferation, migration, and invasion in HCC cells, while increasing apoptosis. Conversely, HULC overexpression promotes tumor growth, metastasis, and the maintenance of the mesenchymal phenotype (Du et al., 2012). Furthermore, HULC has been reported to contribute to angiogenesis-related tumor development via the miR-107/E2F1/SPHK1 axis (Lu et al., 2016).

At the molecular level, HULC largely regulates HCC progression through a ceRNA mechanism. HULC sponges miRNAs such as miR-200a-3p, miR-372, miR-9, and miR-186, increasing the expression of oncogenic targets such as ZEB1, PRKACB, RXRA, and HMGA2, thereby promoting EMT, lipid metabolism, and tumor growth (Wang et al., 2010; Cui et al., 2015; Zhang et al., 2019).

In addition, HULC contributes to metabolic reprogramming, increasing the activity of glycolysis enzymes such as LDHA and PKM2, and supporting the Warburg effect. It also acts as a scaffold for YB-1, facilitating the translation of oncogenic mRNAs and enhancing autophagy-mediated tumor progression by activating the PI3K/AKT/mTOR pathway (Xiong et al., 2017).

Clinically, high HULC levels are associated with advanced disease, intrahepatic metastasis, recurrence, and poor survival. Its detectability in serum makes HULC a potential diagnostic and

prognostic biomarker for HCC; furthermore, its low mutation rate makes it a targetable therapeutic candidate (Liu et al., 2019; Huang.,2025).

H19

H19 is a well-established oncogenic lncRNA that plays a critical role in HCC (Kallen et al., 2013). Approximately 2.3 kb in length, H19 is highly expressed during embryonic development but silenced in most adult tissues; however, its expression is reactivated in chronic liver disease and HCC. H19 belongs to the imprinted H19/IGF2 locus on chromosome 11p15.5, where hypomethylation leads to aberrant H19 upregulation and IGF2 activation in HCC Li et al., 2015).

The oncogenic activity of H19 is primarily mediated through its function as a ceRNA. By sponging multiple microRNAs, H19 derepresses key oncogenic pathways involved in proliferation, migration, invasion, and EMT (Kallen et al., 2013; Zhang et al., 2013). Major regulatory axes include miR-15b/CDC42/PAK1, miR-326/TWIST1, miR-200b-3p/ZEB1, and miR-22–associated EMT in HBV-related HCC (Li et al., 2015). Exosomal H19 further promotes tumor progression by activating LIMK1 and MAPK1 signaling and contributes to immune evasion via regulation of immune checkpoint molecules such as PD-L1 and CD155 (Zhou et al., 2015).

H19 also exerts oncogenic effects through its embedded microRNA miR-675, forming the H19/miR-675/PPARα axis, which

modulates Akt/mTOR signaling, cellular metabolism, and cell survival (Kallen et al., 2013; Wang et al., 2023). In addition, H19 participates in epigenetic regulation through interactions with chromatin modifiers such as EZH2 and, following NSUN2-mediated m5C modification, promotes MYC accumulation via G3BP1 (Sun et al.,2020).

Clinically, H19 is consistently upregulated in HCC and is associated with aggressive tumor features, poor prognosis, and increased recurrence (Li et al., 2017; Liu et al., 2016). Its expression is enriched in $CD90^{+}$ liver cancer stem cells, contributes to chemoresistance, and suppression of H19 enhances sensitivity to sorafenib (Zhou et al., 2015). Due to its tumor-specific expression and multifaceted oncogenic roles, H19 is considered a promising biomarker and therapeutic target in HCC (Kallen et al., 2013; Zhang et al., 2013).

NEAT1 (Nuclear Enriched Abundant Transcript 1)

NEAT1 is a lncRNA (>200 nt) that plays a central role in the formation of nuclear paraspeckles. It exists in two major isoforms: the polyadenylated NEAT1_1 (3.7 kb) and the long NEAT1_2 (~23 kb), which is essential for paraspeckle biogenesis (Clemson et al., 2009; Fox et al., 2018). Although predominantly nuclear, NEAT1 can also localize to the cytoplasm, where it exerts regulatory functions through ceRNA mechanisms.

NEAT1 is frequently overexpressed in HCC, and elevated expression levels are consistently associated with aggressive tumor behavior, poor prognosis, and reduced patient survival (Guo et al., 2015). Functionally, NEAT1 promotes HCC progression mainly by acting as a miRNA sponge. Key oncogenic axes include miR-22-3p/AKT2, which activates PI3K/AKT signaling, and miR-129-5p/PEG3, which contributes to fibrogenic and tumor-promoting processes (Zhou et al., 2022).

NEAT1 further enhances EMT and metastasis via activation of the Wnt/β-catenin/ZEB1 pathway (Expósito-Villén et al., 2018). Its expression is strongly induced under hypoxic conditions through direct transcriptional activation by HIF-1α, linking NEAT1 to metabolic adaptation and poor clinical outcome in HCC. NEAT1 also participates in metabolic reprogramming by modulating pathways such as mTORC1 and AMPK/SREBP-1, thereby influencing glycolysis and lipid metabolism (Choudhry et al., 2015).

In the context of therapy resistance, NEAT1 contributes to sorafenib and Lenvatinib resistance by promoting autophagy and AKT signaling, and it induces radio resistance through mitophagy-related mechanisms (Li et al., 2020). Additionally, NEAT1 supports cancer stem cell (CSC) maintenance by sustaining CD44 expression and regulating self-renewal pathways such as PKA/Hippo signaling (Koyama et al., 2020). NEAT1 is a multifunctional oncogenic lncRNA whose overexpression correlates with poor prognosis in

HCC. Circulating NEAT1 is under investigation as a diagnostic biomarker, and therapeutic strategies targeting NEAT1 represent promising avenues for overcoming metabolic reprogramming and treatment resistance in HCC (Statello et al., 2021).

Conclusion

This section highlights the central role of lncRNAs in disease biology within the context of the etiological diversity and significant molecular heterogeneity of HCC. The spectrum of genetic mutations, epigenetic reprogramming, transcriptomic subclasses, and the dynamic effects of the tumor microenvironment form a multi-layered network that determines the clinical course, prognosis, and treatment response of HCC (Villanueva, 2019; Cancer Genome Atlas Research Network, 2017; Llovet et al., 2021). Within this multidimensional structure, lncRNAs stand out not only as an output of heterogeneity but also as active regulators in shaping the HCC phenotype through a variety of functions dependent on transcriptional regulation, post-transcriptional control, ceRNA interactions, chromatin modification, protein stability, and subcellular localization (Quinn and Chang, 2016; Kopp and Mendell, 2018; Ulitsky and Bartel, 2013; Statello et al., 2021). Indeed, intensively studied examples such as HOTAIR, MALAT1, HULC, H19, and NEAT1 demonstrate that they contribute to the maintenance of key cancer features such as proliferation, invasion, EMT, metabolic reprogramming, hypoxia adaptation, immune evasion, and treatment resistance through lncRNA-centric

mechanisms. Therefore, the functional characterization and holistic evaluation of lncRNAs at the multi-omics level are crucial; more precise identification of subtypes in HCC provides a strong foundation for the development of reliable biomarkers and the design of next-generation therapeutic strategies specifically targeting resistance mechanisms.

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Abbreviations

World Health Organisation (WHO)

hepatocellular carcinoma (HCC)

metabolic dysfunction-associated fatty liver disease (MASLD)

reactive oxygen species (ROS)

CTNNB1 (β-catenin)

Long non-coding RNAs (lncRNAs)

enhancer RNAs (eRNAs)

Intergenic lncRNAs (lincRNAs)

Enhancer RNAs (eRNAs)

ceRNA (miRNA Sponge)

miRNA response elements (MREs)

Polycomb Repressive Complex 2 (PRC2)

lysine-specific demethylase 1 (LSD1)

DNA methyltransferases (DNMTs)

HOTAIR (HOX transcript antisense intergenic RNA)

MALAT1 (Metastasis-Associated Lung Adenocarcinoma Transcript 1)

peripheral blood mononuclear cells (PBMCs)

HULC (Highly Upregulated in Liver Cancer)

NEAT1 (Nuclear Enriched Abundant Transcript 1)

epithelial–mesenchymal transition (EMT)

cancer stem cell (CSC)

DNA methyl transferases (DNMTs)

BÖLÜM 3

Mitochondrial Dysfunction, Diseases and Current Treatment Methods

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Introduction

Mitochondria, commonly designated as the cellular "powerhouses," represent semi-autonomous organelles equipped with their own genome and dedicated machinery for replication, transcription, and translation, facilitating adenosine triphosphate synthesis via oxidative phosphorylation (Li et al., 2024). The functionality of this sophisticated apparatus is indispensable for cellular energy homeostasis, contingent upon a fine-tuned balance of mitochondrial biogenesis, dynamics, and quality control pathways (Meiliana et al., 2021). As dynamic organelles pivotal to cellular energy demands, mitochondria exhibit dysfunction that is progressively acknowledged as a cornerstone in diverse pathologies (Gupta, 2016). Extending beyond ATP production, mitochondria engage in essential processes such as signaling, apoptosis, and calcium homeostasis, thereby affirming their paramount importance to cellular viability and operation (Gupta, 2016; Khan et al., 2022).

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Accordingly, impairments in mitochondrial performance precipitate an extensive array of metabolic, neurodegenerative, and immunological disorders (Valenti & Vacca, 2022). Enhanced recognition of mitochondria as integrative hubs across cellular functions has elucidated their dysfunction as a fundamental driver of numerous diseases, spanning primary and secondary mitochondrial disorders alongside multifactorial conditions featuring prominent mitochondrial deficits (Giulivi et al., 2023). Notably, oxidative phosphorylation defects constitute among the most frequent congenital disorders (Zheng et al., 2023). This malfunction routinely provokes excessive reactive oxygen species generation, culminating in macromolecular damage, energy provision deficits, and perturbed synthesis of vital biomolecules (Sorrentino et al., 2017). Elucidating the complex regulatory mechanisms of mitochondrial dysfunction is thus imperative for formulating potent therapeutic modalities to alleviate sequelae and reestablish cellular equilibrium (Yao et al., 2024). Accumulated mitochondrial DNA mutations emerge as a principal instigator of these aberrations, exerting substantial influence on aging and concomitant pathologies (Somasundaram et al., 2024). Moreover, waning mitochondrial proficiency engenders chronic inflammation, thereby amplifying degenerative processes linked to aging (Sinha et al., 2024). This review seeks to delineate the multifaceted dimensions of mitochondrial dysfunction, scrutinizing its mechanistic underpinnings, disease ramifications, and contemporary therapeutic paradigms (Tomita et al., 2021). In particular, it will expound upon the intrinsic associations between perturbations in mitochondrial quality—including morphology, dynamics, function, and metabolism—and pathologies such as ischemia-hypoxia, inflammatory diseases, viral infections, metabolic derangements, degenerative disorders, and neoplasms (Hong et al., 2024).

Mitochondrial Structure and Function

To comprehend the intricate relationship between mitochondrial impairment and disease pathogenesis, a thorough understanding of the organelle's complex architecture and diverse functional roles is essential. Mitochondria possess a double-membrane structure that compartmentalizes various enzymatic reactions essential for ATP synthesis—facilitated by an electrochemical potential gradient across the inner membrane—and plays a critical role in cellular metabolism (Guo et al., 2023; San-Millán, 2023). Far from being merely static power generators, these vital organelles represent complex entities involved in a harmonious regulation of structural integrity and dynamic processes, such as membrane potential homeostasis, mitophagy, and inter-organelle contacts (Yao et al., 2024). They are pivotal in regulating cellular homeostasis through their integral roles in bioenergetics, metabolic precursor synthesis, calcium regulation, and reactive oxygen species production (Harrington et al., 2023). Indeed, their ancient incorporation into eukaryotic cells for energy production was fundamental for species survival and evolution. Beyond ATP production via oxidative phosphorylation, mitochondria participate critically in other metabolic pathways—such as the citric acid cycle (krebs cycle) and β-oxidation of fatty acids—while also regulating reactive oxygen species production (Figure 1) (Dutta et al., 2024; Jarmuszkiewicz et al., 2023; Li et al., 2024). Moreover, they contribute significantly to cellular homeostasis by modulating intracellular calcium concentrations, serving as a critical buffer for these ions in conjunction with the endoplasmic reticulum and other cellular structures (Song et al., 2024). This intricate interplay between mitochondria and other organelles, particularly in calcium signaling, is crucial for processes like cell proliferation, differentiation, and apoptosis, thereby underscoring their multifaceted role beyond energy generation (Yao et al., 2024). Even

minor alterations in mitochondrial integrity can profoundly impact cellular homeostasis, extending beyond ATP synthesis to affect detoxification of reactive oxygen species, calcium regulation, and apoptotic signaling (Nedel et al., 2024).

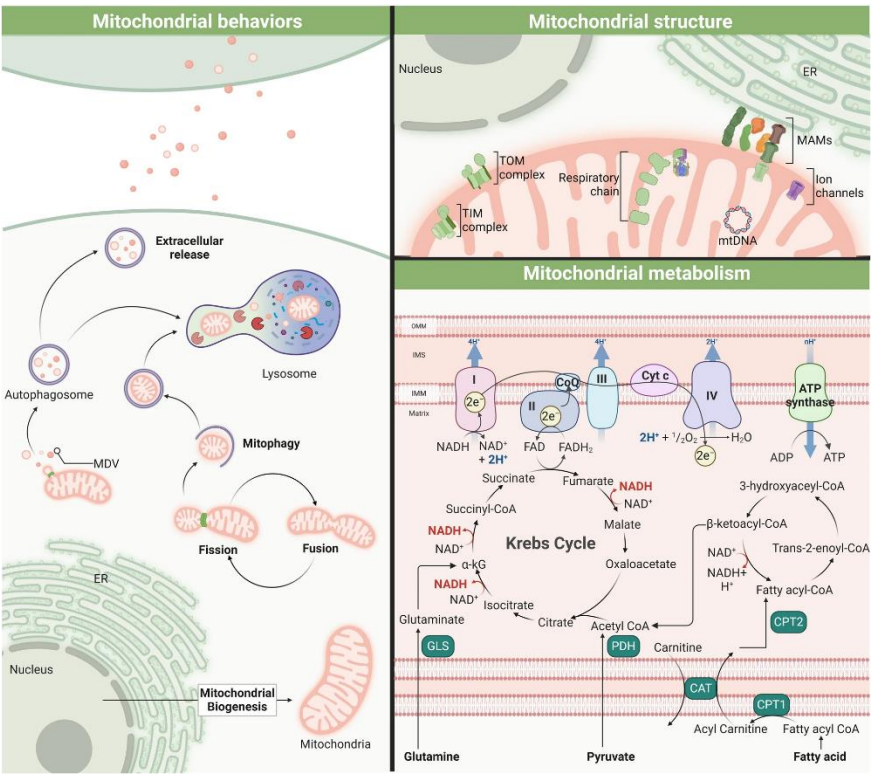

Figure 1. Schematic overview of mitochondrial structure and functions. Mitochondria maintain cellular homeostasis through quality control, energy production, and metabolic regulation. Energy production centers on the respiratory chain, fueled by the Krebs cycle and electron transport (Yao et al., 2024).

As crucial hubs for metabolic integration, these organelles synthesize important intermediates—such as those for amino acid biosynthesis and phospholipid synthesis, both essential for membrane biogenesis and cellular structural integrity—and function as dynamic, interconnected networks that communicate with other

cellular compartments via biomembrane systems (Chen et al., 2023; Koteeswaran & Keerthana, 2022; Zhong et al., 2022).

Mitochondria are postulated to have arisen from endosymbiotic proteobacteria via an ancient symbiotic process, wherein an ancestral eukaryotic host cell engulfed a free-living aerobic bacterium, thereby establishing a mutually advantageous partnership that profoundly influenced cellular evolution. This endosymbiosis bestowed a significant evolutionary benefit upon eukaryotic cells by enabling efficient aerobic respiration, which utilizes oxygen for oxidative phosphorylation to yield substantially greater ATP quantities than anaerobic fermentation alone—a development pivotal to the advent and diversification of complex multicellular organisms (Chen et al., 2020). This primordial origin elucidates their distinctive double-membrane configuration—the outer membrane derived from the host cell's phagocytic vesicle and the inner membrane preserving the bacterium's plasma membrane, thereby facilitating cristae formation indispensable for electron transport chain complexes—as well as the retention of their autonomous circular DNA, a compact, intron-deficient, bacterial-like genome that encodes 13 essential respiratory chain proteins alongside tRNAs and rRNAs, with the preponderance of mitochondrial proteins now being nuclear-encoded and imported (Valera-Alberni & Cantó, 2018; Yin & Cadenas, 2015).

Mitochondrial Dysfunction

Causes of Mitochondrial Dysfunction

Mitochondrial dysfunction represents a widespread cellular aberration marked by diminished ATP synthesis, heightened reactive oxygen species production, and perturbed calcium homeostasis, collectively undermining cellular bioenergetics and signaling pathways (Tan & Finkel, 2020). This condition occupies a pivotal position in the pathogenesis of myriad disorders, ranging from

neurodegenerative to cardiovascular ailments (Cai et al., 2024). Precipitating factors encompass genetic alterations in mitochondrial or nuclear DNA, environmental toxicants, nutritional deficits, and persistent oxidative stress (González-Chapa et al., 2023). Aberrant mitochondrial fission and fusion frequently culminate in the retention of impaired mitochondria evading mitophagy, thereby liberating proinflammatory elements such as mtDNA and ROS into the cytosol, which instigate innate immune activation (Picca et al., 2020; Somasundaram et al., 2024). Characteristic features include morphological alterations like organelle swelling and cristae disruption, coupled with biochemical anomalies such as defective oxidative phosphorylation and calcium handling (Ryter et al., 2018). Such deficits curtail energy generation, augment susceptibility to apoptosis (Somasundaram et al., 2024), and sustain oxidative stress via disequilibrium between ROS and antioxidants, resulting in lipid peroxidation, protein carbonylation, and genomic injury (Cui et al., 2024). Within neuroinflammatory contexts, this engenders a self-reinforcing cycle of mitochondrial impairment, inflammation, and neuronal degeneration, particularly evident in Alzheimer's, Parkinson's, and Huntington's diseases, wherein mtDNA vulnerability—stemming from its adjacency to ROS-generating sites and absence of histones—intensifies the pathology (Blagov et al., 2024; Qin et al., 2024; Yusoff & Khair, 2024).

Molecular Mechanisms of Dysfunction

Key molecular mechanisms underlying mitochondrial dysfunction involve aberrations in mitochondrial DNA (mtDNA), which, due to its proximity to the electron transport chain, is highly susceptible to reactive oxygen species-induced damage, leading to a high mutation rate and clonal expansion of damaged mtDNA (Cai et al., 2016). This accumulation of mutations can subsequently impair the electron transport chain, further increasing reactive oxygen species production and exacerbating cellular nitroxidative stress

(Akbar et al., 2016). Furthermore, excessive free radicals generated by compromised mitochondrial energy production can damage the inner mitochondrial membrane, impairing metabolism and leading to neuronal dysfunction (Qin et al., 2024). This energetic crisis, alongside heightened oxidative stress, culminates in the breakdown of neural networks and neurotransmission disruption, manifesting in symptoms characteristic of neurodegenerative disorders (Aran & Singh, 2023; Micucci et al., 2025). Such mitochondrial dysfunction is particularly pronounced in neurodegenerative diseases like Parkinson's and Alzheimer's, where neuronal mitochondria exhibit accelerated accumulation of molecular damage and impaired bioenergetic capacity (Raefsky & Mattson, 2016). This initial mitochondrial inhibition precipitates an energetic crisis and elevated oxidative stress, culminating in the accumulation of oxidative DNA damage and mutations, and ultimately leading to neuronal cell death (Souza-Pinto et al., 2008). Mitochondrial dynamics, including fusion and fission, play a critical role in maintaining mitochondrial health, and their dysregulation is frequently observed in neurodegenerative pathologies, contributing to fragmented mitochondria and impaired bioenergetic function (Alqahtani et al., 2023). Notably, mitochondrial DNA is exceptionally vulnerable to oxidative damage because it lacks protective histones and is situated in close proximity to the electron transport chain, a primary source of reactive oxygen species within the cell (Rekatsina et al., 2019; Spencer et al., 2010). This inherent susceptibility results in a higher mutation rate in mitochondrial DNA compared to nuclear DNA, contributing significantly to mitochondrial dysfunction and disease progression (Cha et al., 2015). Such damage to mitochondrial DNA leads to instability of genes and proteins, disrupting the electron transport chain, and consequently increasing reactive oxygen species production, which ultimately compromises bioenergetic functions and promotes cell damage (Liu et al., 2017). This vulnerability of mtDNA to oxidative damage establishes a feedback loop where

increased reactive oxygen species production further impairs mitochondrial function and genomic integrity (Cha et al., 2015). In addition to DNA damage, mitochondrial dysfunction also involves alterations to mitochondrial lipids and proteins, which can lead to structural instability and impaired enzymatic activity (Bhat et al., 2015). These molecular changes collectively contribute to an overall decline in mitochondrial performance, severely impacting cellular energy homeostasis and signaling pathways crucial for neuronal survival (Noushad et al., 2019).

Impact on Cellular Processes

The intricate interplay between mitochondrial dysfunction and cellular processes extends to profound effects on cellular aging, where the accumulation of DNA damage, particularly in non-replicating cells such as neurons, becomes detrimental, leading to senescence or apoptosis and subsequent tissue function loss (Pignataro et al., 2017). This age-related decline is further exacerbated by the mitochondrial impairment observed in various neurodegenerative diseases, where the accumulation of damaged or mutated mitochondrial DNA due to increased reactive oxygen species production contributes to altered mitochondrial mass and impaired neuronal function (Akbar et al., 2016). Moreover, aberrations in mitochondrial DNA copy number, a marker for mitochondrial abundance, have been correlated with diminished physical performance and cognitive decline, underscoring the broader systemic impact of mitochondrial health beyond direct energy production (Asghar et al., 2022). This highlights how compromised mitochondrial integrity can trigger a cascading failure across multiple cellular systems, impacting overall physiological resilience and contributing to the etiology of age-related diseases (Cha et al., 2015). Such cellular damage extends beyond neurons, impacting a variety of tissues and organs, thereby contributing to the systemic manifestations observed in metabolic syndrome,

cardiomyopathies, and renal failure (Roede & Jones, 2010). These widespread systemic effects emphasize the critical role of mitochondrial integrity in maintaining overall physiological homeostasis and preventing the onset and progression of a diverse range of chronic diseases (Somasundaram et al., 2024; Wallace, 2013).

Mitochondrial Diseases

Classification of Mitochondrial Disorders

Mitochondrial diseases encompass a heterogeneous group of disorders arising from inherited or acquired mitochondrial dysfunction, often affecting high-energy demand organs like the brain, heart, and skeletal muscle (Xu et al., 2025). These disorders manifest with a wide spectrum of clinical presentations, reflecting the pervasive role of mitochondria in cellular metabolism and the varying tissue-specific energetic requirements (Camara et al., 2009). The genetic basis of mitochondrial diseases is complex, involving mutations in either mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) encoding mitochondrial proteins, which can lead to diverse biochemical defects in the electron transport chain or other mitochondrial functions (Camara et al., 2009). Mitochondrial disorders are broadly categorized based on their genetic origin, distinguishing between those caused by mutations in mitochondrial DNA (mtDNA) and those stemming from defects in nuclear DNA (nDNA) genes that encode mitochondrial proteins (Wallace et al., 2010). Mitochondrial DNA mutations are exclusively maternally inherited, whereas nuclear DNA mutations can follow autosomal dominant, autosomal recessive, or X-linked patterns of inheritance (Casanova et al., 2023). These distinct inheritance patterns underscore the complexity of diagnosing mitochondrial diseases and highlight the need for comprehensive genetic screening (Yao et al., 2024). Furthermore, mitochondrial disorders can also be classified

into primary and secondary forms, with primary mitochondrial diseases being inherited genetic disorders directly affecting mitochondrial structure or function, whereas secondary mitochondrial dysfunction can arise from various non-genetic factors or be a consequence of other underlying conditions (Akl et al., 2022). Primary mitochondrial diseases are characterized by direct genetic defects in mitochondrial or nuclear DNA impacting proteins involved in oxidative phosphorylation, with over 1,000 identified mutations exhibiting diverse inheritance patterns (Abdul-Fatah et al., 2022). This extensive genetic heterogeneity makes diagnosis challenging, as symptoms can vary widely in severity and presentation, affecting nearly any organ system and manifesting at any age (McCormick et al., 2018; Meiliana et al., 2019). Conversely, secondary mitochondrial dysfunction, though not directly inherited, can significantly contribute to a range of chronic diseases and age-related decline (Uittenbogaard & Chiaramello, 2014). These secondary forms can be triggered by external stressors such as toxins, infections, or ischemia, which induce oxidative stress and subsequently impair mitochondrial function (Khajuria et al., 2021). For instance, conditions like diabetes, neurodegenerative disorders, and cardiovascular diseases often exhibit secondary mitochondrial dysfunction, highlighting its pervasive involvement in the pathophysiology of numerous common illnesses (Wallace & Chalkia, 2013). While primary mitochondrial disorders are definitively linked to genetic alterations impacting oxidative phosphorylation, the landscape of mitochondrial diseases has expanded to include "secondary mitochondrial dysfunctions" resulting from mutations in nuclear genes unrelated to OXPHOS assembly or activity (Gouiza et al., 2024; Roberta & Falchetti, 2018). This expanded understanding recognizes that a wide array of genetic defects, even those not directly affecting the core machinery of oxidative phosphorylation, can indirectly compromise

mitochondrial health and lead to clinical manifestations resembling primary mitochondrial disorders (Schlieben & Prokisch, 2020).

Genetic Basis of Mitochondrial Diseases

The complex genetic architecture of primary mitochondrial diseases involves mutations in either mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) that disrupt the intricate balance of mitochondrial biogenesis and function (Schlieben & Prokisch, 2020). Nuclear DNA-encoded mitochondrial proteins are crucial for various mitochondrial processes, including oxidative phosphorylation, DNA replication, and protein synthesis, with mutations in over 228 nuclear genes now linked to human mitochondrial disorders (Koopman et al., 2012). These genes encode structural proteins of the oxidative phosphorylation system, as well as factors critical for mitochondrial DNA replication and maintenance, transcription, translation, protein import, and lipid environment, among others (You et al., 2024). Given this intricate genetic landscape, the phenotypic expression of primary mitochondrial disorders is remarkably diverse, ranging from mild myopathies to severe multi-systemic failures, often complicated by factors such as heteroplasmy and modifier genes (Macken et al., 2021; Scheibye-Knudsen et al., 2014). Moreover, the specific combination of mutant to wild-type mitochondrial DNA, known as heteroplasmy, significantly influences disease severity and tissue distribution, further contributing to the clinical variability observed in affected individuals (Schwenzer, 2013). Furthermore, the impact of mitochondrial DNA background on nuclear gene expression indicates a complex interplay that influences disease progression not only through mitochondrial function but also via crosstalk with nuclear DNA (Braganza et al., 2019). This intricate genetic landscape, involving mutations in more than 350 genes across both mitochondrial and nuclear genomes, underscores the significant heterogeneity in the clinical presentation and inheritance patterns of

primary mitochondrial diseases (McCormick et al., 2018). Specifically, while pathogenic variants in the multi-copy mitochondrial DNA (mtDNA) are exclusively maternally inherited and their clinical impact is often modulated by heteroplasmy levels, nuclear DNA (nDNA) mutations, of which over 250 genes have been implicated in mitochondrial disease, can exhibit diverse Mendelian inheritance patterns (Figure 2) (Theunissen et al., 2018; Wen et al., 2025). These nuclear DNA mutations, which are the most common genetic cause of primary mitochondrial disease, are typically inherited in an autosomal recessive manner, particularly in childhood-onset cases, although autosomal dominant and X-linked patterns are also observed (McCormick et al., 2018).

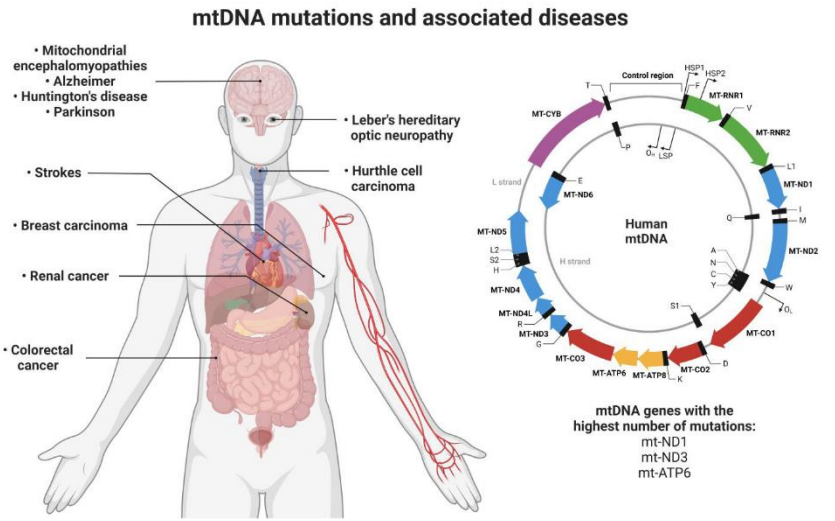

Figure 2. Illustration of the mitochondrial genome, highlighting genes with the highest mutation rates. This figure identifies mtDNA regions prone to mutations that often link to specific disease phenotypes (Faria et al., 2023).

The genetic landscape is further complicated by the fact that over 400 genes, both mitochondrial and nuclear in origin, have been identified as causative for mitochondriopathies, with more than half of the 190 known primary disease genes leading to defective assembly of OXPHOS complexes or the mitoribosome (Hock et al., 2020; Schlieben & Prokisch, 2020). Remarkably, over 1136 human genes are currently associated with the mitochondrial proteome, emphasizing the extensive genetic contributions to mitochondrial function and dysfunction (Grigalionienė et al., 2023). The sheer number of genes involved highlights the challenge in pinpointing specific genetic defects and developing targeted therapies for mitochondrial diseases, necessitating advanced genomic sequencing approaches (Grigalionienė et al., 2023).

Clinical Manifestations of Mitochondrial Diseases

Given the ubiquitous nature of mitochondria across various cell types, mitochondrial diseases can affect virtually any organ system and present with a broad spectrum of clinical symptoms, often involving tissues with high energy demands such as the brain, muscle, heart, and liver (Tinker et al., 2021). This broad systemic involvement contributes to the highly variable and often complex clinical presentations, ranging from isolated organ dysfunction to severe multi-systemic disorders (Ambrose et al., 2024; Chow et al., 2016). The diversity in symptoms necessitates a comprehensive diagnostic approach, often involving a combination of clinical evaluation, biochemical assays, and advanced genetic testing (Ambrose et al., 2024). The age of onset also varies widely, from infancy to adulthood, further complicating diagnosis and treatment strategies (Nogueira et al., 2024). For instance, early-onset mitochondrial disorders frequently present with severe, progressive multi-systemic involvement, whereas adult-onset forms may manifest with more subtle or isolated neurological, muscular, or endocrine symptoms (Aldossary et al., 2022). Neurological

manifestations, such as encephalopathy, seizures, and developmental regression, are particularly common due to the brain's substantial energy requirements, while cardiomyopathy and renal insufficiency are frequently observed in pediatric patients (Kirby & Thorburn, 2008; Luciani et al., 2021). Beyond these, symptoms can also include optic atrophy, hearing loss, diabetes mellitus, and gastrointestinal dysmotility, reflecting the mitochondria's critical role in maintaining cellular homeostasis across diverse physiological systems (Lopriore et al., 2022).

Diagnosis of Mitochondrial Diseases

Given the heterogeneous clinical presentations and complex genetic underpinnings, diagnosing mitochondrial diseases poses a significant challenge, necessitating a multifaceted approach that integrates clinical, biochemical, imaging, and genetic investigations. The absence of a definitive gold standard for diagnosis further complicates this process, often leading to prolonged diagnostic odysseys for affected individuals (Kerr et al., 2020).

The inherent heterogeneity and multi-systemic nature of mitochondrial diseases present significant diagnostic challenges, often requiring a multifaceted approach that integrates clinical assessment, biochemical analyses, and advanced genetic testing (Khan et al., 2020). The variability in clinical presentation, coupled with the involvement of both nuclear and mitochondrial DNA mutations, often leads to diagnostic delays and misdiagnoses (Rius et al., 2023). This complexity is further exacerbated by the fact that many common symptoms, such as migraine or diabetes, are not specific to mitochondrial disorders and can overlap with other systemic or neurological conditions (Schlieben & Prokisch, 2020). Consequently, the non-specific nature of many presenting symptoms, such as unexplained combinations of neuromuscular and non-neuromuscular issues, progressive deterioration, and fluctuating

symptomatology, frequently complicates the diagnostic process (Dimmock & Lawlor, 2016). Therefore, a comprehensive evaluation encompassing clinical symptoms, imaging reports, biochemical markers, and muscle biopsy findings, alongside genetic mutations, is often essential for an accurate diagnosis (Zhao et al., 2024). Molecular genetic testing, specifically, is becoming increasingly crucial for definitive diagnosis, often revealing pathogenic mutations in either mitochondrial or nuclear genomes (Clarke et al., 2013). Muscle biopsies, revealing abnormalities such as ragged red fibers or cytochrome c oxidase deficiency, coupled with biochemical testing of blood, urine, and cerebrospinal fluid for metabolites like lactate and pyruvate, remain vital for confirming the functional impact of suspected mitochondrial dysfunction (Balogiannis, 2019). However, despite advancements in diagnostic techniques, considerable diagnostic delays persist, underscoring the need for improved recognition of canonical features and early flagging of at-risk patients (Tinker et al., 2025).

Current Treatment Approaches for Mitochondrial Diseases

Symptomatic Treatments

These treatments often involve pharmacological agents aimed at managing specific symptoms such as seizures, pain, or cardiac arrhythmias, alongside nutritional interventions to optimize energy metabolism and mitigate secondary deficiencies (El-Hattab et al., 2017). For instance, antiepileptic drugs are prescribed to control seizures, while therapies targeting cardiac dysfunction, such as beta-blockers or ACE inhibitors, are employed when cardiomyopathy is present (Koňaříková et al., 2020). Furthermore, physical therapy and occupational therapy are crucial for addressing motor delays and hypotonia, enhancing mobility, and improving overall functional independence (El-Hattab et al., 2017). Moreover, specialized diets, such as the ketogenic diet, are utilized in specific

conditions like pyruvate dehydrogenase deficiency, though careful consideration of contraindications, such as fatty acid oxidation disorders, is paramount (Akl et al., 2022). Similarly, a metabolic approach involving endurance training has shown promise for patients with mtDNA mutations and deletions, while a ketogenic diet may benefit those with complex I deficiency (Koňaříková et al., 2020). In addition to dietary and lifestyle adjustments, emerging therapies are being developed to target mitochondrial dysfunction more directly, with many currently undergoing rigorous evaluation in clinical trials (Yao et al., 2024). These interventions aim to improve mitochondrial biogenesis, reduce oxidative stress, and enhance mitochondrial respiratory chain function (Hirano et al., 2018). Pharmacological agents designed to modulate mitochondrial processes, such as pyruvate or N-acetyl cysteine, have shown promise in preclinical models by supporting metabolic pathways or reducing oxidative damage (Akl et al., 2022). However, due to the high heterogeneity of mitochondrial diseases, therapeutic trials have historically been largely ineffective and inadequately designed, necessitating rigorous, double-blinded, placebo-controlled studies to validate the efficacy of these novel approaches (Schon et al., 2010).

Mitochondrial-Targeted Therapies

This category of therapies represents a more precise approach, focusing on directly improving mitochondrial function or mitigating specific mitochondrial defects at a molecular level, moving beyond generalized supportive care. These strategies often include mitochondrial-targeted drugs designed to enhance bioavailability at the site of action, lipophilic antioxidants, or transcriptional modulators aiming to restore mitochondrial homeostasis (Figure 3) (Falk, 2010). Examples of such non-tailored therapeutic targets include strategies to activate mitochondrial biogenesis, regulate mitophagy and mitochondrial dynamics, bypass biochemical defects, and even implement mitochondrial

replacement therapy or modulate hypoxia responses (Hirano et al., 2018). For instance, compounds like bezafibrate and resveratrol are being investigated for their ability to stimulate mitochondrial biogenesis, thereby increasing the cellular content of healthy mitochondria (Davison & Rahman, 2017).

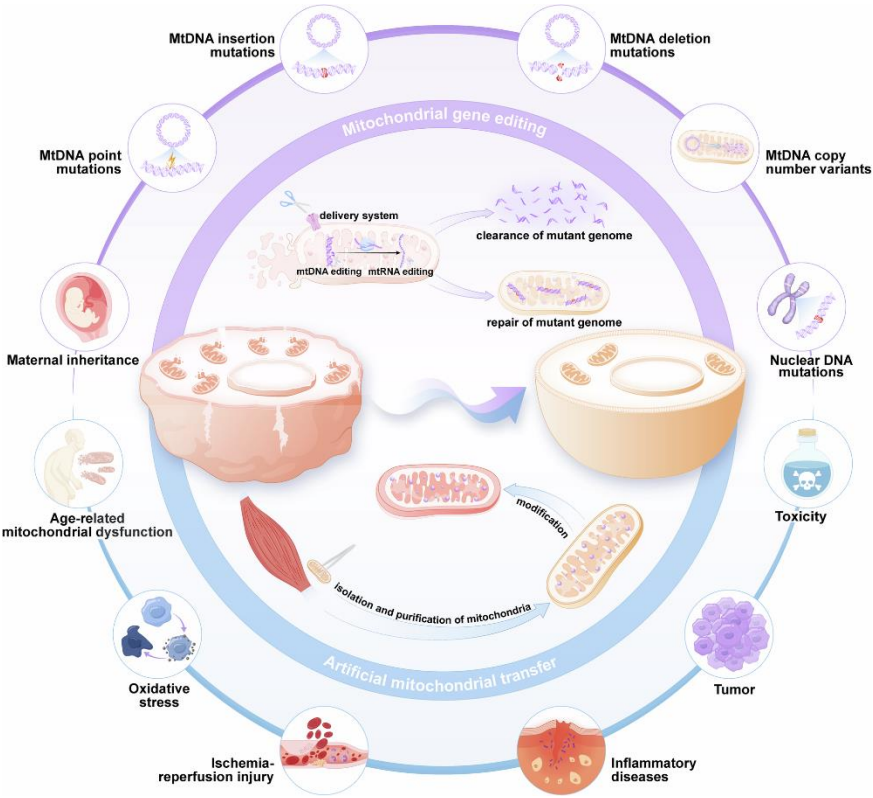

Figure 3. Schematic of engineered mitochondria for disease therapy. Mitochondrial gene editing corrects abnormalities by eliminating or repairing mutant mtDNA, while artificial mitochondrial transfer restores function by introducing healthy mitochondria (Li et al., 2025).

Moreover, pharmacological chaperones, which assist in the correct folding of mitochondrial proteins, are another area of active research, particularly for diseases caused by misfolded enzymes. Another promising avenue involves the development of agents that can bypass defective electron transport chain components, such as the use of NDI1 or AOX to provide alternative electron transfer pathways (Viscomi, 2016). Furthermore, gene therapy approaches, particularly those utilizing adeno-associated viruses, are being explored to deliver wild-type copies of mutated mitochondrial genes or other therapeutic genes to affected tissues, addressing the genetic root of many mitochondrial diseases (Viscomi, 2016).

Gene Therapy Approaches

Gene therapy holds significant promise for treating mitochondrial disorders by directly addressing the underlying genetic defects (Soldatov et al., 2022). This approach involves introducing functional genes into cells to compensate for defective ones, potentially correcting the molecular pathology at its source (El-Hattab et al., 2017). This can be achieved through gene replacement strategies, where a healthy copy of a gene is delivered to replace a mutated one, or through gene editing technologies that aim to correct specific genetic errors within the mitochondrial or nuclear DNA (Garone & Viscomi, 2018). One method, allotopic gene therapy, involves introducing nuclear-encoded versions of mitochondrial proteins into the nucleus, which are then imported into the mitochondria to replace defective ones (Rahman & Rahman, 2018). Additionally, emerging research suggests that gene therapy can modulate mitochondrial biogenesis through the delivery of master regulators like peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (Hanaford et al., 2022). For instance, compounds such as PXL770 and metformin activate AMPK, subsequently boosting PGC-1α expression and enhancing mitochondrial DNA copy number (Yao et al., 2024). Beyond

pharmacological activation, direct gene therapy approaches are also being explored to deliver PGC-1α or other transcriptional activators to enhance mitochondrial biogenesis, which can be critical for preserving tissue homeostasis (Zhao et al., 2024). Moreover, other gene therapy strategies focus on using viral vectors, such as adeno-associated viruses, to deliver therapeutic genes, offering a mechanism for either replacing mutated genes or expressing proteins that can mitigate mitochondrial dysfunction (Hanaford et al., 2022). These viral vectors, particularly adeno-associated viruses, are favored due to their ability to remain episomal, minimizing the risk of insertional mutagenesis (Viscomi, 2016). Furthermore, advancements in gene editing technologies like CRISPR/Cas9 offer the potential for precise correction of mitochondrial DNA mutations, which represents a highly targeted approach to treating these complex disorders.

Nutritional and Lifestyle Interventions

Dietary supplements, including cofactors of oxidative phosphorylation, are often employed to support ATP production and mitigate symptoms in mitochondrial disorders. For instance, supplementation with coenzyme Q10, riboflavin, or thiamine can be beneficial in certain mitochondrial disease subtypes by supporting electron transport chain function and metabolic pathways (Khajuria et al., 2021). Although traditionally these nutritional interventions were the primary treatment, their efficacy has often lacked robust evidence from controlled clinical trials (Nightingale et al., 2016). However, recent research indicates that certain compounds, such as thiamine, lipoic acid, and dichloracetate, can activate pyruvate dehydrogenase, thereby reducing lactate accumulation by converting pyruvate into acetyl-CoA (Soldatov et al., 2022). This conversion helps to optimize substrate utilization for mitochondrial respiration, potentially improving energy metabolism in affected individuals. Moreover, therapies targeting the activation of AMP-activated

protein kinase and Sirtuin-1, such as AICAR and resveratrol, respectively, promote mitochondrial biogenesis through the upregulation of PGC-1α (Bakare et al., 2021; El-Hattab et al., 2017; Yao et al., 2024). In particular, nicotinamide riboside has been shown to augment nicotinamide adenine dinucleotide biosynthesis, which is crucial for various mitochondrial processes, including ATP production and DNA repair (Yao et al., 2024).

Emerging Therapeutic Strategies

Mitochondrial Transplantation

This innovative approach involves isolating healthy mitochondria from a donor and transplanting them into a recipient's cells, aiming to replace or augment dysfunctional mitochondria (Sinha et al., 2024). While still largely experimental, mitochondrial transplantation holds promise for conditions where existing mitochondria are severely compromised or in situations requiring rapid restoration of cellular energy production. This technique has shown preclinical efficacy in models of ischemia-reperfusion injury and other mitochondrial-related pathologies by directly providing functional organelles to ATP-deficient cells. Specifically, this involves the isolation of mitochondria from healthy tissues, followed by their delivery into recipient cells, often via direct injection or intravenous infusion, where they integrate and contribute to cellular bioenergetics (Yao et al., 2024). However, challenges remain in optimizing mitochondrial delivery, ensuring their long-term survival, and demonstrating consistent therapeutic benefits in complex mitochondrial diseases (Tinker et al., 2021). Further investigation is needed to address potential immunological responses to transplanted mitochondria and to establish standardized protocols for donor selection and mitochondrial preparation to ensure optimal efficacy and safety (Liu et al., 2023).

CRISPR-Cas9 Gene Editing

CRISPR-Cas9 gene editing represents a revolutionary advancement in the pursuit of precise genomic interventions, offering the potential to correct specific mutations implicated in mitochondrial diseases (Soldatov et al., 2022). This technology allows for targeted modification of nuclear DNA, which encodes the majority of mitochondrial proteins, to restore protein function or mitigate pathogenic effects. Moreover, recent developments indicate that mitochondrially targeted Cas9 systems can directly cleave and edit mitochondrial DNA, offering a more direct approach for correcting mtDNA mutations (Koňaříková et al., 2020). However, such mitochondrial manipulation techniques raise significant ethical considerations that warrant careful deliberation and regulatory oversight (Fasullo & Catalá, 2021). In addition to these considerations, the efficiency and specificity of gene editing within the mitochondria, especially given the heteroplasmic nature of many mtDNA mutations, remain significant technical hurdles that necessitate further research (Song et al., 2024).

Small Molecule Modulators

Pharmacological interventions, particularly small molecule modulators, are emerging as a promising avenue to directly influence mitochondrial function, addressing specific pathways that become dysregulated in mitochondrial diseases. These molecules can target various aspects of mitochondrial physiology, such as oxidative phosphorylation, mitochondrial dynamics (fission and fusion), and quality control mechanisms like mitophagy, thereby offering diverse therapeutic strategies (Hong et al., 2024; Khajuria et al., 2021). For instance, compounds that enhance mitochondrial biogenesis, optimize ATP production, or mitigate oxidative stress are actively being investigated for their therapeutic potential (Yao et al., 2024). For example, novel small molecules are being developed to

selectively inhibit specific mitochondrial protein targets that contribute to disease pathogenesis, such as those involved in aberrant mitochondrial fission or dysfunctional calcium handling (Schon et al., 2010). Furthermore, other small molecule drugs are designed to modulate key processes governing mitochondrial quality, including morphology and dynamics, function and metabolism, and protein expression and regulation (Hong et al., 2024). This includes compounds that enhance the activity of mitochondrial respiratory chain complexes or those that improve mitochondrial calcium homeostasis, both critical for maintaining cellular energy balance and preventing excitotoxicity. Additionally, drugs targeting mitochondrial fission, such as Mdivi-1, P110, and dynasore, aim to prevent excessive fragmentation by inhibiting DRP1 assembly and GTPase activity, offering protective effects against oxidative stress-induced mitochondrial damage (Hong et al., 2024).

Conclusion

Throughout this review, we have charted a significant evolution in scientific understanding: the recasting of the mitochondrion from a cellular "powerhouse" to a semi-autonomous, central signaling hub. These dynamic organelles are now recognized as indispensable for the fine-tuned balance of cellular energy homeostasis, metabolism, and overall viability. Consequently, mitochondrial dysfunction is no longer viewed as a niche concern but as a cornerstone mechanism in a vast spectrum of human pathologies. Its impact extends from rare primary genetic disorders to common, multifactorial conditions like neurodegeneration, where mitochondrial deficits are a prominent feature. This foundational understanding sets the stage for a critical analysis of the field's current clinical landscape and its promising future trajectory toward precision medicine.

At the core of mitochondrial pathology lies a self-reinforcing cycle of damage that serves as a unifying principle across numerous diseases. This vicious cycle often begins with an underlying genetic vulnerability, particularly within the mitochondrial DNA (mtDNA). Lacking the protective histones of nuclear DNA and situated in close proximity to the electron transport chain—a primary source of reactive oxygen species (ROS)—mtDNA is uniquely susceptible to oxidative injury. Initial damage triggers a cascade of increased ROS production, which in turn inflicts further harm upon mtDNA, as well as mitochondrial lipids and proteins. This destructive feedback loop culminates in a profound energetic crisis, perturbed cellular signaling (most notably calcium homeostasis), and a state of sustained, chronic inflammation. This inflammatory state is driven, in part, by the liberation of proinflammatory elements like mtDNA and ROS into the cytosol, which instigates innate immune activation. Ultimately, this cycle drives cellular senescence, apoptosis, and the progressive functional decline characteristic of diseases affecting high-energy-demand tissues, such as the brain. The profound molecular entrenchment of this cycle underscores the immense challenge of clinical intervention.

The diagnosis and treatment of mitochondrial diseases currently represent a formidable clinical challenge. The diagnostic process is frequently a prolonged "odyssey" for patients, complicated by immense genetic heterogeneity—with pathogenic mutations identified in more than 350 genes across both the nuclear and mitochondrial genomes—and non-specific, multi-systemic clinical presentations that can mimic other conditions. This complexity makes definitive and timely diagnosis exceptionally difficult.

Consequently, the current therapeutic paradigm remains largely supportive rather than curative. Treatments are centered on symptomatic management, such as the use of antiepileptic drugs for

seizures, and nutritional interventions like coenzyme Q10 to support metabolic pathways. While these strategies, along with lifestyle adjustments, can offer palliative relief, they do not address the root genetic or molecular defects. Critically, many of these interventions lack robust, validating evidence from rigorous, double-blinded, placebo-controlled clinical trials. This highlights an urgent and unmet need for more effective, mechanism-based strategies that can disrupt the underlying pathophysiology. It is this need that provides the impetus for the profound promise of emerging, targeted therapeutic approaches.

The field of mitochondrial medicine is at an inflection point, poised to transition from an era of generalized supportive care to one defined by precision and molecular engineering. A new wave of emerging therapeutic strategies is moving beyond symptom management to directly address the underlying defects that cause disease. By aiming to correct these faults at the genetic and functional levels, these novel approaches offer the potential for transformative, rather than merely palliative, outcomes, heralding a new vision for the future of treatment.

Gene-based therapies represent a paradigm shift with the potential to offer definitive cures for mitochondrial diseases. One primary strategy is gene replacement, including allotopic gene therapy, which introduces nuclear-encoded versions of mitochondrial proteins that are subsequently imported into the organelle to compensate for mutated genes. This is often achieved using viral vectors, such as adeno-associated viruses (AAV), to deliver the therapeutic genetic payload. An even more revolutionary approach is CRISPR-Cas9 gene editing. This technology offers the unprecedented ability to precisely correct pathogenic mutations themselves, whether they reside in the nuclear DNA encoding mitochondrial proteins or, using newly developed mitochondrially targeted systems, directly within the mtDNA itself. While these

technologies hold immense promise, significant technical hurdles and important ethical considerations remain to be addressed before they can be widely implemented in the clinic.

Alongside genetic interventions, several other advanced strategies are being developed to restore mitochondrial function at the organellar and cellular levels. Two of the most promising modalities include:

Mitochondrial Transplantation: This innovative and direct approach involves isolating healthy, functional mitochondria from donor tissue and introducing them into affected cells, often accomplished via direct injection or intravenous infusion. The goal of this technique is to augment or entirely replace the dysfunctional mitochondrial population, thereby restoring cellular bioenergetics and function. While still largely experimental, it holds significant potential for conditions involving severe energy deficits.

Small Molecule Modulators: This pharmacological strategy utilizes highly specific chemical compounds to modulate mitochondrial physiology. These agents can be designed to achieve a variety of therapeutic effects, such as enhancing mitochondrial biogenesis (e.g., resveratrol), altering mitochondrial dynamics by inhibiting excessive fission (e.g., Mdivi-1), or even bypassing specific defects within the electron transport chain to restore ATP production, such as the therapeutic use of NDI1 or AOX to provide alternative electron transfer pathways.

These advanced strategies reflect a sophisticated, multi-pronged effort to re-establish cellular equilibrium by targeting the organelle from multiple angles.

A deep understanding of mitochondrial dysfunction has become fundamental to deciphering the pathogenesis of a remarkably wide array of human ailments, from the natural process of aging and the progression of neurodegenerative disorders to the

complexities of metabolic syndrome. The future of the field is characterized by a determined and accelerating shift away from merely managing symptoms and toward engineering cures. As the scientific community continues to overcome the technical and ethical challenges associated with next-generation therapies, targeting the mitochondrion—the central hub of cellular fate—is set to become an increasingly powerful and central strategy in the practice of 21st-century medicine.

BÖLÜM 4

EPIGENETIC MODIFICATIONS AND THEIR EFFETCS ON OUR LIVES

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Assoc. Prof. Dr. Funda KARABAĞ [2]

Introduction

Epigenetics refers to heritable and reprogrammable biological processes that regulate gene functions without changing the nucleotide sequence of DNA (Martin et al. 2007)). These processes are recognized as a mediator of the interaction between the genome and environmental factors and lifestyle (Doğan et al. 2016). Epigenetic mechanisms also direct the differentiation of cells by playing an active role especially during embryonic development. These mechanisms include DNA methylation reorganization of chromatin structure, histone modifications (e.g. acetylation and methylation of arginine and lysine residues) and the functions of non-coding RNAs [Berry et al. 2019 Studies have shown that DNA methylation is particularly effective on stress genes, while non-

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coding RNAs regulate the repression or enhancement of gene expression. The stress experienced by the mother during pregnancy can cause the baby to show stress reactions after birth. This is due to increased methylation, expecially in the glucocorticoid receptor (NR3C1) gene, making the child more sensitive to stress hormones. Another situation in which histone modification and DNA methylation have an effect is when the mother, who is starved or malnourished during pregnancy, becomes susceptible to appetite control and insulin deficiency, which may lead to the silencing of some genes in the baby. Not only genetics but also environmental factors and epigenetic mechanisms play a role in the emergence of human behavior. İn some people, unexplaned, panic attacks, fears, phobias, and chronic pain may be caused by unresolved pain in the family history, and epigenetic processes that change gene expression play an active role in the emergence of these symptoms (Can et al. 2016)

HISTORY OF EPIGENETICS

Epigenetics had long history before it became an important sub-branch of genetics. In 1940, developmental biologist Cornard Waddington coind the term ‘epigenetics. Cornard used to term epigenetics to describe the interactions in the process of genotype to phenotype transformation. In 1975, Arthur Riggs and Robin Holliday proposed that DNA methylation can permanently alter gene expression. This idea raised the possibility that epigenetic changes could be inherited through cell divisions. By the 1990s epigenetics was redefined as a branch of science that studies mechanisms of inherited gene regulation. Studies conducted during this period

revealed that processes sucs as X chromosome inactivation and genomic imprinting, especially in mammals regulate gene expression whithout a change in DNA sequence.

Table 1 Important milestones in the history of epigenetics

1940s	**Conrad Waddington's definition of epigenetics as environment-gene interactions that induce developmental phenotypes**
1975	**Holliday and Pugh's determination of DNA methylation**
1988	**X chromosome inactivation and DNA methylation**
1990s	**Imprinted genes, allelic expression, and DNA methylation**
1995	**Histone modifications and chromatin structure**
2000s	**Small non-coding RNAs**
2005	**Epigenome mapping**

(Can and Aslan, 2016)

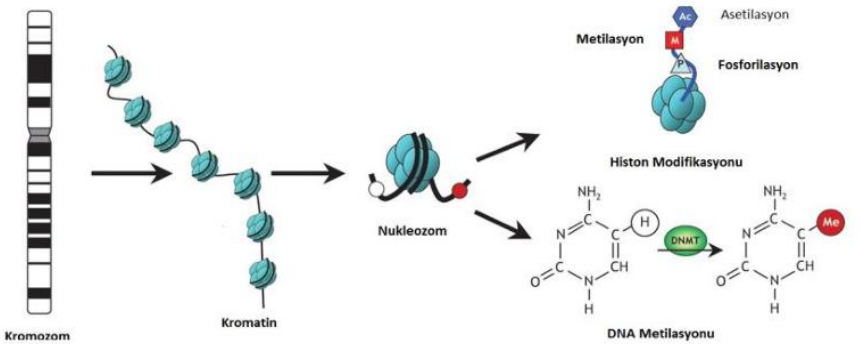
In this context, epigenetics has become the study of heritable but reversible changes in gene expression through mechanisms such as DNA methylations, modifications of histone proteins and chromatin reorganization. In 1997, the clone sheep Dolly demonstrated that differentiated cells can be reprogrammed. This also showed that epigenetics memory could be reversed. The 2003 completion of the Human Genome Project raised the question of how genetic information is organized and read, which increased interest in epigenetics. In 2008, the 'Epigenome Atlas' project was launched to create epigenetic profiles of various human cells. In recent years, it has been intensively studied in the treatment of different diseases, neuroscience and psychology. The impact of epigenetics shows that it will affect not only the individual but also the next generations. This approach has shown that genetic regulation is much more dynamic than previously thought. In particular, the idea that

environmental factors can affect these epigenetic mechanisms and change the phenotype of the individual and even the gene expression profiles of subsequent generations has increased the importance of epigenetics in biology and medicine (Can et al. 2016).

EPIGENETIC MECHANISMS

Epigenetics, which regulates gene expression without changing the DNA sequence, enables cells to gain different functions and can also have an effect on behavior and psychological states. We can list these mechanisms as follows; DNA methylation/Histone modifications/Non-coding RNAs

Figure 1 Epigenetic mechanisms

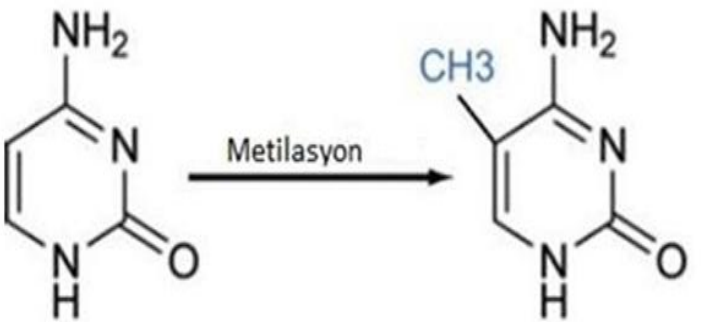


(Yokuş, 2013)

DNA Methylation

DNA methylation is an epigenetic modification that occurs when a methyl group (-CH3) is added to certain bases in the DNA molecule (usually cytosine). The Figure methyl group is usually added to the 5th carbon atom on CpG dinucleotides (i.e. the regions where a cytosine base is followed by a guanine base)

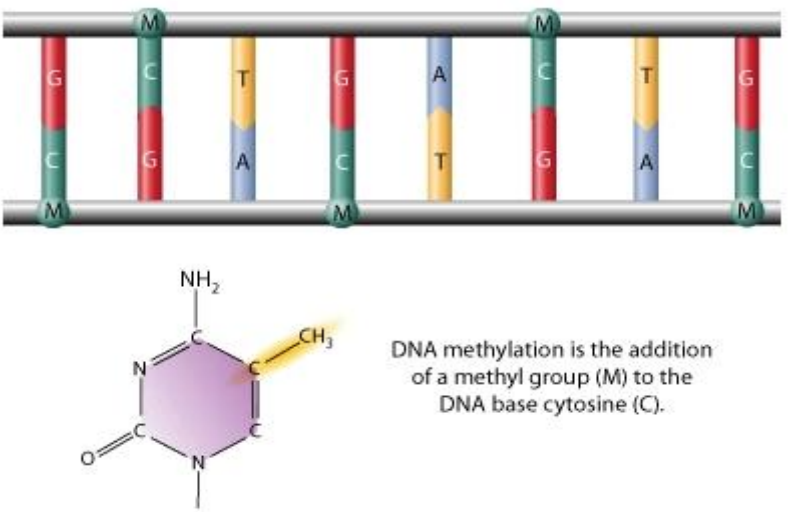
Figure:2 Methyl group (CH3) addition to DNA base



(Doğan et al. 2016)

This addition is done by the enzyme DNA methyltransferase (DNMT). Among the DNA methyltransferase enzymes, DNMT1 retains the existing methyl groups and transfers them to the new strand in the same way during cell division. DNMT3A and DNMT3B, on the other hand, create different patterns by adding new methyl groups.

Figure:3 Methylated cytosine and DNA methylation



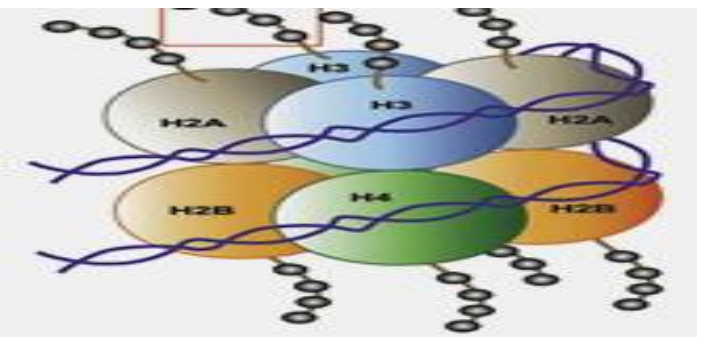
(Can and Aslan, 2016)

Normally, promoter regions are unmethylated active gene regions, but when methylated, these gene regions are silenced (Memik, 2025). When DNA regions are methylated, the binding of transcription factors is inhibited. In addition, methyl-CpG binding proteins (e.g. MeCP2) bind to methylated regions, attract complexes that deacetylate histones and compress chromatin, and silence gene expression by increasing heterochromatin formation (Yokuş, 2013).

Histone Modifications

In eukaryotic cells, DNA is packaged together with five different histone proteins to form structures called nucleosomes. The N-terminal tails of histone proteins extend outward from the nucleosome and undergo various posttranslational modifications such as acetylation, methylation, phosphorylation form (Murray et al. 2014) All of these modifications change the electrostatic charge of histones, affecting whether the chromatin structure becomes tight or loose. So if the chromatin structure is loose (euchromatin) genes can be active; if is tight (heterochromatin) genes are repressed. As a result, it is thought to change the accessibility of the protein complex that regulates transcription to promoter regions on DNA (Güneş et al. 2013).

Figure:4 Histone proteins, each containing two copies



(Can and Aslan, 2016)

Histone Acetylation

Histone acetylation occurs when the enzyme histone acetyltransferase (HAT) adds an acetyl group (-COCH3) to the amino acid lysine. Lysine is a positively charged amino acid and forms a tight bond with the negative charge of DNA. The addition of an acetyl group to the amino acid lysine neutralizes this positive charge and reduces the DNA-histone interaction. Thus, chromatin relaxes, transcription factors can gain easier access to DNA and increase gene expression (Nestler, 2013). This event is reversible and the removal of acetyl groups added to histone proteins by the enzyme histone deacetylase (HDAC) leads to histone deacetylation. Acetylation and deacetylation enzymes are involved in important functions in the genome such as cell cycle, proliferation, differentiation, cell death, DNA replication and mitosis (Boskovic et al. 2018).

Histone Methylation

Histone methylation is the addition of one or two methyl groups to the amino acids lysine or arginine by the histone methyltransferase enzyme (HMT). Unlike acetylation, histone methylation does not involve a charge change, but it can alter protein interactions (İzmirli, 2013). Depending on the binding site, it can activate transcription or cause gene repression. The removal of the methyl group from the amino acids lysine or arginine by the histone demethylase (HDM) enzyme is histone demethylation (Berry et al. 2019).

Histone phosphorylation

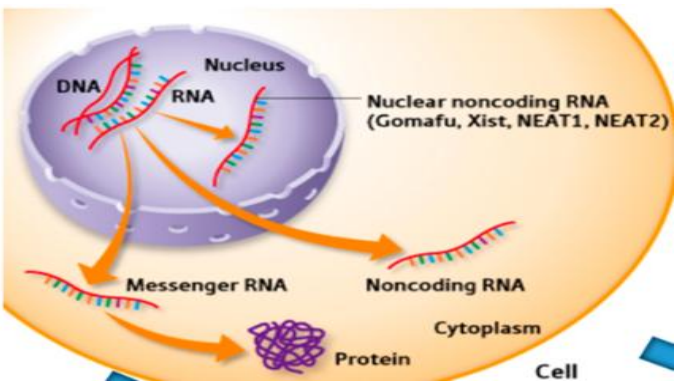
It is the addition of a phosphate group (-PO4) to serine, threonine or tyrosine amino acids. The addition of the phosphate group to amino acids is carried out by protein kinases (PK), while the removal of the phosphate group from amino acids is carried out by protein

phosphatases (Izmirli, 2013). The addition of a phosphate group to histone proteins increases the negative charge of histones, which leads to loosening of the chromatin structure. Phosphorylation plays an important role in processes such as cell cycle, DNA damage response and apoptosis. These modifications can work alone or in combination to act as epigenetic markers. As a result, the readability of the genome or the transcriptional status can be determined (Yokuş, 2013).

Non-coding RNA

Non-coding RNAs (ncRNAs) are types of RNAs that do not code for proteins but are involved in gene expression and many other epigenetic processes. Non-coding RNAs suppress the translation process or increase the degradation of target mRNAs, thereby increasing cell they can play a decisive role on life span and survival (Bodur et al. 2010). Therefore, at the levels of expression observed Changes, potential biomarkers is defined as and considered among the targets for diagnosis,prognosis or treatment (Seven, 2024).

Figure:5 Non-coding RNA mechanism



Can and Aslan 2016

Non-coding RNAs have also assumed critical roles in the regulation of cellular functions. Studies have shown that small RNA classes such as siRNA, piRNA and microRNA are also effective in gene regulation in addition to classical non-coding RNAs with basic functions such as tRNA and rRNA (Murray et. al, 2014). Examples belonging to the long non-coding RNA (incRNA) group include molecules such as Xist and HOTAIR, which are known to play a role in the epigenetic control of gene expression (Can et al. 2016).

Briefly, non-coding RNA types and their effects on epigenetic mechanisms;

Micro RNA (miRNA): Binds to the target mRNA and enables its degradation, represses translation, or controls the production of epigenetic regulators by targeting epigenetic enzymes such as DNA methyltransferase (DNMT) (Saydam et al. 2010).

Small Interfering RNA (siRNA): Suppresses target mRNA by cutting it. It may be involved in the initiation of other mechanisms such as DNA methylation and histone modifications (Güngör et al. 2015).

Long non-coding RNA (IncRNA): It can regulate gene expression in multiple ways: It can both increase and repress transcription (Güngör et al. 2015).

It enables the reorganization of chromatin structure by binding to DNA, histones and proteins. Xist inactivates the X chromosome in female mammals, while HOTAIR acts on gene silencing by directing histone methyltransferases.

Some Examples of Epigenetic Mechanism in Our Lives

One example that supports epigenetic regulation is a study of two genetically identical mice. In the experiment, differences emerged as a result of the expression of the agoti gene. One of the mice has a normal black and brown coat, while the other has a yellow coat. Over time, observations showed that the yellow-coated mouse gained more weight and fat than the other mouse tissue and have a higher risk of developing diseases such as diabetes and cancer (Can and Aslan, 2014). Looking at the genetic mechanisms underlying this difference, it was found that the gene called 'agoti', which determines hair color, was active in yellow-haired mice and repressed in black and brown-haired mice. Therefore, it is understood that the differences between these mice, whose genetic structures are exactly the same, are only due to changes at the epigenetic level. This suggests that environmental factors and epigenetic mechanisms can be highly influential on the phenotypes and disease susceptibility of individual In the later stages of the study, researchers added Bisphenol A (BPA), a common pollutant, to the diet of mother mice to test the effect of environmental factors-particularly the mother's diet during pregnancy on gene expression in the offspring (Can and Aslan, 2014).

Figure: 6 Twin mice

(Can and Aslan 2016)

BPA is a chemical commonly found in plastic bottles, food packaging and baby bottles As a result of the experiment, a significant increase in the number of yellow and obese individuals was observed in the offspring of mother mice exposed to BPA. This suggests that BPA is an agoti gene by disrupting its epigenetic control, leading to overactivity of the gene. In particular, decreased DNA methylation levels in the promoter region of the gene resulted in the gene continuing to function even in a state where it should normally be silenced. Following these findings, some nutritional supplements were added to the diet of pregnant mice to reduce the harmful effects of BPA: One group received vitamin B9 (folic acid). The other group was given genistin, a natural compound derived

from soybeans. The proportion of yellow-colored and obese mice decreased significantly, while the proportion of healthy offspring with black-brown hair increased significantly. This suggests that the mother's nutrition during pregnancy can directly influence the functioning of the offspring's genes, i.e. their epigenetic profile.

The mechanism underlying this change is DNA methylation. Folic acid is a compound that provides methyl groups. When folic acid was added to the mother's diet, methyl groups were added to the agoti gene in the offspring mice, leading to repression of gene expression. In brown mice, because the agoti gene was methylated, the gene was silenced, resulting in mice with normal coat color and low risk of disease. In yellow-haired mice, the agoti gene was not methylated and the gene continued to function. This resulted in mice with yellow hair and a predisposition to obesity. As can be seen from this research result, although the genetic structure is fixed, environmental influences especially the mother's dietary habits and chemical exposures during pregnancy have changed the genetic expression of the offspring through epigenetic means (Can and Aslan, 2016).

Another epigenetic example; conditions such as anxiety, phobia, anger, addiction, the source of health problems experienced by an individual are not in the person's own life history. Most of the time, they can be hidden in the unresolved traumas of previous generations. Epigenetically, this transmission begins when stress,

especially during pregnancy, affects the baby's physiology and gene expression through the placenta. For example, if a person has an unexplained fear, recurring relational problems or certain health problems, this may be an echo of the traumas experienced by their ancestors, if there is no cause in their own life. Epigenetic experiments with mice for example, the development of a similar condition in the descendants of mice conditioned with the smell of fear showed the scientific aspect of the experiments (Ulucan, 2022).

One of the most striking and instructive examples of epigenetics is the famine in the Netherlands during World War II and the epigenetic effects of this event. In late 1944, the people in the Netherlands under Nazi siege were exposed to a great food shortage. This period is called 'Hongerwinter' (Hunger winter). Daily calorie intake was reduced to 500-800 calories, and pregnant women and infants were particularly severely affected. The children of women whose pregnancies coincide with a period of starvation look genetically normal at birth, but later in life: (Murray et. al, 2014)

• Obesity

• Type 2 diabetes

• Cardiovascular diseases

• Metabolic Syndrome

• Schizophrenia and depression become more susceptible.

Hypomethylation (inadequate methylation) has been observed in these individuals especially in the IGF2 (Insulin-like growth factor 2) gene.

The IGF2 gene plays an important role in embryonic development and cell division. If the methylation level is low, this gene can be overactive, leading to unbalanced growth, fat storage and insulin imbalances. This is not only the case in individuals who have been exposed to starvation, but also in their children, who are predisposed to similar diseases. It is thought that epigenetic changes on the germ line (egg/sperm) may be responsible for its transmission to future generations (Ulucan 2022).

When it comes to the effect of environment on genetics, it is also worth mentioning identical twins. Identical twins (monozygotic twins) develop from the same zygote and therefore have 100% identical genetic sequences. However, they may be exposed to different environmental conditions throughout life. One may grow up in a more stressful environment, while the other may grow up in a less stressful environment with a healthier diet. These different environmental conditions affect epigenetic mechanisms. For example, the genetic profiles of identical twins at the age of 3 years and at the age of 50 years were compared and the results:

The 3-year-old twins had almost identical DNA methylation patterns. However, twins who reach the age of 50 have been found to have very different epigenetic differences. These differences

occurred between histone modifications and DNA methylation patterns and caused some genes to be turned on in one sibling and off in the other. in the past few years. These changes have manifested themselves in behavioral, disease susceptibility and metabolic conditions. For example, one twin may be more introverted and the other more extroverted, one may have cancer and the other a neurological disorder, and there may be differences in obesity, insulin resistance and cholesterol levels. Based on these examples, it can be said that genetic structure alone does not determine the fate of an individual. Epigenetics is like a dynamic layer of regulation written over the genetic material and is shaped by environmental interactions. Having the same genes does not mean having the same diseases or the same psychological profiles (Ulucan 2022).

CONCLUSION

As a result of all these studies, we have learned how epigenetic mechanisms direct the genetic expression of individuals through environmental factors. Epigenetic processes, which decide when and at what level genes work without any change in the DNA sequence, are realized by mechanisms that operate at the molecular level such as DNA methylation, histone modifications and non-coding RNAs (Sun et al. 2021). Twin studies reveal how epigenetic differentiation can occur despite genetic identity. Differences in epigenetic patterns with age in identical twins show that individual life experiences, diet, stress levels and environmental exposures can directly affect gene expression. In mouse experiments, it has been shown that the

mother's diet during pregnancy alters phenotypic traits of the offspring, such as susceptibility to obesity and hair color, through epigenetic pathways. The example of the Dutch Hunger Winter showed that extreme nutrient deprivation during pregnancy leaves epigenetic traces that affect not only the exposed individuals but also their subsequent generations (Murray et. al, 2014). These examples, where epigenetic changes are shaped by environmental factors, clearly show that genetic structure alone does not determine biological destiny. Lifestyle factors such as nutrition, exposure to toxic substances, stress levels and physical activity can have permanent and sometimes intergenerational effects on the epigenome. In conclusion, epigenetics provides a powerful scientific framework that explains how an individual's life experiences are reflected at the biological level. This system, which reorganizes the way genetic information is read with environmental inputs, is one of the most important tools for modern medicine to address not only genetic-based diseases, but also behavioral ones, It also constitutes an important model in approaches to mental and metabolic conditions. In the future, epigenetics will be used more effectively in the fields of early diagnosis, preventive medicine and individualized treatment, and environmental factors will be taken into account more in the fight against diseases.

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A Chronobiological Perspective on the Role of Royal Jelly in Maintaining Metabolic Homeostasis

BÖLÜM 5

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Gizem SÖNMEZ OSKAY[2]

1. Introduction

A discipline known as "chronobiology," which has long been affirmed to represent an intrinsic property of life itself, has also been concerned with clinically interesting issues such as sleeping disorders. A key conceptual achievement in this body of research has been the recognition of the existence of the negative transcriptional feedback loop, in which the bound form of the CLOCK/BMAL1 protein complex to E-box elements has defined the molecular mechanism of biological clocks. Thus, the term "chrononutrition" was first used in 2005 in a Japanese textbook of nutrition compiled by Oda & et al. in 2005, followed by the publication of the world's first book titled "Chrononutrition" in 2009. Although it has been a short while since the birth of this novel discipline in the realm of nutritional sciences, it has been attracting increasingly more attention, at least in research circles, for its potential in explaining, at least in appearance, the accelerating incidence of obesity and related "metabolic syndromes" despite a general decrease in dietary energy intake (Oda, 2015). The findings have formed the basis of understanding the disruption of biological timing as it relates to the development of modern metabolic diseases.

Biological rhythms represent deeply historical underpinnings, with some of the first recordings by the French scientist de Mairan in the 1700s. He noted that diel leaf movements of plants continued in the absence of sunlight, suggesting this was due to an endogenous internal clock rather than simply a response to the sun itself (Vitaterna & et al., 2020). Even though modern chronobiology began to take shape some 50 years ago with the foundational work of Colin Pittendrigh on fruit flies and Jürgen Aschoff on humans, along with the identification of stages of sleep by Nathaniel Kleitman, these early discoveries presented that biological timing is an evolutionarily conserved trait. Accordingly, evolution has equipped mammals with robust systems to anticipate changes in the environment brought about by the rotation of the Earth (Rutter & et al., 2002). Currently, a "circadian rhythm" can be defined as an innate, entrainable, self-sustaining oscillation of approximately 24 hours that persists under constant conditions. While these rhythms are endogenous, they are synchronized with environmental clues known as Zeitgebers (German for time givers), including light, temperature, and eating patterns (Guan and Lazar, 2021). Light is the main Zeitgeber synchronizing the suprachiasmatic nucleus with the environment, while other cues, such as food intake and stress, provide modulation of the interaction between the SCN and peripheral clocks (Lassi & et al., 2021).

The mammalian circadian clock at the molecular level is mediated by a cell-autonomous transcription-translation feedback loop (TTFL). In more specific detail, the core mechanism consists of the heterodimerization of the positive regulators 'CLOCK' and 'BMAL1' (also known as ARNTL), complex binding to E-box sequences in the promoters of target genes, and driving the expression of negative regulators, Period and Cryptochrome genes (Sahar & Sassone-Corsi, 2012). In this process, PER and CRY proteins are accumulated in the cytoplasm, which then

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translocate into the nucleus to inhibit CLOCK:BMAL1 activity and, thus, repress their own transcription and complete the feedback loop. It takes about 24 hours to proceed with a cycle (Schrader & et al., 2024). A secondary regulatory loop further reinforces the robustness of the clock via nuclear receptors such as the REV-ERBs (for example, NR1D1) and RORs, repressing and activating 'BMAL1' transcription, respectively. These core clock genes are responsible for sustaining rhythmicity and provide regulation to thousands of downstream "clock-controlled genes" implicated in many vital physiological functions, including energy balance and metabolism (Choi & et al., 2021; Harmer & et al., 2001).

The combination of circadian biology with metabolic health has led to the development of chrono-nutrition. It is now clear that most metabolic processes are controlled by the circadian rhythms, while feeding times, in turn, influence the clock. For example, it has been established that the composition of the gut microbiota varies rhythmically during the day, thereby indicating a two-way relationship between the clock and metabolic homeostasis (Lotti & et al., 2023). Studies in animals and in humans have demonstrated that time-restricted feeding is related to beneficial effects on body weight, lipid profile, glucose metabolism, and insulin sensitivity, and these effects support the importance of the timing of eating in metabolic health (Rothschild & et al., 2014). Thus, it is apparent that the ingestion of food in the daytime and at night will result in varied metabolic outcomes (Moran-Ramos & et al., 2016).

In addition to eating patterns, a growing body of literature shows that individual bioactive food constituents can directly modulate the circadian system, validating the novel notion of “chrononutraceuticals” (Franzago & et al., 2023; Hu & et al., 2025). Circadian desynchronization is a fundamental contributor to cases of metabolic dysfunction, mainly hepatic steatosis and NAFLD. In this context, Royal Jelly (RJ) has emerged as a promising chrononutraceutical due to its ability to modulate circadian gene expression and improve metabolic homeostasis. In this respect, in estrogen-deficient models, the application of RJ has been shown to counter liver injury and lipid accumulation through the upregulation of antioxidant enzymes SOD and GSH-Px and the restoration of circadian gene expression of the *Per1* and *Per2* genes (You & et al., 2020). In addition to circadian regulation, RJ protects against diet-induced steatosis due to its ability to modulate the AMPK pathway and inhibit lipogenesis. Moreover, active molecules such as 10-HDA help in promoting healthy aging due to the inhibition of inflammatory cascades and MMPs. In this respect, RJ has been proved to maintain tissue integrity based on the inhibition of MMPs and inflammatory molecules (Oršolić & Jembrek, 2024). Although there is increasing evidence for its metabolic advantages, its potential function in a chrononutrition concept, in which timing combines with circadian rhythm regulation, has yet to be sufficiently investigated.

Although scientific data have increased in recent years to prove the metabolic advantages offered by Royal Jelly, the role that this nutrient might play in the context of chrononutrition, in which circadian rhythms regulate gene expression, has yet to be fully investigated. Under these circumstances, the current book chapter intends to provide a well-organized and broad spectrum of circadian regulation of metabolism within the context of Chrono-Nutrition, especially concerning Royal Jelly as a bioactive molecule having the ability to influence circadian gene expression. In positioning the royal jelly as a chrononutraceutical, this chapter has underscored the centrality of timing in the determination of metabolic health and healthy aging.

2. Circadian Regulation of Metabolism and the Therapeutic Potential of Royal Jelly

Circadian rhythms are observed in various metabolic pathways, such as glucose and fatty acid metabolism, and endocrine secretion, including insulin and various hormones (Duez & Staels, 2010). At the molecular level, the circadian clock controls metabolic functions by coordinating the regulation of neurotransmitters, hormones, amino acids, and lipids (Han & et al., 2022). Most genes involved in glucose and lipid metabolism, particularly the key enzymes of central metabolic pathways, are directly regulated by the circadian clock. Destruction of the central clock

components causes extensive metabolic dysfunctions. For instance, genetic ablation of central clock components has demonstrated that mice bearing mutated Clock genes exhibit reduced feeding rhythmicity, hyperphagia, obesity, defective gluconeogenesis, insulin resistance, and lipid metabolic disturbances (Oike & et al., 2014). Notably, the circadian clock and metabolic interactions are bidirectional, where metabolic information regulates the circadian clock (Kim, 2019). The strength and phase of the interaction depend on endocrine signals and tissue-specific forms and are, on average, self-sustained in a 24-hour cycle corresponding to the turn of the Earth (Kim, 2019). Given this critical interplay between biological timing and metabolic functions, natural compounds capable of modulating these processes have seen considerable interest. Among these natural compounds, RJ stands out as a promising functional food source owing to its complex bioactive properties.

Honey bees produce various products to ensure that their hive is always bustling with activity all year round. Honey bees require pollen, royal jelly, honey, and bee bread to survive. Honey bees also require other products, such as propolis, which they produce from plants, to shield their hive from pathogens. Honey bees' products have been extracted by human beings since ancient times, as they understand that they are good health products. Honey bees' products are also used in traditional medicine, specifically as apitherapy products (Rzetecka & et al., 2024). It aims to improve human health by using all bee products, from honey to bee venom. Apitherapy products and their bioactive components exhibit a wide array of characteristics and functions, such as antioxidant, immunomodulatory, antibacterial, and anticancer properties, indicating their potential for the treatment of various diseases (Gupta & Stangaciu, 2014; Szabat & et al., 2019). In recent years, RJ has emerged as a highly sought-after commercial product in the food, pharmaceutical, and cosmetic industries.

RJ is a creamy, yellowish-white liquid that nurse honey bees produce via their mandibular and hypopharyngeal glands to feed the queen bee and young larvae in the hive. Queen larvae consume royal jelly their whole lives and eventually turn into queen bees. In this way, queen bees are bigger than worker honey bees (about twice as big), have developed reproductive organs, and live longer (approximately 5–6 years, compared to the 35–40 days of nurse worker bees) Khazaei & et al., 2018). Doolittle queen honey bee breeding is a good way to produce abundant RJ. While there are currently no official figures on the royal jelly market, it is clear that China is the world's largest producer and exporter of RJ. Over 60% of the world's royal jelly comes from China, and almost all of the 2.000 metric tons produced annually goes to the Japanese, US, and European markets. Japan, Korea, and Taiwan are among the important countries in terms of both exports and production. As for other RJ producers in the world, most are in Eastern Europe, but some are also reported in Western Europe and, most importantly, in Mexico (Ramadan & Al-Ghamdi, 2012).

RJ is a natural substance made by honey bees that has a lot of promise for use in medicine. The pH of RJ ranges from 3.6 to 4.2. Water constitutes the primary component at 60–70% (w/w), followed by proteins at 9–18% (w/w) and total carbohydrates at 10–16% (w/w) (Ramanathan & 2018). RJ contains numerous beneficial compounds, including major royal jelly proteins, peptides, fatty acids, and more. The variability is mostly influenced by the abundance of flora, which is unique to each geographic region, along with the feeding season (Civelek, 2022). The Major Royal Jelly Proteins (MRJPs1-9) class includes the primary proteins in RJ. They make up around 90% of all the proteins. MRJP1, also known as apalbumin 1, has gotten the most attention in the MRJP family because it is important for the development of honeybee queens and for keeping queen larvae at the front of the colony. MRJP1, which can be either a monomer (mono-MRJP1) or an oligomer (oligo-MRJP1), is the most abundant mildly acidic glycoprotein in royal jelly. It makes up 48% of the water-soluble proteins. The 57 kDa MRJP1 monomer, also known as royalactin, is the primary factor initiating queen differentiation (Li & et al., 2021). A recent study has comprehensively demonstrated the antioxidant, antibacterial, anticancer, hypotensive, hypolipidemic, cell growth-promoting, wound-healing, anti-aging, neuroprotective, anti-inflammatory, and immunomodulatory pharmacological properties of MRJPs and their derived

peptides (Mureşan & et al., 2022). About 80 to 90% of the lipid fraction is made up of free fatty acids. The rest is made up of neutral lipids and sterols. 10-hydroxide-2-decenoic acid (10-H2DA), an unsaturated fatty acid, and 10-hydroxydecanoic acid (10-HDA), its saturated counterpart, are important and unique lipid components of RJ that are mainly responsible for many of the biological processes that happen with RJ (Stojanovic & et al., 2021). 10-HDA has many biological effects, including lowering blood sugar, reducing inflammation, and anti-cancer (Zhi & et al., 2025). RJ also has organic acids, flavonoids, carotenoids, phenolic acids, and minerals. The phenolic compounds and flavonoids in RJ, such as chrysin, acacetin, apigenin, quercetin, hesperidin, and kaempferol, are what give it its antioxidant properties. Flavonoids exhibit strong antioxidant properties and may influence various signaling pathways, including the regulation of the cell cycle, enhancement of apoptotic pathways, and inhibition of cell viability. This indicates that they have anticancer effects (Ayna & Darendelioğlu, 2022). Besides, the main bioactive components of RJ have a variety of biological effects, such as promoting cell growth and wound healing, modulating the immune system, blood pressure, controlling blood vessel dilation, protecting liver and kidney function, protecting nerve cells, controlling sex hormones, and increasing fertility (Huang & et al., 2025). As mentioned in Figure 1, the biological activities of RJ consist of antioxidant, neuroprotective, anti-inflammatory, and anti-diabetic activities, among others, all of which together make RJ a functional food for healthy aging.

Current research supports the promising potential of RJ in the treatment of metabolic diseases (El-Seedi & et al., 2024; Zhi & et al., 2025). Several studies have demonstrated that RJ lowers glucose and cholesterol levels in diabetic rats; its bioactive components, MRJPs and 10-HDA, help reduce insulin resistance. Additionally, it modulates immune responses, boosts the production of anti-inflammatory cytokines, and decreases the secretion of inflammatory substances. Findings from recent reports on the role of RJ for unmasking various associations in metabolic regulation have been summarized in Table 1.

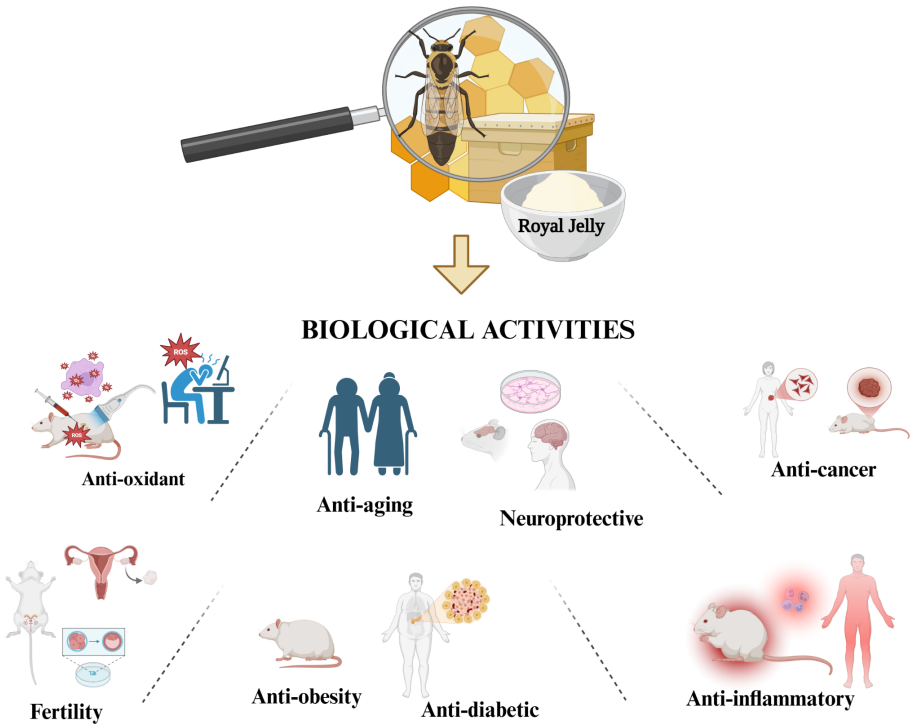

Figure 1. Schematic representation of the broad spectrum of biological activities associated with RJ.

Table 1. Summary of *in vitro and vivo* RJ studies

RJ or bioactive components	***Study type, length, and groups***	***Study design***	***Results***	***References***
10-HDA	48 Sprague–Dawley rats (180–200 g)	3 different doses (30, 60, and 120 mg/kg/day)	Lowering serum lipid levels, reducing liver damage, and decreasing oxidative stress	Zhi & et al., (2025).
RJ	30 male Wistar rats, (6 -week- old, 120 ± 10 g) HFD rodent model	RJ dose: 300 mg/kg	decrease in the release of IL-6 and TNF-α, the phosphorylation of AMPK, SREBP1, and ACC-1 increased, along with higher levels of mRNA and protein for HSL and ATG.	Felemban & et al., (2024)
RJ	50 male Wistar rats, (3-week-old, 50–70 g) HFD rodent model	100 mg/kg/day RJ	decreased IL-1β and CRP levels, and leptin concentrations	Mesri Alamdari & et al., (2024)
RJ	C57BLKS/JIar-+Leprdb/+Leprdb (db/db) (7-week-old)	5% RJ dose	Modifying the epigenome in the small intestine may enhance metabolic health and reduce the expression of genes associated with cancer.	Kobayashi & et al., (2024)
RJ MCFAs	male diabetic homozygous db/db mice NAFLD model	3 different doses (0.2, 1, and 5% RJ)	reduction in the expression of genes linked to fatty acid metabolism, fibrosis, and inflammation in the liver	Kobayashi & et al., (2023)
MRJPs	The male C57BL/6J mice (6-week-old) HFD rodent model	low dose of MRJPs (250 mg/kg/day); high dose of MRJPs (500 mg/kg/day)	The frequency of obesity and insulin resistance reduced.	Zhu & et al., (2022)
MRJPs	*in vitro* HepG2 cells and L02 cells	pre-incubation (24 hr) with MRJPs (0.2, 0.5, and 1.0 g/L)	reducing triglyceride levels in cells	Zhang & et al., (2021)
RJ	Sixty-five female SD rats (180–200 g) OVX rodent model	RJ (150, 300, and 450 mg kg^{-1} day^{-1}, 8 weeks)	The blood lipid profile improved, and both hepatic steatosis and liver damage were reduced.	You & et al., (2020)
RJ	obese/diabetic KK-Ay mice	RJ (10 mg/kg) was administered by oral gavage	suppressed the mRNA expression of glucose-6-phosphatase (G6Pase)	Yoshida & et al., (2017)

The first research presented in Table 1 investigated the anti-hyperlipidemic mechanism of 10-HDA by human network pharmacology, molecular docking analysis, animal experiments, and plasma metabolomics. 10-HDA was shown to significantly reduce blood cholesterol levels, ameliorate hepatic pathological damage, and decrease oxidative stress levels in the experimental rats. Forty-one significant metabolites and ten central targets were crucial in the expression of anti-hyperlipidemic effects, including tumor necrosis factor (TNF), insulin (INS), and epidermal growth factor receptor (EGFR). Twenty-four pathways, such as tryptophan, the citrate cycle, and arachidonic acid metabolism, were recognized as significant factors in the mitigation of hyperlipidemia by 10-HDA (Zhi & et al., 2025).

In the second study presented in Table 1, rats were administered RJ orally at a dose of 300 mg/kg, and minimal increases in body weight, fat weight, BMI, Lee index, abdominal circumference (AC), and fat index (AI) were observed. RJ increased blood glycerol and adiponectin levels while decreasing leptin, IL-6, and TNF-α levels. RJ also inhibited IL-6 and TNF-α production in isolated white adipose tissue (WAT). In the study, when examined at the tissue level, HFD + RJ rats were found to have smaller fat cell size compared to HFD rats. In WAT of HFD rats, RJ increased the levels of HSL and ATG mRNA and protein, as well as the phosphorylation of AMPK, SREBP1, and ACC-1. RJ also elevated PGC-α mRNA levels, decreased PPARγ protein levels, and inhibited the transcription of PPARγ, SREBP1, and C/EBPαβ in the adipose tissue of these rats. The study results highlight that RJ promotes lipolysis and inhibits adipose tissue adipogenesis in an obese rat model, achieving this effect through its AMPK-dependent action (Felemban & et al., 2024).

Another study investigated how RJ, tocotrienol-rich fraction (TRF), and their combinations affected blood sugar levels and inflammation in an obese rat model. The rats were made obese by the administration of the high-fat diet (HFD) and subsequently placed on the calorie-restricted diet (CRD). The study aimed to determine the combined effects of these two compounds via the mechanism of irisin. Fifty obese rats, placed on the HFD, received either RJ, TRF, or the combination of both RJ and TRF, along with the CRD, for eight weeks. At the end of the study period, the rats had the following parameters measured: body weight, fasting blood sugar (FBS), irisin, insulin, C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-1 beta (IL-1β), leptin, adiponectin, and insulin resistance (IR). After eight weeks of treatment, the rats under the administration of the RJ and the combination of RJ+TRF demonstrated significant weight reduction, compared to the rats under the CRD. Conversely, the rats under the administration of TRF alone did not indicate significant changes in weight. Among the rats under the CRD, the administration of the two supplements, RJ and RJ+TRF, showed significant elevation of the irisin level. Besides, TRF alone did not affect irisin levels. Adding RJ, TRF, and their combinations to CRD significantly reduced levels of glucose, inflammation, and leptin. The mediation analysis showed that irisin was the factor that helped regulate RJ's ability to control blood sugar levels. Novel nutrients RJ and TRF may help with obesity-related diseases, according to this study's findings. According to these findings, irisin is the mechanism via which RJ achieves its positive glycemic-regulating effects (Mesri Alamdari & et al., 2024).

In a recent study, the changes caused by RJ in the small intestine epigenome of male db/m and db/db mice were investigated using mRNA sequencing and CUT&Tag techniques. Although body weight didn't change, the treatment with RJ improved insulin sensitivity and lipid metabolism. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses showed enrichment in metabolic activities, cellular components, and molecular functions. In G2M checkpoint genes, RJ increased H3K27me3 and decreased H3K23Ac levels. The genes *Smc2*, *Mcm3*, *Ccnd1*, *Rasal2*, *Mcm6*, and *Mad2l1* are known to play a role in

metabolism and the development of cancer. Changes in the epigenome of small intestine epithelial cells are linked to better metabolic health and a decrease in the expression of genes related to cancer. The data suggest that RJ could be a potential treatment for metabolic disorders (Kobayashi & et al., 2024).

In a study evaluating the therapeutic efficacy of RJ's unique medium-chain fatty acids (MCFAs) in the treatment of non-alcoholic fatty liver disease (NAFLD), three mouse groups were compared: db/m mice given only a standard diet, and db/db mice given only a standard diet along with varying amounts of RJ (0.2, 1, and 5%). RJ reduced inflammation, fibrosis, and fatty acid metabolism-related gene expression, and improved NAFLD activity scores. Reducing the expression of genes linked to inflammation and nutrient absorption transporters, RJ controlled inflammatory responses in the small intestine that were connected to innate immunity. RJ increased seven taxa, including those producing short-chain fatty acids, operational taxonomic units, and the concentration of *Bacteroides.* When RJ was administered, the concentration of MCFAs in the blood and liver increased. The MCFAs consisted of 10-hydroxy-2-decenoic acid, 10-hydroxydecanoic acid, 2-decenedioic acid, and sebacic acid. The MCFAs from RJ decreased saturated fatty acid accumulation in HepG2 cells. The MCFAs also reduced the expression of genes responsible for fatty acid metabolism and fibrosis. The prevention of NAFLD was done through the use of RJ and its associated MCFAs, which reduced dysbiosis and controlled the (Kobayashi & et al., 2023).

Researchers examined the potential of MRJPs, water-soluble proteins found in RJ, to alleviate NAFLD in a rat model. Findings indicate that the administration of 250-500 mg/kg/day of MRJPs may prevent the development of obesity, lipid abnormalities, fatty liver, and insulin resistance. The study further reported that the administration of 500 mg/kg of MRJPs reduced inflammation, oxidative stress, and lipid metabolism, which was mediated by the changes in the metabolic pathways of alpha-linolenic acid, linoleic acid, and arachidonic acid, and the synthesis of unsaturated fatty acids. In addition to that, MRJPs showed antioxidant and anti-inflammatory activities in inhibiting markers of oxidative stress and inflammatory factors. On the whole, the experimental results manifest that MRJPs have the potential to be "multi-component-multi-target-multi-pathway" mediators in the process of NAFLD development and should be an ideal health food for treating NAFLD (Zhu & et al., 2022).

A study evaluated the effect of MRJPs on lipid accumulation and oxidative stress in liver cells. After that, the researchers induced hepatocytes with oleic acid (OA) with a concentration of 1.0 mM for 24 hr to generate stable liver cell models. Results showed that pre-administration (24 hr) with MRJPs (concentrations of 0.2, 0.5, and 1.0 g/L) significantly decreased lipid droplets, the level of triglycerides, and the concentration of alanine and aspartate aminotransferase in the cell culture supernatant of model cells. Pre-administration with MRJPs (concentrations of 0.2, 0.5, and 1.0 g/L) for 24 hr also significantly increased SOD levels and mitochondrial membrane potential compared with the OA group. Results also indicated that MRJPs significantly elevated the expression levels of Silent Information Regulator 2 Associated Protein 3 (SIRT3), mitochondrial SOD2, and cytochrome c oxidase subunit IV proteins in OA-induced HepG2 cells. Results demonstrate that MRJPs reduce lipids at the cellular level; it could be applicable for developing polypeptide drugs and could be a chance for MRJPs to be a choice for NAFLD prevention and treatment (Zhang et al., 2021).

Another study examined the impact of RJ on OVX-induced NAFLD in rats. The rats were given a dosage of 150, 300, and 450 mg/kg/day for a period of eight weeks. The RJ treatment relieved the rats of their levels of anxiety, the blood lipid levels in OVX rats were improved, the

lipid in the liver of the rats was reduced in amount, the damage in the liver was reduced, and the antioxidant activity of the RJ treatment shows it to be a possible enhancer of the antioxidant enzymes in the liver. The qRT-PCR shows the expression of *Per1* and *Per2* in the OVX rats. Based on these findings, hypothesize the possible efficacy of RJ in the treatment of NAFLD as described (You & et al. 2020).

The research also investigated if RJ can assist in reducing the weight and lowering blood sugar in type 2 diabetic patients. The KK-Ay mice utilized in the research were obese and suffered from diabetes. RJ was given by mouth in a dose of 10 mg/kg. The study looked at body weight, blood sugar, and insulin levels. After four weeks of RJ treatment, blood sugar levels went down, but the weight gain was not statistically significant. Insulin resistance didn't change after RJ treatment. The levels of hepatic glucose-6-phosphatase (G6Pase) mRNA went down after RJ. This enzyme is necessary for gluconeogenesis. RJ made the levels of adiponectin receptor-1 (AdipoR1), abdominal fat adiponectin, and phosphorylated AMP-activated protein kinase higher in KK-Ay mice. This made the levels of hepatic G6Pase go down. RJ supplementation did not move Glut4 around, but it did raise the levels of pAMPK in skeletal muscle. Long-term RJ treatment may help lower high blood sugar levels by raising the levels of AdipoQ and AdipoR1 mRNA and pAMPK proteins. Besides, these proteins stop the production of G6Pase (Yoshida & et al., 2017).

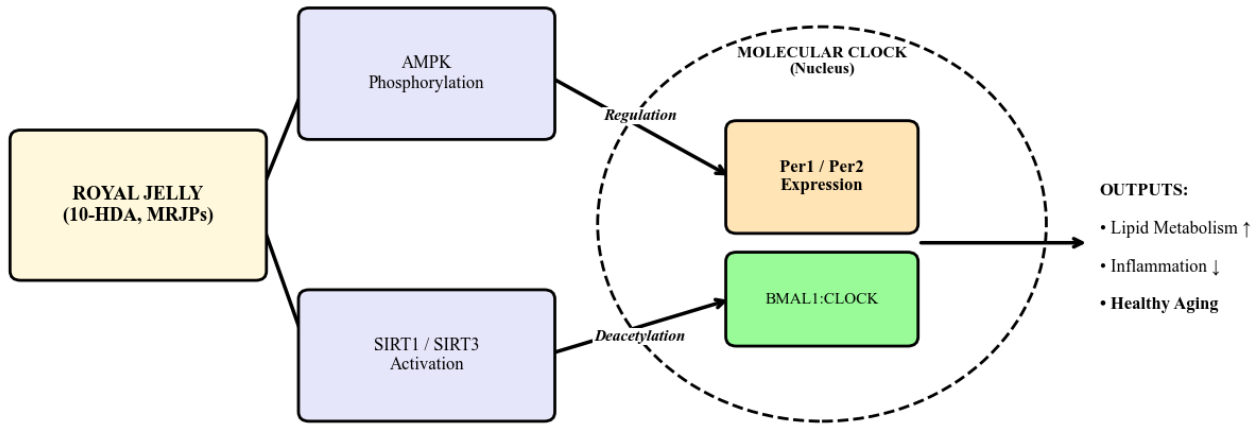

Figure synthesized based on data from You & et al. (2020), Zhang & et al. (2021), and Felemban & et al. (2024).

Figure 2. Proposed conceptual framework illustrating the potential molecular mechanism of RJ as a chrononutraceutical.

Although the metabolic properties of RJ are well-documented in the above literature, the mechanism connecting it to the Circadian Rhythm has not yet been fully explained. However, through the synthesis of the data in Table 1, a mechanism of action can be hypothesized (Figure 2). Figure 2 summarizes recent literature evidence to propose a pathway in which RJ bioactive components activate metabolic sensors (AMPK-SIRT1) to restore circadian gene expression amplitude. Our synthesis of the existing literature indicates that the pathway between RJ and the biological clock could indeed be mediated by energy sensors. As outlined in previous sections, the activation of principal components of RJ, namely 10-HDA, results in the activation of AMPK (Felemban & et al., 2024; Yoshida & et al., 2017), a master regulator known to stimulate SIRT1 signaling. Additionally, RJ has been shown to upregulate SIRT3 expression (Zhang & et al., 2021). Given that chronobiology describes the molecular clock as being regulated by these sensors, the scientific plausibility of the mechanism by which the biological clock system might work through

the use of RJ exists. Specifically, by activating these sensors, RJ likely reinforces the robustness of core clock genes (eg, *Per1* and *Per2*), a conclusion that aligns with the findings from animal studies discussed earlier (You & et al., 2020).

3. Conclusion

Synchronization of the metabolic processes and the internal biological clock is essential for maintaining homeostasis within the body and preventing the development of modern metabolic diseases. As explored in the chapter, the novel area of chrononutrition involves not just the regulation of eating times, as described earlier, but also encompasses the novel field of chrononutraceuticals, which involve bioactive molecules that can modulate the biological clock. In light of the novel area of chrononutrition, RJ is not only considered a nutritional supplement, but also as an agent that can normalize the biological clock that is out of phase.

The evidence synthesis presented in this chapter shows that the bioactive compounds in RJ, specifically 10-HDA and MRJPs, display therapeutic effects in two ways. Firstly, they directly interact with major metabolic sensors, namely AMPK and SIRT1, to increase mitochondrial function and decrease oxidative stress. Secondly, these metabolic sensors function as molecular mediators, and they can reset the amplitude of major clock genes, namely *Per1* and *Per2*. This has very important therapeutic implications in reducing dysmetabolism resulting from desynchrony in biological rhythms, like hepatic steatosis, insulin resistance, and inflammation.

In conclusion, in light of the above discussion, it is evident that Royal Jelly is a novel approach towards healthy aging. The combination of both metabolic regulation and circadian rhythm synchronization makes it a functional food. On the contrary, the specific effects of Royal Jelly on the genes of the biological clock shall be addressed in further research to position Royal Jelly as a functional food. The fact is that the biological clock represents a promising target in the fight against aging. Indeed, targeting the biological clock via functional nutrients like Royal Jelly represents a pivotal frontier in combatting age-related metabolic decline.

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BÖLÜM 6
ENGINEERED CELL MEMBRANE–CAMOUFLAGED NANOPARTICLES: ADVANCES, CURRENT TRENDS, AND FUTURE DIRECTIONS

Gözde Yeşiltaş[1], Rizvan İmamoğlu[2], and Serkan Yaman[3]

1. Introduction

Cell membrane-camouflaged nanoparticles (CMNPs) holds a huge potential for variety of applications in biomedical research and medicine (Gao & Zhang, 2015). Combining synthetic nanomaterials engineering flexibility with the functions of the cell membranes, these nanoparticles have become a very popular tool owing to their potential in targeted therapy, biomimetic immune modulation, drug delivery, and detoxification (He et al., 2024; Jiménez-Jiménez, Manzano, & Vallet-Regí, 2020). The interactions and applications of CMNPs have been extensively investigated in fields such as phototherapy, immunotherapy, cancer chemotherapy, and in-vivo imaging (Chengfang Wang & Wu, 2022; Z. Zeng & Pu, 2020).

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Recent developments have focused on functionalizing CMNPs to increase their efficacy and specificity in drug delivery (Ai et al., 2020; Bu et al., 2023). Studies have highlighted the importance of using CMNPs for precision cancer therapy, where these nanoparticles hold promise as effective drug delivery vehicles (He et al., 2024). Moreover, developing genetically engineered CMNPs with customized membrane receptors has opened new avenues for high-performance drug lead discovery (Bu et al., 2023). The use of CMNPs in targeting tumors for drug delivery and photothermal therapy (PTT) has also attracted attention and demonstrated their capability in combating cancer (Feng & Zheng, 2023; Wu et al., 2019). Furthermore, the applications of CMNPs in inflammation, cancer, chronic/acute diseases, and immunoregulation have been investigated, demonstrating these nanoparticles' versatility in addressing a broad scope of medical disorders (Angsantikul, Thamphiwatana, Gao, & Zhang, 2015; X. Zhen, Cheng, & Pu, 2019). Additionally, distinctive utilization of this technology such as circulating tumor cell isolation via the use of engineered cell membrane coated magnetic nanoparticles and therapeutic delivery to targeted tissues highlights the sensitivity and specificity that can be achieved (Zou, Wang, Wang, Wang, & Zhang, 2020). Therefore, research on CMNPs has shown significant advances in exploiting cell membranes' unique properties in diverse biomedical applications. From targeted therapy and disease treatment to advanced imaging, CMNPs offer a versatile platform with tremendous potential to advance healthcare and precision medicine.

1.1. Overview of Nanoparticles in Biomedical Applications

Distinctive features and versatile structures of nanoparticles lead to being used as one of the most popular drug carrier tools in biomedical research and medical applications. Various types of nanoparticles (i.e. organic, inorganic, magnetic, degradable, non-degradable, and metallic etc.) have been extensively studied for their capability in imaging, chemo-drug delivery, immunomodulation and tissue engineering applications (Dubey & Kaur, 2024). These

nanoparticles have developed into proper instruments for improving healthcare, providing a variety of uses ranging from medicines and diagnostics to tailored payload delivery platforms (Molinelli et al., 2024). The use of nanocarrier systems in biomedicine is rapidly developing, and continuous progress is being made in synthesis methods, surface modifications, and applications (Bashir et al., 2022). For instance, gold nanoparticles have been the focus of research due to their biocompatibility and convenience of functionalization, making them suitable across multiple biomedical fields (Yousefi, Oudadesse, Akbarzadeh, Wers, & Lucas-Girot, 2014). Similarly, biosourced material based nanoparticles such as chitosan have extensively used as a multifunctional platform for tissue engineering and payload delivery applications thanks to their biocompatibility and biodegradability (Nikolic, 2023). These nanoparticles present a promising strategy for advancing novel therapeutic approaches and treatment modalities within the biomedical sector (Sangaiya & Jayaprakash, 2018). In bone tissue engineering, nanohydroxyapatite and bioactive glasses demonstrate significant potential in facilitating bone regeneration and repair processes (Farkaš & de Leeuw, 2021). These inorganic nanoparticles are perfect candidates for scaffolds and implants in regenerative medicine because of their distinctive physical and biological characteristics (Sheikhpour, Arabi, Kasaeian, Rokn Rabei, & Taherian, 2020). By mimicking the natural components of bone, these nanoparticles improve tissue regeneration and healing processes. Moreover, magnetic nanoparticles have found several application field from multiple imaging modalities to drug delivery systems due to their unique manipulable features (Bernal-Chávez et al., 2021). These features enables precise delivery of payloads to affected tissues and organs, while also serving as contrast agent to be used in magnetic resonance imaging (MRI) systems (Lee, Kim, & Cheon, 2013). The versatility of magnetic nanoparticles in combining imaging and therapeutic functions makes them valuable tools in precision medicine and personalized healthcare (Rizeq, Younes, Rasool, & Nasrallah, 2019). Within the framework of

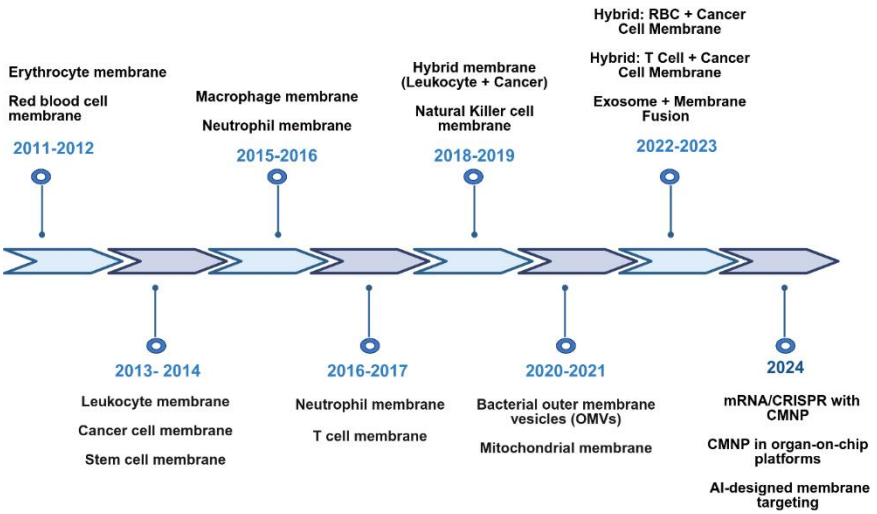
nanotoxicology, it is crucial to consider the safety and biocompatibility of nanoparticles intended for biomedical applications (Gusev, 2022). Understanding the potential toxicity of nanoparticles, such as metal-based and carbon-based nanoparticles, is crucial to ensure their safe application in medicine (Ahmed Ansari et al., 2014). Comprehensive assessments of the toxicity profiles of nanoparticles are essential to reduce risks and advance their biomedical applications (Akinfenwa & Hussein, 2023). The design and engineering of nanoparticles for biomedical purposes involves considering synthesis methods, surface modifications, and physicochemical characterization to optimize their performance (Karnwal et al., 2024). Surface engineering of nanoparticles is critical in improving their biocompatibility, targeting capabilities, and stability for specific biomedical applications (Upadhyay, Tamrakar, Thomas, & Kumar, 2023). By tailoring the properties of nanoparticles through surface modifications, researchers can fine-tune their interactions with biological systems and increase their efficacy in diagnosis and therapy (Patil et al., 2019).

Nanoparticles have the potential for groundbreaking applications in fields such as targeted regenerative medicine, tissue engineering, biomedical imaging, and drug delivery. Their diverse properties and functionalities, such as magnetic response, biocompatibility, and tunable surface properties, make them valuable tools to address various healthcare challenges. Continued research efforts in nanoparticle synthesis, characterization, and application will pave the way for innovative solutions in personalized medicine, disease diagnosis, and treatment strategies.

1.2. Historical Background and Development of CMNPs

CMNPs have influenced significant advances in diverse fields, for example, drug delivery, nanotechnology, and environmental science.

Figure 1. Timeline of Cell Membrane-Coated Nanoparticle (CMNP) Research and Development.



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CMNPs have been comprehensively studied for their applications in cancer diagnostic and therapeutic approaches (C.-H. Xu et al., 2020). These nanoparticles have been used in chemotherapy, phototherapy, in vivo imaging, and immunotherapy, demonstrating their versatility and potential in the medical field (C.-H. Xu et al., 2020). Moreover, CMNPs have also been investigated for their roles in water pollution control, especially in wastewater treatment, demonstrating their applicability beyond healthcare settings (Janet Joshiba, Senthil Kumar, Christopher, & Govindaraj, 2019). Studies have investigated synthetic nanomaterials' catalytic properties and stability on chitosan-encapsulated magnetic nanoparticles (CMNPs) and highlighted their potential to enhance catalytic performance in various applications (Liu, Zhou, Wang, & Wang, 2016). Furthermore, recent research has demonstrated the therapeutic potential of CMNPs in immune modulation, chronic/acute diseases (i.e. kidney damage) and cancer treatment.

These developments emphasize the wide range of applications for CMNPs in the healthcare sector. Regarding the treatment of cancer, CMNPs become more popular research field for their role in targeted payload carrier systems. In addition, combinatorial studies such as magnetic nanoparticle incorporated doxorubicin-loaded thermosensitive liposomes for cetuximab targeted therapy against EGFR-expressing breast cancer cells have demonstrated the efficacy and the specificity of CMNPs against target cell types (Dorjsuren et al., 2020). Additionally, studies have demonstrated the utility of cell membrane-camouflaged nanoparticles as a biomimetic platform for cancer PTT and have shown the promise of CMNPs in innovative cancer treatment modalities (Alimohammadvand, Zenjanab, Mashinchian, Shayegh, & Jahanban-Esfahlan, 2024). The historical background of CMNPs is intertwined with drug delivery systems and nanotechnology development. Over the years, there has been a significant shift toward targeted delivery approaches for cancer therapies, with a particular focus on effective nanoparticles such as CMNPs to enhance drug efficacy and reduce off-target effects (Rosenblum, Joshi, Tao, Karp, & Peer, 2018). This trend reflects the continuous development and improvement of CMNPs to meet the increasing demands for precise and effective payload delivery systems in oncology. Moreover, the development of CMNPs is in line with the broader historical context of advances in nanomedicine and biotechnology. The use of CMNPs offers an innovative drug delivery strategy that increases biocompatibility and targeting capabilities by exploiting the inherent properties of cell membranes (S. Zhang et al., 2024). This innovative strategy marks a significant milestone in nanomedicine, opening new possibilities for personalized and targeted therapies in a variety of disease conditions. In conclusion, the historical background and development of CMNPs have been marked by significant advances in drug delivery, cancer therapy, and environmental applications. From their origins in improving enzyme immobilization to their current roles in targeted payload carrier systems for cancer therapy, CMNPs have shown tremendous potential to revolutionize various fields. The

continued evolution of CMNPs highlights their importance in advancing precision medicine and addressing complex challenges in the healthcare and environmental sectors.

1.3. Advantages of CMNPs in Disease Diagnosis and Therapy

In the last decade, the use of cell membrane derived biomimetic CMNPs for disease therapy and diagnosis achieved considerable advances owing to their exceptional properties and capabilities. Several studies have highlighted advances and applications of CMNPs in disease therapy and diagnosis, shedding light on their potential clinical applications (Ma et al., 2019; Shan et al., 2020). These studies have demonstrated that CMNPs are effective in targeted drug delivery, improving therapeutic outcomes, and enhancing treatment efficacy in conditions such as cancer, stroke, and bacterial infections.

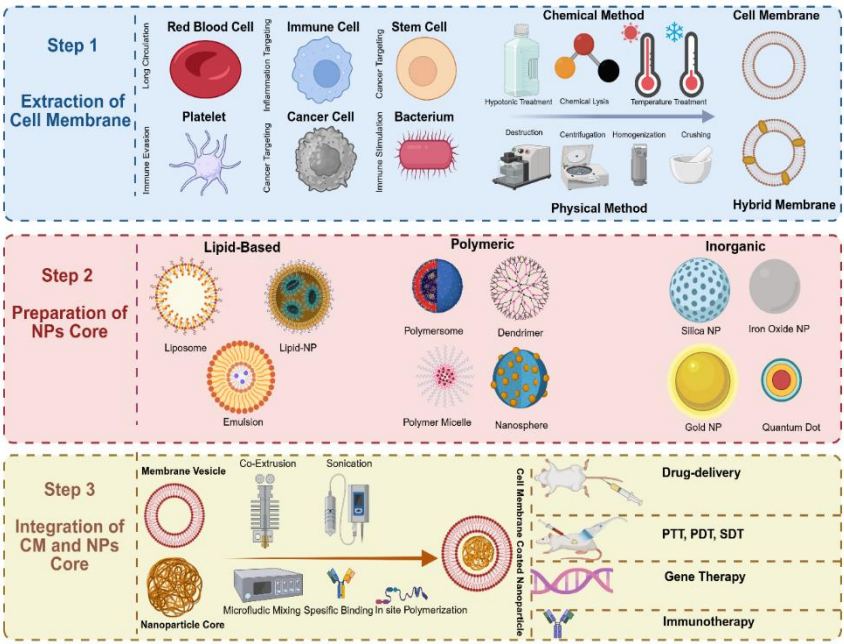
CMNPs become prominent via directing therapeutic payloads to target sites within the body with high precision. For example, it has shown that the CMNPs loaded with specific chemo-drugs effectively target cancer cells and inhibit their growth. Trametinib loaded T cell membrane coated CMNPs can be given as an example in this manner. Yaman et al. reported the use of trametinib loaded CMNPs against melanoma cancer. In this study, gp100 melanoma- antigen bound HLA-A2 specific T-cell receptor (TCR) expressing lymphocyte membranes coated onto trametinib loaded PLGA nanoparticles. Nanoparticles covered with the T cell membranes were able to target cancer cells *in vitro* and *in vivo.* In *in vitro* targeting, T-MNPs group was uptaken by the target cells threefold more compared to control group NPs. Animal studies also demonstrated the targeting capabilities of T-MNPS by accumulating two fold more in to DM-6 melanoma tumors on mice model (Yaman, Ramachandramoorthy, et al., 2020)**.** This targeted drug delivery approach minimizes off target adverse effects and increases the therapeutic outcome. Other than cancer targeting, CMNPs have also been used to deliver drugs to the site of stroke and have shown improved therapeutic outcomes compared to traditional drug

delivery methods (Ma et al., 2019). Moreover, CMNPs are also useful tools to manipulate the tumor microenvironment for the purpose of enhancing the efficacy of chemotherapy. Researchers have successfully altered the tumor microenvironment by delivering hedgehog pathway inhibitors using CMNPs, improving the delivery of chemotherapeutic agents to pancreatic carcinoma, and resulting in significant tumor growth inhibition (T. Jiang et al., 2018). This tumor microenvironment manipulation capability highlights the versatility and potential of CMNPs to improve cancer therapy efficiency. In addition, CMNPs have been investigated as dual-modal cancer therapy by combining neutrophil and macrophage membranes for synergistic cancer cell killing. Studies have shown that hybrid CMNPs manufactured from both neutrophil and macrophage membranes can be used for glioma therapy, leading to orchestrated tumor-microenvironment responsive capability and improved cancer cell killing (Yaman, Chintapula, Rodriguez, Ramachandramoorthy, & Nguyen, 2020). This dual-modal therapy approach demonstrates the multifunctionality of CMNPs in cancer treatment and its' potential to overcome the limitations of conventional therapy modalities. In addition, CMNPs have been investigated for their antimicrobial properties and their potential in combating bacterial infections has been demonstrated. Studies have reported that CMNPs exhibit highly effective antimicrobial activity against bacteria such as Staphylococcus aureus, highlighting their role as novel antibacterial agents (Y. Jiang et al., 2023). Overall, studies on CMNPs in disease therapy and diagnosis highlight their importance in revolutionizing nanomedicine. From precise payload delivery to tumor microenvironment modification to antimicrobial applications, CMNPs offer a wide range of benefits that could potentially transform the healthcare and environmental remediation landscape. With ongoing advancements in this field, the full potential of CMNPs becomes increasingly evident for the improvement of advanced therapies and addressing complex medical challenges.

2. Characteristics of Cell Membrane-Coated Nanoparticles

Consisting of synthetic cores wrapped in natural cell membranes, these nanoparticles offer a promising platform for various biomedical purposes. The interactions and applications of these CMNPs have spread to different biomedical fields, including payload delivery, immunomodulation and, detoxification (Yaman et al., 2024). By combining the cell membrane functions with the flexibility of synthetic nanomaterial engineering, these biomimetic nanoparticles exhibit enhanced capabilities for targeted drug delivery and novel therapeutics (Fernández-Borbolla, García-Hevia, & Fanarraga, 2024). The most important feature of CMNPs is to imitate natural functions of cell membranes, enabling targeted drug delivery and tissue-specific therapeutics (Chenguang Wang, Li, Zhang, & Huang, 2024).

Figure 2. *Features and Fabrication Process of CMNPs.*



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These nanoparticles also have the ability to retain the beneficial synthetic nanomaterial characteristics in terms of drug delivery while exploiting complex cellular functions that are difficult to mimic. (Fernández-Borbolla et al., 2024). Moreover, macrophage membrane functionalized nanoparticles has shown promising results for the infectious disease treatment by taking advantage of macrophage membranes for targeted therapy (Zhao, Li, Jiang, Gu, & Liu, 2021). The incorporation of different cell types and synthetic nanocarriers highlights their multifunctionality across multiple biomedical domains, including photo-thermal therapy, payload delivery, imaging, immunotherapy, immunomodulation, vaccination and theranostic applications (Dan Wang et al., 2022). By fusing plasma membranes of white blood cells to synthetic nanoparticle cores, white blood CMNPs have been developed and have demonstrated dynamic and diverse functions for cancer therapy and immune system modulation applications (Yu et al., 2024). Moreover, the functionalization of CMNPs has been the subject of recent research aimed at improving their capabilities for specific biomedical tasks (Jingchao Li et al., 2018). These functionalized nanoparticles preserve native surface antigens and cell membrane functions while possessing additional properties such as intrinsic targeting based on cell source, immune evasion and, extended circulation (L. Sun, Li, Yang, & Li, 2022). In the context of infectious diseases, biomimetic CMNPs have shown promising results for the management of viral infections by exploiting the unique properties of cell membranes for targeted therapy (Fan, Li, Deng, Bady, & Cheng, 2018). Additionally, It has also shown that cell membrane coating contributed to reduce nanoparticle-induced inflammatory responses, enabling evasion of immune and reticuloendothelial system clearance (W. Chen et al., 2016). The application of CMNPs holds a broad treatment applications extending from payload delivery to tissue-specific applications, as demonstrated via beta-cell membrane cloaked nanofiber scaffolds to support cellular function and proliferation (X. Jiang et al., 2024). In addition, genetically engineered cells also has created another novel

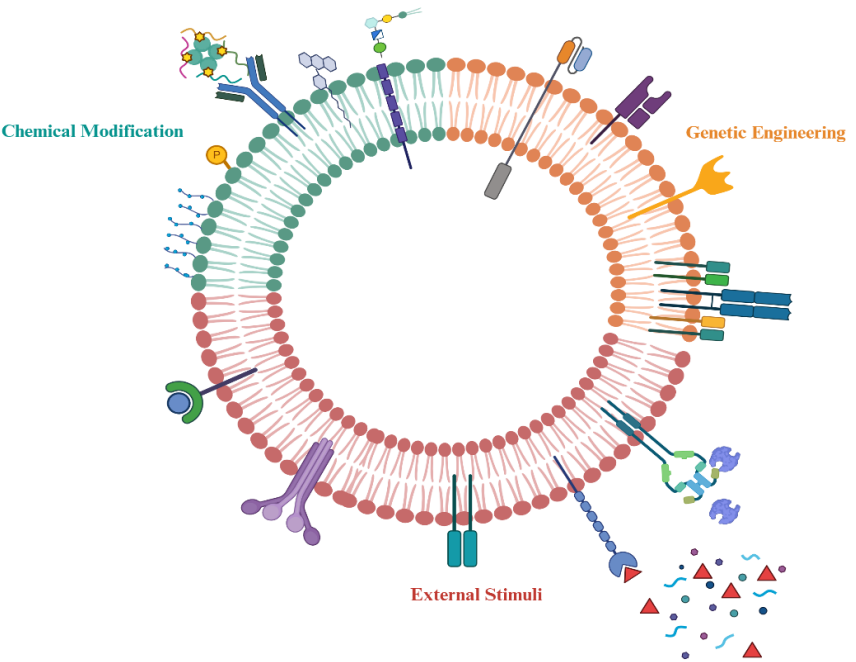
platform for membrane-coated nanoparticles which can be used for precision cancer therapies, demonstrating the potential of these nanoparticles in precision medicine and cancer diagnostics (Dongdong Wang et al., 2018). In this field Yaman et. al have reported the engineering HER-2 specific chimeric antigen receptor (CAR) engineered T lymphocytes and the use of their membranes as coating material onto polymeric nanoparticles. For this, antiHER-2 CAR-T lymphocytes were transduced with lentiviral gen delivery method and isolated CAR-T lymphocyte membranes used as a coating layer on cisplatin loaded poly D, l-lactide-*co*-glycolic acid NPs. Membrane-coated NPs showed prolonged payload release for more than 21 days in physiological circumstances. As their targeting abilities, CAR-T-MNPs demonstrated superior selective accumulation in Her2+ A549 cells compared to control group by increasing the selective uptake in two-fold. Same trend was also confirmed via *in vivo* biodistribution studies. *In vitro* cell killing studies conducted on multiple cell lines revealed that cisplatin-loaded CAR-T-MNPs inhibited the growth of HER2+ cells. Similarly, in vivo therapeutic studies using a subcutaneous lung cancer model in nude mice resulted higher chemotoxicity towards the target cells while leaving minimal toxic effects on non-target cells. Therefore, cellular engineering approach with various receptors such as T-cell receptors (TCRs), chimeric antigenic receptors (CARs), single chain fragment variable domains (ScFvs) and/or nanobodies can bind target moieties from heterogeneous cancer cell population and can improve payload drug targeting personalized medicine (Yaman et al., 2024). CMNPs have also been investigated for their combinatorial targeted payload delivery and PTT by utilizing the intrinsic functions of cell membranes for enhanced biocompatibility and therapeutic efficacy (Craig, Lee, Mun, Torre, & Luther, 2014). By camouflaging hollow copper sulfide nanoparticles with erythrocyte-cancer hybrid membranes, long-circulating lifetime and homotypic targeted photothermal/chemotherapy of melanoma were achieved, highlighting the versatility of these biomimetic nanoparticles in

cancer therapy (Craig et al., 2014). Overall, CMNPs offer a versatile platform with diverse applications in payload delivery, imaging and, targeted therapy.

2.1. Structural and Functional Features of CMNPs

The structural and functional properties of CMNPs encompass a range of features. By exploiting these properties of cell membranes and combining them with the synthetic nanomaterials' versatility, biomimetic nanoparticles offer a promising avenue to advance precision medicine and biomedical research. CMNPs display a biomimetic surface which preserves cell source properties so allows NPs to surpass physiological barriers (cell-specific targeting, immune - systemic clearance etc.) (Yao, Zhang, Wang, & Zhang, 2023).

Figure 3. *Functionalization Strategies Demonstration of CMNPs*



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This inherent ability of CMNPs to mimic the properties of source cells is crucial to their functionality and enables them to interact effectively with biological systems. The fabrication process on the other hand is critical in tailoring mentioned properties of CMNPs especially for sophisticated applications. Nanoparticles coated with macrophage and platelet cell membranes offer versatile targeting and treatment opportunities in biomedical applications. For instance, nanoparticles functionalized with platelet membranes provided targeting by binding to multiple biological components of atherosclerotic plaques and enabled non-invasive detection of plaques with magnetic resonance imaging. Wei et al. have shown that the platelet membrane functionalized polymeric nanoparticles was able to localize atherosclerotic regions. In addition, their platform was also targeting subclinical atherosclerotic regions. They have demonstrated the ability of platelet derived membrane coated biomimetic nanoparticles *in vivo* via magnetic resonance imaging on atherosclerotic animal models (Wei et al., 2018). In another study, Hu et al. also shown that the platelet bio-interfaced CMNPs was able to mimic immunomodulatory and adhesion properties of platelets on different disease models. They have quantified targeting and payload delivery capabilities of manufactured CMNPs in coronary restenosis and systemic infection animal models. Consequently, their findings states that the platelet membrane is a versatile tool to be used for different disease scenarios to deliver payloads on affected regions (C.-M. J. Hu et al., 2015). Understanding the physiological behavior of CMNPs is important to optimize their performance in imaging, drug delivery, and other biomedical applications. In addition, as reviewed, core-shell nanoparticles composed of synthetic cores and biomimetic membrane layer important in different applications such as imaging, extended payload release, and magnetic manipulations (isolation, drug release thermal therapy etc.) (S. Zeng et al., 2023). The tunability of properties in CMNPs via their core-shell structures opens up avenues for innovative applications in various fields.

In conclusion, the structural and functional properties of CMNPs cover a wide range of features that make them versatile and valuable in various applications. Understanding the fabrication processes, functional properties, and physiological behavior of CMNPs is crucial to exploiting their full potential in fields such as imaging, theranostic and targeted delivery. The biomimetic structure, unique fabrication techniques, and tunable magnetic properties of CMNPs position them as promising candidates for addressing advanced technologies and complex challenges in various industries.

2.2. Surface Properties and Biocompatibility

The surface properties and biocompatibility of CMNPs are important issues in the field of nanomedicine and drug delivery systems. By mimicking the functional attributes of natural cell membranes—such as immune evasion, prolonged circulation, and target recognition—CMNPs enhance biocompatibility and enable surface tunability for advanced biomedical use (Song et al., 2022). Modification of nanocarriers with cell membrane components has been shown to increase biocompatibility and promote cell proliferation (Y. Jiang et al., 2023). These modifications enable CMNPs to inherit receptors, surface adhesive molecules, and functional proteins from the original cell membrane, making them versatile systems that can be used in a wide variety of biomedical applications (Bhattacharya & Beninger, 2024). The biocompatibility of CMNPs is a key focus of research, and researchers currently investigating their immune escape, payload delivery & capacity, and biological functionality on nanomaterials. (Spanjers & Städler, 2020). Nature's use of cell membrane properties, including surface chemistry, geometry, and mechanical properties, influences the design and efficacy of CMNPs in drug delivery systems (M. Zhang, Cheng, Jin, Zhang, & Wang, 2021). Moreover, surface modification of nanoparticles with the cell membrane plays an important role in nanometer-scale therapeutics, affecting aspects such as immunotherapy, drug delivery systems, and biomimetics (Seon et

al., 2015). Surface engineering plays a vital role in improving the biocompatibility of various materials, including nanoparticles. These modifications are designed to improve interactions between carriers and biological systems, facilitate better integration, and minimize adverse reactions. Furthermore, grafting materials with agents such as dermatan sulfate has proven effective in enhancing bio-integration and overall biocompatibility (C.-M. J. Hu et al., 2011). In the nanomedical field, CMNPs have received significant attention because of their unique properties that mimic natural cell membranes. These CMNPs provide benefits to nano carriers such as reticuloendothelial system evasion, extended circulation time, and the ability to recognize specific targets, all of which contribute to improved biocompatibility and surface properties (Song et al., 2022). Surface modification of nanoparticles with cell membrane components has been shown to significantly increase biocompatibility, leading to increased cell proliferation and overall compatibility (Dongdong Wang et al., 2018). Studies on CMNPs have highlighted their potential in targeted drug delivery systems, where surface properties are critical for precise delivery and effective therapeutic outcomes. CMNPs exhibit versatile properties that make them suitable for various biomedical applications, especially in cancer treatment, by inheriting surface molecules and proteins from cell membranes (Bhattacharya & Beninger, 2024). The biocompatibility of CMNPs has been extensively analyzed, focusing on their stability, modulation of immune responses, and capacity to carry higher drug loads while maintaining biological functionality (Spanjers & Städler, 2020). In summary, surface engineering and modification techniques are vital in advancing the biocompatibility of materials used in biomedical applications, improving their integration with biological systems, and improving therapeutic outcomes.

2.3. Stability and Circulation Time in the Bloodstream

The stability and circulation time of CMNPs in the bloodstream are critical factors affecting their efficacy in drug

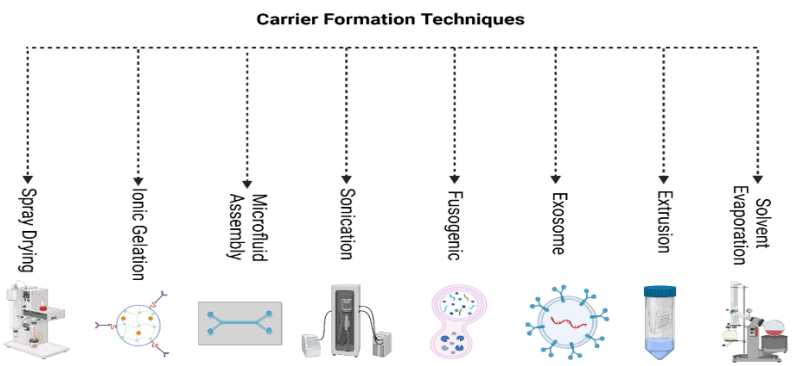
delivery and therapeutic applications. Recent studies have shown that CMNPs exhibit significantly longer circulation times compared to conventional nanoparticles, primarily due to their biomimetic properties and structural characteristics. One of the primary mechanisms contributing to the prolonged circulation time of CMNPs is their ability to evade the immune system. For example, erythrocyte membrane-coated nanoparticles (RBCNPs) have shown improved stability and extended half-lives in circulation due to their structural rigidity and the presence of immunomodulatory markers such as CD47, which help prevent their recognition and clearance by phagocytic cells (Dehaini et al., 2017; Zhou et al., 2018). This is confirmed by the findings that RBC membrane-coated nanoparticles can achieve circulation times of up to 22.2 h, which is significantly longer compared to their uncoated counterparts (Q. Xu et al., 2015). Furthermore, the composition and density of the surface coating play an important role in determining the pharmacokinetics of CMNPs. Dense polyethylene glycol (PEG) coatings have been shown to increase blood circulation time by minimizing opsonization, the process by which nanoparticles are tagged for clearance by the immune system (Abbina et al., 2020). Dynamic remodeling of protein opsonins at the nano-bio interface further influences circulation dynamics, suggesting that the surface chemistry of nanoparticles is a critical determinant of their blood residence time (Bertrand et al., 2017). In addition to immune evasion, the design of CMNPs improves the targeting and delivery of therapeutic agents. For example, CMNPs can accumulate chemotherapeutic drugs efficiently such as cyclopamine to cancer cells in the tissues and increase therapeutic efficacy while using significantly lower doses compared to traditional delivery methods (Hadjidemetriou et al., 2015). This ability is depends on the enhanced retention and permeability effect facilitated by the long circulation time of these nanoparticles (Abbina et al., 2020). Moreover, the formation of a protein corona around nanoparticles when they enter the bloodstream can alter their pharmacokinetics. Studies show that the protein corona can affect the interaction of nanoparticles with cells

and tissues, thereby affecting their distribution and clearance rate (Hyötyläinen & Riekkola, 2004; T. Jiang et al., 2018). The ability of CMNPs to form a suitable protein corona can improve their stability and circulation time, making them more effective as drug delivery systems. In summary, the stability and circulation time of CMNPs in the bloodstream are significantly enhanced by their biomimetic properties, surface modifications, and ability to evade immune recognition. These factors contribute to their potential as effective drug-delivery vehicles, especially in oncology and other therapeutic areas.

3. Extraction Methods for Cell Membranes

CMNPs have shown up as a promising strategy for drug delivery systems in various biomedical applications. The synthesis of CMNPs typically involves three main steps: cell membrane isolation, coating of these membranes onto nanoparticles, and finally formulation of the surface properties of the resulting CMNPs (Yao et al., 2023)**.** Various physical methods such as sonication, homogenization, density gradient centrifugation, extrusion, and microfluidic fractionation is being applied for controlled fusion and or coating of these membranes on nanoparticles (Yaman, Chintapula, et al., 2020).

Figure 4. *The Demonstration of Extraction Methods for Cell Membranes.*



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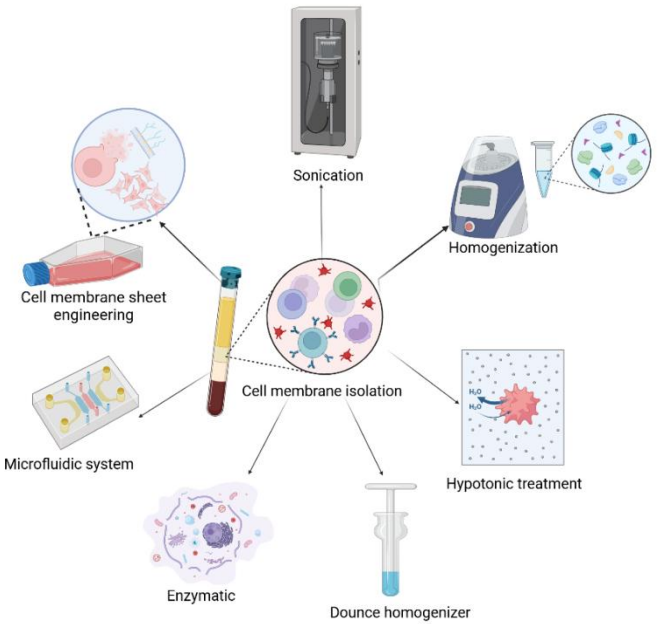
These methods aim to improve the stability and functionality of CMNPs for targeted payload delivery. Removal of cell membranes is a critical step in the production of CMNPs. Various membrane-derived CMNPs have been developed for applications such as cancer PTT (Wu et al., 2019). Purification of membranes is essential to ensure the efficacy of CMNPs in drug delivery systems. Techniques such as liquid-liquid extraction, solid phase extraction, membrane-based techniques, and others have been used for membrane extraction and purification (Kang et al., 2017). These methods help to obtain high-quality membranes for the successful production of CMNPs. Moreover, the use of CMNPs holds promise in targeted cancer therapy. By coating nanoparticles with cell membranes, researchers have been able to increase the efficacy of drug delivery systems for cancer therapy (H. S. Kim et al., 2021). The expression of specific cell-cell interaction mediators was preserved during the production of CMNPs, indicating the potential of these nanoparticles to inhibit angiogenesis and promote hypoxic cell-cell packing (Waugh, 2013). This targeted approach has significant potential for precision cancer therapy. Therefore, extraction methods for cell membranes to form CMNPs play a vital role in the development of advanced drug delivery systems. By leveraging innovative techniques for membrane extraction and fusion with nanoparticles, researchers can increase the stability, functionality, and targeting capabilities of CMNPs for various biomedical applications, including cancer treatment and photothermal therapies (PTT).

3.1. Techniques for Isolating Cell Membranes

Isolating cell membranes for CMNPs involves various techniques to remove and fuse cell membranes with nanoparticles. Techniques such as extrusion, sonication, co-extrusion, and microfluidic electroporation are widely used for membrane extraction and fusion.

Figure 5. *The Illustration of Techniques for Isolating Cell Membranes.*



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These methods are crucial for generating promising CMNPs in cancer therapy (Spanjers & Städler, 2020). The isolation and biochemical analysis of trans-Golgi network-endosomal membranes have been facilitated by an improved protocol that offers wide applications in biomedical and cell biology research (Rao et al., 2017). Genetic engineered cell membranes coated onto magnetic nanoparticles have been created for various applications. These nanoparticles exhibit properties such as reduced nonspecific binding achieved through simple extrusion and expression of specific antibodies on the membrane and high-purity isolation of target cells (Dongdong Wang et al., 2018). Red blood CMNPs have been used to prolong the circulation time of CMNPs with consensus on the synthesis procedure involving membrane extraction, fusion with nanoparticles, and surface engineering (Yao et al., 2023). Functionalization strategies for CMNPs, including chemical

modification, genetic engineering, and external stimuli, have been highlighted for precision cancer therapy (He et al., 2024). White blood CMNPs have been investigated for various medical applications, including immune modulation, payload delivery and isolation of circulating tumor cells (Yu et al., 2024). Studies on cell membrane-camouflaged nanoparticles have shown that erythrocyte membranes can decrease protein adsorption and maintain the biological properties of CMNPs (Wu et al., 2019). Moreover, coating nanoparticles with erythrocyte membranes has been shown to minimize protein adsorption and improve tumor imaging (W. Zhang et al., 2017). Techniques such as magnetic and folate functionalization have enabled the rapid isolation of cell-derived micro-vesicles and improved tumor targeting (Ye, Sun, Pei, Sun, & Wu, 2017). Membrane isolation techniques have been developed for a variety of cell types, including tissue culture cells and bladder epithelial cells. Consequently, techniques for isolating cell membranes for CMNPs include a combination of excision and fusion methods, genetic engineering, and functionalization strategies. These approaches have shown promising results in cancer therapy, drug delivery, immune modulation, and tumor imaging, highlighting the potential of CMNPs in various biomedical applications.

3.2. Purification Processes for High-Quality Membrane Extraction

Purification processes are important to ensure high quality of membrane extraction for various applications including purification of membrane proteins and other compounds. Innovative methods have been developed to increase the efficiency and effectiveness of purification processes for membrane extraction. One of these methods involves the use of emulsion liquid membranes, which are practical and promising for the purification of certain compounds due to their high surface interface area for mass transfer and simultaneous extraction and stripping (Parhi, 2013). This approach offers high solute transfer flux and recyclability of raw materials,

making it a sustainable option for purification processes. In the area of membrane protein purification, techniques such as supported liquid membranes (SLM) have been investigated for their efficiency and cost-effectiveness. SLM techniques offer advantages such as simultaneous extraction and stripping, low solvent inventory, high throughput, and reduced operating costs (Karlova et al., 2021). Additionally, the use of styrene maleic acid (SMA) for membrane protein extraction has shown promising results in providing stable membrane proteins that can be further purified and analyzed using various biochemical methods (Kumar, Khan, & Arafat, 2020). These developments highlight the importance of continuous innovation in membrane technologies to address water scarcity and quality challenges. Additionally, the use of SMALPs (styrene-maleic acid-lipid particles) for detergent-free purification of ATP-binding cassette (ABC) transporters showcases a novel approach to membrane protein purification (Brahma & Raghuraman, 2022). The potential for many scientific applications of SMALPs encapsulating ABC transporters is demonstrated by their ability to be purified by affinity chromatography. Strategies like using dual detergent methods have been suggested in the context of membrane protein purification as a way to boost the stability and purity of membrane proteins at a reasonable cost (Hering et al., 2020). This innovative strategy offers a practical solution to the challenges associated with conventional purification methods. Furthermore, developing rapid and efficient purification techniques such as the “tea bag” method for high-end purification of membrane proteins highlights the continuous efforts to streamline purification processes and improve the quality of purified membrane proteins (Outram & Zhang, 2018). Purification of membrane proteins is important for various downstream applications including structural studies and biochemical analyses. Techniques such as electrophoretic characterization of detergent-treated plasma membrane fractions have been instrumental in studying carrier proteins and integral proteins while preserving their native structures and functionality. These methods are crucial for elucidating the roles and functions of

membrane proteins in biological systems. In conclusion, new methods and approaches are being developed quickly, which is changing the area of purification procedures for high-quality membrane extraction, particularly in the context of membrane proteins and other substances. Researchers are always looking for ways to increase purified membrane components' effectiveness, stability, and purity, from emulsion liquid membranes to SMALPs and dual detergent methods. These advancements have applications in biotechnology, pharmaceuticals, environmental science, and fundamental membrane biology research.

3.3 Challenges and Solutions in Membrane Extraction

Membrane extraction, especially in the context of CMNPs, involves complex techniques such as membrane isolation and fusion, including methods such as sonication, extrusion, co-extrusion, and microfluidic assembly (Spanjers & Städler, 2020). These techniques are crucial to ensure the successful integration of cell membranes with core nanoparticles to form functional CMNPs. In addition, the synthesis procedure for CMNPs typically involves three main steps: membrane extraction, membrane fusion with nanoparticles, and surface engineering of the resulting CMNPs (Yao et al., 2023). This standardized approach has facilitated the production of CMNPs and facilitated their widespread application in various fields. One of the main challenges in membrane extraction is the efficient extraction of specific compounds or substances. Studies have shown that membrane extraction techniques can be highly effective in removing volatile fatty acids from solutions, with significant recovery percentages achieved in short time frames (Niu, Wang, Zhang, Meng, & Cai, 2012). This highlights the potential of membrane extraction for targeted compound removal. Moreover, the adsorption or extraction behavior of CMNPs is influenced by the composition of the carbon shell, where oxygen-containing species and graphitic carbon play important roles in determining interactions with analytes (Ramos-Mandujano et al., 2021). Understanding these factors is essential to optimize membrane extraction processes for specific

applications. Another important challenge in membrane extraction is the need for safe and scalable workflows, especially in areas such as environmental science and pathogen RNA extraction. Innovative approaches such as the MAVRICS system have been developed to directly inactivate and lyse pathogens in wastewater samples, followed by RNA extraction using magnetic nanoparticles (Saba et al., 2018). This not only increases the safety of the process by reducing biohazard risks but also simplifies the overall processing procedures. Similarly, the development of effective sorbents such as chitosan-coated magnetic nanoparticles has enabled the removal of contaminants such as Reactive Orange 107 dye from aqueous media with high removal potential (S. Li et al., 2020). These developments highlight the importance of developing robust and safe membrane removal techniques for various applications. Furthermore, implementing CMNPs in drug delivery systems has brought new challenges and opportunities. Using cell membrane coatings on nanoparticles, researchers have increased drug delivery efficiency and targeted specific diseases such as cancer and urological disorders (Yao et al., 2023). The use of CMNPs for targeted drug delivery, such as delivering Notch-1 inhibitors to inhibit angiogenesis, demonstrates the potential of this technology in precision medicine (Waugh, 2013). Moreover, incorporating engineered neural stem cell membranes onto nanoparticles has shown promising results in targeted drug delivery for stroke treatment, highlighting the versatility of CMNPs in addressing complex medical conditions (Ijaz et al., 2024).

4. Engineering of Cell Membrane-Coated Nanoparticles

Recently, CMNPs have emerged as a promising strategy, especially in nanomedicine, and offer a wide range of opportunities for engineering synthetic nanomaterials with cell membrane functionalities for various biomedical applications. These biomimetic nanoparticles have shown significant potential in targeted drug delivery, detoxification, immune modulation, and vaccine development (Zheng, Zhang, Huang, Zhou, & Gao, 2022).

In addition, these nanoparticles can mimic the properties of specific cell types by using cell membranes as coatings. Thus, they can increase circulation time, immune escape, and targeted delivery to particular tissues or cells (Cheng, Abdullah, & Buechler, 2024). Recent developments in this field have focused on functionalizing CMNPs to improve their performance. For example, genetically engineered membrane-coated nanoparticles have been developed to express specific membrane receptors such as fibroblast growth factor receptor 4 (FGFR4) for high-performance drug discovery (Ding Wang et al., 2023). Furthermore, genetically modifying cell membranes to express viral fusion proteins has been explored to modulate the intracellular localization of cargos delivered by these nanoparticles (Hameed, Nabi-Afjadi, Gu, & Wu, 2023). The versatility of membrane-coated nanoparticles has been demonstrated by their use in various medical applications, including cancer phototherapy, drug delivery to inflamed lungs, and combating urological diseases (Kroll, Fang, & Zhang, 2017; Yao et al., 2023). These nanoparticles have also been investigated for their potential to isolate high-purity circulating tumor cells, demonstrating their use in precision medicine and diagnostics (Hameed et al., 2023). Overall, the engineering of CMNPs represents an advanced approach in nanomedicine and offers a sophisticated tool for targeted payload delivery, vaccine development, and therapeutic interventions in various biomedical applications.

4.1. Methods for Coating Nanoparticles with Cell Membranes

In nanotechnology, coating nanoparticles with cell membranes is a critical area of research, especially for biomedical applications. This method increases the biocompatibility of nanoparticles which allows for immune system escape and improve targeting capabilities. Various techniques and materials have been studied for effective coating of nanoparticles with cell membranes such as sonication, co-extrusion, microfluidic assembly, and electroporation. Each process has its unique advantages and disadvantages to the characteristics of resulting biomimetic

nanoparticle. One of these coating methods is the use of erythrocyte membranes, thanks to their natural properties that prevent uptake by macrophages, and this has been shown to significantly extend the circulation time of nanoparticles in living organisms. Similarly, such coating techniques enable nanoparticles to function more effectively in biological systems (Fang, Kroll, Gao, & Zhang, 2018; C. Li, Hu, He, & He, 2024). The process typically involves the removal of erythrocyte membranes under hypotonic conditions and subsequent extrusion of these membranes onto synthetic nanoparticle cores (Duan et al., 2023; C. Li et al., 2024). This technique advantageous in terms of protecting the membrane integrity. In addition, surface proteins which take role in CMNPs physiological tasks can be well protected in hypotonic treatment technique (Duan et al., 2023; Yu et al., 2024). Thus, this method offers significant advantages in various biomedical applications by enabling nanoparticles to function more effectively in biological systems. Recent developments have also brought their use for membrane coating of alternative cell types, such as macrophages and cancer cells, which can offer additional functions customized to specific therapeutic needs. This strategy increases the targeted therapeutic capabilities of nanoparticles, allowing them to function more effectively in biological systems. In particular, the innate immune properties of macrophage cells and the specific targeting abilities of cancer cells significantly increase the biocompatibility and treatment efficacy of nanoparticles (Ben-Akiva et al., 2020; Cao et al., 2020). For example, nanoparticles coated with macrophage membranes were effectively internalized by tumor cells, which increased therapeutic efficacy (Ben-Akiva et al., 2020). The potential of this approach is further highlighted by the flexibility of cell membranes (i.e genetic, chemical modification etc.) that can enrich the capabilities of core nanoparticle. For instance, allowing the integration of specific membrane receptors via membrane coating improves core particles' targeting ability. Thus, such nanocarriers tenders more precise and effective approach to cancer therapy (Cao et al., 2020). The physicochemical properties of nanoparticles can be affected by the curvature of the coated

membranes. Anisotropic nanoparticles can be effectively coated with erythrocyte membranes, maintaining both stability and functionality despite changes in curvature (D. Zhang, Ye, Wei, Luo, & Xiao, 2019). This flexibility is of great importance for membrane-coated nanoparticles to be used in wide variety of applications such as drug carrier systems and biosensors. Thus, the ability to design nanoparticles in different shapes and sizes increases their potential in the biomedical field. Such coatings allow nanoparticles to be used more effectively in targeted therapy and diagnostic processes (Ai et al., 2020; Zhou, Fan, Lemons, & Cheng, 2016). In addition, the integration of cell membranes with synthetic nanoparticles enables the development of multifunctional platforms. For example, the incorporation of cell membrane coatings with hydrogels has paved the way for research aimed at creating localized delivery systems that can respond to specific stimuli (Jianzhuang Li et al., 2023). This method not only increases the therapeutic potential of nanoparticles but also allows the design of systems that can interact dynamically in biological environments and respond to environmental changes. Thus, such innovative approaches can significantly improve therapeutic processes by increasing the delivery rate and efficacy of drugs. As a result, the methods of coating nanoparticles with cell membranes are quite diverse and are constantly evolving, which has important consequences for advance the capabilities of nanocarriers in biomedical applications. The use of natural cell membranes offers a unique advantage in the design of nanoparticles that mimic biological systems, thus increasing their effectiveness and safety in therapeutic contexts. These coating techniques facilitate the evasion of nanoparticles from immune detection, allowing more successful results in targeted therapeutic processes, thus creating new opportunities in the treatment of diseases.

4.2. Design and Optimization of CMNPs for Targeted Delivery

Recently, the development of CMNPs for payload carriers and delivery systems has attracted great attention. CMNPs have the potential to increase therapeutic efficacy while reducing side effects.

Unique properties of cell membranes allow them to improve circulation time and provide specific targeting capabilities. To increase the targeting capabilities of CMNPs, one of the most important methods is to use membranes derived from specific cell types that naturally interact with target tissues. For example, it has been shown that engineered neural cell membrane coated nanoparticles can effectively target areas affected by stroke using chemotactic uptake mechanisms (X. Xu et al., 2018). This approach emphasizes the importance of selecting appropriate source cells for membrane extraction according to the desired therapeutic target. Similarly, macrophage-derived membrane CMNPs lead to increase *in vivo* circulation time. This enables long-term therapeutic effect (Cao et al., 2020). The physicochemical features of nanoparticles holds crucial role in their performance. It has been emphasized that the size of erythrocyte derived CMNPs significantly affect their circulation blood vessels and migration in target tissues, suggesting that smaller sizes may be more advantageous for targeted delivery (Desai, Tambe, Pofali, & Vora, 2024). Moreover, incorporating genetic modifications into membrane proteins can further enhance the targeting capabilities of CMNPs. For example, genetically engineered macrophage membranes can be tailored to express specific receptors that facilitate binding to target cells, thereby improving the overall efficacy of the drug delivery system (Cao et al., 2020). Furthermore, dual membrane-coated nanoparticles derived from different sources have emerged as a up and coming approach to combine the advantages of different cell types. These hybrid systems can combine properties of multiple cell membranes to improve targeting and therapeutic efficacy. It has been shown that anisotropic nanoparticles coated with RBC membranes can maintain their stability and functionality even with increased curvature, which is beneficial for navigating complex biological environments (D. Zhang et al., 2019). This versatility enables the design of multifunctional nanoparticles that can address various therapeutic challenges. In addition to selecting membrane sources and nanoparticle properties, the functionalization of CMNPs is crucial to

optimizing their performance. It has been reported that incorporating specific ligands onto the surface of CMNPs can significantly enhance their targeting capabilities and enable active targeting of cancer cells expressing specific markers (D. Zhang et al., 2019). Many methods, including chemical conjugation and genetic engineering, achieve this functionalization. This provides flexibility in designing nanoparticles tailored to specific therapeutic needs. Moreover, integrating CMNPs with other materials, such as hydrogels or magnetic nanoparticles, can enhance their functionality and therapeutic applications. For example, incorporating CMNPs into hydrogels has been explored to create localized payload delivery systems responding to particular stimuli, thereby increasing the sensitivity of the treatment (Jianzhuang Li et al., 2023). This approach not only increases treatment potential of nanoparticles but also enables dynamic interactions with biological environments. Consequently, the design and optimization of CMNPs for targeted delivery involves a multifaceted approach that includes the selection of appropriate cell membrane sources, careful evaluation of nanoparticle properties, and strategic functionalization. By integrating advanced engineering techniques into unique features of cell membranes, CMNPs can be developed to achieve enhanced targeting and therapeutic efficacy in a variety of biomedical applications.

4.3. Functionalization and Customization of CMNPs for Specific Applications

The customization and functionalization of CMNPs represent a significant advancement in enhancing drug delivery efficiency and specificity, especially in the nanomedicine field. This section covers several strategies to customize CMNP functionalities supported by literature. One of the most promising strategies involves using membranes derived from specific cell types that naturally target specific tissues. For example, photo-responsive organic or inorganic carriers with cancer cell membranes reveals that the tumor targeting and treatment outcome improved significantly in

photodynamic therapy (D. Zhang et al., 2019). This biomimetic approach exploits the natural properties of cancer cell membranes, allowing for selective binding to tumor cells and improved therapeutic outcomes. Similarly, cancer CMNPs have shown superior targeting capabilities compared to those coated with red blood cell membranes (M. Chen, Chen, & He, 2019; Deng et al., 2018). In addition to selecting appropriate membrane sources, incorporating genetic modifications into membrane proteins can further enhance the targeting capabilities of CMNPs. Recent studies have highlighted the potential of genetically engineered macrophage membranes to improve *in vivo* nanoparticle residence time and thus increase the therapeutic efficacy (Cao et al., 2020). This genetic manipulation allows the expression of desired receptors on the membrane surface, facilitating targeted interactions with disease sites. In addition, hybrid membrane coated systems have emerged as a versatile platform to combine the advantages of different cell types. For example, erythrocyte and cancer cell membrane coated hybrid nanoparticles exhibited long retention time and enhanced accumulation in melanoma cells (J.-D. Li & Yin, 2023). This hybrid approach not only improves biocompatibility but also enables the integration of multiple functionalities such as immune evasion and targeted therapy. CMNPs can be functionalized by integrating additional nanomaterials such as hydrogels or magnetic nanoparticles. It has been stated that combining CMNPs with hydrogels can create localized payload delivery systems that respond to specific stimuli, thus increasing the sensitivity of the therapy (Jianzhuang Li et al., 2023). This integration allows for dynamic interactions with biological environments, improving the overall therapeutic potential of nanoparticles. Moreover, the customization of CMNPs can be tailored to specific therapeutic needs by changing their core materials. For example, using biodegradable polymers such as PLGA in combination with cell membrane coatings has increased drug delivery efficiency and reduced systemic toxicity (C. Sun et al., 2023). This strategy not only improves the pharmacokinetics of drug-loaded nanoparticles but also provides

controlled release profiles that are crucial for effective therapy. In conclusion, the functionalization and customization of CMNPs involves an interdisciplinary approach, selection of appropriate cell membrane sources, genetic modifications, hybridization, and integration of additional materials. By utilizing these strategies, researchers can develop advanced nanoparticle systems that exhibit enhanced targeting capabilities and therapeutic efficacy in a variety of biomedical applications.

5. Biomedical Applications of Cell Membrane-Coated Nanoparticles

CMNPs play an important role in biomedical applications. These nanocarriers have the potential to deeply impact the payload delivery, immunotherapy, and other therapeutic areas due to their enhanced biocompatibility and targeting capabilities. In targeted cancer therapy, nanoparticles have been developed that target tumor growth using membranes derived from cancer cells. In this way, treatment efficacy is improved by increasing the cellular uptake of chemotherapeutic agents. CMNPs also holds a crucial place in immunotherapy; the use of T-cell membrane-encapsulated agonists emphasizes the potential to increase immune responses against tumors. The use of CMNPs is also being investigated against infectious diseases, and the ability to increase the immune system's response to infected tissues is highlighted. As a result, CMNPs offer various biomedical applications, including targeted cancer therapy, immunotherapy, infectious disease management, and regenerative medicine. Their unique properties and innovative customization strategies make these nanoparticles a promising basis for developing therapeutic interventions.

5.1. CMNPs in Drug Delivery Systems

CMNPs have appeared as a promising platform for payload delivery systems that utilize inherent cell membrane properties to enhance efficacy and specificity of therapeutic agents. One of the most significant advantages of CMNPs is their ability to improve targeted drug delivery. For example, nanoparticles coated with

engineered neural stem cell membranes effectively target and deliver drugs to the brain, particularly in the treatment of stroke (Ma et al., 2019). This approach utilizes the inherent chemotactic properties of neural stem cells to enable precise localization of therapeutic agents to injured brain tissues. The findings suggest that CMNPs can be customized for a variety of neurological conditions other than stroke, demonstrating their versatility in drug delivery applications. In cancer treatment, CMNPs have shown significant promise. For example, doxorubicin and cetuximab-coated thermosensitive liposomes loaded with magnetic nanoparticles specifically target EGFR-expressing breast cancer cells (Dorjsuren et al., 2020). This combination therapy enhances chemotherapeutic delivery and demonstrates the potential of CMNPs to integrate multiple therapeutic modalities by utilizing the magnetic properties of nanoparticles for improved targeting and efficacy. Additionally, CMNPs can be engineered to respond to specific stimuli. For example, biomimetic nanoparticles can deliver hedgehog pathway inhibitors to modulate the tumor microenvironment and enhance chemotherapy efficacy for pancreatic cancer (T. Jiang et al., 2018). The ability to alter the tumor microenvironment through targeted drug delivery significantly advances cancer treatment strategies. Beyond cancer and neurological applications, CMNPs have attracted attention due to their potential in treating urological diseases, highlighting their capacity to protect encapsulated drugs from physiological detrimental effects (Yao et al., 2023). This targeted approach is important for improving therapeutic efficacy in prostate and bladder cancer treatments. Customizing CMNPs through surface engineering significantly increases their efficacy; for example, lipid nanoparticles can mimic low-density lipoproteins (LDL) for improved delivery to endothelial cells (Rafiei & Haddadi, 2017). Additionally, the incorporation of biodegradable materials enhances pharmacokinetics and biodistribution. Studies on docetaxel-loaded PLGA nanoparticles have shown improved drug delivery to tumor sites, with biodegradable polymers allowing controlled release and maximizing therapeutic potential (S. Zhen & Li, 2019). The use of

biodegradable polymers increases the safety of drug delivery systems and maximizes treatment efficacy by enabling controlled release of therapeutic agents. In conclusion, CMNPs are a versatile and effective platform for drug delivery in a wide range of applications, improving targeted delivery, responsiveness to stimuli, and integrating multiple therapeutic modalities to improve treatment outcomes.

5.2. CMNPs in Gene Therapy and CRISPR Technology

CMNPs have been the focus of interest in gene therapy and CRISPR technology due to their role in improving the delivery of gene editing tools, especially the CRISPR/Cas9 system. CMNPs facilitate targeted delivery of CRISPR components such as Cas9 protein and guide RNA (gRNA), increasing specificity and efficiency. For example, modified gRNA can be effectively delivered to prostate cancer cells using aptamer-cationic liposomes. Additionally, CMNPs can be engineered for improved stability and targeting, making them a promising strategy for advancing gene therapy and CRISPR applications (S. M. Kim et al., 2018). This approach highlights the potential of CMNPs to improve the targeting of gene editing tools and thereby increase their therapeutic efficacy. CMNPs can increase the in vivo stability and bioavailability of CRISPR components. Direct self-assembly of Cas9 ribonucleoprotein complexes has been shown to facilitate efficient gene editing by reducing off-target effects compared to traditional DNA plasmid delivery systems (Wright et al., 2015). Encapsulation of these complexes within CMNPs protects them from degradation, ensuring functionality during delivery to target cells. Additionally, CMNPs can be customized to contain specific targeting ligands such as aptamers or antibodies, increasing the precision of gene editing by targeting CMNPs to specific cell types. This targeted approach is particularly useful in cancer therapy, where precise editing of oncogenes can improve treatment outcomes. Moreover, CMNPs can aid in the rational design of split-Cas9 enzyme complexes for efficient delivery to target cells (Wollebo et al., 2015). CMNPs hold

significant promise in viral gene therapy, particularly in the context of the CRISPR/Cas9 system. Studies have shown that CMNPs can effectively deliver CRISPR components to eliminate polyomavirus JC infection, inhibiting viral replication (Chaverra-Rodriguez et al., 2018). This versatility highlights the potential of CMNPs to address a variety of viral infections through gene editing strategies. Additionally, CMNPs have been successfully used to deliver CRISPR-Cas9 ribonucleoproteins to arthropod ovaries, facilitating heritable germline gene editing and expanding the applications of CRISPR technology beyond mammalian cells (Yin et al., 2018). Moreover, CMNPs can minimize off-target effects in gene therapy. Studies have shown that partially DNA-guided Cas9 can reduce off-target activity, and combining this approach with CMNPs can increase the safety and efficiency of gene editing (T. Hu et al., 2023). Therefore, CMNPs play an important role in advancing gene therapy and CRISPR technology. CMNPs help specific gene editing technology via various ways such as alleviation of the associated risks by improving targeted delivery, stability, and efficiency, making them a promising platform for future developments in genetic engineering and therapeutic applications.

5.3. CMNPs Applications in Cancer Therapy and Immunotherapy

CMNPs offer an innovative approach to enhance drug delivery and improve cancer treatment and immunotherapy targeting. One of the most important applications of CMNPs is the efficient delivery of chemotherapeutic agents. For example, cetuximab-coated thermosensitive liposomes loaded with doxorubicin and magnetic nanoparticles were developed to target breast cancer cells expressing EGFR. This targeting increases the efficacy of the treatment while avoiding systemic toxicity (Dorjsuren et al., 2020). This targeted approach increases the efficacy of chemotherapy while minimizing systemic toxicity. The use of magnetic nanoparticles improves therapeutic efficacy by increasing the concentration of drugs at the tumor site by directing external

magnetic fields. In addition, CMNPs make an important contribution to immunotherapy by enhancing the immune system's ability to recognize and target cancer cells. Biomimetic cell-derived nanoparticles have the potential to activate immune responses while reducing systemic immunotoxicity (Yang et al., 2023). CMNPs, which mimic the properties of immune cells, can effectively stimulate the immune system, leading to enhanced antitumor responses. In addition, these nanoparticles can be designed to carry immune checkpoint inhibitors. For example, a biomimetic nano-vaccine that induces tumor infiltration of T cells and regulates the immunosuppressive tumor microenvironment has been investigated (Xiong et al., 2021). This approach addresses the challenges of the immunosuppressive nature of tumors while improving the efficacy of immunotherapy. The combination of CMNPs with photothermal therapy (PTT) holds promise in cancer treatment. Cancer-erythrocyte hybrid membrane-camouflaged magnetic nanoparticles developed for ovarian cancer have produced synergistic effects that activate specific immune responses while delivering localized heat to the tumor, enhancing overall therapeutic (Valkema, Mostert, Lagarde, Wijnhoven, & van Lanschot, 2023). Furthermore, CMNPs may enhance the potential of cell membrane-camouflaged nanocarriers to deliver CRISPR/Cas9 components that can regulate genes associated with tumor growth and immune evasion (Ijaz et al., 2024). This innovative approach may increase treatment sensitivity by targeting the genetic basis of tumors. The role of CMNPs in regulating the tumor microenvironment is also critical, emphasizing the importance of immune checkpoint inhibitors in unleashing antitumor responses (L.-Y. Zhang et al., 2020). Researchers can enhance the efficacy of immunotherapy by delivering these inhibitors directly to the tumor site. In conclusion, CMNPs offer a promising solution for cancer treatment with their ability to enhance drug delivery, evoke immune responses, and regulate the tumor microenvironment.

6. Challenges and Future Perspectives

The integration of EVs with CMNPs offers a promising avenue for biomedical applications, especially in the field of targeted drug delivery and diagnostics. EVs that naturally encapsulate bioactive molecules can be engineered to enhance their therapeutic efficacy and specificity when combined with CMNPs that possess unique optical and magnetic properties. This synergistic approach has the potential to enable more effective targeting of diseased tissues such as tumors while minimizing off-target effects. For example, studies have shown that EVs modified with CMNPs can facilitate the direct delivery of chemotherapeutic agents to cancer cells, thereby increasing treatment efficacy and reducing systemic toxicity. Furthermore, the integration of CMNPs into EVs may contribute to the development of personalized medicine by enabling real-time monitoring of therapeutic responses. However, to fully realize the clinical potential of these hybrid systems, challenges such as standardization of EV isolation methods, manufacturing scalability, and regulatory requirements need to be overcome. Future research should focus on optimizing the engineering of EVs and CMNPs to improve their therapeutic capabilities and explore their applications in regenerative medicine and immunotherapy. Furthermore, although engineered EV-CMNP hybrid systems hold great potential for targeted therapy and diagnosis, several challenges remain, such as standard fabrication, immune-compatibility, drug loading efficiency, and clinical scalability. In the future, innovative approaches such as programmable surfaces, synthetic EV analogues, and CRISPR-loaded nano-systems may accelerate the clinical transition of these systems.

6.1. Current Barriers in CMNP and EV Research

Research around CMNPs and extracellular vesicles (EVs) faces several major hurdles that limit their potential in biomedical applications (Tao & Guo, 2020). First, their heterogeneous structure and the presence of similar-sized particles such as lipoproteins in biological samples create difficulties in isolating and characterizing

EVs. This complexity complicates the analysis and quantification processes that are critical for understanding the biological roles and therapeutic applications of EVs. Furthermore, the limited amount of EVs, especially those obtained from the central nervous system (CNS), restricts their availability for research and clinical use (Zhong et al., 2024). The lack of standard protocols for EV extraction and characterization leads to variability across studies, making it difficult to compare findings and establish reliable clinical applications (Ferreira-Faria et al., 2022). In addition, there are challenges in the transition from preclinical studies to clinical applications, such as regulatory barriers and the need for high-throughput manufacturing methods. The low application efficiency of EVs in clinical settings is due to their complex biological interactions and the need for targeted delivery systems to enhance their therapeutic efficacy . Therefore, researchers are seeking innovative solutions to increase the scalability and functionality of EVs and CMNPs and to develop robust methodologies for clinical translation. Overcoming these challenges is critical to unlocking the full potential of CMNPs and EVs in therapeutic and diagnostic applications.

6.2. Regulatory and Ethical Issues

The use of CMNPs in biomedical research and applications is fraught with important regulatory and ethical issues to ensure safe and effective outcomes. The need for comprehensive guidelines for the production, characterization, and clinical application of these nanomaterials is highlighted by the fact that current regulations often lag behind technological advances. This creates uncertainties about the safety and efficacy of CMNPs in therapeutic contexts. For example, the special properties of CMNPs may pose unforeseen risks, and therefore, rigorous preclinical testing and transparent reporting of potential adverse effects are required (Y. Zeng et al., 2022). Researchers need to ensure that participants are fully informed about how their biological material will be used and the potential risks and benefits (De Wever & Hendrix, 2019). Data

privacy and ownership issues also present complexities in the context of biobanking and the use of electronic health records for research purposes. Ethical frameworks must guide the responsible use of patient data by balancing public health interests with individual rights (Bull & Bhagwandin, 2020). As the field of nanomedicine evolves, it is imperative to establish robust ethical guidelines and regulatory frameworks that protect participants while encouraging innovation and trust in these new technologies (Graur et al., 2011). Consequently, the regulatory and ethical dimensions of CMNPs require a collaborative approach among researchers, regulatory agencies, and ethicists to ensure that advances in nanomedicine are achieved responsibly and equitably.

6.3. Future Trends and Emerging Technologies

The field of nanomedicine is rapidly evolving with technological advances and increasing understanding of the therapeutic and diagnostic uses of nanoscale materials. Future trends will focus on several key areas, including the development of innovative drug delivery systems, advanced imaging techniques, and the integration of artificial intelligence (AI) and machine learning (ML). In particular, the use of laser-generated nanoalloys as theranostic platforms for cancer treatment stands out as a promising direction. These nanoalloys offer unique optical properties for both imaging and targeted therapy, enabling more precise treatment modalities (Geller et al., 2014). Furthermore, photonic nanomedical applications are supported by fiber optic developments that enable light to penetrate deeper into tumors, increasing the efficacy of light-based therapies (Dean, Beggs, & Keane, 2010). Nanomedical applications extend beyond oncology to include acute brain injuries and neuroprotective strategies. Studies show that nanoparticles can directly deliver chemotherapeutic drugs to targeted brain regions by crossing the blood–brain barrier (BBB) (Kjellström & Fridlund, 2010). Clinical translation of nanomedicines continues to be a critical focus with efforts to facilitate commercialization pathways. Current trends emphasize the importance of understanding disease-

specific properties in the design of personalized nanomedicines (Amendola, 2024). Furthermore, the emergence of products that mimic existing nanomedicines, referred to as "nano-similars," poses regulatory challenges to ensure safety and efficacy (de Oliveira et al., 2021). In regenerative medicine, nanomedicines are being investigated for their potential to enhance drug delivery and improve therapeutic indices while minimizing off-target effects (Kandell, Waggoner, & Kwon, 2020). The COVID-19 pandemic has accelerated nanotechnology innovations, particularly in the development of mRNA vaccines, highlighting the potential of nanomedicine to meet urgent public health needs (Y. Xu et al., 2020). As the field advances, the integration of AI and automation in nanomedicine design and testing is expected to increase reproducibility and drive innovation. These technologies can facilitate the rapid identification of effective formulations and optimization of delivery systems (Zaslavsky, Bannigan, & Allen, 2023). As a result, the future of nanomedicine is poised for significant advances with emerging technologies and innovative approaches that promise to improve the efficacy and safety of therapeutic interventions. Continued interdisciplinary collaboration and investment in research are critical to overcoming current challenges and fully realizing the potential of nanomedicine in clinical applications.

7. Conclusion

Engineered membrane-coated nanoparticles are innovative tools with the potential to revolutionize biomedical applications due to their biomimetic surface properties and functional versatility. CMNPs present significant advantages in areas such as cancer treatment, immunotherapy, infection control, and tissue engineering, such as target-specific transport, immune evasion, and long circulation time. CMNPs boost drug delivery, improve targeting, and increase treatment efficacy by utilizing the natural properties of cell membranes. The structural and functional properties, biocompatibility, and stability of these nanoparticles make them a

versatile platform for the treatment of various diseases, such as cancer, neurological diseases, and infections. CMNPs, which are developed by coating biological membranes obtained from various sources such as tumor cells, immune cells, platelets or bacterial membranes, show superior performance in terms of both homotopic targeting and directing therapeutic responses. For example, CMNPs used in cancer treatment mimic the surface properties of tumor cells, optimize targeted drug delivery and minimize side effects throughout the treatment process. This both increases treatment efficacy and improves the quality of life of patients. The historical development of CMNPs has paved the way for innovative extraction methods and engineering techniques that allow the production of customized nanoparticles for specific therapeutic needs. This process contributes to the development of more effective and safe treatment methods in nanomedical applications. EV technologies play an important role as supporting platforms that increase the functionality of CMNPs. Engineered EVs are evaluated in hybrid structures to increase the targeting ability or biological transport capacity in some CMNP systems. These hybrid membrane-coated nanoparticles can both manage immune system interactions and guide treatment by carrying multiple cellular features at the same time. For example, hybrid structures using tumor cell membrane and EVs together enable both homotopic targeting and immune modulation on the same platform. However, CMNPs as the basic carrier structure have the flexibility to carry both theranostic agents and genetic materials in a multimodal manner thanks to their synthetic core. Hybrid membrane coating strategies can further develop multimodal structures, enabling synergistic applications in targeted therapy, imaging, and immunotherapy. In the future, CMNPs and especially their hybrid membrane-coated versions are expected to become more prominent in personalized treatment approaches. CMNPs coated with membranes specific to specific disease states can be customized according to individual patient profiles, making targeted treatment processes more effective and safer. However, some important challenges need to be overcome for

these technologies to be able to move into clinical applications, such as scalable production, long-term biocompatibility, safety assessments, and clarification of regulatory processes. In conclusion, the integration of CMNPs and their hybrid membrane-coated forms into biomedical systems has the potential to create a comprehensive transformation from disease diagnosis to treatment. When supported by interdisciplinary collaboration, advanced engineering techniques and sustainable research investments, it is inevitable that these biomimetic systems will gain a central place in clinical treatment protocols and become trend-setting technologies in nanomedicine.

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Dear Dr. Purwar and the Team,

Based on our conversation at the last meeting we would like to hear back from you about the LV batch order deliveries. As we already signed the Term Sheet, we delivered the intention of our production validations next month. Therefore the timing of the Lentiviral Vector (LV) delivery process is critical for us to align with the validation studies planned for January and the CAR-T Summit we aim to hold in February.

In relation to the process already initiated, could you please provide information on the earliest possible delivery date? Additionally, we would appreciate your advice on what steps can be taken to expedite the process as much as possible. Your guidance on this matter would be very helpful."

GEÇİCİ KAPAK

Kapak tasarımı
devam ediyor.