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Extrachromosomal DNA: Molecular perspectives in aging and neurodegenerative diseases

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SUMMARY Extrachromosomal DNA (ecDNA) refers to a class of circular, non-chromosomal DNA that has recently gained widespread attention due to its potential role in aging and neurodegenerative diseases. The generation of ecDNA is closely associated with processes such as double-strand breaks, micronuclei formation, and the breakage-fusion-bridge (BFB) cycle, all of which are integral to regulation of gene expression, genetic stability, and clonal evolution. In neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease, the aberrant formation of ecDNA is closely linked to defects in DNA repair, alterations in synaptic plasticity, and neuronal dysfunction. The distinct distribution and functional roles of ecDNA in these conditions make it a potential diagnostic biomarker and therapeutic target. This review provides an overview of the mechanisms underlying ecDNA formation and its functions in the nervous system. Additionally, it explores the clinical potential of ecDNA in disease diagnosis, targeted therapy, and personalized medicine, offering new insights for future research and treatment strategies.

Keywords extrachromosomal DNA (ecDNA), aging, neurodegenerative diseases, DNA repair, micronuclei, breakage-fusion-bridge cycle

1. Introduction

With the advancement of life sciences research, extrachromosomal DNA (ecDNA) has emerged as an important biological phenomenon that is attracting increasing attention. From normal physiological activities to pathological conditions, ecDNA plays a critical role in regulating gene expression, maintaining cellular genetic stability, and influencing disease progression. Recent studies on the role of ecDNA in aging and neurodegenerative diseases have provided new insights into the understanding of these complex diseases.

2. Biological characteristics and mechanisms of ecDNA formation

ecDNA refers to circular DNA molecules that originate from chromosomes and lack centromeres and telomeres. These DNA fragments are generated through various mechanisms, including double-strand DNA breaks, asymmetric chromosome segregation, micronuclei formation, and the breakage-fusion-bridge (BFB) cycle (*1-3*). Studies have shown that ecDNA generation significantly increases under stress or during repair of DNA damage (*4*). For example, drug-induced stress, such as methotrexate treatment, can trigger the amplification of the *DHFR* gene in the form of ecDNA, thereby enhancing cellular resistance to the drug (*5*).

ecDNA is typically distributed unevenly within subcellular compartments, a feature that exacerbates genetic diversity between cell clones and provides a selective advantage for cellular evolution (*6*). In tumors, ecDNA containing oncogenes such as *MYC*, *EGFR*, and *HER2* has been closely linked to tumor progression (*7,8*). However, ecDNA is not limited to cancer cells. Small polydispersed circular DNAs (spcDNA) have also been found in normal tissues, such as muscle and blood, although their functions remain largely unexplored (*9,10*).

The formation of ecDNA within cells is closely associated with DNA repair mechanisms. Studies have shown that double-strand breaks (DSBs) are one of the primary triggers for ecDNA formation (*11*). Under stresses such as chemotherapy or radiation exposure, non-homologous end joining and microhomology-mediated end joining (MMEJ) DNA repair mechanisms may incorrectly stitch together DNA fragments, resulting in circular structures (*12-14*). Additionally, micronuclei formation and the BFB cycle are also key mechanisms in

ecDNA generation. In micronuclei, residual chromosome fragments may transform into ecDNA due to replication delays or chromatin breaks.

In normal cells, ecDNA, such as spcDNA and t-circles, primarily originates from repetitive sequences and may play a role in regulating genomic stability and maintaining telomere integrity (*9,15*). In cancer cells, however, ecDNA is typically larger and contains oncogenes or enhancer elements. Studies have shown that the amplification of genes such as *MYC* and *HER2* is closely associated with the malignancy of tumors (*16,17*). Due to their circular structure, ecDNAs exhibit higher gene expression activity and genetic instability, which provide cells with an adaptive advantage in response to the microenvironment (*18*).

3. EcDNA and genetic stability in aging

Aging is a biological process closely associated with the accumulation of DNA damage (*19*). Studies have shown that, as individuals age, the capacity for DNA repair gradually declines, while DNA damage, and DSBs in particular, increases significantly (*20*). This damage may lead to the formation of ecDNA through inaccurate repair pathways and can also impact chromosomal stability. Extrachromosomal ribosomal DNA circles (ERCs), which were first discovered in model organisms like yeast, have been directly linked to the aging process (*21,22*). In aging yeast cells, ERCs accumulate in large quantities and accelerate the aging process by disrupting cellular metabolism and gene expression (*23*).

In human neurons, the generation of DSBs is considered a normal physiological process that is involved in the expression of early response genes (ERGs) (*24*). With advancing age, however, the DNA repair capacity of neurons declines, and the generation of ecDNA may have profound effects on neuronal function. Research has shown that small ecDNAs containing regulatory gene fragments play a role in modulating gene expression and epigenetic modifications. This may help explain the significance of ecDNA in neural plasticity.

4. Role of ecDNA in neurodegenerative diseases

In neurodegenerative diseases, the accumulation of DNA damage and the impairment of DNA repair are considered to be key factors in disease pathogenesis. Aberrant generation of ecDNA has been linked to the onset and progression of diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD).

4.1. AD

In patients with AD, elevated levels of markers of DNA damage such as γH2AX have been observed in neurons and glial cells, suggesting that dysfunctional DNA repair plays a critical role in disease progression (*25*). Studies have shown that β-amyloid can inhibit DNA-PK-dependent non-homologous end joining (NHEJ) repair, leading to the accumulation of DNA damage (*26*). The generation of ecDNA in this context may exacerbate neuronal dysfunction by affecting gene expression or epigenetic modifications. Additionally, AD mouse models have revealed increased ecDNA formation after exposure to a new environment, but these DNA fragments are poorly repaired, suggesting that ecDNA could serve as a novel marker of defects in DNA repair (*27*).

4.2. PD

In PD, key proteins, including α-synuclein, are closely linked to DNA damage and ecDNA formation (*28*). Overexpression of α-synuclein has been found to induce both single-strand and double-strand DNA breaks and to interfere with the repair of DSBs (*29*). EcDNA detected in patients with PD may contain gene sequences that regulate synaptic plasticity, thereby impacting the functional stability of dopaminergic neurons.

4.3. HD

In HD, the mutant huntingtin protein (mHTT) impairs NHEJ repair, resulting in the accumulation of DSBs in primary neurons and increased formation of ecDNA (*30-32*). Studies have shown that mHTT interacts with the Ku70 protein, hindering the repair of DSBs and subsequently increasing the amount of ecDNA (*32*). These ecDNA fragments may further disrupt gene expression networks, contributing to neuronal degeneration.

5. Clinical and research implications

The role of ecDNA in aging and neurodegenerative diseases opens new avenues for disease diagnosis and treatment. First, the specific sequences and structures of ecDNA can serve as molecular biomarkers for early disease diagnosis (*33,34*). Monitoring the abnormal presence of ecDNA in the brain tissue of patients with AD may provide insights into disease progression, helping to inform personalized treatment strategies. Moreover, interventions aimed at modulating ecDNA formation or clearance or influencing the regulation of gene expression associated with ecDNA, could represent novel therapeutic approaches. Studies have shown that targeted deletion of ecDNA carrying oncogenes can reduce cancer invasiveness, and this strategy may potentially be adopted to regulate abnormal gene expression in neurodegenerative diseases.

Furthermore, drugs that enhance DNA repair capacity

may protect neurons from aging and degenerative damage by reducing ecDNA formation (*35,36*). NAD+ supplementation strategies have been found to improve DNA repair and delay disease progression in mouse models of AD and PD (*27*). Use of next-generation sequencing (NGS) technology to analyze patient-specific ecDNA profiles can assist in formulating personalized treatment plans (*37*). Additionally, ecDNA sequencing can provide valuable data with which to understand the molecular characteristics of various diseases (*38*).

Finally, researchers can, through use of CRISPR technology to precisely simulate the mechanisms of ecDNA formation, better construct cell or animal models of human diseases, thereby advancing drug development (*11*). The progress of these studies and technologies not only provides new tools for functional research on ecDNA but also opens new avenues for the diagnosis and treatment of neurodegenerative diseases.

6. Future directions

Despite significant progress in ecDNA research over the past years, many unanswered questions remain. How does ecDNA precisely regulate neural plasticity? Which types of ecDNA are critical for neuronal function? What are the mechanisms of interaction between ecDNA and gene expression regulation in disease states? Future research could further integrate single-cell sequencing, CRISPR/Cas9 technology, and high-resolution microscopy to explore the mechanisms of ecDNA formation and its functions in the nervous system. In neurodegenerative diseases in particular, understanding the biological role of ecDNA will provide new insights into the molecular pathogenesis and potential therapeutic strategies.

7. Conclusion

As a unique molecular phenomenon, ecDNA bridges the complex relationships between DNA damage, repair, gene amplification, and cellular function. In aging and neurodegenerative diseases, the generation and accumulation of ecDNA may be key drivers of pathological processes. Gaining a deeper understanding of the mechanisms and functions of ecDNA may offer valuable insights to improve human health.

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