

MEDICAL BIOLOGY AND GENETICS IN THE GENOMIC ERA

EDITOR: ASSISTANT PROFESSOR MUSTAFA ÇAKIR



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Assistant Professor MUSTAFA AKIR

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BACTERIAL GENETICS

Elif SEVİM¹, Ali SEVİM²

INTRODUCTION

Bacterial genetics is the foundation of modern molecular biology and biotechnology. One of the most important milestones in the historical development of this field was the pioneering study published by François Jacob and Jacques Monod in 1961 on the genetic regulatory mechanisms of protein synthesis (operon model). Jacob and Monod demonstrated that genes not only encode protein structures but also possess regulatory systems, including operons, repressors, and inducers, which control the timing and extent of gene expression in response to environmental stimuli (Jacob and Monod, 1961).

Bacterial cells possess a high degree of genomic flexibility that enables them to adapt to rapid physical and chemical changes in their environments. One of the most striking aspects of bacterial evolution is that genetic material can be transferred not only vertically (from parent to offspring) but also horizontally among different species, genera, and even kingdoms (De Sousa et al., 2023; Tokuda and Shintani, 2024). Horizontal gene transfer (HGT) allows bacterial populations to benefit from a collective genetic pool, a phenomenon referred to as “open-source evolution” (Frost et al., 2005). Through HGT, bacterial cells can acquire entirely new metabolic capabilities, virulence factors, or antibiotic resistance genes within a single generation (Partridge et al., 2018).

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The dynamic balance between stability and instability of the bacterial genome forms the basis of the evolutionary process (Darmon and Leach, 2014). Although DNA replication must be carried out with high fidelity, the instability generated by horizontal gene transfer and mobile genetic elements plays a critical role in enabling bacteria to adapt to new ecological niches (Thierauf et al., 2009; Darmon and Leach, 2014).

BACTERIAL GENOME STRUCTURE

Traditionally, bacterial genomes consist of a chromosome composed of a single, circular, double-stranded DNA molecule; however, some bacteria may possess linear chromosomes or multiple chromosomes. Unlike eukaryotic DNA, bacterial DNA is not enclosed within a membrane-bound nucleus but is located in a condensed cytoplasmic region called the nucleoid, where it is organized in a supercoiled form by histone-like proteins (e.g., such as HU and H-NS) (Snyder et al., 2020; Willey et al., 2023; Madigan et al., 2024).

Bacterial DNA replication begins at a single region on the circular chromosome called the “origin of replication” (*oriC*) and proceeds bidirectionally (Snyder et al., 2020). Although replication is an extremely precise and rapid process, it can be interrupted by various environmental and internal stressors. Stalling or collapse of replication forks can lead to a condition known as “genome instability” in bacterial genomes. Replication-associated stalling, double-strand breaks, and single-strand gaps can result in genomic rearrangements, deletions, duplications, and translocations (Darmon and Leach, 2014). To overcome this instability and restart replication, bacterial cells activate complex DNA repair mechanisms, such as homologous recombination (*RecA*-mediated) and the SOS response. This balanced instability can, on the one hand, threaten cell survival, while on the other hand, it acts as a driving force that increases genetic diversity and promotes evolution-

ary adaptation (Darmon and Leach, 2014; Snyder et al., 2020; Tokuda and Shintani, 2024).

Genes within the bacterial genome are generally organized in a highly compact manner. A gene contains the genetic information required for the synthesis of a specific RNA or protein product. In bacteria, it is common for multiple genes to be co-expressed under the control of a shared promoter and regulatory region. This type of gene organization is referred to as an operon. The concept of the operon represented an important milestone in understanding bacterial gene regulation through the classical studies of Jacob and Monod. In particular, the model established through the lac operon in *Escherichia coli* provided important insights into how bacteria regulate gene expression in response to changes in environmental conditions. The regulation of gene expression in bacteria enables the efficient use of energy and resources. For example, under conditions in which a particular nutrient is unavailable, repression of genes encoding enzymes involved in the metabolism of that nutrient prevents the cell from producing unnecessary proteins. Conversely, when the appropriate substrate is available, the expression of the relevant genes can be increased (Jacob and Monod, 1961; Snyder et al., 2020).

From a functional perspective, the bacterial genome can be classified into the core and accessory genomes. The core genome contains genes shared by most bacteria within the same species or population and are essential for basic cellular functions. In contrast, the accessory genome consists of genes present in specific strains that can often confer traits such as environmental adaptation, virulence, metabolic capabilities, or antimicrobial resistance. This framework is important for explaining why bacteria belonging to the same species can exhibit different phenotypic and clinical characteristics. For example, two strains of the same bacterial species may possess different resistance genes or virulence factors. Some of these genes may

have been acquired through plasmids and other mobile genetic elements (Madigan et al., 2024; Snyder et al., 2020).

BACTERIAL HORIZONTAL GENE TRANSFER

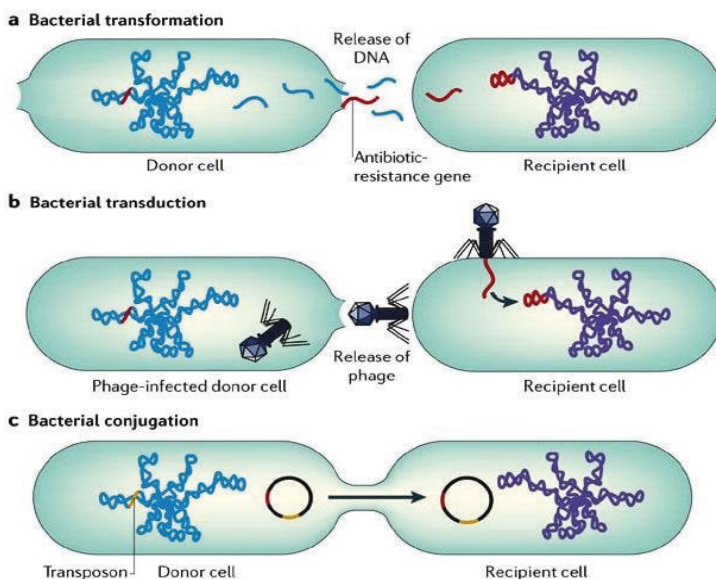
Horizontal gene transfer (HGT) involves the transfer of genetic material from one cell to another within the same generation, unlike vertical gene transfer, which refers to the transfer of genetic material from a parent cell to a daughter cell. HGT is one of the most important mechanisms by which bacteria rapidly acquire new genetic traits. HGT occurs through three main mechanisms: transformation, conjugation, and transduction (Table 1) (Frost et al., 2005; De Sousa et al., 2023).

Table 1. Comparison of horizontal gene transfer mechanisms in bacteria

	Transformation	Transduction	Conjugation
DNA source	Free DNA in the environment	Phage containing donor bacterial DNA	Donor bacterial DNA
Mechanism	Natural or artificial competence	Capsid packaging and injection	Relaxosome-mediated transfer
Cell-to-cell contact	Not required	Not required	Required
Chromosomal integration	RecA-mediated recombination	Homologous recombination or integration	Replication and circularization

Transformation

Transformation is the process by which free (naked) DNA molecules present in the environment are taken up by a bacterial cell and integrated into its own genome (Figure 1) (Madigan et al., 2024; Willey et al., 2023).



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Figure 1. Major mechanisms of horizontal gene transfer in bacteria: transformation, transduction, and conjugation (Furuya and Lowy, 2006).

Some bacteria can enter a genetically programmed state of “natural competence” which enables them to take up DNA from the extracellular environment. This process is regulated by complex protein nanomachines located on the cell surface and composed of structures resembling Type IV pili (Johnston et al., 2014; Dubnau and Blokesch, 2019). In the Gram-positive model organism *Bacillus subtilis*, natural competence and DNA uptake mechanisms have been extensively characterized (Maier, 2020). Extracellular double-stranded DNA (dsDNA) is bound by competence proteins, such as *ComEA*. Subsequently, one strand of the DNA is degraded by nucleases, whereas the other strand is transported into the cell through the *ComEC* channel (Dubnau and Blokesch, 2019; Maier, 2020). The single-stranded DNA (ssDNA) entering the cell is protected by *ComA/RecA* proteins and integrated into the bacterial

chromosome through homologous recombination (Johnston et al., 2014; Maier, 2020). In Gram-negative bacteria, additional secretin channels, such as *PilQ*, facilitate the passage of DNA across the outer membrane (Dubnau and Blokesch, 2019). The regulation of competence varies considerably among bacterial species; for example, quorum sensing mechanisms trigger competence in *Streptococcus pneumoniae*, whereas peptide pheromones and nutrient limitation regulate this process in *Bacillus subtilis* (Johnston et al., 2014).

Bacteria that are not naturally competent (e.g., *Escherichia coli*) can be induced to take up exogenous DNA in the laboratory using various physical and chemical methods (Hanahan, 1983; Ren et al., 2019). Hanahan's classic study in 1983 demonstrated that the transformation efficiency of *E. coli* could be dramatically increased using divalent cations, particularly calcium, rubidium, and manganese chloride (Hanahan, 1983). Electroporation is widely used today, in addition to chemical competence. This method employs high-voltage electrical pulses to create transient pores in the cell membrane, facilitating DNA uptake. Artificial transformation methodologies are a cornerstone of modern recombinant DNA technology and genetic engineering (Ren et al., 2019).

Conjugation

Conjugation is the transfer of genetic material between two bacterial cells through direct physical contact (Figure 1) (Snyder et al., 2020).

This transfer is generally mediated by Type IV Secretion Systems (T4SS), which are encoded by conjugative plasmids present in the "donor" cell. The T4SS is a large multiprotein complex found in both Gram-negative and Gram-positive bacteria that crosses the cell envelope and functions as a tunnel between the donor and recipient cells. The conjugation process begins when a relaxase enzyme binds to the *oriT* (origin of transfer) region on the plasmid and cleaves one strand of the

DNA through a process known as nicking. The relaxase enzyme remains covalently attached to the cleaved DNA strand, and this complex, known as the relaxosome, is transferred to the recipient cell through the T4SS (Virolle et al., 2020; Paillard et al., 2025).

Conjugative transfer involves the transfer of DNA and the delivery of specific proteins into the recipient cell. Mamun et al. (2021) demonstrated that single-stranded DNA-binding proteins (SSBs) encoded by the F plasmid are transferred to the recipient cell during conjugation and suppress the SOS stress response triggered by the accumulation of single-stranded DNA in the recipient cell. This enables conjugation to proceed smoothly without inducing substantial stress in the recipient cells, thereby increasing the efficiency of plasmid dissemination within the bacterial population (Mamun et al., 2021). Conjugation is one of the most widespread mechanisms facilitating interspecies and even interkingdom gene flow in laboratory settings and natural microbial communities (Virolle et al., 2020).

Transduction

Transduction is the transfer of genetic material between bacterial cells mediated by bacteriophages (bacterial viruses) (Figure 1) (Thierauf et al., 2009; Willey et al., 2023). Transduction is classically divided into two main types:

Generalized Transduction: This occurs when the bacterial chromosome is randomly fragmented during the lytic cycle of a phage, and some of these DNA fragments are mistakenly packaged into phage heads. When the resulting “transducing particle” infects a new cell, it transfers DNA from the previous host instead of phage DNA (Thierauf et al., 2009).

Specialized Transduction: This occurs when a temperate phage is incorrectly excised from the bacterial chromosome upon exiting the lysogenic cycle, resulting in the incorporation and

transfer of specific bacterial genes located adjacent to the phage integration site into the phage genome (Snyder et al., 2020).

Mechanisms discovered in recent years have further expanded the scope of transduction. Phage-inducible chromosomal islands (PICIs) are mobile genetic elements integrated into the host bacterial genome that can be activated following infection by a helper phage and subsequently packaged and transferred at high efficiency. Lateral transduction has challenged the conventional understanding of the rates of horizontal gene transfer. In some temperate phages, replication of phage DNA begins before the phage DNA is excised from the host chromosome. Consequently, large bacterial chromosomal regions, sometimes spanning hundreds of kilobases downstream of the prophage, are replicated together with the phage genome and subsequently packaged and transferred at very high frequencies. Lateral transduction can greatly exceed the transfer rates of classical mobile genetic elements, resulting in a substantial increase in bacterial chromosomal mobility (Humphrey et al., 2021; Borodovich et al., 2022).

Phages also play an important role in gene transfer in the human microbiota. In particular, the high abundance of bacteriophages in the intestinal microbiota suggests that phage-mediated gene transfer may influence the genetic composition and evolution of bacterial communities (Borodovich et al., 2022).

BACTERIAL MOBILE GENETIC ELEMENTS (MGE)

Mobile genetic elements (MGEs) are DNA elements capable of moving within a genome or mediating the transfer of genetic material between different cells. These elements are fundamental components of the dynamic nature of bacterial genomes. Through MGEs, bacteria can acquire new genes, alter the positions of existing genes within their genomes, and trans-

fer genetic traits to other bacteria (Frost et al., 2005; Tokuda and Shintani, 2024).

Mobile genetic elements include insertion sequence elements, transposons, integrons, plasmids, bacteriophages, and integrative and conjugative elements (ICEs) (Table 2).

Tablo 2. Major mobile genetic elements

MGE	Basic Feature	Characteristics	Medical Importance
Insertion Sequence (IS) Element	Small transposable DNA element	Can affect gene expression	Genome rearrangement
Transposon	Can move within the genome	May carry resistance/virulence genes	Dissemination of resistance genes
Integron	Captures and expresses gene cassettes	Often carries multiple resistance genes	Multidrug resistance
Plasmid	Extrachromosomal DNA	May carry resistance, virulence, and metabolic genes	Rapid HGT
Bacteriophage	Bacterial virus	Can carry bacterial DNA	Transduction
Integrative and Conjugative Element (ICE)	Integrates into the chromosome and can be transferred	May carry resistance, virulence, and metabolic genes	Chromosomal gene transfer

Insertion Sequence Elements (IS)

Insertion sequences (IS elements) are among the simplest transposable DNA elements found in bacterial genomes. They typically consist of a gene encoding the transposase enzyme required for transposition, flanked by terminal repeat sequences. Movement of IS elements within the genome can alter gene function by inserting them into coding or regulatory regions of genes. In addition, the association of two different IS elements or IS-like sequences can contribute to the formation of more complex transposons (Mahillon and Chandler, 1998).

Transposons

Transposons are genetic elements capable of moving from one location to another within the genome. Some transposons may carry genes that encode antimicrobial resistance or other adaptive traits. Their importance lies in their ability to facilitate the transfer of resistance genes between different genetic structures of bacteria. For example, a resistance gene may initially be located on a transposon and subsequently transferred to a plasmid or chromosome. This enables both the movement of resistance genes within the genome and their horizontal transfer between bacterial species (Partridge et al., 2018).

Integrans

Integrans are specialized genetic systems that enable bacteria to capture genetic material from the environment and organize it into expressible gene cassettes. Key components of integrans include integrase enzyme, recombination site, and an adjacent region where gene cassettes are incorporated. Integrans are particularly important in the context of antimicrobial resistance. The presence of multiple distinct resistance genes within the same integron or within genetic structures associated with integrans can contribute to the acquisition of resistance to multiple classes of antimicrobials by a single bacterium. This is particularly important in the emergence and dissemination of multidrug-resistant gram-negative bacteria. However, integrans should not be considered independent, self-mobilizing elements in the classical sense. Their dissemination among different bacterial populations is largely facilitated by their association with other mobile genetic elements, such as plasmids and transposons (Mazel, 2006).

Integrative and Conjugative Elements (ICEs)

Integrative and conjugative elements (ICEs) are mobile genetic elements that can integrate into the bacterial chromosome, but under appropriate conditions, can excise from the

chromosome and be transferred to another bacterium through conjugation-like mechanisms. ICEs function as a bridge between chromosomal DNA and extrachromosomal mobile genetic elements. Some ICEs may carry genes associated with antimicrobial resistance, virulence, and metabolic adaptation. Therefore, ICEs are considered important vehicles in the evolution of bacterial genome (Johnson and Grossman, 2015).

Plasmids

Plasmids are predominantly circular, double-stranded DNA molecules that replicate independently of the bacterial chromosome. Some plasmids are not essential for bacterial survival; however, the genes they carry may provide important advantages to the cell under specific environmental conditions. One of the most important characteristics of plasmids is their ability to transfer between different cells. In particular, conjugative plasmids mediate horizontal gene transfer between bacteria by encoding the genetic systems required for their transfer (Smillie et al., 2010; Snyder et al., 2020).

To be maintained within the cell, plasmids must possess independent replication mechanisms. Regulation of plasmid replication ensures that an appropriate number of plasmid copies is maintained within the cell. Some plasmids are present at low copy numbers, whereas others are present at high copy numbers. The maintenance of plasmids within bacterial cells depends not only on replication but also on mechanisms that ensure their distribution to daughter cells. Some plasmids have evolved specialized systems that facilitate the partitioning of plasmid molecules into newly formed daughter cells during cell division (Smillie et al., 2010).

Based on their transfer capabilities, plasmids can be classified as conjugative or mobilizable plasmids. Conjugative plasmids carry a substantial portion of the genetic machinery required for transfer, whereas mobilizable plasmids may not possess a complete transfer system of their own and can uti-

lize proteins provided by another conjugative system. Plasmid transfer can facilitate the rapid dissemination of genetic traits within bacterial communities. In particular, gene transfer between different bacterial species in dense bacterial populations or healthcare settings can contribute to the spread of antimicrobial resistance traits (Virolle et al., 2020).

Plasmids carrying antimicrobial resistance genes are of particular importance in clinical settings. Resistance plasmids may carry a single resistance gene or multiple genes conferring resistance to different classes of antimicrobials. The presence of multiple resistance genes on the same plasmid can provide bacteria with a selective advantage when exposed to different antimicrobial agents. These genes are often associated with other mobile genetic elements, such as transposons and integrons. Thus, plasmids can serve as platforms for the dissemination of not only individual resistance genes but also multiple resistance determinants (Partridge et al., 2018).

Because plasmids exist outside the bacterial chromosome, they allow genetic traits to be acquired and lost rapidly. Under changing environmental conditions, a particular plasmid may provide a selective advantage to a bacterium, whereas it may be lost when it no longer confers a fitness benefit to the bacterium. This dynamic contributes to the ability of bacterial genomes to respond rapidly to environmental selective pressure (Smillie et al., 2010; Tokuda and Shintani, 2024).

CONCLUSION AND RECOMMENDATIONS

Bacterial genetics encompasses the fundamental mechanisms that enable these unicellular organisms to be among the most successful and adaptable forms of life on Earth. The dynamic nature of the bacterial genome and the activity of mobile genetic elements are among the major factors underlying the ability of bacteria to rapidly adapt to changing environmental conditions. However, this flexibility can also facilitate

the emergence and dissemination of antimicrobial resistance and novel virulence traits, thereby posing a significant public health challenge. In the future, comprehensive investigation of bacterial genomes and mobile genetic elements will be critical for elucidating the pathways through which resistance genes are disseminated and for developing novel infection-control strategies.

The progression from the classical operon model proposed by Jacob and Monod to contemporary approaches, such as lateral transduction and artificial transformation, has demonstrated the remarkable genomic flexibility of bacteria. Through horizontal gene transfer and mobile genetic elements, bacteria function as dynamic systems that continuously modify and update their genomes, enabling an “open-source” evolution.

Understanding the implications of these processes in medicine and biotechnology represents one of the most powerful tools available for combating infectious diseases and advancing next-generation genetic engineering applications. Continued genomic surveillance, molecular characterization of mobile genetic elements, and integration of genomic data with epidemiological information will be essential for monitoring the emergence and dissemination of antimicrobial resistance and developing effective strategies to control its spread.

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BIOMARKERS AND THERAPEUTIC TARGETS IN NEURODEGENERATIVE DISEASES

Muazzez Derya-Andeden¹, Pınar Altın-Çelik², Naciye Elif Tokgüner³

INTRODUCTION

Neurodegenerative diseases are a group of progressive and multifactorial disorders that impair the structure and function of neurons, ultimately leading to their degeneration and loss. As populations age, these disorders are becoming an increasingly important global health concern. Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) are among the most prominent examples. Although they differ in their clinical presentation, they are generally associated with gradual neuronal loss that results in declining cognitive and/or motor function over time. The mechanisms underlying neurodegenerative diseases have not yet been fully clarified. Nevertheless, a number of interconnected biological processes are thought to contribute to their onset and progression. Among these, neuroinflammation has attracted particular attention. Neuroinflammation primarily involves the activation of microglia and astrocytes in response to tissue injury or pathological changes in the central nervous system. While this response may initially be protective, persistent or dysregulated activation can become detrimental. Chronic neuroinflammatory signaling has been associated with synaptic dysfunction,

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neuronal injury, and the further progression of neurodegeneration. For this reason, neuroinflammation is considered a central area of interest in current neurodegenerative disease research (Selvam and Ayyavoo, 2024).

The global burden of neurodegenerative diseases is increasing steadily, largely in parallel with rising life expectancy worldwide. Among these disorders, AD represents the greatest share, currently affecting more than 50 million people globally, with prevalence expected to increase markedly by 2050. PD, the second most common neurodegenerative disorder, affects nearly 10 million individuals worldwide. Although ALS (ALS) is less common, its rapid progression and poor prognosis impose a severe burden on both patients and their families. Beyond their clinical consequences, these disorders also create a substantial socioeconomic impact through healthcare expenditures, reduced productivity, and long-term caregiving demands, which together amount to hundreds of billions of dollars annually (Chen et al., 2018).

Despite extensive research over several decades, the precise causes of AD, PD, and ALS have not yet been fully elucidated. Current therapeutic approaches are largely limited to symptomatic management, and no curative treatment is currently available for these conditions. This major therapeutic limitation has driven growing interest in the identification of reliable biomarkers for early diagnosis, as well as in the discovery of novel therapeutic targets aimed at the underlying mechanisms of disease rather than the relief of symptoms alone (Houldsworth, 2024).

The diagnosis and treatment of neurodegenerative diseases remain particularly challenging because of their biological and clinical complexity. Similar pathological mechanisms may give rise to different clinical phenotypes, while distinct proteinopathies can be difficult to distinguish in practice. These challenges highlight the need for robust biomarkers that can

improve early detection, monitor disease progression, predict therapeutic response, and serve as surrogate endpoints in clinical trials (Konickova et al., 2023).

At the same time, major advances in molecular biology, neuroimaging, and systems biology have significantly expanded our understanding of neurodegenerative processes. High-throughput omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, have revealed complex networks of dysregulated pathways involved in disease pathogenesis. As a result, a growing number of candidate biomarkers and therapeutic targets have been identified, offering new opportunities for earlier diagnosis and more mechanism-based treatment strategies (Singh et al., 2019).

Classification and Types of Biomarkers

Biomarkers are measurable indicators of biological processes, disease states, or responses to therapeutic interventions. In the field of neurodegenerative diseases, they have attracted increasing attention because of their potential value in early diagnosis, disease monitoring, and therapeutic evaluation. In many patients, clinical symptoms become apparent only after substantial neuronal damage has already occurred, which makes early detection particularly difficult. In this context, biomarkers provide an important advantage by offering measurable signals of ongoing pathological or physiological changes. These markers may take different forms, including proteins, genetic alterations, and imaging-based findings. In addition to their diagnostic value, reliable biomarkers also contribute to therapeutic development by enabling more accurate monitoring of disease progression and treatment response (Niedzielska et al. 2016).

In neurodegenerative diseases, biomarkers are commonly classified into four major groups according to their clinical utility. Diagnostic biomarkers are used to identify the presence of disease, especially during preclinical or early symptomatic stages before overt manifestations are fully established. These

biomarkers are particularly helpful when different disorders present with overlapping clinical features. For example, characteristic cerebrospinal fluid profiles involving tau and amyloid-beta may help distinguish AD from other forms of dementia, even before pronounced cognitive decline becomes evident (Jellinger, 2010).

Prognostic biomarkers, in contrast, provide information about the likely course and progression rate of a disease. They can assist clinicians in estimating future outcomes, guiding clinical decision-making, and informing patients and families more accurately about disease trajectory. Such markers are also valuable in clinical trials, where they support patient stratification according to expected disease progression. Plasma glial fibrillary acidic protein (GFAP), for instance, has emerged as a promising prognostic biomarker for predicting the transition from mild cognitive impairment to Alzheimer's dementia, thereby supporting earlier intervention and improved care planning (Pereira et al., 2021).

Staging biomarkers indicate the current phase or severity of a disease and are therefore highly relevant for treatment planning and inclusion in clinical trials. These markers reflect the degree of neuronal injury and functional decline present at a given stage of the disease. Among them, neurofilament light chain (NfL) measured in blood or cerebrospinal fluid has become one of the most informative examples, as its levels correlate with the extent of neurodegeneration across a range of neurological disorders and can provide a quantitative estimate of disease stage (Gaetani et al., 2019).

Treatment response biomarkers are used to assess the biological effects of therapeutic interventions and often function as pharmacodynamic indicators of target engagement. Their value lies not only in monitoring whether a therapy is exerting its intended biological action, but also in supporting dose optimization during drug development. In some cases, biomarker chang-

es may be detected before clear clinical improvement becomes evident. For this reason, shifts in CSF biomarker profiles after experimental treatment may provide early indications of therapeutic benefit or lack of efficacy, thereby contributing to more efficient evaluation of candidate therapies (Nowak et al., 2024).

Biomarkers in neurodegenerative diseases can also be categorized according to their source. Clinical biomarkers include findings obtained from neurological examination and neurophysiological testing. Biochemical biomarkers are measured in cerebrospinal fluid, serum, plasma, or tissue samples. Neuroimaging biomarkers rely on advanced imaging modalities such as magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT). In addition, genetic biomarkers comprise both inherited and acquired alterations, while molecular biomarkers include a broad range of proteins, lipids, metabolites, and nucleic acids, including microRNAs (Jack et al., 2018).

Increasingly, emphasis has been placed on the integration of different biomarker types through a multimodal approach. The combination of fluid biomarkers with imaging findings and clinical assessments offers a broader and often more accurate representation of disease status than any single modality alone. This integrated perspective is particularly valuable in neurodegenerative diseases, where pathogenesis is multifactorial and heterogeneous, and it can improve both diagnostic performance and prognostic assessment (Hansson, 2021).

Blood-Based Biomarkers

Blood-based biomarkers have attracted considerable attention in recent years because they are easily accessible, minimally invasive, and suitable for repeated measurement. In contrast to cerebrospinal fluid sampling, which requires lumbar puncture, blood collection is a routine clinical procedure that can be performed more frequently and with less discomfort for

patients. These practical advantages make blood-based biomarkers especially valuable for large-scale screening, longitudinal follow-up, and broader use in clinical settings, including primary care (Blennow et al., 2023).

Among the blood-based biomarkers studied in neurodegenerative diseases, plasma glial fibrillary acidic protein (GFAP) has emerged as a particularly promising candidate for AD. GFAP is an intermediate filament protein mainly expressed in astrocytes, and elevated plasma levels are generally considered to reflect astrocytic activation and neuroinflammatory processes. Recent studies have shown that increased plasma GFAP levels may predict the transition from mild cognitive impairment to Alzheimer's dementia, suggesting that this marker could be useful for identifying disease progression at relatively early stages (Teunissen et al., 2024).

The relevance of plasma GFAP is not limited to indicating astrocyte injury alone. Astrocytes have diverse roles in the central nervous system, including the maintenance of synaptic homeostasis, support of the blood-brain barrier, and regulation of inflammatory signaling. Therefore, elevated GFAP levels may also reflect reactive astrogliosis, a process whose effects may vary depending on disease stage and pathological context. For this reason, interpreting GFAP in clinical practice requires consideration of its temporal dynamics and biological context throughout disease progression (Escartin et al., 2021).

Neurofilament light chain (NfL) is another important blood-based biomarker with broad relevance across neurodegenerative disorders. Neurofilaments are major structural components of the neuronal cytoskeleton, and their release into extracellular fluids occurs following axonal damage. NfL can be quantified in both cerebrospinal fluid and blood, and blood concentrations generally correlate well with those measured in CSF. Although NfL is not specific to a single disease, it is widely regarded as a marker of neuroaxonal injury and therefore pro-

vides useful information about the extent of neurodegeneration in disorders such as AD, PD, and ALS (Khalil et al., 2018).

In AD, higher blood NfL levels have been associated with greater disease severity, increased brain atrophy, and cognitive decline. In PD, NfL levels are typically lower than those observed in AD, but still tend to be elevated relative to healthy individuals, with higher concentrations often associated with faster motor deterioration. In ALS, NfL levels are often markedly increased, consistent with the rapid and severe degeneration of motor neurons. Because of this pronounced elevation, NfL may also help distinguish ALS from clinically similar conditions and may provide prognostic information regarding disease course and survival (Verde et al., 2019).

The development of ultrasensitive immunoassay platforms, particularly single-molecule array technologies, has substantially improved the detection of low-abundance proteins in blood. These methods have made it possible to measure analytes at concentrations that were previously detectable mainly in cerebrospinal fluid, thereby expanding the clinical potential of blood-based biomarkers. At present, ongoing work is focused on assay standardization, the definition of reference ranges across different populations, and the establishment of clinically meaningful cut-off values for diagnostic and prognostic use (Rissin et al., 2010).

In addition to GFAP and NfL, several other blood-based biomarkers are under investigation. Among these, phosphorylated tau species, especially p-tau181 and p-tau217, can now be measured in plasma and have shown considerable promise for the diagnosis and staging of AD. Blood-based alpha-synuclein measurement is also being explored as a possible tool for PD, although technical limitations remain. Beyond these protein markers, circulating inflammatory mediators, oxidative stress-related parameters, and metabolomic signatures may provide further insight into disease-related biological processes (Ashton et al., 2021).

Cerebrospinal Fluid Biomarkers

Because cerebrospinal fluid is in close contact with the central nervous system, it occupies a central role in biomarker research for neurological diseases. In many settings, CSF is still regarded as the reference biological fluid for biomarker analysis, as it more directly reflects ongoing biochemical and pathological changes in the brain than peripheral blood does. Although CSF collection is more invasive than blood sampling, lumbar puncture has become a routine procedure in specialized neurology clinics and is generally considered well tolerated (Duits et al., 2016).

Tau protein is present in several forms in CSF, and these different species provide complementary information about AD pathology. Total tau is generally interpreted as a marker of neuronal injury and has been associated with the extent of neurodegeneration. In contrast, phosphorylated tau (p-tau), particularly p-tau181, p-tau217, and p-tau231, is considered more specific for Alzheimer-type pathology because tau hyperphosphorylation is a defining feature of neurofibrillary tangle formation. In addition, ratios such as p-tau/total tau or p-tau/A β 42 improve the specificity of biomarker-based differentiation between AD and other dementias (Blennow and Zetterberg, 2018).

Recent studies have further shown that distinct tau phosphorylation sites may carry different clinical significance. Among them, p-tau217 appears to have a particularly strong relationship with amyloid PET positivity and may become abnormal earlier in the disease course than p-tau181. These observations suggest that using a panel of tau-related biomarkers, rather than relying on a single measure, may provide a more comprehensive assessment of AD pathology. Moreover, CSF tau measurements correlate with tau PET findings, supporting their use as surrogate indicators of tau burden in the brain (Janelidze et al., 2020).

Amyloid-beta peptides in cerebrospinal fluid, particularly A β 42 and the A β 42/A β 40 ratio, play a central role in the biomarker-based evaluation of AD. In contrast to what might be expected, CSF A β 42 levels are reduced in patients with AD, which is generally interpreted as a consequence of amyloid deposition and sequestration within brain plaques. The A β 42/A β 40 ratio is especially useful because it compensates for interindividual differences in total amyloid production and thereby improves diagnostic performance. These changes occur early in the course of the disease, often long before the onset of clinical symptoms, making them especially valuable for identifying at-risk individuals and for selecting participants for prevention-oriented studies (Hansson et al., 2018).

Alpha-synuclein has also been widely investigated in CSF as a biomarker for PD and other synucleinopathies. In general, total alpha-synuclein levels tend to be lower in patients with PD than in healthy controls, possibly because of its aggregation and deposition in Lewy bodies. However, the diagnostic usefulness of total CSF alpha-synuclein remains limited because of the considerable overlap between patients and control groups. More informative approaches appear to include the detection of oligomeric or aggregated alpha-synuclein species, as well as seed amplification assays designed to identify pathological alpha-synuclein conformations with high analytical sensitivity and specificity (Shahnawaz et al., 2017).

TDP-43 (TAR DNA-binding protein 43) is the major pathological protein associated with ALS and frontotemporal dementia. Although TDP-43 pathology is a prominent feature of these disorders, its measurement in CSF remains technically challenging. Studies evaluating total TDP-43 levels have produced inconsistent findings, which has led to increasing interest in phosphorylated, aggregated, or otherwise modified forms that may more accurately reflect underlying pathology. In addition, TDP-43 fragments and post-translationally modified variants are being explored as potentially more specif-

ic biomarkers for ALS and related TDP-43 proteinopathies (Kasai et al., 2009).

Among inflammatory CSF biomarkers, soluble TREM2 (sTREM2) and YKL-40 have attracted growing attention as indicators of microglial and astrocytic activation, respectively. TREM2 is a microglial receptor involved in the regulation of inflammatory signaling and phagocytic function, and its soluble form has been found to be elevated in the CSF of patients with AD, where it has also been associated with disease progression. YKL-40, also known as chitinase-3-like protein 1, is secreted by activated astrocytes and has been reported to increase in the CSF of patients with several neurodegenerative disorders. Together, these biomarkers provide insight into the neuroinflammatory component of neurodegeneration and may also prove useful as companion markers in studies of immunomodulatory therapies (Ewers et al., 2020).

The use of CSF biomarkers within structured diagnostic panels has now become a standard approach, particularly in AD research. One of the most widely adopted models is the AT(N) framework, which classifies biomarkers according to amyloid deposition (A), tau pathology (T), and neurodegeneration (N). This framework supports both diagnosis and disease staging and can be applied using CSF biomarkers alone or together with imaging-based measures, making it useful in both clinical and research contexts (Jack et al., 2018).

Imaging Biomarkers

Neuroimaging has become an essential tool in the diagnosis and follow-up of neurodegenerative diseases because it allows noninvasive assessment of brain structure, function, and molecular pathology. Unlike fluid biomarkers, imaging approaches provide spatial information, making it possible to visualize regional patterns of atrophy, metabolic disturbance, and protein deposition that characterize different neurodegenerative disorders (Frisoni et al., 2010).

Magnetic resonance imaging (MRI) provides detailed anatomical information and can reveal atrophy patterns associated with specific neurodegenerative conditions. In AD, atrophy of the hippocampus and medial temporal lobe is among the earliest and most prominent findings. Volumetric MRI measurements of these regions have been associated with cognitive decline and may help predict progression from mild cognitive impairment to dementia. In addition, advanced MRI techniques such as diffusion tensor imaging (DTI) and functional MRI (fMRI) offer further insight into white matter integrity and alterations in functional connectivity (Jack et al., 1999).

In PD, structural MRI findings are often less pronounced, but specialized imaging sequences can detect abnormalities in the substantia nigra. For example, neuromelanin-sensitive MRI may visualize the loss of dopaminergic neurons in this region and has therefore been proposed as a potential marker of disease severity. In ALS, MRI can demonstrate corticospinal tract degeneration and changes in the motor cortex, thereby contributing to diagnosis and longitudinal disease assessment (Pyatigorskaya et al., 2014).

Positron emission tomography (PET) enables *in vivo* visualization of molecular pathological processes. Amyloid PET tracers, including Pittsburgh Compound B (PiB), florbetapir, and florbetaben, bind to amyloid plaques and allow the detection of cerebral amyloid deposition in living patients. Amyloid PET positivity may be observed years before the onset of overt clinical symptoms and is now widely used in research settings and increasingly in clinical practice to confirm amyloid pathology and support diagnostic decision-making (Klunk et al., 2004).

Tau PET imaging, using tracers such as flortaucipir and newer second-generation compounds, provides information on the distribution of neurofibrillary tangles in the brain. Compared with amyloid PET, tau PET generally shows a clos-

er relationship with cognitive impairment, which is consistent with the stronger link between tau pathology and neuronal dysfunction. In addition, the regional pattern of tau accumulation often parallels Braak staging and can therefore contribute to disease staging (Ossenkoppele et al., 2018).

PET has also become an important approach for investigating neuroinflammation *in vivo*. In AD, PD, and ALS, tracers targeting the translocator protein (TSPO), such as [11C] PK11195 and [18F]-FEPPA, have been used to assess microglial activation. More recently, monoamine oxidase B (MAO-B) tracers have provided further insight into astrocytic activation, particularly in reactive astrocytes. Together, these imaging approaches help characterize the spatial and temporal dynamics of neuroinflammation and may also be useful for evaluating the biological effects of anti-inflammatory therapies (Chiotis et al., 2022).

Multimodal PET/MRI integrates the molecular information provided by PET with the high-resolution anatomical detail of MRI within a single imaging session. Simultaneous PET/MRI acquisition has been proposed as a reproducible and informative approach for the evaluation of motor neuron disease and other neurodegenerative disorders, as it allows molecular, structural, and functional data to be assessed together. By combining these complementary imaging modalities, this approach may enhance diagnostic accuracy and provide a more comprehensive view of disease status (Costagli et al., 2020; Catana, 2019).

Despite their considerable promise, imaging biomarkers still face several important limitations. These include high costs, restricted access to PET scanners and radiotracers, exposure to ionizing radiation, and the need for specialized expertise in both image acquisition and interpretation. Ongoing efforts are focused on standardizing imaging protocols, improving automated analysis methods, and reducing practical barriers.

ers to broader implementation. As these challenges are gradually addressed, imaging biomarkers are likely to assume an increasingly important role in both clinical practice and therapeutic development (Villemagne et al., 2018).

Oxidative Stress Biomarkers

Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense mechanisms and is considered one of the major contributors to neurodegeneration. The brain is especially susceptible to oxidative injury because of its high metabolic demand, abundant lipid content, and relatively limited antioxidant capacity. Excess oxidative stress has been linked to protein misfolding, lipid peroxidation, DNA damage, and mitochondrial dysfunction, all of which play important roles in the pathogenesis of AD, PD, and ALS (Singh et al., 2019).

F2-isoprostanes are prostaglandin-like compounds generated through the non-enzymatic peroxidation of arachidonic acid in membrane lipids. They are widely regarded as reliable indicators of lipid peroxidation and oxidative stress *in vivo*. These molecules can be measured in CSF, plasma, and urine, with CSF levels being especially informative for oxidative processes occurring in the central nervous system. Increased levels of F2-isoprostanes have been reported in AD, PD, and ALS, and in some studies they have been associated with disease severity and progression. For this reason, they are also being investigated as potential outcome measures in trials of antioxidant-based therapies (Montine et al., 2002).

Another important product of lipid peroxidation is 4-hydroxynonenal (4-HNE), which accumulates in several neurodegenerative conditions. Because 4-HNE is highly reactive, it can form adducts with proteins, thereby altering their function and promoting aggregation. Immunohistochemical studies have shown increased accumulation of 4-HNE-modified proteins in brain regions affected by AD, PD, and ALS. Although

direct measurement of 4-HNE in biological fluids is challenging because of its short half-life and chemical reactivity, protein-bound 4-HNE adducts may still serve as useful indicators of oxidative damage (Perluigi et al., 2012).

8-Hydroxy-2'-deoxyguanosine (8-OHdG) is a commonly used marker of oxidative DNA damage. It is generated when reactive oxygen species attack guanine residues in DNA. Elevated levels of 8-OHdG have been detected in CSF, blood, and brain tissue from patients with neurodegenerative diseases. This marker reflects damage to both nuclear and mitochondrial DNA, with mitochondrial injury being particularly relevant given the central role of mitochondrial dysfunction in neurodegeneration. Urinary 8-OHdG has also been proposed as a noninvasive biomarker, although its specificity for central nervous system pathology is limited because systemic oxidative stress may also contribute to its levels (Lovell and Markesbery, 2007).

NADPH oxidase 4 (NOX4) has gained increasing attention as both a source of reactive oxygen species and a potential biomarker and therapeutic target in neurodegenerative diseases. NOX4 is expressed in astrocytes as well as other brain cell types, where it contributes to the production of superoxide and hydrogen peroxide. Recent studies have highlighted its role as an important mediator of astrocyte-derived oxidative stress in AD and PD. Increased NOX4 expression has been associated with enhanced oxidative damage and the activation of downstream inflammatory signaling pathways. In preclinical models, both pharmacological inhibition and genetic suppression of NOX4 have shown neuroprotective effects, suggesting that NOX4 may represent a promising target for therapeutic intervention (Sorce et al., 2024; Zhang et al., 2023).

Transcriptomic studies have also identified oxidative stress-related gene signatures that may be relevant to neurodegenerative disease progression. In one study focusing on AD,

111 differentially expressed genes related to oxidative stress were identified, and nine hub genes were further validated by quantitative RT-PCR. Among these, NEFL was found to be up-regulated, whereas SST, NPY, and GAP43 were downregulated. Such expression profiles may serve as molecular biomarkers for disease detection and may also be useful for monitoring responses to antioxidant-based interventions (Li et al., 2022).

The analysis of oxidative stress biomarkers remains technically challenging, largely because sample collection and processing must be carefully controlled to avoid artificial oxidation. In addition, assay standardization and the establishment of reliable reference ranges remain important unresolved issues. Even so, these biomarkers provide valuable insight into the mechanisms underlying neurodegeneration and may also help identify potential targets for therapeutic intervention (Dalle-Donne et al., 2006).

Neuroinflammation Biomarkers

Chronic neuroinflammation is now widely recognized as a major feature of neurodegenerative diseases. It involves not only the activation of microglia and astrocytes, but also the infiltration of peripheral immune cells and the release of pro-inflammatory mediators. Neuroinflammation may arise as a response to neuronal injury, but it can also actively contribute to ongoing degeneration, thereby creating a self-sustaining cycle of damage. For this reason, biomarkers related to neuroinflammation are important not only for understanding disease mechanisms, but also for identifying patients who may benefit from immunomodulatory treatment strategies (Wyss-Coray, 2006).

Cytokines and chemokines are among the principal mediators of neuroinflammation. Pro-inflammatory cytokines such as interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α) have been reported at elevated levels in the cerebrospinal fluid and blood of patients with AD, PD, and ALS. In PD in particular, several studies

have documented increased concentrations of IL-1 β , IL-6, TNF- α , and high-sensitivity C-reactive protein (hsCRP) in patient cohorts. These inflammatory markers have been associated with disease progression and have also been explored as possible indicators of treatment response (Qin et al., 2016; Wijeyekoon et al., 2020).

The origin of these mediators is heterogeneous and includes activated microglia, reactive astrocytes, infiltrating peripheral immune cells, and, in some contexts, neurons themselves. Their biological effects depend not only on absolute levels but also on the balance between pro-inflammatory and anti-inflammatory signals. Cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF- β) are generally regarded as anti-inflammatory and neuroprotective, and the relationship between these factors and pro-inflammatory cytokines may provide more meaningful information than isolated measurement of individual mediators (Chakrabarty et al., 2015).

Markers of microglial activation include soluble TREM2 (sTREM2), as discussed earlier in the context of CSF biomarkers, along with other proteins released during microglial activation. TREM2 is a receptor involved in the regulation of microglial responses to amyloid plaques and other pathological stimuli. Genetic variation in TREM2 has been linked to increased risk of AD, underscoring the importance of microglial function in disease development. Elevated CSF sTREM2 levels are considered to reflect microglial activation and have been associated with disease progression, making sTREM2 a relevant candidate for monitoring neuroinflammatory activity and for stratifying patients in studies of TREM2-targeted therapies (Ewers et al., 2019).

Biomarkers of astrocytic activation include YKL-40 (chitinase-3-like protein 1) and GFAP, both of which have been reported to increase in CSF and plasma in several neurodegenerative disorders. YKL-40 is secreted by activated astrocytes

and has been found at elevated levels in AD, PD, and ALS. Likewise, plasma GFAP, as noted earlier, has shown value in predicting the transition from mild cognitive impairment to Alzheimer's dementia and is considered an early indicator of astrocytic activation and neuroinflammatory change (Hansson et al., 2023).

Imaging-based biomarkers of neuroinflammation complement fluid biomarkers by adding spatial information about the distribution of glial activation within the brain. PET tracers targeting TSPO and MAO-B allow the *in vivo* assessment of microglial and astrocytic activation, respectively. These tracers have been applied in studies of AD, PD, and ALS to investigate the spatial and temporal dynamics of neuroinflammatory processes. For example, PET imaging with [11C] PK11195 and [18F]-FEPPA has demonstrated increased microglial activation in brain regions affected by PD pathology (Koshimori et al., 2015).

Increasing evidence also suggests that neuroinflammation does not occur uniformly across patients or across different stages of disease. Some individuals appear to exhibit a predominantly pro-inflammatory profile, whereas others show a more balanced or even relatively anti-inflammatory pattern. This heterogeneity has important therapeutic implications, since immunomodulatory interventions may be more beneficial in patients with pronounced inflammatory activity. In this context, biomarker-guided stratification may support precision medicine approaches by helping match patients to the most appropriate treatment strategies (Heneka et al., 2015).

MicroRNA Biomarkers

MicroRNAs (miRNAs) are short non-coding RNA molecules, typically 20 to 24 nucleotides in length, that regulate gene expression at the post-transcriptional level. They are involved in a wide range of biological processes, including neuronal development, synaptic plasticity, and cellular stress re-

sponses. Altered miRNA expression has been implicated in the pathogenesis of neurodegenerative diseases, and circulating miRNAs detected in blood and cerebrospinal fluid have emerged as promising biomarker candidates (Bartel, 2004).

One of the main reasons miRNAs are attractive as biomarkers is their relative stability in biological fluids. They are protected from degradation either through encapsulation within extracellular vesicles (EVs) or through association with RNA-binding proteins. In addition, some miRNAs are capable of crossing the blood-brain barrier, either freely or within EVs, allowing brain-derived miRNAs to be detected in peripheral blood. Distinct miRNA expression profiles have been associated with different neurodegenerative disorders, suggesting potential utility in differential diagnosis (Turchinovich et al., 2011).

In AD, several miRNAs have been reported to be dysregulated in both CSF and blood. Exploratory studies comparing miRNA expression profiles among patients with AD and other forms of dementia have identified disease-associated signatures that may assist in differentiation. In addition, extracellular vesicles derived from microglia in CSF have been shown to carry AD-associated miRNAs capable of activating Toll-like receptor 8 (TLR8) and inducing TNF release in recipient cells. These observations suggest that EV-associated miRNAs in CSF may function not only as biomarkers, but also as active participants in the propagation of neuroinflammatory processes (Winkler et al., 2022).

Among the miRNAs associated with AD, miR-29, miR-107, miR-125b, and miR-146a are among the most frequently discussed. These molecules regulate genes involved in processes such as amyloid metabolism, tau phosphorylation, neuroinflammatory signaling, and synaptic function. Alterations in their expression have been linked to disease severity as well as cognitive decline. In general, panels composed of multiple miRNAs

appear to provide greater diagnostic accuracy than the evaluation of individual miRNAs alone (Sorensen et al., 2016).

In PD, candidate miRNA biomarkers have been investigated in blood, cerebrospinal fluid, and even saliva. MiR-7, miR-153, and miR-133b are among the miRNAs involved in the regulation of alpha-synuclein expression and have been reported to be dysregulated in patients with PD. Other miRNAs, including miR-34b/c and miR-124, are associated with dopaminergic neuron function and survival. However, further longitudinal studies are needed to clarify whether these alterations precede clinical onset and whether they can reliably predict disease progression (Junn and Mouradian, 2012).

In ALS, miRNA profiles measured in blood and CSF have also shown potential value for both diagnosis and prognosis. MiR-206, which is upregulated in skeletal muscle after denervation, has been detected in the serum of ALS patients and may reflect the extent of motor neuron degeneration. Additional miRNAs, such as miR-338-3p and miR-149, have also been linked to the rate of disease progression. If validated further, miRNA markers associated with survival may become useful tools for patient stratification in clinical trials (Freischmidt et al., 2013).

The biological roles of dysregulated miRNAs in neurodegenerative diseases remain an active area of investigation. Some miRNAs may contribute directly to disease mechanisms by promoting protein aggregation, oxidative stress, or inflammatory signaling, whereas others may represent compensatory responses intended to limit neuronal injury. A better understanding of these regulatory functions may also open new therapeutic avenues, including the development of miRNA mimics and inhibitory molecules such as antagomirs (Eacker et al., 2013).

At the same time, several methodological challenges continue to limit progress in miRNA biomarker research. These include variability in sample collection and processing, the lack of standardized normalization strategies, and the need for large

and well-characterized validation cohorts. Even with these limitations, miRNAs remain promising minimally invasive biomarkers because they capture important aspects of the gene regulatory networks involved in neurodegeneration (Pritchard et al., 2012)

Therapeutic Targets in Neurodegeneration

Alongside the identification of robust biomarkers, increasing attention has been directed toward therapeutic targets that address the underlying mechanisms of neurodegeneration. Rather than concentrating only on symptomatic improvement, recent research has increasingly focused on the development of disease-modifying strategies capable of slowing or preventing disease progression. In this context, a number of molecular targets have emerged as promising candidates for more effective treatment approaches (Cummings et al., 2020).

One of these targets is RIPK1 (receptor-interacting protein kinase 1), which has been recognized as an important mediator of necroptosis, a regulated form of cell death implicated in ALS and other neurodegenerative conditions. Genetic and transcriptomic studies have suggested that RIPK1-driven necroptotic pathways are active in ALS-associated glial cells, particularly astrocytes and oligodendrocytes. In preclinical studies, pharmacological inhibition of RIPK1 delayed symptom onset and reduced motor dysfunction in SOD1-G93A mice, a commonly used ALS model. In addition, RIPK1 inhibition has been shown to modulate glial responses and alter CSF biomarker profiles, providing preliminary support for its therapeutic potential in ALS (Ito et al., 2016).

RIPK1 appears to contribute to neurodegeneration through the formation of the necrosome complex, which promotes cell death and the release of inflammatory mediators. For this reason, RIPK1 inhibition may offer dual benefits by limiting both neuronal loss and neuroinflammatory activity. Clinical studies of RIPK1-targeting agents are ongoing, and

biomarkers such as NfL and inflammatory cytokines are being evaluated to assess target engagement and biological response (Degtarev et al., 2019).

Another important target is the NLRP3 inflammasome, a multiprotein complex that activates caspase-1 and thereby promotes the maturation and release of pro-inflammatory cytokines such as IL-1 β and IL-18. NLRP3 activation has been implicated in AD, PD, and ALS, where it appears to contribute to chronic neuroinflammation. In experimental models, inhibition of NLRP3 has shown neuroprotective effects, including reductions in amyloid- and tau-related pathology in AD models and attenuation of alpha-synuclein-associated inflammatory responses in PD models (Heneka et al., 2013).

NF- κ B (nuclear factor kappa B) is another major regulator of neuroinflammatory signaling. As a transcription factor, it controls the expression of numerous genes involved in inflammatory responses, including cytokines, chemokines, and adhesion molecules. Dysregulated NF- κ B activation has been reported across several neurodegenerative diseases and is thought to contribute to sustained inflammatory signaling. Although NF- κ B has long been considered an attractive therapeutic target, its broad role in normal immune regulation makes selective and safe inhibition particularly challenging (Kaltschmidt et al., 1997).

NOX4 (NADPH oxidase 4), as noted earlier, represents both a biomarker-related molecule and a potential therapeutic target in neurodegenerative disease. Reactive oxygen species generated by NOX4 contribute to oxidative stress and inflammatory signaling in astrocytes and other brain cell populations. Experimental studies have shown that pharmacological inhibition or genetic deletion of NOX4 can reduce oxidative injury and neuroinflammation in models of AD and PD. In parallel, small-molecule NOX4 inhibitors are being developed and evaluated for their possible neuroprotective effects (Sorce et al., 2024).

Another target that has attracted interest is cPLA2 (cytosolic phospholipase A2), encoded by the PLA2G4A gene. cPLA2 has been proposed as an upstream regulator of vascular inflammation in AD, particularly in association with cerebral amyloid angiopathy (CAA). Its activation promotes the release of arachidonic acid and the subsequent production of pro-inflammatory eicosanoids. In the context of anti-amyloid immunotherapy, cPLA2-related inflammatory activity may contribute to amyloid-related imaging abnormalities (ARIA), an important treatment-associated adverse effect. For this reason, cPLA2 inhibition has been suggested as a potential strategy to complement anti-amyloid approaches, while imaging-based and biochemical methods are being developed to monitor cPLA2 activity in vivo (Chowdhury et al., 2024).

TREM2 (triggering receptor expressed on myeloid cells 2) is a microglial receptor that regulates cellular responses to amyloid plaques and other pathological stimuli. Loss-of-function variants in TREM2 are associated with increased risk of AD, suggesting that normal TREM2 activity plays a protective role. Experimental strategies aimed at enhancing TREM2 signaling, either through agonistic antibodies or increased receptor expression, have shown encouraging findings in preclinical studies. These approaches are intended to support beneficial microglial functions, including amyloid phagocytosis and debris clearance, while reducing maladaptive inflammatory responses (Ulrich et al., 2017).

Mitochondria-targeted therapeutic strategies have also gained attention because mitochondrial dysfunction is a central feature of many neurodegenerative diseases. Agents such as MitoQ and SS-31 (elamipretide) are designed to deliver antioxidant activity directly to mitochondria, where they can help scavenge reactive oxygen species and preserve mitochondrial membrane integrity. Clinical studies in PD and related conditions have yielded some encouraging results, although overall

findings remain mixed. In addition, activators of the Nrf2 pathway, including dimethyl fumarate and sulforaphane, have been explored as neuroprotective agents because they enhance endogenous antioxidant defense mechanisms (Smith et al., 2021).

Taken together, the multifactorial nature of neurodegenerative diseases suggests that therapies directed at a single pathway may not always be sufficient. Combination strategies targeting multiple mechanisms may therefore offer greater therapeutic benefit. In this setting, precision medicine approaches informed by biomarkers and multi-omics data may help identify the most suitable treatment combinations for individual patients (Hampel et al., 2018).

Novel Treatment Strategies

Beyond traditional small-molecule drugs, several innovative treatment strategies have emerged from recent research, leveraging advances in nanotechnology, immunotherapy, and nutritional science. These approaches offer new mechanisms of action and the potential to address aspects of neurodegeneration that have been difficult to target with conventional therapies (Nowak et al., 2023).

Nanologomers represent a novel class of brain-penetrant therapeutic agents designed to modulate multiple targets simultaneously. A nanologomer cocktail designated NI112 has shown remarkable efficacy in preclinical models of AD and ALS. In AD organoids, NI112 reduced neuroinflammation and decreased amyloid-beta 42 levels. In TDP-43 ALS organoids, it improved mean firing rate, indicating enhanced neuronal function. In SOD1-G93A mice, NI112 restored grip strength and improved motor performance. In AD mouse models, it improved cognitive function. These multimodal pharmacodynamic effects suggest that nanologomers targeting NF- κ B and NLRP3 pathways can address multiple aspects of neurodegeneration simultaneously (Ewans et al., 2024).

The mechanism of action of nanoligomers involves the delivery of oligonucleotide-based therapeutics that modulate gene expression or protein function. Their nanoscale formulation enables crossing of the blood-brain barrier, a major obstacle for many CNS drugs. The ability to target multiple pathways with a single formulation represents a significant advance over traditional single-target therapies (Gao, 2016).

N-Acetylcysteine (NAC) is a well-known antioxidant and precursor to glutathione, the major intracellular antioxidant. Recent research has revealed a novel mechanism by which NAC may protect against neurodegeneration: inhibition of disulfidptosis, a newly described form of cell death driven by disulfide stress. Bioinformatic analyses combined with experimental studies in MPTP-induced PD mice demonstrated that NAC inhibited disulfidptosis and provided neuroprotection. Disulfidptosis-related genes, including SNCA (alpha-synuclein) and NDUFS1, were identified as potential biomarkers and therapeutic targets. This finding opens new avenues for repurposing NAC and developing novel therapies targeting disulfide metabolism (Liu et al., 2024).

Regulatory T-cell (Treg) therapy represents an immunomodulatory approach to ALS treatment. Tregs are a subset of T lymphocytes that suppress excessive immune responses and maintain immune homeostasis. In ALS, Treg function is impaired, contributing to systemic inflammation and disease progression. Two small clinical trials have tested ex vivo expanded autologous Treg infusions in ALS patients. These trials demonstrated safety and were connected to suppression of pro-inflammatory lipid peroxides and promising clinical signals, including stabilization of disease progression in some patients. Larger trials are needed to confirm efficacy, but these early results support immunomodulation as a viable therapeutic axis in ALS (Thonhoff et al., 2018).

Lipid transport enhancement has emerged as a novel strategy for ALS treatment. The major facilitator superfamily domain-containing protein 2A (MFSD2A) is a transporter that facilitates the uptake of docosahexaenoic acid (DHA), an omega-3 fatty acid critical for neuronal membrane integrity and function. Overexpression of MFSD2A combined with DHA supplementation produced synergistic behavioral and histological benefits in SOD1-G93A mice. This combination reduced systemic inflammation, as evidenced by decreased TNF- α , IL-6, and IL-1 β levels, and reduced oxidative stress, with restored glutathione peroxidase (GSH-Px) activity and lower malondialdehyde (MDA) levels. Mechanistic studies implicated TRIM21 and glycolytic regulation in these protective effects. This approach highlights the potential of targeting lipid metabolism and transport to support neuronal health (Zhang et al., 2025).

Antioxidant therapies continue to be explored, with a focus on precision targeting and patient stratification. Multi-omics approaches are being used to identify patients with high oxidative stress burdens who may be most likely to benefit from antioxidant interventions. Mitochondria-targeted antioxidants, Nrf2 activators, and dietary antioxidants such as resveratrol and curcumin are under investigation in clinical trials. While early trials of broad-spectrum antioxidants have been disappointing, more targeted approaches guided by biomarkers may yield better results (Espay et al., 2020).

Gut-brain axis modulation has gained attention as a potential therapeutic strategy, particularly in PD. The gut microbiome influences neuroinflammation, oxidative stress, and alpha-synuclein pathology through multiple mechanisms, including production of short-chain fatty acids, modulation of immune responses, and direct effects on the enteric nervous system. Probiotic and prebiotic interventions, fecal microbiota transplantation, and dietary modifications are being explored as ways to modulate the gut-brain axis and potentially slow disease progression (Sampson et al., 2016).

Repurposed drugs offer the advantage of known safety profiles and accelerated development timelines. Angiotensin-converting enzyme (ACE) inhibitors, traditionally used for hypertension, have shown neuroprotective effects in preclinical models. Cilazapril and benazepril reduced oxidative damage and alpha-synuclein aggregation in 6-OHDA-induced PD models, with statistically significant effects ($p < 0.05$). These findings suggest that ACE inhibitors may have multi-target protective effects, potentially through modulation of the renin-angiotensin system in the brain (Labandeira – Garcia et al., 2021).

The translation of these novel strategies from preclinical models to clinical practice faces numerous challenges, including the complexity of human disease, the heterogeneity of patient populations, and the difficulty of conducting long-term trials in slowly progressive conditions. Biomarker-guided trial designs, adaptive trial methodologies, and collaborative research networks are essential to accelerate the development of effective treatments (Cummings et al., 2021).

CONCLUSION AND FUTURE DIRECTIONS

The landscape of biomarkers and therapeutic targets in neurodegenerative diseases has evolved dramatically in recent years. Advances in analytical technologies, neuroimaging, and systems biology have enabled the identification of numerous biomarkers that reflect diverse aspects of disease pathology, from protein aggregation and neuronal injury to oxidative stress and neuroinflammation. Blood-based biomarkers such as plasma GFAP and NfL offer accessible tools for screening and monitoring, while CSF biomarkers provide more direct insights into CNS pathology. Imaging biomarkers visualize molecular and structural changes in living patients, complementing fluid-based measures (Zetterberg and Burnham, 2019).

The integration of multiple biomarker modalities into comprehensive diagnostic and prognostic frameworks represents a

major step forward. The AT(N) system for AD exemplifies this approach, categorizing biomarkers by the pathological process they reflect. Similar frameworks are being developed for PD and ALS, recognizing that these conditions are also characterized by multiple, interacting pathological mechanisms (Höglinger et al., 2017).

Biomarkers are not only diagnostic tools but also essential for drug development. They enable patient stratification, ensuring that clinical trials enroll individuals most likely to benefit from a given intervention. They serve as pharmacodynamic markers, demonstrating target engagement and biological activity. They can function as surrogate endpoints, potentially accelerating regulatory approval by providing evidence of efficacy before clinical benefits become apparent. The use of biomarkers in adaptive trial designs allows for more efficient testing of multiple therapies and doses (Cummings et al., 2019).

The therapeutic targets discussed in this chapter—RIPK1, NLRP3, NF- κ B, NOX4, cPLA2, TREM2, and mitochondrial pathways—reflect a shift from symptomatic treatment to disease modification. These targets address fundamental mechanisms of neurodegeneration, including cell death pathways, inflammation, oxidative stress, and metabolic dysfunction. The development of drugs targeting these pathways is progressing, with several candidates in clinical trials (Cummings et al., 2021).

Novel treatment strategies, including nanoligomers, NAC, Treg therapy, lipid transport enhancement, and gut-brain axis modulation, offer innovative approaches that complement traditional pharmacology. These strategies leverage insights from diverse fields, including nanotechnology, immunology, nutrition, and microbiome research. The success of these approaches will depend on rigorous preclinical validation, careful clinical trial design, and the use of biomarkers to guide patient selection and monitor treatment effects (Gitler et al., 2017).

Despite this progress, significant challenges remain. The heterogeneity of neurodegenerative diseases, both between and within diagnostic categories, complicates the development of universally effective treatments. The blood-brain barrier continues to limit drug delivery to the CNS. The slow progression of many neurodegenerative diseases requires long and expensive clinical trials. The lack of curative therapies for any major neurodegenerative disease underscores the difficulty of translating preclinical findings to clinical benefit (Mehta et al., 2017).

Future directions in this field will likely emphasize precision medicine approaches that match patients to therapies based on their individual molecular profiles. Multi-omics technologies—integrating genomics, transcriptomics, proteomics, metabolomics, and microbiomics—will enable comprehensive characterization of disease subtypes and identification of predictive biomarkers. Artificial intelligence and machine learning will play increasingly important roles in analyzing complex datasets and identifying patterns that predict disease progression and treatment response (Hampel et al., 2018).

Combination therapies targeting multiple pathways simultaneously may prove more effective than single-target approaches, given the multifactorial nature of neurodegeneration. Biomarker-guided adaptive trials will enable efficient testing of combination regimens. The development of disease-modifying therapies will require collaboration among academia, industry, regulatory agencies, and patient advocacy groups (Cummings et al., 2018).

Early intervention, ideally in the preclinical phase before irreversible neuronal loss occurs, offers the best hope for preventing or delaying neurodegenerative diseases. This will require widespread implementation of screening programs using accessible biomarkers, such as blood tests, and the availability of effective preventive therapies. Lifestyle interventions, including diet, exercise, cognitive engagement, and management of vas-

cular risk factors, will remain important components of a comprehensive approach to brain health (Livingston et al., 2020).

In conclusion, the field of neurodegenerative disease research stands at a pivotal moment. The tools to detect and monitor these diseases have never been more sophisticated, and the pipeline of potential therapies has never been more robust. While challenges remain formidable, the convergence of biomarker science, mechanistic understanding, and therapeutic innovation provides genuine hope that the coming years will bring meaningful advances in the prevention and treatment of AD, PD, ALS, and related disorders. The ultimate goal—to preserve cognitive and motor function, maintain quality of life, and extend healthy lifespan—is within reach if the scientific community, healthcare systems, and society as a whole commit to this endeavor (Gomes et al., 2018).

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THE ROLE OF ENDOPLASMIC RETICULUM STRESS IN NEURODEGENERATIVE DISEASES

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INTRODUCTION

Neurodegenerative diseases are a significant public health problem affecting millions of people worldwide. Moreover, they are progressive diseases with increasing mortality and morbidity rates each year. This group of diseases includes major conditions such as Alzheimer's Disease (AD), Parkinson's Disease (PD), Amyotrophic Lateral Sclerosis (ALS), and Huntington's Disease (HD) (Divya et al., 2025). As the aging population increases, the incidence of these diseases in society is also increasing significantly. Despite their increasing prevalence and clinical heterogeneity, neurodegenerative diseases are commonly characterized by progressive neuronal loss and synaptic dysfunction. Among these diseases, Alzheimer's disease is considered the most common neurodegenerative disease, accounting for approximately 60–70% of all dementia cases, while Parkinson's disease ranks second (Azam et al., 2021; Hansson, 2021).

Numerous risk factors, including genetic predisposition, aging, environmental exposures, lifestyle factors (diet and physical activity), smoking, and alcohol consumption, contribute to the development of neurodegenerative diseases. Nevertheless, one of the most prominent common features in the pathogen-

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esis of these diseases is the disruption of protein homeostasis. The accumulation and aggregation of misfolded proteins exert toxic effects on neuronal cells, leading to impaired cellular functions and ultimately cell death (Bi et al., 2022). Disease-specific protein pathologies are among the key determinants of this process. Amyloid beta (A β) plaques and hyperphosphorylated tau proteins in Alzheimer's disease; α -synuclein aggregates in Parkinson's disease; mutant huntingtin protein in Huntington's disease; and the accumulation of proteins such as superoxide dismutase 1 (SOD1) and TAR DNA-binding protein 43 (TDP-43) in ALS are among the major molecular determinants of neuronal degeneration (Sanghai and Tranmer, 2023; Zaib et al., 2023). The misfolding and accumulation of these proteins overload cellular quality control systems, thereby directly affecting endoplasmic reticulum (ER) functions in particular (Hasan et al., 2024).

The pathogenesis of neurodegenerative diseases involves not only protein aggregation but also multiple interconnected cellular stress mechanisms. These include mitochondrial dysfunction, increased oxidative stress, lysosomal and autophagic dysfunction, neuroinflammation, and dysregulation of apoptotic mechanisms. ER stress, which plays a central role in these processes, is a critical mechanism that arises when the capacity for intracellular protein folding is exceeded, leading to a disruption of cellular homeostasis (Sanghai and Tranmer, 2023).

The ER is an organelle that performs vital functions such as protein synthesis and folding, as well as the regulation of calcium homeostasis and lipid biosynthesis. The accumulation of misfolded or unfolded proteins in the ER lumen triggers a condition known as 'ER stress', and the cell develops an adaptive response to this situation known as the unfolded protein response (UPR). The UPR initially acts as a protective mechanism for the cell, aiming to enhance protein folding capacity, reduce protein synthesis and eliminate misfolded proteins. However, when stress becomes chronic, this adaptive response

gives way to pro-apoptotic signalling pathways and contributes to neuronal cell death (Ghemrawi and Khair, 2020; Hasan et al., 2024). Studies conducted in recent years have shown that ER stress is not merely a consequence in the pathogenesis of neurodegenerative diseases, but also an active mechanism that accelerates the progression of the disease. In particular, chronic UPR activation reinforces the neurodegenerative process by leading to impaired synaptic function, increased neuroinflammation and worsening mitochondrial dysfunction (Ghemrawi and Khair, 2020). In this context, this section will comprehensively address the molecular mechanisms of ER stress, its association with neurodegenerative diseases, and potential therapeutic strategies.

Structure and Functions of the Endoplasmic Reticulum

The ER is a dynamic membrane network that extends along the outer membrane of the nuclear envelope and throughout the cytoplasm (Obara et al., 2023). Comprising tubular and cisternae-like structures, this organelle plays a central role in the synthesis, folding and transport of intracellular proteins. The ER is divided into two main sections, both structurally and functionally: the rough endoplasmic reticulum (RER) and the smooth endoplasmic reticulum (SER) (Figure 1). The RER, which is lined with ribosomes, typically exhibits a structure comprising flat, stacked cisternae and is responsible, in particular, for the synthesis and post-translational modification of proteins destined for secretion, lysosomal degradation or membrane integration. The RER lumen is rich in chaperone proteins and quality control mechanisms that support protein folding. In contrast, the ribosome-free SER exhibits a more tubular organisation and is characterised by highly folded membrane structures. The SER is involved in metabolic functions such as lipid and phospholipid biosynthesis, steroid production, detoxification processes and calcium storage. In particular, its spe-

cialized form, the sarcoplasmic reticulum, plays a critical role in the regulation of Ca^{2+} homeostasis in muscle cells (Perkins and Allan, 2021; Schwarz and Blower, 2015)

The ER network is organised by proteins such as REEP, atlastin, Climp63 and Lnp, which stabilise steeply sloping membranes, facilitate membrane fusion and regulate lumen width, and is an energy-dependent structure. This dynamic tubular and cisternal network forms a complex matrix containing three-way junctions and is continuously remodeled in response to the metabolic demands of the cell, cytoskeletal interactions, and interorganelle contact sites (Perkins and Allan, 2021; Scott et al., 2024). Another characteristic of the tubular ER in particular is its ability to form membrane contact sites (MCSs) with all other organelles and the cell membrane. Through these physical interactions, the ER can act as a channel, enabling organelles to exchange ions, lipids and metabolites, and to carry out cooperative metabolic processes along and beyond organelle boundaries without disrupting the cytoplasm as a whole (Arruda and Parlakg  l, 2023).

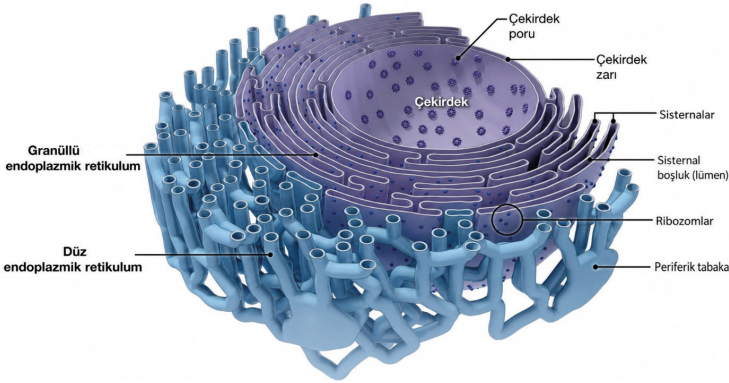


Figure 1. Structure of the Endoplasmic Reticulum (Goyal ve Blackstone, 2013).

Throughout evolution, the structure and functions of the ER have been conserved across species; however, its abundance and characteristics vary according to cellular functions. For example, cells with a high protein secretory capacity, such as

pancreatic acinar cells that secrete digestive enzymes or intestinal goblet cells, contain abundant RER sheets. Cells involved in the synthesis of steroid hormones or lipids, such as Leydig cells and adipocytes, predominantly contain SER tubules. In contrast, cells such as hepatocytes and enterocytes perform pleiotropic functions depending on metabolic demands, including high rates of protein and lipid synthesis and secretion. These cells exhibit a heterogeneous and dynamic ER organization (Arruda and Parlakgöl, 2023).

All secretory and luminal proteins targeted to organelles of the endomembrane system, including the Golgi apparatus, lysosomes, endosomes, and the plasma membrane, are translocated into the ER lumen through a protein-conducting channel (Sun et al., 2021). This process begins with the initiation of protein translation in the cytoplasm. The signal sequence located at the amino terminus of the newly synthesized polypeptide chain and composed of hydrophobic amino acids is recognized and bound by the signal recognition particle (SRP), a cytosolic ribonucleoprotein complex consisting of proteins and 7S RNA. SRP binding temporarily arrests translation, allowing the ribosome–mRNA–polypeptide complex to be targeted to the ER membrane. After this complex binds to the SRP receptor located in the ER membrane, the polypeptide chain is translocated through the Sec61 translocon channel into the ER lumen in the case of secretory and luminal proteins or becomes integrated into the membrane through its hydrophobic regions to form an integral membrane protein (Cooper, 2023; Schwarz and Blower, 2015). The polypeptide chain is transferred directly from the ribosome into the Sec61 channel, and upon completion of translation, the signal peptide is cleaved by signal peptidase, allowing the protein to be released into the ER lumen (Rapoport, 2007).

Proteins translocated into the ER lumen acquire their appropriate three-dimensional structures with the assistance of chaperones and folding enzymes. During this process, pro-

teins undergo post-translational modifications such as disulfide bond formation, N-linked glycosylation, and oligomerization. Chaperones in the ER bind to newly synthesized unfolded proteins, preventing misfolding, promoting proper folding, and determining the intracellular fate of proteins. In addition to general chaperones such as Hsp70 and Hsp90, the ER contains specialized ER-specific chaperones and auxiliary proteins involved in processes such as the quality control of N-linked glycosylation and disulfide bond formation (Wiseman et al., 2022). Proteins that successfully pass folding and quality-control mechanisms are transported from the ER to the Golgi apparatus via coat protein complex II (COPII)-mediated vesicular transport (Braakman and Bulleid, 2011; Sun et al., 2021). Misfolded or defective proteins, however, are retained in the ER and targeted for degradation through ER-associated degradation (ERAD) mechanisms (Schwarz and Blower, 2015).

On the other hand, phospholipids and cholesterol, which are major structural components of cellular membranes, are largely synthesized in the ER and subsequently transported to other organelles. In this process, a dynamic transition zone located between the ER and the Golgi apparatus, known as the ER–Golgi intermediate compartment (ERGIC), plays an important role. This region, which is rich in tubular structures and transport vesicles, acts as an intermediate station where lipids and proteins are involved in advanced processing and targeting processes. Lipids synthesised in the ER are transported to the Golgi apparatus via the ERGIC pathway and, from there, are distributed to different compartments within the cell via vesicular transport or inter-organelle contact regions (Fagone and Jackowski, 2009; Schwarz and Blower, 2015).

The ER is a central player in intracellular calcium ion (Ca^{2+}) metabolism. It both stores calcium at high concentrations and regulates numerous cellular processes by releasing it in a controlled manner. Under physiological conditions, there is a marked difference in Ca^{2+} concentration between the ER (100

μM to 1 mM) and the cytosol (approximately 100 nM). This difference provides a suitable environment for both protein folding and rapid Ca^{2+} signalling. The uptake of Ca^{2+} into the ER is mediated by the energy-dependent SERCA pump, whilst its release from the ER into the cytosol occurs primarily via IP_3 receptors and ryanodine receptors; the Sec61 translocon channel also contributes to this process (Daverkausen-Fischer and Pröls, 2022; Krebs et al., 2015). Ca^{2+} released from the ER acts as a critical signal in the regulation of metabolic processes and apoptotic responses, particularly at the mitochondria-endoplasmic reticulum junctions (MAM); however, excessive increases or decreases in Ca^{2+} balance can disrupt mitochondrial function, leading to impairments in energy production, increased oxidative stress and cell death. Disruption of ER Ca^{2+} levels also affects protein folding, triggering ER stress and the UPR; whilst this response is initially protective, its chronic activation contributes to increased apoptosis and the development of various pathological conditions (Krebs et al., 2015; Marchi et al., 2018).

ER Stress and UPR Mechanisms

The ER acts as a quality control organelle for the proteins it produces and allows only normal proteins to exit its vesicles. The continuity of ER function is of critical importance for the maintenance of cellular proteostasis and the continuation of metabolic activities. Protein quality control systems, chaperones, ATPases, glucose-regulated protein 94 (Grp94), BiP (a member of the Hsp70 family) and the ubiquitin-proteasome and lysosome-autophagy systems all play a role in maintaining proteostasis (Chen et al., 2023). Conversely, various physiological and pathological conditions-such as a decrease in ER calcium levels, an increased secretory load, oxidative stress or the expression of mutant proteins-exceed the ER's protein folding capacity, leading to the accumulation of misfolded proteins within the ER; this condition is defined as 'ER stress' (Carreras-Sureda et al., 2018; Zhang et al., 2025).

When ER stress occurs, cells initiate a series of stepwise response pathways, including the UPR and ERAD, to adapt to changes in protein folding. The UPR is a response mechanism that regulates fundamental processes such as the suppression of protein synthesis, reprogramming of gene expression, and determination of cell fate (Chen et al., 2023). Abnormally high levels of misfolded proteins in the ER activate the UPR as a rapid response to restore cellular function (Gerakis and Hetz, 2018). Initially cytoprotective, this response represents a multifaceted adaptive program aimed at reducing the burden of misfolded proteins and enhancing protein-folding capacity. Within this context, protein synthesis is temporarily suppressed, while the expression of ER chaperones and folding enzymes is increased. Subsequently, ERAD mechanisms are activated to remove defective proteins. The ERAD system retrotranslocates misfolded proteins into the cytosol and targets them for degradation through the ubiquitin–proteasome system (Carreras-Sureda et al., 2018; Ghemrawi and Khair, 2020; Xie et al., 2025). However, when the severity or duration of ER stress increases and homeostasis cannot be restored, UPR signaling shifts from an adaptive phase toward a pro-apoptotic response. In this process, chronic UPR activation may result in increased inflammation, mitochondrial dysfunction, and the induction of cell death (Fagone and Jackowski, 2009).

The UPR is regulated by three major ER transmembrane proteins: PKR-like ER kinase (PERK), inositol-requiring transmembrane kinase/endoribonuclease 1 α (IRE1 α), and activating transcription factor 6 (ATF6) (Figure 2). Under normal conditions, these proteins are associated with binding immunoglobulin protein (BiP; glucose-regulated protein 78 [GRP78]), an ER-resident chaperone that acts as a central regulator of ER stress sensors, and remain in an inactive state (Ghemrawi and Khair, 2020; Ong and Logue, 2023). During ER stress, BiP dissociates from these sensors to bind unfolded proteins, thereby activating PERK, IRE1, and ATF6 (Carrara

et al., 2015). Activated PERK undergoes autophosphorylation and subsequently phosphorylates eukaryotic translation initiation factor 2 α (eIF2 α), thereby reducing global protein synthesis. However, translation of activating transcription factor 4 (ATF4) is increased, and this factor can induce target genes such as C/EBP homologous protein (CHOP), potentially triggering apoptosis under conditions of prolonged stress (Chen et al., 2023; Han et al., 2013). On the other hand, under ER stress, IRE1 dimerizes and becomes activated. IRE1 functions as both a protein kinase and an endoribonuclease and can participate in RNA degradation through its RNase activity to reduce protein synthesis. Among its target molecules, the transcription factor X-box binding protein 1 (XBP1) is particularly important for cell survival. Activated IRE1 removes an intron from XBP1 mRNA, converting it into spliced XBP1 (XBP1s). XBP1s translocates to the nucleus, where it transcriptionally activates a series of genes responsible for restoring ER folding capacity and acts as an important regulator of the UPR (Iurlaro and Muñoz-Pinedo, 2016). The third sensor, ATF6, is also a transmembrane protein but functions as a transcription factor following its cleavage. Although ATF6 is constitutively localized in the ER, it translocates to the Golgi apparatus in response to ER stress, where it is cleaved to release its transcriptionally active form. ATF6 induces genes encoding chaperones, CHOP, and XBP1 and regulates both UPR and ERAD mechanisms (Chen et al., 2023; Iurlaro and Muñoz-Pinedo, 2016).

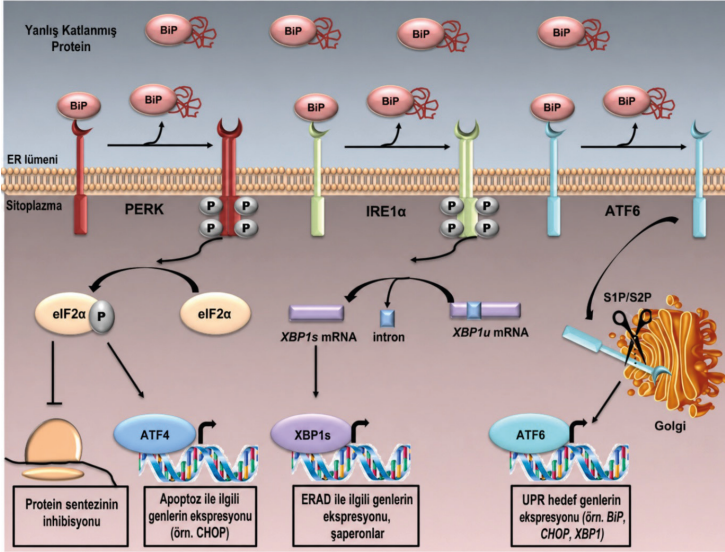


Figure 2. Induction of ER Stress and the Resulting Cellular Mechanisms (Ghemrawi ve Khair, 2020).

ERAD Mechanism and Alternative Degradation Pathways

ERAD is a protein quality-control system that is essential for the degradation of misfolded proteins in the endoplasmic reticulum (Zhang et al., 2025). Proteins that fail to fold properly in the ER must be recognized and removed through ERAD mechanisms to maintain cellular homeostasis. This process involves a complex sequence of events extending from the ER to the cytoplasm, comprising the recognition of misfolded proteins, their retrotranslocation across the ER membrane, poly-ubiquitination via E3 ligases, and finally their degradation by the 26S proteasome (Wiseman et al., 2022).

First, misfolded proteins are recognised by lectins (OS9, ERLEC1) and chaperones (BiP); these proteins are then transported out of the membrane into the cytosol via retrotranslocation through complexes such as SEL1L–HRD1. Here, the valosin-containing protein (VCP/p97) ATPase facilitates the

extraction of substrates from the ER membrane and unfolds their misfolded structures, preparing them for proteasomal degradation. Concurrently, these proteins are polyubiquitinated by E3 ubiquitin ligases and degraded via the ubiquitin–proteasome system (Lemmer et al., 2021).

In addition to ERAD, ER proteins prone to misfolding can also be targeted for degradation via alternative mechanisms such as ER-phage, which interacts with conventional autophagic vesicle formation subcomponents. In this process, ER fragments are targeted for degradation via micro-ER-phagy (direct uptake of the ER by the lysosome) or macro-ER-phagy (transport of the ER to lysosomes via autophagosomes). This mechanism contributes both to the regulation of total ER volume and to the elimination of ERAD-resistant protein aggregates. In recent years, the identification of ER-phage-specific receptors such as FAM134B and RTN3L has demonstrated that this mechanism plays a critical role, particularly in the removal of proteins prone to aggregation or resistant to ERAD (Chino and Mizushima, 2020; Wiseman et al., 2022).

ER stress is closely interlinked with oxidative stress, mitochondrial dysfunction, autophagy and inflammatory responses, and plays a central role in the multifaceted regulation of cell death. Increased levels of reactive oxygen species (ROS) trigger inflammatory responses via NF- κ B activation, whilst promoting apoptosis by increasing both protein synthesis and oxidative load via CHOP target genes (e.g. GADD34 and ERO-1 α). At the same time, ER-derived Ca²⁺ release is transferred to the mitochondria, particularly via IP3R, resulting in mitochondrial Ca²⁺ overload, changes in membrane permeability and cytochrome c release, thereby activating intrinsic apoptotic pathways. The activation of the ASK1–JNK axis via the IRE1 branch of the UPR and its interaction with caspase-12 contribute to the shift of the stress response towards a pro-apoptotic direction; whilst the regulation of autophagy genes via the eIF2 α /ATF4 pathway demonstrates that the cellular response

can be bidirectional (protective or detrimental). Furthermore, the ER and MAMs are critical for the coordination of Ca^{2+} and lipid homeostasis, and the reciprocal interaction between these two organelles plays a decisive role in inflammasome activation and the determination of cellular fate. In this context, the bidirectional interaction between the ER and mitochondria is considered a central regulatory mechanism in the integration of cellular stress responses (Chen et al., 2023; Iurlaro and Muñoz-Pinedo, 2016).

ER Stress in Neurodegenerative Diseases

Neurodegenerative diseases are complex disorders of the nervous system that affect many people worldwide and occur particularly in older age. Although the pathophysiology of these diseases has not yet been fully elucidated, factors such as genetic alterations, oxidative stress, inflammatory responses, protein aggregation, and protein misfolding are known to contribute to disease development (Divya et al., 2025).

The most common neurodegenerative diseases include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and multiple sclerosis (MS). These diseases are characterized by the accumulation of abnormally conformational protein aggregates in neurons and/or neuroglial cells, leading to impairments in memory, cognitive, and/or motor functions (Karvandi et al., 2023). The major proteins involved in the pathogenesis of neurodegenerative diseases include amyloid- β , tau, α -synuclein, prion protein, and TAR DNA-binding protein 43 kDa (TDP-43). In addition, proteins associated with trinucleotide repeat expansions, as well as neuroserpin, ferritin, and proteins associated with familial cerebral amyloidoses, are involved particularly in hereditary neurodegenerative diseases. One of the common pathophysiological features of these diseases is the intracellular accumulation of misfolded and aggregation-prone proteins. This condition overwhelms the protein-folding capac-

ity of the ER, leading to the development of ER stress and activation of the UPR (Bi et al., 2022; Kovacs, 2018).

Neurons are particularly vulnerable to disruptions in protein-folding processes due to their high levels of protein synthesis and synaptic activity. Therefore, the accumulation of misfolded proteins following various cellular stress mechanisms makes ER stress more pronounced in neuronal cells and accelerates cellular damage (Figure 3). Although the UPR activated in response to ER stress initially constitutes a protective response aimed at restoring proteostasis, under conditions of chronic or unresolved stress, it contributes to the progression of the neurodegenerative process by triggering apoptosis and cell death (Hetz and Mollereau, 2014; Kovacs, 2018). These common mechanisms suggest that ER stress plays a central role in the pathogenesis of neurodegenerative diseases and that targeting these pathways may enable the development of a common therapeutic strategy for different neurodegenerative disorders (Bi et al., 2022).

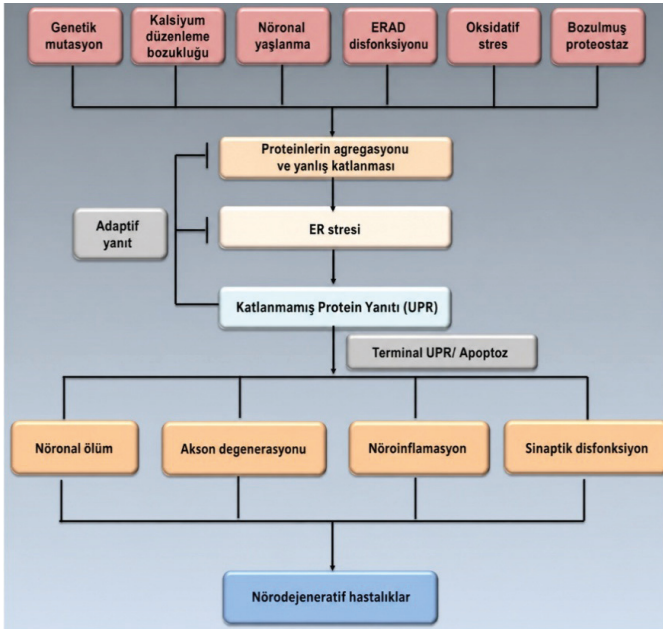


Figure 3. ER Stress, UPR, and Neurodegeneration (Ghemrawi ve Khair, 2020).

Relationship Between Alzheimer's Disease and ER Stress

AD is a severe neurodegenerative disorder characterized by a gradual decline in cognitive function and is the leading cause of dementia. According to data published by the World Health Organization in 2021, more than 50 million individuals worldwide are living with dementia (Cabral-Miranda and Hetz, 2018; Gauthier, S., Rosa-Neto, P., Morais, 2021). Individuals affected by AD typically develop episodic memory impairment, followed by language difficulties, impairments in executive and visuospatial functions, and ultimately a range of cognitive symptoms. Amyloid- β ($A\beta$), one of the characteristic features of AD, begins to accumulate and aggregate in cortical extracellular plaques approximately 10–30 years before the onset of dementia. Other major pathological features include neuronal neurofibrillary tangles (NFTs) containing hyperphosphorylated tau (p-tau) (Eciroglu-Sarban et al., 2025; Hansson, 2021).

$A\beta$ peptides are generated through the cleavage of amyloid precursor protein (APP), a transmembrane protein, via different enzymatic pathways. In the non-amyloidogenic pathway, in which APP is cleaved by alpha (α)-secretase, non-toxic products are generated, whereas the amyloidogenic pathway involving beta (β)-secretase (BACE-1) and gamma (γ)-secretase results in the formation of neurotoxic $A\beta$ peptides. In particular, presenilin-1 (PSEN-1) mutations promote this pathological process and are associated with familial AD. The disruptions occurring during this process lead to the formation of toxic oligomers and protein aggregation, thereby adversely affecting synaptic transmission and neuronal survival. Furthermore, although $A\beta$ plaques are typically characterised as a hallmark of Alzheimer's disease, studies have shown that $A\beta$ oligomers and intracellular $A\beta$ accumulations may have more potent neurotoxic effects than amyloid plaques (Breijyeh et al., 2020; Cabral-Miranda and Hetz, 2018).

Tau proteins are microtubule-associated proteins abundantly expressed in neurons of the vertebrate central and peripheral nervous systems. Hyperphosphorylation of tau proteins, which are involved in microtubule formation, stability, and axonal transport, at various serine and threonine residues is one of the characteristic features of AD. Hyperphosphorylation and abnormal aggregation of tau, together with its insufficient clearance and intracellular accumulation, result in the formation of NFTs and contribute to neurotoxicity in AD (Bakota and Brandt, 2016; Gao et al., 2018). NFTs typically begin to develop approximately a decade after the onset of A β plaque formation. Studies have demonstrated that A β oligomers may trigger NFT formation. Moreover, NFTs have been reported to show a stronger correlation with cognitive decline. A recent study demonstrated that, with the formation of amyloid plaques and NFTs, A β and tau oligomer levels reach an equilibrium, whereas accumulated toxicity continues to increase over time. Exceeding a certain toxicity threshold is associated with neuronal death (Kuznetsov, 2025).

The characteristic features of AD include misfolded protein aggregates, increased free radical formation and metabolic disturbances occurring in affected neurons. Alzheimer's disease and endoplasmic reticulum (ER) stress have been studied in neuronal cell cultures, various cell lines and animal models. These studies have demonstrated that, in addition to the accumulation of A β and tau aggregates, these proteins directly affect ER function, thereby disrupting cellular proteostatic balance (Gerakis and Hetz, 2018). The ER senses these alterations in neuronal homeostasis, and consequently, multiple signs of ER stress are observed in AD brains. Under these conditions, the UPR is activated, involving signaling cascades that mediate the necessary adjustments in metabolism and gene expression to maintain neuronal homeostasis (Uddin et al., 2020).

The relationship between ER stress and A β is largely explained by disturbances in calcium (Ca²⁺) homeostasis. A β has

been shown to disrupt intracellular Ca^{2+} balance through interactions with glutamatergic receptors, thereby triggering ER stress. In addition, $\text{A}\beta$ can increase Ca^{2+} release from the ER, leading to the production of reactive oxygen species (ROS) and neuronal cell death through caspase-3 activation (Uddin et al., 2020). Furthermore, during AD, the persistent accumulation of $\text{A}\beta$ or p-tau leads to abnormal levels of ER stress, contributing to synaptic dysfunction and neurodegeneration. Mitochondrial defects and free radical production also contribute substantially to ER stress and may induce oxidation of ER proteins, possibly by altering the activity of resident chaperones belonging to the protein disulfide isomerase (PDI) family (Gerakis and Hetz, 2018). In addition, tau accumulation disrupts ER protein homeostasis, activates the inflammatory cytokine pathway (NF- κ B) through PERK phosphorylation, and activates the UPR by impairing ERAD pathways, thereby leading to ER stress. $\text{A}\beta$ peptides, a hallmark of AD, have been shown to induce ER stress by interfering with protein folding and calcium homeostasis within the ER. Persistent ER stress, if unresolved, can lead to sustained UPR activation, which shifts from a protective response to a pro-apoptotic response and ultimately results in neuronal death (Hasan et al., 2024).

Relationship Between Parkinson's Disease and ER Stress

PD is the second most common neurodegenerative disorder after Alzheimer's disease (AD) and poses a significant threat to public health. The pathogenesis of the disease involves severe neuronal loss, resulting in a complex clinical picture characterised by both motor and non-motor symptoms (Dextera and Jenner, 2013). Risk factors such as ageing, genetic predisposition and environmental factors all play a role in the onset of the pathogenic process; however, the mechanism underlying the development of PD and the onset of neuronal loss has not yet been fully elucidated. Progressive asymmetric slowness of movement (bradykinesia), rigidity, tremor and gait disturbance

are the prominent clinical features of PD. Key challenges in understanding the disease include identifying new pathways involved in its development and progression, and linking these mechanisms to the heterogeneous clinical manifestations and disease course (Morris et al., 2024).

Parkinson's disease is characterised by the progressive loss of dopaminergic neurons, particularly in the substantia nigra pars compacta (SNc), and by the presence of Lewy bodies, which consist of α -synuclein protein aggregates. Over the course of the disease, aggregates of misfolded α -synuclein gradually impair synaptic function, trigger neuroinflammation and lead to neuronal degeneration (Burré et al., 2024; Dextera and Jenner, 2013). Significant progress has been made in understanding the mechanisms contributing to the death of nigral dopaminergic cells, including mitochondrial dysfunction, oxidative stress, altered protein processing and inflammation. However, it is not known whether the same processes are responsible for neuronal loss in non-dopaminergic brain regions (Burré et al., 2024). In PD, various genetic and environmental triggers jointly activate mutually reinforcing mechanisms, which in turn form a complex yet identifiable pathophysiological network leading to dopaminergic neuron loss and both motor and non-motor symptoms (Matsui and Takahashi, 2024).

In addition to the currently identified pathologies in the development of PD, ER stress and UPR activation also emerge as recurrent findings. Recent clinical and experimental studies indicate that ER stress may be one of the early pathological events and also accelerates neuronal death in the later stages (Zeng et al., 2024). It has been demonstrated that markers of UPR activation (p-PERK, p-eIF2 α and p-IRE1 α) are elevated in dopaminergic neurons of the SNpc in patients with PH and correlate with misfolded α -synuclein. Furthermore, studies have shown that BiP levels are reduced in certain regions of post-mortem brain tissue from PH patients, suggesting that UPR alterations may be localised to the region of neurodegen-

eration, and consequently that protein folding function is impaired in disease-specific regions (Baek et al., 2019; Heman-Ackah et al., 2017; Wang et al., 2023).

The molecules most commonly used to establish a PH model in experimental studies are 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenylpyridinium (MPP) / 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone, or transgenic animal models. These molecules have repeatedly been shown to induce ER stress under both in vitro and in vivo conditions (Wang et al., 2023). Several studies have reported that pharmacological and genetic interventions targeting ER stress exert neuroprotective effects in PD models (Kovaleva and Saarma, 2021). In one such study, α -synuclein was shown to suppress ER-to-Golgi trafficking in a PD model by inhibiting ATF6 processing through direct physical interactions and indirectly through the limited incorporation of ATF6 into COPII vesicles. Impaired ATF6 signaling, together with reduced ERAD function and increased pro-apoptotic signaling, was shown to contribute directly to disease pathology (Credle et al., 2015). Another study demonstrated that activation of the UPR pathway in the PD brain was partially associated with the accumulation of α -synuclein within the ER. In both in vitro and in vivo models, α -synuclein was shown to directly activate the UPR pathway by binding to the misfolded protein sensor/UPR activator GRP78/BiP, while α -synuclein accumulation within the ER triggered activation of the PERK-dependent branch of the UPR (Bellucci et al., 2011). Furthermore, in a neurotoxin-induced mouse model of PD, targeting translational attenuation through the PERK/p-eIF2 α pathway with the PERK inhibitor GSK2606414 was reported to protect dopaminergic neurons, increase the levels of dopaminergic and synaptic proteins, and restore motor function (Mercado et al., 2018).

In summary, the relationship between α -synuclein aggregation and impaired ER function is particularly strong and has been consistently demonstrated in both human tissues and ex-

perimental models. In PD, α -synuclein overexpression and accumulation induce the UPR and impair protein transport from the ER to the Golgi apparatus, whereas chronic or irreversible ER stress may ultimately lead to cell death through apoptosis (Kovaleva and Saarma, 2021; Mercado et al., 2018).

Relationship Between Huntington's Disease and ER Stress

HD is an autosomal dominant neurodegenerative disorder caused by the expansion of CAG trinucleotide repeats in the HTT gene encoding the huntingtin protein. It is characterized by polyglutamine (polyQ) expansion and leads to progressive motor and cognitive impairments (Shacham et al., 2019; Tabrizi et al., 2020). CAG trinucleotide repeats of 36 or more are pathogenic, and longer repeat expansions generally result in an earlier age of disease onset. Polyglutamine expansion causes abnormal folding of HTT, leading soluble HTT monomers to associate and form oligomers. Mutant HTT (mHTT) affects multiple cellular functions and ultimately leads to cell death; it disrupts transcription, interferes with immune and mitochondrial functions, and undergoes abnormal post-translational modifications. The accumulation of cytoplasmic aggregates and nuclear inclusions throughout the brain is one of the major pathological features of HD (Maity et al., 2022; Shacham et al., 2019; Tabrizi et al., 2020).

Similar to other neurodegenerative diseases, HD is associated with ER stress in relation to aging and the accumulation of misfolded proteins. Although mHTT plays a role in HD pathogenesis, wild-type HTT is known to be required for ER-to-Golgi transport and secretory vesicle fusion at the plasma membrane. The relationship between HD and ER stress was first demonstrated by the observation of abnormal expansion of the endoplasmic reticulum in striatal cells carrying mHTT (Maity et al., 2022). Subsequent studies have demonstrated that polyQ-containing mutant huntingtin proteins induce neu-

ronal cell death, particularly through activation of the IRE1–TRAF2–ASK1–JNK signaling pathway (Nishitoh et al., 2002).

PERK signaling within the UPR is important for the regulation of autophagy. Phosphorylation of eIF2 α has been shown to inhibit ER stress-induced caspase-12 activation and ER stress-mediated cell death (Maity et al., 2022). In contrast, an experimental study demonstrated that PolyQ72 aggregates induced autophagy through the PERK/eIF2 α signaling pathway, whereas the eIF2 α A/A mutation could induce cell death through caspase-12 activation. Consequently, autophagy functions as a protective defense mechanism against ER stress-induced cell death by clearing polyQ72 aggregates (Kouroku et al., 2006).

These findings indicate that ER stress plays an important role in HD pathogenesis not only as a mechanism that triggers cellular damage but also by activating the PERK/eIF2 α -mediated autophagic response, thereby facilitating the removal of mutant protein aggregates and maintaining neuronal survival.

Conclusion

ER stress is a cellular mechanism that plays a central role in the pathogenesis of neurodegenerative diseases and is of critical importance in maintaining protein homeostasis. In various neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS), ER stress has been shown to be markedly activated alongside the accumulation of misfolded proteins, disruption of calcium homeostasis, oxidative stress, mitochondrial dysfunction, and neuroinflammation. Specifically, unfolded protein response (UPR) signaling pathways activated as a result of chronic ER stress initially induce protective adaptive responses; however, under conditions of prolonged activation, they trigger apoptotic mechanisms, thereby contributing to neuronal loss.

At present, there is no effective approach for the definitive treatment of neurodegenerative diseases. Furthermore, a better understanding of the molecular mechanisms underlying endoplasmic reticulum (ER) stress—one of the common features of many neurodegenerative diseases—is crucial for the early diagnosis of these diseases, the development of biomarkers, and the formulation of targeted treatment strategies. In particular, a detailed elucidation of the interactions between endoplasmic reticulum (ER) stress, mitochondrial dysfunction, autophagy and neuroinflammation will contribute to the development of more effective and multi-targeted therapeutic approaches in the future.

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NF- κ B/NLRP3 INFLAMMASOME INTERACTION: MOLECULAR MECHANISMS AND SIGNIFICANCE IN DISEASES

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INTRODUCTION

Inflammation is a fundamental defense mechanism that activates the innate immune system, developed by the body in response to various harmful stimuli such as pathogen invasion, injury, tissue trauma, or cellular damage. This complex biological process is vital for eliminating invading pathogens, repairing damaged tissues, and restoring cellular homeostasis. One of the key mechanisms involved in regulating the inflammatory response is inflammasome complexes, which detect intracellular danger signals and are important components of the innate immune system. Inflammasomes play critical roles in initiating and maintaining the inflammatory response by enabling the maturation of pro-inflammatory cytokines such as interleukin (IL)-1 β and IL-18. Within the inflammasome family, the most extensively studied complex, and one that has been shown to play a role in the pathogenesis of numerous diseases, is the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome.

This physiological response, which protects the organism and supports tissue repair under physiological conditions, can transform into a pathological process and damage healthy tis-

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sues when activated inappropriately, excessively, or chronically. Today, it is known that dysregulation of inflammatory pathways plays a significant role in the development of metabolic diseases such as atherosclerosis, type 2 diabetes, and obesity; neurodegenerative disorders such as Alzheimer's and Parkinson's disease; various autoimmune diseases; and different types of cancer. Recent findings have revealed that the reciprocal and bidirectional interaction between the NLRP3 inflammasome and the nuclear factor kappa B (NF- κ B) signaling pathway plays a crucial role in regulating inflammatory processes. As will be discussed in detail in the following sections, this regulatory relationship has made the NLRP3/NF- κ B axis a key molecular target in the pathogenesis of many inflammatory and degenerative diseases.

STRUCTURE AND ACTIVATION OF THE NLRP3 INFLAMMASOME

Structural Components and Organization of the NLRP3 Inflammosome

Inflammosomes are multi-protein complexes located in the cytoplasm of cells that are activated upon detection of pathogens or cellular damage signals (Dadkhah & Sharifi, 2025; Ge et al., 2024). NLRP3, the most extensively studied protein within this family, acts as a central sensor in the body's innate immune system (Y. Chen et al., 2023; Kodi et al., 2024). NLRP3 is a protein composed of three main domains: the N-terminal pyrin domain (PYD), which interacts with the ASC adapter protein; the centrally located NACHT (or NOD) domain, which enables self-oligomerization via ATPase activity; and the C-terminal leucine-rich repeat (LRR) domain, associated with signal recognition and auto-inhibition (Figure 1a). The establishment of an active NLRP3 inflammasome complex requires three essential components: a sensor molecule (NLRP3), an adapter protein (ASC), and an effector enzyme (Pro-caspase-1) (Ren et al., 2024) (Figure 1b).

- **Sensor (NLRP3 Protein):** The NLRP3 protein acts as a sensor within this inflammasome complex. Its NACHT domain provides the energy required for complex assembly via ATP hydrolysis and is essential for oligomerization (Swanson et al., 2019a). When activated, the sensor protein self-oligomerizes via the NACHT domain and recruits ASC proteins via the PYD domain.
- **Adapter (ASC Protein):** The ASC (Apoptosis-associated speck-like protein containing a CARD) is a bimodal molecule that acts as a bridge between the sensor and the effector. It contains an N-terminal PYD and a C-terminal CARD (Caspase recruitment domain). Activated NLRP3 recruits ASC proteins via PYD-PYD interaction, and these proteins assemble to form macromolecular foci called “ASC foci (specks)” (Ren et al., 2024).
- **Effector (Pro-caspase-1):** ASC incorporates pro-caspase-1, an inactive enzyme, into the complex via CARD, causing the enzyme to self-cleave and become active caspase-1 (Kodi et al., 2024). Caspase-1 is a key enzyme responsible for cytokine maturation and the programmed cell death pathway called pyroptosis (Dadkhah & Sharifi, 2025).

However, recent research has revealed that NEK7, a mitotic kinase and a fourth component of the inflammasome, is an essential and unique component for NLRP3 activation (Figure 1b) (Swanson et al., 2019a; J. Xu & Núñez, 2023). NEK7 binds to the LRR and NACHT domains of NLRP3, facilitating complex assembly and caspase-1 activation (Blevins et al., 2022; Paik et al., 2021a). This structural assembly serves as a platform through which the cell translates “danger” signals into a biological response.

Molecular Pathways of Inflammasome Activation

Three main pathways have been identified for the activation of the NLRP3 inflammasome (Dadkhah & Sharifi, 2025; Ren

et al., 2024): the canonical pathway, the non-canonical pathway, and the alternative pathway.

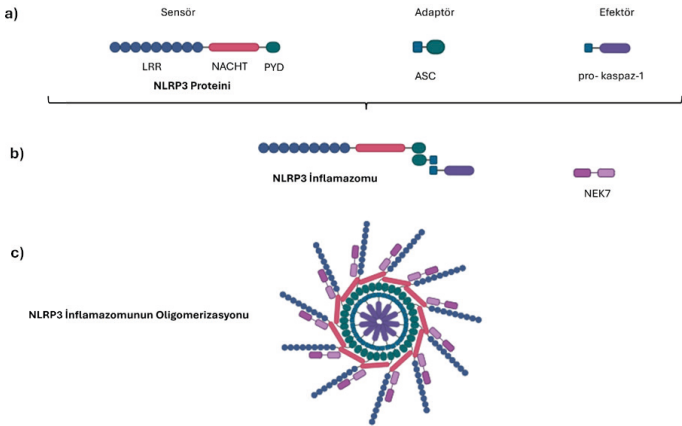


Figure 1. a) Components of the NLRP3 inflammasome b) Structure of the NLRP3 inflammasome c) Oligomerization of the NLRP3 inflammasome (Generated by the author using BioRender.com)

Canonical activation pathway: Canonical activation is a process applicable to most cell types (especially macrophages) and described by a two-step signaling process (priming and activation) (Dadkhah & Sharifi, 2025). The first signaling step (also called Signal 1), the “priming” step, begins with the binding of PAMPs (e.g., LPS) or cytokines (TNF, IL-1 β) to Toll-like receptors (TLRs) or cytokine receptors (Yang et al., 2019). This interaction activates the NF- κ B signaling pathway, increasing the transcriptional expression of NLRP3, pro-IL-1 β , and pro-IL-18. Furthermore, priming triggers post-translational modifications (such as deubiquitination and phosphorylation) to make NLRP3 ready for activation (Gupta et al., 2025; Swanson et al., 2019a). Following the priming phase, the physical assembly of the complex begins with a second signal (also called Signal 2), the “activation” stimulus. When the cell, sensitized by the first signal, is exposed to a second stimulus, oligomerization and assembly of the NLRP3 inflammasome complex occurs (Figure 1c). The critical cellular events identified at this stage are:

- **Potassium (K⁺) Efflux:** This is a fundamental signal common and necessary for most activators. The binding of extracellular ATP to the P2X7 receptor or the pore formation of bacterial toxins reduces intracellular K⁺ concentration, which triggers the conformational change of NLRP3. In addition, the increase in calcium (Ca²⁺) and the removal of chloride (Cl⁻) are also important ionic events supporting assembly (Y. Chen et al., 2023).
- **Mitochondrial Dysfunction:** Cellular stimuli damage mitochondria, leading to the production of mitochondrial reactive oxygen species (mtROS) and the leakage of oxidized mitochondrial DNA (mtDNA) into the cytoplasm. The leaked mtDNA can directly bind to NLRP3, initiating its activation. Furthermore, the translocation of the mitochondrial lipid cardiolipin to the outer membrane also serves as a scaffold for NLRP3 activation (Kodi et al., 2024).
- **Lysosomal Damage and Cathepsin B:** Crystals (uric acid, cholesterol, amyloid- β) phagocytosed by the cell lead to the degradation of lysosomes. Lysosomal enzymes, particularly Cathepsin B, released into the cytoplasm as a result of this damage, directly or indirectly interact with NLRP3, triggering its activation (Ren et al., 2024).
- **Disintegration of the Trans-Golgi Network (dTGN):** It has been shown that the Trans-Golgi network is disrupted (dTGN) by the action of various activators, and NLRP3 binds to PI4P molecules on these dispersed vesicles, forming an assembly platform (Yang et al., 2019).

Assembly and Result: Active NLRP3 recruits the adapter protein ASC; ASC, in turn, binds pro-caspase-1, forming the inflammasome complex. Ultimately, active caspase-1 matures cytokines (IL-1 β , IL-18) and causes the cell to swell by taking up water through Gasdermin D (GSDMD) pores, ultimately leading to lysis via pyroptosis (Dadkhah & Sharifi, 2025; Gupta et al., 2025; Kelley et al., 2019).

Non-canonical activation pathway: This pathway occurs via caspase-4/5 in humans and caspase-11 in mice, rather than caspase-1. Intracellular LPS (lipopolysaccharide) from Gram-negative bacteria is directly sensed by caspase-4/5/11. Activation of these caspases cleaves GSDMD, creating pores in the cell membrane and triggering pyroptosis (Yang et al., 2019). Potassium outflow from GSDMD pores activates the canonical NLRP3 inflammasome as a secondary signal. This situation leads to caspase-1 activation and consequently IL-1 β release (Swanson et al., 2019a).

Alternative Activation Pathway: This pathway has been specifically identified in human monocytes and exhibits different characteristics from the canonical pathway. Activation of this pathway is not dependent on potassium efflux; furthermore, the formation of ASC foci (specks), GSDMD pores, and pyroptosis are not observed (He et al., 2016).

Other pathways demonstrated in studies are ZBP1-dependent and necroptotic pathways. During influenza A virus (IAV) infection, ZBP1, a sensor, detects Z-RNA. ZBP1 recruits RIPK3 and caspase-8, coordinating NLRP3 activation and subsequent IL-1 β release (Sharma & Kanneganti, 2021a). ZBP1 is also a necroptosis effector. The relationship between necroptosis and the NLRP3 inflammasome has been described as follows: when activated, MLKL, the necroptosis driver, disrupts membrane integrity, causing potassium release, and in this way can activate the NLRP3 inflammasome. However, the detailed mechanisms related to these pathways still need to be elucidated (Huang et al., 2021; Sharma & Kanneganti, 2021a; J. Xu & Núñez, 2023).

In conclusion, NLRP3 activation can occur via one of these different molecular pathways depending on the type of stimulus and the type of cell.

Caspase-1, IL-1 β , and IL-18 Production

Following activation signals, NLRP3 monomers oligomerize via the NACHT domain, incorporating the adapter protein ASC and subsequently pro-caspase-1 into the complex.

Caspase-1 Activation: Pro-caspase-1 molecules assembled within the inflammasome form the active caspase-1 enzyme via proximity-induced autocatalytic cleavage (autoproteolysis). Active caspase-1 has a heterotetramer structure (Ren et al., 2024; Zheng et al., 2022).

Cytokine Maturation: Active caspase-1 cleaves pro-interleukin-1 β and pro-interleukin-18, which are in an inactive form within the cell, at specific points. As a result of this process, the biologically active cytokines IL-1 β and IL-18 are produced (Dadkhah & Sharifi, 2025; Meier et al., 2024).

Gasdermin D and Pyroptosis: Caspase-1 also cleaves the Gasdermin D protein, releasing its N-terminal fragment (GSDMD-NT). These fragments travel to the plasma membrane, oligomerize, and form large membrane pores approximately 10-21 nm in diameter (J. Wang et al., 2025).

Release and Cell Death: Since the mature cytokines do not contain a signal peptide, they are released outside the cell through these pores. The loss of membrane integrity causes the cell to swell by taking up water and eventually rupture through pyroptosis. This process is a fundamental defense mechanism that allows the body to release pathogens that cannot be destroyed by phagocytosis, exposing them to other elements of the immune system (Dadkhah & Sharifi, 2025; Huang et al., 2021).

NF- κ B and NLRP3 INTERACTION

In the immune system's effort to maintain homeostasis, the molecular interaction between the nuclear factor kappa-B (NF- κ B) signaling pathway and the NLRP3 inflammasome can be described as both the "architect" and "executor" of the inflammatory response (Kaszycki & Kim, 2025). NF- κ B is a central

transcription factor that translates stimuli from the cell's external environment (PAMPs) or internal damage (DAMPs) into a genetic command by transmitting them to the nucleus; NLRP3 translates this genetic instruction into a functional protein platform, coordinating the release of effector cytokines (IL-1 β and IL-18) and pyroptotic cell death (Dadkhah & Sharifi, 2025; Mo et al., 2025; J. Wang et al., 2025).

The Role of NF- κ B in NLRP3 Expression and Molecular Bridges

For the NLRP3 inflammasome to function properly, the amount of intracellular components must exceed a certain threshold, and NF- κ B is a key determinant in this process. In resting myeloid cells (especially macrophages and monocytes), NLRP3 protein levels and pro-IL-1 β amounts are below the threshold required to trigger an inflammatory response (Sharma & Kanneganti, 2021a; Zhou et al., 2010). At this point, NF- κ B acts as the absolute regulator of the priming phase. The binding of PAMPs or cytokines to TLRs (Toll-like receptors) or cytokine receptors on the cell surface triggers the phosphorylation and degradation of I κ B, an inhibitor of NF- κ B, via adapter proteins such as MyD88 and TRIF (Kodi et al., 2024; Ren et al., 2024). The released active NF- κ B p65/p50 dimers translocate to the nucleus and directly bind to the promoter regions of the NLRP3 and IL1B genes, initiating mRNA transcription (Boaru et al., 2015). New research suggests that this transcriptional control is not limited to NF- κ B alone; FADD and Caspase-8 are shown to support NLRP3 expression by acting as a scaffold that stabilizes NF- κ B activation independently of their apoptotic functions (P. Chen & Li, 2024; Y. Chen et al., 2023; Kodi et al., 2024).

Activation of NLRP3 via NF- κ B-Dependent Post-Translational Regulation

Activation of the NF- κ B signaling pathway not only promotes the transcription of target genes but also regulates post-translational modifications (PTMs) that constitutive-

ly prepare (license) the NLRP3 protein for activation (Paik et al., 2021a; J. Xu & Núñez, 2023). During NF- κ B-induced signaling, JNK1 kinase phosphorylates the Ser198 residue in the NACHT domain of NLRP3 (P. Chen & Li, 2024; Mangan et al., 2018). This phosphorylation is essential for the deubiquitination enzyme BRCC3 to clear ubiquitin chains in the LRR domain of NLRP3 (Yang et al., 2019). Consequently, NF- κ B-dependent signaling rescues NLRP3 from its auto-inhibitory state, bringing it to a “signal-receiving” state (Swanson et al., 2019a).

Epigenetic and Environmental Regulation of NF- κ B/NLRP3 Interaction

Current literature highlights that the interaction between NLRP3 and NF- κ B is further complicated by epigenetic modifications (Kaszycki & Kim, 2025; Kodi et al., 2024). For example, DNA hypomethylation in the NLRP3 promoter in chronic inflammatory conditions facilitates easier access of NF- κ B to this region and triggers excessive NLRP3 production. In addition, histone acetylation (H3 acetylation) provides the “open chromatin” structure necessary for NF- κ B to transcribe target genes. Nuclear receptors such as Rev-erba and ROR γ , discovered in recent years, have been shown to repress or stimulate the NF- κ B/NLRP3 axis depending on the circadian rhythm; This also explains, at the molecular level, why inflammatory responses vary depending on the time of day (Mo et al., 2025; Zheng et al., 2022).

The Delicate Balance in Positive and Negative Feedback Mechanisms

The interaction between NLRP3 and NF- κ B can involve “vicious cycles” that can escalate inflammation uncontrollably. The most striking example of this interaction is the mature IL-1 β cytokine released as a result of NLRP3 activation. Mature IL-1 β released as a result of NLRP3 activation binds to IL-1 receptors in the cell, reactivating the NF- κ B pathway; this creates a positive feedback loop resulting in further NLRP3 and

pro-cytokine synthesis (Mangan et al., 2018; Mo et al., 2025; Zheng et al., 2022). Increased ROS resulting from oxidative stress stimulate NF- κ B, thereby increasing NLRP3 expression; active NLRP3 disrupts mitochondrial homeostasis, leading to further ROS production and continuously fueling the inflammatory response (Moustakli et al., 2025; M. Wang et al., 2023). TXNIP (Thioredoxin-interacting protein) acts as a sensor in this feedback loop by detecting the increase in ROS levels and interlocking the NF- κ B/NLRP3 axis (He et al., 2022; Zheng et al., 2022). However, the NF- κ B signaling pathway may also paradoxically assume a negative regulatory role to limit inflammation. According to Zhong et al. (2016), NF- κ B activation leads to the accumulation of the autophagy receptor p62/SQSTM1 (Zhong et al., 2016). p62, by clearing damaged mitochondria (via mitophagy), which is one of the main signals activating NLRP3, limits the overactivation of the inflammasome and the resulting tissue damage. This “NF- κ B-p62-mitophagy” axis is a critical control point for inflammation to have a self-limiting nature.

NF- κ B/NLRP3 AXIS IN DISEASES

The balance between the NLRP3 inflammasome and the NF- κ B signaling pathway is vital for maintaining cellular homeostasis; chronic dysregulation in this interaction underlies many systemic and organ-specific diseases encountered by modern medicine.

The balance between these two systems normally has a self-limiting structure; Indeed, the NF- κ B signaling pathway not only triggers inflammation but also provides a protective “brake” mechanism that prevents NLRP3 overactivation by stimulating mitophagy (p62/SQSTM1-mediated) pathways that clear damaged mitochondria (Zhong et al., 2016). However, chronic accumulation of endogenous danger signals such as cholesterol crystals, β -amyloid aggregates, supersaturated fatty acids, and uric acid crystals can lead to continuous activation of

the NLRP3/NF- κ B axis. In this case, the protective inflammatory response is replaced by metainflammation, a low-grade and chronic form of inflammation, leading to progressive damage in healthy tissues (Y. Chen et al., 2023; Dadkhah & Sharifi, 2025). Consequently, the loss of homeostatic coordination between NF- κ B readiness signaling and NLRP3 activation strength leads to; It constitutes the fundamental biological pathology of a wide spectrum of diseases ranging from neurodegenerative processes such as Alzheimer's and Parkinson's to metabolic breakdowns such as atherosclerosis and type 2 diabetes.

Neuroinflammatory Diseases

The central nervous system (CNS) is highly sensitive to NLRP3 inflammatory responses. Neuroinflammation is defined as both a consequence and a contributing factor to neurodegenerative disorders (W. Xu et al., 2025).

- **Alzheimer's Disease (AD):** Microglia cells in the brain perceive accumulating β -amyloid ($A\beta$) plaques and tau protein clusters as a danger signal (Y. Chen et al., 2023). Following an NF- κ B-mediated priming step, $A\beta$ fibrils activate NLRP3, leading to IL-1 β release and a chronic neuroinflammatory vicious cycle; This situation accelerates synaptic loss and cognitive decline (W. Xu et al., 2025).
- **Parkinson's Disease (PD):** In the pathogenesis of PD, α -synuclein protein clusters stimulate the NLRP3 inflammasome in microglia, creating a pro-inflammatory environment that leads to the loss of dopaminergic neurons (W. Xu et al., 2025).
- **Multiple Sclerosis (MS) and Stroke:** In the MS model, dysregulation of the NLRP3/NF- κ B axis promotes T cell infiltration and demyelination (Ren et al., 2024). After ischemic stroke, ROS generated during reperfusion injury strongly stimulates this axis, playing a role in the development of cerebral edema and blood-brain barrier damage (W. Xu et al., 2025).

Metabolic Diseases

Metabolic disorders are characterized by low-grade chronic inflammation that is pathogen-free but triggered by cellular stress signals and is called “metaflammation” (Sharma & Kanneganti, 2021a). • Type 2 Diabetes and Obesity: Saturated fatty acids (such as palmitate) and high glucose levels activate the NF- κ B pathway in adipocytes and macrophages, increasing NLRP3 expression (Dadkhah & Sharifi, 2025). Released IL-1 β directly disrupts insulin receptor signaling, leading to insulin resistance and pancreatic β -cell dysfunction (Meier et al., 2024).

- Liver Diseases: Activation of this axis in Kupffer cells of the liver is a critical control point in the progression of simple steatosis to the more serious condition of steatohepatitis (NASH) and eventually fibrosis (Y. Chen et al., 2023).
- Gout: Monosodium urate (MSU) crystals accumulating in the joints are one of the most potent endogenous activators of the NLRP3 inflammasome and are responsible for the typical inflammatory picture of a gout attack (Dadkhah & Sharifi, 2025).

Cardiovascular Diseases

The role of inflammation in atherosclerosis and heart failure has been proven by clinical studies (Sharma & Kanneganti, 2021a). • Atherosclerosis: Cholesterol crystals and oxidized LDL in the vessel wall stimulate the NF- κ B/NLRP3 axis in macrophages, increasing “foam cell” formation and plaque instability (Dadkhah & Sharifi, 2025). This process predisposes to narrowing of the vessel lumen and acute thrombotic events.

- Myocardial Infarction and Heart Failure: DAMPs released from damaged tissue after a heart attack activate NLRP3, leading to adverse ventricular remodeling and fibrosis (Zheng et al., 2022). Inhibition of NLRP3 has been shown to limit damage to heart tissue and preserve contractile function (Kodi et al., 2024).

Cancer

The role of the NF- κ B/NLRP3 axis in cancer is a double-edged sword depending on the type and stage of the tumor. In many malignancies such as lung, breast, and prostate cancer, NLRP3-mediated IL-1 β and IL-18 release promotes angiogenesis, invasion, and immunosuppression (through the accumulation of MDSC cells) in the tumor microenvironment, thus stimulating tumorigenesis (Dadkhah & Sharifi, 2025; Sharma & Kanneganti, 2021a). In contrast, in some cancer types such as colorectal cancer, NLRP3 activation exhibits a tumor-suppressive property by playing a protective role in maintaining intestinal epithelial integrity and coordinating anticancer immune responses (NK and T cell activation) (Y. Chen et al., 2023).

Kidney Diseases

- Diabetic Kidney Disease (DKD): C-reactive protein (CRP) has been shown to exacerbate renal inflammation and fibrosis by linking the NLRP3 and NF- κ B pathways via Smad3. Smad3 deficiency has been found to inhibit NLRP3 activation by reducing NF- κ B signaling and macrophage infiltration in the kidney (Y. Wang et al., 2025).
- Lupus Nephritis: Vitamin D receptor (VDR) activation has been reported to improve lupus nephritis by modulating the NF- κ B/NLRP3/Caspase-1 axis and suppressing NF- κ B transport to the nucleus (Ren et al., 2024).

Autoimmune and Endocrine Disorders

- Autoimmune Thyroiditis (AIT): Excessive iodine intake has been shown to trigger NLRP3 inflammasome by activating the ROS and NF- κ B signaling pathway in thyroid follicular cells, leading to pyroptosis and cell death. Furthermore, phthalate (DBP) exposure has also been found to cause thyroid damage via AKT/NF- κ B/NLRP3 signaling (Ren et al., 2024).

- Sjögren's Syndrome (SS): In patients with severe SS, extracellular DNA accumulation has been shown to fuel systemic NLRP3 activation and enhance NF- κ B-related inflammatory responses (Dadkhah & Sharifi, 2025).

Eye and Skin Diseases

- Dry Eye Disease: The SM934 molecule, derived from artemisinin, has been reported to alleviate dry eye disease by regulating the TLR4/NF- κ B/NLRP3 signaling pathway (Ge et al., 2024). MicroRNA-146a has also been shown to negatively regulate this pathway via NF- κ B (Kaszycki & Kim, 2025).
- Diabetic Keratopathy (DK): Increased advanced glycation end products (AGEs) resulting from chronic high glucose levels have been found to cause pyroptosis in corneal epithelial cells by hyperactivating the TLR4/NF- κ B and NLRP3 pathways (Ge et al., 2024).
- Psoriasis: Inhibitors such as BAY 11-7082 have been reported to reduce psoriasis symptoms by simultaneously blocking both the NF- κ B and NLRP3 pathways (Kodi et al., 2024).
- Gastrointestinal and Lung Diseases
 - Inflammatory Bowel Disease (IBD): NEK7 protein has been shown to modulate pyroptosis and bowel damage in IBD models by interacting with NLRP3 via NF- κ B signaling (X. Chen et al., 2019).
 - Acute Lung Injury: Treatments such as Sanzi Yangqin decoction have been found to improve lung damage by regulating the TLR2/NF- κ B/NLRP3 signaling pathway (Gan et al., 2025).

Other Pathological Conditions

- Neuropathic Pain: In conditions such as lumbar disc herniation, simultaneous activation of NF- κ B and NLRP3 has been shown to increase pain signals in the dorsal root ganglia (Kodi et al., 2024). Furthermore, NF- κ B p65 has been

found to contribute to chronic pain by regulating the microRNA-183/TXNIP/NLRP3 axis (P. Chen & Li, 2024).

- Sepsis: NF- κ B manages the priming phase of the NLRP3 inflammasome in macrophages; it also provides an internal control mechanism that restricts excessive NLRP3 activation via the NF- κ B/p62/mitophagy cycle (Zhong et al., 2016).

All this information proves that the NLRP3/NF- κ B axis is a central regulator not only in general metabolic or neuroinflammatory processes but also in specific inflammatory and autoimmune processes specific to organs such as the kidney, thyroid, eye, lung, and intestine.

THERAPEUTIC APPROACHES

The central role of the NLRP3/NF- κ B axis in numerous chronic and acute inflammatory diseases has necessitated the development of innovative treatment strategies targeting different steps of this pathway. Current therapeutic approaches range from small molecules directly targeting the NLRP3 protein to suppression of NF- κ B signaling pathways that govern the priming phase, and combined strategies that utilize these mechanisms together. NLRP3 Inhibitors

Direct pharmacological inhibition of the NLRP3 inflammasome is considered the most specific method for halting the overproduction of cytokines (IL-1 β and IL-18) and pyroptotic cell death (Dadkhah & Sharifi, 2025).

- Small Molecules Targeting the NACHT Domain: The best-known compound in this group, MCC950 (CRID3), strongly inhibits ATP hydrolysis and thus the physical assembly of the inflammasome by binding to the Walker B motif in the NACHT domain (Yang et al., 2019). However, clinical trials of MCC950 were halted due to unexpected hepatotoxicity; this led researchers to develop safer derivatives (e.g., N14 or B6 in a non-sulfonylurea structure) (P. Chen &

Li, 2024). Similarly, the compound CY-09 blocks ATPase activity by directly binding to the Walker A motif, while OLT1177 (Dapansutrile) exhibits a safe pharmacokinetic profile and is progressing in phase studies for gout and heart failure (P. Chen & Li, 2024; Yang et al., 2019).

- **Disruptors of NEK7 Interaction:** Blocking the interaction with NEK7, a mandatory licensing factor for NLRP3 activation, is a strategic target (Paik et al., 2021a). Oridonin, a naturally occurring compound, irreversibly halts NEK7-NLRP3 coupling by covalently binding to the Cys279 residue of NLRP3 (Yang et al., 2019). Furthermore, the anticancer drug RRx-001 has also been shown to block this interaction via Cys409 (Kodi et al., 2024).
- **Approaches to Other Domains:** Recent research has shown that molecules such as TH5487 and SU0268, which target the pyrin domain (PYD) of NLRP3 and disrupt its interaction with the ASC adapter protein, are effective in limiting oxidized DNA (oxDNA)-induced activation (Cabral et al., 2025).

NF- κ B Targeted Strategies

Suppression of the NF- κ B pathway, which constitutes the “priming (Signal 1)” phase of inflammasome activation, aims to limit the inflammatory response in the early stages by reducing NLRP3 expression and pro-cytokine production (Kodi et al., 2024).

- **Signal Transmission Inhibitors:** BAY 11-7082, in addition to stopping the transport of NF- κ B to the nucleus by inhibiting IKK β activity, also exerts a direct inhibitory effect by alkylating cysteine residues in the ATPase region of NLRP3 (Kodi et al., 2024). TAK-242 (Resatorvid), on the other hand, interrupts the NF- κ B/NLRP3 axis at the highest level by blocking the TLR4 receptor and shows protective effects in conditions such as sepsis (Zhang et al., 2023).

- **Natural Products and Phytotherapy:** Polyphenols such as curcumin, luteolin, and isoliquiritigenin (ISL) reduce pro-inflammatory gene expression by suppressing NF- κ B stimulation and indirectly restrict inflammasome activation by maintaining mitochondrial homeostasis (Blevins et al., 2022).
- **Epigenetic and RNA-Based Approaches:** Endogenous regulators such as miR-223 and miR-146a are being evaluated as therapeutic tools by directly targeting NLRP3 mRNA or negatively regulating the NF- κ B pathway (J. Wang et al., 2025).

Combined Therapy Approaches

Given the complex pathophysiology of diseases, combined approaches that simultaneously control multiple points of the pathway or parallel mechanisms, rather than targeting only one molecule, promise higher efficacy.

- **Simultaneous Blockade of Signal 1 and Signal 2:** In experimental studies, the combination of NF- κ B inhibitors (e.g. TAK242) and direct NLRP3 inhibitors has been shown to suppress the inflammatory response more effectively than treatments administered alone.
- **Inhibition at Different Steps of the Signaling Pathway:** In acute lung injury models, the combination of MCC950, a direct NLRP3 inhibitor, and Anakinra, an IL-1 receptor antagonist, provides a synergistic improvement by disrupting both the establishment of the complex and the paracrine loops of released cytokines (new NLRP3 synthesis via NF- κ B) (X. Wang et al., 2021).
- **Repurposing Strategies:** Disulfiram, used in the treatment of alcohol dependence, has been shown to both inhibit pyroptosis by blocking Gasdermin D pores and reduce the localization of NLRP3 to the Trans-Golgi network by inhibiting its palmitoylation. This dual mechanism of action makes disulfiram suitable for sepsis and COVID-19. This makes it a potential treatment candidate for diseases characterized by excessive inflammatory responses, such as (J. Xu et al., 2024).

CONCLUSION AND FUTURE PERSPECTIVES

Although research on the NLRP3 inflammasome and NF- κ B signaling pathway has gained tremendous momentum in the last decade, a universal and unified molecular mechanism for the activation of this complex has still not been identified (Dadkhah & Sharifi, 2025). Which common signaling pathway converges different stimuli such as intracellular potassium efflux, mitochondrial damage, and ROS, and in which regions and in what order these events occur within the cell, are among the fundamental questions that still await clarification. Uncertainties regarding the precise function of the leucine-rich repeat (LRR) domain of NLRP3 and the high-resolution structural organization of the protein in its active state remain one of the biggest obstacles to rational drug design (He et al., 2016). Future strategies should focus not only on direct protein inhibition but also on methods such as deubiquitination, phosphorylation, and acetylation. Research will focus on approaches that specifically target post-translational modifications (PTMs) (Paik et al., 2021a). Furthermore, epigenetic interventions via microRNAs and long non-coding RNAs (lncRNAs) will allow for more precise control of the inflammatory response at the gene level. For central nervous system diseases, the development of small molecules that can effectively cross the blood-brain barrier and the elimination of potential off-target effects are key to clinical success. Elucidating the mechanisms underlying the protective and disease-progression-promoting effects of NLRP3 in some cancer types and inflammatory bowel diseases is critical for optimizing treatment strategies and identifying patient groups that may respond to treatment. In this regard, instead of completely suppressing the inflammasome, the discovery of modulators that restore homeostasis and targeting specific checkpoints such as NEK7 interaction are expected (He et al., 2016). High-level biomarker-based patient classification is also crucial. Identifying individuals exhibiting NLRP3 activity and combining NLRP3 inhibitors with other targeted therapies in these patients could be an important part of personalized treatment strategies in the future.

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MOLECULAR PATHOGENESIS OF POLYENDOCRINE METABOLIC OVARIAN SYNDROME: THE ROLE OF ANGIOGENESIS, OXIDATIVE STRESS, AND INFLAMMATION

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INTRODUCTION

Polyendocrine Metabolic Ovarian Syndrome (PMOS) is one of the most common endocrine disorders in women of reproductive age. It is not only a gynecological disease but also a complex syndrome with significant metabolic and systemic consequences. For many years, this disease was defined as Polycystic Ovary Syndrome (PCOS). However, in line with recent international consensus studies, the term “Polyendocrine Metabolic Ovarian Syndrome” (PMOS) has been proposed to more accurately reflect the multi-systemic endocrine and metabolic characteristics of the syndrome. Nevertheless, the term PCOS is still widely used in the literature, and in this section, both terms will be considered synonymous. PMOS, whose prevalence is reported to vary between 5–21% according to different diagnostic criteria, is a complex syndrome affecting millions of women worldwide and is considered a significant public health problem due to the reproductive, metabolic, and psychosocial burden it creates. Clinically, PMOS involves an-ovulation, menstrual irregularities,

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PMOS, reported to have a prevalence ranging from 5% to 21% according to different diagnostic criteria, is a complex syndrome affecting millions of women worldwide and considered a significant public health problem due to its reproductive, metabolic, and psychosocial burden. Clinically, PMOS is characterized by anovulation, menstrual irregularities, and infertility, and is considered responsible for a large proportion of cases of infertility due to ovulation disorders (anovulatory infertility). However, the effects of the syndrome are not limited to the reproductive system. Clinical findings such as male-pattern hair growth (hirsutism), acne, and male-pattern hair loss (androgenic alopecia) due to hyperandrogenism negatively affect the quality of life of patients; while insulin resistance and accompanying high insulin levels (hyperinsulinemia) predispose to the development of long-term metabolic complications such as type 2 diabetes, obesity, impaired blood lipid levels (dyslipidemia), hypertension, and fatty liver disease associated with metabolic dysfunction. Furthermore, it is reported that women with PMOS have an increased risk of cardiovascular disease and experience more frequent psychological problems such as anxiety, depression, and negative body image. This multifaceted clinical picture indicates that the disease should be considered not only as a hormonal disorder but also as a complex syndrome with systemic effects.

Although the etiology and pathogenesis of PMOS are not fully elucidated, it is accepted that it arises as a result of the interaction of genetic predisposition, epigenetic regulation, and environmental factors. While dysregulations in the hypothalamus-pituitary-ovarian axis (neuroendocrine axis disorders), hyperandrogenism, and insulin resistance are currently defined as the main pathophysiological components of the disease, recent findings have revealed that changes occurring in the ovarian microenvironment play a critical role in the development and progression of the disease. In particular, chronic low-grade inflammation, increased oxidative stress, and abnormal angio-

genesis processes are closely associated with impaired follicular development, decreased oocyte quality, and loss of ovarian function. Increasing evidence suggests that these three fundamental mechanisms are not independent processes; Conversely, it demonstrates the formation of a complex molecular network in which these processes mutually influence each other and support disease progression. Therefore, a better understanding of the relationship between angiogenesis, oxidative stress, and inflammation in PMOS is of great importance for elucidating the molecular pathogenesis of the disease, identifying new biomarkers, and developing mechanism-based therapeutic approaches. In this section, the molecular mechanisms involved in PMOS pathogenesis will be discussed in light of current literature, and the interactions between these processes will be evaluated in detail.

NOMENCLATIVE CHANGE: FROM PCOS TO PMOS

Historically described by Stein and Leventhal in 1935, this heterogeneous clinical condition, referred to as “Polycystic Ovary Syndrome” (PCOS), has been the focus of various debates regarding its nomenclature for many years (Escobar-Morreale, 2018; Su et al., 2025). Although the traditionally used term “polycystic” suggests the presence of pathological cysts in the ovaries, the structures observed in the disease are not true cysts, but immature antral follicles whose development has stalled (Escobar-Morreale, 2018). This naming error leads to unfounded fears and deep confusion among patients and their families, such as the possibility of cancer. Moreover, this narrow gynecological focus obscures the multi-systemic nature of the syndrome, leading physicians to perceive the disease as limited only to the reproductive organs, thus delaying diagnostic processes for years (up to 70% of patients live without a diagnosis) and fragmenting clinical management (Teede et al., 2026).

In line with these criticisms, the first institutional initiatives to change the name of the disease were raised in workshops organized by the US National Institutes of Health (NIH) in 2012 (Teede et al., 2026). However, for many years, an inclusive terminology that all stakeholders could agree on could not be developed, and the name change could not be implemented. Terms such as “functional ovarian hyperandrogenism,” which were put forward during this process, neglected the metabolic aspect of the syndrome; Alternatives such as “metabolic reproductive syndrome,” “syndrome XX,” or “cardiometabolic ovarian syndrome” have not fully encompassed phenotypes that do not exhibit androgen excess (especially phenotype D) (Escobar-Morreale, 2018). On the other hand, it has been argued that completely changing the name of the syndrome would require the re-establishment of terminology that has been known and recognized by the public and the World Health Organization (WHO) for years. To permanently overcome these obstacles, a global nomenclature consortium was established in 2023, spearheaded by the UK-based patient association Verity, Monash University, and the Androgen Excess & PCOS Society (AE-PCOS), and a comprehensive evaluation process was initiated with funding provided in September 2024 (Teede et al., 2026). Between April 2025 and January 2026, a multi-stage international consensus process was conducted, involving over ten thousand patients and approximately four thousand healthcare professionals from around the world. Scientific accuracy, clinical applicability, ease of communication, and cultural appropriateness were adopted as key criteria in this process. At the end of the process, a consensus was reached on a new terminology that more accurately reflects the endocrine and metabolic characteristics of the syndrome, which are not limited solely to ovarian findings.

As a result of the evaluations, the new terminology was created by combining the terms “Polyendocrine,” which indicates that the disease is not merely an ovarian disorder but a

multi-systemic one; “Metabolic,” which emphasizes metabolic components such as insulin resistance, compensatory hyperinsulinemia, obesity, type 2 diabetes, and cardiovascular risk, which are present in most patients; and “Ovarian,” which describes ovarian dysfunction. Thus, following an international vote in February 2026, the new name for the syndrome was adopted as Polyendocrine Metabolic Ovarian Syndrome (PMOS). The primary aim of this change is to develop terminology that more accurately reflects the pathophysiology of the disease, is more easily understood by patients and healthcare professionals, and can create a common language in clinical practice (Teede et al., 2026).

BASIC PATHOPHYSIOLOGY OF PMOS

The pathogenesis of PMOS is based on complex interactions between hormonal dysregulation, metabolic disorders, and changes in ovarian function (Lonardo et al., 2024). To understand this complex picture, it is necessary to examine the molecular interactions between the core components of the syndrome: hyperandrogenism, insulin resistance, and follicular developmental disorder.

Hyperandrogenism

Hyperandrogenism is the most prominent and diagnostically critical endocrine feature of PMOS. The primary source of androgen excess observed in the syndrome is the ovaries, specifically the theca cells of the ovaries (E Joham et al., 2022). Under physiological conditions, androgen synthesis from theca cells is controlled by the pulsatile release of hypothalamic gonadotropin (GnRH) and the resulting luteinizing hormone (LH) secreted from the pituitary gland (Lonardo et al., 2024). LH directly stimulates the expression and activity of the cytochrome P450c17 (CYP17A1) enzyme, the rate-limiting step in androgen synthesis from cholesterol in theca cells (Yuan et al., 2025). In the pathophysiology of PMOS, the ovaries show hypersen-

sitivity to LH stimulation (Sanchez-Garrido & Tena-Sempere, 2020). Theca cells isolated from PMOS patients continue to constitutively overexpress androgen biosynthesis enzymes such as CYP11A1, CYP17A1, 3 β -HSD2, and AKR1C3 (17 β -HSD5), even when cultured in vitro. This suggests a persistent increase in steroid hormone production in theca cells. This excess androgen secretion from the ovaries also disrupts feedback mechanisms at the hypothalamic level (Sanchez-Garrido & Tena-Sempere, 2020). Androgen excess reduces the suppressive effect of progesterone and estradiol on the hypothalamus, leading to more frequent GnRH secretion (Helvaci & Yildiz, 2024a). Increased GnRH secretion, in turn, shifts the hormonal balance between LH and FSH in favor of LH. Elevated LH levels create a vicious cycle that further increases androgen production in theca cells, contributing to the persistence of the disease (Sanchez-Garrido & Tena-Sempere, 2020).

Insulin Resistance

Insulin resistance and the resulting hyperinsulinemia are among the main metabolic disorders seen in 50–95% of women with PMOS and approximately 75% of non-obese patients (Zhao et al., 2023). The molecular mechanism of insulin resistance observed in PMOS, unlike obesity or type 2 diabetes, is based on post-receptor defects in insulin receptor signaling. Excessive serine phosphorylation (and decreased tyrosine phosphorylation) in insulin receptor substrate-1 (IRS-1) and the insulin receptor itself inhibit the PI3K/Akt pathway responsible for metabolic effects, thereby preventing glucose uptake (GLUT4 translocation) in target tissues (Lonardo et al., 2024). However, this insulin resistance in PMOS has a tissue-specific character. Skeletal muscle, adipose tissue, and liver are resistant to the metabolic effects of insulin, while steroid-producing ovarian and adrenal tissues retain insulin sensitivity completely (Zhao et al., 2023). Thus, increased insulin secretion to keep blood glucose levels under control can directly contribute to

the development of pathological changes in the ovaries. Insulin directly stimulates steroidogenesis and CYP17A1 activity by directly binding to its own receptors in theca cells or via insulin-like growth factor-1 (IGF-1) receptors in the ovary, and dramatically increases ovarian androgen secretion by forming a strong synergy with LH. In addition, hyperinsulinemia directly suppresses sex hormone-binding globulin (SHBG) synthesis in the liver (E Joham et al., 2022). This decrease in SHBG levels increases the proportion of biologically active free testosterone, which is not bound to proteins in the blood, further exacerbating the androgenic effect and metabolic dysfunction in the tissues (Su et al., 2025).

Follicular Development Disorder

The most typical histological and ultrasonographic feature of PMOS ovaries is the accumulation of numerous small antral follicles (2–9 mm) whose development stops in the early stages and which fail to mature into follicles capable of ovulation (E Joham et al., 2022). Underlying this follicular arrest process are hormonal and growth factor imbalances in the ovarian microenvironment (Sanchez-Garrido & Tena-Sempere, 2020). Androgen excess increases the initiation of growth in resting follicles in the early stages, while preventing these follicles from maturing normally in later stages (Stener-Victorin et al., 2020). The relatively low levels of FSH compared to LH levels (increased LH/FSH ratio) and the resistance of the follicles to FSH stimulation are another critical factor in inhibiting dominant follicle selection (Lonardo et al., 2024). The granulosa cells of these numerous small, immature antral follicles secrete 2 to 3 times more anti-Müllerian hormone (AMH) than normal. Excessively high local AMH levels further reduce the sensitivity of the follicles to FSH and block the activity of the CYP19A1 (aromatase) enzyme, which converts androgens to estrogens. Suppression of aromatase prevents the conversion of androgens to estrogens, thus perpetuating the hyperandro-

genic state in the ovarian microenvironment. Finally, local androgen excess and hyperinsulinemia lead to premature luteinization of the granulosa cells in the small follicles. With premature luteinization, the cells mature prematurely.

ANGIOGENESIS DISRUPTION IN PMOS

Angiogenesis, the budding and development of new capillaries from existing vessels, is one of the most dynamic and delicate processes cyclically repeated in ovarian physiology. In PMOS pathophysiology, imbalances of intraovarian local factors and systemic metabolic deviations lead to serious disruptions in ovarian angiogenesis mechanisms (Palomba et al., 2021). This section will detail the physiological vascularization processes in a normal ovary, the basic cellular pathways governing these processes, and how the angiogenic balance in PMOS takes on a pathological character.

Normal Ovarian Angiogenesis

In a healthy female reproductive cycle, the ovary is one of the rare tissues where regular and dynamic angiogenesis occurs. Essential reproductive processes such as follicle development, ovulation, and the formation and regression of the corpus luteum require the continuous remodeling of the ovarian vascular network (Abraham Gnanadass et al., 2021). Primordial and primary follicles do not possess their own independent vascular network; they obtain their nutrients and oxygen through passive diffusion from the stromal vessels in the ovarian cortex (Rosenfield & Ehrmann, 2016). However, as the follicle grows and progresses to the secondary and early antral stages, a follicle-specific capillary network begins to form at the outer boundary of the theca cell layer, simultaneously with the proliferation of the theca cells. Under normal conditions, this capillary network does not cross the basal membrane and reach the avascular granulosa cell layer. Granulosa cells and the oocyte obtain the oxygen and nutrients they need through diffu-

sion from this vascular network in the theca layer. Immediately following ovulation, with the breakdown of the basal membrane, these theca-derived vessels rapidly migrate towards the granulosa layer, leading to intense vascularization of the corpus luteum; this is essential for the efficient secretion of steroid hormones such as progesterone into the systemic circulation. Therefore, cyclical ovarian angiogenesis is a fundamental physiological mechanism that determines oocyte quality, follicular development rate, and consequently, reproductive potential (Parent et al., 2025; Zhang et al., 2022).

VEGF and Ovarian Vascularization

The most dominant molecular stimulant in initiating and maintaining vascularization processes in the ovarian microenvironment is the Vascular Endothelial Growth Factor (VEGF) family, particularly VEGFA (Palomba et al., 2021). In ovarian tissue, VEGFA is synthesized by granulosa cells, theca cells, and interstitial stroma. VEGFA synthesis is strongly stimulated by local gonadotropin stimulation, hypoxia, pro-inflammatory cytokines, and androgens that constitutively increase in the ovary (Rosenfield & Ehrmann, 2016). The synthesized VEGFA ligand binds to VEGFR2, a tyrosine kinase receptor located on the surface of endothelial cells, initiating angiogenic signal transduction. This binding triggers proliferation, migration, survival, and increased vascular permeability in endothelial cells through the formation of fenestrated vascular structures (Patel, 2018). This vascular density and vascularization quality in the ovary are monitored by the CD31 (PECAM-1) glycoprotein, expressed at endothelial cell-cell junctions and considered the gold standard for vascular integrity and endothelial cell density. CD31 expression levels directly reflect the surface area of the functional capillary bed and the microvascular density in the ovary. Vascularization regulated via the VEGFA/VEGFR2 pathway not only provides nourishment to the follicles but also determines the tissue stiffness of the ovarian stroma. Under normal conditions, the balanced structure of the cap-

illary network maintains stromal stiffness at an optimal level. This physical environment prevents the uncontrolled proliferation of theca, granulosa, and stroma cells by providing physiological control of the Hippo signaling pathway, which restricts cell growth; thus, it prepares the ground for the healthy selection of the dominant follicle (Rosenfield & Ehrmann, 2016).

Disruption of Angiogenic Balance in PMOS ovaries

In PMOS ovaries, angiogenic processes, which should be carried out in a physiologically delicate balance, take on a pathological character. Hyperandrogenism and increased inflammation, the main components of the syndrome, lead to a significant increase in VEGFA levels and vascular density (CD31-positive vascular area) in the ovarian tissue (Palomba et al., 2021; Stener-Victorin et al., 2024). The direct effects of androgens and the indirect effects of high insulin levels developing due to insulin resistance increase androgen production in the theca cells, leading to increased VEGFA gene expression (Rosenfield & Ehrmann, 2016). This situation causes excessive stromal vascularization (ovarian hypervascularization) in PMOS ovaries. The increase in vascular density, as indicated by CD31, leads to structural changes in the ovarian cortex, contributing to tissue thickening and hardening (Stener-Victorin et al., 2024). A thickened and vascularized ovarian cortex disrupts the regulatory effect of the Hippo signaling pathway on follicle development, leading to early follicular development arrest and the accumulation of small, immature antral follicles (Rosenfield & Ehrmann, 2016). Furthermore, pro-inflammatory cytokines leaking from this pathological vascular network impair intraovarian microcirculation, directly threatening oocyte quality and developmental competence (Zhang et al., 2022). Another critical molecular reason for the increased angiogenesis observed in PMOS is the inadequacy of anti-angiogenic defense mechanisms in parallel with the increase in pro-angio-

genic factors. In this context, Thrombospondin-1 (TSP-1), a potent endogenous inhibitor of angiogenesis, limits VEGFA effects under physiological conditions by blocking VEGFR2 activation and inducing endothelial cell apoptosis. However, in the chronic hyperandrogenic, insulin-resistant, and low-grade inflammatory ovarian microenvironment of PMOS, TSP-1 expression is suppressed or functionally insufficient (Palomba et al., 2021). The decreased inhibitory effect of TSP-1 leads to an uncontrolled increase in VEGFA/VEGFR2 signaling and a disruption of the angiogenic balance towards excessive vascularization. Increased vascularization and increased vascular permeability due to VEGFA observed in PMOS increase the risk of developing Ovarian Hyperstimulation Syndrome (OHSS), which can be life-threatening, especially in patients undergoing fertility treatments (Stener-Victorin et al., 2024). In this process, triggered by LH and hCG stimulation, excessively high VEGFA levels overstimulate VEGFR2 receptors, increasing vascular permeability. As a result, fluid within the vessels can leak into surrounding tissues and body cavities, leading to serious circulatory disorders and life-threatening clinical conditions (Patel, 2018). Therefore, disruption of angiogenic balance in PMOS is not only central to follicular development arrest and infertility pathogenesis, but also constitutes one of the most serious clinical risk factors for the syndrome.

OXIDATIVE STRESS AND INFLAMMATION IN PMOS

For many years, reproductive system disorders and metabolic irregularities in the pathophysiology of PMOS were considered independent processes. However, current molecular evidence clearly reveals that a chronic, low-grade systemic inflammation and accompanying oxidative stress underlie PMOS (Deng et al., 2024). These two processes stand out as common mechanisms that disrupt the homeostasis of the ovarian microenvironment, leading to both decreased oocyte quality and hor-

monal imbalance. This section will address the effects of mitochondrial reactive oxygen species on ovarian function, the role of the inflammatory response in these processes, and the interactions between oxidative stress and inflammation.

Reactive Oxygen Species (ROS) and Oxidative Damage

A healthy ovarian microenvironment relies on a delicate balance between ROS, which are essential for normal cellular functions and follicle development, and antioxidant defense systems that neutralize these molecules. In PMOS pathophysiology, however, the excessive increase of pro-oxidant factors and the suppression of the antioxidant capacity to clear them trigger oxidative stress in ovarian tissue. The primary source of the overproduction of reactive oxygen species such as superoxide anion and hydrogen peroxide is the loss of normal mitochondrial function (Yan et al., 2024). In PMOS granulosa cells, the level of Sirtuin 3 (SIRT3), a critical mitochondrial protein that regulates mitochondrial homeostasis, the respiratory chain, and deacetylation processes, is significantly lower (Zhang et al., 2022). SIRT3 deficiency impairs the function of the mitochondrial respiratory chain, reducing energy production efficiency and increasing mitochondrial-derived ROS production. Another critical source fueling intracellular ROS production is the pathological activation of NADPH oxidase 2 (NOX2) and NOX4 enzyme complexes (Yan et al., 2024). Excessively accumulated ROS attack cellular lipids, genetic material, and proteins, creating irreversible oxidative damage. Oxidative stress leads to lipid damage in the cell membranes of granulosa cells, increasing malondialdehyde (MDA) levels. Simultaneously, accumulation of 8-hydroxy-2'-deoxyguanosine (8-OHdG), an indicator of DNA damage in oocytes and granulosa cells, is observed. Increased 8-OHdG levels in follicular fluid directly reduce oocyte quality, disrupt mitotic spindle integrity, and significantly decrease embryo quality and transfer success rates in clinical in-vitro fertilization (IVF) stages (Yan et al., 2024). In addition to this

process, Advanced Glycation End Products (AGEs), known as “glucotoxins,” which accumulate in PMOS, completely disrupt intracellular redox balance by binding to their specific surface receptors, RAGE (Sadeghi et al., 2022). AGE-RAGE interaction increases intracellular free radical production, while inhibiting the transport of GLUT4 to the cell membrane and glucose uptake in granulosa cells, thereby increasing insulin resistance. This contributes to the deepening of impaired follicle development. In PMOS, the activation of the Keap1-Nrf2/ARE signaling pathway, which protects cells against oxidative stress, is suppressed. Inhibition of the migration of the Nrf2 transcription factor to the nucleus reduces the expression of critical antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1), leaving ovarian tissue completely vulnerable to oxidative destruction (Yan et al., 2024).

Chronic Low-Grade Inflammation

PMOS is a state of insidious immune activation characterized by systemic low-grade chronic inflammation beyond reproductive dysfunction (Su et al., 2025). This is a chronic inflammatory state that remains constantly active in the background and wears down the ovarian microenvironment, unlike acute inflammation in clinical infections. One of the main causes of inflammation in PMOS is dysfunction of the adipose tissue surrounding the internal organs, which can be seen even in women of normal weight (Lonardo et al., 2024). Hyperandrogenism leads to the growth of fat cells around the internal organs and the accumulation of immune cells such as macrophages, lymphocytes, and neutrophils in these tissues. These cells increase systemic inflammation and also affect ovarian tissue by releasing inflammatory molecules such as C-reactive protein (hs-CRP), monocyte chemoattractant protein-1 (MCP-1), and MIP-1 α (Helvacı & Yildiz, 2024a). Indeed, peripheral blood counts of patients reveal markers of chronic inflammation such as leukocytosis, an increase in the neutrophil-lymphocyte ratio

(NLR), and an increase in mean platelet volume (MPV) (Su et al., 2025). In the ovarian microenvironment, chronic inflammation manifests as abnormal infiltration of macrophages and lymphocytes into the ovarian stromal tissue and follicular fluid (Deng et al., 2024). One of the most critical external factors that keeps this local inflammation constantly active is the increased intestinal wall permeability resulting from disruption of the gut microbiota flora (Escorcia Mora et al., 2025). Bacterial lipopolysaccharides (LPS) that pass from the gut into the circulation activate TLR4 found in granulosa cells, causing inflammation to persist in the ovary. Similarly, high homocysteine levels, which are common in PMOS, contribute to the initiation and maintenance of inflammation in tissues by increasing monocyte and macrophage activation (Su et al., 2025).

NF- κ B Activation

One of the regulatory mechanisms that plays a central role in the conversion of cellular danger signals such as oxidative stress, leaking LPS from the gut, free fatty acids, and AGEs into an inflammatory response is the Nuclear Factor Kappa B (NF- κ B) signaling pathway (Lonardo et al., 2024). Under physiological conditions, NF- κ B is a transcription factor that exists in an inactive state in the cytoplasm, bound to inhibitor proteins. In PMOS, however, it is activated by the stimulation of receptors such as TLR4, RAGE, and IL-1R1, by being released from these inhibitors (Yan et al., 2024). The released and activated NF- κ B (especially the RelA/p65 subunit) rapidly migrates to the nucleus, instructing the cell to synthesize pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α). In addition, NF- κ B activation triggers the formation of an inflammatory protein complex known as the NLRP3 inflammasome within the cell, which rapidly enters the nucleus and induces the synthesis of target pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α). The accumulation and activation of the NLRP3 protein around the mitochondria in granulosa cells causes the procaspase-1 protein

to be cleaved and converted into the active caspase-1 enzyme. The activated caspase-1 activates the Gasdermin D (GSDMD) protein, initiating inflammatory cell death called pyroptosis. Molecules released from the cells during this process further strengthen the inflammatory response. Increased pyroptosis in granulosa cells leads to the cessation of follicular development (follicular arrest) and contributes to the development of fibrosis by increasing collagen accumulation in the ovarian stroma (Wei et al., 2026). Pro-inflammatory Cytokines

In PMOS, the cytokine network is altered by the dominance of molecules that increase inflammation. Three key cytokines associated with granulosa cell apoptosis, steroidogenesis disorders, and metabolic dysregulation stand out:

- **IL-1 β (Interleukin-1 Beta):** A pro-inflammatory cytokine that is one of the main regulators of inflammation, matured via caspase-1 activated by the NLRP3 inflammasome and released during pyroptosis. IL-1 β increases NF- κ B activation by stimulating IL-1R receptors in granulosa cells and ensures the continuation of the inflammatory process. High follicular IL-1 β levels suppress LH and FSH signaling pathways, inhibiting dominant follicle development (Sadeghi et al., 2022).
- **IL-6 (Interleukin-6):** This is the key cytokine that is most significantly elevated in both the serum and follicular fluid of PMOS patients and triggers CRP production from the liver. One of the important effects of IL-6 on reproductive functions is the suppression of the expression and activity of the aromatase (CYP19A1) enzyme, which is responsible for the conversion of androgens to estrogens in granulosa cells. Suppression of aromatase directly leads to the pathological accumulation of androgens in the ovarian microenvironment and thus to hyperandrogenism, the most fundamental feature of PMOS (Deng et al., 2024).
- **TNF- α (Tumor Necrosis Factor Alpha):** TNF- α , which is secreted intensely by macrophages residing in the ovary,

directly triggers apoptosis by binding to TNFRI receptors in oocytes and granulosa cells. Furthermore, it disrupts steroidogenesis in the theca and granulosa cells, leading to premature regression (luteolysis) of the corpus luteum (Deng et al., 2024). At the metabolic level, TNF- α directly blocks insulin receptor tyrosine kinase phosphorylation in skeletal muscle and adipose tissue, leading to systemic and local insulin resistance (IR) at the post-receptor level; this completely prevents the translocation of GLUT4 protein to the cell membrane (Su et al., 2025).

Interaction Between ROS and Inflammation: A Vicious Cycle Model

In PMOS pathophysiology, oxidative stress and chronic inflammation are not independent processes but form a mutually reinforcing cycle. The mechanism of this molecular vicious cycle proceeds through the following steps:

1. Increased ROS accumulation in the ovarian microenvironment directly activates the NF- κ B pathway, triggering its migration to the nucleus.
2. NF- κ B activation dramatically increases the expression and release of TNF- α , IL-6, and IL-1 β cytokines in granulosa cells and intraovarian macrophages.
3. These released cytokines bind to the receptors (TLR4/IL-1R1/TNFRI) of neighboring cells, damaging the mitochondria of the cells and stimulating the production of more ROS.
4. Hyperandrogenism, added to this cycle at the systemic level, makes immune cells overly sensitive to glucose, triggering glucose-stimulated ROS production and NOX4 activation, thus further fueling inflammation.
5. Oxidative stress stimulates serine/threonine phosphorylation on the insulin receptor and insulin receptor substrate-1 (IRS-1), inhibiting tyrosine phosphorylation necessary for healthy insulin delivery and making insulin resistance permanent.

6. The high amount of compensatory insulin secreted with increased insulin resistance stimulates LH receptors in ovarian theca cells, further increasing androgen synthesis and restarting the cycle. 7. This continuous stimulation by inflammatory cytokines and androgens completely suppresses the SIRT1/SIRT3 and Nrf2 antioxidant pathways that protect mitochondria. The loss of protective proteins disrupts the balance between division and fusion processes in mitochondria, leading to the accumulation of damaged mitochondria. These accumulated mitochondria, in turn, increase ROS production. This vicious cycle of “ROS-NF κ B-Cytokine-Mitochondrial damage,” which thickens the ovarian stromal tissue, leads to pyroptotic and apoptotic death of granulosa cells, reduces oocyte quality, and completely blocks dominant follicle development, is the most destructive axis in the molecular pathogenesis of PMOS (Zhang et al., 2022). Breaking this molecular cycle is the most critical scientific goal for developing rational treatment strategies targeting the cause, going beyond simply alleviating symptoms in the treatment of the syndrome.

INTERACTIONS OF CELLULAR MECHANISMS PLAYING A ROLE IN PMOS PATHOPHYSIOLOGY

In the ovarian microenvironment, follicular development, steroidogenesis, and ovulation processes require the perfect coordination of hormonal, angiogenic, metabolic, and immunological signaling pathways. In PMOS pathogenesis, these delicate balances are completely disrupted by the intersection of three fundamental pathological processes in ovarian tissue: impaired angiogenesis, oxidative stress, and chronic low-grade inflammation (Su et al., 2025). These three fundamental processes are not independent mechanisms but are interconnected through cellular and molecular interactions that maintain the disruptions in the ovarian microenvironment.

Effect of Oxidative Stress on Angiogenesis

Disruption of intracellular redox balance in favor of pro-oxidants and ROS accumulation play a primary role as a trigger in the pathological activation of angiogenic signaling pathways. Increased oxidative stress and ROS accumulation are key factors triggering pathological activation of angiogenic signaling pathways. Excessive ROS levels detected in granulosa cells due to mitochondrial dysfunction and SIRT3 deficiency disrupt intracellular metabolic homeostasis, mimicking or directly stimulating hypoxia-related pathways. In particular, cellular oxidative stress increases the stabilization of HIF-1 α protein at the transcriptional level. Stabilized HIF-1 α migrates to the nucleus, binds to the promoter region of the VEGFA gene, the most potent mediator of angiogenesis, and causes its overexpression (Yan et al., 2024). In addition, excessive ROS accumulation directly impairs endothelial cell integrity and microvascular function (Saadati et al., 2025). ROS-induced endothelial damage increases capillary permeability by disrupting the distribution and function of CD31 (PECAM-1) protein, which plays a crucial role in maintaining vascular stability (Rosenfield & Ehrmann, 2016). Oxidative damage suppresses the Keap1-Nrf2/ARE defense system, reducing the effectiveness of anti-angiogenic factors such as TSP-1, which controls vessel formation (Yan et al., 2024). Decreased TSP-1 leads to uncontrolled activation of the VEGFA/VEGFR2 signaling pathway, increasing abnormal neovascularization in the ovarian stroma (Rosenfield & Ehrmann, 2016).

Effect of Inflammation on Angiogenesis

Immune cells migrating to the ovarian stroma and the pro-inflammatory cytokines they secrete are local stimuli that play a significant role in increasing angiogenesis (Lonardo et al., 2024). NF- κ B transcription factor, located at the focus of chronic low-grade inflammation, when activated and translocated to the nucleus, not only increases the gene expression

of inflammatory cytokines but also directly stimulates VEGFA transcription, triggering endothelial cell proliferation and migration. IL-6 and TNF- α , released from intraovarian macrophages and granulosa cells, support pathological angiogenesis by increasing the expression of VEGFA and its receptor VEGFR2 (Yan et al., 2024). IL-6 increases vascularization in ovarian theca cells while enhancing the steroidogenic effect of LH, thereby increasing androgen synthesis. Similarly, TNF- α stimulates the expression of adhesion molecules (ICAM-1, Vcam1) in endothelial cells, triggering increased migration of leukocytes and monocytes into the ovarian microenvironment and continuously fueling angiogenic inflammation. Decreased levels of Stanniocalcin-1 (STC-1), which suppresses inflammation in PMOS ovaries, lead to weakening of protective mechanisms that limit angiogenic and inflammatory processes (Deng et al., 2024). Consequently, the pro-angiogenic microenvironment created by chronic inflammation destroys the ovarian stromal architecture by increasing intrastromal capillary density far beyond physiological limits.

MOLECULAR TARGETING THERAPY APPROACHES

Traditionally, symptomatic treatments such as oral contraceptives are widely used in the control of reproductive functions and menstrual irregularities in PMOS, but they are insufficient in targeting the underlying metabolic, inflammatory, and oxidative mechanisms of the syndrome. In recent years, data obtained at the clinical and molecular levels have shifted the direction of treatment towards cellular targets directly addressing the cause (Lonardo et al., 2024). Accordingly, therapeutic approaches targeting specific molecular pathways that will improve the intraovarian microenvironment, increase insulin sensitivity, and suppress oxidative stress and chronic low-grade inflammation have gained great importance.

Metformin

Metformin is an insulin sensitizing biguanide derivative widely used in the management of metabolic and endocrine disorders in PMOS, but it is not licensed for this indication in most countries (Zhao et al., 2023). Metformin, which enters the cell via the organic cation transporter 1 (OCT1) channel, exerts its molecular-level effects through both AMP-activated protein kinase (AMPK)-dependent and AMPK-independent mechanisms. The primary cellular target of metformin is the inhibition of mitochondrial respiratory chain Complex I; this reduces ATP production and increases cellular AMP levels, triggering direct and indirect activation of the AMPK signaling pathway and regulating glucose metabolism. In this way, it reduces glucose production in the liver, limits glucose absorption from the intestine, and improves insulin sensitivity by increasing glucose utilization in muscle and adipose tissue. At the ovarian level, it modulates FSH signaling in granulosa cells while suppressing the activity of enzymes responsible for androgen synthesis in theca cells, thereby reducing ovarian androgen production (Saadati et al., 2025). Metformin also increases insulin sensitivity in the endometrium and supports the molecular processes necessary for embryo implantation. In addition, it contributes to the alleviation of low-grade inflammation and fibrotic changes by reducing the expression of pro-inflammatory cytokines and some enzymes involved in tissue remodeling (Palomba et al., 2021). Clinical studies have shown that metformin improves insulin resistance, particularly in overweight and obese PMOS patients. However, when used in combination with agents such as GLP-1 receptor agonists, SGLT2 inhibitors, or DPP-4 inhibitors, it may provide more significant benefits on weight loss, insulin resistance, androgen levels, and menstrual regulation (Yan et al., 2024).

Antioxidants

Mitochondrial dysfunction and associated increase in ROS, particularly in granulosa cells in PMOS, contribute to de-

creased oocyte quality, impaired follicular development, and increased cell death. Therefore, antioxidant therapies aimed at reducing oxidative stress and restoring cellular redox balance are attracting attention.

- **Coenzyme Q10 (CoQ10):** CoQ10, involved in mitochondrial energy production, supports cellular energy metabolism by reducing ROS formation. In clinical studies, positive effects on follicle development, ovulation rates, and endometrial thickness have been reported, especially in clomiphene-resistant patients (Palomba et al., 2021).
- **Melatonin:** A powerful antioxidant, melatonin protects mitochondrial functions, reduces oxidative damage in granulosa cells, and limits cell death. It can also show beneficial effects on androgen levels, insulin resistance, and menstrual irregularities (Voros et al., 2026).
- **Resveratrol:** A naturally occurring polyphenol, resveratrol activates antioxidant defense systems while suppressing inflammatory signaling pathways. Experimental studies have shown it to reduce ROS production, limit inflammation, and decrease follicle loss (Wei et al., 2026).
- **N-Acetylcysteine (NAC):** A precursor to glutathione, a key intracellular antioxidant, NAC reduces oxidative stress while increasing insulin sensitivity. It has been shown to increase ovulation and pregnancy rates, particularly in clomiphene-resistant patients (Palomba et al., 2021).
- **Vitamin D3 and Selenium:** Vitamin D3 supports mitochondrial function, reducing oxidative damage, while selenium increases antioxidant capacity and suppresses the production of inflammatory cytokines. Both agents have shown positive effects on ovarian function in experimental and clinical studies (Zhao et al., 2023).

Low-grade chronic inflammation seen in PMOS negatively affects follicle development by disrupting insulin signaling pathways and increasing inflammatory processes in ovarian

tissue. Therefore, therapies targeting inflammation are considered a potential approach in the management of the disease (Deng et al., 2024).

- **Salicylates (Salsalate):** Salsalate reduces the inflammatory response by suppressing the NF- κ B signaling pathway, one of the key regulators of inflammation. Studies have shown that it can improve insulin sensitivity, reduce androgen production, and support ovulation (Yan et al., 2024).
- **Anti-TNF- α Treatments:** TNF- α inhibitors such as etanercept have reduced inflammation in ovarian tissue, lowered androgen levels, and partially improved follicle development disorders in experimental studies (Deng et al., 2024; Yuan et al., 2025).
- **Natural Anti-inflammatory Nutraceuticals:** Curcumin suppresses inflammatory signaling pathways, reducing the production of cytokines such as TNF- α and IL-6, alleviating insulin resistance, and potentially having positive effects on metabolic parameters. Some studies have reported that the combination of curcumin and metformin is more effective than single treatments in terms of lipid profile and hormonal balance (Yan et al., 2024).
- **Omega-3 Fatty Acids:** Omega-3 fatty acids, including EPA and DHA, reduce inflammation in adipose tissue, lower systemic inflammation markers, and support insulin sensitivity (Melo et al., 2022).

NEW TREATMENT TARGETS

Due to the limitations of traditional treatments, the effects of novel therapeutic agents used in the treatment of diabetes and obesity on PMOS are increasingly being investigated.

- **GLP-1 Receptor Agonists (Liraglutide, Semaglutide):** These drugs support insulin secretion, reduce appetite, and provide significant weight loss. Studies show that they can reduce insulin resistance, visceral fat, and androgen levels, especially in obese PMOS patients. When used in combination with metformin,

their metabolic benefits can be further enhanced (Norman et al., 2007; Rosenfield & Ehrmann, 2016).

- **SGLT2 Inhibitors (Empagliflozin, Canagliflozin):** They improve blood sugar control by reducing glucose reabsorption from the kidneys and alleviate insulin resistance. They can also support weight loss and reduce inflammation. Clinical studies show that these drugs can have positive effects on metabolic parameters and menstrual regularity (Li et al., 2019; Zhao et al., 2023).
- **DPP-4 Inhibitors (Sitagliptin, Saxagliptin):** These agents, which prolong the effect of incretin hormones, can improve glucose metabolism and contribute to the regulation of menstruation, especially when used in combination with metformin (Saadati et al., 2025).
- **Ferroptosis and the SIRT1-Nrf2-GPX4 Axis:** In recent years, ferroptosis, a form of cell death characterized by iron-dependent lipid peroxidation, has become an important area of research in PMOS. Supporting the SIRT1-Nrf2-GPX4 signaling pathway, which plays a role in this process, is considered a promising approach in terms of reducing oxidative damage and protecting granulosa cells. Furthermore, GPX4 levels, lipid peroxidation products, and markers associated with iron metabolism are among the biomarker candidates that could be used in the future to predict oocyte quality and the success of assisted reproductive techniques (Voros et al., 2026).

CONCLUSION

While PMOS was previously considered only as an ovarian dysfunction, it is now recognized as a systemic endocrine and metabolic disease extending beyond the reproductive system. Renaming the syndrome as “Polyendocrine Metabolic Ovarian Syndrome” (PMOS) more accurately reflects the multifaceted nature of the disease, which is related to the neuroendocrine, metabolic, and reproductive systems. PMOS is not merely a

hormonal disorder; it is a complex clinical picture associated with long-term health risks such as cardiometabolic diseases, type 2 diabetes, metabolic dysfunction-related fatty liver disease (MASLD), and endometrial cancer. The information presented in this section demonstrates that angiogenesis disorders, oxidative stress, and chronic inflammation are not independent processes in the pathogenesis of PMOS. Increased ROS production due to mitochondrial dysfunction activates inflammatory signaling pathways, increasing cytokine production; the resulting inflammatory environment promotes hyperandrogenism and abnormal blood vessel formation. Together, these processes disrupt the ovarian microenvironment, leading to a cessation of follicle development, decreased oocyte quality, and ovulation disorders. A better understanding of these mechanisms at the molecular level offers new opportunities in the diagnosis and treatment of PMOS. Inflammatory cytokines, oxidative stress markers, and ferroptosis-related molecules are among the potential biomarkers that could be used in the future to assess disease activity and reproductive potential. Similarly, new therapeutic approaches targeting oxidative stress, inflammation, and cellular energy metabolism aim not only to control symptoms but also to correct the underlying mechanisms of the disease. In conclusion, elucidating the molecular links established between angiogenesis, oxidative stress, and inflammation in the pathophysiology of PMOS remains an important area of research, both for the development of new biomarkers and for the creation of personalized treatment strategies.

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THE ROLE AND IMPORTANCE OF NANOPARTICULARS IN CANCER TREATMENT

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INTRODUCTION

Cancer is known as one of the most dangerous diseases in the world, causing millions of deaths every year. Current cancer treatments include chemotherapy, radiotherapy, and surgical interventions, but these drugs often kill healthy cells and cause toxicity in patients. In cancer treatment, nanoparticles are one of the innovative drug delivery systems that increase treatment success and reduce drug side effects by enabling more effective delivery of drugs to the target tissue. Different nanodevice systems such as liposomes, polymeric nanoparticles, dendrimers, gold nanoparticles, and magnetic nanoparticles are used in various cancer treatment approaches such as chemotherapy and immunotherapy.

The use of nanoparticle-based treatment systems holds promise for the development of safer, more effective, and personalized cancer treatments in the future.

2. Basic Properties of Nanoparticles

Nanoparticles are microscopic particles with dimensions of 100 nm and smaller, forming the basis of nanotechnology. The remarkable properties of nanometer-sized structures and

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their potential for future use were first brought to attention by Faraday in 1957 (Altammar, 2023). The concept of nanometer, used to define dimensions at the nanoscale, was first used by Richard Zsigmondy in 1925. Zsigmondy observed the structural properties of gold nanoparticles using an ultra-microscope he developed to study the heterogeneous structure and properties of colloidal solutions, and was awarded the Nobel Prize for this work (Li et al., 2014). Thanks to these properties, nanoparticles are used in many fields such as medicine, biotechnology, and environmental science.

2.1. Classification of Nanoparticles

Nanoparticles used in cancer treatment are generally divided into three main groups according to their structural properties and composition: organic, inorganic, and hybrid nanoparticles. Each nanoparticle group; They possess different advantages and characteristics in terms of features such as drug delivery capacity, biocompatibility, targeting ability, and imaging (Patra et al., 2018; Rodrigues et al., 2025).

2.1.1. Organic Nanoparticles

Organic nanoparticles are particles containing carbon-based natural or synthetic organic molecules with dimensions less than 100 nm. This group includes various carrier systems such as liposomes, polymeric nanoparticles, dendrimers, micelles, solid lipid nanoparticles, nanostructured lipid carriers, protein nanoparticles, and polysaccharide-based nanoparticles. Organic nanoparticles have an important place in targeted therapy applications due to their properties such as biocompatibility, controlled drug release, long circulation time, and the ease with which their surfaces can be functionalized and degraded (Mehnert and Mäder, 2001; Xie et al., 2023).

2.1.2. Inorganic Nanoparticles

Inorganic nanoparticles are small, carbon-free structures with high stability, unique optical properties, hydrophilic,

non-toxic characteristics, and biocompatibility with living systems. Their stability, magnetic properties, optical behavior, and surface reactivities are superior to those of organic nanoparticles. This group includes gold nanoparticles, silver nanoparticles, iron oxide nanoparticles, silica nanoparticles, titanium dioxide, zinc oxide nanoparticles, and quantum dots (Alshammari et al., 2023; Parra-Nieto et al., 2021).

2.1.3. Hybrid Nanoparticles

Hybrid nanoparticles are nanocarriers developed to combine the advantages of individual nanocarriers, such as high biocompatibility and multiple drug loading, into a single system (Rajana et al., 2022; Wang et al., 2014). Hybrid nanoparticle systems developed today include lipid-polymer hybrid nanoparticles, core-shell nanoparticles, magnetic-polymer hybrid nanoparticles, and organic-inorganic composite nanoparticles (He et al., 2015).

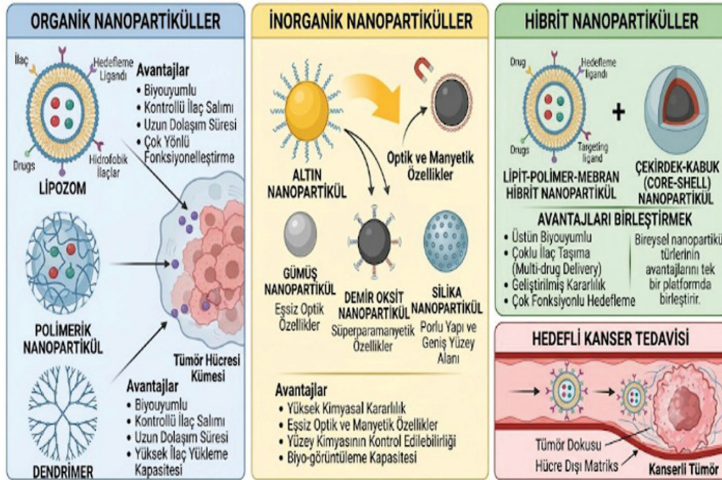


Figure 1. Advantages of organic, inorganic, and hybrid nanoparticle classes used in cancer treatment and their applications in targeted tumor therapy. (Image prepared using BioRender.com.)

3. Advantages of Nanoparticles in Cancer Treatment

Advances in nanotechnology have presented significant opportunities for the controlled delivery of drugs used in cancer treatment to the target area, increasing their effectiveness, and reducing treatment-related side effects (Luo et al., 2010; Zhang et al., 2012). Traditional treatment methods can cause serious systemic toxicity because they cannot demonstrate sufficient selectivity between tumor cells and healthy cells. Therefore, the properties of these systemic carriers provide an advantage.

3.1. Targeted Drug Delivery

One of the most important advantages of nanoparticles is their ability to selectively deliver anticancer drugs to tumor tissue. This allows the drugs used to concentrate selectively in the tumor region, increasing treatment effectiveness while reducing toxicity.

Nanoparticles reach tumor tissue through two main mechanisms: passive targeting and active targeting. Passive targeting occurs due to the enhanced permeability and retention (EPR) effect resulting from increased permeability of tumor vessels and insufficient lymphatic drainage. In active targeting, antibodies, peptides, aptamers, or various ligands are attached to the surface of the nanoparticle, enabling specific binding to cancer cells that overexpress certain receptors (Kalyane et al., 2019).

Nanoparticles can be delivered to cancer cells through two main mechanisms: passive and active targeting. In passive targeting, newly formed vessels in tumor cells have an irregular structure with large spaces between endothelial cells. In addition, lymphatic drainage is insufficient, leading to accumulation in the tumor region. This situation is described as the “enhanced permeability and retention (EPR) effect”. In active targeting, ligands that can recognize tumor cell-specific biomarkers are attached to the surface of the nanoparticles, binding to tumor cells. Thus, while the intracellular concentration of the

drug increases, toxic effects that may occur in healthy cells can be significantly reduced, contributing to increased treatment efficacy and a reduction in chemotherapy-related side effects (Lamprecht et al., 2001; Singh and Lillard, 2009).

3.2. Controlled Drug Release and Pharmacokinetic Improvement

Another advantage of nanoparticle systems is that they enable the controlled and sustainable release of anticancer drugs. While anticancer drugs used in traditional drug applications enter the bloodstream quickly, are rapidly metabolized, and eliminated from the body, nanoparticles release the drug in a controlled manner over a certain period, contributing to the maintenance of therapeutic drug concentration for a longer time and reducing the need for frequent dosing (Guzman et al., 1996).

3.3. Reduction of Systemic Toxicity

Nanoparticle drug delivery systems enable the selective accumulation of anticancer drugs used in treatment within the tumor microenvironment, while significantly reducing drug exposure in healthy tissues. Thus, while a large portion of the drug exerts its effect in the target region, toxic effects and side effects that may occur in normal tissues are minimized (Xuan et al., 2023).

4. Clinical Applications and Current Developments

In cancer treatment, nanoparticle-based drug delivery systems are among the nanotechnological approaches that are no longer limited to experimental research but are also beginning to be used in clinical applications. Lipid, polymer, and protein-based nanoparticles, in particular, are of great clinical importance due to their properties of increasing the solubility of anticancer drugs, ensuring long-term retention in circulation, supporting accumulation in tumor tissue, and reducing systemic toxicity (Shi et al., 2017).

One of the first nanoparticle-based cancer drugs to reach clinical application and receive clinical use approval is the liposomal formulation of doxorubicin. This system allows the drug to remain in the tumor microenvironment for a longer period and show fewer side effects compared to free doxorubicin (Barenholz, 2012). Another example is nab-paclitaxel, an albumin-bound nanoparticle form of paclitaxel, which has found clinical application in various cancer types such as breast, pancreatic, and lung cancers that do not require standard solvents (Gradishar, 2006).

In recent years, nanoparticle technologies have not only been limited to the delivery of chemotherapeutic agents, a traditional treatment method, but have also been used in next-generation treatment approaches such as immunotherapy and gene therapy. Lipid nanoparticles, in particular, have played a significant role in the development of RNA-based therapies by enabling the transport of nucleic acids into cells, thus entering clinical applications (Dilliard et al., 2021; Mitchell et al., 2021).

In short, the increasing use of nanoparticle-based therapies in the clinical field has led to the development of new delivery systems tailored to various tumor types and individual patients. Targeted nanoparticle systems, in particular, aim to selectively deliver drugs directly to diseased tissue by enabling the recognition of tumor cell-specific biomarkers, thus reducing unwanted effects in healthy tissues (Hrkach et al., 2012; Wang et al., 2010). All these developments highlight the importance of nanoparticle technologies in developing targeted, selective, safe, and patient-specific cancer therapies.

CONCLUSION AND RECOMMENDATIONS

In conclusion, advances in nanoparticle technologies offer numerous advantages such as targeted drug delivery, controlled release, increased bioavailability, overcoming microenvironmental barriers, reduced systemic toxicity, overcoming

drug resistance, enabling combination therapies, and developing patient-specific treatment strategies. However, to expand the clinical use of these systems in cancer treatment, not only therapeutic efficacy but also factors such as long-term safety, pharmacokinetic effects, standardization of production processes, and cost must be considered. Furthermore, to translate promising results from laboratory and preclinical studies into clinical applications, the development of nanoparticles specific to different tumor types and patients, the optimization of their targeting mechanisms, and the demonstration of their efficacy and safety through extensive clinical trials will contribute to their establishment as future cancer treatment options.

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FASN IN CANCER METABOLISM AND CELL DEATH MECHANISMS

Ayşe Nur COŞKUN¹

INTRODUCTION

Cancer is not merely a group of diseases characterized by uncontrolled cell proliferation and genetic alterations; it is also a complex biological process involving extensive reprogramming of cellular metabolism. Tumor cells undergo substantial alterations in multiple metabolic pathways, particularly glucose, amino acid, and lipid metabolism, in order to meet the energy and biosynthetic demands required for rapid proliferation (Martin-Perez et al., 2022). This metabolic reprogramming not only supports biomass production in cancer cells but also contributes to the regulation of processes such as adaptation to oxidative stress, cellular signaling, maintenance of membrane integrity, and evasion of cell death. Therefore, cancer metabolism is now recognized as a fundamental component of tumor biology and an important area of research for the identification of novel therapeutic targets (You et al., 2023).

Among these metabolic alterations, increased de novo fatty acid synthesis, also known as de novo lipogenesis (DNL), is a prominent feature observed in numerous cancer types. While extracellularly derived fatty acids represent an important source in normal cells, cancer cells may develop an increased dependence on endogenous fatty acid synthesis to meet their proliferative demands. In this process, fatty acid synthase (FASN) occupies a central position in the synthesis of long-chain fat-

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ty acids from metabolic intermediates such as acetyl-CoA and malonyl-CoA. Increased FASN expression has been reported in breast, colorectal, prostate, ovarian, lung, pancreatic, and several other cancers, while elevated FASN levels have been associated with aggressive biological characteristics of tumors (Vanauberg et al., 2023).

The role of FASN in cancer cells is not limited to sustaining lipid biosynthesis. Fatty acid synthesis mediated by FASN contributes to the biosynthesis of cellular membranes and membrane-associated structures, thereby supporting the maintenance of proliferative capacity. In addition, FASN activity has been shown to interact with mTOR, HER2, and other oncogenic signaling networks and may contribute to the regulation of fundamental cancer-related processes, including cell proliferation, invasion, metastasis, angiogenesis, and therapeutic resistance. These multifaceted functions indicate that FASN should be considered not merely a metabolic enzyme but also an important metabolic regulator capable of influencing the biological behavior of tumor cells (Menendez and Lupu, 2007).

In contrast to the ability of increased FASN activity to support cancer cell survival, inhibition of this enzyme can disrupt cellular metabolic homeostasis and induce various stress responses. Pharmacological or genetic inhibition of FASN has been shown not only to reduce lipid biosynthesis but also to contribute to the accumulation of malonyl-CoA and other metabolic intermediates, disruption of redox homeostasis, increased levels of reactive oxygen species, and the development of mitochondrial stress. These alterations have been associated with outcomes such as cell-cycle arrest and programmed cell death (Buckley et al., 2017).

In particular, the increasingly detailed characterization of the relationship between FASN and cellular death mechanisms in recent years has renewed interest in the therapeutic potential of this enzyme in cancer treatment. FASN inhibition has

been reported to trigger the intrinsic apoptotic pathway in some models, characterized by disruption of mitochondrial membrane integrity, accumulation of reactive oxygen species, cytochrome c release, and caspase activation. In addition, interactions between FASN and BCL-2 family proteins have been proposed to play a role in regulating the mitochondrial “priming” state, which determines the susceptibility of tumor cells to apoptotic stimuli. Thus, FASN may be regarded as more than a single enzyme within a metabolic pathway; rather, it represents one of the key points of connection between metabolic status and cell fate (Flavin et al., 2010; Menendez et al., 2024).

These characteristics of FASN have accelerated research aimed at therapeutically targeting the enzyme. FASN inhibitors with distinct pharmacological properties, including cerulenin, C75, and orlistat, have been shown in preclinical models to suppress tumor cell proliferation and increase cell death (Benjamin et al., 2015). However, early-generation FASN inhibitors had several limitations, including insufficient selectivity, unfavorable pharmacokinetic properties, and toxicity, which represented important barriers to their clinical translation. The development of more selective FASN inhibitors has subsequently renewed momentum in this field. In particular, inhibitors such as denifanstat (TVB-2640), which have reached clinical development, have strengthened the translational potential of FASN targeting (Menendez et al., 2024). Current studies also suggest that combining FASN inhibition with chemotherapeutic and targeted therapies may enhance treatment responses (Zhao et al., 2013; Li et al., 2025).

In this context, FASN targeting has emerged as a promising approach for suppressing cancer cell survival by directly targeting the metabolic dependencies of tumor cells. However, the response to FASN inhibition is influenced by numerous factors, including tumor type, FASN expression levels, metabolic flexibility, redox capacity, and the activation status of cellular death pathways, highlighting the need for a more detailed

investigation of this approach at the molecular level. In particular, elucidating the relationships among FASN inhibition, metabolic stress, AMPK activation, oxidative stress, mitochondrial apoptotic signaling, and response to chemotherapy is important for determining the potential of FASN as a target in precision cancer therapy.

This chapter will address the biological role of FASN within the reprogrammed lipid metabolism of cancer cells. The structure and function of FASN, its regulation in cancer cells, the metabolic and molecular consequences of FASN inhibition, and its relationship with cellular death mechanisms will be examined. In addition, the potential of FASN-targeted approaches for combination with existing therapies and recent developments in clinical applications will be evaluated. Finally, the position of FASN in cancer metabolism will be discussed in terms of its role as both a metabolic target and a regulator of cellular death.

Reprogramming of Lipid Metabolism in Cancer Metabolism

The rapid and uncontrolled proliferation of cancer cells requires not only increased energy production but also the continuous synthesis of biomolecules necessary for the generation of new cells. Therefore, tumor cells reprogram lipid metabolism in addition to glucose and amino acid metabolism (Jiang et al., 2025). In particular, *de novo* lipogenesis is considered an important metabolic adaptation that enables cancer cells to sustain their proliferative capacity. Recent studies have shown that these alterations in lipid metabolism not only support membrane biosynthesis but also influence redox homeostasis, cellular signaling, treatment resistance, and susceptibility to cell death (Huang and Freter, 2015).

In a substantial proportion of normal adult tissues, fatty acids are largely obtained from the circulation, whereas endogenous fatty acid synthesis is markedly activated in many cancer cells. ATP citrate lyase (ACLY), acetyl-CoA carboxylase

(ACC), and fatty acid synthase (FASN) function as key enzymes in this process. While ACC catalyzes the conversion of cytoplasmic acetyl-CoA into malonyl-CoA, FASN utilizes acetyl-CoA and malonyl-CoA to synthesize palmitate as its major end product. Thus, FASN serves as a central enzyme that catalyzes the final, multistep reactions of *de novo* fatty acid synthesis (Zaidi et al., 2012).

Increased lipid synthesis in cancer cells is particularly important for the formation of new membranes during rapid proliferation. In addition, alterations in the composition of phospholipids and other lipid species may affect membrane fluidity, the organization of receptors within the membrane, and signal transduction. Therefore, the reprogramming of lipid metabolism is not merely a passive adaptation that meets the energy requirements of cancer cells but rather an active component of the tumor phenotype.

FASN Structure and Its Role in *De Novo* Fatty Acid Synthesis

Fatty acid synthase (FASN) is a key enzyme involved in *de novo* fatty acid synthesis in human cells. Human FASN is a homodimeric, multifunctional enzyme complex with a molecular mass of approximately 270 kDa. Its structure contains distinct catalytic activities, including acetyl/malonyl transferase, β -ketoacyl synthase, β -ketoacyl reductase, dehydratase, enoyl reductase, acyl carrier protein, and thioesterase activities (Fhu and Ali, 2020). This organization enables the sequential reactions of fatty acid synthesis to occur within a single molecular complex.

In FASN-mediated fatty acid synthesis, acetyl-CoA serves as the initial substrate, whereas malonyl-CoA provides the carbon units required for chain elongation. NADPH functions as the electron donor in the reductive steps of the reaction. Palmitate, the primary end product of the synthesis process, is subsequently utilized in the synthesis of various fatty acids, phospholipids, and other lipid molecules (Sethi et al., 2025).

Therefore, FASN activity represents an important metabolic control point in directing cellular carbon flux toward lipid biosynthesis. In cancer cells, increased FASN expression is considered in association with enhanced de novo lipogenesis and is linked to the metabolic requirements of tumor cells. The overexpression of FASN in numerous tumor types has consequently made this enzyme an important target in cancer metabolism.

Regulation of FASN Activity in Cancer Cells

FASN expression and activity are closely associated with the cellular energy status, growth signals, and nutrient availability. Therefore, FASN represents an important point of convergence between metabolic signals and oncogenic signaling pathways (Menendez and Lupu, 2007).

SREBP-1 and FASN

Sterol regulatory element-binding protein 1 (SREBP-1) is one of the major transcriptional regulators of lipid biosynthesis. Activation of SREBP-1 can increase the expression of enzymes involved in lipogenesis, particularly FASN, as well as ATP citrate lyase (ACLY) and acetyl-CoA carboxylase (ACC) (Eberlé et al., 2004).

In particular, activation of the PI3K/AKT/mTOR axis can support SREBP-1-mediated lipogenic programs, thereby contributing to increased lipid synthesis in cancer cells (Zhao et al., 2022). Thus, the PI3K/AKT/mTOR → SREBP-1 → FASN axis is considered one of the important links between oncogenic signaling and metabolic reprogramming (Su and Koeberle, 2024).

AMPK and Cellular Energy Homeostasis

AMP-activated protein kinase (AMPK) is one of the major metabolic regulators responsible for sensing the cellular energy status (Hardie et al., 2006). Under conditions of energy deficiency, AMPK activation generally contributes to the suppres-

sion of ATP-consuming anabolic processes while promoting energy-generating catabolic pathways.

With respect to lipid metabolism, AMPK can reduce fatty acid synthesis by inhibiting ACC activity. However, the role of AMPK in cancer cells is highly complex. Under certain conditions, AMPK may exert tumor-suppressive effects, whereas under conditions of energy limitation, it may also contribute to the ability of tumor cells to cope with metabolic stress and maintain cell survival (Keerthana et al., 2023). Therefore, the relationship between FASN and AMPK should be evaluated in the context of tumor type and metabolic conditions.

Interaction Between Oncogenic Signaling Pathways and FASN

FASN activity has been shown to be regulated not only by metabolic requirements but also by growth factor receptors and oncogenic signaling pathways (Zhang et al., 2021). Signals transmitted through EGFR, HER2, PI3K/AKT/mTOR, and various steroid hormone receptors may contribute to the activation of the lipid biosynthesis program (Wan et al., 2025).

This observation supports the consideration of FASN not merely as a metabolic enzyme in cancer cells, but also as a functional link between oncogenic signaling and metabolic adaptation.

Molecular Consequences of FASN Targeting in Cancer Cells

Suppression of FASN activity can induce a series of metabolic and molecular alterations by reducing the lipid biosynthetic capacity of cancer cells. These changes primarily arise from decreased production of newly synthesized lipids required for proliferation and disturbances in the levels of intermediates involved in lipid metabolism.

Inhibition of FASN activity reduces de novo lipogenesis and consequently affects the cellular levels of palmitate and other fatty acids. This disruption of lipid biosynthesis may alter membrane lipid composition and cellular lipid homeostasis, thereby leading to increased metabolic and oxidative stress. Elevated cellular stress may ultimately suppress proliferation and promote cell death through the activation of cell-cycle checkpoints and apoptotic signaling pathways (Ventura et al., 2015; Menendez and Lupu, 2007) (Figure 1).

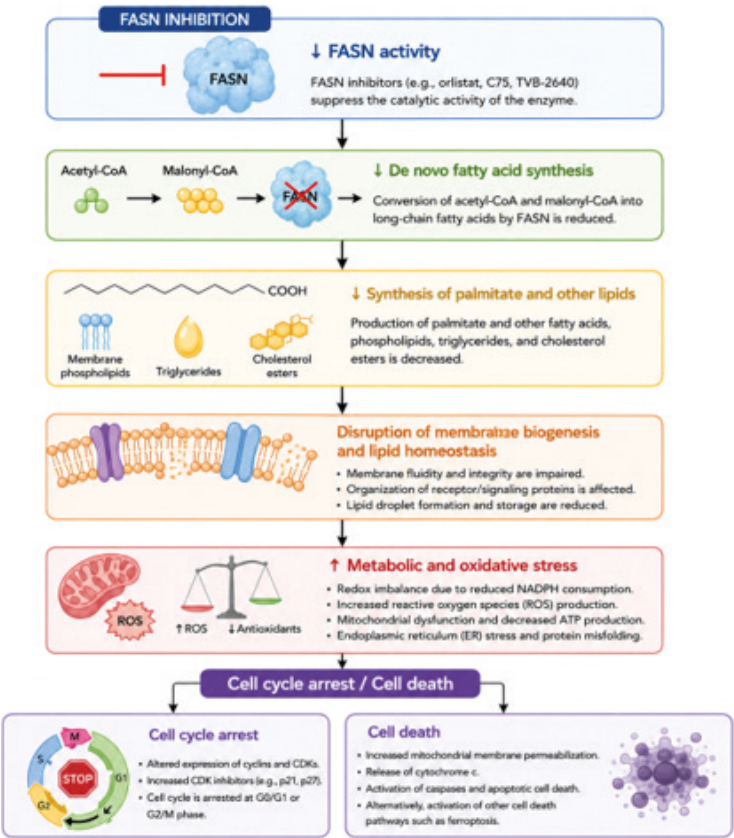


Figure 1. Proposed effects of FASN inhibition on lipid metabolism and cell fate in cancer cells. Inhibition of FASN reduces de novo fatty acid synthesis and the production of palmitate and other lipids, leading to impaired membrane biogenesis and disrupted lipid homeostasis. The resulting metabolic and oxidative stress contributes to cell cycle arrest and activation of cell death pathways.

Figure 1. Potential effects of FASN inhibition on lipid metabolism and cellular death in cancer cells.

Suppression of FASN activity reduces *de novo* fatty acid synthesis and palmitate production, leading to disruptions in membrane biosynthesis and lipid homeostasis. The accompanying increase in metabolic and oxidative stress may contribute to the emergence of various cellular responses, particularly cell-cycle arrest and apoptotic cell death.

The ability of FASN inhibition to suppress cell-cycle progression and enhance apoptotic cell death has been demonstrated in different cancer models (Lupu and Menendez, 2006). However, the resulting cellular response may vary depending on the characteristics of the inhibitor used, the tumor type, and the metabolic flexibility of the cell (Martinez-Outschoorn et al., 2017).

FASN Inhibition and Apoptotic Cell Death

Apoptosis is one of the fundamental mechanisms of programmed cell death that enables the controlled elimination of cells. Cancer cells may gain a survival advantage by suppressing apoptotic signals. Therefore, targeting the metabolic dependencies of tumor cells represents an important strategy that may help reset the apoptotic threshold (Elmore, 2007).

FASN inhibition has been reported to affect mitochondrial function and enhance apoptotic signaling. Disruption of lipid homeostasis, oxidative stress, and alterations in mitochondrial membrane integrity may contribute to the activation of the intrinsic apoptotic pathway (Schroeder et al., 2021).

In this process, BCL-2 family proteins play a critical role in regulating mitochondrial membrane permeability. A shift in the balance between pro-apoptotic and anti-apoptotic proteins toward a pro-apoptotic state may facilitate cytochrome c release and subsequent activation of the caspase cascade (Tsujimoto, 2003).

Recent studies suggest that FASN targeting may particularly affect the mitochondrial apoptotic threshold and that combin-

ing FASN inhibitors with agents targeting apoptotic pathways, such as BH3 mimetics, warrants further investigation. Recent experimental evidence from pancreatic ductal adenocarcinoma models indicating that FASN inhibition may increase susceptibility to mitochondrial apoptosis supports this approach.

FASN Targeting and Oxidative Stress

There is a strong link between lipid metabolism and cellular redox homeostasis (Aon et al., 2014). Suppression of FASN activity may alter metabolic balance in cancer cells and contribute to the accumulation of reactive oxygen species.

Because cancer cells may already be under substantial metabolic stress due to rapid proliferation, inhibition of lipid biosynthesis may further limit their capacity to cope with stress. Increased oxidative stress can cause damage to DNA, proteins, and membrane lipids, thereby enhancing cellular death signals.

However, the relationship between FASN inhibition and oxidative stress is neither simple nor unidirectional. The antioxidant capacity of cancer cells, NADPH metabolism, and their ability to utilize alternative lipid sources may substantially determine their response to FASN inhibition. Therefore, metabolic flexibility is considered an important factor in determining sensitivity to FASN-targeted therapies.

The Relationship Between FASN and Ferroptosis

The relationship between FASN and cell death is not limited to apoptosis. In recent years, the role of lipid metabolism in iron-dependent cell death mechanisms such as ferroptosis, which is characterized by lipid peroxidation, has been increasingly investigated.

Ferroptosis is fundamentally driven by the oxidation of membrane lipids and an insufficient cellular capacity to defend against lipid peroxidation. Because fatty acids synthesized by FASN can influence the lipid composition of cell membranes and, consequently, their susceptibility to oxidative damage, a

functional relationship between FASN activity and ferroptotic cell death has been proposed (Qian et al., 2025).

Recent studies suggest that FASN may link lipid remodeling, ferroptosis evasion, and therapeutic resistance. Therefore, FASN targeting is important not only for suppressing lipid synthesis but also for altering the susceptibility of cancer cells to specific cell death pathways.

FASN Targeting and Chemotherapy Response

One of the most notable aspects of FASN targeting in cancer therapy is the potential of metabolic inhibition to alter responses to existing treatments. Cancer cells can adapt to the stress conditions induced by chemotherapy, targeted therapies, or radiotherapy by reprogramming their metabolic pathways. Therefore, targeting metabolic dependencies may provide a complementary approach for overcoming treatment resistance.

In addition to limiting lipid synthesis and thereby reducing the proliferative capacity of cancer cells, FASN inhibition is thought to increase their susceptibility to apoptotic signals. Recent reviews have reported that FASN blockade supports the development of combination strategies that may enhance the efficacy of chemotherapeutic and targeted agents.

From this perspective, the combined use of FASN inhibitors and chemotherapeutic agents represents an important example of the “metabolic targeting + conventional cytotoxic therapy” approach. However, the efficacy of these combinations depends on tumor type, inhibitor selectivity, dosage, and the ability of tumor cells to utilize alternative metabolic pathways.

FASN Inhibitors

Early studies targeting FASN began with the use of natural or semisynthetic inhibitors. Compounds such as cerulenin and C75 have been shown to suppress FASN activity, resulting in reduced proliferation and increased cell death in cancer cells. However, the pharmacological properties and specificity

of these early inhibitors have been important factors limiting their clinical application.

Orlistat has also been investigated as a compound capable of targeting, particularly, the thioesterase activity of FASN. The anticancer effects of orlistat have been examined in different cancer cell models, and metabolic alterations associated with FASN inhibition have been reported to affect cell proliferation and survival (Kridel et al., 2004). Nevertheless, the systemic bioavailability of orlistat and its potential FASN-independent effects should be taken into consideration in terms of clinical translation.

The development of more selective FASN inhibitors has represented an important advance in the evaluation of FASN as a therapeutic target. Denifanstat (TVB-2640), which targets the ketoacyl reductase activity of FASN, is one of the leading examples of selective FASN inhibitors that have reached clinical development. Although FASN inhibitors, particularly denifanstat, have been evaluated in early-phase clinical studies across several solid tumors, as of 2025, no agent directly targeting FASN has received FDA approval for cancer treatment. Identifying appropriate patient populations, developing predictive biomarkers, and accounting for metabolic heterogeneity among tumors remain important areas of research for improving the clinical success of FASN-targeted therapies (Huang et al., 2025).

Current Approaches and Future Perspectives in FASN-Targeted Therapies

The involvement of FASN in multiple metabolic and signaling pathways makes this enzyme an important candidate not only for single-agent targeting but also for combination therapies. Current research is investigating the use of FASN inhibition in combination with chemotherapy, targeted therapies, apoptotic pathway modulators, and immunotherapies (Flavin et al., 2010).

However, the metabolic flexibility of cancer cells represents one of the major challenges associated with FASN inhibition. Tumor cells may compensate for FASN inhibition by increasing lipid uptake from the extracellular environment or activating alternative metabolic pathways. Therefore, the future success of FASN-targeted therapies may depend not only on determining FASN expression levels but also on accurately characterizing the metabolic dependencies and lipid utilization patterns of individual tumors.

In this context, the combined assessment of FASN expression or activity, lipid metabolic profiles, metabolic dependencies, and molecular markers associated with cell death mechanisms may become increasingly important for personalized therapy. Current literature also indicates that biomarker-based patient selection and metabolic profiling may be critical for future clinical studies of FASN targeting.

Conclusion

FASN represents an important link between increased *de novo* lipogenesis and cellular proliferation, metabolic adaptation, and cell death mechanisms in cancer cells. Increased FASN activity contributes to the provision of lipids required for rapid proliferation, whereas inhibition of the enzyme can disrupt lipid homeostasis, leading to metabolic stress, oxidative stress, cell cycle arrest, and activation of different cell death pathways.

Therefore, the role of FASN in cancer metabolism should not be evaluated solely in terms of fatty acid synthesis but should instead be considered in conjunction with metabolic reprogramming, mitochondrial function, the apoptotic threshold, ferroptosis, and therapeutic resistance. Although combining FASN inhibitors with chemotherapeutic and targeted agents represents a promising approach for therapeutically exploiting metabolic dependencies, further mechanistic and clinical stud-

ies are required because of tumor heterogeneity and metabolic flexibility.

Within this framework, FASN can be considered one of the key intersection points between the metabolic requirements and cellular fate of cancer cells and remains a promising target for the development of biomarker-driven, combination-based metabolic therapeutic strategies in the future.

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EFFECTS OF ALLANTOLACTONE ON IMMUNE CELL PRIMING IN DRAINED LYMPH NODES IN A MULTIPLE SCLEROSIS MODEL

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1. Introduction

Multiple sclerosis (MS) is a chronic, immune-mediated disease of the central nervous system (CNS) and one of the major causes of neurological disability in young and middle-aged adults (Guan, S. et. al., 2026). The disease is characterized by a complex pathological process involving inflammatory demyelination, axonal injury, oligodendrocyte dysfunction, and, particularly during progressive stages, neurodegenerative changes (Boutitah B. et al., 2025). Although the major pathological consequences of MS occur within the CNS, the initiation and regulation of autoimmune responses involve coordinated events in peripheral immune organs. Consequently, MS is increasingly considered a disease in which interactions between peripheral immune activation and CNS-resident responses determine the development and progression of neurological pathology (Charabati, Wheeler, Weiner, & Quintana, 2023).

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The immunological component of MS is largely driven by autoreactive lymphocytes that recognize components of the myelin sheath or other CNS-associated antigens. Under physiological conditions, potentially autoreactive immune cells are controlled by central and peripheral mechanisms of immune tolerance (Rutkowska, A. 2026). However, disruption of these regulatory mechanisms can permit the activation and expansion of autoreactive lymphocytes. Activated immune cells may subsequently acquire the functional and migratory characteristics required to reach the CNS, where they contribute to an inflammatory environment involving infiltrating leukocytes, microglia, astrocytes, and other resident or recruited cells. The resulting inflammatory response can promote myelin damage and interfere with neuronal function, ultimately contributing to the neurological manifestations associated with MS (van Noort, Baker, Kipp, & Amor, 2023).

The experimental autoimmune encephalomyelitis (EAE) model has been extensively used to investigate the immunological processes associated with MS. EAE reproduces several important features of CNS-directed autoimmune inflammation and provides a controlled experimental framework for studying disease initiation, immune-cell activation, leukocyte migration, CNS inflammation, and tissue injury. In commonly used MOG_{35–55}-induced EAE models, immunization with the myelin oligodendrocyte glycoprotein-derived peptide initiates an antigen-specific immune response in peripheral lymphoid tissues. The subsequent activation and expansion of autoreactive T cells are followed by their migration toward the CNS and the development of neuroinflammatory pathology. Thus, EAE provides an important opportunity to examine the events that occur before and during the establishment of CNS inflammation (Bonaldo, Casile, Montarolo, & Bertolotto, 2023).

An important feature of EAE pathogenesis is that the initial activation of autoreactive lymphocytes occurs outside the CNS. Secondary lymphoid organs, including regional drain-

ing lymph nodes (DLNs), provide an essential environment in which antigen-specific immune responses are initiated and regulated. Following immunization, antigen-presenting cells process and present antigenic peptides to T lymphocytes, initiating an adaptive immune response that is subsequently shaped by the local immunological environment. The resulting cellular response determines the magnitude and characteristics of the effector immune population that can subsequently participate in CNS inflammation. Therefore, examination of regional lymphoid tissues during the early phase of EAE can provide valuable information regarding peripheral immune activation before the disease becomes predominantly associated with CNS infiltration and tissue pathology (van Noort et al., 2023).

The balance between inflammatory and regulatory immune responses is particularly important in autoimmune diseases. CD4⁺ T lymphocytes represent a major component of this balance and can develop into functionally distinct populations depending on the signals received during activation. In the context of neuroinflammation, inflammatory helper T-cell responses have been strongly associated with disease development, whereas regulatory populations contribute to the maintenance of peripheral immune tolerance. Consequently, alterations in the relative abundance or functional state of these populations may influence the subsequent course of autoimmune inflammation. Assessment of CD4⁺ T-cell responses in DLNs therefore represents a useful approach for investigating whether a candidate compound affects the peripheral immune component of EAE (Charabati et al., 2023).

In addition to cellular composition, the functional activity of immune cells is determined by intracellular signaling networks that integrate antigenic, co-stimulatory, and cytokine-derived signals. These signaling systems regulate cellular proliferation, differentiation, survival, cytokine production, and acquisition of effector functions. Dysregulation of such pathways can contribute to the persistence of inflammatory immune responses.

Accordingly, analysis of selected intracellular signaling molecules can complement phenotypic evaluation and provide additional information regarding the potential immunomodulatory effects of therapeutic candidates (Charabati et al., 2023).

The identification of compounds capable of modifying pathological immune responses without broadly compromising immune function remains an important objective in MS research. Current therapeutic approaches for MS primarily aim to reduce inflammatory activity, prevent new lesion formation, and delay disease progression. Nevertheless, limitations associated with efficacy, adverse effects, treatment accessibility, and the management of progressive disease continue to encourage the investigation of additional pharmacological strategies. In this context, naturally occurring bioactive compounds have attracted scientific interest because many exhibit biological activities, including anti-inflammatory and antioxidant effects (Dalla Costa, Leocani, Rodegher, Chiveri, Gradassi, & Comi, 2024).

Alantolactone (ALT) is a naturally occurring sesquiterpene lactone found in plants of the *Inula* genus, including *Inula helenium* L. It has attracted attention because of its broad range of experimentally observed biological activities. Previous studies have described anti-inflammatory, antioxidant, antimicrobial, and antiproliferative properties of ALT in different experimental systems. These effects have been associated with modulation of cellular responses involved in inflammation, oxidative stress, and cell survival. Such findings have raised the possibility that ALT may have broader immunomodulatory potential beyond the experimental models in which it has been investigated (Liu, Bian, Duan, Zhuang, Sui, & Yang, 2021).

The biological interest of ALT is also related to the characteristic chemical structure of sesquiterpene lactones. These compounds contain reactive structural features that can participate in interactions with cellular proteins and thereby influence intracellular responses. Importantly, the biological effects of

ALT appear to depend on the experimental context, cell type, dose, and duration of exposure. Therefore, observations obtained from cancer, inflammatory, or oxidative-stress models cannot necessarily be directly extrapolated to autoimmune neuroinflammation. Specific experimental evaluation is required to determine whether ALT can influence the immune processes relevant to EAE (Liu et al., 2021).

Despite increasing interest in the pharmacological properties of ALT, its effects on the early peripheral immune response during experimental autoimmune neuroinflammation remain comparatively underexplored. In particular, limited information is available regarding whether ALT administration during the initial phase of MOG₃₅₋₅₅-induced EAE modifies immune-cell responses within regional draining lymph nodes. This question is relevant because modulation of peripheral immune activation may represent one potential mechanism through which an intervention could influence the subsequent development of CNS inflammation. At the same time, the absence of a substantial effect within peripheral lymphoid tissues would also provide valuable information by suggesting that potential biological effects may involve later stages of disease or compartments other than the initial lymphoid response (Liu et al., 2021; Bonaldo et al., 2023).

The present study was therefore designed to investigate the effects of ALT during the early phase of MOG₃₅₋₅₅-induced EAE, with particular emphasis on immune responses within the draining inguinal lymph nodes. The study evaluated CD4⁺ T-cell populations, selected inflammatory and regulatory cytokine responses, and intracellular signaling-associated markers in order to characterize the peripheral immune response following ALT administration. By focusing on the early lymphoid phase of EAE, this approach provides a defined experimental framework for examining whether ALT influences peripheral immune activation before the subsequent development of pronounced CNS-associated inflammatory pathology (Bonaldo et al., 2023).

Understanding the effects of ALT at this stage may contribute to a more comprehensive evaluation of its potential immunomodulatory properties and help clarify whether its biological activity is associated with early peripheral immune regulation. The findings may also provide a basis for future investigations examining the relationship between peripheral immune responses and subsequent CNS inflammation in EAE. In this regard, characterization of the DLN response represents an important first step toward understanding the potential role of ALT in experimental autoimmune neuroinflammation (Cakir et al., 2019).

2.Pathophysiology of Multiple Sclerosis and Peripheral Immunological Priming

The development of autoimmune neuroinflammation in MS and EAE involves a coordinated sequence of peripheral and central immunological events. In MOG_{35–55}-induced EAE, antigen-specific immune responses are initiated in secondary lymphoid organs, followed by T-cell expansion and differentiation, acquisition of migratory properties, and subsequent entry into the CNS. The characteristics of this initial peripheral response can influence the magnitude and composition of the autoreactive lymphocyte pool that subsequently participates in CNS inflammation (Lassmann, 2018; Dendrou et al., 2015).

2.1. Dendritic Cell Activation and Antigen Presentation in Draining Lymph Nodes

Peripheral immune priming begins following administration of the encephalitogenic MOG_{35–55} peptide in Complete Freund's Adjuvant (CFA). CFA provides a strong inflammatory stimulus through its mycobacterial components and promotes activation of innate immune cells at the immunization site. Pertussis toxin (PTx), commonly incorporated into EAE induction protocols, further facilitates disease development by modifying inflammatory and vascular processes that support subsequent immune-cell trafficking (Stromnes and Goverman, 2006).

Antigen-presenting cells (APCs), particularly dendritic cells (DCs), capture antigen and undergo maturation in response to inflammatory signals. During this process, DCs increase their expression of MHC class II molecules and co-stimulatory molecules such as CD80 and CD86, thereby acquiring an enhanced capacity to activate naïve T lymphocytes. Mature DCs subsequently migrate toward regional draining lymph nodes through afferent lymphatic vessels, where they encounter antigen-specific CD4⁺ T cells (Ruddle, 2014; Sallusto, 2016).

Within the T-cell-rich paracortical region of the lymph node, antigen presentation initiates T-cell priming through the integration of three major classes of signals. The first is T-cell receptor recognition of peptide–MHC class II complexes. The second involves co-stimulatory interactions, particularly CD28 engagement by CD80/CD86. The third consists of cytokine-mediated signals that influence the subsequent functional differentiation of activated CD4⁺ T cells. The strength and composition of these signals are important determinants of the balance between inflammatory effector and regulatory responses.

Thus, the draining lymph node is not simply a site of lymphocyte accumulation but a dynamic immunological environment in which antigen presentation, cellular interactions, and soluble mediators collectively shape the developing autoimmune response. This makes the draining lymph node particularly suitable for investigating early pharmacological effects on peripheral immune activation.

2.2. CD4⁺ T-Cell Differentiation and the Th1/Th17–Treg Balance

Following activation, naïve CD4⁺ T cells can differentiate into functionally distinct populations according to the cytokine environment and intracellular transcriptional programs established during priming. In EAE, Th1- and Th17-associated responses are strongly linked to inflammatory pathology, whereas regulatory T cells (Tregs) contribute to suppression

of excessive autoreactive responses (Dendrou et al., 2015; Korn et al., 2009).

Th1 differentiation is principally associated with IL-12-dependent signaling and activation of STAT4, followed by induction of the transcription factor T-bet. T-bet promotes expression of IFN- γ and establishes the characteristic Th1 functional program. Th1-associated responses can enhance inflammatory cellular recruitment and activation within target tissues and have historically been considered an important component of EAE pathogenesis (Kaplan et al., 1996; Szabo et al., 2000).

Th17 cells represent another major inflammatory population in autoimmune neuroinflammation. In murine systems, TGF- β together with IL-6 contributes to initial Th17 differentiation, with STAT3 and ROR γ t playing central roles in establishment of the Th17 transcriptional program. Subsequent exposure to inflammatory cytokines, particularly IL-1 β and IL-23, promotes expansion and functional maturation of pathogenic Th17 populations. Importantly, IL-23 is more appropriately considered a factor supporting the maintenance and pathogenic differentiation of Th17 cells rather than simply an initiating cytokine (Bettelli et al., 2006; Mangan et al., 2006; Cua et al., 2003; Chung et al., 2009).

The functional heterogeneity of Th17 cells is particularly relevant to EAE. Some inflammatory T cells can simultaneously express IL-17 and IFN- γ or produce GM-CSF, indicating that autoimmune effector populations cannot always be defined by a single cytokine. Such plasticity may contribute to differences in pathogenic potential during neuroinflammation (El-Behi et al., 2011; Hirota et al., 2011).

In contrast, Foxp3-expressing Tregs constitute a major mechanism for maintaining peripheral tolerance. TGF- β and IL-2-dependent signaling contribute to the development and stability of regulatory T-cell populations, with Foxp3 serving as a central transcriptional regulator of their suppressive phe-

notype (Chen et al., 2003; Fontenot et al., 2003). Consequently, the relationship between inflammatory effector populations and Tregs is an important determinant of the immunological environment during EAE. Rather than representing a simple binary switch, the Th17/Treg relationship is a dynamic balance influenced by cytokine availability, signal intensity, cellular context, and disease stage.

2.3. Intracellular Signaling Networks Governing T-Cell Responses

The phenotypic differentiation of CD4⁺ T cells is accompanied by activation of interconnected intracellular signaling pathways. Among these, the JAK/STAT, MAPK, and PI3K/Akt pathways serve as important signaling nodes linking extracellular receptor stimulation to transcriptional, metabolic, and functional changes.

STAT3 is particularly important for the development and maintenance of Th17-associated responses. Cytokines such as IL-6, IL-21, and IL-23 activate receptor-associated JAKs and promote STAT3 phosphorylation. Activated STAT3 then contributes to transcriptional programs involving ROR γ t and inflammatory cytokine production (Yang et al., 2007). Because STAT3 activity is closely associated with inflammatory T-cell differentiation, changes in STAT3 phosphorylation can provide an informative indicator of altered cytokine signaling during peripheral immune activation.

MAPK pathways provide additional regulatory inputs. p38 MAPK participates in inflammatory cytokine production and T-cell responses, while ERK1/2 is strongly associated with T-cell receptor signaling, proliferation, and cellular survival. Experimental studies have demonstrated that p38 activity can influence autoimmune neuroinflammation, emphasizing the importance of MAPK signaling in the development of pathogenic immune responses (Noubade et al., 2011).

The PI3K/Akt pathway represents another major signaling axis activated downstream of antigen receptor and co-stimulatory signals. Akt contributes to cellular metabolism, proliferation, survival, and differentiation, thereby linking immune-cell activation with the metabolic requirements of proliferating effector lymphocytes. The combined activity of these pathways enables T cells to integrate antigenic and cytokine-derived information rather than relying on a single signaling cascade (Luu et al., 2019).

2.4. Egress from Lymph Nodes and Migration toward the CNS

After sufficient activation and differentiation, encephalitogenic T cells leave secondary lymphoid tissues and enter the circulation. Sphingosine-1-phosphate receptor 1 (S1PR1) is an important regulator of lymphocyte egress, while transient changes in CD69 and S1PR1 expression help coordinate retention within lymphoid tissues during activation and subsequent migration (Baeyens and Cyster, 2016).

Circulating effector T cells subsequently interact with the CNS vascular compartment through coordinated chemokine and adhesion mechanisms. Integrins such as $\alpha 4\beta 1$ and $\alpha L\beta 2$ facilitate interactions with endothelial adhesion molecules, whereas chemokine receptors contribute to directional migration toward inflammatory signals. Th1- and Th17-associated populations can exhibit distinct trafficking characteristics, including involvement of CXCR3, CCR5, and CCR6-dependent pathways (Reboldi et al., 2009).

Following entry into the CNS, autoreactive lymphocytes encounter local antigen-presenting cells and inflammatory mediators, initiating or amplifying local immune responses. These events ultimately contribute to recruitment of additional immune cells and activation of CNS-resident cells, thereby promoting demyelinating and neuroinflammatory pathology. The sequential nature of these events emphasizes why the peripher-

al priming phase represents an important stage for investigating potential immunomodulatory interventions.

3. Alantolactone: Chemistry, Bioactivity, and Immunomodulatory Potential

Natural products represent an important source of pharmacologically active structures, particularly for the development of compounds capable of modulating complex inflammatory processes. Sesquiterpene lactones constitute a chemically diverse group of plant-derived secondary metabolites with reported anti-inflammatory, antioxidant, antimicrobial, and antiproliferative activities (Ghantous et al., 2010; Newman and Cragg, 2020).

3.1. Botanical Origin and Chemical Characteristics

Alantolactone (ALT) is a naturally occurring eudesmanolide-type sesquiterpene lactone found primarily in *Inula* species, including *Inula helenium* L. and *Inula racemosa*. *Inula helenium*, commonly known as elecampane, has a long history of traditional medicinal use, while modern phytochemical investigations have identified ALT as one of its biologically relevant constituents (Seca et al., 2014).

ALT has the molecular formula $C_{15}H_{20}O_2$ and a molecular weight of approximately 232.32 g/mol. Its characteristic α,β -unsaturated γ -lactone structure provides an electrophilic center capable of interacting with nucleophilic groups, particularly thiol-containing residues in proteins. This chemical property is considered an important contributor to the biological activity of many sesquiterpene lactones.

Protein thiol reactivity can alter the activity of signaling proteins involved in inflammation and cellular stress responses. Experimental studies have consequently investigated ALT in relation to pathways such as NF- κ B, MAPK, STAT3, and cellular redox regulation. However, the biological consequenc-

es of these interactions are highly dependent on cellular context, exposure level, and the specific molecular environment.

3.2. Anti-Inflammatory and Immunomodulatory Properties

A number of experimental studies have demonstrated that ALT can attenuate inflammatory responses. In activated macrophage models, ALT has been associated with reduced expression of inflammatory mediators, including inducible nitric oxide synthase and cyclooxygenase-2, together with modulation of NF- κ B, MAPK, and AP-1-associated signaling (Chun et al., 2012). These observations suggest that ALT can influence inflammatory signaling at multiple levels.

STAT3 has also emerged as a potential molecular component of ALT activity. Studies in cancer and inflammatory models have reported inhibition of STAT3 activation following ALT exposure, accompanied by changes in downstream cellular responses (Khan et al., 2013; Chun et al., 2015). These findings are of particular interest in the context of autoimmune inflammation because STAT3 is also involved in inflammatory T-cell differentiation.

ALT may additionally influence cellular redox homeostasis. Depending on concentration and cellular context, electrophilic sesquiterpene lactones can modify redox-regulated proteins and alter the balance between adaptive antioxidant responses and oxidative stress. In experimental cancer models, ALT-associated oxidative stress has been linked to mitochondrial dysfunction and apoptosis (Cui et al., 2018). However, these effects should not be directly extrapolated to immune cells or EAE without experimental confirmation.

3.3. Rationale for Investigating Alantolactone in EAE

The reported ability of ALT to modulate inflammatory and stress-related signaling provides a biological rationale for investigating its effects in autoimmune neuroinflammation. Sev-

eral pathways influenced by ALT—including NF- κ B, MAPK, and STAT3-associated signaling—also participate in the activation and functional differentiation of immune cells. Nevertheless, observations obtained from macrophages, cancer cells, or other experimental systems cannot by themselves establish that ALT modifies autoreactive T-cell priming *in vivo*.

Therefore, direct evaluation of peripheral lymphoid tissues is necessary to determine whether ALT alters the early immune response during EAE. Analysis of draining lymph nodes provides an opportunity to assess CD4⁺ T-cell populations, cytokine-associated responses, and selected intracellular signaling pathways within the physiological context of an ongoing autoimmune response. Such an approach can distinguish a potential effect on peripheral immune priming from effects that may occur later during leukocyte trafficking or CNS inflammation.

Accordingly, investigation of ALT during the early phase of MOG_{35–55}-induced EAE provides a mechanistically relevant framework for determining whether this natural sesquiterpene lactone can influence the peripheral immunological events that precede CNS disease.

4. Experimental Evaluation of Alantolactone-Induced Changes in Peripheral Immune Responses

The experimental approach was designed to investigate whether ALT modifies the early peripheral immune response associated with EAE development. Female C57BL/6 mice were immunized subcutaneously with MOG_{35–55} peptide emulsified in CFA containing inactivated *Mycobacterium tuberculosis*, while pertussis toxin was administered intraperitoneally on days 0 and 2. ALT was administered intraperitoneally at 15 mg/kg once daily from the day of immunization. On day 7, DLNs were collected and analyzed by multiparameter flow cytometry.

This experimental design provided a direct assessment of the cellular and intracellular signaling environment within a major site of peripheral immune activation. CD4⁺ T-cell populations, effector and regulatory cytokine profiles, and selected phosphorylation-dependent signaling pathways were simultaneously examined. Because the available dataset consisted of group means and standard errors without individual animal-level measurements, the findings are presented as descriptive experimental observations, with statistical significance reported as provided.

4.1. Peripheral CD4⁺ T-Cell Landscape during Early EAE Development

Analysis of DLNs demonstrated that ALT administration did not produce a statistically significant remodeling of the overall CD4⁺ T-cell compartment or the principal inflammatory CD4⁺ phenotypes examined. Total CD4⁺ T cells represented 26.93% of lymph-node leukocytes in control animals and 30.73% in ALT-treated animals. The IFN- γ -producing CD4⁺IL-17⁻ population increased descriptively from 0.18% to 0.55%, whereas the CD4⁺IL-17+IFN- γ ⁺ population changed from 0.071% to 0.058%. The CD4⁺IL-17+IFN- γ ⁻ population showed a small numerical increase from 1.22% to 1.41%. None of these differences reached statistical significance ($p>0.05$).

These observations provide an important experimental perspective on the peripheral phase of EAE. Under the present conditions, ALT did not cause a broad depletion or expansion of the major CD4⁺ T-cell populations within DLNs during the evaluated early phase. The numerical changes observed in individual populations therefore appear insufficient to establish a robust alteration in T-cell polarization.

Table 1. CD4+ T-cell populations in draining inguinal lymph nodes on day 7. Values are expressed as mean percentages with SE as provided in the dataset. All comparisons were non-significant ($p>0.05$).

Cell population / phenotype	Control (%)	ALT 15 mg/kg (%)
CD4+ total T cells	26.93 $\pm$ SE	30.73 $\pm$ SE
CD4+ IL-17 $^-$ IFN- γ $^+$ (Th1)	0.18 $\pm$ SE	0.55 $\pm$ SE
CD4+ IL-17+ IFN- γ $^+$ (Th17/Th1)	0.071 $\pm$ SE	0.058 $\pm$ SE
CD4+ IL-17+ IFN- γ $^-$ (Th17)	1.22 $\pm$ SE	1.41 $\pm$ SE

4.2. Regulatory and Cytokine-Associated Immune Profiles

The analysis of cytokine-associated CD4+ populations further demonstrated that ALT did not significantly alter the measured regulatory and alternative helper T-cell phenotypes. The CD4+IL-10+IL-22 $^-$ population remained highly similar between groups (0.79% versus 0.76%). In contrast, CD4+IL-10 $^-$ IL-22+ cells showed a descriptive reduction from 2.22% to 1.36% following ALT administration. CD4+IL-6+ cells remained comparable between groups (0.38% versus 0.43%).

Foxp3+ regulatory T cells exhibited a modest numerical increase from 2.9% in controls to 3.6% following ALT treatment. Although this observation may be compatible with a tendency toward preservation of regulatory immune populations, the difference was not statistically significant and therefore should not be interpreted as evidence of enhanced Treg differentiation.

Table 2. Regulatory and cytokine-associated CD4+ T-cell populations. Values are expressed as mean percentages with SE as provided in the dataset. All comparisons were non-significant ($p>0.05$).

Cell population / phenotype	Control (%)	ALT 15 mg/kg (%)
CD4+ IL-10+ IL-22 $^-$	0.79 $\pm$ SE	0.76 $\pm$ SE
CD4+ IL-10 $^-$ IL-22+	2.22 $\pm$ SE	1.36 $\pm$ SE
CD4+ IL-6+	0.38 $\pm$ SE	0.43 $\pm$ SE
CD4+ Foxp3+ (Treg)	2.9 $\pm$ SE	3.6 $\pm$ SE

Together, these findings suggest that ALT administration at the selected dose did not substantially redirect the balance between inflammatory and regulatory CD4+ populations in DLNs at this stage of EAE. The numerical reduction in IL-22-associated cells and increase in Foxp3+ cells may nevertheless provide hypotheses for future experiments involving larger cohorts and multiple disease stages.

4.3. Intracellular Signaling Landscape of Peripheral Immune Cells

The examination of phosphorylation-dependent signaling pathways provided an additional layer of information regarding the biological response to ALT. The mean fluorescence intensity of phosphorylated p38 MAPK was 2744 in control animals and 3054 following ALT administration. Similarly, p-ERK1/2 increased descriptively from 241 to 274 MFI, whereas p-Akt1/2 remained almost identical between groups (2858 versus 2861 MFI). None of these differences was statistically significant. For p-STAT3, the available dataset indicated no significant difference, although a numerical control MFI was not provided.

Table 3. Intracellular phosphorylation-dependent signaling in draining lymph nodes. Values represent mean fluorescence intensity with SE as provided. All reported comparisons were non-significant (p>0.05).

Phosphoprotein	Control (MFI)	ALT 15 mg/kg (MFI)
p-STAT3	Not provided	No significant difference
p-p38 MAPK	2744 ± SE	3054 ± SE
p-ERK1/2	241 ± SE	274 ± SE
p-Akt1/2	2858 ± SE	2861 ± SE

The stability of these signaling readouts suggests that ALT did not broadly suppress the phosphorylation state of STAT3, p38 MAPK, ERK1/2, or Akt within the analyzed lymph-node leukocyte population at day 7. This finding is particularly relevant in light of previous experimental studies reporting anti-in-

flammatory effects of ALT through NF- κ B, MAPK, STAT3, and redox-associated mechanisms (Chun et al., 2012; Chun et al., 2015; Cui et al., 2018). Rather than contradicting these observations, the present data indicate that pharmacological responses to ALT may depend strongly on the biological context in which they are evaluated.

4.4. Emerging Biological Interpretation of the Findings

The combined cellular and signaling data support a model in which ALT does not primarily act by producing a generalized suppression of early peripheral lymph-node immune activation under the present experimental conditions. This interpretation is consistent with the complexity of EAE, in which disease development involves sequential interactions among antigen-presenting cells, T-cell differentiation, lymphocyte trafficking, CNS entry, and local inflammatory responses (Gold et al., 2006; Constantinescu et al., 2011).

An important implication is that the absence of significant alterations in DLNs should not be interpreted as evidence that ALT lacks biological activity during EAE. Previous studies have demonstrated that ALT can modulate inflammatory transcriptional and signaling networks in macrophages and tumor cells, including NF- κ B, MAPK, AP-1, STAT3, and oxidative-stress-associated pathways (Chun et al., 2012; Chun et al., 2015; Cui et al., 2018). However, the cellular composition, drug exposure, intracellular concentration, and signaling environment of lymph-node leukocytes differ substantially from those experimental systems.

The present observations therefore introduce a potentially important concept for understanding ALT activity in neuroinflammatory disease: its biological effects may be tissue-, cell-, and stage-dependent rather than uniformly immunosuppressive. An effect that is not detectable in total DLN leukocytes

at day 7 could emerge during later effector phases or within CNS-associated populations. Similarly, a selective alteration in CD4⁺ T cells may remain undetectable when phosphorylation is measured across heterogeneous lymph-node leukocytes.

Future investigations should consequently integrate longitudinal sampling of DLNs with analysis of spinal cord and brain tissues, antigen-specific T-cell responses, lineage-restricted phospho-flow, leukocyte migration markers, and inflammatory or redox-associated pathways. Such an approach would clarify whether the numerical trends observed here represent early indications of a biological response or simply normal experimental variability.

Overall, the present findings extend the current understanding of ALT by demonstrating that, at 15 mg/kg during the early peripheral phase of MOG35–55-induced EAE, its influence is not characterized by a broad alteration of CD4⁺ T-cell composition or major phosphorylation-dependent signaling pathways in draining lymph nodes. This provides a useful reference point for defining where and when ALT may exert its immunomodulatory effects during the subsequent stages of neuroinflammation.

9. References

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