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Prof. Dr. Hasan AKGÜL

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CONTENTS

CHAPTER 1

PANNEXIN CHANNELS FROM NEURODEVELOPMENT TO NEUROLOGICAL DISEASE

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1. Introduction

Cell-to-cell communication is essential for embryonic development and tissue homeostasis, and its dysregulation may contribute to various diseases. This coomunication is mediated through several signaling mechanisms, including autocrine, paracrine, endocrine, and gap junction signaling (Su et al. 2024). In particular, gap junctions play a key role in embryogenesis; by facilitating intracellular communication between cells, they help regulate cellular behaviours such as proliferation, migration and differentiation.

Gap junctions channels are formed by connexin (Conx) proteins, which are essential mediators of direct intercellular communication (Matsuuchi & Naus 2013). In addition to Conxs, which constitute the principal components of vertebrate gap junction channels, pannexins (Panxs) have emerged as an important family of transmembrane channel proteins over the past two decades.In addition to connexins, the Panx transmembrane channels described by Panchin et al. have gained significant importance in recent years (Panchina et al. 2000). Panxs are a family of vertebrate proteins defined by their homology with the innexins (Inxs) of invertebrates (Panchina et al. 2000). In contrast to Inxs, Panxs predominantly function as large-pore membrane channels mediating communication between intracellular and extracellular compartments (Bao et al. 2004; Shestopalov & Panchin 2008). Panxs have been implicated in several key neurodevelopmental processes, including cell migration, neuronal differentiation, and neural circuit formation. Although recent studies have documented Panx expression in various embryonic brain regions, their contribution to nervous system development remains only partially understood. Consequently, further research is needed to elucidate their precise roles and molecular mechanisms in neurodevelopment. In this chapter, we provide a comprehensive overview of pannexins in nervous system development, with particular emphasis on their embryological functions, development regulationi and clinical relevance in neurological disorders.

2. Structure and Physiological Functinos of Pannexin Channels

2.1 Molecular Structure of Pannexins

The mammalian pannexin family comprises three isoforms, Panx1, Panx2, and Panx3. Whereas Panx1 and Panx3 are encoded on chromosome 11, Panx2 is encoded on chromosome 22 and constitutes the largest family member due to its extended C-terminal domain (Baranova et al. 2004). Panx proteins have a four-transmembrane (4TM) topology and consist of two

extracellular loops (ECL1/ECL2), cytoplasmic N- and C-termini, and an intracellular loop (Bhat & Sajjad 2021). Post-translational N-glycosylation of panxs occurs within their extracellular loops and plays a pivotal role in regulating protein trafficking and cellular localization. The glycosylation sites differ among panxs isoforms, occurring at Asn254 in the second extracellular loop (ECL2) of Panx1, Asn86 in the first extracellular loop (ECL1) of Panx2, and Asn71 within ECL1 of Panx3 (Penuela et al. 2009). N-glycosylation is required for the efficient trafficking of Panx1 and Panx3 to the plasma membrane. In contrast, Panx2 predominantly resides within intracellular compartments, including the endoplasmic reticulum and Golgi apparatus, independently of its glycosylation state (Sanchez-Pupo et al. 2018). The regulation of panxs channels extends beyond glycosylation. It includes several additional post-translational modifications, such as phosphorylation, deacetylation, S-nitrosylation, ubiquitination, and lipidation, which collectively regulate channel function and cellular localization (Chiu et al. 2021; D'Hondt et al. 2013; Lohman et al. 2012; Penuela et al. 2014).

The three panx isoforms exhibit distinct tissue distribution profiles. Panx1 is ubiquitously expressed throughout the body, whereas Panx2 shows a more limited expression pattern, predominantly in the nervous system and selected peripheral organs (Baranova et al. 2004). In contrast, Panx3 is mainly localized to tissues involved in epithelial, vascular, and skeletal functions, including the skin, inner ear, mammary gland, testis, blood vessels, small intestine, and skeletal system (O'Donnell & Penuela 2021).

Advances in Cryo-electron microscopy (Cryo-EM) have provided detailed insights into the structural organization of panx channels, demonstrating that they are composed of seven subunits surrounding a central pore (Hussain et al. 2024; Jin et al. 2020; Zhang et al. 2023). This architecture enables the selective passage of small molecules, including amino acids, nucleotides, and ions. Consistent with their broad tissue distribution, panx channels have been implicated in numerous physiological processes across multiple organ systems, such as the nervous, cardiovascular, respiratory, renal, digestive, urinary, and musculoskeletal systems (Jiang et al. 2025).

2.2 **Expression of Panx Isoforms in the Nervous System**

The distribution of panx isoforms within the nervous system is highly isoform-specific. Panx1 exhibits widespread expression throughout both the central and peripheral nervous systems, whereas Panx2 is predominantly confined to selected CNS regions, including the cerebellum,

brainstem, and spinal cord. Unlike Panx1 and Panx2, Panx3 appears to be absent from neural tissues, suggesting distinct physiological roles among panx family members.

2.2.1. Neurons

The localisation of Panx1 within the nervous system has been demonstrated in various studies. A study by Zoidl and colleagues indicated that Panx1 exhibits postsynaptic localisation by accumulating in postsynaptic terminals in the rodent hippocampus and in cortical principal neurons (Zoidl et al. 2007). Recent studies using human cerebral organoids have demonstrated that Panx1 exhibits a dynamic expression pattern during neurodevelopment. During neural induction and neuroepithelial expansion, Panx1 becomes highly enriched at the apical membrane domain of neuroepithelial rosettes and buds, where it co-localizes with key adherens junction proteins such as β-catenin and N-cadherin. As development progresses, Panx1 expression shifts toward neuronal populations, suggesting stage-specific roles in neuroepithelial organization and neuronal maturation (Noort et al. 2024). Panx2 exhibits a dynamic subcellular distribution during neurogenesis. In early neural progenitor cells, Panx2 is predominantly localized to the endoplasmic reticulum and Golgi apparatus, whereas neuronal maturation is accompanied by its redistribution to the plasma membrane, where it is re-expressed in mature neurons (Swayne et al. 2010). Unlike Panx1 and Panx2, Panx3 is generally considered absent from the nervous system under physiological conditions. However, a compensatory upregulation of Panx3 has been reported in the vomeronasal organ of Panx1-deficient mice, where it was detected in autonomic nerve fibers and non-sensory epithelial regions, suggesting a potential adaptive role following Panx1 loss (Whyte-Fagundes et al. 2018).

2.2.2 Astrocytes

Panx1 is expressed in astrocytes and has been implicated in astrocyte-mediated inflammatory signaling, where its activation contributes to inflammasome activity and the propagation of neuroinflammatory responses following brain injury (Tichauer & Rovegno 2025). Panx2 was detected in the cytoplasm of immature primary astrocytes, whereas its expression decreased substantially as astrocytes matured in vitro, indicating a potential role for Panx2 during early stages of astrocyte development and differentiation (Le Vasseur et al. 2014). Whyte-Fagundes

et al. demonstrated that Panx3 expression is significantly increased in the vomeronasal organ of Panx1-deficient mice. The preservation of ATP release and sensory behaviors in these animals suggests that Panx3 may partially compensate for the loss of Panx1, indicating functional overlap between pannexin isoforms in neural tissues (Whyte-Fagundes et al. 2018). Although Panx3 expression in the nervous system is generally absent or very limited, this study provides supportive evidence that Panx3 may contribute to neural tissue function under compensatory conditions.

2.2.3. Oligodendrocytes

The functional expression of Panx1 channels in oligodendrocytes was first described by Domercg et al. (2010). In this study, it was reported that both Panx1 and Panx2 were detected in oligodendrocytes derived from O41 cell cultures using immunocytochemistry and RT-PCR, but Panx3 could not be detected (Domercq et al. 2010) (Vejar et al. 2019). Using primary oligodendrocyte cultures, the authors demonstrated that oxygen-glucose deprivation (OGD) promotes ATP release and oligodendrocyte death through a mechanism involving Panx1 channel activation and subsequent P2X7 receptor signaling. However, the research is rather limited, and there is a need for further regional studies.

2.2.4. Microglia

Pannexin channels have been implicated in the regulation of microglial development and morphology. In particular, Panx1-mediated ATP release in response to glutamatergic signaling contributes to the remodeling of dendritic architecture and the dynamic surveillance activity of microglial processes (Fontainhas et al. 2011). Microglia predominantly express Panx1, whereas Panx2 expression remains relatively low, particularly under stress-related conditions (Rigato et al. 2012). Evidence indicates that amyloid-β triggers a feed-forward hemichannel signaling cascade, whereby ATP and glutamate released from microglia and astrocytes activate neuronal hemichannels, thereby amplifying neurotoxic signaling and promoting neuronal loss. Consequently, modulation of pannexin-dependent signaling in both glial and neuronal populations has emerged as a potential therapeutic strategy for protecting neurons against amyloid-β-induced damage (Orellana et al. 2011). The expression of Panx3, however, has not been demonstrated in oligodendrocytes.

2.2.5. Schwann Cells

Schwann cells are the principal glial cells of the peripheral nervous system (PNS) that ensheath axons. They are broadly categorized into myelinating Schwann cells, which form myelin around individual axonal segments, and non-myelinating (Remak) Schwann cells, which support axonal maintenance, neuronal survival, peripheral nerve repair, and neuromuscular junction remodeling (Gherghiceanu et al. 2020). In the PNS, Panx1 is abundantly expressed in Schwann cells and is markedly upregulated following nerve injury. Immunofluorescence analyses demonstrated that Panx1 colocalizes with the Schwann cell marker S100β, supporting a role for Schwann cell Panx1 in neuroinflammatory responses and neuropathic pain development (Wang et al. 2022). Although no data is currently available on Panx2 and Panx3 in Schwann cells, further research into these proteins is required.

2.2.6. Satellite Glial Cells (SGCs)

Satellite glial cells (SGCs) are specialized glial cells that envelop the soma of primary sensory neurons within peripheral sensory ganglia, forming a distinct functional unit with the associated neuron. Beyond their structural and metabolic support functions, SGCs actively regulate neuronal homeostasis through bidirectional communication and the release of signaling molecules, thereby influencing neuronal excitability (Qiu et al. 2024).
Retamal et al. demonstrated that SGCs express Conx43, whereas Panx1 is predominantly localized in sensory neurons of the nodose ganglion. Their findings further suggest that Conx43 hemichannels and Panx1 channels contribute to paracrine signaling between SGCs and neurons, thereby modulating sensory neuronal activity and excitability (Retamal et al. 2014). Emerging evidence suggests that SGC activation following inflammation or nerve injury contributes to altered neuron–glia communication. As with Schwann cells, further research is needed on Panx2 and Panx3.

3. Pannexins in Neurological Disorders

3.1. Alzheimer’s disease

Alzheimer’s disease (AD) is a progressive, age-related neurodegenerative disorder characterized by a gradual decline in cognitive functions, particularly learning and memory. Although the disease is classically associated with extracellular amyloid-β (Aβ) plaque

deposition and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein, growing evidence suggests that early synaptic dysfunction and synaptic loss are more closely correlated with cognitive impairment than these traditional neuropathological hallmarks (Nelson et al. 2012). In the study conducted by Flores-Muñoz et al. (2020), the role of Panx1 channels in the early synaptic alterations associated with AD was investigated using APP/PS1 transgenic mice. The authors demonstrated that hippocampal Panx1 expression and activity were significantly increased in AD mice and positively correlated with Aβ accumulation. Furthermore, acute pharmacological blockade of Panx1 with probenecid improved synaptic plasticity, restored dendritic spine density, and reduced p38 MAPK activation, suggesting that Panx1 contributes to early synaptic dysfunction during AD progression (Flores-Munoz et al. 2020). In particular, this study has demonstrated that Panx1 is overexpressed and functionally hyperactive in the hippocampus of APP/PS1 mice. Panx1 immunoreactivity was detected both in neurons and in reactive astrocytes surrounding amyloid plaques, and the expression of this protein increased with age in parallel with Aβ accumulation; this suggests that Panx1 may contribute to Aβ-associated neurotoxicity. Consistent with experimental findings in AD models, Panx1 channels are increasingly recognized as key mediators of neuroinflammation through ATP-dependent activation of purinergic signaling pathways in neurons and glial cells (Seo et al. 2021).

3.2. Epilepsy

Epilepsy is a chronic neurological disorder characterized by a persistent predisposition to generate recurrent, unprovoked seizures resulting from abnormal and excessive neuronal activity within the brain (Fisher et al. 2014). Li et al. demonstrated that Panx1 expression is significantly increased in focal cortical dysplasia (FCD)-associated epileptic lesions at both the mRNA and protein levels. Moreover, Panx1 was prominently localized in dysmorphic neurons, balloon cells, and reactive astrocytes, and its protein expression positively correlated with seizure frequency, suggesting a potential role in epileptogenesis (Li et al. 2017). Also, while Panx1 was broadly upregulated across FCD lesions and correlated with seizure frequency, Panx2 expression was selectively increased in FCDIIb lesions and was primarily detected in SOX2- and MASH1-positive balloon cells, suggesting distinct roles for Panx1 and Panx2 in epileptogenesis and lesion pathogenesis, respectively (Li et al. 2017). In another study, Jiang and colleagues reported a significant increase in Panx1 in the temporal cortex of patients with temporal lobe epilepsy; in contrast, Panx2 expression remained unchanged. This finding

supports the notion that Panx1 plays a potential role in epileptogenesis (Jiang et al. 2013). Taken together, the available evidence indicates that Panx1 is a key mediator of seizure-related neuronal dysfunction and neuroinflammation, supporting its involvement in the initiation and progression of epileptic pathology.

3.3. Pain

Experimental studies have demonstrated that the loss of Panx1, either globally or specifically in neurons, reduces CFA-induced pain hypersensitivity, highlighting a critical role for Panx1 in the maintenance of chronic pain states (Xing et al. 2024) . In a this study, neuronal Panx1 deletion was shown to impair neuronal differentiation in vitro, accompanied by suppression of Wnt/β-catenin signaling, reduced cellular excitability, and a diminished response to ATP stimulation (Xing et al. 2024). On the other hand, cortical spreading depression (CSD) is a phenomenon in which a decrease in brain activity occurs following slowly spreading depolarisation waves (Charles & Baca 2013). Karatas et al. (2013) demonstrated that cortical spreading depression (CSD) induces neuronal Panx1 channel activation, leading to caspase-1 activation and HMGB1 release. The resulting inflammatory signaling was proposed to promote astrocyte activation through the NF-κB pathway (Karatas et al. 2013).

4. Conclusion

Pannexin channels have emerged as important regulators of nervous system development and function, extending their roles far beyond their classical involvement in ATP release and intercellular signaling. Accumulating evidence indicates that pannexin isoforms contribute to key neurodevelopmental processes, including neural progenitor maintenance, neuronal differentiation, neuron–glia communication, and the establishment of functional neural networks. Among the family members, Panx1 is the most extensively studied and appears to play a central role in both physiological and pathological processes within the nervous system. Increasing evidence further implicates Panx1 in the pathogenesis of several neurological disorders, including Alzheimer’s disease, epilepsy, and chronic pain, primarily through its involvement in neuroinflammatory and purinergic signaling pathways. In contrast, the functions of Panx2 and particularly Panx3 remain incompletely understood, highlighting important gaps in our current knowledge. Future studies integrating developmental, molecular, and translational approaches will be essential to clarify the precise roles of pannexin channels in

neural development and disease and to evaluate their potential as novel therapeutic targets for neurological disorders.

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CHAPTER 2

THE METTL SUPERFAMILY PROTEINS IN CANCER: EMERGING ROLES OF M6A WRITERS IN TUMORIGENESIS

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Abbreviations

AML: Acute Myeloid Leukemia

BRCA: Breast Cancer

CCA: Cholangiocarcinoma

CRC: Colorectal Cancer

DDLPS: Dedifferentiated Liposarcoma

ESCC: Esophageal Squamous Cell Carcinoma

HCC: Hepatocellular Carcinoma

HNSCC: Head and Neck Squamous Cell Carcinoma

ICC: Intrahepatic Cholangiocarcinoma

KIRC: Kidney Renal Clear Cell Carcinoma

KIRP: Kidney Renal Papillary Cell Carcinoma

LMS: Leiomyosarcoma

OSCC: Oral Squamous Cell Carcinoma

STS: Soft Tissue Sarcoma

Introduction

Post-transcriptional modifications control the activity of several cellular pathways. N6-methyladenosine (m6A) is the most prevalent internal post-transcriptional modification in messenger ribonucleic acid (mRNA) in the majority of eukaryotic organisms, including yeast, insects, plants, mammals, and some viruses (Huang et al., 2020). m6A is primarily engaged in several aspects of RNA metabolism, such as mRNA degradation, stability, splicing, transportation from the nucleus, translation, RNA-protein interactions, and embryonic development (Wang et al., 2014; Roundtree et al., 2017; Wang et al., 2015; Liu et al., 2015; Mendel et al., 2018). By this way, these post-transcriptional covalent modifications of RNA serve as a "second dimension of code" for dynamic control, accurately regulating the spatiotemporal specificity of gene expression (Boccaletto et al., 2018; He, 2010). In addition to its role as an RNA regulatory modulator, m6A also plays an essential role in gene transcription, DNA demethylation and chromatin accessibility (Deng et al., 2022).

A family of RNA m6A methyltransferases, comprising methyltransferase-like 3 (METTL3), METTL5, METTL4 and METTL16 act as “writers” of m6A RNA methylation (Bokar et al., 1997; Warda et al., 2017; vanTran et al., 2019; Goh et al., 2020), which can be eliminated by "eraser" proteins such as AlkB homolog 5 (ALKBH5) and fat mass and obesity-associated protein (FTO) (Zheng et al., 2013). Certain "reader" proteins such as the YTH domain-containing proteins, can recognize and bind to the m6A-modified RNA, affecting RNA

processing, translation, and stability (Wang et al., 2014). Together, writer, eraser, and reader proteins regulate several metabolic processes of m6A-containing RNA by adding, removing, or recognizing the m6A modifications and preserving the dynamic balance of m6A modification (Jia et al., 2011; Liu et al., 2014; Bokar et al., 1997). Here we present a comprehensive view on the role of METTL3, METTL4, METTL5, METTL14 and METTL16 and their involvement in cancer.

METLL Superfamily Proteins

METTL proteins are members of the seven-beta-strand (7BS) methyltransferase subfamily, which constitutes METTL proteins and SET-domain (Su(var)3–9, enhancer-of-zeste, trithorax) protein methyltransferases (Petrossian et al., 2011). Out of the 34 known human METTLs (Campeanu et al., 2021), 11 members of the METTL family of proteins participate in modifications, including 7-methylguanosine (m7G), m6A, 5-methylcytosine (m5C), and other modifications that impact related signaling pathways to control transcript splicing and translation (Boccaletto et al., 2018).

METTL proteins typically have a pro-oncogenic role in cancer, but they also have various context-dependent outcomes. Tumorigenesis and METTL-mediated epigenetic changes play intricate and varied functions that include several regulatory pathways, diversified regulatory mechanisms, and distinct regulatory consequences (Su et al., 2022). The positive effect on cancer of aberrant m6A modification made by some of the METTLs is associated with different cellular pathways (e.g., RAS, c-Myc, and histone deacetylase (HDAC) pathways); tumor immune microenvironment (e.g., cytokine signaling); or controling the specific “reader” proteins (Zou et al., 2022; Han et al., 2023). Since several METTLs show carcinogenic and tumor suppressor effects, there are various functions of METTL family members across tumor types (Su et al., 2022).

METTL3

The major writer of the m6A modification on mRNA in eukaryotic cells is the METTL3 and METTL14 heterodimer as the multicomponent m6A methyltransferase complex (MCT) (Liu et al., 2014). In the nucleus, this complex's primary function is to methylate nascent pre-mRNA (Ke et al., 2017). Although both METTL3 and METTL14 have methyltransferase domains, the principal role of METTL14 is to help METTL3 attach to RNA substrates since it lacks a S-

adenosylmethionine (SAM)-binding motif in its catalytic site (Wang et al., 2016). METTL3 facilitates m6A modifications in approximately 95% of cellular mRNAs and targets a range of non-coding RNA (ncRNA) species, such as tRNAs, snRNAs, miRNAs, and lncRNAs (Poh et al., 2022). The MCT complex has different additional accessory proteins, such as the Wilms tumor 1-associated protein (WTAP), which primarily provides stabilization of the complex that promotes m6A modification on RNA and lacks methyltransferase activity itself (Ping et al., 2014). CBL proto-oncogene-like protein 1 (CBLL1/HAKAI), RNA-binding motif proteins (RBM15/RBM15B), zinc finger CCCH domain-containing protein 13 (ZC3H13), and Virus-like m6A methyltransferase-associated protein (VIRMA/KIAA1429) are a group of additional proteins that are involved in the m6A modification process in addition to WTAP (Schwartz et al., 2014) (Patil et al., 2016) (Wen et al., 2018) (Knuckles et al., 2018) (Růžičkaet al., 2017). These MCT-associated proteins affect stability, substrate selection, catalytic efficiency, and substrate binding (Yue et al., 2018).

METTL3 has mostly oncogenic effects by involving several different cellular pathways for the different cancer types that have hematological tumors. In gastrointestinal cancers, METTL3 has several effects, especially on colorectal cancer (CRC), gastric cancer, hepatocellular carcinoma (HCC), and esophageal squamous cell carcinoma (ESCC) diseases. In CRC cells, it sustains the expression of the oncogenic SOX2 gene via the m6A in the IGF2Bp2-dependent pathway (Li et al., 2019). Additionally, by associating with the mRNA of the glycolysis-related genes HK2 and glucose transporter 1 (GLUT1), METTL3 mediates m6A-dependent translational control of GLUT1, increasing glucose absorption that contributes to CRC carcinogenesis (Shen et al., 2020; Chen et al., 2021). METTL3 has a dual role in gastric cancer as a result of its canonical and non-canonical functions. Through the m6A modification, by suppressing the production of miR-22-3p and miR-320a, METTL3 increases cell proliferation due to THAP7-AS1 overexpression (Liu et al., 2021). Besides, by interacting with PABPC1, it enhances interaction with eIF4F and RNA looping, thus promoting translation initiation (Wei et al. 2022). By suppressing the expression of SOCS2 via a mechanism that relies on YTHDF2, METTL3 has been demonstrated to enhance the growth of HCC and play a role in the progression of HCC (Chen et al., 2018). Research has demonstrated that METTL3 contributes positively to ESCC metastasis and dissemination by promoting oncogenic EGR1 as a downstream target of the m6A modification (Liao et al., 2022).

METTL3 also plays a part in cancer types such as breast cancer (BRCA), prostate cancer, and endometriosis that are linked to the reproductive system or hormones. Enhanced m6A

methylation mediated by METTL3 has been linked to increased tumorigenicity in BRCA, potentially by altering the stability and translation of genes that play a role in cell cycle progression and metastasis (Li et al., 2025). In a m6A-dependent way, METTL3 stimulates the expression of the oncoprotein HBXIP. A positive feedback loop is created when HBXIP suppresses the tumor suppressor miRNA let-7g (Cai et al., 2018). The METTL3 can also result in the invasion of cancer cells in prostate cancer cells. In prostate cancer tissues with bone metastases, METTL3 overexpression is found. This can raise the amount of ITGB1 expression to encourage cell adhesion to collagen I, which improves cell motility and bone metastasis (Li et al., 2020). METTL3 knockdown promotes the development of endometriosis by increasing cell invasion and migration in a way reliant on the METTL3/m6A/miR126 axis (Li et al., 2021).

In glioblastoma stem-like cells (GSCs), METTL3 expression was shown to be both raised and decreased throughout differentiation, indicating a correlation with the clinical aggressiveness of malignant gliomas (Visvanathan et al., 2018). Moreover, in oral squamous cell carcinoma (OSCC), METTL3 interacts with the 3′ UTR (next to the stop codon) of the c-Myc mRNA to add m6A alterations, hence boosting the stability of c-Myc through the reader protein YTHDF1 (Zhao et al., 2020). METTL3 increases the expression of CDC25B, which triggers CDK1/cyclin B to start the G2/M transition via m6A, promoting the development of head and neck squamous cell carcinoma (HNSCC) (Guo et al., 2022). In a study, the expression of cell cycle-associated proteins such as phospho-CDC2, CDK2, cyclin D2, cyclin D3, E2F1, and phospho-Rb was markedly downregulated by METTL3 knockdown, which caused the cell cycle to enter the G1 phase and prevented the proliferation and colony formation of uveal melanoma cells (Luo et al., 2020). A study discovered that METTL3 protein is found in the cytosol differently than METTL14 and WTAP in lung cancer cells. As a result of the non-canonical function of METTL3, it promotes translation of some oncogenic genes, such as BRD4, by interacting with eIF3h. The N-terminus end of METTL3 can form an mRNA loop with eIF3 in both its catalytically inactive and wild-type forms (Lin et al., 2016; Choe et al., 2018). According to recent findings, METTL3 functions as an oncogene in acute myeloid leukemia (AML) cells and can function as a chromatin-based regulatory factor by being attracted to the chromatin by the CAATT-box binding protein CEBPZ, which causes m6A deposition on the corresponding mRNA transcripts. It is found at the SP1 and SP2 transcription start sites (Barbieri et al., 2017).

METTL4

METTL4 is a SAM containing methyltransferase protein located in the nucleus and mediating m^6A methylation of U2 small nuclear RNA (snRNA) (Goh et al., 2020; Chen et al., 2020). METTL4 preferentially acts on Am, while many METTL family proteins prefer A. Yet this preference appears to differ across species, drosophila METTL4 seems to prefer A in U2 snRNA because of its difference in loop structure (Luo et al., 2022). Since U2 snRNA is an important component of the major spliceosome, methylation of this molecule affects the splicing of mRNAs (Goh et al., 2020; Donmez et al., 2004). It is important to add that the methylated adenosine (A_{30}) is localized in the upstream of the branch point recognition sequence, supporting the idea of METTL4 mediated U2 snRNA methylation affecting the mechanism of spliceosome complex (Donmez et al., 2004). Furthermore, it was observed that the depletion of METTL4 protein in human cells results in 3' splice-site weakness and an increase in exon inclusion (Goh et al., 2020).

METTL4 has been studied in various cancer types, including glioma and soft tissue sarcoma. According to the study of Zhang et. al., the high expression of METTL4 was related with poor prognosis, and they stated the high expression of METTL4 had poorer overall survival compared to the low METTL4 expression group of. METTL4 appears to be associated with cell cycle and immune infiltration, but not with apoptosis. They observed cell cycle arrest in G0/G1 and higher mitochondrial reactive oxygen species levels in METTL4 knockout cells. Zhang et. al. stated that METTL4 can be used as an immunotherapeutically relevant biomarker considering its role in regulating immune filtration of glioma (2025). A study made in soft tissue sarcoma (STS) revealed that high diploid copy number of METTL4 is linked to better overall survival in STS and its subtypes leiomyosarcoma (LMS) and dedifferentiated liposarcoma (DDLPS). In addition, diploid copy number was found to be closely related with higher infiltration fraction of resting mast cells. Wang et. al., pointed out that the copy number variation of METTL4 can be used as a biomarker for STS. Moreover, low copy number variation of METTL4 in STS suggested being sensitive to Temozolomide and Olaparib (Wang et al., 2021). A study made by Hsu et. al., showed 6mA levels of mammalian tumor cells increase in hypoxic conditions mediated by METTL4. More importantly, the increased activity of METTL4 results in activating metastasis-inducing genes, mechanism of this pathway and regulation of METTL4 expression was shown to be linked to HIF-1α, the results of the study making METTL4 a potential therapeutic target for hypoxia involved tumors (Hsu et al., 2022).

METTL5

METTL5 contains a SAM-binding domain and mediates m^6A methylation of 18S ribosomal RNA (rRNA) at the position A_{1832}. It localizes within the nucleolus where the ribosome biogenesis takes place (Van Tran et al., 2019). METTL5 interacts with TRMT112, which improves its stability by nearly 100-fold (Chen et al., 2020 ; van Tran et al., 2019). In addition, METTL5 knockdown in human 293T cells causes the loss of 70% of m6A methylation in 18S rRNA (Van Tran et al., 2019). Methylation of 18S rRNA at the position of A_{1832} affects translation specificity, since the methylated adenosine is in a close area to ribosome decoding center where tRNA interacts with mRNA (Sepich-Poore et al, 2022).

In many cancer studies, METTL5 expression highlights the relation of rRNA modifications to cancer development. The high expression of METTL5 in intrahepatic cholangiocarcinoma (ICC) was studied by Dai et. al., they stated overexpression of METTL5 in ICC is related to poor survival. They observed that reducing the 18S m6A modification prevents the ICC growth and metastasis. The reason is thought to be the translation of G-quadruplex (G4) motif containing oncogenic signals were affected (Dai et al., 2023). Another study made by Xia et. al. in hepatocellular carcinoma (HCC) showed the high expression of METTL5 affects the glucose metabolism, therefore promotes the proliferation and metastasis of HCC. The mechanism was suggested to be about c-Myc stabilization stimulated by METTL5 activity. They later discovered that ubiquitin specific peptidase 5 (USP5) is also a part of the pathway of regulating the glucose metabolism and enhancing the tumor growth. Regarding all this information, using METTL5 as a biomarker and a therapeutic target was suggested to be possible in future (Xia et al., 2023). Rong et. al. discovered that METTL5 is necessary for proper S6K phosphorylation in HEK293T and HeLa cells, leading them to hypothesize METTL5 is important for tumor growth. Therefore, they tested on breast cancer cells by using siRNAs to knock down METTL5, their results showed the tumor growth was significantly reduced compared to the control group (Rong et al., 2020). Moreover, studies made on HCC, kidney renal clear cell carcinoma (KIRC) and kidney renal papillary cell carcinoma (KIRP) showed METTL5 overexpression results in poor survival by immune environment regulation (Wang & Peng, 2023; Zhang et al., 2022).

METTL14

Despite having no enzymatic activity, METTL14 is involved in mRNA binding and targeting by MCT. It is the major stabilizing unit of the MTC and has a significant role in the methylation activity by forming the fully functional methyltransferase heterodimer complex (Wang et al., 2016; Śledź et al., 2016). At a 1:1 ratio, METTL3 and METTL14 form stable complexes. METTL3 contains the primary methyltransferase catalytic domain, whereas METTL14 identifies RNA substrates and acts as an RNA-binding platform (Liu et al., 2014). Additionally, METTL14 contributes to the regulation of histone modification in addition to forming a complex with METTL3 to alter different kinds of RNAs (Wang et al., 2018).

METTL14 increases the carcinogenic characteristics of METTL3 in a m6A modification way because it is a methylation partner of the METTL3 protein. However, because of its context-dependent actions, it can behave as a tumor suppressor. This phenomenon may be explained by variations in the specificity of RNA substrates or tissue-specific expression patterns (Wu et al., 2025). It is related to ESCC, cholangiocarcinoma, endometrial cancer, pancreatic cancer, CRC, renal adenocarcinoma, gastric cancer, BRCA, AML, and viral-caused cancers.

A point that distinguishes METTL14 from METTL3 is the R298P mutation of METTL14. Mutant METTL14 (R298P)-faciliated m6A alterations at aberrant motifs enhance the methylation efficiency at adjacent canonical sites, which reduces the stability of associated mRNAs, such as c-MET. A mutational hotspot at the highly conserved amino acid residue R298 within METTL14 was found via a pan-cancer investigation. The majority of patients with endometrial cancer were found to have the METTL14 R298P mutation. Despite decreasing methylation activity, particularly at canonical motifs, the writer complex harboring the mutant METTL14 (R298P) painted m6A with aberrant patterns. METTL14's homozygous R298P mutation inhibits the growth of cancer cells, but the heterozygous mutation increases it (Miyake et al., 2023).

METTL14 has several effects on gastrointestinal malignancies, particularly CRC, gastric, cholangiocarcinoma, and ESCC disorders. METTL14 knockout reduced the m6A level of lncRNA XIST and increased CRC’s ability to proliferate (Yang et al., 2020). METTL14 is downregulated in gastric cancer, and its overexpression suppresses proliferation by blocking the PI3K/AKT/mTOR pathway (Liu et al., 2021). METTL14 and STUB1 expression were shown to be strongly negatively correlated across most malignancies, especially cholangiocarcinoma (CCA), according to a pan-cancer study based on the The Cancer Genome

Atlas (TCGA) database (Zeng et al., 2023). TRIB2 expression is suppressed by the m6A RNA methyltransferase METTL14, which is downregulated in ESCC (Liu et al., 2021).

METTL14 causes the AKT pathway to be activated and m6A methylation to decrease, which encourages endometrial cancer cells to proliferate and migrate. According to a study that sequenced clinical specimens of endometrial cancer, METTL14 has a hot spot R298P mutation that causes roughly 70% of endometrial cancer to decrease m6A methylation, which promotes cell proliferation (Liu et al., 2018). In BRCA stem-like cells, Aurora kinase A (AURKA) inhibits the ubiquitylation-mediated degradation of METTL14 protein, hence positively regulating its expression (Peng et al., 2021).

Other cancer types that METTL14 impacts include AML and viral-caused cancer types. Depletion of METTL14 mostly decreased m6A abundance on its target mRNAs such as MYB and MYC, adversely affecting their mRNA stability and translation, inhibiting the self-renewal of leukemia stem/initiating cells (LSCs/LICs) and promoting the myeloid differentiation of AML cells (Weng et al., 2018). The latent oncoprotein Epstein-Barr nuclear antigen 3C (EBNA3C), which is encoded by the virus, transcriptionally activates METTL14 in malignancies associated with the Epstein-Barr virus (EBV) (Lang et al., 2019). However, alphaherpesvirus kinases have been shown to phosphorylate METTL3, METTL14, and WTAP. The complex is rendered inactive as a result of its phosphorylation, and m6A levels are nearly completely lost (Jansens et al., 2022).

METTL16

METTL16 is another catalyst of m6A modification that controls mRNA stability and splicing. Recent research indicated that METTL16 has non-canonical capabilities similar to those of the METTL3 protein in addition to its canonical methylation role (Pendleton et al., 2017). The canonical role of METTL16 displays a stronger substrate selectivity than the METTL3/METTL14 complex, usually identifying secondary structure characteristics of RNA including U6 snRNA, MAT2A mRNA, and certain ncRNAs (Doxtader et al., 2018). One of the noncanonical functions of METTL16 is stabilizing the mRNA of SAM synthetase (MAT2A) to preserve methylation metabolic equilibrium, creating a bidirectional regulation loop known as "modification-metabolism." (Zhang et al., 2022). METTL16 methylates adenosine bases in many RNA hairpin loops when SAM levels are sufficient. Hairpin 1 (HP1) methylation inhibits terminal intron splicing, resulting in intron retention and degradation.

However, as cellular SAM levels drop, METTL16 coupled to HP1 attracts splicing machinery to the final intron, eliminating it and enabling the production of the full-length mature mRNA (Scarborough et al., 2021).

A significant amount of endogenous METTL16 is found in the cytoplasm, in contrast to METTL3–METTL14, which are mostly found in the nucleus. However, METTL16 can also be found in the cell nucleus as a writer of m6A modification (Nance et al., 2020). METTL16 stimulates translation in the cytosol without the need for m6A. Through its MTase domain, METTL16 directly interact with rRNA and the eukaryotic initiation factors, boosting the translation of more than 4,000 mRNA transcripts and enabling the translation-initiation complex formation.

Because of its dual functions as a translation-initiation facilitator and a m6A writer, METTL16 has a broad impact on cancer diseases (Su et al., 2022). METTL16 has a significant impact on gastrointestinal cancers such as gastric, pancreatic, CRC, HCC, and cholangiocarcinoma. METTL16 influences the progression of the cell cycle by upregulating Cyclin D1 (CCND1), which contributes to gastric cancer. Cyclin D1 promotes the growth of gastric cancer cells by speeding up the G1/S phase transition. METTL16 modifies the methylation of CCND1 mRNA to increase its stability. That's why the patients with gastric cancer had elevated expression of METTL16 (Wang et al., 2021). Additionally, due to its association with "cuproptosis," or copper-induced cell death, METTL16 also influences gastric cancer. METTL16-mediated cuproptosis was linked to elevated copper levels and lactylation of METTL16 was found to be a crucial control mechanism that improves its activity in m6A modification in mRNA molecule of FDX1 (Sun et al., 2023). By controlling the mRNA stability of MROH8, METTL16 facilitates its translation. Tata-binding protein (TBP), a transcription factor that binds to the promoter and increases transcription of CAPN2, which encourages the growth and spread of pancreatic cancer cells, is degraded by MROH8. As a result, METTL16 dramatically reduced pancreatic cancer tumor growth and spread (Yi et al., 2024). Through m6A modification, METTL16 increases the expression of its downstream target SOGA1, which in turn causes the important glycolytic regulator PDK4 to be upregulated in CRC (Wei et al., 2023). For HCC, it was found that METTL16 knockdown dramatically reduced HCC cell migration and invasion. Through its methyltransferase domain, it directly engages with eIF3a/b, helping to assemble the translation initiation complex (Su et al., 2022). METTL16 also suppresses ferroptosis, or iron-induced cell death, in cholangiocarcinoma by stabilizing ATF4 mRNA in a m6A modification manner (Zhao et al., 2025).

Other effects of METTL16 contribute to cancers related to the reproductive system or hormones, including BRCA and ovarian cancer. Suppression of METTL16 slows the growth of BRCA. Because METTL16 increases the stability of FBXO5 mRNA by m6A alteration, which encourages the growth of cancer cells and Epithelial-Mesenchymal Transition (EMT) in BRCA (Wang et al., 2024). On the other hand, due to their high iron concentration, cancer cells are more vulnerable to ferroptosis. METTL16 increases the expression of GPX4 through the m6A alteration, which inhibits ferroptosis and causes proliferation of BRCA (Ye et al., 2023). By binding to MALAT1 and encouraging its breakdown, METTL16 lowers the expression of the β-catenin protein. The tumor-suppressive effects in ovarian cancer are mediated by this METTL16–MALAT1–β-catenin axis (Li et al., 2023).

Unlike other METTLs, METTL16 has the ability to methylate lncRNAs, which gives it authenticity. The m6A modification faciliated by METTL16 has also been found in lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) (Cusenza et al., 2023). Additionally, in lung carcinogenesis, the interaction between METTL16 and translation repressor eIF4E2 prevents eIF4E2 from being recruited to the mRNA cap structure, which facilitates selective protein synthesis and eIF4E's recognition to the cap (Wang et al., 2023). In the AML, branched-chain amino acid (BCAA) metabolism is reprogrammed by METTL16, which increases the expression of BCAT1 and BCAT2 (Han et al., 2023). By controlling cell proliferation via a m6A-dependent mechanism, METTL16 behaves like a tumor suppressor in bladder cancer. METTL16 reduces PMEPA1 mRNA stability, inhibits tumor cell proliferation, and suppresses PMEPA1-mediated autophagy via attaching to the m6A site in the 3′-UTR region of mRNA molecule of PMEPA1. Also, HIF-2α promotes the growth of tumors by interacting with the promoter of METTL16 and suppressing its transcription. This demonstrates that the HIF-2α–METTL16–PMEPA1–autophagy axis is a crucial regulator of the growth of bladder cancer (Yu et al., 2024).

Conclusions

The knowledge of epitranscriptomic control in cancer biology is completely transformed by the finding of reversible m6A methylation. The m6A writer proteins METTL3, METTL4, METTL5, METTL14, and METTL16 are important regulators of cancer cell proliferation, and metastasis. METTL16 functions in several ways, broadening the scope of m6A control, whereas METTL3 and METTL14 work together in the conventional methyltransferase complex. The context-dependent nature of these proteins, which may alternate between oncogenic and tumor-suppressive activities depending on the particular RNA substrates and the cellular mechanisms, is a recurrent motif across many tumor types. METTL14 and METTL3 have comparable functions inside the same tumor. In certain cases, they are similar to how working from the same side yields the same outcome. However, these two proteins function differently in cancers. Tumor heterogeneity, their distinct protein structures, noncanonical functions and, occasionally, various research models might be the reasons for the conflicting functions (Liu et al., 2022). As in AML, m6A writers and erasers can be paradoxically increased simultaneously, much like DNA methylation regulators. This finding demonstrates that localized subsets of RNA transcripts, rather than global alterations, are what cause m6A's effect on carcinogenesis. Therefore, reconciling the contradictory roles of METTL3, METTL14, and METTL16 in cancer development requires a knowledge of their context-specific targets (Deng et al., 2023). In addition, both METTL4 and METTL5 have emerged as important RNA methyltransferases with potential roles in cancer progression and patient prognosis. Studies have shown that altered expression of these genes is associated with tumor growth, metastasis, immune regulation, and survival outcomes across different cancer types. METTL4 appears to be involved in cell cycle regulation (Zhang et al., 2025) and hypoxia-mediated tumor progression (Hsu et al., 2022) while METTL5 contributes to oncogenic translation (Dai et al., 2023) and tumor proliferation (Rong et al., 2020). Further studies are needed to understand the molecular mechanisms of these proteins and their roles in certain cancer types better.

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CHAPTER 3

SIGMA RECEPTORS AND THEIR RELATIONSHIP WITH CANCER: AN EVALUATION FROM THE PERSPECTIVE OF MEDICINAL CHEMISTRY

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Sigma Receptors

Sigma receptors constitute a class of receptors whose biological roles have not yet been fully elucidated. They were first identified in 1976 by Martin and colleagues. These receptors were previously considered a type of opioid receptor but have been classified as a separate group due to their low affinity for naloxone and naltrexone (Piechal, A., Jakimiuk, A., & Mirowska-Guzel, D., 2021). Sigma receptors possess at least two binding sites, designated as Sigma-1 (σ1) and Sigma-2 (σ2). They are distributed throughout the central nervous system (CNS) and various peripheral tissues. However, they are overexpressed in many tumour cells. The specific functional roles of the two subtypes have been progressively elucidated, gaining clinical significance (Sparatore, F., & Sparatore, A., 2023).

Sigma-1 is a chaperone protein consisting of 223 amino acids and with a molecular weight of approximately 25 kDa. Its crystal structure, characterised by Kruse and colleagues in 2016, revealed a transmembrane domain for each monomer. Sigma-1 is an endoplasmic reticulum chaperone protein localised to membranes associated with the mitochondria. Based on current studies, it appears that the various functions of Sigma-1 (Figure 1) are directed towards maintaining calcium homeostasis within the cell (Martin, P. et al., 2021).

Sigma-1 is encoded by the Sigma-1 receptor gene, which is located on chromosome 9 in humans and chromosome 4 in mice. It shares no homology with any other mammalian protein. Sigma-1 was first identified at the molecular level in guinea pig liver. It has subsequently been cloned from a human placental choriocarcinoma cell line, rat brain and mouse. Sigma-1 modulates the activity of various proteins, such as the inositol trisphosphate receptor (IP3R), various ion channels and small G-proteins, by interacting with them. Although there is no confirmed endogenous ligand for Sigma-1, neuroactive steroids including the hallucinogen N,N-dimethyltryptamine, progesterone and dehydroepiandrosterone sulphate and endogenous N-alkylamines are considered potential candidates. Another receptor with a similar ligand-binding profile is the Sigma-2 receptor. However, it is not structurally or genetically related to Sigma-1 (von Rüden et al., 2026).

Sigma-2 (Figure 1) has a molecular weight of 18–21.5 kDa, is located on chromosome 17, and was previously thought to be a component of progesterone receptor membrane component 1 (PGRMC1). Sigma-2 consists of 176 amino acids and belongs to a protein family whose prototypical member is the emopamil-binding protein (EBP). However, unlike EBP, which possesses steroid isomerase activity, Sigma-2 lacks any enzymatic activity. Sigma-2 has four

transmembrane domains and three small beta-strand extensions (Malar, D. S. et al., 2023; Nguyen, N. et al., 2023).

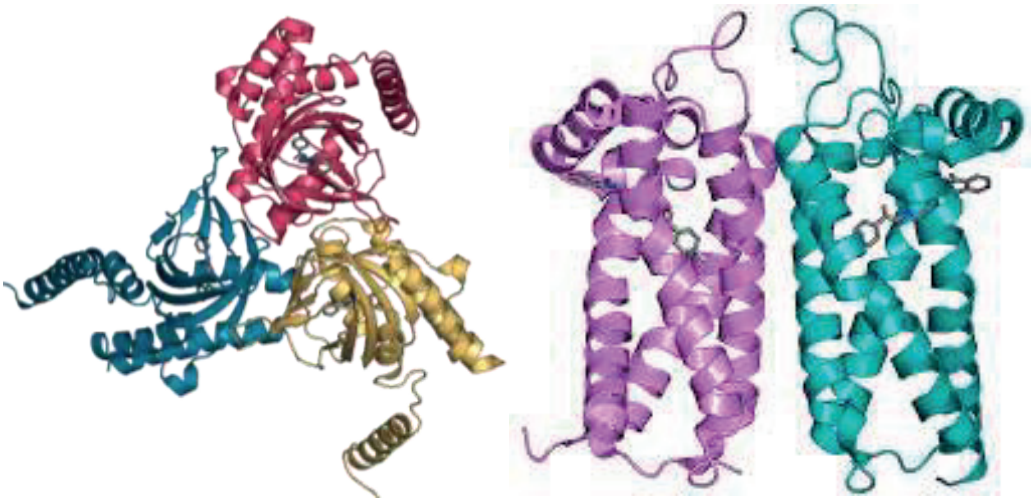

Figure 1. Structures of the Sigma 1 and Sigma 2 receptor proteins

Sigma-2, also known as transmembrane protein 97 (TMEM97), is a four-domain transmembrane receptor. It is expressed in multiple cell types susceptible to degeneration, including neurons, and regulates cellular functions such as cholesterol biosynthesis, membrane transport, autophagy, lipid-dependent protein transport, and receptor stabilisation at the cell surface, all of which are disrupted in disease (Lizama, B. N. et al., 2023).

Sigma Receptors and Diseases

Our understanding of what Sigma-1 receptors are has advanced significantly over the past 40 years. Today, Sigma-1 receptors are recognised as a unique class of chaperone proteins that regulate protein folding, oxidative stress and cellular homeostasis, and they play a role in numerous pharmacological processes, making them an established therapeutic target. Sigma-1 and Sigma-2 receptors differ in terms of protein size, tissue expression, and pharmacological and drug selectivity profiles. These receptors are widely distributed in the central nervous system and peripherally in areas involved in pain modulation, memory, emotions and motor function. In the periphery, they are primarily expressed in the heart, liver, spleen, lungs, kidneys, adrenal glands and the gastrointestinal system (Agha, H., & McCurdy, C. R., 2021).

The Sigma-1 receptor regulates inositol trisphosphate receptors to control calcium signalling between the endoplasmic reticulum and the mitochondria. Sigma-1 receptors form a complex with an endoplasmic reticulum protein known as binding immunoglobulin protein (BiP). The dissociation of these complexes is primarily determined by the endogenous ligand neurosteroid

and affects calcium ion channels. Neurosteroids that act as agonists of the Sigma-1 receptor include pregnenolone sulphate (PREGS) and dehydroepiandrosterone sulphate (DHEAS). Progesterone is a representative neurosteroid that acts as an antagonist. However, these neurosteroids also modulate N-Methyl-D-Aspartate (NMDA) and Gamma-Aminobutyric Acid (GABA) receptors (Park, G. W. et al., 2025).

It is known that alterations in the Sigma-1 receptor play a role in the pathological development of certain neurological disorders, including ischaemic stroke. The Sigma-1 receptor is a chaperone protein that modulates multiple aspects of cellular functions, such as cell death, autophagy/apoptosis, neuronal differentiation and inflammation. Alterations in the receptor's functions have been implicated in several neurological and psychiatric disorders, including depression, addiction, schizophrenia and Parkinson's disease (Zhang, G. et al., 2023). Sigma-1 receptors are multifunctional proteins localised on the membranes of the endoplasmic reticulum; they play a critical role in cellular signal transduction by interacting with receptors, ion channels, lipids and kinases. Changes in their function and expression can lead to various diseases, including depression or memory disorders. Consequently, modulation of Sigma-1 receptors may be beneficial in the treatment of these central nervous system disorders. Sigma-1 receptors are proteins that have attracted significant attention as potential targets for drugs treating a wide range of medical conditions. The significant therapeutic potential of these ligands stems from the fact that the receptors are involved in the development of numerous disorders (such as retinal diseases, alcohol and substance dependence, and pain). Studies have shown that Sigma-1 receptors exist as monomers, dimers and higher-order oligomers. However, ligand-binding properties are only evident in the oligomeric state. Interestingly, the presence of an agonist supports the monomeric and dimeric forms of the receptors. An antagonist, on the other hand, promotes higher-order receptor oligomers (Sałaciak, K., & Pytka, K. 2022).

The importance of Sigma-1 in critical roles such as the regulation of mitochondrial and endoplasmic reticulum (ER) functions has made it a therapeutic target for various pathological conditions or diseases in which these organelles exhibit dysfunction. In recent years, it has been reported that the activation or inactivation of Sigma-1 reduces pathological phenotypes in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis. This highlights the interest in Sigma-1 therapy for the most common neurodegenerative disorders. Furthermore, Sigma-1 plays a role in other diseases as well. These include heart conditions, chronic pain, depression, addiction and cancer (Couly, S. et al., 2022). The SARS-CoV-2 protein binds directly to Sigma-1 receptors. A study has

demonstrated the autophagosome process induced by SARS-CoV-2. It was found that the reduction in autophagosome size mediated by SARS-CoV-2 is strongly associated with the Sigma-1 receptor, and it has been suggested that the Sigma-1 receptor could be a potential therapeutic target for developing drugs against the coronavirus (Zhang, C. et al., 2024).

Sigma-1 receptors have a very broad pharmacological profile. Their ligands are compounds with different chemical structures and pharmacological effects: antidepressants (fluvoxamine, sertraline, imipramine), neuroleptics (haloperidol, chlorpromazine), analgesics (pentazocine), anxiolytics (afobazol), anticonvulsants (phenytoin), cough suppressants (dextromethorphan, carbethapentan) and antihistamines (chlorphenamine), narcotic drugs (methamphetamine and cocaine) and drugs used in the treatment of neurodegenerative diseases (amantadine, memantine, donepezil). The common structural features of ligands include a cationic amino group and at least one aromatic ring. Typical neuroleptics (haloperidol, fluphenazine, chlorpromazine, trifluoperazine) have a high affinity for Sigma-1 receptors (Milenina, L. S. et al., 2022).

Sigma Receptors and Cancer

Cancer is a major public health problem worldwide and the second leading cause of death in the United States. Cancer treatment has changed significantly over the years. The first modern treatment approach dates back to the late 1800s with the discovery of X-rays. Since then, remarkable scientific and medical advances have yielded countless approaches, leading to increasingly specific and effective treatments. Both Sigma receptor subtypes (Sigma-1 and Sigma-2) play a role in cancer and have frequently been used as targets for the development of multi-targeted, directed ligands to create synergy with anti-tumour activity via other targets (Abatematteo, F. S. et al., 2021). Cancer is a multifactorial disease that can be combated by targeting various signalling pathways. Hem-Oxygenase-1 and Sigma receptors are overexpressed in various cancers, including prostate and brain cancer, and contribute to the spread of the disease. Cancer remains a major cause of death worldwide, and most antineoplastic agents in clinical practice have developed severe side effects alongside multi-drug resistance. Consequently, the identification of new molecules capable of enhancing or restoring the efficacy of anticancer drugs by acting on classical or novel molecular targets is a major focus of interest. Among the countless pathways involved in the formation, growth and survival of cancer cells, the heme-oxygenase system and Sigma receptors play significant roles (Romeo, G. et al., 2021).

Both Sigma receptor subtypes have been proposed as therapeutic targets for various types of cancer, and numerous studies have provided evidence that their selective ligands (agonists and antagonists) exhibit antiproliferative and cytotoxic activity (Sereti, E. et al., 2021). As Sigma receptors are highly expressed in various cancer cells, radioactively labelled Sigma receptor ligands have been developed as imaging and therapeutic probes for cancer. Initially, research into the Sigma-1 receptor focused on its relationship with brain functions such as signal transduction, pain modulation, memory, recognition and emotions. Research has aimed to gain insights into neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and multiple sclerosis (MS). For example, in preclinical models, Sigma-1 receptor agonists have been reported to be effective protectors against these neurodegenerative diseases. Furthermore, the Sigma-1 receptor has attracted attention in the field of oncology research, as it is known to be highly expressed in various cancer cells. Furthermore, it has been reported that Sigma-1 receptor antagonists inhibit the proliferation of cancer cells. Consequently, radioactively labelled Sigma-1 receptor ligands have been developed as imaging probes not only for neurodegenerative diseases but also for the diagnosis of cancer (Mishiro, K. et al., 2022).

The Sigma-1 receptor plays a significant role in ion channel activity, apoptosis and proliferation. Its localisation in various integral membranes, its interaction with diverse protein classes and its regulation of different cellular pathways upon activation make the Sigma-1 receptor a potential target for the metabolic modulation of cancer cells. The Sigma-1 receptor is highly expressed in various cancers, including prostate, colon, melanoma, breast and lung cancer (Oflaz, F. E. et al., 2022). Increased expression in tumour cell lines plays a role in programmed cell death. Consequently, they represent promising targets for innovative cancer treatments and tumour diagnosis (Kronenberg, E. et al., 2021).

Sigma receptors and ion channels play a critical role in key cellular adaptation processes that increase the aggressiveness of tumour cells and promote metastasis. Consequently, they are attracting considerable interest as potential therapeutic targets in cancer treatment. The Sigma-2 receptor remains an important molecular target in tumour biology. The high expression of Sigma-2 in proliferating cells compared to quiescent cells makes it an important clinical biomarker for determining the proliferative status of solid tumours using functional Positron Emission Tomography (PET) imaging. The ability of Sigma-2 selective ligands to neutralise cell volume via both apoptotic and non-apoptotic pathways suggests that this receptor could be a potential target for cancer chemotherapeutic agents (Fotakopoulos, G. et al., 2024). The

Sigma-2 receptor is overexpressed on rapidly proliferating tumour cells compared to untransformed host tissues. Initially, Sigma-2 ligands were successfully used as tumour-targeting imaging probes capable of identifying solid tumours. Subsequently, it was found that a structurally diverse family of high-affinity Sigma-2 ligands is rapidly internalised into cancer cells. This has enabled the design of cancer-selective therapies capable of delivering maximum therapeutic benefits whilst minimising systemic toxicity (Takchi, R. et al., 2024). The Sigma-2 receptor/transmembrane protein 97 (TMEM97) is up-regulated in cancer cells compared to normal cells (showing increased expression). Conventional Sigma-2 receptor agonists induce apoptosis and autophagy, making them of interest in cancer therapy. Due to its upregulation in cancer cell lines, it is of interest in oncology (Liu, C. et al., 2021).

Characteristics of Sigma Receptor Inhibitors Used in Treatments

Inhibitors targeting Sigma receptors are generally defined as molecules with a multi-faceted pharmacological profile, rather than as single-target receptor antagonists in the classical sense. A significant proportion of these agents used in clinical and preclinical studies were initially developed as antipsychotics, antidepressants or analgesics; however, it was subsequently demonstrated that they exhibit high affinity for Sigma receptors (Walker et al., 1990; Milenina et al., 2022).

σ1 Receptor Inhibitors

σ1 receptor inhibitors are generally compounds that act as antagonists or exhibit antagonistic properties. Typical antipsychotics such as haloperidol, fluphenazine and chlorpromazine bind to σ1 receptors with nanomolar affinity, thereby suppressing the effects of these receptors on intracellular calcium signalling and ion channel modulation (Milenina et al., 2022). However, due to their effects on the dopaminergic and adrenergic systems, these agents have a wide range of side effects.

NE-100 and BD-1063 (Figure 2), which are frequently used in preclinical studies, stand out as selective σ1 receptor antagonists. It has been demonstrated that NE-100 inhibits σ1 receptor-mediated behavioural and neurochemical responses, whilst BD-1063 potentiates the analgesic effect, particularly in neuropathic pain and inflammatory processes (Okuyama et al., 1994; Espinosa-Juárez et al., 2021). These agents are of great importance as pharmacological tools in elucidating the role of Sigma receptor function in pathological processes.

Siramesine (Lu 28-179) (Figure 2), although initially developed as an antidepressant, has attracted attention in the field of oncology due to its apoptotic and cytotoxic effects as a σ1 receptor antagonist. In particular, it has been reported to induce cell death via lysosomal membrane permeabilisation and mitochondrial dysfunction (Skuza et al., 2006; Fotakopoulos et al., 2024).

β2-Receptor Inhibitors

As σ_2 receptors (TMEM97) are overexpressed in proliferating cells, their inhibitors have been shown to possess properties that are particularly relevant to anticancer therapy. PB28 is one of the selective ligands that exhibits high affinity for the σ_2 receptor and has been associated with the induction of mitochondrial stress, endoplasmic reticulum (ER) stress and apoptosis in cancer cells (Abate et al., 2020; Takchi et al., 2024).

σ2 receptor antagonists such as ISO-1 and CM-398 (Figure 2) have been associated with cell cycle arrest, disruption of cholesterol homeostasis and autophagic cell death in proliferative tumour cells. In particular, it has been reported that CM-398 possesses potential for use in both imaging and therapeutic applications due to its capacity for selective binding to tumour tissues (Wilson et al., 2022; Mach et al., 2013).

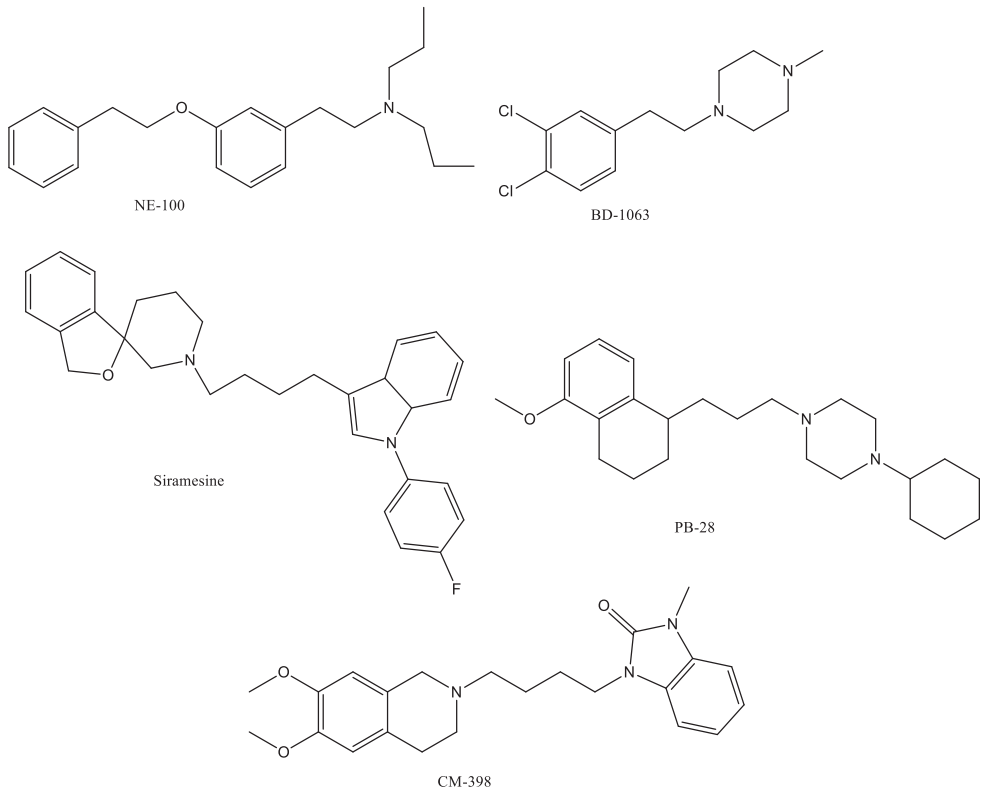

Figure 2. The chemical structure of Sigma receptor inhibitors

In general, the fact that σ2 receptor inhibitors exhibit a more selective effect in tumour cells compared to normal tissues suggests that molecules targeting these receptors may offer high therapeutic efficacy with lower systemic toxicity (Mach et al., 2013; Abatematteo et al., 2021).

Table 1. Sigma receptors and their indications

Compounds Name	Target Sigma Receptor	Interaction Type	Development Stage	Therapeutic Area Under Investigation
Siramesine (Skuza G et al., 2006) **(Lu 28-179)**	σ_1	Antagonist	Preclinical / Phase II	Depression, oncology
Rimcazole (Katz JL et al., 2003)	σ_1 / σ_2	Antagonist	Preclinical	Neurodegenerative diseases
PB28 (Abate C et al., 2020)	σ_2	Selective ligand / inhibitor	Preclinical	Anticancer
BD-1063 (Espinosa-Juárez JV et al., 2021)	σ_1	Selective antagonist	Preclinical	Neurological disease models
NE-100 (Okuyama S. et al., 1994)	σ_1	Selective antagonist	Preclinical	Neuropsychiatric disorders
SA4503 (Tagashira H et al., 2025)	σ_1	Agonist	Preclinical	Stroke, neuroprotection
CM-398 (Wilson LL et al., 2022)	σ_2	Selective ligand	Preclinical	Cancer imaging/treatment
ISO-1 (Naruoka T et al., 2013)	σ_2	Antagonist	Preclinical	Anticancer

In vivo and *in vitro* Experimental Studies on the Sigma Receptor

The multifaceted roles of Sigma receptors in cellular homeostasis, the stress response and proliferation have made these receptors the subject of intensive research in both *in vitro* cell culture models and *in vivo* animal models.

In vitro studies

In *in vitro* experimental studies, the effects of σ_1 and σ_2 receptor ligands have been evaluated in various cell models, including neuronal cell lines (PC12, SH-SY5Y), glioblastoma cells (U87), breast cancer (MCF-7) and cervical cancer (HeLa) cells. It has been demonstrated that selective σ_2 receptor ligands, particularly due to their high expression in proliferative cells, are associated with cell cycle arrest and the activation of mitochondrial apoptotic pathways (Vilner et al., 1995; Zeng et al., 2012).

It has been reported that PB28 and similar σ2 ligands trigger cell death via an increase in reactive oxygen species, endoplasmic reticulum stress and lysosomal dysfunction; this effect is significantly lower in normal cells (Abate & Niso, 2017; Takchi et al., 2024). It has been shown that σ_1 receptor inhibitors disrupt intracellular Ca^{2+} balance, thereby reducing the mitochondrial membrane potential and activating apoptotic signalling (Hayashi & Su, 2007; Oflaz et al., 2022).

In an interactome analysis, it has been clarified through laboratory-level interaction maps that the mechanisms of action of already known drugs, such as fluvoxamine and donepezil, are associated with σ_1 (Mastropasqua, Ludwig & Abate, 2025). It has been demonstrated that Sigma receptor agonists alleviate brain damage following experimental focal cerebral ischaemia in various species. In one study, the hypothesis that 4-phenyl-1-(4-phenylbutyl)piperidine (PPBP), a potent and prototypical σ_1 receptor agonist, protects neurons via a mechanism involving Bcl-2, an anti-apoptotic (cell death-inhibiting) protein, was tested. It has been demonstrated that, in vitro, PPBP reduces cell death via a mechanism involving receptor-dependent protection of protective genes such as Bcl-2 (Yang et al., 2007).

All σ_1 receptor structures associated with agonists (pentazocine) or antagonists (haloperidol) consistently show that the ligands are primarily surrounded by hydrophobic residues and that these residues are in contact with the hydrophobic regions of the ligands. Furthermore, all these synthetic ligands contain a positively charged amine region that interacts directly with the E172/E169 residue of the σ_1 receptor, consistent with proposed pharmacophore models. It is

known that these neurosteroids exert opposing effects on memory and nerve transmission in the living body. Crystal structure analysis has demonstrated at the atomic level that this contrast stems from their opposite binding to the receptor (Fu et al., 2024).

In vivo Studies

In vivo studies have predominantly been conducted in rodent models, and the neurological, analgesic and anticancer effects of Sigma receptor ligands have been investigated using behavioural tests, biochemical analyses and histopathological evaluations. It has been reported that σ_1 receptor antagonists reduce markers of oxidative stress and enhance neuronal survival in models of ischaemic stroke and neurodegenerative diseases (Matsumoto et al., 2014; Zhang et al., 2023).

In a study, the *in vitro* Ca^{2+} concentration-modulating activities and *in vivo* behavioural effects of the enantiomers of methylphenylpiracetam, a novel positive allosteric modulator of σ_1 receptors, were compared. It was found that the R-configuration enantiomers of methylphenylpiracetam are more active positive allosteric modulators than the S-configuration enantiomers of the σ_1 receptor, and the σ_1 receptor modulator activity of the compounds correlated with memory-enhancing effects (Vavers et al., 2015).

In a study evaluating pain-related behaviours (allodynia and hyperalgesia), it was found that σ_1 receptor agonists (e.g. pentazocine) reduced opioid analgesic activity, whilst σ_1 receptor antagonists (e.g. haloperidol) potentiated opioid analgesia and were found to possess anti-allodynic effects in various pain models (Agha & McCurdy, 2021).

In animal models bearing tumours, it has been shown that σ_2 receptor inhibitors result in a significant reduction in tumour volume and are associated with increased caspase activation, DNA fragmentation and apoptosis markers (Mach et al., 2013; Vilner et al., 1995). Furthermore, it has been reported that radioactively labelled σ_2 ligands accurately reflect tumour proliferation in PET imaging; this makes σ_2 receptors a functional biomarker of proliferation (Mach et al., 2013; Mishiro et al., 2022).

When these experimental data are considered together, it is clear that Sigma receptors represent versatile therapeutic targets of high translational value in both central nervous system disorders and oncological treatment strategies.

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CHAPTER 4

REGULATION OF VASCULAR HOMEOSTASIS BY THE ANGIOPOIETIN FAMILY AND ITS IMPLICATIONS IN DISEASE

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Introduction

Angiopoietins constitute a subgroup of the vascular growth factor family and are critically involved in the regulation of vascular biology, particularly in processes such as angiogenesis and the maintenance of vascular stability. These proteins are primarily responsible for the formation of blood and lymphatic vessels, modulation of vascular permeability, angiogenic remodelling, stabilization of vascular structures, and preservation of functional vascular integrity. Recent evidence indicates that angiopoietins are not only essential for normal vascular development but also play significant roles in various pathological conditions. Notably, dysregulation of the angiopoietin system in diseases such as cancer, inflammation, sepsis, and diabetic retinopathy has been shown to influence vascular permeability, vascular remodelling, and pathological angiogenesis. There are now four known angiopoietins: Angiopoietin-1, Angiopoietin-2, Angiopoietin-3, and Angiopoietin-4. These molecules primarily mediate their biological effects through the TEK receptor (Tie2 receptor tyrosine kinase) located on endothelial cell surfaces. Among them, Angiopoietin-1 (Ang-1) is essential for preserving vascular integrity by promoting vascular stabilization, enhancing endothelial cell survival, and decreasing vascular permeability. In contrast, Angiopoietin-2 (Ang-2) typically acts as a functional antagonist of Angiopoietin-1, thereby destabilizing blood vessels and rendering endothelial cells more responsive to remodelling and angiogenic stimuli (Eklund & Saharinen, 2013; Akwii et al., 2019; Fagiani & Christofori, 2013; Fiedler & Augustin, 2006). Angiopoietin-3 (Ang-3) in mice and Angiopoietin-4 (Ang-4) in humans are considered species-specific orthologs. Although both Ang-3 and Ang-4 are considered to contribute to vascular development and the regulation of vascular function, their roles remain less clearly defined compared with the two principal members of the angiopoietin family (Valenzuela et al., 1999). Evidence suggests that, similar to Ang-4, Ang-3 functions as a Tie2 receptor agonist rather than an antagonist; however, this activity appears to be limited to its corresponding species (6). Furthermore, Beaudet et al. (2010) demonstrated in an experimental glioma model that Ang-3 promotes tumour growth by inducing destabilization of the cerebral endothelium, leading to changes in the tumour microenvironment that support glioma cell progression. Conversely, according to Xu et al. (2004), elevated expression of Ang-3 may inhibit the ability of some carcinomas, such as Lewis lung carcinoma and TA3 mammary carcinoma, to spread to other parts of the body, especially the lungs. Lung adenocarcinoma, glioblastoma, and ovarian cancer are among the tumour types where Ang-4 has been found to be highly expressed. This increased expression is believed to be associated with its role in regulating tumour vasculature and the

lymphatic system. From a mechanistic perspective, Ang-4 is thought to promote cancer progression by influencing the TIE2 receptor tyrosine kinase, along with downstream signalling pathways like ERK1/2 and PI3K/AKT. As a result, Ang-4 has been proposed as a possible biomarker for the onset, spread, and poor prognosis of cancer, and its suppression may be a promising oncology treatment approach (Zhou et al., 2024). In contrast to other angiopoietin family members, the biological roles of Ang-3 and Ang-4 are still unclear, as was previously mentioned.

Ang-1 is considered one of the most thoroughly studied members of the angiopoietin family. It serves as an essential growth factor that supports vascular formation, promotes vessel maturation, and helps maintain the structural and functional stability of the endothelium. Its biological effects are mainly mediated through binding to the Tie2 (TEK) receptor expressed on the surface of endothelial cells. This interaction leads to activation of the Tie2 receptor and initiates multiple intracellular signalling cascades. These pathways regulate essential processes such as endothelial cell survival, maintenance of vascular integrity, and stabilization of the vascular network (Brindle et al., 2006). A major function of Ang-1 is the promotion of vascular stability. Ang-1 reduces vascular permeability by strengthening endothelial cell intercellular connections through Tie2 activation. In addition, it exerts anti-apoptotic effects on endothelial cells, prolonging their survival and contributing to the continuity and integrity of the vascular structure. Moreover, Ang-1 strengthens the association between endothelial cells and supporting cells, including pericytes and vascular smooth muscle cells, which in turn promotes vascular maturation and improves functional stability (Gamble et al., 2000; Satchell et al., 2004). The biological actions of Ang-1 are largely mediated through the activation of several critical intracellular signalling cascades. Among these, the PI3K/Akt pathway plays a central role in enhancing endothelial cell survival by stimulating anti-apoptotic mechanisms. Another key pathway is the MAPK/ERK signalling cascade, which is involved in regulating cellular proliferation, migration, and vascular remodelling. According to reports, the pro-apoptotic activity of p38 MAPKs can be overridden by the strong anti-apoptotic effects of the ERK and PI3K pathways, resulting in a general decrease in apoptosis in response to Ang-1. Furthermore, Ang-1/Tie2 signalling is also implicated in the regulation of inflammation-associated pathways, including the NF-κB signalling pathway. Through these mechanisms, Ang-1 contributes to the maintenance of endothelial junction integrity and the control of vascular permeability (Kim et al., 2000; Papapetropoulos et al., 2000; Harfouche et al., 2003; Cai et al., 2021).

Under normal physiological conditions, Ang-1 plays a predominant role in preserving vascular stability, particularly when Ang-2 levels remain low. Through activation of the Tie2 receptor, Ang-1 reinforces endothelial cell–cell junctions and supports overall vascular integrity. In contrast, Ang-2 generally counteracts Tie2 signalling, leading to reduced vascular stability and increased endothelial sensitivity to inflammatory stimuli. Ang-2, a key protein within the angiopoietin signalling system, is primarily stored in Weibel–Palade bodies of endothelial cells and is rapidly mobilized upon stimulation, including inflammatory signals, hypoxic conditions, or activation by growth factors (Akwii et al., 2019; Fiedler et al., 2004). It serves as a key regulatory molecule, particularly in pathological angiogenesis. By antagonizing the Tie2 receptor, Ang-2 weakens intercellular junctions between endothelial cells and promotes destabilization of the vascular wall, thereby facilitating the action of other angiogenic mediators. Importantly, the effects of Ang-2 are context-dependent. In the presence of vascular endothelial growth factor (VEGF), Ang-2 strongly enhances angiogenesis. However, in the absence of VEGF, Ang-2 activity may instead lead to vascular regression (Holopainen et al., 2012; Yancopoulos et al., 2000; Lobov et al., 2002). Ju et al. (2014) showed that Ang-2 is released from endothelial cells through exosomal pathways. This release is negatively regulated by the PI3K/Akt/eNOS signalling pathway, while it is promoted via the syndecan-4/syntenin pathway. Increased Ang-2 levels are linked to disruption of endothelial barrier integrity and elevated vascular permeability. Such alterations promote the extravasation of inflammatory cells and intensify inflammatory responses. Furthermore, Ang-2 facilitates leukocyte adhesion and transmigration by increasing the expression of adhesion molecules in endothelial cells. Upregulation of Ang-2 has been associated with numerous pathological conditions. Increased vascular permeability, inflammatory processes, sepsis, and tumour angiogenesis have all been linked to elevated Ang-2 concentrations. Within the tumour microenvironment, Ang-2 may promote the formation of abnormal vascular structures, thereby supporting tumour growth and metastatic spread. Consequently, Ang-2 is currently regarded as a promising target for anti-angiogenic therapeutic strategies (Yan et al., 2017; De Palma & Naldini, 2011). Fiedler et al. (2006) further demonstrated that Ang-2 regulates endothelial cell responsiveness to inflammatory stimuli by increasing their sensitivity to TNF-α. This effect appears to be partly driven by its influence on TNF-α–induced adhesion molecule expression in endothelial cells, which in turn promotes increased leukocyte adhesion. On the basis of these observations, the authors proposed that Ang-2 functions as an autocrine mediator in controlling endothelial inflammatory responses. In this context, Ang-2 may be regarded as a central regulator of

vascular reactivity, creating a permissive environment that amplifies the effects of pro-inflammatory cytokines.

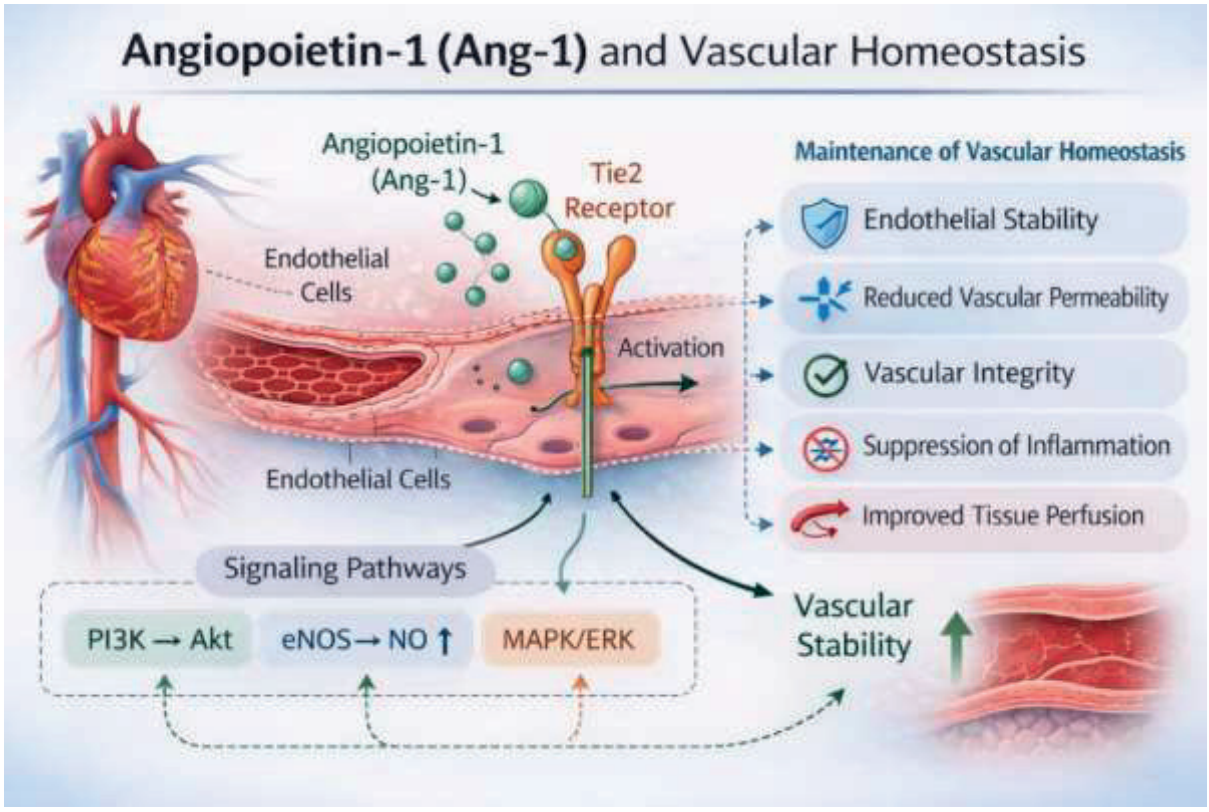

Figure 1. Role of Ang-1 in the maintenance of vascular homeostasis through Tie2 receptor signalling.

Ischemic heart disease (IHD) represents a major proportion of cardiovascular diseases, which remain one of the leading causes of mortality and morbidity on a global scale. The pathophysiology of IHD involves multiple interconnected biological processes, including endothelial dysfunction, inflammation, impaired angiogenesis, and vascular remodelling. A key molecular system involved in the regulation of these mechanisms is the angiopoietin–Tie receptor signalling axis. In particular, Ang-2 is recognized as an important regulator of vascular homeostasis and pathological angiogenesis (Augustin et al., 2009; Patel et al., 2008). Under normal physiological conditions, Ang-1 promotes vascular stability through activation of the Tie2 receptor, thereby maintaining endothelial barrier integrity and exerting anti-inflammatory effects. In contrast, Ang-2 predominantly functions as a natural antagonist of Ang-1, leading to reduced vascular stability and enhanced endothelial activation. Consequently, elevated Ang-2 levels are closely associated with vascular inflammation and aberrant angiogenic responses (Saharinen et al., 2017). In ischemic heart disease, conditions such as hypoxia, oxidative stress,

and elevated pro-inflammatory cytokines contribute to enhanced levels of Ang-2. Stored within endothelial cells, Ang-2 is rapidly released into the vascular microenvironment, particularly from Weibel–Palade bodies. Elevated Ang-2 levels compromise endothelial stability, enhance vascular permeability, promote adhesion of inflammatory cells to the vascular wall, and establish a microenvironment that supports the development of atherosclerotic plaques (Clajus et al., 2012; Lee et al., 2018). The actions of Ang-2 in ischemic heart disease are mediated through several signalling pathways, with Tie2 receptor signalling playing a central role. Binding of Ang-2 to the Tie2 receptor counteracts the stabilizing effects of Ang-1, resulting in weakened endothelial intercellular junctions. This leads to increased vascular permeability and facilitates inflammatory cell infiltration. Moreover, inhibition of Tie2 signalling may promote vascular remodelling and contribute to pathological angiogenesis processes (Lukasz et al., 2017). Ang-2 also maintains a strong functional interaction with VEGF. During ischemia, hypoxic conditions lead to an upregulation of VEGF, which, together with Ang-2, plays a critical role in modulating neovascularization. In this context, Ang-2 potentiates VEGF-driven signalling, promoting endothelial cell proliferation and migration and thereby supporting new vessel formation. Nevertheless, the vessels formed under these conditions are generally structurally immature and highly permeable. This increased permeability can aggravate inflammatory responses and further contribute to myocardial tissue damage (Lobov et al., 2002; Augustin et al., 2009). Another key pathway influenced by Ang-2 is the inflammation-associated NF-κB signalling cascade. Ang-2 elevation promotes inflammatory activation in endothelial cells through stimulation of NF-κB signalling, leading to elevated levels of adhesion molecules and pro-inflammatory cytokines. This mechanism may accelerate atherogenesis by promoting the adhesion and recruitment of monocytes and neutrophils to the vascular endothelium (Rathnakumar et al., 2016; Lukasz et al., 2012). In addition, Ang-2 exerts regulatory influences on the PI3K/Akt and MAPK signalling pathways. These cascades are essential for controlling cellular proliferation, migration, and survival, and they play a pivotal role in vascular remodelling following myocardial ischemia. Through modulation of these pathways, Ang-2 can influence both adaptive (compensatory) angiogenesis and maladaptive vascular remodelling processes (Harfouche & Hussain, 2006; Li et al., 2018). Clinical research has shown that patients with acute coronary syndrome, myocardial infarction, and chronic ischemic heart disease had considerably higher levels of circulating Ang-2. Elevated Ang-2 concentrations are commonly regarded as indicators of endothelial injury and vascular inflammation. Ang-2 levels have also been linked to the severity of the disease, the degree of cardiac damage, and the prognosis in general. For these reasons, Ang-2 is considered a key

molecule in cardiovascular research, serving both as a potential early diagnostic biomarker and a therapeutic target (Patel et al., 2008; Lee et al., 2018; Pöss et al., 2015; Aarsetøy et al., 2020).

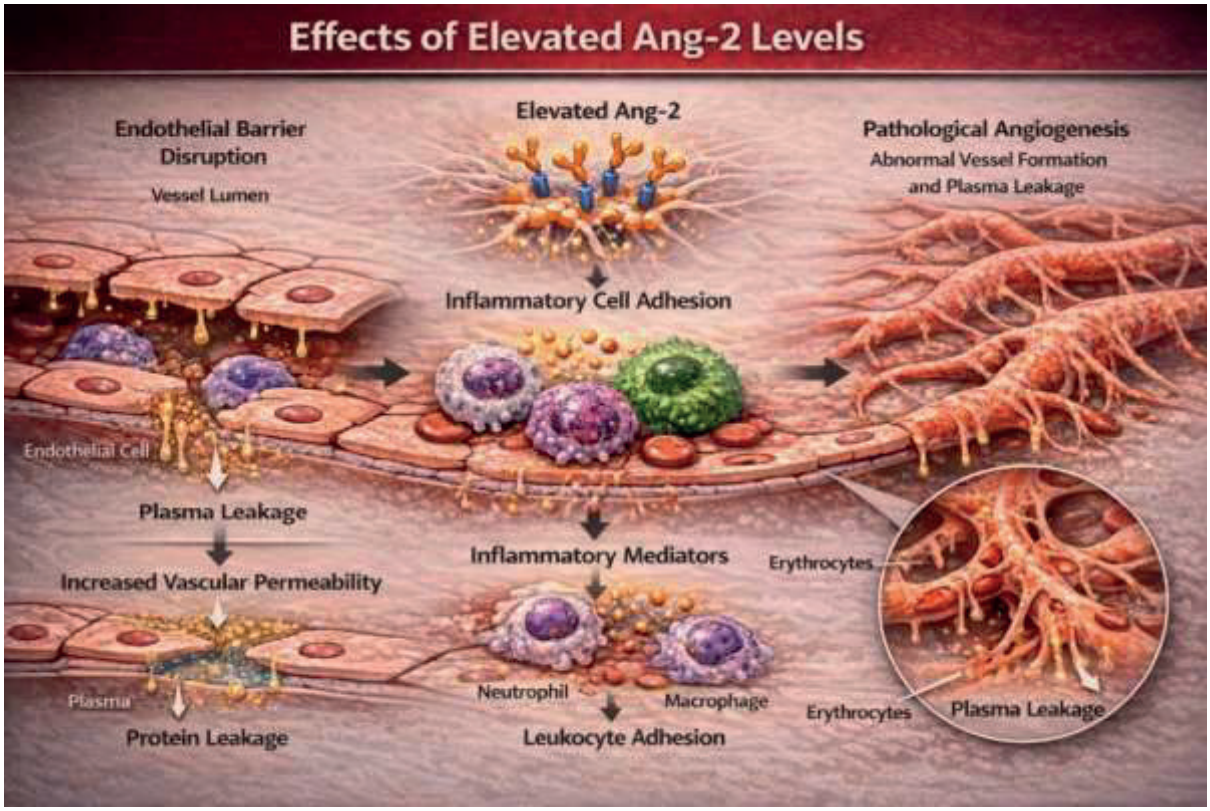

Figure 2. Ang-2-Mediated Endothelial Destabilization: A Central Driver of Vascular Leakage, Inflammation, and Pathological Angiogenesis

Elevated Ang-2 levels within the tumour microenvironment increase vascular permeability by disrupting tight junctions between endothelial cells. This process enhances the activity of pro-angiogenic mediators such as VEGF and promotes the development of new blood vessels, which are often structurally abnormal and functionally immature. Although Ang-2 primarily acts on endothelial cells, it also exerts indirect effects on tumour cells. The abnormal vascular architecture induced by Ang-2 contributes to the activation of hypoxia-inducible factor (HIF) signalling in tumour cells, which in turn upregulates VEGF and other pro-angiogenic mediators, establishing a self-amplifying positive feedback loop. In addition to supporting tumour growth, Ang-2-driven vascular remodelling facilitates tumour cell intravasation into the circulation, thereby increasing metastatic potential (Szarvas et al., 2008; Helfrich et al., 2009; Saharinen et al., 2011). Ang-2 enhances the adhesion and transmigration of inflammatory cells to the vascular endothelium by upregulating adhesion molecules such as ICAM-1 and VCAM-1 in endothelial cells. The recruited myeloid cells, including tumour-associated macrophages, subsequently release additional pro-angiogenic cytokines and matrix metalloproteinases. This

cascade contributes to vascular destabilization and extracellular matrix remodelling, thereby promoting tumour progression and establishing a microenvironment that supports metastatic spread (Scholz et al., 2015).

Conclusion

In conclusion, Ang-1 and Ang-2 emerge as the principal regulators of vascular homeostasis, exerting opposing yet complementary effects on vascular stability, permeability, and remodelling through their interactions with the Tie2 receptor and associated signalling pathways, thereby playing critical roles in both physiological processes and the pathogenesis of diseases, particularly cancer; nevertheless, further studies are needed to clarify the comparatively less understood roles of Ang-3 and Ang-4 and to assess their therapeutic potential.

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CHAPTER 5

PERIODONTITIS AS A RISK FACTOR IN ATHEROSCLEROTIC HEART DISEASE

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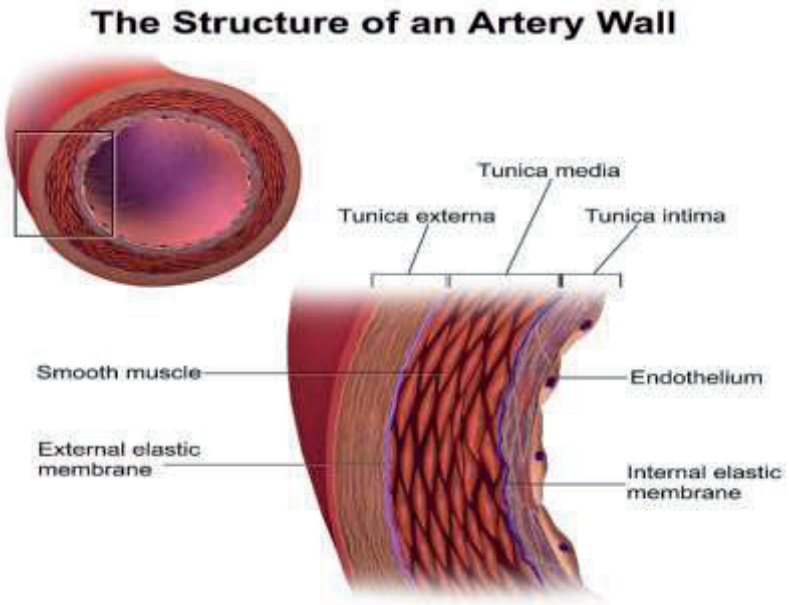
1. Atherosclerosis

Atherosclerosis is one of the major causes of death and disability in developed countries (Jebari-Benslaiman et al., 2022). Studies predict that cardiovascular diseases, especially atherosclerosis, will continue to hold a significant place. Although many risk factors are effective in the development of atherosclerosis, it produces different clinical findings depending on the circulatory region it affects. Atherosclerosis of the coronary arteries leads to myocardial infarction and angina pectoris. Atherosclerosis of the central nervous system arteries results in stroke and transient ischemic attack, peripheral atherosclerosis results in intermittent claudication and gangrene, and splanchnic circulation involvement results in mesenteric ischemia (Claessen et al., 2025). Atherosclerosis is a degenerative disease that mostly affects elderly individuals, causing significant symptoms by causing mechanical effects on blood circulation. The development of the disease is insidious and slow, but it can also progress rapidly in parallel with the increase in risk factors (Björkegren & Lusis, 2022).

The normal arterial structure consists of three separate layers. The innermost layer surrounding the vascular lumen is the tunica intima, above which is the tunica media, and the lamina elastica interna separating the tunica intima. The middle layer, the tunica media, consists mostly of smooth muscle cells. It also contains a high percentage of elastin. The outermost layer, the tunica adventitia (externa), is a connective tissue containing the vasa vasorum and nerves. The lamina elastica externa is also located between the tunica media and the adventitia (Libby & Soehnlein, 2025).

Figure 1: Histologically normal arterial structure (Libby & Soehnlein, 2025).



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2. Onset of Atherosclerosis

Atherosclerosis begins with a process affecting the intima layer of elastic arteries. It typically occurs over a period of many years, often spanning decades (Moss et al., 2023). Although the

cause is unknown, coronary arteries, carotid, cerebral and renal arteries, as well as the aorta and lower extremity

arteries, are more frequently affected, while some arteries, such as the internal mammary artery, are almost unaffected by this process. This process, which begins in the womb, develops over the years (Mortensen et al., 2023). First, lipid-laden macrophages and T lymphocytes accumulate in the subendothelial layer. In the second stage, non-stenotic fatty streaks begin to form in the first decade of life. Then, these intracellular lipid deposits begin to proliferate and are covered with a fibrous layer. Ulceration, calcification, and erosion develop in this plaque structure. As inflammation within the plaque increases, rupture occurs due to increased pressure within it (Freeman et al., 2023).

3. Important Cells in the Development of Atherosclerosis

3.1. Endothelial Cells

Endothelial cells are the most abundant tissue in the body, lining the entire vascular system. Normal endothelium acts selectively between the blood and the vessel wall, facilitating the release of many mediators. Although many factors affect the endothelium, many substances secreted by the endothelium are in a state of balance. Unhealthy endothelium increases permeability in both directions and acquires a procoagulant property.

Nitric oxide (NO) is synthesized by endothelial cells under the control of the endothelial nitric oxide synthase enzyme. NO is a potent vasodilator and platelet aggregation inhibitor.

Intracellular adhesion molecule-1 (ICAM-1), which is effective in the atherosclerosis process, reduces inflammatory cell aggregation by eliminating the expression of vascular cell adhesion molecule-1 (VCAM-1) chemoattractant genes (Zhang et al., 2023).

3.2. Smooth Muscle Cells

Smooth muscle cells, the most fundamental building blocks of progressive atherosclerosis lesions, originate from the tunica media. Smooth muscle cells are one of the cells that synthesize connective tissue, such as fibroblasts, in addition to contraction and the synthesis of collagen and elastic fibers. They are an important part of the advanced atherosclerotic process (Francis, 2023).

3.3. Macrophages

They act as scavengers in inflamed tissue, removing foreign substances from the tissue through phagocytosis. They play an important role in cell defense along with neutrophils. They also contribute to the oxidation of harmful molecules such as oxidized low-density lipoprotein (LDL) through lipoxygenase enzyme systems. Various growth factors (Interleukin-1, PDGF (platelet-derived growth factor), FGF (fibroblast growth factor), EGF (epidermal growth factor), TGF alpha and beta (transforming growth factor) and toxic substances such as oxygen free radicals are released into the environment, further increasing inflammation and LDL oxidation. In the development of atherosclerosis, oxidized LDL that is phagocytosed but not hydrolyzed accumulates in the form of lipid-filled granules and forms the foam cell. The

macrophage, having lost this function, then undergoes necrosis, releasing its lipid content into the environment (Farias-Itao et al., 2022).

3.4. Platelets (Thrombocytes)

Platelets are the most important cells in the formation of thrombosis. They provide coagulation through mediators released from the granules within them. These mediators include PDGF, EGF, FGF, TGF alpha and beta. At the beginning of atherosclerosis, collagen is released as a result of damage, and platelets come to the area and contribute to this coagulation with the mediators they secrete. A type of LDL coated with a protein called lipoprotein (a), which is very similar to plasminogen, also contributes to this prothrombotic environment (Baaten, Nagy, Bergmeier, Spronk, & van der Meijden, 2024).

3.5. T Lymphocytes

Both CD-8 and CD-4 type T lymphocytes are seen at every stage of atherosclerosis. Although the exact mechanism in atherosclerosis is unknown, it may be related to immunity. More detailed studies are needed on this subject (Fonzar et al., 2022).

4. Atherosclerotic Plaques

4.1. Stable Plaque

• Low lipid content

• Rich in collagen

• Thick fibrous capsule

• Increased smooth muscle cells and macrophages

4.2. Sensitive Plaque

• High lipid content

• Poor in collagen

• Thin fibrous capsule

• Decreased smooth muscle cells and macrophages

Stable plaque has low inflammatory activity and can progress to active sensitive plaque with intense inflammatory cell activity with certain stimuli (Dawson, Lum, Nerleker, Nicholls, & Layland, 2022) (Moss et al., 2023)

5. Atherosclerotic Cardiovascular Risk Factors

5.1. Age

In men, age over 45, and in women, age over 55 is a risk factor for ischemic heart disease. Although it can occur at any age, its frequency gradually increases after these ages.

5.2. Gender

In men, the risk is higher until the menopausal period, while in the postmenopausal period, this risk equalizes. This is because estrogen in the premenopausal period has been shown to be protective against cardiovascular diseases. 5.3. Family History

Having cardiovascular disease in first-degree male relatives before the age of 55, and in first-degree female relatives before the age of 65, is a risk factor.

5.4. Smoking

Smoking and tobacco use significantly increase the risk of cardiovascular disease compared to non-smokers. This is because it increases LDL oxidation, accelerates endothelial damage and the inflammatory process. This risk decreases if smoking is stopped. (Freeman et al., 2023) (Figtree et al., 2023) (Figtree et al., 2023) (Figtree et al., 2023)

5.5. Hypertension

Hypertension is highly prevalent in the population. In the study conducted in Turkey, the prevalence of hypertension in the adult population is as high as 34%. The risk of coronary heart disease is increased in hypertensive patients compared to normotensive individuals. At the same time, mortality and morbidity are higher in hypertensive patients compared to normotensive patients (Yumuk, 2005).

5.6. Dyslipidemia

High levels of low-density lipoprotein (LDL) and low levels of high-density lipoprotein (HDL) are risk factors for the development of atherosclerosis.

5.7. Diabetes Mellitus

Diabetes mellitus is considered a risk factor for atherosclerotic cardiovascular disease and is also considered equivalent to coronary artery disease. When looking at mortality rates in diabetic patients, it has been shown that 75% are due to cardiovascular causes (Jebari-Benslaiman et al., 2022)

Furthermore, it has been observed that insulin resistance, which is the period when the clinical picture of diabetes has not yet developed, increases the risk of myocardial infarction by 3-4 times (Jebari-Benslaiman et al., 2022).

Metabolic syndrome, which is a combination of obesity, insulin resistance, and hyperlipidemia, is a risk factor for cardiovascular disease.

5.8. Sedentary Lifestyle and Obesity

A sedentary lifestyle is a risk factor for ischemic heart disease. Regular and moderate-intensity exercise reduces the risk of cardiovascular disease.

5.9. Psychological Factors

A stressful lifestyle and depression increase the risk of cardiovascular disease.

6. Relationship Between Atherosclerosis and Periodontitis

Periodontitis is a chronic inflammatory disease characterized by the destruction of surrounding periodontal tissues. The disease causes irreversible damage to the periodontal ligament, cementum, and alveolar bone tissues, leading to tooth loss (Tonetti, Greenwell, & Kornman, 2018). Periodontitis not only affects the supporting tissues of the tooth locally but is also considered a risk factor for systemic diseases and conditions. Inflammation resulting from the host response can cause effects at the systemic level (Hegde & Awan, 2019; Otomo-Corgel, Pucher, Rethman, & Reynolds, 2012).

The cause-and-effect relationship between cardiovascular diseases and periodontitis is still not fully understood. However, with increasing epidemiological studies, it is increasingly accepted that periodontitis is a risk factor. Periodontal pathogens and their products can dynamically access the bloodstream from the inner part of the ulcerated periodontal pocket (20). Epidemiological studies support the link between periodontitis and systemic diseases and provide evidence that periodontal treatments can have a positive effect on the course of these diseases (Carra, Rangé, Caligiuri, & Bouchard, 2023; Dietrich, Sharma, Walter, Weston, & Beck, 2013; Kebschull, Demmer, & Papapanou, 2010).

Although periodontitis cannot be proven to be an independent risk factor for atherosclerotic diseases, it has been reported that it may contribute to the likelihood of developing the disease in the presence of other risk factors. There are many mechanisms that explain this relationship.

6.1. Increased Systemic Inflammation

Proinflammatory mediators such as IL-1β, IL-6, TNF-α, and prostaglandin E2, released as a result of the host response to periodontal pathogens, are not limited to periodontal tissues but pass into the systemic circulation. These mediators cause dysfunction in the vascular endothelium, facilitating endothelial damage, which is the first step in atherosclerotic plaque formation. IL-6 increases C-reactive protein (CRP) production in the liver; high CRP levels are associated with both the development of AKD and the risk of unstable plaque. TNF-α and IL-1β promote intimal thickening by stimulating proliferation in vascular smooth muscle cells (Khatri, Thakare, Ganvir, & Khatri, 2025; Shetty et al., 2023).

6.2. Bacteremia

It has been shown that periodontal pathogens enter the bloodstream even with daily activities such as brushing, flossing, and chewing in individuals with periodontitis. Although this bacteremia is temporary, it can damage the vascular endothelium when it recurs frequently (Iwai, 2009; Molina, Coque, Maldonado, & Herrera, 2023)(26, 27). In particular, pathogens such as Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Fusobacterium nucleatum have been shown to adhere to endothelial cells and cause changes in these cells such as apoptosis, inflammation, and adhesion molecule expression (Hou et al., 2019; Ijaz, 2024).

6.3. Activation of the Innate Immune System

Periodontal pathogens and their structural components contribute to the development of a systemic inflammatory response by activating pathogen recognition receptors (PAMPs). In particular, lipopolysaccharides (LPS) found in the cell walls of gram-negative periodontal bacteria activate the immune response via Toll-like receptors (TLRs) found in host cells. Among these receptors, TLR-2 and TLR-4 play a significant role in the recognition of periodontal pathogens (Folwaczny, Glas, Török, Limbersky, & Folwaczny, 2004; Song et al., 2017).

The recognition of LPS via TLRs in periodontal tissues leads to the activation of transcription factors such as nuclear factor kappa B (NF-κB). As a result, pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) are released by macrophages, monocytes, and dendritic cells. These mediators not only maintain inflammation in periodontal tissues but also pass into the systemic circulation and affect the vascular endothelium (Hans & Hans, 2011; Zou et al., 2022).

This continuous stimulation of the innate immune system can lead to chronic low-grade systemic inflammation and contribute to the initiation of processes such as endothelial dysfunction, oxidative stress, and inflammatory cell infiltration, which play a significant role in the development of atherosclerosis.

6.4. Endothelial Dysfunction

One of the earliest pathophysiological events in the development of atherosclerosis is endothelial dysfunction. Systemic inflammation resulting from periodontal infection can disrupt the normal functions of endothelial cells (34). During periodontitis, nitric oxide (NO) levels decrease as a result of increased inflammatory cytokines and bacterial products. Nitric oxide is a powerful vasodilator and an important molecule that inhibits platelet aggregation and leukocyte adhesion. A decrease in NO levels leads to increased vasoconstriction, platelet aggregation, and inflammatory cell adhesion in the vessel wall (35). In addition, during periodontal infection, the expression of adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin increases in endothelial cells. These molecules facilitate the attachment of monocytes and T lymphocytes to the vessel wall, initiating the early stages of atherosclerotic plaque formation (36, 37).

6.5. Molecular Mimicry

Another biological mechanism by which periodontal pathogens may contribute to the pathogenesis of atherosclerosis is immune-mediated vascular damage that develops through molecular mimicry. This mechanism is explained by the phenomenon of molecular mimicry, which is based on the structural similarity between bacterial components and host cell proteins (38, 39). In particular, heat shock proteins produced by P. gingivalis show similarity to HSPs in human endothelial cells. When it produces antibodies against bacterial HSPs, these antibodies also attack endothelial cells, creating cross-reactivity and causing endothelial damage (38, 40, 41).

6.6. Pro-thrombotic State and Platelet Activation

Periodontitis can contribute to the development of a pro-thrombotic state through systemic inflammatory response and hemostatic changes. P. gingivalis and Streptococcus sanguis can cause direct platelet aggregation by expressing "platelet aggregation-associated protein" (42, 43).

6.7. Impairments in Lipid Metabolism

Cytokines released into the systemic circulation as a result of periodontal inflammation contribute to the oxidation of low-density lipoproteins. Oxidized LDLs are taken up by macrophages, contributing to the formation of "foam cells" and thus to the growth of atherosclerotic plaque (Martinelli, Palmer, Wilson, & Newton).

In conclusion, current clinical, epidemiological, and molecular evidence shows a significant association between periodontitis and atherosclerotic cardiovascular diseases. Periodontal inflammation can contribute to the formation and progression of the atherosclerotic process through various mechanisms, including increased systemic inflammatory response, development of endothelial dysfunction, changes in lipid metabolism, and activation of thrombotic processes. Therefore, periodontitis should be considered not merely a local oral infection, but a condition closely related to systemic health. Thus, early diagnosis, prevention, and effective treatment of periodontal diseases are seen as a crucial component of holistic health approaches, not only for maintaining oral health but also for reducing cardiovascular risk.

6.8 Demonstration of Periodontal Bacteria in Atherosclerotic Plaques

Studies on atherosclerotic plaque samples have shown that periodontal pathogens can be found not only in the oral cavity but also in its tissues. DNA of periodontal bacteria such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, and *Fusobacterium nucleatum* has been detected in atherosclerotic plaques. These findings suggest that periodontal infections can reach vascular tissues via the systemic circulation and play a direct role in the atherosclerotic process (45,46).

In conclusion, current clinical, epidemiological, and molecular evidence indicates a significant association between periodontitis and atherosclerotic cardiovascular disease. Periodontal inflammation can contribute to the development and progression of the atherosclerotic process through various mechanisms, including increased systemic inflammatory response, development of endothelial dysfunction, changes in lipid metabolism, and activation of thrombotic processes. Therefore, periodontitis should be considered not merely a local oral infection, but a condition closely related to systemic health. Consequently, early diagnosis, prevention, and effective treatment of periodontal diseases are seen as a crucial component of holistic health approaches, not only for maintaining oral health but also for reducing cardiovascular risk.

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CHAPTER 6

NEUROMUSCULAR PROTECTION AND RATIONAL MAGNESIUM STRATEGIES: FROM PATHOPHYSIOLOGY TO PERSONALIZED SUPPLEMENTATION APPROACHES

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1. Introduction

Magnesium is an essential intracellular cation that serves as a cofactor in more than three hundred enzymatic reactions and plays a central role in maintaining the electrical stability of excitable tissues in the human body (Bertinato et al., 2015; Gröber et al., 2015). The proper functioning of the nervous and muscular systems (the neuromuscular axis) depends on the precise balance of intracellular and extracellular magnesium concentrations. Recent clinical and epidemiological studies have demonstrated that magnesium intake is insufficient across large segments of the population due to modern dietary habits and agricultural practices, thereby predisposing individuals to pathological conditions characterized by neuromuscular hyperexcitability. Within neuromuscular protection strategies, magnesium functions as a natural calcium antagonist that modulates neural transmission and optimizes the contraction–relaxation mechanisms of muscle fibers (Gröber et al., 2015). In particular, its ability to prevent glutamate-mediated neuronal overexcitation and the subsequent development of excitotoxic damage through voltage-dependent blockade of N-methyl-D-aspartate (NMDA) receptors in the central nervous system (Vink & Nechifor, 2011) has made this mineral a critical focus in the management of common clinical conditions such as migraine prophylaxis and muscle cramps. However, contemporary magnesium supplementation practices have evolved beyond a standardized approach toward a personalized paradigm.

The distinct pharmacokinetic properties, bioavailability profiles, and tissue affinities of various magnesium chelates (e.g., magnesium glycinate, magnesium malate, and others) contribute to the design of individualized supplementation protocols tailored to patients' specific neurological or muscular symptoms.

1.1. Importance of Magnesium in Human Health

As the second most prevalent cation located within the intracellular environment and fourth most abundant cation in the human body, magnesium (Mg) is fundamentally essential for maintaining human physiological equilibrium (Al Alawi et al., 2018). It serves as an indispensable enzymatic cofactor, driving more than three hundred distinct biochemical pathways that govern vital cellular behaviors, ranging from metabolic energy transduction to the stability of nucleic acids (Gröber et al., 2015). Because of its extensive involvement in molecular operations, systemic clinical deficiency—frequently referred to as hypomagnesemia—is directly linked to a broad spectrum of pathological vulnerabilities, including severe neuromuscular disorders, systemic metabolic dysfunction, and cardiovascular anomalies (Al Alawi et al., 2018).

From a functional perspective, the physiological role of magnesium spans multiple organic systems, making its adequate intake a cornerstone of preventive medicine. It acts as a critical determinant in skeletal preservation, where it works alongside calcium and vitamin D to sustain structural bone density (Al Alawi et al., 2018). Furthermore, within the cardiovascular framework, the ion acts as an endogenous calcium channel blocker, thereby modulating vascular tone, peripheral resistance, and myocardial conduction (Gröber et al., 2015). On a neuromuscular level, magnesium acts as a vital modulator of synaptic transmission, a mechanism further detailed below. Consequently, ensuring optimal magnesium status is no longer viewed merely as a dietary requirement, but as a primary therapeutic approach to mitigating chronic systemic diseases.

1.2. Neuromuscular System and Magnesium Relationship

The integrity of the neuromuscular apparatus depends heavily on the homeostatic balance of magnesium. Within the neuromuscular junction and peripheral pathways, magnesium acts as a physiological gatekeeper of cellular excitability (Gröber et al., 2015). By occupying voltage-gated calcium channels on presynaptic terminals, it modulates calcium influx—the primary trigger for acetylcholine exocytosis into the synaptic cleft (Pilchova et al., 2017). Consequently, adequate extracellular magnesium exerts a stabilizing, sedative effect on peripheral nerves, preventing spontaneous, uncoordinated depolarization.

On the postsynaptic side, magnesium regulates muscle biophysics through its natural antagonism with calcium. While calcium binds to troponin C to initiate muscle contraction, magnesium competes for these sites to promote myofibrillar relaxation (Potter et al., 1981). Furthermore, magnesium is a mandatory cofactor for the SERCA pump, which actively sequesters cytosolic calcium back into the sarcoplasmic reticulum to terminate contraction (Pilchova et al., 2017). When magnesium levels fall low, this enzymatic clearance fails, leading to persistent cytosolic calcium accumulation, continuous cross-bridge activation, and clinical symptoms ranging from fasciculations to severe muscle cramps.

1.3. The Concept of Neuromuscular Protection

Neuromuscular protection aims to preserve the physiological and structural integrity of central neural circuits and peripheral muscular tissues from damage caused by hyperfunctional stress or metabolic deficiencies. Central to this paradigm is regulating excitotoxicity, a destructive cascade driven by excessive extracellular glutamate accumulation and subsequent uncontrolled calcium influx into neurons (Kirkland et al., 2021). Magnesium serves as an

essential endogenous neuroprotective agent by providing a voltage-dependent blockade of the NMDA receptor channel pore (Kirkland et al., 2021). Under resting conditions, this physical barrier prevents catastrophic intracellular calcium overloads, shielding neuronal networks from apoptotic signaling, mitochondrial degradation, and structural decay.

Beyond the central nervous system, neuromuscular protection extends to peripheral motor units and vascular interfaces, where preservation requires mitigating localized neuro-inflammation and oxidative stress exacerbated by insufficient magnesium levels (Maier et al., 2022). Optimal magnesium concentrations suppress the systemic overproduction of pro-inflammatory cytokines—such as IL-6 and TNF-—which otherwise induce endothelial dysfunction and lower the threshold for localized muscle injury (Maier et al., 2022). Furthermore, magnesium-mediated protection preserves the microvascular blood supply to nerve terminals and muscle fibers, preventing ischemic stress and maintaining consistent ATP generation (Fiorentini et al., 2021). Consequently, maintaining systemic magnesium homeostatic stability serves as a foundational defense mechanism against both chronic neurodegenerative decline and acute physical trauma.

2. Biological Foundations of Magnesium and its Role in Neuromuscular Physiology

2.1. Magnesium Distribution and Homeostasis in the Human Body

In a healthy adult, total body magnesium stores fluctuate between approximately 22 to 26 grams (Fiorentini et al., 2021). The anatomical distribution of this mineral is highly compartmentalized: roughly 60% is sequestered within the crystalline lattice of the skeletal system, acting as both a structural component and a dynamic functional reservoir (Al Alawi et al., 2018). Approximately 38% resides within soft tissues—primarily inside metabolically active cells of skeletal muscle and organs like the heart and liver—whereas less than 1% to 2% is found in the extracellular fluid compartment (Fiorentini et al., 2021). Serum magnesium levels are tightly regulated within a narrow physiological window of 0.75 to 0.95 mmol/L through a continuous, dynamic equilibrium maintained by the bones, intestines, and kidneys (Al Alawi et al., 2018).

2.2. Absorption, Distribution, and Excretion Mechanisms

The systemic availability of magnesium is dictated by an intricate balance between gastrointestinal uptake and renal clearance. Dietary magnesium absorption occurs predominantly within the small intestine—specifically the distal jejunum and ileum—via two distinct transport pathways: a passive, paracellular bulk transport driven by electrochemical

gradients, and a saturable, active transcellular mechanism mediated by specialized ion channels known as TRPM6 and TRPM7 (Transient Receptor Potential Melastatin member 6 and 7) (Schlingmann et al., 2002). Once absorbed into the bloodstream, approximately one-third of serum magnesium is bound non-specifically to plasma proteins like albumin, while the remaining 60% to 70% exists as biologically active, free ionized Mg^{++}(Al Alawi et al., 2018). Renal excretion serves as the primary regulator of total body magnesium homeostasis. Under normal physiological conditions, the glomeruli filter a substantial portion of plasma magnesium; however, roughly 90% to 95% of this filtered load is meticulously reabsorbed along the nephron, primarily within the thick ascending limb of the loop of Henle via claudin-mediated paracellular pathways, allowing the kidneys to rapidly adjust excretion rates in response to dietary fluctuations (Schlingmann et al., 2002).

2.3. Nerve Transmission and the Role of Magnesium at the Neuromuscular Junction

At the neuromuscular junction (NMJ), magnesium acts as a fundamental endogenous stabilizer of excitable membranes, modulating the precise chemical communication between motor neurons and skeletal muscle fibers. The physiological release of the neurotransmitter acetylcholine (ACh) from presynaptic axon terminals requires a rapid influx of calcium (Ca^{++}) through voltage-gated P/Q-type calcium channels (Pilchova et al., 2017). Magnesium directly opposes this mechanism by competing with calcium for the binding sites of these presynaptic channels, physically hindering calcium entry and subsequent exocytosis of ACh vesicles into the synaptic cleft (Pilchova et al., 2017). By acting as a natural brake on neurotransmitter release, adequate extracellular magnesium concentrations raise the depolarization threshold of the motor endplate, safeguarding the neuromuscular apparatus against erratic, spontaneous nerve firing and continuous synaptic fatigue.

2.4. Importance of Magnesium in Muscle Contraction and Relaxation

The biophysical machinery responsible for myofibrillar shortening and lengthening requires a constant, delicate interplay between calcium activation and magnesium-mediated enzymatic termination. While the elevation of intracellular calcium triggers muscle contraction by binding to troponin C and exposing actin-myosin binding sites, magnesium acts as an essential relaxation factor (Potter et al., 1981). Magnesium physically competes with calcium for these specific regulatory sites on troponin C, promoting a conformational change that shifts the muscle fiber back into a resting state (Potter et al., 1981). Furthermore, muscle relaxation

is an energy-dependent process that relies on the sarcoendoplasmic reticulum calcium-ATPase (SERCA) pump to actively transport cytosolic calcium back into intracellular storage sites (Pilchova et al., 2017). Magnesium is a mandatory catalytic cofactor for this SERCA pump, as well as for the Na^+/K^+-ATPase pump; it forms the essential biologically active complex (Mg-ATP) required to fuel these active transport systems, preventing persistent cytosolic calcium accumulation and subsequent rigid muscle contractures.

3. Magnesium Deficiency and Neuromuscular Pathophysiology

3.1. Etiology of Magnesium Deficiency

Nutritional inadequacies, chronic medical conditions, and lifestyle factors represent the primary elements driving the development of systemic magnesium depletion. Although magnesium is widely distributed in various unrefined food sources, modern agricultural strategies, soil exhaustion, and the extensive industrial processing of dietary items have substantially lowered the magnesium content in standard diets (Rosique-Esteban et al., 2018). Beyond insufficient dietary consumption, the physiological etiology of hypomagnesemia is structurally divided into impaired gastrointestinal absorption and accelerated renal excretion. Chronic intestinal malabsorption syndromes, including celiac disease, inflammatory bowel disease (IBD), and surgical bowel resections, fundamentally disrupt the active TRPM6 and passive paracellular transport pathways responsible for magnesium uptake in the enterocytes (Fiorentini et al., 2021). Furthermore, the prolonged administration of specific pharmacological agents significantly exacerbates renal wasting. In particular, loop and thiazide diuretics inhibit magnesium reabsorption within the thick ascending limb of Henle's loop and the distal convoluted tubule, while proton pump inhibitors (PPIs) disturb the luminal pH required for optimal intestinal channel function, creating a state of chronic latent magnesium deficiency (William & Danziger, 2016).

3.2. Cellular Changes Developing in Magnesium Deficiency

At the cellular level, an insufficient concentration of magnesium ions (Mg^{++}) alters membrane structural dynamics and disrupts vital intracellular metabolic operations. Because intracellular Mg^{++} serves as an obligatory counter-ion for adenosine triphosphate (Mg\ATP), its depletion directly impairs the functionality of energy-dependent enzymatic complexes, most notably the Na^+/K^+-ATPase pump (Workinger et al., 2018). The failure of this primary active transport system leads to an anomalous accumulation of intracellular sodium alongside a severe loss of intracellular potassium, causing a persistent state of cellular depolarization.

Additionally, magnesium serves as the natural, voltage-dependent blocker of the NMDA receptor-operated calcium channels. In a magnesium-depleted microenvironment, this protective electrostatic plug is removed, culminating in an uncontrolled, sustained influx of extracellular calcium into the cytosol (Kirkland et al., 2021). This sustained elevation of intracellular calcium triggers toxic enzymatic cascades, promotes structural mitochondrial damage, and initiates apoptotic cellular pathways within both neural structures and myofibers.

4. Use of Magnesium in Neuromuscular Diseases and Clinical Conditions

4.1. Muscle Cramps and Muscle Spasms

Muscle cramps are common neuromuscular symptoms characterized by sudden, involuntary, and painful contractions of skeletal muscles. They are particularly prevalent among older adults, pregnant women, and individuals exposed to prolonged physical exertion. Because magnesium plays a crucial role in maintaining normal neuromuscular function, magnesium supplementation has long been proposed as a therapeutic strategy for cramp prevention and management. However, current evidence suggests that the efficacy of magnesium supplementation varies according to the underlying etiology of the cramps. A recent Cochrane review concluded that magnesium supplementation does not provide clinically meaningful benefits for idiopathic nocturnal leg cramps in the general population (Garrison et al., 2020). Nevertheless, individuals with documented magnesium deficiency or pregnancy-associated cramps may derive greater benefit from supplementation. Contemporary clinical practice therefore favors a targeted rather than universal supplementation strategy, emphasizing the identification of underlying electrolyte disturbances, hydration status, medication-related causes, and nutritional deficiencies before initiating magnesium therapy (Moretti et al., 2021). In this context, magnesium should be viewed as one component of a comprehensive neuromuscular management approach rather than a standalone treatment.

4.2. Migraine and Primary Headache Disorders

Migraine is a complex neurological disorder involving cortical spreading depression, trigeminovascular activation, neurogenic inflammation, and altered neuronal excitability. Increasing evidence suggests that magnesium deficiency may contribute to several of these pathophysiological processes. Studies have demonstrated reduced magnesium concentrations in serum, cerebrospinal fluid, and brain tissue among migraine sufferers, particularly in patients experiencing migraine with aura and menstrual migraine (Dominguez et al., 2025). Clinical trials have shown that oral magnesium supplementation, typically administered at doses ranging

from 400 to 600 mg daily, may reduce attack frequency, headache days, and migraine severity in selected patients (Varga et al., 2025). Consequently, magnesium has been incorporated into several evidence-based migraine prevention recommendations as a low-cost and generally well-tolerated adjunctive option. Despite these promising findings, variations in study design, magnesium formulations, and patient populations necessitate cautious interpretation of the available evidence. Therefore, magnesium supplementation should be considered as part of an individualized migraine prevention strategy rather than a universal therapeutic intervention.

4.3. Exercise-Induced Muscle Damage and Fatigue

Exercise-induced muscle damage (EIMD) is a physiological response to intense or unaccustomed physical activity and is characterized by structural muscle disruption, inflammation, oxidative stress, and transient declines in performance. Given magnesium's involvement in ATP production, protein synthesis, and cellular energy metabolism, adequate magnesium status is essential for optimal exercise recovery. Emerging evidence suggests that magnesium supplementation may attenuate post-exercise muscle soreness, reduce markers of muscle damage, and support recovery processes following strenuous exercise (Tarsitano et al., 2024). Furthermore, magnesium contributes to the maintenance of normal muscle function and may help preserve neuromuscular efficiency during prolonged physical activity. However, while supplementation appears beneficial in individuals with suboptimal magnesium status, its direct effects on athletic performance outcomes remain inconsistent across clinical studies, showing no definitive ergogenic advantage in magnesium-replete athletes (Newhouse & Finstad, 2000). Current evidence therefore supports maintaining adequate magnesium intake as part of a broader nutritional strategy aimed at promoting recovery and reducing exercise-related neuromuscular stress and muscle damage rather than as a direct performance enhancer (Córdova et al., 2019).

4.4. Fibromyalgia and Chronic Pain Syndromes

Fibromyalgia is a complex chronic pain disorder characterized by widespread musculoskeletal tenderness, persistent fatigue, and heightened subjective stress, which are frequently refractory to conventional pharmacological treatments. From a neuropathophysiological standpoint, magnesium serves as a crucial physiological regulator of central pain transmission through its voltage-dependent blockade of N-methyl-D-aspartate (NMDA) receptors in the central nervous system, thereby dampening neural excitotoxicity and central sensitization pathways that drive chronic pain amplification (Kirkland et al., 2018).

Clinical literature reviews reveal that certain patient cohorts exhibit diminished magnesium levels within multiple tissue compartments, showing a negative correlation with clinical symptom intensity, although the broader epidemiological evidence regarding systemic deficiency remains inconsistent (Boulis et al., 2021). Nonetheless, targeted clinical management utilizing oral magnesium supplementation has been demonstrated to help decrease overall pain severity and alleviate stress parameters, indicating that appropriate magnesium replenishment is a highly tolerable and valuable supportive strategy within a multimodal management approach for chronic pain diseases (Boulis et al., 2021).

4.5. Restless Legs Syndrome

Restless Legs Syndrome (RLS), also known as Willis-Ekbom Disease, is a neurological sensorimotor disorder characterized by an irresistible urge to move the legs, typically exacerbated during periods of rest or inactivity and demonstrating a distinct circadian pattern that peaks at night. Given that the underlying pathophysiology of RLS involves central dopaminergic dysfunction and peripheral neuromuscular hyperexcitabilty, restoring intracellular electrolyte equilibrium is of notable therapeutic interest. Early open-label clinical evaluations indicated that oral magnesium administration could significantly decrease periodic limb movements during sleep (PLMS) and improve overall sleep efficiency in patients suffering from restless legs-related insomnia (Hornyak et al., 1998). More recently, randomized controlled data have further substantiated these clinical outcomes, demonstrating that targeted magnesium supplementation effectively mitigates the subjective severity of RLS symptoms, making it a viable, well-tolerated non-pharmacological adjunctive therapy to enhance sleep quality and reduce sensorimotor distress (Jadidi et al., 2022). 5.1. Oral Magnesium Preparations

The clinical efficacy of oral magnesium supplements is determined by two distinct parameters: the elemental magnesium content of the preparation and its bioavailability. These two properties are often inversely related; salts with high elemental content (e.g., magnesium oxide) generally show low solubility and absorption, whereas organic salts can be absorbed more efficiently despite a lower elemental content. Magnesium salts are traditionally classified as inorganic (oxide, chloride, sulfate) and organic/chelated (citrate, glycinate, malate, lactate, aspartate) forms. The general consensus is that organic forms have higher bioavailability than inorganic ones (Blancquaert et al., 2019; Schuchardt & Hahn, 2017).

5.1.1. Magnesium Oxide

Magnesium oxide (MgO) has the highest elemental magnesium content, at approximately 60%; it therefore provides the most elemental magnesium per tablet and is inexpensive. However, it is practically insoluble in water, and its absorption depends largely on gastric acid–dependent dissolution. Lindberg et al. (1990), together with single-dose cross-over studies, confirmed that magnesium citrate produces a higher serum and urinary magnesium response than oxide (Kappeler et al., 2017; Lindberg et al., 1990).

Unabsorbed magnesium osmotically retains water in the lumen and produces a laxative effect; for this reason MgO is also used at high doses to treat constipation and as an antacid (Ranade & Somberg, 2001).

5.1.2. Magnesium Citrate

Magnesium citrate is among the best-studied and most widely used organic salts, with an elemental magnesium content of approximately 16%. It is highly water-soluble and remains largely in a soluble complex at intestinal pH: Lindberg et al. (1990) showed that about 65% of citrate is present as a soluble magnesium–citrate complex under in vitro conditions, with no such complexation for oxide, making citrate more soluble and more bioavailable (Kappeler et al., 2017). Its absorption is dose-dependent, with fractional absorption falling at high doses (Blancquaert et al., 2019). A potent osmotic laxative also used for pre-colonoscopy bowel preparation, citrate is overall a well-tolerated, cost-effective option with a comparatively strong evidence base. It is preferred in hypomagnesemia from inadequate intake, malabsorption, or chronic medication use (e.g., PPIs or diuretics), and is commonly recommended for nocturnal leg cramps, myofascial pain, fibromyalgia symptoms, and migraine prophylaxis (to reduce neuronal excitability).

5.1.3. Magnesium Glycinate

Magnesium glycinate (bisglycinate/diglycinate) is the form in which a magnesium ion is chelated to two glycine molecules; its elemental magnesium content is approximately 14%. Its proposed advantage is that it can be absorbed partly intact via the dipeptide transport pathway, thereby avoiding competition with ionic metals for carriers in the small intestine (Schuette et al., 1994).

Consequently, the principal clinical value of glycinate lies less in very high bioavailability than in its minimal laxative effect and good gastrointestinal tolerability; it is therefore preferred in patients requiring high-dose repletion or sensitive to gastrointestinal

adverse effects. On the grounds of the calming effect of the glycine moiety, it is also used for sleep and anxiety indications, although the evidence base for these uses remains limited (Rawji et al., 2024; Siebrecht, 2013).

5.1.4. Magnesium Malate

Magnesium malate is formed from the combination of magnesium with malic acid (malate); its elemental magnesium content is approximately 15%, and it generally shows good gastrointestinal tolerability. The basis of its clinical interest is that malate is an intermediate metabolite of the Krebs cycle and contributes to cellular ATP production; it has therefore been proposed particularly in the context of fatigue and fibromyalgia. The theoretical framework was put forward by Abraham and Flechas (1992); however, current systematic appraisals conclude that the use of magnesium and malic acid in fibromyalgia makes little or no difference to pain and depressive symptoms. Magnesium malate should therefore be regarded as a well-tolerated general repletion option in clinical practice, and the claims for energy and fibromyalgia indications should be treated as low-quality evidence.

5.2. Intravenous Magnesium Administration

The intravenous (IV) route bypasses the gastrointestinal absorption step entirely and provides complete bioavailability; it is therefore preferred in severe, symptomatic, or emergency presentations. The most commonly used salt is magnesium sulfate (1 g $MgSO_4$ ≈ 98.6 mg elemental magnesium ≈ 8.12 mEq). The principal indications are repletion of severe/symptomatic hypomagnesemia, prevention and treatment of seizures in pre-eclampsia and eclampsia, torsades de pointes and refractory ventricular arrhythmias due to hypomagnesemia, and adjunctive treatment in severe acute asthma exacerbation. In pre-eclampsia/eclampsia, magnesium sulfate is the agent of choice, having demonstrated superiority over diazepam and phenytoin (Altman et al., 2002).

6. Personalized Magnesium Supplementation Approaches

6.1. Identifying At-Risk Groups

Because magnesium levels cannot be measured reliably, the first step in the clinical approach is to identify individuals at high risk of deficiency, such as those with gastrointestinal disorders, those with type 2 diabetes, individuals with alcohol use disorder, older adults, and users of magnesium-depleting drugs (long-term PPIs, diuretics) (NIH, 2022). In older adults this risk is further accentuated by the combination of inadequate dietary intake, reduced

absorption, increased renal excretion, and polypharmacy (NIH, 2022). Because magnesium acts as a physiological calcium antagonist, its deficiency reduces its inhibitory effect at the neuromuscular junction, so that signs of neuromuscular excitability such as tremor, muscle cramps, hyperreflexia, and tetany may emerge (Barna et al., 2021; NIH, 2022). In practice, therefore, risk assessment should evaluate the patient's dietary history, current medications, and clinical findings as a whole; and where clinical suspicion is high despite a normal serum magnesium, further investigations such as erythrocyte/ionized magnesium measurement or a loading (tolerance) test should be considered (NIH, 2022).

6.2. Magnesium Supplementation in Athletes

Magnesium plays a critical role in energy metabolism and muscle function, and it has been suggested that the requirement of those who exercise intensely may be 10–20% higher than that of sedentary individuals. Nevertheless, since no significant benefit has been demonstrated for the general population in preventing exercise-associated muscle cramps, the practical approach should be to aim for adequate intake through food and to treat only documented deficiencies, rather than blind high-dose supplementation (Garrison et al., 2020). By contrast, the situation differs for non-exercise-related nocturnal cramps; sufficiently prolonged use (e.g., 60 days or >8 weeks) of forms with improved cellular absorption (e.g., magnesium oxide monohydrate) can significantly improve the frequency and duration of cramps and the quality of sleep (Barna et al., 2021).

7. Rational Magnesium Use and the Role of the Clinical Pharmacist

The principles of rational drug use apply to magnesium supplementation as well: the right indication, the right product, the right dose and duration, the right patient. The clinical pharmacist is central to this process, undertaking a stewardship role that ensures the rational use of magnesium by questioning the indication, screening for interactions, monitoring safety, and distinguishing marketing claims from evidence.

7.1. Evidence-Based Supplementation Decisions

The decision must rest first on the indication. The strength of evidence varies markedly with the purpose of use: repletion of documented deficiency is the strongest; support directed at disease course in conditions such as diabetes is moderate and mixed; and symptomatic claims such as cramps or sleep have the weakest evidence (Garrison et al., 2020; Veronese et al., 2016). Bearing in mind that serum magnesium poorly reflects status, the decision should rest not on a

single value but on risk assessment and the clinical picture; food sources should be prioritized where possible, and unnecessary/excessive supplementation should be avoided (NIH, 2022).

7.2. Drug–Magnesium Interactions

Magnesium interactions are bidirectional: some drugs impair magnesium status, while magnesium can reduce the absorption of certain drugs or modify their effects pharmacodynamically (Table 7.1). The most clinically common are hypomagnesemia due to long-term PPI and diuretic use, and reduced absorption of drugs such as tetracyclines, fluoroquinolones, bisphosphonates, and levothyroxine (NIH, 2022). PPI-induced hypomagnesemia develops through impairment of both active (TRPM6/7) and paracellular absorption in the intestine; it typically arises with long-term use and resolves within a few days of discontinuation (William & Danziger, 2016). A point directly connected to the theme of this chapter is that magnesium potentiates the effect of neuromuscular blockers; magnesium use before surgery/anesthesia must therefore always be disclosed (NIH, 2022).

Table 1. Clinically important drug–magnesium interactions and their management.

Interaction type	Drug class (example)	Mechanism / consequence	Clinical recommendation
A. Drugs that deplete magnesium stores (risk of hypomagnesemia)			
Proton-pump inhibitors	Omeprazole, pantoprazole, esomeprazole	Impaired intestinal absorption (TRPM6/7) with long-term use	Monitor serum Mg with prolonged use; avoid unnecessary/long use
Diuretics	Loop (furosemide), thiazide (HCTZ)	Increased renal Mg excretion	Monitor Mg status; K-sparing diuretics (spironolactone) retain Mg
Nephrotoxic/tubule-acting agents	Cisplatin, aminoglycosides, amphotericin B, calcineurin inhibitors, EGFR inhibitors	Tubular injury or renal Mg wasting	Monitor Mg throughout therapy and replete as needed
B. Drugs whose absorption magnesium reduces (separate dosing required)			
Antibiotics	Tetracyclines, fluoroquinolones	Chelation → reduced antibiotic absorption	Take the antibiotic 2 h before or 4–6 h after Mg
Bisphosphonates	Alendronate, risedronate	Reduced absorption	Separate by at least 2 h
Thyroid hormone	Levothyroxine	Reduced absorption via divalent cation binding	Separate by at least 4 h
C. Pharmacodynamic interactions			

Interaction type	Drug class (example)	Mechanism / consequence	Clinical recommendation
Neuromuscular blockers	Agents used in anesthesia	Mg potentiates neuromuscular blockade	Disclose Mg use to the anesthesiologist; use caution
Calcium-channel blockers	Amlodipine, diltiazem, verapamil	Additive hypotension	Monitor blood pressure
Digoxin / QT-prolonging drugs	Digoxin, certain antidepressants	Hypomagnesemia increases arrhythmia risk	Correct and monitor Mg and K levels

HCTZ: hydrochlorothiazide; TRPM6/7: transient receptor potential melastatin type 6/7; QT: ECG QT interval.

7.4. Safety and Adverse-Effect Management

The most common adverse effect of oral magnesium is gastrointestinal (diarrhea, cramping, nausea) and is dose- and form-dependent; it is more frequent with forms that have a marked laxative effect, such as oxide and citrate. In individuals with normal renal function, hypermagnesemia is rare with dietary or usual supplement doses; the risk increases mainly in renal failure and with very high-dose laxative/antacid use (>5,000 mg/day) (NIH, 2022). Signs of toxicity generally follow a pattern that progresses as the serum level rises: early loss of deep tendon reflexes, then hypotension, facial flushing, and nausea; and at advanced stages muscle weakness, respiratory depression, bradyarrhythmia, and cardiac arrest. For this reason, reflexes, respiration, and the ECG are monitored during parenteral use, and intravenous calcium is kept available as an antidote (NIH, 2022). Monitoring serum magnesium in at-risk groups (especially renal failure) is the foundation of safe use.

7.5. Patient Education and Counseling

Effective counseling ensures that supplementation is both safe and genuinely beneficial. The main points the clinical pharmacist should emphasize are that dividing the dose across the day or taking it with food reduces gastrointestinal adverse effects; that it should be separated by an appropriate time interval from interacting drugs (antibiotics, bisphosphonates,

levothyroxine); that adverse effects such as diarrhea should be reported; and that the supplement does not replace medical treatment. Keeping expectations aligned with the evidence is also important: the evidence base for popular uses such as cramps and sleep is weak (Garrison et al., 2020). During medication reconciliation the clinical pharmacist can identify magnesium-depleting drugs (long-term PPIs, diuretics) and recommend monitoring or, where appropriate, dose reduction/discontinuation; provide over-the-counter product counseling; and support rational use by distinguishing marketing claims from evidence (NIH, 2022; William & Danziger, 2016).

8. Future Perspectives

The most fundamental limitation in the magnesium field is the inability to measure status reliably, making better biomarkers the key area for future progress; ionized magnesium, erythrocyte/intracellular measurements, and the loading (tolerance) test may reflect true stores more accurately, though none is satisfactory alone (NIH, 2022; de Baaij et al., 2015). Personalized nutrition is a second direction, with genetic variants in transporters such as TRPM6, the microbiome, and individual response markers expected to guide decisions (de Baaij et al., 2015), while higher-bioavailability chelates and extended-release formulations await confirmation of clinical superiority in comparative randomized trials (Blancquaert et al., 2019). Adequately powered trials are still needed in muscle cramps, athletic performance, and diabetes outcomes, together with clearer thresholds defining true deficiency (Garrison et al., 2020; Veronese et al., 2016). From a neuromuscular-protection standpoint, magnesium's potential role is promising but must be advanced within an evidence-based, rational framework.

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