Cytogenetic and Genome Research

Review Article

Cytogenet Genome Res
DOI: 10.1159/000541563

Received: June 15, 2024
Accepted: September 19, 2024
Published online: September 30, 2024

Exploring the Role of Extrachromosomal Circular DNA in Human Diseases

Yali Peng[a] Huihui Tao[a, b, c] Guoying Wang[a] Mengyao Wu[a] Tinatin Xu[a] Chunmei Wen[a] Xuejia Zheng[d] Yong Dai[a, b, c, d]

[a]School of Medicine, Anhui University of Science and Technology, Huainan, China; [b]Key Laboratory of Industrial Dust Deep Reduction and Occupational Health and Safety of Anhui Higher Education Institutes, Huainan, China; [c]Anhui Province Engineering Laboratory of Occupational Health and Safety, Huainan, China; [d]The First Hospital of Anhui University of Science and Technology, Huainan, China

Keywords
Extrachromosomal circular DNA · Autoimmune disease · Tumor · Hereditary disease · Biomarker · Mechanism of action

Abstract
Background: Extrachromosomal circular DNA (eccDNA) has emerged as a central focus in molecular biology, with various types being found across species through advanced techniques, including high-throughput sequencing. This dynamic molecule exerts a significant influence on aging and immune function and plays pivotal roles in autoimmune diseases, type 2 diabetes mellitus, cancer, and genetic disorders. ***Summary:*** This comprehensive review investigates the classification, characteristics, formation processes, and multifaceted functions of eccDNA, providing an in-depth exploration of its mechanisms in diverse diseases. ***Key Messages:*** The goal of this review was to establish a robust theoretical foundation for a more comprehensive understanding of eccDNA, offering valuable insights for the development of clinical diagnostics and innovative therapeutic strategies in the context of related diseases.

Introduction

Extrachromosomal circular DNA (eccDNA) is a circular double-stranded DNA found outside the chromosomes. The discovery of eccDNA dates back to the last century. However, it was only recently that we have begun to have a deeper understanding of its complex role in cellular function, with the development of high-throughput sequencing and molecular biology techniques. Currently, various techniques have been used to detect and characterize eccDNA. Although these methods have greatly advanced research in this field, they still face challenges in terms of specificity, sensitivity, and high-throughput analysis [1]. The broad functions and impacts of eccDNA in cellular physiology are progressively being elucidated. EccDNA not only participates in cell proliferation and differentiation but also regulates cellular senescence and death. It also plays a crucial role in various diseases. For example, in cancer, eccDNA promotes tumor development by altering expression profiles [2], and its potential as a diagnostic biomarker and therapeutic target in autoimmune diseases has gained widespread attention [3]. This review delineates the historical identification of eccDNA, detection techniques, and changes in expression profiles. Furthermore, it explores the molecular

Yali Peng and Huihui Tao contributed equally to this work.

karger@karger.com
www.karger.com/cgr

Correspondence to:
Huihui Tao, 475433093@qq.com
Yong Dai, daiyong22@aust.edu.cn

Karger

mechanisms of eccDNA in promoting cancer, autoimmune diseases, and hereditary diseases, aiming to elucidate its specific roles in disease processes and to discover novel biomarkers for clinical diagnosis while providing a theoretical foundation for drug development in clinical treatment.

Overview of EccDNA

Evolution of EccDNA Research

Recently, eccDNA, a special type of DNA structure found inside cells, has gradually become a research hotspot in the fields of molecular biology and genomics. In 1965, Hoota and Bassel [4] demonstrated the presence of circular DNA in the DNA of porcine spermatozoa by sedimentation analysis and electron microscopy, which was the first time that circular DNA was demonstrated in the chromosomes of higher organisms. Subsequently, circular DNA called double minutes (DMs) was found in human tumor cells [5, 6]. Although the size of cyclic DNA was identified and estimated using electron microscopy, information regarding its quantity and sequence was limited. In 1976, trace amounts of cyclic DNA were successfully extracted and purified from HeLa cells for the first time using cesium chloride-ethidium bromide density gradient centrifugation [7]. However, for the purification and extraction of closed-loop DNA, the cesium chloride-ethidium bromide density gradient centrifugation method suffers from the disadvantages of requiring a large number of genomic samples and being susceptible to contamination with linear DNA degradation [8, 9]. The introduction of two-dimensional agarose gel electrophoresis enhances cyclic DNA purification [10]. Cyclic DNA is primarily derived from chromosomes inside the nucleus and from mitochondria and chloroplasts outside the nucleus. In 1990, extranuclear cyclic DNA of endogenous chromosomal origin was named eccDNA [11]. Recently, advancements have been made in the purification, tracking, and analysis of eccDNA. Super-resolution confocal imaging and high-throughput DNA sequencing have made it feasible to identify and quantify eccDNA in a wider range of species, including humans, yeast, Drosophila, and some plants [12].

Classification of EccDNA

EccDNA is used as a general term to refer to circular DNA molecules in the cell nucleus [13], whose structure shares morphological similarities with mitochondrial DNA (mtDNA) and chloroplast DNA [14, 15]. The majority of eccDNA (approximately 60%) comprise unique sequences. Furthermore, over 90% of genomic loci contain direct repeat sequences made up of multiple bases [16]. EccDNA is extensively distributed throughout the entire genome. However, it has a higher concentration in certain specific regions [17], often including untranslated regions and areas with a high guanine–cytosine (GC) content [18]. Compared with chromosomal DNA, eccDNA predominantly contains active histone marks, which endow it with a more accessible chromatin landscape. Notably, eccDNA exhibits an unusual structural plasticity compared with chromosomal DNA. The active histone modifications of eccDNA confer it with a more open chromatin structure, facilitating the accessibility of gene regulatory elements. This allows eccDNA to reintegrate into chromosomes and form homogeneously staining regions (HSRs) [19]. This type of DNA is morphologically diverse, with both single- and double-stranded structures. Their size varies from a few hundred base pairs (bp) for small ones to several megabases (Mb) for large ones [20, 21]. EccDNA can be classified into several categories based on their size and sequence characteristics. First, small polydispersed circular DNA (spcDNA) ranges between 100 and 10,000 bp in size, with a diameter of 0.05–2.0 μm. SpcDNA is ubiquitous in a wide range of eukaryotic cells and has been associated with the onset of genetic instability [22]. The second type of eccDNA is called episomes, which are submicroscopic in size and created by removing linear DNA from chromosomes, circularization, and amplification. The third type is microDNAs, which range from 200 bp to 3,000 bp in size and are enriched in genes, exons, and the 5′ untranslated regions of CpG islands. All normal cells of species contain microDNAs [18, 23]. The fourth type is called telomeric circles. Telomeric circles are a unique form of eccDNA that can be double-stranded (t-circles) or single-stranded (c-circles) and are present in a broad range of organisms. They range from 100 bp to 30,000 bp in size. Lastly, there exist extrachromosomal DNA (ecDNA) and DMs. The former has a size of 100 kilobase pairs to 3 Mb pairs, whereas the latter is amplified at the Mb pair level. Both ecDNA and DM do not contain telomeres and filaments [24]. Moreover, ecDNA has many whole genes and regulatory regions. Altogether, the importance of ecDNAs in genetic and cell biology research is highlighted by their different structures and functions and their extensive distribution throughout animals [25].

Roles of EccDNA in Physiology and Immunology

Physiologic Function of EccDNA

The high expression of eccDNA in muscle tissue is linked to a special phenomenon. The *TTN* gene, which produces the most transcribed protein in the muscle, has high transcript levels in muscle areas with high eccDNA

expression. This gene encodes the titin protein. At rest, the muscle expresses titin, which suggests that elevated levels of eccDNA could improve host cell function [26]. Furthermore, eccDNA is linked to the aging process. For example, the accumulation of *CUP1* eccDNA is caused by the transcriptional activity of the tandemly duplicated *CUP1* gene [27]. In mouse research, eccDNA has been suggested as a possible marker of senescence. Previous studies have examined the eccDNA of long inbred strains of mice, senescence-prone series and senescence-resistant series [28, 29]. Although eccDNA appeared more quickly in senescence-prone mice, it underwent amplification with normal aging in senescence-resistant mice [30]. In age-related disorders (ARDs) and physiological aging, reduced exclusion mechanisms cause eccDNA to accumulate in the nucleus of eukaryotic cells. EccDNA generally travels along the nuclear actin toward the nuclear pore complex (NPC) in young, healthy cells, where it is excluded from the nucleus. However, malfunctioning NPCs, aberrant NPC composition, and increased nuclear actin rods may decrease eccDNA exclusion from the nucleus in senescence and ARDs. Consequently, elevated levels of eccDNA can be observed in senescence and ARDs. These results point to a possible significance of eccDNA in aging, illness, and cell function [31].

Immunization Mechanisms of EccDNA

Gene Ontology enrichment analyses, along with in vivo studies in mouse models, have revealed that eccDNA can trigger innate immune responses via the Sting1 (interferon gene stimulator) pathway. Furthermore, the high GC content of eccDNA is believed to play a significant role in triggering the innate immune system, particularly by means of the cGAS-STING signaling cascade [32]. In a pivotal study, researchers generated bone marrow-derived dendritic cells and macrophages to explore their immune reactions to three distinct DNA configurations: linear genomic DNA, eccDNA, and poly(dG:dC). The focus was particularly on the production of cytokines, including interferon (IFN)α, IFNβ, interleukin-6, and tumor necrosis factor (TNF). The results showed that cyclic DNA forms, notably eccDNA, markedly enhanced immune activation. Moreover, introducing a cut in eccDNA for linearization revealed that linearized eccDNAs behaved similarly to linear DNAs. Unlike cyclic DNA, linear DNA was unable to activate cytokines. This phenomenon supports that cyclic eccDNA has potent immunostimulatory activity [33] and may induce primary B-cell and T-helper type 2 responses [34]. The close association of cyclic DNA with the immune response and related signaling pathways suggests that eccDNA is an important stimulator of the immune response. MtDNA is also a cyclic DNA that plays a crucial role in triggering innate immunity. A previous study demonstrated that purified mtDNA could induce inflammation and arthritis in mice, and its addition to mouse splenocytes increased TNF-α secretion [35]. MtDNA is also recognized as an activator of Toll-like receptor 9 (TLR9), primarily due to its CpG motifs that stimulate TLR9 signaling [36, 37]. Moreover, mtDNA can integrate into the nucleus in the event of chromosomal damage, thereby contributing to the development of human pathological conditions [38, 39]. A previous study induced DNA double-stranded breaks (DSBs) using doxorubicin (DOX) and subsequently performed fluorescence in situ hybridization analysis with mtDNA probes to compare DOX-treated and untreated groups. The results showed that DOX treatment increased the levels of mtDNA insertion into chromosomes [40] (Fig. 1).

Complex Mechanisms of EccDNA Formation

The formation of eccDNA is intricate and varied, and numerous theories have been proposed to explain its origins. Although the exact mechanisms are still unclear, there are four types of eccDNA formation processes: homologous recombination, nonhomologous end joining, DNA replication, and R-loop formation [2, 16, 41–43]. Beyond these, several specific ways of eccDNA formation have been recognized, including the breakage-fusion-bridge (BFB) cycle, chromothripsis model, episome model, and translocation-deletion-amplification mechanism (Fig. 2).

BFB Cycle

The BFB cycle, which was first proposed by McClintock [44] in 1951 while studying the mechanism of variable sites in maize, is the classical model for eccDNA formation. Triggered by external stimuli, the BFB cycle unfolds when the body's DNA repair mechanisms fail to rectify double-stranded DNA breaks in time. This failure can lead to the loss of chromosomal telomeres during replication, which prompts the fusion of the broken ends of sister chromatids into a dicentric chromosome. This formation eventually transitions into a late-replicating anaphase bridge, characterized by the presence of two centromeres, which, upon breaking, results in the unequal distribution of genetic material to daughter cells [45]. This causes the extensive focal amplification of DNA ladders and numerous deletions. The

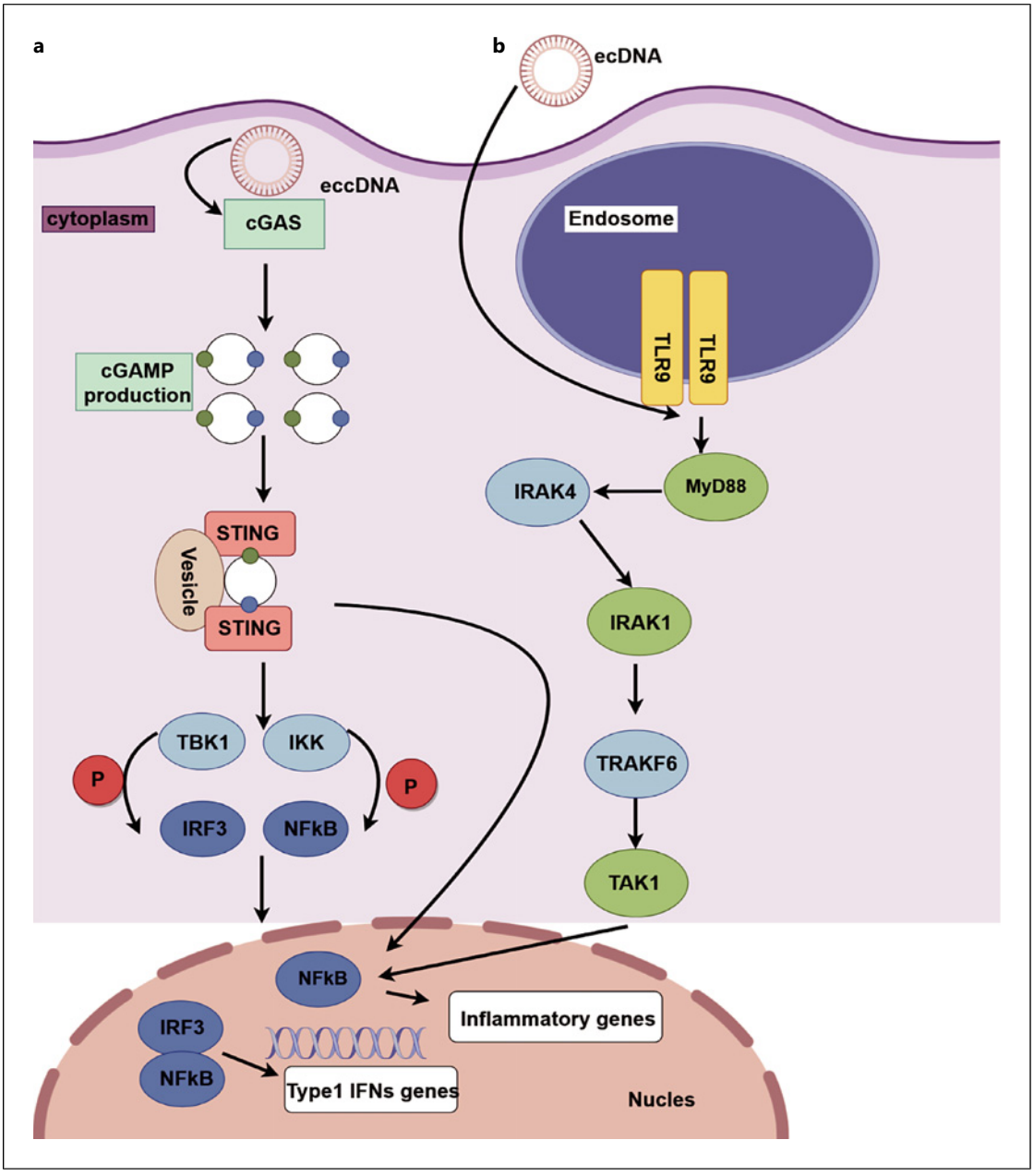

Fig. 1. EccDNA initiating immune responses. **a** EccDNA activates the cGAS-STING pathway, thereby initiating an innate immune response. **b** EcDNA significantly enhances the signaling transduction of TLR9-MyD88-NF-kB in plasma, thereby promoting the secretion of pro-inflammatory cytokines.

formation of telomere-free bridges can be amplified through the replication process. This cycle is repeated to extend telomere-free bridges, which eventually detach and circularize into eccDNA. This is followed by chromosome splitting or entry into another BFB cycle [46]. The BFB cycle causes severe genetic abnormalities and various chromosomal aberrations in cancer cells [47].

Chromothripsis

The chromothripsis model is based on the observation that tens to hundreds of genomic rearrangements occur in a one-time cell crisis in patients with chronic lymphocytic leukemia [48]. According to this model, catastrophic events shatter chromosomes into numerous pieces, generating a multitude of sequence fragments.

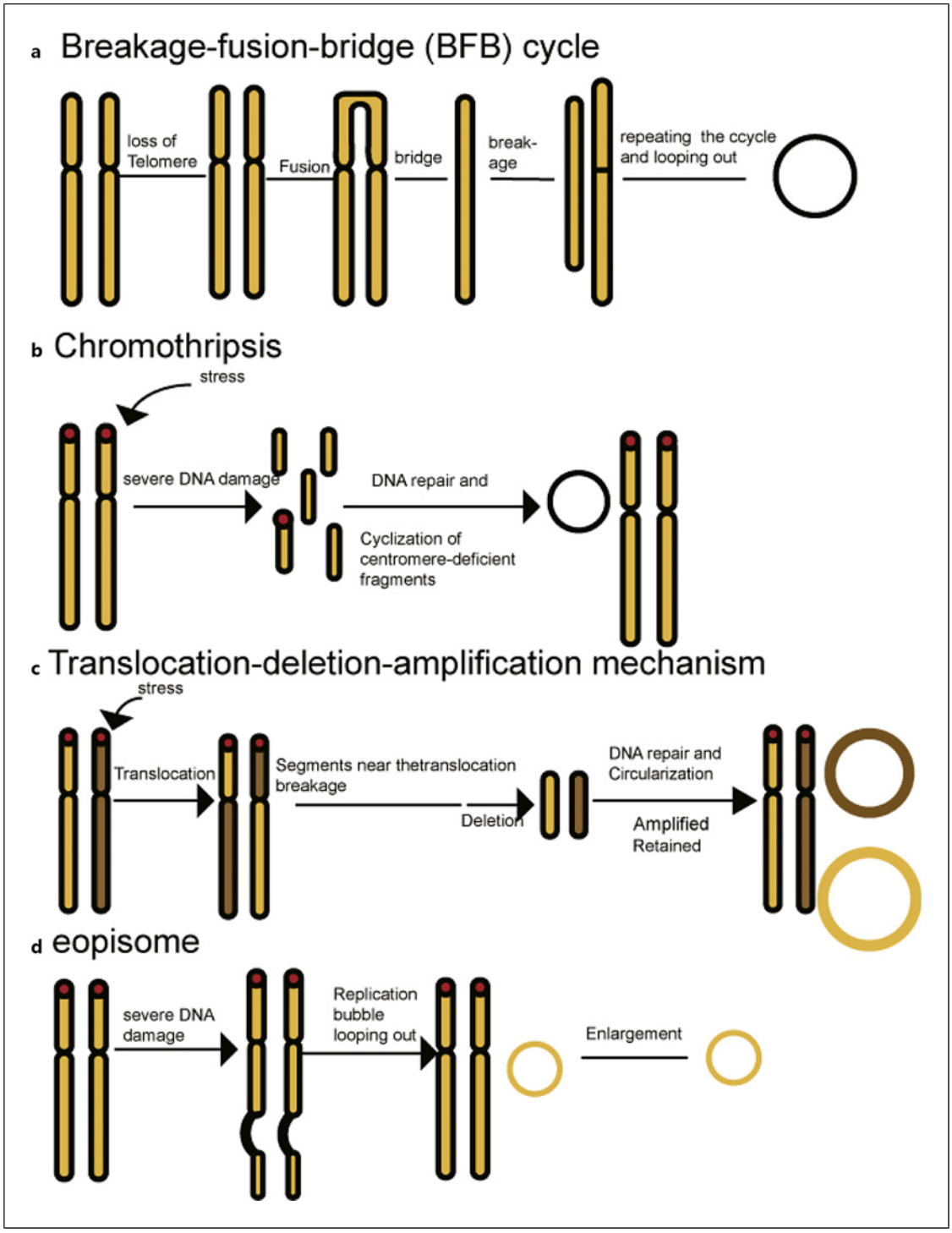

Fig. 2. The mechanism of eccDNA formation. **a** The double-strand DNA breaks and telomere loss, where the broken ends of the two sister chromatids fuse together, leading to the formation of a dicentric chromosome and subsequently evolving into a late replication bridge. This is further amplified by replication to form eccDNA. **b** The chromosomes, when shattered by catastrophic events, break down into fragments. Afterward, these fragments may recombine in unconventional ways, forming eccDNA. **c** The translocation-deletion-amplification mechanism is often triggered by exogenous stimuli, with gene rearrangements occurring near translocation sites. Fragments near the translocation breakpoints may be repaired or removed by the DNA repair system, but if amplified or retained, they form ecDNA and HSRs. **d** Small, extrachromosomal, circular molecules can mediate gene amplification and have been termed "episomes." Episomes might represent an unrecognized type of structural variation within the genome, produced through intragenomic recombination events to generate eccDNA.

Although the repair system removes most of these DNA fragments, some randomly connected fragments persist. These persistent fragments lead to locally aggregated DNA rearrangements [45]. During mitosis, these irregularities can precipitate incorrect chromosome segregation, setting the stage for chromothripsis – a catastrophic cycle of fragmentation and faulty reassembly. Moreover, this process may generate rearranged entities, including eccDNA [49]. Shoshani et al. [50] demonstrated that this process can be initiated by the catalytic subunits of poly (ADP-ribose) polymerases and DNA-dependent protein kinases. Intriguingly, the induction of chromothripsis in tumors has been linked to methotrexate treatment, with a notable dosage dependency. This particular phenomenon has been found in various tumors, including pancreatic cancer, neuroblastoma, prostate cancer, pediatric medulloblastoma, and small-cell lung cancer [51].

Translocation-Deletion-Amplification Mechanism

In 1996, Barr et al. [52] introduced the translocation-deletion-amplification model and verified that oncogenes are typically activated in a sequential manner triggered by exogenous stimuli. This model also elucidated the co-amplification of the oncogene *MYC* alongside AT motif-binding factor 1 (*ATBF1*) [53]. Furthermore, it highlighted the co-amplification and overexpression of tumor-related genes *HMGIC* and *MDM2* as eccDNA in carcinoma ex pleomorphic adenoma, showcasing the model's power for predicting the fate of DNA fragments near translocation breakpoints. By contrast, those that are retained or amplified can form ecDNA and HSRs [53].

Episome Model

The episome model, which was proposed by Carroll et al. in 1987, is one of the most important models of eccDNA biogenesis. Carroll et al. found that small, circular extrachromosomal molecules can mediate gene amplification and named these molecules "episomes" [54]. Episomes could be a kind of structural variation in the genome that is underappreciated. They are generated through a process where eccDNA, which is produced from recombination events within the genome, further recombines with other eccDNA fragments. This suggests that DNA cyclization might play a crucial role in the mobility of retrotransposable elements across the genome [55]. Recombination processes that eliminate sequences containing replication start sites and nearby genes produce episomes, which are tiny precursors of autonomously replicating ecDNA. These episomes either undergo amplification, leading to ecDNA production, or integrate into chromosomes, generating HSRs [56]. Storlazzi et al. [57] highlighted the creation of DM chromosomes containing *MYC* genes in leukemia cases through excision and amplification processes. Carroll et al. indicated that the *CAD* episome gradually increases in size over time. Furthermore, they have confirmed that these episomes are in fact DM precursors. The primary difference between the two lies in their size, with DM demonstrating greater stability [56]. Moreover, HSRs and DMs are both specific chromosomal alterations found in cancer cells, which are capable of transforming into one another under certain conditions. During the cloning process of human neuroblastoma cells and melanocyte culture, previous studies have observed that an increase in HSRs is often accompanied by a decrease in DMs, and vice versa [58, 59]. These studies further support that the episome model is a significant mechanism for eccDNA generation.

Association of EccDNA with Diseases

Role of EccDNA in Autoimmune Diseases

There are about 80 different autoimmune diseases, which are unified by a common pathology of the immune system erroneously attacking the body's own tissues. EccDNA plays a significant role in autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and potential autoimmune disease-type 2 diabetes mellitus (T2DM) [60, 61] (Table 1). EccDNA can be detected in cells at the early stages of the development of autoimmune diseases, opening up the possibility for early diagnosis. Compared with traditional biomarkers, eccDNA can be analyzed in the blood or other bodily fluids, enabling the noninvasive detection and diagnosis of autoimmune diseases even before clinical symptoms appear [62].

This is particularly evident in RA, wherein ecDNA plays a particularly compelling role. Compared with healthy individuals, patients with RA have elevated levels of plasma ecDNA, underscoring its potential as a biomarker for this autoimmune disease. In a previous study, a significant reduction (16%) in plasma ecDNA concentration was observed among patients following 3 months of treatment with biological disease-modifying antirheumatic drugs, highlighting the relevance of ecDNA in RA monitoring [63]. Furthermore, ecDNA was found to significantly stimulate TLR9-MyD88-NF-κB signaling in the plasma of patients with RA, thereby promoting the secretion of pro-inflammatory cytokines [32].

Cell-free DNA (cfDNA), primarily in linear forms, plays a pivotal role in the onset of immune diseases,

Table 1. Characterization of cyclic extracellular DNA in autoimmune diseases and its implications

Types of autoimmune diseases	EccDNA characteristics	EccDNA impact	Specific marker
Rheumatoid arthritis (RA)	Plasma eccDNA levels are higher in RA patients than in healthy controls	Stimulation of TLR9-MyD88-NF-KB signaling promotes pro-inflammatory cytokine secretion	Yes
Systemic lupus erythematosus (SLE)	The amount of eccDNA in HC was significantly smaller than that in DNASE1L3-deficient SLE and both have different eccDNA distributions	The unique eccDNA gene BARX2 in DNASE1L3-deficient SLE, which is involved in skeletal muscle morphogenesis and connective tissue development	Yes
Type 2 diabetes mellitus (T2DM)	Differences in the number and distribution of eccDNA between patients with T2DM and the healthy population were observed	EccDNA-associated genes are significantly enriched in the phosphatidylinositol signaling pathway in T2DM patients	Yes

which is in contrast to eccDNA derived from linear DNA fragments. CfDNA was first reported in human plasma by Mandel and Metais [64] in 1948 [64, 65]. In 1966, elevated levels of cfDNA were linked to SLE [66]. In SLE, the pathogenicity of anti-DNA autoantibodies is well established. The enzyme deoxyribonuclease 1-like 3 (DNASE1L3) plays a crucial role in mitigating SLE progression by efficiently eliminating cfDNA, thus reducing the production of anti-DNA autoantibodies. Consequently, individuals lacking DNASE1L3 exhibit an increased risk of developing SLE [67]. Advanced differential analysis of eccDNA using the DifCir method in DNASE1L3-deficient patients with SLE revealed unique gene region excision patterns distinct from those in healthy individuals [68]. Furthermore, the presence of cell-free eccDNA (cf-eccDNA) from specific genes was consistently observed in all SLE samples lacking DNASE1L3, unlike in healthy controls [69]. These findings suggest that the abundance, size distribution, and gene-specific cf-eccDNA profiles could serve as valuable biomarkers for SLE activity.

The research conducted by Kong et al. [70] marks a pivotal advancement in our understanding of T2DM through the lens of eccDNA. By collecting, purifying, and sequencing eccDNA from serum samples of patients with newly diagnosed T2DM and healthy controls, the research uncovered differences in the quantity and distribution of eccDNA across the two groups, especially in chromosomes 1, 14, 16, and X. These findings suggest a potential association between T2DM and eccDNA changes within specific chromosomal regions. Moreover, the study identified 598 significantly upregulated eccDNAs and 856 significantly downregulated eccDNAs in the serum of patients with T2DM compared with healthy controls. The researchers analyzed the significantly upregulated eccDNA, named *SORBS1*, and measured its expression in cells from a cellular model of insulin resistance characterized by reduced phosphorylation of key signaling molecules in the insulin signaling pathway. The eccDNA *SORBS1* circle level was significantly increased within the insulin resistance cell model. Moreover, Kong et al. [70] demonstrated that the level of eccDNA *SORBS1* circles in an insulin-resistant cell model was associated with apoptotic DNA fragmentation. This study not only reveals the potential mechanism of eccDNA action in T2DM but also suggests the potential of eccDNA and the genes it carries as possible biomarkers or therapeutic targets in diseases. The insights obtained beckon for further investigations into the contribution of eccDNA to T2DM pathogenesis and its utility in developing strategies for disease prevention, diagnosis, and treatment [70].

Role of EccDNA in Tumors

Tumor Promotion by EccDNA

EccDNA has emerged as a critical player in cancer biology, driving tumor initiation and progression through mechanisms that markedly deviate from traditional genetic inheritance. EccDNA is associated with oncogene amplification, and it does not follow traditional chromosomal mechanisms of inheritance. The absence of telomeres in eccDNA facilitates unequal distribution among daughter cells during cell division, potentially leading to an augmented copy number of oncogenes in some cells compared with their progenitors. The uniqueness of this genetic mechanism allows eccDNA to rapidly adapt to environmental changes, leading to a rapid increase in oncogene copy number [12, 19].

Alteration of oncogenes and enhancers: eccDNA also alters oncogenes and enhancers to further promote cancer development. Promotion of drug resistance in

cancer cells: eccDNA enhances drug resistance in cancer cells by upregulating drug target genes and transporter proteins [25]. Intercellular messaging: eccDNA may transmit messages between cells through extracellular vesicles, thus forming more complex biological networks and promoting tumor heterogeneity. Compared with small RNAs, eccDNA has a longer lifespan, more stable structure, and the capacity to carry a wealth of genetic data, enabling it to deliver more information in intercellular communication [51].

Changes in EccDNA Expression Profiles in Tumors

Investigating the pathological significance of eccDNA in tumors is one of the most rigorously pursued areas of study. EccDNAs are increasingly recognized for their potential pivotal role in cancer initiation and advancement. The variations in their abundance and size distribution could mirror the distinct molecular characteristics of tumor cells. Research across various cancers, including neuroblastoma, glioblastoma, colorectal cancer, ovarian cancer, breast cancer, lung cancer, gastric cancer, liver cancer, and esophageal cancer, has demonstrated the significant pathological effects of eccDNA on these tumors [51, 71–74].

In a previous study on high-grade serous ovarian cancer (HGSOC), a particularly aggressive form of ovarian cancer often diagnosed at the metastatic stage, eccDNA has been implicated in the genomic remodeling associated with the disease [75]. Cen et al. [76] provided a detailed characterization of eccDNA expression profiles in primary and metastatic tissues of HGSOC, encompassing analyses of their chromosomal origins, expression levels, length variations, and GC content. They found that eccDNAs from primary and metastatic tissues originated from all chromosomes. However, they observed a significant difference in the expression frequencies of eccDNAs, with metastatic tissues showing a wider range (32,113–48,817) compared with primary tissues (12,871–7,053). The results of size distribution analysis revealed that the average sizes of eccDNAs in primary and metastatic tissues were 388 and 379 bp, respectively. Furthermore, the eccDNA sequences of both tissues showed enrichment in GC content, suggesting that eccDNA tended to possess higher GC content than other genomic regions. These findings suggest that an enriched GC content is a common feature of eccDNA, indicating that eccDNA plays a crucial role in the genesis, development, and metastasis of HGSOC. The analysis also revealed 464 differentially expressed eccDNAs in metastatic tissues compared with primary tissues [76].

Wu et al. [77] comparatively analyzed the size distribution, chromosomal origin, formation, and expression patterns of eccDNA between patients with lung adenocarcinoma (LUAD) and healthy populations, including healthy pregnant women. They found that the average size of eccDNA in samples was under 800 bp, predominantly clustering around sizes of 191 and 320 bp. Interestingly, the eccDNA quantity in patients with LUAD was fivefold higher than that in pregnant women. Moreover, the eccDNA length was more frequently near 200 bp in patients with LUAD than in healthy individuals [77]. Parallel observations were noted in non-small-cell lung cancer (NSCLC), where an average of 945 and 1,005 eccDNAs were detected in normal lung tissues and NSCLC tissues, respectively, underscoring the prevalence of eccDNAs in NSCLC. In a previous study that examined the functions of differentially expressed eccDNA-related genes in normal lung tissues of NSCLC by bioinformatics analysis, the Kyoto Encyclopedia of Genes and Genomes results suggested that these genes played an important regulatory role in the nicotinic acid and nicotinamide metabolic pathways [71].

In a study on gastric cancer, eccDNA gene expression levels were compared between gastric cancer tissues and normal adjacent tissues. The abundance of eccDNA was found to be much higher in tumor tissues than in normal adjacent tissues. This finding highlights the importance of eccDNA in tumor development. The researchers also examined the length distribution of eccDNA between tumor tissues and normal adjacent tissues. They found that in both tissue samples, the presence of eccDNA with a length of 364 bp was significantly higher in the gastric cancer block than in paracancerous tissues. This specificity suggests that eccDNA of this particular size may have a pivotal role in gastric cancer progression. Furthermore, the researchers discovered that eccDNAs smaller than 250 bp were significantly more prevalent in paracancerous tissues than in tumor tissues, whereas eccDNAs ranging from 250 bp to 1,000 bp were more frequently found in tumor tissues than in paracancerous tissues. This pattern implies that gastric tumors are prone to generating longer eccDNAs, which can encompass broader functional genomic sequences, potentially contributing to cancer development and progression. In the chromosome distribution analysis, tumor tissues were found to have a higher density of eccDNA on chromosomes 8 and 20 compared with paracancerous tissues. Conversely, the density of eccDNA was significantly higher in paracancerous tissues than in tumor tissues on chromosomes 9 and 21. This finding reflects the increased instability of eccDNA expression in tumors [72] (Table 2).

Table 2. Study and analysis of cyclic extracellular DNA characterization and expression in different cancer types

Type of study	EccDNA characteristics	Frequency of expression	Key findings
High-grade plasma ovarian cancer (HGSOC)	The average size is 388 bp (range 370–399 bp), mean eccDNA of metastatic tissue 379 bp (range 371–419 bp)	Original substitute (32,113–48,817) is higher than transfer (12,871–7,053)	The decrease of DNMT1circle10302690-10302961 is associated with poor prognosis in HGSOC patients
Lung adenocarcinoma (LUAD)	The average size is less than 800 bp, concentrated at 191 bp and 320 bp	Patients (6,512–455,371) is lower than healthy gravidas (216,064–490,941)	Host genes that downregulate eccDNA in LUAD are mainly involved in cardiomyopathy, axon guidance, calcium signaling pathway, and hormone secretion
Non-small cell lung cancer (NSCLC)	Length distribution ranges from 0.01 kb to 1,000 kb	NSCLC (mean expression 1,005) is higher than normal (mean expression 945)	The KEGG results showed that differentially expressed eccDNAs played an important regulatory role in the nicotinate and nicotinamide metabolism pathways
Gastric cancer	The average size is 364 bp	Gastric cancer (mean expression 248,283) is higher than adjacent tissues (mean expression 35,379)	Inheritance of GCT overexpressed eccMIRs favors host cell growth, survival, and progression

Role of EccDNA in Hereditary Diseases

Hereditary diseases, which are characterized by genetic alterations, manifest through familial patterns and profoundly impact individual and familial health. Alzheimer's disease is a hereditary neurodegenerative disorder, and certain inherited disorders affect DNA repair and chromosome stability, which may be related to eccDNA formation and accumulation. In 2023, Smalheiser [78] proposed insightful scientific hypotheses concerning eccDNA formation in Alzheimer's disease and its potential mechanisms of action. Since neuron stimulation triggers immediate early gene transcription to cause DSBs, eccDNA can be formed on DSBs. Therefore, Smalheiser [78] suggested that activated neurons may produce eccDNA during the repair of DSB sites. He also suggested that eccDNA may regulate the translation and transcription of other genes involved in synaptic plasticity, learning, and memory. EccDNA can also masquerade as exosomes released by neurons and thus regulate gene expression. Thus, activity-induced eccDNA may provide new experimental tools for studying the physiology and pathology of Alzheimer's disease and may provide biomarkers that reflect neuronal activity and health status. Turnover targeting eccDNA or its RNA transcripts may provide a new therapeutic strategy [78].

Dyskeratosis congenita (DC) is a genetically heterogeneous syndrome leading to bone marrow failure and increased cancer risk. The loss of function of the *RTEL1* gene is thought to be a contributing factor to DC. In our study, we found that a heterozygous mutation at the R1264H locus in the *RTEL1* gene leads to the production of a large number of extrachromosomal T-loops, resulting in telomere shortening. The clinical features of DC may be a consequence of defects in telomere maintenance and function, and although the exact mechanism behind the link between eccDNA and DC remains unclear, a link between the two has been demonstrated [62].

Theoretically, the impact of eccDNA on hereditary diseases is manifested by the fact that it can carry genes or gene fragments, whose generation and accumulation may lead to gene expression changes, thus affecting individuals' genetic diversity and disease susceptibility. In some cases, gene mutations carried in eccDNA may directly lead to the development of hereditary diseases. Second, eccDNAs can also be involved in regulating host gene expression. For example, some eccDNAs may contain enhancer or repressor sequences that can affect the expression level of specific genes, and this regulatory mechanism may play a key role in the development of hereditary diseases.

EccDNA and Other Diseases

Research on the association of eccDNA with many types of diseases has revealed its potential roles and mechanisms in a wide range of pathologies, providing new insights into understanding its role in disease

progression. In a study on gouty arthritis, eccDNA was found to have a new potential biological role. Gouty arthritis is an inflammatory disease that is usually triggered by the deposition of crystals in tissues and joints; moreover, it is the most common form of arthritis among men [79]. Scientists have sequenced and examined eccDNA from the plasma of healthy people and patients with acute gout. They found that acute gout and healthy control plasma samples had 57,216 and 109,683 eccDNAs, respectively. These sequences, sourced from all chromosomes in patients with gout and healthy individuals, revealed a notable observation; no significant differences were identified across most chromosomes, except for chromosome 18, where eccDNA frequencies notably diverged between the gout and control groups. Numerous distinct eccDNAs were found with a pair of separate peaks in the size distribution, measuring 201 and 339 bp. Importantly, genes linked to these unique eccDNAs were identified, pointing toward a potential involvement in inflammation or immune response pathways.

In a previous study investigating eccDNA in the anterior lens capsule, eccDNA was found to be very common in the anterior lens capsule of the human eye. Using functional enrichment analysis for the differentially mapped genes of eccDNA, the researchers identified differences in eccDNA and gene expression in the anterior lens capsule of patients with nuclear cataract alone and patients with nuclear cataract with high myopia. Moreover, they discussed the mechanism of eccDNA in patients with nuclear cataract with high myopia [80]. In another study that analyzed the characteristics, formation processes, and potential functions of eccDNA in osteoporotic or normal bone tissues, eccDNA was found to be widespread in these tissues and might play a key role in the age-related development pathway of osteoporosis [81].

Conclusion

EccDNA has been widely investigated for its role in cell proliferation, differentiation, senescence, and death in various physiological and pathological processes. However, there are numerous challenges in eccDNA research, including limitations in detection technologies and a comprehensive understanding of its biological functions. With the rapid advancement of science and technology, the future exploration of eccDNA is expected to overcome existing limitations, ushering in new research domains and therapeutic avenues. Cutting-edge genome editing technologies, including the CRISPR-Cas system, are anticipated to enable precise manipulation and functional studies of eccDNA, unraveling its specific roles in cellular life activities. Furthermore, the advancements in high-throughput sequencing technology will enhance the sensitivity of eccDNA detection, providing robust tools for studying the relationship between eccDNA and diseases.

These advancements lay the foundation for further exploration of the biological functions and applications of eccDNA. In the field of disease treatment, research on eccDNA is poised to revolutionize the diagnosis and treatment of various diseases, including cancer and autoimmune diseases. The utilization of eccDNA as a biomarker holds the potential for early disease diagnosis, prognostic assessment, and monitoring of therapeutic effects. Exploring targeted therapeutic strategies against eccDNA, including the specific inhibition of eccDNA production or function, may offer novel solutions for diseases resistant to traditional methods. However, realizing these potential applications requires overcoming challenges, including improving the accuracy and specificity of eccDNA detection, gaining a deeper understanding of the mechanisms of eccDNA in different diseases, and developing safe and effective interventions.

Moreover, interdisciplinary collaboration will be pivotal in advancing eccDNA research. Integrating knowledge and technologies from diverse fields, including molecular biology, genomics, and bioinformatics, will provide substantial momentum to unravel the mysteries of eccDNA. In conclusion, eccDNA research is entering a new era filled with potential and challenges. By deepening our understanding of the biological functions of eccDNA and exploring applications in diseases using cutting-edge technologies, eccDNA research is poised to advance basic science and bring innovative therapeutic strategies to clinical medicine, thereby improving patient prognosis and quality of life.

Conflict of Interest Statement

All authors declare no conflicts of interest.

Funding Sources

No funding has been received.

Author Contributions

Yong Dai and Huihui Tao: conceptualization. Yali Peng, Mengyao Wu, and Guoying Wang: data curation. Yali Peng, Tiantian Xu, Xuejia Zheng, and Chunmei Wen: formal analysis. Yali Peng and Huihui Tao: writing – original draft. Yong Dai: writing – review and editing.

References

1 Wang Y, Wang M, Zhang Y. Purification, full-length sequencing and genomic origin mapping of eccDNA. Nat Protoc. 2023;18(3):683–99. https://doi.org/10.1038/s41596-022-00783-7

2 Ling X, Han Y, Meng J, Zhong B, Chen J, Zhang H, et al. Small extrachromosomal circular DNA (eccDNA): major functions in evolution and cancer. Mol Cancer. 2021;20(1):113. https://doi.org/10.1186/s12943-021-01413-8.

3 Mondelo-Macía P, Castro-Santos P, Castillo-García A, Muinelo-Romay L, Diaz-Peña R. Circulating free DNA and its emerging role in autoimmune diseases. J Pers Med. 2021;11(2):151. https://doi.org/10.3390/jpm11020151.

4 Hotta Y, Bassel A. Molecular size and circularity of DNA in cells of mammals and higher plants. Proc Natl Acad Sci U S A. 1965;53(2):356–62. https://doi.org/10.1073/pnas.53.2.356

5 Lubs HA Jr, Salmon JH. The chromosomal complement of human solid tumors. II. Karyotypes of glial tumors. J Neurosurg. 1965;22:160–8. https://doi.org/10.3171/jns.1965.22.2.0160

6 Cox D, Yuncken C, Spriggs AI. Minute chromatin bodies in malignant tumours of childhood. Lancet. 1965;1(7402):55–8. https://doi.org/10.1016/s0140-6736(65)90131-5

7 Radloff R, Bauer W, Vinograd J. A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in HeLa cells. Proc Natl Acad Sci U S A. 1967;57(5):1514–21. https://doi.org/10.1073/pnas.57.5.1514

8 Jones RS, Potter SS. L1 sequences in HeLa extrachromosomal circular DNA: evidence for circularization by homologous recombination. Proc Natl Acad Sci U S A. 1985;82(7):1989–93. https://doi.org/10.1073/pnas.82.7.1989

9 Ohki R, Oishi M, Kiyama R. Preference of the recombination sites involved in the formation of extrachromosomal copies of the human alphoid Sau3A repeat family. Nucleic Acids Res. 1995;23(24):4971–7. https://doi.org/10.1093/nar/23.24.4971

10 Brewer BJ, Fangman WL. The localization of replication origins on ARS plasmids in S. cerevisiae. Cell. 1987;51(3):463–71. https://doi.org/10.1016/0092-8674(87)90642-8

11 Gaubatz JW. Extrachromosomal circular DNAs and genomic sequence plasticity in eukaryotic cells. Mutat Res. 1990;237(5–6):271–92. https://doi.org/10.1016/0921-8734(90)90009-g

12 Turner KM, Deshpande V, Beyter D, Koga T, Rusert J, Lee C, et al. Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. Nature. 2017;543(7643):122–5. https://doi.org/10.1038/nature21356.

13 Wu P, Liu Y, Zhou R, Li T, Yu X, Liu L, et al. Extrachromosomal Circular DNA: A New Target in Cancer. Front Oncol. 2022;12(1):814504. https://doi.org/10.3389/fonc.2022.814504.

14 Saccone C, Gissi C, Lanave C, Larizza A, Pesole G, Reyes A, et al. Evolution of the mitochondrial genetic system: an overview. Gene. 2000;261(1):153–9. https://doi.org/10.1016/s0378-1119(00)00484-4.

15 Sugiura M. The chloroplast genome. Essays in biochemistry. 1995;30:49–57.

16 Paulsen T, Kumar P, Koseoglu MM, Dutta A. Discoveries of extrachromosomal circles of DNA in normal and tumor cells. Trends Genet. 2018;34(4):270–8. https://doi.org/10.1016/j.tig.2017.12.010.

17 Møller HD, Ramos-Madrigal J, Prada-Luengo I, Gilbert MTP, Regenberg B. Near-random distribution of chromosome-derived circular DNA in the condensed genome of pigeons and the larger, more repeat-rich human genome. Genome Biol Evol. 2020;12(1):3762–77. https://doi.org/10.1093/gbe/evz281

18 Shibata Y, Kumar P, Layer R, Willcox S, Gagan JR, Griffith JD, et al. Extrachromosomal microDNAs and chromosomal microdeletions in normal tissues. Science. 2012;336(6077):82–6. https://doi.org/10.1126/science.1213307

19 Verhaak RGW, Bafna V, Mischel PS. Extrachromosomal oncogene amplification in tumour pathogenesis and evolution. Nat Rev Cancer. 2019;19(5):283–8. https://doi.org/10.1038/s41568-019-0128-6

20 Wu S, Turner KM, Nguyen N, Raviram R, Erb M, Santini J, et al. Circular ecDNA promotes accessible chromatin and high oncogene expression. Nature. 2019;575(7784):699–703. https://doi.org/10.1038/s41586-019-1763-5.

21 Wu S, Bafna V, Chang HY, Mischel PS. Extrachromosomal DNA: an emerging hallmark in human cancer. Annu Rev Pathol. 2022;17:367–86. https://doi.org/10.1146/annurev-pathmechdis-051821-114223.

22 Cohen S, Lavi S. Induction of circles of heterogeneous sizes in carcinogen-treated cells: two-dimensional gel analysis of circular DNA molecules. Mol Cell Biol. 1996;16(5):2002–14. https://doi.org/10.1128/mcb.16.5.2002

23 Paulsen T, Shibata Y, Kumar P, Dillon L, Dutta A. Small extrachromosomal circular DNAs, microDNA, produce short regulatory RNAs that suppress gene expression independent of canonical promoters. Nucleic Acids Res. 2019;47(9):4586–96. https://doi.org/10.1093/nar/gkz155.

24 Lin CC, Meyne J, Sasi R, Moyzis RK. Apparent lack of telomere sequences on double minute chromosomes. Cancer Genet Cytogenet. 1990;48(2):271–4. https://doi.org/10.1016/0165-4608(90)90131-s.

25 Luo J, Li Y, Zhang T, Xv T, Chen C, Li M, et al. Extrachromosomal circular DNA in cancer drug resistance and its potential clinical implications. Front Oncol. 2022;12:1092705. https://doi.org/10.3389/fonc.2022.1092705.

26 Møller HD, Mohiyuddin M, Prada-Luengo I, Sailani MR, Halling JF, Plomgaard P, et al. Circular DNA elements of chromosomal origin are common in healthy human somatic tissue. Nat Commun. 2018;9(1):1069. https://doi.org/10.1038/s41467-018-03369-8.

27 Hull RM, King M, Pizza G, Krueger F, Vergara X, Houseley J. Transcription-induced formation of extrachromosomal DNA during yeast ageing. PLoS Biol. 2019;17(12):e3000471. https://doi.org/10.1371/journal.pbio.3000471.

28 Takeda T, Hosokawa M, Takeshita S, Irino M, Higuchi K, Matsushita T, et al. A new murine model of accelerated senescence. Mech Ageing Dev. 1981;17(2):183–94. https://doi.org/10.1016/0047-6374(81)90084-1.

29 Hosokawa M, Kasai R, Higuchi K, Takeshita S, Shimizu K, Hamamoto H, et al. Grading score system: a method for evaluation of the degree of senescence in senescence accelerated mouse (SAM). Mech Ageing Dev. 1984;26(1):91–102. https://doi.org/10.1016/0047-6374(84)90168-4.

30 Yamagishi H, Kunisada T, Takeda T. Amplification of extrachromosomal small circular DNAs in a murine model of accelerated senescence. A brief note. Mech Ageing Dev. 1985;29(1):101–3. https://doi.org/10.1016/0047-6374(85)90051-x

31 Qiu G-H, Zheng X, Fu M, Huang C, Yang X. The decreased exclusion of nuclear eccDNA: from molecular and subcellular levels to human aging and age-related diseases. Ageing Res Rev. 2021;67:101306. https://doi.org/10.1016/j.arr.2021.101306.

32 Karami Fath M, Akbari Oryani M, Ramezani A, Barjoie-Mojarad F, Khalesi B, Delazar S, et al. Extra chromosomal DNA in different cancers: individual genome with important biological functions. Crit Rev Oncol Hematol. 2021;166:103477. https://doi.org/10.1016/j.critrevonc.2021.103477.

33 Wang Y, Wang M, Djekidel MN, Chen H, Liu D, Alt FW, et al. eccDNAs are apoptotic products with high innate immunostimulatory activity. Nature. 2021;599(7884):308–14. https://doi.org/10.1038/s41586-021-04009-w.

34 Marichal T, Ohata K, Bedoret D, Mesnil C, Sabatel C, Kobiyama K, et al. DNA released from dying host cells mediates aluminum adjuvant activity. Nat Med. 2011;17(8):996–1002. https://doi.org/10.1038/nm.2403.

35 Collins LV, Hajizadeh S, Holme E, Jonsson IM, Tarkowski A. Endogenously oxidized mitochondrial DNA induces in vivo and in vitro inflammatory responses. J Leukoc Biol. 2004;75(6):995–1000. https://doi.org/10.1189/jlb.0703328.

36 Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature. 2010;464(7285):104–7. https://doi.org/10.1038/nature08780.
37 Zhang Q, Itagaki K, Hauser CJ. Mitochondrial DNA is released by shock and activates neutrophils via p38 map kinase. Shock. 2010;34(1):55–9. https://doi.org/10.1097/SHK.0b013e3181cd8c08
38 Turner C, Killoran C, Thomas NST, Rosenberg M, Chuzhanova NA, Johnston J, et al. Human genetic disease caused by de novo mitochondrial-nuclear DNA transfer. Hum Genet. 2003;112(3):303–9. https://doi.org/10.1007/s00439-002-0892-2.
39 Borensztajn K, Chafa O, Alhenc-Gelas M, Salha S, Reghis A, Fischer AM, et al. Characterization of two novel splice site mutations in human factor VII gene causing severe plasma factor VII deficiency and bleeding diathesis. Br J Haematol. 2002;117(1):168–71. https://doi.org/10.1046/j.1365-2141.2002.03397.x.
40 Harutyunyan T, Al-Rikabi A, Sargsyan A, Hovhannisyan G, Aroutiounian R, Liehr T. Doxorubicin-induced translocation of mtDNA into the nuclear genome of human lymphocytes detected using a molecular-cytogenetic approach. Int J Mol Sci. 2020;21(20):7690. https://doi.org/10.3390/ijms21207690.
41 Wang M, Chen X, Yu F, Ding H, Zhang Y, Wang K, et al. Extrachromosomal circular DNAs: origin, formation and emerging function in cancer. Int J Biol Sci. 2021;17(4):1010–25. https://doi.org/10.7150/ijbs.54614.
42 Cao X, Wang S, Ge L, Zhang W, Huang J, Sun W. Extrachromosomal circular DNA: category, biogenesis, recognition, and functions. Front Vet Sci. 2021;8:693641. https://doi.org/10.3389/fvets.2021.693641.
43 Liao Z, Jiang W, Ye L, Li T, Yu X, Liu L. Classification of extrachromosomal circular DNA with a focus on the role of extrachromosomal DNA (ecDNA) in tumor heterogeneity and progression. Biochim Biophys Acta Rev Cancer. 2020;1874(1):188392. https://doi.org/10.1016/j.bbcan.2020.188392.
44 McClintock B. Chromosome organization and genic expression. Cold Spring Harb Symp Quant Biol. 1951;16:13–47. https://doi.org/10.1101/sqb.1951.016.01.004
45 Ly P, Cleveland DW. Rebuilding chromosomes after catastrophe: emerging mechanisms of chromothripsis. Trends Cell Biol. 2017;27(12):917–30. https://doi.org/10.1016/j.tcb.2017.08.005
46 Zakov S, Kinsella M, Bafna V. An algorithmic approach for breakage-fusion-bridge detection in tumor genomes. Proc Natl Acad Sci U S A. 2013;110(14):5546–51. https://doi.org/10.1073/pnas.1220977110
47 Murnane JP. Telomeres and chromosome instability. DNA repair. 2006;5(9–10):1082–92. https://doi.org/10.1016/j.dnarep.2006.05.030
48 Rausch T, Jones DTW, Zapatka M, Stutz AM, Zichner T, Weischenfeldt J, et al. Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. Cell. 2012;148(1–2):59–71. https://doi.org/10.1016/j.cell.2011.12.013.
49 Zhang CZ, Spektor A, Cornils H, Francis JM, Jackson EK, Liu S, et al. Chromothripsis from DNA damage in micronuclei. Nature. 2015;522(7555):179–84. https://doi.org/10.1038/nature14493.
50 Shoshani O, Brunner SF, Yaeger R, Ly P, Nechemia Arbely Y, Kim DH, et al. Chromothripsis drives the evolution of gene amplification in cancer. Nature. 2021;591(7848):137–41. https://doi.org/10.1038/s41586-020-03064-z.
51 Wang T, Zhang H, Zhou Y, Shi J. Extrachromosomal circular DNA: a new potential role in cancer progression. J Transl Med. 2021;19(1):257. https://doi.org/10.1186/s12967-021-02927-x.
52 Barr FG, Nauta LE, Davis RJ, Schafer BW, Nycum LM, Biegel JA. In vivo amplification of the PAX3-FKHR and PAX7-FKHR fusion genes in alveolar rhabdomyosarcoma. Hum Mol Genet. 1996;5(1):15–21. https://doi.org/10.1093/hmg/5.1.15.
53 Van Roy N, Vandesompele J, Menten B, Nilsson H, De Smet E, Rocchi M, et al. Translocation-excision-deletion-amplification mechanism leading to nonsyntenic coamplification of MYC and ATBF1. Genes Chromosomes Cancer. 2006;45(2):107–17. https://doi.org/10.1002/gcc.20272.
54 Carroll SM, Gaudray P, De Rose ML, Emery jf, Meinkoth JL, Nakkim E, et al. Characterization of an episome produced in hamster cells that amplify a transfected CAD gene at high frequency: functional evidence for a mammalian replication origin. Mol Cell Biol. 1987;7(5):1740–50. https://doi.org/10.1128/mcb.7.5.1740-1750.1987.
55 Møller HD, Larsen CE, Parsons L, Hansen AJ, Regenberg B, Mourier T. Formation of extrachromosomal circular DNA from long terminal repeats of retrotransposons in Saccharomyces cerevisiae. G3. 2015;6(2):453–62. https://doi.org/10.1534/g3.115.025858.
56 Carroll SM, DeRose ML, Gaudray P, Moore CM, Needham Vandevanter DR, Von Hoff DD, et al. Double minute chromosomes can be produced from precursors derived from a chromosomal deletion. Mol Cell Biol. 1988;8(4):1525–33. https://doi.org/10.1128/mcb.8.4.1525-1533.1988.
57 Storlazzi CT, Lonoce A, Guastadisegni MC, Trombetta D, D'Addabbo P, Daniel G, et al. Gene amplification as double minutes or homogeneously staining regions in solid tumors: origin and structure. Genome Res. 2010;20(9):1198–206. https://doi.org/10.1101/gr.106252.110.
58 Balaban-Malenbaum G, Gilbert F. Double minute chromosomes and the homogeneously staining regions in chromosomes of a human neuroblastoma cell line. Science. 1977;198(4318):739–41. https://doi.org/10.1126/science.71759
59 Trent JM, Thompson FH, Ludwig C. Evidence for selection of homogeneously staining regions in a human melanoma cell line. Cancer Res. 1984;44(1):233–7.
60 Rose NR. Prediction and prevention of autoimmune disease in the 21st century: a review and preview. Am J Epidemiol. 2016;183(5):403–6. https://doi.org/10.1093/aje/kwv292
61 Tsai S, Clemente-Casares X, Revelo X S, et al. Are obesity-related insulin resistance and type 2 diabetes autoimmune diseases? Diabetes. 2015;64(6):1886–97. https://doi.org/10.2337/db14-1488.
62 Ballew BJ, Joseph V, De S, Sarek G, Vannier JB, Stracker T, et al. A recessive founder mutation in regulator of telomere elongation helicase 1, RTEL1, underlies severe immunodeficiency and features of Hoyeraal Hreidarsson syndrome. PLoS Genet. 2013;9(8):e1003695. https://doi.org/10.1371/journal.pgen.1003695.
63 Lauková L, Konečná B, Vlková B, Mlynarikova V, Celec P, Stenova E, et al. Anti-cytokine therapy and plasma DNA in patients with rheumatoid arthritis. Rheumatol Int. 2018;38(8):1449–54. https://doi.org/10.1007/s00296-018-4055-8.
64 Mandel P, Metais P. Nuclear acids in human blood plasma. C R Seances Soc Biol Fil. 1948;142(3–4):241–3.
65 Tan EM, Kunkel HG. Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. J Immunol. 1966;96(3):464–71. https://doi.org/10.4049/jimmunol.96.3.464
66 Dunaeva M, Buddingh' BC, Toes REM, Luime JJ, Lubberts E, Pruijn GJM, et al. Decreased serum cell-free DNA levels in rheumatoid arthritis. Auto Immun Highlights. 2015;6(1–2):23–30. https://doi.org/10.1007/s13317-015-0066-6.
67 Mathapathi S, Chu CQ. Contribution of impaired DNASE1L3 activity to anti-DNA autoantibody production in systemic lupus erythematosus. Rheumatol Immunol Res. 2022;3(1):17–22. https://doi.org/10.2478/rir-2022-0003
68 Sin ST, Deng J, Ji L, Yukawa M, Chan RW, Volpi S, et al. Effects of nucleases on cell-free extrachromosomal circular DNA. JCI insight. 2022;7(8):e156070. https://doi.org/10.1172/jci.insight.156070.
69 Gerovska D, Araúzo-Bravo MJ. Systemic lupus erythematosus patients with DNASE1L3-deficiency have a distinctive and specific genic circular DNA profile in plasma. Cells. 2023;12(7):1061. https://doi.org/10.3390/cells12071061
70 Kong X, Wan SJ, Chen TB, Jiang L, Xing YJ, Bai YP, et al. Increased serum extrachromosomal circular DNA SORBS1(circle) level is associated with insulin resistance in patients with newly diagnosed type 2 diabetes mellitus. Cell Mol Biol Lett. 2024;29(1):12. https://doi.org/10.1186/s11658-023-00530-0.

71 Yang Y, Yang Y, Huang H, Song T, Mao S, Liu D, et al. PLCG2 can exist in eccDNA and contribute to the metastasis of non-small cell lung cancer by regulating mitochondrial respiration. Cell Death Dis. 2023;14(4):257. https://doi.org/10.1038/s41419-023-05755-7.
72 Jiang X, Pan X, Li W, Han P, Yu J, Li J, et al. Genome-wide characterization of extrachromosomal circular DNA in gastric cancer and its potential role in carcinogenesis and cancer progression. Cell Mol Life Sci. 2023;80(7):191. https://doi.org/10.1007/s00018-023-04838-0.
73 Zou S, Chen S, Rao G, Zhang G, Ma M, Peng B, et al. Extrachromosomal circular MiR-17-92 amplicon promotes HCC. Hepatology. 2024;79(1):79–95. https://doi.org/10.1097/hep.0000000000000435.
74 Sun Z, Ji N, Zhao R, Liang J, Jiang J, Tian H, et al. Extrachromosomal circular DNAs are common and functional in esophageal squamous cell carcinoma. Ann Transl Med. 2021;9(18):1464. https://doi.org/10.21037/atm-21-4372.
75 Lheureux S, Gourley C, Vergote I, Oza AM. Epithelial ovarian cancer. Lancet. 2019;393(10177):1240–53. https://doi.org/10.1016/s0140-6736(18)32552-2.
76 Cen Y, Fang Y, Ren Y, Hong S, Lu W, Xu J. Global characterization of extrachromosomal circular DNAs in advanced high grade serous ovarian cancer. Cell Death Dis. 2022;13(4):342. https://doi.org/10.1038/s41419-022-04807-8.
77 Wu X, Li P, Yimiti M, Ye Z, Fang X, Chen P, et al. Identification and characterization of extrachromosomal circular DNA in plasma of lung adenocarcinoma patients. Int J Gen Med. 2022;15:4781–91. https://doi.org/10.2147/ijgm.S363425.
78 Smalheiser NR. Mobile circular DNAs regulating memory and communication in CNS neurons. Front Mol Neurosci. 2023;16:1304667. https://doi.org/10.3389/fnmol.2023.1304667
79 So AK, Martinon F. Inflammation in gout: mechanisms and therapeutic targets. Nat Rev Rheumatol. 2017;13(11):639–47. https://doi.org/10.1038/nrrheum.2017.155
80 Wen K, Zhang L, Cai Y, Teng H, Liang J, Yue Y, et al. Identification and characterization of extrachromosomal circular DNA in patients with high myopia and cataract. Epigenetics. 2023;18(1):2192324. https://doi.org/10.1080/15592294.2023.2192324.
81 Zhu Q, Chen R, Kuang M, Zhang W, Wang D, Han S. Identification and characterization of extrachromosomal circular DNA in age-related osteoporosis. Aging. 2023;15(24):15489–503. https://doi.org/10.18632/aging.205388.