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The influence of the liposomal form of curcumin and microRNA on the course of Alzheimer's disease

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Infiltration of inflammatory cytokines, oxidative stress, and chronic inflammation are associated with the onset and progression of neurodegenerative and oncological diseases. For this reason, treatment with drugs with antioxidant and anti-inflammatory properties may be appropriate to prevent or slow the progression of these disorders. Alzheimer's disease is associated with the occurrence and progression of cerebral amyloidosis in the foci of cholinergic neurons of the neocortex and hippocampus. It is the neurons of the neocortex and hippocampus that suffer the most from the toxicity of Aβ oligomers, which subsequently form senile plaques, leading to the destruction of neuronal networks and the activation of nonspecific inflammation. Polyphenols, such as flavonoids, are characterised by potent antioxidant and anti-inflammatory properties. Curcumin exhibits a wide spectrum of pharmacological activity against many chronic diseases and is able not only to inhibit cell proliferation but also to induce apoptosis by modulating several pro-inflammatory factors. Curcumin's bioavailability is limited by its low solubility in water, so loading curcumin into appropriate nanocarriers can improve its efficacy, local deposition and distribution. Targeted delivery of pharmaceutically active ingredients is a pressing issue and, given the significant results in proteome research, is emerging as a leading issue in the study of RNA regulatory mechanisms, in particular, the divergent functions of small RNAs (microRNAs). MicroRNAs are key modulators of the genome due to their ability to influence most of the genes that code for proteins in the body. Gene therapy regulates or blocks the overexpression of a single gene at the post-transcriptional level using the corresponding microRNA. The major unresolved problem in the application of microRNAs has been the targeted delivery of these molecules. Therefore, to address the challenge of targeted delivery of microRNA to the central nervous system, it is proposed to use administration via lipid-based delivery nanosystems. The review presents the main characteristics and mechanisms of action of curcumin and microRNA on the chronic inflammatory process that consistently accompanies the progression of amyloidosis, as well as their direct inhibitory effect on the excessive formation of β-amyloid peptides. It has been shown that microRNA miR-101 can specifically interact, via its "seed" region, with the messenger RNA of the amyloid precursor protein (AβPP), thereby repressing the overexpression of the AβPP gene and preventing its amyloidogenic processing.

Keywords: curcumin; microRNA; Alzheimer's disease; β-amyloid peptide; τ-proteins.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by memory loss, cognitive impairments, and behavioural changes, ultimately leading to complete loss of independence. Currently, treatment for AD remains largely symptomatic and does not address the underlying mechanisms of neurodegeneration. Existing drugs are primarily used in the late stages of the disease. They are ineffective due to their low permeability through the blood-brain barrier (BBB), as well as significant side effects. All this creates an urgent need for early detection of the disease, new drugs that have a targeted effect and minimal toxicity, and an unconventional approach to treatment protocols.

A promising approach is the use of nanoliposomes – lipid nanoparticles (LNPs) – which can efficiently encapsulate and deliver pharmaceutically active ingredients (APIs) across the BBB. LNPs can be modified for targeted delivery of APIs to specific brain regions, protecting the liposomal content from degradation and providing controlled release of the APIs. A promising therapeutic strategy involves the combined use of curcumin (Cur) and microRNA (miRNA, miR) encapsulated in nanoliposomes.

Curcumin is the main curcuminoid of turmeric (*Curcuma longa*) with pronounced anti-inflammatory, antioxidant, and neuroprotective properties. Curcumin can influence key pathogenic mechanisms of AD, including reducing β-amyloid (Aβ) aggregation, modulating tau protein phosphorylation, chelating metals, and decreasing neuroinflammation and oxidative stress. However, the clinical use of Cur is limited by its low bioavailability, which can be significantly improved through encapsulation in nanoliposomes.

miRNAs regulate gene expression through two main mechanisms: by fully binding to the 3′ untranslated region (3′ UTR) of target mRNAs, leading to mRNA degradation and suppression of gene expression, or by partially binding to the 3′ UTR to inhibit the translation process, thereby reducing gene expression. In the case of AD, miRNAs, particularly miR-101, regulate the expression of the amyloid precursor protein (APP), suppressing Aβ formation. Reduced levels of miR-101 in the brain during AD are associated with enhanced neurodegenerative processes. Administration of exogenous miR-101 can decrease Aβ production and influence neuroinflammation, paving the way for targeted therapy.

Thus, the development and application of LNPs loaded with Cur and miR-101 combine multifactorial action with high delivery efficiency and minimised side effects. This approach may open new opportunities for targeted treatment of AD.

Structure of liposomes

Both natural and synthetic phospholipids are used to create liposomes. Natural phospholipids include PC, PE, PI, and PS. Among synthetic phospholipids used in liposome formulations, the most commonly employed are dioleoylphosphatidylcholine, distearoylphosphatidylcholine, and dioleoylphosphatidylethanolamine (Tiwari et al., 2020). Cholesterol is also an important component of liposome membranes and can be incorporated into their structure at high concentrati-

ons, in a molar ratio to phosphatidylcholine of 1:1 or even 2:1. As an amphipathic molecule, cholesterol embeds itself in the bilayer membrane in such way that its hydroxyl group is oriented toward the aqueous environment, while its aliphatic chain aligns parallel to the acyl chains of the phospholipids within the membrane (Fig.1). Cholesterol increases the distance between the choline heads of phospholipids and eliminates typical electrostatic and hydrogen interactions, which provides increased stability and lower permeability of the liposomal membrane (Dwivedi et al., 2014).

Liposomes can be divided into several different types based on their composition and application, including:

– Conventional liposomes. Conventional liposomes are the first generation of liposomes. They consist of lipid bilayer molecules surrounding an aqueous core and serve as the foundation for all subsequent liposome types.

– Immunoliposomes. Immunoliposomes are vesicles specifically designed to actively target drug substances within the body.

– Long-circulating liposomes. Surface modification or PEG modification of liposomes is called PEGylation of liposomes, and the modified liposomes are called long-circulating liposomes or stealth liposomes. Compared to conventional liposomes, PEG-liposomes can avoid phagocytosis and circulate for a long time in the systemic circulation.

– Cationic liposomes. Cationic liposomes can be obtained by adding a cationic phospholipid to the bilayer membrane. This allows a high rate of DNA incorporation, and for this reason, such liposomes may be more suitable for gene and antisense therapy.

– Stimuli-responsive. Liposomes can be easily functionalised by incorporating functional materials, such as stimulus-responsive elements. Their structure, configuration, and other properties can change in response to specific stimuli *in vivo* or *in vitro*, such as variations in temperature, light, or pH.

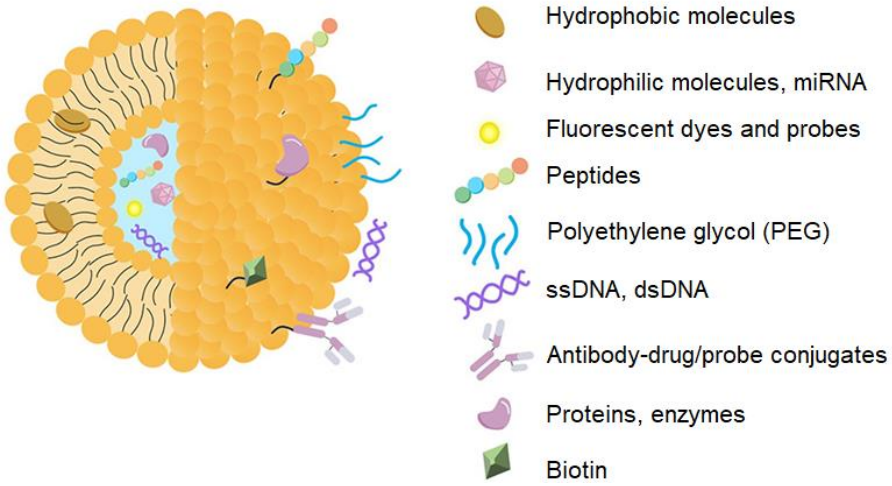


Fig. 1. Classification of liposomes based on composition and application (hydrophobic molecules; hydrophilic molecules, microRNA; fluorescent dyes and probes; peptides; polyethylene glycol (PEG); DNA; antibodies, antibody conjugates; peptides, enzymes; biotin)

Methods of liposome synthesis

Hydration of phospholipids in water, in the presence of external energy (such as sonication, shaking, heating, or homogenization), induces interactions between lipid molecules and water, ultimately leading to the formation of bilayer vesicles. This allows the system to achieve thermodynamic equilibrium in the aqueous environment. The formation of bilayer structures occurs for several reasons: reduction of energetically unfavourable interactions between hydrophilic and hydrophobic regions through vesicle closure, decrease in the system's free energy, and the attainment of maximum stability of the supramolecular self-assembled structure (Sharma et al., 2018).

There are several effective methods for preparing liposomes, which differ in the mechanism of vesicle formation, particle size, encapsulation efficiency, and complexity of implementation. (Shulga, 2013; Mishra et al., 2018).

The thin film hydration method is one of the most common methods for obtaining liposomes. Initially, phospholipids and cholesterol are dissolved in an organic solvent (e.g., chloroform or methanol), forming a homogeneous lipid emulsion. The solvent is removed by evaporation under reduced pressure, resulting in a thin lipid film on the walls of the flask. An aqueous solution (such as distilled water or a buffer) is added, and the film is hydrated. The resulting emulsion is stirred, filtered, and liposomes are formed by extrusion or sonication. (Mishra et al., 2018).

The reverse-phase evaporation method enables the achievement of high API encapsulation efficiency. In this method, lipids are dissolved in an organic solvent together with an aqueous solution containing the substance to be encapsulated. The resulting water-in-oil emulsion is evaporated under reduced pressure, which leads to the formation of liposomes with the inclusion of the active substance (Sawant et al., 2021). The extrusion method is used to obtain liposomes with a uniform and controlled size. After the formation of multilayer vesicles by the thin film hydration method, the emulsion is repeatedly passed through polymer membranes with a defined pore diameter (e.g., 100 nm) using an extruder. This allows the most uniform size distribution of liposomes to be achieved (Sawant et al., 2021).

Sonication is used to reduce the size of liposomes or to prepare unilamellar vesicles. The suspension obtained by one of the basic methods is treated with ultrasound at a frequency of 20–24 kHz, which leads to the destruction of larger vesicles and the formation of smaller structures (Mishra et al., 2018).

The detergent removal method is used to encapsulate hydrophobic substances. Lipids are dissolved in a detergent solution along with a hydrophobic compound. The detergent is then gradually removed by dialysis or chromatography, resulting in the formation of stable liposomes (Sharma et al., 2018).

The freeze-thaw method increases the stability of liposomes and the efficiency of retaining encapsulated substances. The liposome emulsion is subjected to multiple cycles of freezing at temperatures below the phase transition temperature of lipids with subsequent thawing. This treatment contributes to better encapsulation and reduced losses of the active substance (Sawant et al., 2021).

Advantages and limitations of using liposomes

Liposomes have several important advantages, and one of their key properties is targeted drug delivery, which enables the transport of active substances directly to affected tissues or cells, thereby reducing side effects. By improving bioavailability, liposomes can encapsulate water-insoluble APIs, enhancing their effectiveness. In addition, the controlled release of pharmaceutical substances ensures prolonged therapeutic action and reduces the frequency of drug administration

(Sawant et al., 2021). Liposomes protect sensitive APIs against degradation by enzymes, changes in pH, or oxidation. Liposomes are highly versatile – their size, composition, and surface characteristics can be effectively modified for specific applications. They reduce the toxicity of pharmaceutical agents, making liposomal formulations suitable for clinical use. In cosmetology, liposomes enhance the penetration of active ingredients through the layers of the skin. In the food industry, liposomes protect flavours, vitamins, and nutrients from degradation, improving product quality and shelf life. In the field of vaccinology, liposomes improve the stability and delivery of antigens, enhancing the immune response. Liposomes are also used as a tool in biomedical experiments, as well as in diagnostics, particularly for disease detection and drug screening. Due to their ability to be customised, liposomes can be adapted to specific needs by changing their physicochemical properties (Dwivedi et al., 2014; Sawant et al., 2021).

Despite their numerous advantages, liposomal delivery systems also have significant limitations. One of the main issues is instability during storage, which can lead to aggregation, leakage of encapsulated substances, or changes in size and structure. In addition, large-scale production of liposomes is a complex and costly process, which limits their widespread use in the pharmaceutical industry. It is also difficult to ensure uniformity in size and composition, which negatively affects the reproducibility of results and delivery efficiency (Sawant et al., 2021).

Liposomes have a short circulation period in the body, as they are rapidly cleared by the immune system, which reduces the duration of the drug's action. Some liposomes may induce an immune response, causing side effects. The limited capacity for loading certain pharmaceutical substances is also a challenge, particularly when large doses of a drug need to be delivered.

The development of liposomal systems is a technically complex process that requires specialised expertise and is often associated with high costs, which affect the final price of the drug. Compatibility issues with certain active ingredients limit the range of potential liposome applications. Moreover, even with successful preclinical studies, not all liposomal formulations successfully pass clinical trials, which complicates their implementation in medical practice (Sawant et al., 2021). Difficulties may arise due to interactions between liposomes and biological systems, which can alter their stability or release control, as well as issues related to biodegradation, especially when synthetic components ("heads") of liposomes are used. In some cases, liposomes have limited applicability and are inferior to other delivery systems. Achieving precise control over the release kinetics of active ingredients and obtaining regulatory approval are additional serious challenges that significantly complicate the development and implementation of liposomal drug formulations (Dwivedi et al., 2014).

Liposomes are a versatile and promising drug delivery system that can radically transform the pharmaceutical industry by enabling targeted delivery, reducing side effects, and enhancing therapeutic efficacy. Despite certain challenges, particularly those related to stability, large-scale production, and cost, which remain relevant, ongoing innovations in liposomal technologies are opening new opportunities for medicine (Sawant et al., 2021).

The role of microRNAs in the initiation of inflammation and oxidative stress

Inflammation significantly contributes to the pathogenesis of neurodegenerative disorders, particularly AD, where neuroinflammation plays a key role. Glial cells, such as microglia and astrocytes, are the main participants in the inflammatory response to Aβ toxicity. Under normal conditions, microglia perform a phagocytic function, clearing the brain of Aβ. However, Aβ aggregates activate microglia, triggering the release of nitric oxide, reactive oxygen species, and pro-inflammatory cytokines, such as IL-1β and TNF-α, which contribute to neuronal damage and the progression of AD (Kinney et al., 2018).

Astrocytes, in response to pathological changes in AD, become reactive and produce toxic molecules, activating inflammatory genes. Their interaction with neurons, particularly through the glutamate / glutamine cycle, is closely linked to energy metabolism and is disrupted in AD (Kinney et al., 2018).

A close relationship between neuroinflammation and autophagy has been revealed. Impaired autophagosome maturation and lysosomal transport lead to the accumulation of autophagic vacuoles, which correlates with Aβ aggregation and autophagy dysfunction. Such an imbalance in microglial and astrocyte activity, combined with disrupted intercellular immune interactions, affects synaptic plasticity, neuronal survival, and cognitive functions (Kwon & Koh, 2020).

Neuroinflammation in AD is accompanied by changes in miRNA expression, which regulate the production of pro-inflammatory cytokines and proteolytic enzymes. For example, overexpression of miR-146a in microglia reduces Aβ levels, improves cognitive functions, and promotes the shift of microglia from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype, decreasing cytokine production and enhancing phagocytosis, although its excessive expression can also exacerbate inflammation by suppressing complement factor H (CFH) (Li et al., 2024).

miR-132 acts as a negative regulator of inflammation – for example, resveratrol increases its expression, thereby reducing inflammatory damage. In contrast, miR-155 enhances neuroinflammation: its elevated levels in 3xTg AD models are associated with hyperactivation of microglia and astrocytes and increased secretion of pro-inflammatory mediators (Kwon & Koh, 2020). Pharmacological modulation of these miRNAs may provide novel strategies to mitigate neuroinflammation and its detrimental effects on neuronal health in AD.

Oxidative stress (OS) is also a key factor in the pathogenesis of AD. It encompasses a range of pathological processes, including increased generation of reactive oxygen species (ROS), mitochondrial dysfunction, impaired neuronal energy metabolism, altered neurotrophic signalling, cellular stress responses, calcium homeostasis imbalance, and defects in autophagy. Unlike DNA, RNA – particularly miRNAs – is especially vulnerable to oxidative damage due to its structure and localisation near mitochondria (Oliver & Reddy, 2019).

Clinical and preclinical studies indicate that oxidative stress (OS) is an early and critical event in the pathogenesis of neurodegeneration, including AD, Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). OS alters the expression of several miRNAs, which in turn regulate the expression of genes involved in responses to oxidative damage (Oliver & Reddy, 2019).

Specifically, it has been shown that the natural compound amphorin stimulates the expression of miR-107, which suppresses BACE1 – a key enzyme in APP processing leading to Aβ formation. This results in reduced Aβ levels and indicates the potential of miR-107 as a therapeutic target in AD. In contrast, miR-125b promotes the expression of both APP and BACE1, contributing to Aβ accumulation and neuronal apoptosis, and also affects inflammatory and oxidative signalling pathways through the regulation of SPHK1 (Li et al., 2024).

Furthermore, miR-125b activates GSK3β and promotes tau protein hyperphosphorylation, which is associated with neurodegeneration. It also affects cell adhesion molecules, such as NCAM, which play a role in AD progression (Li et al., 2024).

miR-146a, a transcriptional target of NF-κB, regulates inflammatory processes, in part by suppressing the expression of complement factor H (CFH), an inhibitor of complement activation. Excessive expression of this miRNA induces tau hyperphosphorylation and alters levels of superoxide dismutase 2 (SOD2), further highlighting its pathogenic role in AD. Thus, inhibition of miR-146a is considered a promising therapeutic approach (Li et al., 2024).

miR-200c exhibits neuroprotective properties under endoplasmic reticulum stress induced by Aβ. It promotes cell survival and neurite outgrowth by suppressing PTEN and modulating insulin signalling pathways, indicating its potential as a therapeutic agent and biomarker in AD (Oliver & Reddy, 2019).

The complex interactions among miRNAs, oxidative stress, and neurodegenerative processes in AD highlight the significant role of miRNAs in cellular and biological responses to oxidative stress.

MicroRNAs, mitochondrial and synaptic dysfunction

Mitochondria play a key role in cellular energy supply, and their dysfunction is a characteristic feature of AD associated with synaptic impairment (Oliver & Reddy, 2019). These alterations include mitochondrial DNA damage, impaired protein import, and decreased activity of the respiratory chain.

Recent studies have revealed the involvement of mitochondrial miRNAs in the regulation of genes associated with energy metabolism and neuronal survival. For example, miR-743a and miR-23a/b suppress the TCA cycle and oxidative phosphorylation, while miR-210, miR-338, and miR-34a target OXPHOS enzymes. miR-16-5p promotes apoptosis by targeting BCL-2 (Li et al., 2024).

Mitochondrial biogenesis is essential for neuronal activity, and miR-455-3p, miR-34a, and miR-23a/b promote this process by alleviating the toxic effects of Aβ through activation of the SIRT1 pathway (Oliver & Reddy, 2019). Thus, miRNAs directly influence mitochondrial dynamics, energy metabolism, and synaptic activity.

Synaptic activity forms the basis of cognitive function. In patients with AD, disturbances in this activity lead to impaired neuronal communication. miRNAs such as miR-132, miR-212, and miR-484 regulate neurotransmitter release and synaptic plasticity (Gowda et al., 2022). Reduced levels of these miRNAs are associated with cognitive deficits and depressive symptoms.

Particular attention has been drawn to mitochondrial miRNAs that influence mitochondrial transport, calcium signaling, and synaptic vesicle formation – processes critical for neurotransmission. Their contribution to synaptic dysfunction in AD requires further investigation (Oliver & Reddy, 2019).

It has been established that miRNAs interact with mitochondrial dysfunction and oxidative stress in a bidirectional manner: on the one hand, they regulate mitochondrial status, while on the other, they themselves are affected by stress (Gowda et al., 2022). This highlights their importance as potential therapeutic targets for diseases associated with mitochondrial impairment.

MicroRNAs as biomarkers and therapeutic targets

miRNAs have attracted considerable attention as regulatory molecules capable of modulating key pathogenic pathways in AD. Unlike complex neuroimaging methods (MRI, PET), the analysis of miRNAs in biological fluids is simpler and less invasive, making them promising biomarkers for early diagnosis and monitoring of AD (Liu et al., 2014).

One of the key miRNAs is miR-132, which shows a consistent decrease in AD. It plays a critical role in adult hippocampal neurogenesis (AHN), essential for memory and cognitive plasticity. In mouse models, administration of miR-132 restored AHN and improved memory. It has also been demonstrated that miR-132 inhibits MAPK1, thereby reducing oxidative stress and iNOS expression. miR-212 and miR-132 are also important, as their levels were found to be decreased in blood exosomes of AD patients, whereas miR-135a, miR-193b, and miR-384 were increased (Liu et al., 2014).

A decrease in miR-101 expression has been observed in the hippocampus of ageing rodents, in the brains of AD patients, and in animal models of the disease. This pattern correlates with cognitive decline, highlighting the significance of miR-101 as a potential biomarker for early diagnosis and a promising therapeutic target. miR-101 acts as a negative regulator of APP expression by binding to specific sequences in the 3'-UTR of its mRNA. This interaction leads to reduced APP synthesis and, consequently, decreased formation of Aβ, which plays a key role in the pathogenesis of AD and the development of amyloid plaques (Barbato et al., 2014).

By combining genomics, proteomics, and bioinformatics approaches, it is possible to construct gene-target networks in models, revealing the regulatory network of miRNAs in AD pathogenesis. This approach allows the study of miRNA regulatory pathways and signaling, providing a comprehensive understanding of the complex mechanisms involved in AD. Ultimately, this knowledge will contribute to the development of targeted therapeutic strategies for AD, offering significant insights into disease pathogenesis and potential treatment avenues (Li et al., 2024).

The potential of miRNAs as therapeutic agents in AD is immense but has not yet been fully realised. The development of miRNA-based therapies will need to address challenges related to delivery, target specificity, and off-target effects. Innovative approaches, such as nanoliposome-based delivery systems, may help overcome these issues, enabling precise modulation of pathological miRNA levels in the brain.

Nanoliposomes for targeted drug delivery

The term “liposome” comes from the Greek words lipos, meaning fat, and soma, meaning body. They are spherical, concentric vesicles composed of a phospholipid shell surrounding an aqueous core. Liposomes were first discovered in the mid-1960s by British haematologist Dr Alec D. Bengham. This discovery was a major milestone in the study of membrane structures and laid the foundation for the further development of liposomes as drug delivery systems (Tiwari et al., 2020).

In the following decades, liposomes attracted considerable attention in pharmacology due to their ability to encapsulate drugs and transport them to specific sites in the body. This discovery was revolutionary in the field of drug delivery, as it allowed controlled release of the drug and reduced side effects (Sawant et al., 2021).

Over time, liposomes have found applications not only in medicine but also in cosmetology, the food industry, and gene therapy. Researchers have developed different types of liposomes, differing in size, composition, and surface modifications, in order to optimise their properties for specific tasks. (Abd El-Alim et al., 2019).

Nanoliposomes are lipid nanoparticles up to 100 nm in size that are widely used as drug carriers capable of penetrating cell membranes (Sawant et al., 2021).

Liposomes can be used as carriers for a variety of APIs, including pharmaceutical drugs, and have significant therapeutic potential. In addition to liposomes, other delivery systems are also used for targeted delivery, such as nanodots, microparticles, micelles, inactivated viruses, cyclodextrins, albumins, chitosans, alginates, polylactides, polyacrylates, and lectins. However, it is liposomal delivery systems that attract the attention of researchers due to their applications in medicine, cosmetology, and modelling the structure of biological membranes (Sharma et al., 2018).

A liposome is a microscopic bubble-like structure (vesicle) whose membrane consists of a phospholipid bilayer. The main components of such membranes are phospholipids, in particular phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS). Phospholipids are amphiphilic molecules with polar hydrophilic "heads" and hydrophobic (non-polar) hydrocarbon "tails" (Shulga, 2013). Due to this structure, phospholipids can spontaneously form bilayer vesicles in an aqueous environment, imitating the structure of cell membranes.

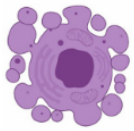
Types of regulated cell death

Current studies demonstrate that neuronal death in AD occurs not only through classical apoptosis, but also through several other RCD mechanisms (Table 1), such as necroptosis, autophagy, ferroptosis, pyroptosis, parthanatosis, and mitochondrial dysfunction (Goel et al., 2022). One of the best-characterised types of RCD is apoptosis. This is a process in which a cell activates intrinsic self-destruction programs involving caspases. In the brain tissue of AD patients, activation of caspase-3, caspase-8, Bcl-2 family proteins, as well as increased expression of Bad, have been observed in the cortex and hippocampus. In transgenic AD mice, inhibition of Bad or caspase-8 reduced apoptosis and improved behaviour. However, not all studies have found a clear association between apoptosis and classical AD markers, questioning its central role (Goel et al., 2022).

Another important mechanism is necroptosis – a form of programmed necrotic cell death that is activated when apoptosis is blocked. Unlike apoptosis, necroptosis is accompanied by rupture of the cell membrane and release of cellular contents into the extracellular space, inducing inflammation. Necroptosis is activated via the TNF-α/RIPK1/RIPK3/MLKL signalling pathway. Activation of necropto-

sis in AD patients correlates with the degree of neurodegeneration and cognitive decline. In APP/PS1 mouse models, inhibition of necroptosis led to reductions in Aβ, hyperphosphorylated tau, and cell death, along with improved cognitive function. Elevated levels of MLKL and pMLKL in brain tissue have been confirmed in both mice and AD patients (Xu et al., 2021).

Table 1
Modes of cell death that contribute to neurodegenerative disease

Types of cell death	Trigger	Effectors	Hallmarks
Apoptosis 	• Excitotoxicity • DNA damage • Receptors activation (TNRF, TRAIL, FAS)	• Caspase 3 • Caspase 7	• Plasma membrane blebbing • Chromatin condensation • Chromatin fragmentation
Necroptosis 	• Cell death receptors activation (TNRF, TRAIL, FAS) • Signal pathways IFN, LPS, TLR3/4	• RIPK1 • RIPK3 • MLKL	• RIPK3/MLKL colocalization • RIPK3, MLKL (pRIPK3, pMLKL) phosphorylation
Pyroptosis 	• DAMPs (ROS, ATP, dsRNA) • PAMPs (viruses, bacteria) • Ca^{2+} accumulation • Antitumor drugs	• NLRP3 • NLRP1 • AIM2 • Caspase 1	• Caspase 1 • Caspases 4, 5, 11 • Gasdermin D (GSDMD)-pore activation • IL1beta, IL18 activation
Ferroptosis 	↓ GPX4 ↓ SLC7A11 ↑ ALCS4	• Lipid peroxidase (LPO)	• Mitochondria disfunction • Endocytotic uptake of Fe^{2} • Lipid raft formation

In AD, the autophagy mechanism – a cellular process normally responsible for the clearance of defective proteins and organelles – is disrupted. Although autophagy is considered a protective mechanism, its excessive activation or blockade at later stages (e.g., impaired fusion of autophagosomes with lysosomes) can lead to the accumulation of toxic structures and trigger cell death.

Accumulation of immature autophagosomes has been observed in AD mice long before the formation of amyloid plaques and neuronal loss. Studies have shown that deletion of the NRBF2 component, which is involved in autophagy regulation, reduces the capacity for long-term potentiation, increases Aβ formation, and impairs memory. In contrast, its overexpression has the opposite effect (Lachance et al., 2019).

Ferroptosis is a relatively recently described type of cell death associated with iron-dependent lipid peroxidation and loss of glutathione peroxidase 4 (GPX4) activity. In AD, iron accumulation has been observed in the hippocampus and cortex, along with increased levels of reactive oxygen species (ROS) and decreased glutathione levels, which are typical markers of ferroptosis activation. This pathway becomes particularly important under conditions of oxidative stress and metabolic dysfunction, which are characteristic of AD. Increased iron levels, decreased GPX4 activity, and increased lipoperoxide oxidation have been found in both patients and models of AD. Blockade of ferroptosis, for example, by administration of liproxstatin-1, improved the condition of neurons (Bao et al., 2021).

Pyroptosis may also play a distinct role in the pathogenesis of AD. This form of cell death is associated with the activation of inflammatory pathways, particularly inflammasomes (NLRP3, NLRP1, AIM2). In AD patients, increased expression of caspase-1 and GSDMD, as well as the release of pro-inflammatory cytokines IL-1β and IL-18, has been observed, indicating the involvement of pyroptosis in neuronal damage. In transgenic mice and cellular models, Aβ and phosphorylated tau have been shown to activate inflammasomes, and their inhibition reduces inflammation and cognitive impairments (Han et al., 2020).

Parthanatosis is activated by excessive PARP-1 activity in response to DNA damage. Aβ induces oxidative stress, PARP-1 activation, calcium translocation, and mitochondrial dysfunction. In mice and cell lines, PARP-1 inhibition prevented microglial activation, memory impairment, and synaptic degeneration (Goel et al., 2022).

The mitochondrial permeability transition pore (mPTP) is formed with the involvement of the protein CypD, particularly under conditions of Aβ overload. This interaction leads to a decrease in mitochondrial membrane potential, respiratory chain dysfunction, and the release of Ca^{2+} and pro-apoptotic proteins. Inhibition or deletion of CypD in mice has been shown to improve synaptic function and memory (Calvo-Rodriguez et al., 2020).

Current evidence suggests that there is a connection between RCD pathways. They can mutually induce or block each other depending on the intensity of stress, cellular microenvironment, metabolic state and expression of regulatory molecules. Proteins such as p53, Beclin-1, TRIB3, RIPK1, LC3, and p62 play a key role in switching between different death pathways (Saleem, 2021). This confirms that AD is not the result of a single pathological process, but the result of a complex interaction between multiple molecular mechanisms of cell death.

Thus, neuronal death in AD is the result of a complex interplay between toxic protein aggregates, impaired cellular metabolism, inflammatory response, and activation of various forms of regulated cell death. Understanding these mechanisms opens new opportunities for the search for therapeutic targets aimed at preserving neuronal integrity.

Anti-inflammatory and antioxidant activity of curcumin

Curcumin has been attributed with anti-inflammatory properties for millennia. Many of its biological effects are related to its ability to suppress both acute and chronic inflammatory processes. Nuclear factor kappa-B (NF-κB) plays a key role in signalling pathways involved in the development of inflammatory diseases and various types of cancer. In an inactive state, NF-κB proteins reside in the cytoplasm, but upon activation – which requires the involvement of several kinases and the phosphorylation and degradation of inhibitors such as IκB – they translocate to the nucleus. Cur has been shown to inhibit TNF-dependent NF-κB activation, as well as other pathways induced by various agents, some of which cause the generation of reactive oxygen species – these radicals are also neutralised by Cur (Hatcher et al., 2008).

COX-2, the inducible form of cyclooxygenase, predominates at sites of inflammation, and numerous studies indicate its critical role in tumour promotion. Cur reduces COX-2 expression and also inhibits the activity of 5-LOX, another enzyme involved in inflammation. It additionally decreases the levels of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8, interferon-γ, and certain chemokines. (Urošević et al., 2022). The anti-inflammatory effect of Cur is manifested by a decrease in iNOS mRNA expression in the liver of mice injected with lipopolysaccharides. Cur reduced this expression by 50–70% after two oral administrations at a dose of 92 ng/g body weight (Chan et al., 1998).

In addition to its anti-inflammatory effect, Cur also actively influences the level of oxidative stress, which plays a key role in the pathogenesis of many diseases, including cancer, diabetes, cardiovascular disorders, neuronal damage, and hypoxia (Noorafshan & Ashkani-Esfahani, 2012).

Cur is classified as a substance that can exhibit both pro-oxidant and antioxidant effects. This is supported by studies showing that Cur is a free radical scavenger, a reducing agent, and an inhibitor of DNA damage, especially in the presence of copper or iron ions (Antunes et al., 2005).

In vitro studies have shown that Cur inhibits the production of nitric oxide (NO) and reactive oxygen species (ROS) in macrophages (Sreejayan, 1997). Cur also suppresses lipoxygenase and cyclooxygenase activity in rat fibroblasts (Lin & Shih, 1994). Moreover, oxidative stimulation of G-proteins in human brain membranes by metabolic

pro-oxidants such as homocysteine and hydrogen peroxide was significantly reduced in the presence of Cur (Jefremov et al., 2007).

Cur reduces cyclophosphamide-induced lung injury by enhancing antioxidant defence. In addition, Cur inhibited lipid peroxidation in liver microsomes and brain homogenates of laboratory rats. (Purkayastha et al., 2009).

Neuronal cell death in Alzheimer's disease

Regulated cell death is an orderly and precisely coordinated set of changes or signalling events that involve both gene expression and protein activity. This process is essential for normal development as well as for maintaining tissue homeostasis. Dysregulation of this pathway leads to cell death through various mechanisms. Pathological changes occurring in neurons are observed in the pathogenesis of various neurodegenerative diseases, including AD (Goel et al., 2022).

The pathological features of AD are primarily associated with the accumulation of two key protein markers: amyloid β-peptides and abnormally phosphorylated tau proteins. The aggregation of these proteins leads to the formation of amyloid-beta plaques and neurofibrillary tangles (NFTs) and induces neuroinflammation and neurodegeneration over several years or decades, which in turn results in cognitive and behavioural impairments. (Goel et al., 2022).

Autopsy results of the brains of patients with AD reveal massive neuronal death, manifested by a significant reduction in cortical volume, a decrease in the size of gyri (by up to 50%), and widening of the sulci. Numerous studies have identified various forms of cell death in neurons. However, the mechanisms of neuronal death in AD remain largely unexplored, as the trigger that leads to the pathological activation of regulated cell death is still unknown (Goel et al., 2022).

AD is a chronic neurodegenerative disease that affects elderly individuals and clinically manifests as a gradual deterioration of memory and cognitive functions, along with various emotional disturbances (Qiu et al., 2009). The disease typically appears after the age of 60 and follows a progressive and irreversible course. As patients' memory and cognitive skills continue to decline, they increasingly require external care and ultimately die within 4–8 years after diagnosis. Notably, brain changes associated with AD begin to develop during the preclinical stage, several decades before the first clinical signs of dementia appear (Dubois et al., 2016).

The vast majority of AD cases are sporadic, while less than 5% are familial forms associated with mutations in the APP, PS1, and PS2 genes. Neuropathologically, the brain of an AD patient is characterised by extracellular accumulation of oligomerised and fibrillar amyloid beta-peptide 42 (Aβ42), surrounded by dystrophic neurites (neuritic plaques), as well as intraneuronal formation of neurofibrillary tangles composed of phosphorylated tau protein. These pathological changes are accompanied by widespread neuronal death, synaptic degeneration, and reactive gliosis in multiple brain regions responsible for memory and cognitive functions (Reitz & Mayex, 2014).

Ageing is a major risk factor for the development of numerous neurodegenerative disorders, including AD, with age-related prevalence nearly doubling every five years after the age of 65. The factors responsible for the transition of some individuals from normal brain ageing to the complex pathogenic cascade characteristic of AD remain largely unknown; however, the presence of shared features between these two processes has attracted considerable scientific interest. In addition to ageing, the development of the sporadic form of the disease is also influenced by numerous genetic and modifiable risk factors (Qiu et al., 2014).

In the study by Goel et al. (2022), the authors proposed that, at the cellular level, the pathogenesis of AD results from the interplay of multiple factors: mitochondrial dysfunction, oxidative stress, pathological protein accumulation (e.g., Aβ42, tau, etc) with associated toxic effects, and an inflammatory response mediated by microglia.

Triggers of neuronal death

Cell death becomes the final solution for a neuron when multiple stresses accumulate to a point that significantly exceeds the cell's ability to repair itself, and this acts as a trigger for the activation of regulated cell death (RCD) signaling cascades, ultimately leading to the development of neurodegenerative diseases, including AD. Most neurodegenerative diseases are associated with the appearance and accumulation of pathological proteins up to the level of high-order aggregate formation. These aggregates act as stressors and induce numerous cytotoxic mechanisms, including increased levels of ROS, reactive nitrogen species, and other highly reactive compounds; synaptic dysfunction; excitotoxicity; impaired protein degradation systems; ER stress; inflammation; re-entry into the cell cycle; DNA damage; and mitochondrial dysfunction, all of which ultimately lead to neuronal cell death (Zhang et al., 2024).

The amyloid cascade hypothesis was previously proposed as the main theoretical concept of AD, in which the Aβ peptide was postulated as the direct agent responsible for progressive neurodegeneration. More recently, this hypothesis has been replaced by the Aβ-oligomer hypothesis, which provides substantial evidence that Aβ oligomers (AβOs), rather than amyloid plaques, play a central role in the pathogenesis of AD. Existing data from various behavioural, neuropathological, and cellular studies indicate that elevated levels of AβOs in the brain have pathogenic consequences. The oligomeric and fibrillar forms of Aβ that arise after its aggregation are toxic. Following the initial transduction, Aβ oligomers exert multiple effects on various subcellular organelles, including mitochondrial impairment, ER stress, and autophagy/lysosomal dysfunction (Cline et al., 2018). Disruption of Aβ processing pathways leads to its deposition, which adversely affects numerous mechanisms such as neurotransmitter release, intracellular signalling cascades, regulation of autophagy, lipid metabolism, and synaptic function, ultimately resulting in neuronal death. Soluble Aβ oligomers and insoluble Aβ aggregates bind to and activate microglia and astrocytes, stimulating a low level of chronic neuroinflammation. In addition, the accumulation of APP leads to increased intracellular calcium levels, which cause excitotoxicity, as well as an increase in pro-apoptotic molecules and heat shock proteins (Goel et al., 2022).

Numerous mechanisms induced by intraneuronal Aβ oligomers are postulated in the etiopathogenesis of AD, including ER stress-induced apoptosis, endosomal/lysosomal leakage, mitochondrial dysfunction, oxidative stress, and synaptic impairment (Goel et al., 2022). It has been demonstrated that Aβ oligomers induce brain insulin resistance in AD, and dysfunction of insulin and IGF-1 receptors leads to further Aβ aggregation and loss of synaptic connections. Neuroinflammation is thought to be one of the earliest pathological responses to intracellular accumulation of Aβ oligomers (Kot et al., 2024). In studies on transgenic APP mice with the E693Δ mutation, it was found that intraneuronal accumulation of Aβ oligomers not only leads to impaired hippocampal synaptic plasticity and memory and down-regulation of the presynaptic protein synaptophysin, but also to abnormal tau phosphorylation, activation of microglia and astrocytes, and neuronal loss. This highlights that Aβ oligomers trigger multiple causal pathways that play a key role in the development of AD pathogenesis (Tomiyama et al., 2010).

The second major pathology in AD is the formation of neurofibrillary tangles (NFTs), which arise as a result of tau protein hyperphosphorylation. In AD, tau becomes phosphorylated at multiple sites, causing it to detach from microtubules, leading to the loss of their structural integrity and disruption of various cellular processes. Subsequently, phosphorylated tau aggregates to form paired helical filaments, which eventually assemble into neurofibrillary tangles. This pathogenic form of tau acts as a stressor for neurons at the molecular level. However, the question of how tau can directly induce neuronal death remains unresolved. Accumulation of hyperphosphorylated tau alters microtubule stability, leading to two consequences: (1) tau interacts with synaptogyrin-3 (a presynaptic protein), resulting in impaired synaptic release, loss of synapses, and neuronal dysfunction, and (2) activation of retrograde neurodegeneration and neuronal death (Goel et al., 2022). It has also been found that tau aggregation is associated with DNA fragmentation, indicating a link to regulated neuronal cell death. Furthermore, at the postsynaptic level, tau stabilises NMDA receptors and induces excitotoxicity. Prolonged activation of NMDA receptors triggers Ca^{2+} release, activates calpain, and causes

mitochondrial dysfunction, ultimately leading to cell death. Data from several studies indicate that tau pathology affects multiple cellular systems, including signalling pathways, transport mechanisms, and the cytoskeleton, while also compromising mitochondrial integrity, resulting in significant neuronal damage (Goel et al., 2022).

A recent *in vivo* study demonstrated the toxic effects of amyloid-β and tau on synaptic function and axonal integrity, respectively, in the etiopathogenesis of AD. The authors showed that the early stages of AD are characterised by synaptic damage induced by amyloid deposits, memory impairment, and alterations in functional connectivity. In contrast, the late stages of AD involve tau-associated axonal damage, cognitive decline, and reduced anatomical connectivity (Pereira et al., 2021).

According to current understanding, under the influence of cytotoxic stressors such as Aβ aggregation, hyperphosphorylated tau, and oxidative stress, mature neurons can regress to a precursor-like state, losing protective mechanisms that normally prevent the activation of regulated cell death pathways. The central trigger of this regression is a metabolic shift: the replacement of mitochondrial oxidative phosphorylation with glycolysis leads to energy instability, which increases neuronal susceptibility to apoptosis and other forms of regulated cell death (RCD). Some experimental models, including induced neurons derived from cells of AD patients, show increased expression of immaturity markers and signs of cell cycle re-entry. However, since not all studies report significant differences, the re-expression of immaturity/cell cycle markers in mature neurons should be interpreted with caution (Hagihara et al., 2019).

Curcumin as a therapeutic compound

Curcumin – a bright yellow spice extracted from the rhizome of *Curcuma longa* L. – is the active component of the plant-based remedy and culinary spice turmeric. Curcumin has long been used in traditional medicine in China, India, and Iran, where it was used to treat numerous diseases, including diabetes, liver disorders, rheumatic diseases, atherosclerosis, infectious diseases, and cancer (Noorafshan & Ashkani-Esfahani, 2012).

Turmeric powder has been used for centuries in cooking, medicine, dyeing fabrics, and cosmetics. This important spice was first introduced to the Western world in the 14th century and is still widely used today. In ancient Indian medicine, turmeric paste was applied to the eyes to treat inflammation and infections; it was also used to treat wounds, bites, burns, and other skin lesions. Curcumin is also credited with the ability to accelerate the healing of childbirth-related tears. Turmeric powder was consumed with hot milk to treat coughs and respiratory illnesses, while roasted turmeric was used as a remedy for dysentery. Additionally, turmeric was employed to alleviate hallucinations caused by opioids or psychoactive substances (Noorafshan & Ashkani-Esfahani, 2012).

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a lipophilic molecule that rapidly penetrates cell membranes. Curcumin affects both the function and structure of the cell membrane and mimics typical processes that occur during apoptosis (Fig. 2). The cellular response to Cur is distinctive, as it induces an immediate and partially reversible loss of membrane integrity, yet cells can recover within a short time. Membrane changes induced by Cur may underlie its biological effects; for example, by altering phosphatidylserine accessibility, Cur can modulate protein kinase C activity (Jaruga et al., 1998).

The molecular structure and density of functional groups make Cur an attractive target for structure-function studies and the optimisation of its derivatives. A typical extract of *Curcuma longa* contains three forms: (I) curcumin, (II) dimethoxycurcumin, and (III) bisdemethoxycurcumin, of which form I is the most abundant. There is debate as to which of the three forms is the most effective antioxidant, anti-inflammatory, and antitumor compound (Rathore et al., 2022).

Since Cur has low bioavailability and selectivity, various approaches have been proposed: the use of adjuvants such as piperine, which inhibits glucuronidation in the liver, the use of liposomal Cur, nanoparticles, phospholipid complexes, and structural analogues (Urošević et al., 2022). Such analogues improved absorption and prolonged plasma half-life. Despite its poor bioavailability, Cur is not toxic to animals or humans, even at high doses (7 g/kg). Studies suggest that the poor bioavailability of Cur is due to poor absorption, rapid metabolism, and rapid elimination from the body (Urošević et al., 2022).

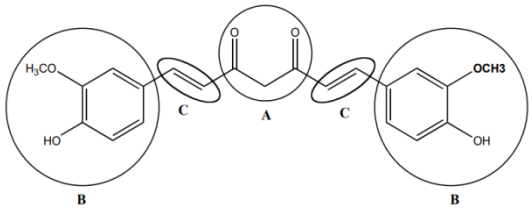


Fig. 2. Parts of the curcumin molecule: (*A*) β-diketone or keto-enol group; (*B*) phenolic groups; (*C*) alkene bridges

The FDA classifies curcumin as "generally safe." However, there is some evidence of potential toxic effects at high doses, including changes in the liver, gastrointestinal tract, and inflammation following long-term administration of high doses of curcumin (Rathore et al., 2022).

Therapeutic effects of curcumin in the nervous system

Cur has been actively investigated for its therapeutic potential in the nervous system, particularly the brain and its associated pathologies. Studies have shown that curcumin effectively inhibits brain tumor formation and induces apoptosis in tumor cells by activating caspases-3 and -7 in human oligodendroglioma cells, as well as in murine neuroblastoma and glioma cell lines (N18, GL261, B16F10). At the same time, a suppression of cyclin D1, NF-κB, Bcl-XL, Akt, and VEGF expression was observed, which accounts for the reduced proliferation, survival, and invasive potential of the cells (Purkayastha et al., 2009). Curcumin also decreased colony formation in semi-solid media, reduced the number of CD133-positive stem cells, and lowered the levels of STAT3 and insulin-like growth factors, thereby contributing to the inhibition of tumour growth. Further studies showed that the nanoformulation of Cur based on glycerol monooleate (GMO) was more effective than free Cur in inhibiting the proliferation, migration, and invasion of glioma cells (Kundu et al., 2012).

In addition to its antiproliferative properties, curcumin exerts a pronounced neuroprotective effect in neurodegenerative diseases. In particular, curcumin binds to aggregated β-amyloid (Aβ) and phosphorylated tau proteins, thereby reducing their toxicity and slowing the progression of neurodegeneration. Curcumin inhibits the formation of large toxic Aβ oligomers, as well as the aggregation of Aβ and tau proteins *in vivo*. Moreover, it modulates key enzymes involved in the pathogenesis of Alzheimer's disease, including acetylcholinesterase (AChE), butyrylcholinesterase (BChE), β-secretase (BACE-1), and glycogen synthase kinase 3β (GSK3β) (Mutsuga et al., 2012).

Curcumin can act as an agonist of the liver X receptor (LXR) and activate the promoter of the ABCA1 gene, leading to increased expression of the corresponding protein and more efficient cholesterol efflux from brain cells (Tian et al., 2012). Oxidative stress, a major contributor to neuronal damage in cerebrovascular disorders, is also attenuated by curcumin. Treatment with curcumin elevates the level of uncoupling protein 2 (UCP2), thereby reducing oxidative stress and protecting neurons under conditions of chronic cerebral ischemia (Liu et al., 2012).

Other therapeutic effects of curcumin

Curcumin inhibits the growth of tumours in the gastrointestinal tract, liver, brain, and neck, and also affects breast stem cells by reducing the number of ALDH-positive cells. By suppressing NF-κB, curcumin decreases the expression of Bcl-2 and Bcl-XL, thereby promoting apoptosis, while COX-2 inhibition is particularly important in colorectal tumours. Curcumin also inhibits Akt, a protein kinase that supports cell survival. The p53 gene is activated by curcumin in basal cell carcinoma, hepatoblastoma, and breast cancer cells; however, in colorectal cancer cells, its level is decreased alongside an increase in

HSP70, indicating a tissue-specific effect. (Noorafshan & Ashkani-Esfahani, 2012).

Angiogenesis is critical for tumour growth, and curcumin disrupts this process, particularly by modulating FGF, VEGF, and angiopoietins. Curcumin also regulates cell adhesion molecules (ELAM-1, ICAM-1, VCAM-1) and proteins involved in metastasis. Furthermore, curcumin affects the cardiovascular system by inhibiting NOS, reducing NO oxidation, decreasing iNOS and eNOS expression in the myocardium of diabetic rats, and mitigating DNA damage. Cur prevents cardiac hypertrophy by inhibiting p300-HAT, improves systolic function in hypertension and infarction, reduces NF-κB activation and levels of MCP-1, IL-6, IL-1, TNF-α, and induces the antioxidant enzyme HO-1 (Noorafshan & Ashkani-Esfahani, 2012).

In a gastric ulcer model, curcumin reduced myeloperoxidase activity, TNF-α, IL-1β, and histological damage. In cases of nonspecific ulcerative colitis, curcumin improved histological outcomes, inhibited NF-κB, and normalised nitric oxide levels. A clinical study demonstrated that 2 g of curcumin per day alleviated ulcerative colitis symptoms and decreased the frequency of relapses. (Giannessi et al., 2008).

In the context of diabetes, curcumin lowers blood glucose levels, improves glucose tolerance, enhances insulin secretion, reduces diabetic nephropathy and fibrosis, and preserves pancreatic β-cells. Curcumin also inhibits aldose reductase, decreases advanced glycation end products (AGEs) and malondialdehyde in the retina, alleviates symptoms of diabetic neuropathy, improves nerve conduction and nerve morphology, and normalises the lipid profile by lowering LDL, triglycerides, and total cholesterol while increasing HDL (Noorafshan & Ashkani-Esfahani, 2012).

In the genitourinary system, curcumin inhibits prostate cancer cell growth by suppressing AR, NF-κB, Akt, COX-2, and Bcl-2 while activating caspase-3. It reduces PSA levels, inhibits LNCaP cell proliferation, disrupts metastatic pathways, and suppresses MMP-2 and MMP-9, which are critical for preventing metastasis. Curcumin also affects kidney and bladder cancer cells by decreasing cell viability and promoting apoptosis (Singh & Singh, 2011).

Overall, curcumin targets numerous biochemical and molecular pathways, including transcription factors, inflammatory cytokines, enzymes, kinases, growth factors, receptors, adhesion molecules, and anti-apoptotic proteins, highlighting its significant therapeutic potential in the treatment of Alzheimer's disease and other pathologies. At the same time, it should be noted that the low bioavailability and limited selectivity of curcumin remain significant barriers to its clinical application, underscoring the need for further research to develop effective pharmaceutical formulations (Chainoglou & Hadjipavlou-Litina, 2020).

The role of microRNAs in the therapy of Alzheimer's disease

Recent studies highlight the potential involvement of microRNAs (miRNAs) in the pathogenesis of Alzheimer's disease (AD) (Shulga et al., 2025). miRNAs are short non-coding RNAs, 20–24 nucleotides in length, that regulate gene expression by inhibiting translation or inducing degradation of target mRNAs. They are generated through processing of primary transcripts in the nucleus, further matured in the cytoplasm, and function as part of the RNA-induced silencing complex (RISC) (Treiber et al., 2019).

miRNAs can fully bind to the 3′ untranslated region (3′ UTR) of target mRNAs, inducing their degradation, or bind partially, thereby repressing translation. Some miRNAs also interact with the 5′ UTR or coding sequences of mRNAs, and in certain cases, may even enhance translation. This versatility makes miRNAs promising therapeutic targets for complex diseases, such as neurological disorders, due to their ability to simultaneously regulate multiple genes (Li et al., 2024).

miRNAs are involved in the pathogenesis of various diseases, including cancer, cardiovascular disorders, and neurological conditions such as Alzheimer's disease (AD). In AD, alterations in miRNA expression levels have been linked to key pathological features (Silvestro et al., 2019), including tau phosphorylation and aggregation, mitochondrial dysfunction, and Aβ production. These alterations in miRNA activity contribute to neuronal dysfunction and cell death, playing a critical role in disease progression. Studying miRNAs in AD provides insights into the underlying molecular mechanisms and highlights their potential as both diagnostic biomarkers and therapeutic targets (Li et al., 2024).

MicroRNAs and pathological features of Alzheimer's disease

miRNAs play an important role in regulating key pathogenetic processes in AD, including Aβ production and tau protein hyperphosphorylation (Li et al., 2024).

Aβ is generated through the amyloidogenic processing of the transmembrane amyloid precursor protein (APP), which undergoes sequential cleavage by β- and γ-secretases. An alternative non-amyloidogenic pathway involves α-secretase, which prevents Aβ formation. Disruption of the balance between Aβ production and clearance leads to its accumulation, neurofibrillary tangle formation, neuronal degeneration, and functional impairment. Aβ levels are closely linked to APP and BACE1 expression. Several miRNAs – including miR-9, miR-29, miR-29a/b-1, miR-124, miR-101, miR-107, miR-298 and miR-328 – are involved in the regulation of Aβ levels (Hébert et al., 2009).

AD patients have decreased expression of miR-149, miR-34a-5p, miR-125b-5p, miR-15b, miR-16, miR-124, and miR-374b-5p in serum and the frontal cortex, which inversely correlates with BACE1 mRNA levels. Introduction of these miRNAs into AD cell models reduces BACE1 levels and Aβ accumulation (Hébert et al., 2009).

Moreover, exogenous overexpression of miRNAs influences Aβ production. For instance, miR-29a expression in transgenic mice reduces BACE1 levels and increases Aβ, whereas miR-195 overexpression in N2a/APP695 cells decreases Aβ levels, and its inhibition leads to their increase. These findings suggest that reduced expression of certain miRNAs may contribute to abnormal BACE1 upregulation and increased Aβ production. Similarly, overexpression of miR-186 in neuronal cells suppresses BACE1 and decreases Aβ levels, highlighting the complex regulatory role of miRNAs in AD pathogenesis (Li et al., 2024).

Clinical data also indicate that miRNAs play a critical role in regulating APP levels. For instance, overexpression of miR-106a and miR-520c significantly reduces APP in HEK-293 cells, suggesting a potential therapeutic avenue for AD. Additionally, miR-128 has been associated with increased Aβ production, whereas inhibition of miR-126 exhibits a neuroprotective effect against Aβ42 (Hébert et al., 2009).

miRNAs altered in AD affect not only Aβ pathology but are also closely associated with tau protein phosphorylation and aggregation. Under normal conditions, tau, encoded by the MAPT gene, stabilises microtubules within neuronal axons. In AD, however, it becomes hyperphosphorylated, loses its affinity for tubulin, and contributes to neuronal dysfunction and degeneration (Li et al., 2024).

Genomic studies of brain tissue from patients with AD have revealed altered expression of more than 250 miRNAs across various cell types and brain regions (Landgraf et al., 2007). In particular, the levels of miR-132 and miR-425-5p were found to be increased, whereas miR-124-3p and miR-512 were decreased. miR-132 plays a crucial regulatory role in the central nervous system (CNS). Mice lacking this microRNA exhibit significant cognitive impairments, while its overexpression promotes neuronal apoptosis and tau protein phosphorylation. Similarly, excessive expression of miR-425-5p activates GSK-3β and enhances tau phosphorylation in AD models. At the same time, miR-124-3p exhibits neuroprotective properties by suppressing tau hyperphosphorylation through the caveolin-1–PI3K/Akt/GSK3β signalling pathway. A reduced level of miR-512 in the later stages of AD has also been associated with tau pathology (Li et al., 2024). These findings indicate that miRNAs are key modulators of AD pathogenesis through their influence on both Aβ formation and tau protein hyperphosphorylation. Alterations in miRNA expression patterns not only affect the expression of APP and BACE1 but also contribute to the accumulation of toxic protein aggregates underlying neurodegenerative changes in the brain.

Conclusion

Abnormal accumulation of amyloid β-peptide in brain cells is associated with pathological processes in Alzheimer's disease. The main pathological manifestations of the disease are amyloidosis and chronic inflammation. Given that curcumin has numerous biochemical and molecular targets, including transcription factors, inflammatory cytokines, enzymes, kinases, growth factors, receptors, adhesion molecules and anti-apoptotic proteins, and that miRNAs regulate the production of pro-inflammatory cytokines and the activity of proteolytic enzymes, it exhibits neuroprotective properties under conditions of oxidative stress induced by Aβ. Therefore, it is relevant to study new therapeutic drugs – aerosols of lipid target systems containing curcumin and microRNAs for the treatment of neurodegenerative diseases, in particular, the determination of the anti-inflammatory and anti-amyloid activity of the liposomal form of curcumin and microRNA, its effectiveness in relation to the dose and duration of action, and after *in vivo* therapy.

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