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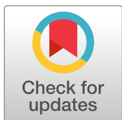
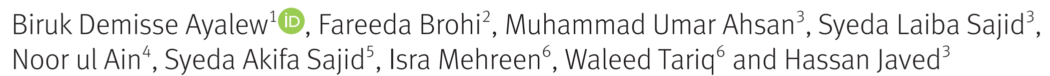


Targeting Extrachromosomal DNA in Cancer: A New Frontier in Precision Oncology



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ABSTRACT

Extrachromosomal DNA (ecDNA) has recently been recognized as a key driver of cancer biology, contributing to oncogene amplification, transcriptional reprogramming, intratumoral heterogeneity, and therapeutic resistance. Unlike chromosomal DNA, ecDNA exists as circular, acentromeric elements that undergo dynamic segregation, cluster into hubs, and engage in enhancer hijacking, thereby maintaining aggressive tumor phenotypes. This narrative review synthesizes current evidence on the molecular mechanisms underlying ecDNA formation, including Chromothripsis, breakage–fusion–bridge (BFB) cycles, and replication stress, and highlights how these events reform cancer genome architecture. We further discuss the rapid advances in detection methods, ranging from next-generation and single-cell sequencing to CRISPR-based capture techniques and liquid biopsy approaches, which have improved the identification and characterization of ecDNA in clinical samples. Increasing evidence links ecDNA to poor prognosis and therapy failure, underscoring its value as a prognostic biomarker and therapeutic target. Finally, we explore emerging strategies to disrupt ecDNA maintenance and function, which may open new avenues in precision oncology. Overall, ecDNA research is redefining our understanding of cancer evolution and offers promising translational opportunities.

Keywords: Extrachromosomal DNA (ecDNA); Liquid biopsy; Oncogene amplification; Precision oncology; Therapy resistance; Tumor heterogeneity

INTRODUCTION

The first identification of ecDNA occurred in the mid-20th century, when scientists spotted double minutes as tiny acentric chromatin bodies in cancer cell metaphase spreads.[1] Scientists initially viewed these cytogenetic anomalies as strange, yet they contained amplified oncogenes, which later established their direct connection to cancer progression.[2] The occurrence of ecDNA spans multiple cancer types because it is a prevalent feature across diverse malignancies, thereby demonstrating its widespread clinical importance.

The initial detection of ecDNA relied mainly on cytogenetic approaches, which used Giemsa banding for identification. These methods provided unbiased detection but suffered from limited resolution and dataset biases, leading to underreported ecDNA prevalence.[3–5] The introduction of high-throughput sequencing technologies brought a revolutionary change in detection methods. The development of next-generation sequencing (NGS) enabled scientists to analyze complete tumor genomes, revealing that ecDNA is widespread across solid tumor types. The extensive analysis of 13,000 cancer genomes through machine learning in 2024 established ecDNA as both widespread and prognostically important, thus advancing ecDNA research to a new critical point.[6] The development of Circle-Seq for detecting small ecDNA and single-cell long-read sequencing now enables researchers to precisely map ecDNA structures from copy-number information to junctional architecture.[7,8] Recent discoveries about ecDNA have elevated it from a scientific novelty to an established characteristic of cancer biology.

Oncogenes are substantially amplified through the functional activities of ecDNA. Numerous studies have found that MYC, EGFR, MYCN, HER2, and DHFR genes are commonly found on ecDNA in various cancer types, including glioblastoma, neuroblastoma, breast, ovarian, and colorectal cancers.[9–15] The ability of ecDNA to harbor multiple copies and its uneven distribution during cell division facilitates tumor diversity while enhancing cellular adaptation to treatment challenges, leading to faster tumor development. "ecDNA-positive cancers" tend to have worse patient outcomes because of their link to poor prognosis and therapy resistance.[16,17]

The structural and epigenetic elements of ecDNA serve to boost its cancer-causing capacity beyond its gene amplification function. The chromatin within ecDNA exists in an open configuration that contains H3K27ac marks, which makes it function as a mobile enhancer. The distinctive structural arrangement of ecDNA permits it to form multiple transcriptional hubs where oncogenes can collaboratively express their functions and sustain oncogenic addiction.[18] Research demonstrates that ecDNA operates as a dynamic regulatory element that transforms transcriptional landscapes in cancer beyond its basic function as an amplified gene carrier.

The genomic and experimental evidence suggest that ecDNA can function as both a potential biomarker of aggressive tumor behavior and a promising therapeutic target. The blood of small-cell lung cancer and colorectal cancer patients contains circulating ecDNA; earlier studies suggest potential associations with tumor burden and treatment response, although the clinical utility of circulating ecDNA remains under investigation.[19,20] The available data demonstrate its promise as a non-invasive liquid biopsy instrument for monitoring disease in real time. Researchers are currently investigating different therapeutic approaches that exploit ecDNA-related weaknesses. Recent preclinical studies indicate that CHK1 inhibition specifically targets the survival of cells that harbor ecDNA,[21]

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suggesting that ecDNA status may serve as a marker for precision treatment decisions.

The new findings establish ecDNA as an essential element that drives cancer development. ecDNA started as a cytogenetic discovery but now functions as a clinically valuable biomarker together with its identified therapeutic weaknesses. This review examines ecDNA's role in causing tumor heterogeneity together with its diagnostic and therapeutic applications for implementing precision oncology strategies.

Methodology

Literature Search Strategy

A structured literature review was conducted to identify studies addressing the biology, detection, and therapeutic targeting of extrachromosomal DNA (ecDNA) in cancer. Electronic databases, including PubMed, Scopus, Web of Science, and the Cochrane Library, were systematically searched from database inception through December 2024. The search strategy combined controlled vocabulary and free-text terms related to extrachromosomal DNA and cancer biology. The core search string used across databases was: ("extrachromosomal DNA" OR ecDNA OR "double minutes" OR eccDNA)

AND (cancer OR tumor OR oncology)

AND (amplification OR replication OR therapy OR detection). Additional keywords were incorporated to capture mechanistic and translational aspects of ecDNA research, including Chromothripsis, Breakage Fusion Bridge (BFB) cycles, enhancer hijacking, ecDNA hubs, transcriptional condensates, therapy resistance, liquid biopsy, next-generation sequencing (NGS), single-cell sequencing, CRISPR-CATCH, replication stress, and homogeneously staining regions (HSRs). Reference lists of relevant review articles and primary research studies were manually screened to identify additional publications not captured in the initial database search.

Study Selection

All retrieved records were initially screened based on titles and abstracts to determine relevance to ecDNA biology or cancer research. Articles meeting the preliminary criteria were evaluated in full text.

Studies were included if they addressed at least one of the following areas:

- Molecular mechanisms of ecDNA formation and maintenance
- Detection technologies and computational methods for ecDNA identification
- Functional consequences of ecDNA in tumor biology
- Clinical associations of ecDNA with prognosis or therapeutic resistance
- Emerging strategies targeting ecDNA-associated vulnerabilities

When multiple publications described overlapping datasets, the most comprehensive or recent study was prioritized.

Inclusion Criteria

Studies were eligible for inclusion if they met the following criteria:

1. Investigated extrachromosomal DNA (ecDNA) or extrachromosomal circular DNA (eccDNA) within cancer biology.
2. Included experimental, preclinical, translational, or clinical data relevant to ecDNA mechanisms or therapeutic implications.
3. Provided mechanistic, diagnostic, prognostic, or therapeutic insights related to ecDNA.
4. Published in peer-reviewed journals and written in English.

Both original research articles and high-quality review articles were considered to provide comprehensive coverage of this rapidly evolving field.

Exclusion Criteria

Studies were excluded if they met any of the following criteria:

- Publications that are unrelated to cancer or oncogene amplification
- Studies without direct evidence on ecDNA or eccDNA
- Publications in languages other than English
- Conference abstracts, editorials, or commentaries lacking primary data or sufficient methodological details

Data Extraction and Evidence Classification

Relevant information from eligible studies was extracted and synthesized narratively. Extracted variables included:

- Cancer type and study model (cell line, animal model, or patient cohort)
- Oncogenes or genomic regions linked to ecDNA
- Mechanisms of ecDNA formation
- Experimental or computational detection techniques
- Clinical correlations, including prognosis or therapeutic resistance
- Therapeutic strategies targeting ecDNA or related vulnerabilities

To enhance interpretability, evidence supporting therapeutic or diagnostic claims was categorized by level, including preclinical studies (cell lines or animal models), translational studies, and clinical research investigations.

Quality Assessment

Although this work is a narrative review, the methodological quality of the included studies was carefully evaluated. Priority was given to studies with strong experimental validation, large-scale genomic analyses, or clinical cohorts. For clinical and observational studies, methodological rigor was assessed based on study design, sample size, and reproducibility of findings. Preclinical studies were evaluated according to experimental design, model relevance, and consistency with independent reports.

Reporting Framework

The literature selection process followed principles aligned with the Preferred Reporting Items for

Systematic Reviews and Meta-Analyses (PRISMA) framework to improve transparency in study identification and screening. A PRISMA-style flow diagram, showing the number of records identified, screened, excluded, and included, is provided in the Supplementary Materials.

DEFINITIONS AND NOMENCLATURE

Extrachromosomal DNA (ecDNA) refers to large, cir\ ular, acentric DNA elements (>100 kb) present outside chromosomes in cancer cells, often carrying oncogenes and regulatory elements. Extrachromosomal circular DNA (eccDNA) refers to small circular DNA molecules, usually less than 1 kb, which arise from genomic regions and generally lack coding sequences. The main differences between extrachromosomal DNA (ecDNA) and extrachromosomal circular DNA (eccDNA) include their size, coding capabilities, and replication characteristics. Large ecDNAs ranging from 100 kb to multiple megabases usually contain oncogenes and drug-resistance genes, along with regulatory elements, and possess the ability to self-replicate and enable clonal selection that benefits cancer cells.[22–25] eccDNA molecules do not replicate autonomously, avoid clonal selection, and are found in both normal and cancerous tissues. Unlike large ecDNAs, eccDNAs typically do not provide a survival advantage to cells.[26]

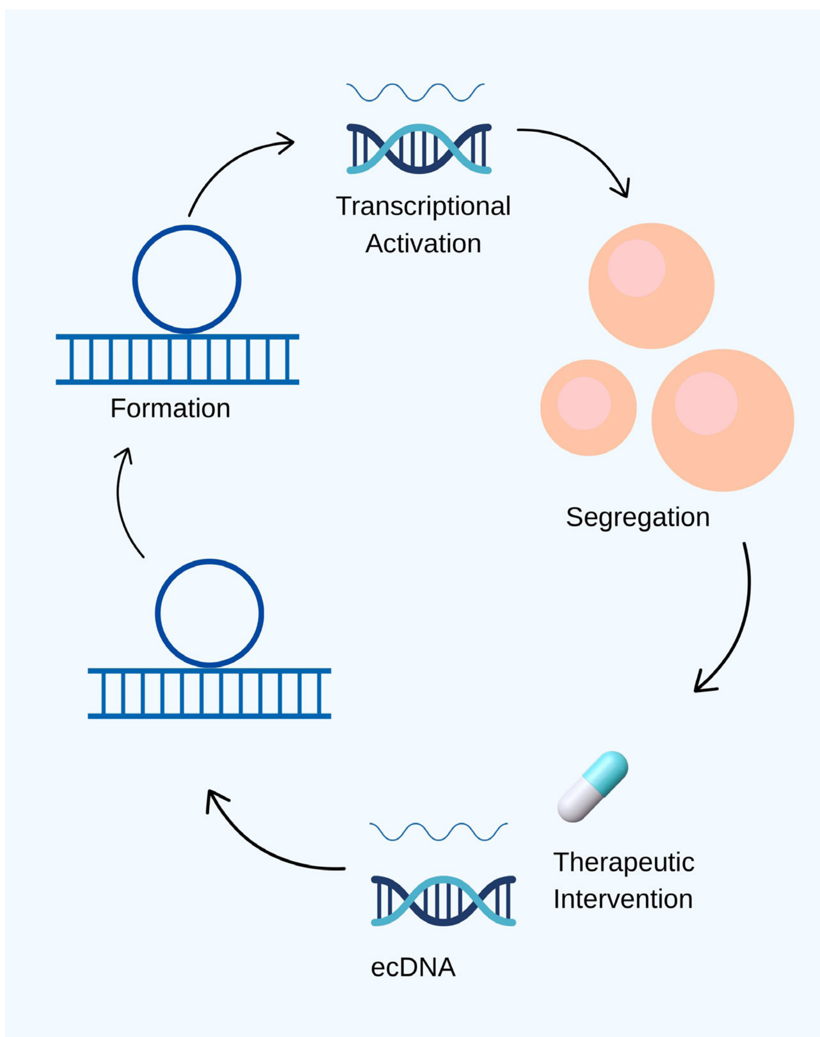

Fig 1 | Graphical abstract- ecDNA life cycle & intervention points

The circular DNA structure of ecDNA lacks both centromeres and telomeres. The lack of centromeres enables ecDNAs to segregate randomly during cell division, resulting in copy numbers ranging from zero to hundreds per cell and creating intratumoral heterogeneity.[23,24] The ability to exist in large numbers enables powerful oncogene amplification, while the circular structure maintains stability and facilitates interactions with regulatory elements.[22–25] The unique structural features of ecDNA set it apart from both chromosomal DNA and eccDNA.

Double minutes are small, cytogenetically visible extrachromosomal DNA fragments and are now recognized as a form of ecDNA. Double minutes, as cytogenetically visible ecDNA fragments, can integrate into chromosomes to form homogeneously staining regions (HSRs), which stabilize oncogene amplification and reduce mitotic heterogeneity.[27,28]

The open chromatin states of ecDNA exhibit exceptional functionality because they maintain high levels of H3K27ac, which promote easy transcriptional access. These DNA elements function as mobile enhancers, enabling multiple ecDNA copies to assemble into nuclear hubs for cooperative interactions. The elevated expression of oncogenes through these mechanisms surpasses chromosomal control, thereby propelling tumor progression, therapeutic resistance, and aggressive tumor characteristics.[29]

These structural and functional distinctions underpin the divergent roles of ecDNA and eccDNA in cancer progression, therapeutic resistance, and their potential as biomarkers (Figure 1).

MECHANISMS OF ecDNA BIOGENESIS

Genomic instability directly results in the production of extrachromosomal DNA (ecDNA). The precise biological mechanisms underlying ecDNA production remain partially unknown, despite evidence linking chromosomal damage and replication stress to its formation. Different models explain the process, which starts with Chromothripsis, followed by breakage–fusion–bridge (BFB) cycles, then replication errors, and finally, improper DNA repair mechanisms.[30] The distinct processes that create ecDNA in cancer cells probably result from the combined effects of multiple mechanisms.

Chromothripsis

Chromothripsis refers to a mutilation phenomenon in which locally clustered chromosomal DNA undergoes extensive fragmentation due to a catastrophic event, followed by haphazard, often incomplete ligation and reassembly of the DNA fragments into one or a few chromosomes. These rearrangements are also associated with DNA fragment deletion and widespread alteration in DNA sequence. During this process, some fragments of chromosomal DNA loop together, forming ecDNA.[31]

Chromothripsis is believed to arise from several mechanisms that remain under discussion to date. One such mechanism is the premature condensation of DNA within micronuclei, which lack proper transcription and replication and undergo multiple breaks due to their highly compacted state. Another involves telomere shortening and the loss of protective functions of RB and TP53 genes, which facilitate the formation of dicentric chromosomes and trigger breakage–fusion–bridge cycles. In addition, TP53 mutations that impair apoptosis and promote uncontrolled proliferation or hyperploidy have been strongly associated with Chromothripsis.[32,33] Chromothripsis-associated DNA fragmentation and ecDNA formation can be observed in a number of tumors, including medulloblastoma,[30] chronic lymphocytic leukemia,[34] osteosarcoma,[35] and glioblastoma.[10]

Breakage–Fusion–Bridge Cycles (BFB Cycles)

Another well-established mechanism of ecDNA formation is the breakage–fusion–bridge (BFB) cycle, which begins with the loss of a chromosome telomere followed by replication, resulting in sister chromatids lacking telomeres. These telomere-deficient chromatids undergo end-to-end fusion, forming a dicentric chromosome and an anaphase bridge. The bridge is subsequently broken, not necessarily at the site of fusion, and the resulting uneven chromatids in daughter cells undergo further replication and additional BFB cycles. The end products of multiple BFB cycles undergo circularization and eventually give rise to ecDNA.[36,37] Several studies indicate that BFB cycles work in association with other mechanisms of chromosomal alteration, such as chromothripsis, to create chromosomal rearrangements and abnormal gene amplification, leading to chemotherapeutic drug resistance and promoting tumor heterogeneity.[38]

Translocation–Deletion–Amplification Mechanism

The translocation–excision–deletion–amplification mechanism explains how aberrant gene amplifications and chromosomal translocations work in concert to encourage the formation of ecDNA. DNA translocations due to underlying external stimuli cause excision of DNA near the breakpoint. This excised portion of DNA undergoes amplification and circularization, giving rise to ecDNA.

This model is supported by the co-amplification of MYC and ATBF1 in SJNB-12 cells via a reciprocal t(8;16) translocation followed by excision and deletion, as well as the co-amplification of HMGIC and MDM2 linked to a t(10;12) (p15;q15) translocation in pleomorphic adenoma carcinoma. Similarly, chromosomal translocations and gene amplifications often co-occur in alveolar rhabdomyosarcoma, producing amplified and non-amplified ecDNA fragments that originate close to the breakpoint. However, the involvement of double-strand DNA breaks emphasizes the need for further research and points toward the involvement of other complex mechanisms associated with ecDNA formation.[39] In vivo amplification of the PAX3–FKHR and PAX7–FKHR fusion genes in alveolar rhabdomyosarcoma has also been reported.[40]

Fork Stalling and Template Switching

Another possible mechanism of ecDNA formation occurs when the DNA replication fork stalls at a lesion on template strands. In this situation, the lagging strand may detach and invade a nearby active replication fork, where it temporarily participates in DNA synthesis. Following repeated rounds of separation and reinvasion, the lagging strand finally rejoins its original template. Because the newly synthesized DNA is not fully complementary to the original strand, mismatches occur, leading to bulged single-stranded DNA regions. These single-stranded loops may then be replicated and develop into circular double-stranded structures, eventually giving rise to ecDNA.[41]

During replication, topoisomerase I and II reduce torsional stress, and topoisomerase II also resolves double-strand breaks. Key events in the template-switching model of ecDNA biogenesis, including fork stalling and collapse, can be favored by replication stress caused by impaired or dysregulated topoisomerase activity.[42]

DNA Repair Pathway Involvement

A major source of ecDNA originates from DNA double-strand breaks (DSBs). Repair pathways through non-homologous end joining (NHEJ) or microhomology-mediated end joining (MMEJ) operate as error-prone mechanisms that connect fragments into circular DNA and typically produce chromosomal scars.[43] Homologous recombination (HR) functions as a fidelity-based repair mechanism that can excise repetitive DNA to produce specialized circular DNA molecules, such as rDNA- and telomeric-derived eccDNAs. Replication-based processes include polymerase slippage, which creates DNA loops that are removed, and transcription-related R-loops that form single-stranded loops that become cut and circularized. These repair and replication processes together contribute to ecDNA biogenesis.[43]

Methods to Detect ecDNA/eccDNA

The precise identification of ecDNA and eccDNA, together with detailed characterization, remains crucial for studying their structural properties, population numbers, and biological effects in both cancerous and noncancerous cells. A variety of methods exist, including cytogenetic visualization along with high-throughput sequencing and computational analysis; however, each technique presents its own advantages and disadvantages (Figure 2). Tables 1 and 2 present a summary of essential methods used to detect and analyze ecDNA/eccDNA.

3D ORGANIZATION AND 'eCDNA HUBS'

Formation Of ecDNA Hubs in Nuclear Space

The three-dimensional mammalian DNA structure describes the manner in which the genome is folded

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Table 1 | Tools and Methods for ecDNA Detection

Tool	Method	Advantages	Disadvantages	Specifity
1. Fluorescence Microscopy-Based Tools				
FISH[21]	Experimental detection: The method involves applying fluorescent probe hybridization to metaphase spreads for direct observation of ecDNA	Direct visualization; distinguishes extrachromosomal vs chromosomal DNA	Limited resolution; requires actively cycling cells; low throughout	Moderate
EcSeg[21]	The Computational image analysis enables automated detection and quantification of ecDNA signals in FISH images	This method demonstrate better recall and F1 score performance compared to manual signal detection	Different input types require fine-tuning while the method shows potential to misinterpret specific signals.	Moderate
DAPI staining[17]	Experimental staining: DAPI staining in metaphase spreads enables visualization of double minutes during experimental procedures.	Simple; allows visualization of double minutes	Non-specific; cannot identify sequences; low resolution	Low
EcDetect[17]	Computational image analysis of DAPI-stained metaphase spread images undergo computational analysis to measure ecDNA presence levels.	Improves detection and quantification	The method depends on image quality levels while computational errors can occur.	Low
2. Sequencing-Based Tools				
Tool	**Method**	**Advantages**	**Disadvantages**	**Specificity**
Amplicon Reconstructor[83]	Computational reconstruction by combining WGS and optical mapping data	High-fidelity ecDNA reconstruction; breakpoints are resolved accurately	High cost; requires multiple platforms	Very High
Whole Genome Sequencing (WGS)[17,47]	Experimental sequencing of the whole genome to detect structural variants and circular DNA	Genome-wide detection; structural variant calling	Expensive; need high coverage	High
Amplicon Architect[17]	Computational reconstruction of circular amplicons from WGS data	Reconstructs ecDNA from low-cost short-read WGS	Data may misclassify; manual cutoff required	High
Circle-Seq[84]	Experimental enrichment and sequencing of circular DNA using exonuclease digestion and rolling-circle amplification	Enriches circular DNA; detects low-abundance ecDNA	Complex library prep; bias to extra small circles	High
Circle-Seq analysis[84]	Computational mapping and annotation of Circle-Seq data to identify eccDNA/ecDNA	Confirms circular DNA sequence; allows for annotation	Requires computational expertise	High
Single-cell DNA-seq[85]	Experimental sequencing of individual cells to detect ecDNA heterogeneity	Detects ecDNA per single cell and identifies heterogeneity	Low coverage per cell; expensive	Moderate–High
Single-cell DNA-seq analysis[85]	Computational analysis of single-cell DNA-seq data to detect and then annotate ecDNA	Detects ecDNA within single cell and annotates its identification	Computationally intensive; pipeline-dependent	Moderate–High
ATAC-seq[86]	Experimental transposase-accessible chromatin sequencing to capture open chromatin regions of ecDNA	Detects accessible ecDNA; helps identify ecDNA regions	Limited to accessible DNA; detection is indirect	Moderate–High
ATAC-seq analysis[86]	Computational analysis of sequencing reads to identify ecDNA regions	Identifies ecDNA from read files	Computational filtering required	Moderate–High
Circle-finder/Circle-Map[87]	Computational short-read paired-end mapping pipelines for eccDNA detection	Detects eccDNA from short-read sequencing	Limited for large ecDNA; enrishmeust occur prior to identification	Moderate–High
3. CRISPR-Based Tools				
Tool	**Method**	**Advantages**	**Disadvantages**	**Specificity**
ecTag[68]	Experimental in vivo visualization using CRISPR-Cas and Casilio system	Allows live-cell imaging of ecDNA	Requires prescise breakpoint design; limited to loci that canbe targeted	High
CRISPR-CATCH[88]	Experimental enrichment and profiling of megabase-sized ecDNA	Characterizes ecDNA at base resolution and epigenetic landscape	Only applicable for large ecDNA molecules; complex	High

and arranged in space within the small nuclear compartment. DNA coils around histones to form nucleosomes at the first level of chromatin structure. Higher-order folding leads to chromosome territories, compartments (A/B), and topologically associating domains (TADs).[44] Within these domains, proteins such as CTCF and cohesion manage cis-regulatory interactions, including enhancer–promoter loops, which control gene expression. Most interactions occur within the same DNA molecule, although inter-chromosomal contacts can occur. Endogenous gene loci are covalently linked to one another along the length of a chromosome and are engaged by regulatory elements in cis within their topologically associating domains (TADs). During interphase, chromosomes are dispersed to occupy distinct territories, while they acquire their highest condensed form during mitosis.[45]

Table 2 | Tools and methods for eccDNA detection[97–103]

Pipeline/Computational method	Wet lab/experimental method	Benefits	Drawbacks	Sensitivity
AmpliconArchitect[89]	Whole Genome Sequencing (Short Reads, WGS-SR)	• Offers genome-wide coverage for ecDNA and focal amplifications. • Can resolve amplicon structures and copy number profiles.	• Needs deep sequencing (expensive). • limited resolution with short reads; can't completely resolve highly complex or repetitive ecDNA structures.	Moderate–High (sequencing depth dependent; will miss low-abundance circles).
Circle-Map[90]	Circle-Seq-SR, 3SEP-SR (enzymatic linear DNA removal, circular DNA enrichment, and short-read sequencing)	• Enriches specifically for circular DNA, enhancing detection sensitivity. • Single-nucleotide resolution mapping by guided realignment.	• Restricted to circles passing enrichment steps (bias towards smaller eccDNA). • Loses quantitative data on absolute abundance.	High for small/medium eccDNA (particularly microDNAs), lower for very large circles.
Circle_finder[91]	ATAC-Seq-SR, Circle-Seq-SR, 3SEP-SR	• Multi-approach compatible (can employ both chromatin accessibility [ATAC-seq] and circle enrichment [Circle-Seq, 3SEP]). • Supports large-scale discovery of thousands of eccDNA in cancers.	• ATAC-seq only detects open chromatin eccDNA with bias in results. • Circle-Seq/3SEP create enrichment bias, particularly for small circles. • Needs high sequencing depth for accurate detection.	Moderate–High (ideal when enrichment-based Circle-Seq/3SEP employed; moderate for ATAC-seq).
ECCsplorer[92]	Circle-Seq-SR, 3SEP-SR	Easy-to-use modular pipeline; combines several detection approaches (mapping, clustering, split-read); efficient with short-read data.	Inefficient resolution on repetitive regions; sequencing depth-dependent performance.	High on short-read datasets but less efficient with repetitive complexes.
ecc_finder (asm, map)[93]	Circle-Seq-SR, 3SEP-SR, Circle-Seq-LR, 3SEP-LR	Compatible with both short- and long-read data; offers assembly-based and mapping-based detection; high-performance across species.	Computationally demanding for large genomes; assembly mode can be a source of false positives	High (SR + LR support enhances detection sensitivity).
eccDNA_RCA_nanopore[94]	Circle-Seq-LR, 3SEP-LR	Preserves full-length eccDNAs; high resolution for structural identification; unmasks immunostimulatory activity.	Specialized nanopore sequencing required; susceptible to sequencing artifacts if corrected	Extremely high for long circular DNA detection.
NanoCircle[95]	Circle-Seq-LR, 3SEP-LR	Optimized for human germline eccDNAs; precise detection with nanopore long reads; facilitates recombination hotspot analysis.	Requires high-quality long-read data; less tested in cancer settings.	Extremely high for long eccDNA, particularly germline-related.
CReSIL[96]	WGS-LR, Circle-Seq-LR, 3SEP-LR	Particularly designed for long-read WGS data; minimizes false positives with split-read + coverage features; precise structural resolution	Demands big long-read datasets; high computational expense.	Extremely high with long-read input; superior to short-read pipelines.

The open chromatin state of ecDNA, together with its minimal higher-order compaction, enables transcriptional machinery to better access oncogene loci.[46] The circular architecture of ecDNA allows it to make frequent cis-interactions; however, these molecules do not scatter randomly but instead form micron-sized clusters called ecDNA hubs or "extrasomes." The aggregation of ecDNA molecules into hubs depends on protein interactions, which simultaneously drive oncogene activity within these clusters.[18]

Role in Enhancer Hijacking and Oncogene Overexpression

The concentration of ecDNA in ecDNA hubs facilitates the formation of new enhancer–gene connections, resulting in oncogene overexpression. Unlike chromosomal loci limited by linear structure, ecDNA simultaneously multiplies enhancer elements while creating new pathways for gene regulation. Through this process, oncogenes can connect with distant or previously isolated enhancers, which leads to significant overexpression.[48]

The PVT1–MYC fusion represents promoter hijacking, which breaks free from feedback controls to maintain MYC expression across different cancer types, such as medulloblastomas.[18,49]

Trans-Regulatory Interactions

The self-sustaining gene deregulation mechanisms of ecDNA have been observed in viral–human hybrid ecDNAs, which demonstrate the flexible nature of ecDNA-driven gene deregulation. The distinctive capability of hubs to execute trans-regulatory interactions represents their main distinguishing characteristic. EcDNA molecules that carry enhancer elements but lack oncogenes work together with oncogene-encoding ecDNAs to increase transcription levels.[18] In gastric cancer cells (SNU16), such "altruistic" enhancer-only ecDNAs drive FGFR2 expression through trans enhancer–promoter interactions. The organization of hubs allows them to achieve oncogene activation that surpasses the capabilities of a single ecDNA molecule, thus setting them apart from chromosomal structures.[50]

Involvement Of Transcriptional Condensates

The transcriptional condensate function of ecDNA hubs leads them to attract coactivators like BRD4 and

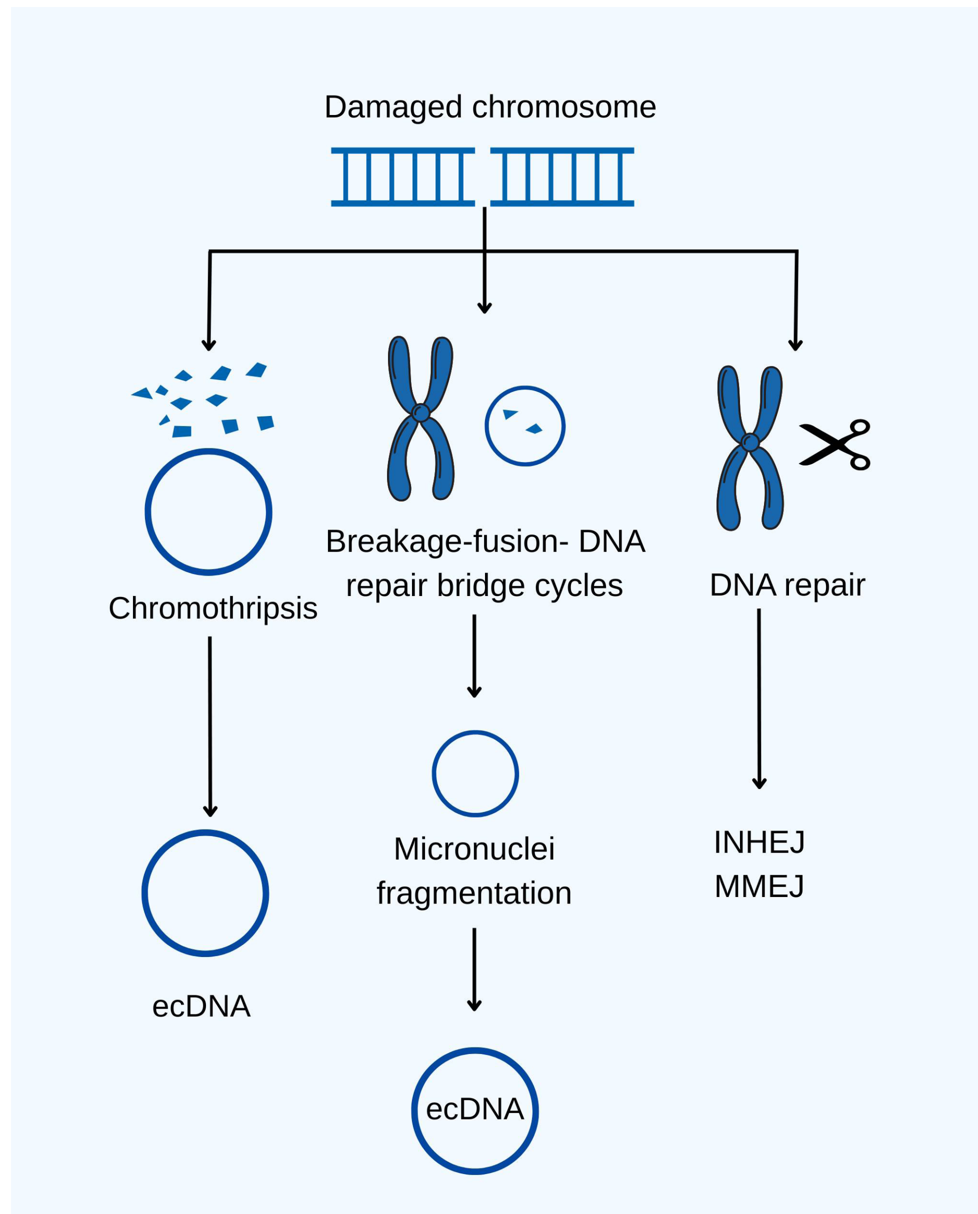

Fig 2 | Mechanisms of ecDNA formation

Mediator. The phase separation properties of these proteins enable them to bring together RNA polymerase II and other transcription machinery at hubs, which maintains elevated levels of transcription. The inhibition of BRD4 function through BET inhibitors has proven effective in breaking down hubs and decreasing MYC expression, thus revealing possible treatment opportunities.[51,52]

Mitotic Partitioning and Its Consequences for Heterogeneity

During mitosis, ecDNAs distribute in a random pattern that distinguishes them from chromosomal amplicons. The unequal distribution of ecDNA during cell division produces varying ecDNA copy numbers among daughter cells, which drives intratumoral heterogeneity and promotes evolutionary adaptability. The spatial arrangement of ecDNA hubs functions as both an enhancer of oncogenic transcription and a driver of tumor evolution and therapy resistance, making them essential targets for precision oncology treatments.[53]

Implications for Therapy Resistance

The understanding that ecDNA hubs function through protein-based interactions exposes potential vulnerabilities. Studies demonstrate that BET inhibitors (such as JQ1) effectively break down MYC ecDNA hubs and inhibit ecDNA-based gene expression in colorectal and gastric cancer cells. The dependency of ecDNA hubs on BRD4 and other oncogene-specific proteins suggests that discovering their tethering and stabilization proteins could reveal new cancer-type-specific therapeutic targets (Figure 3).[54]

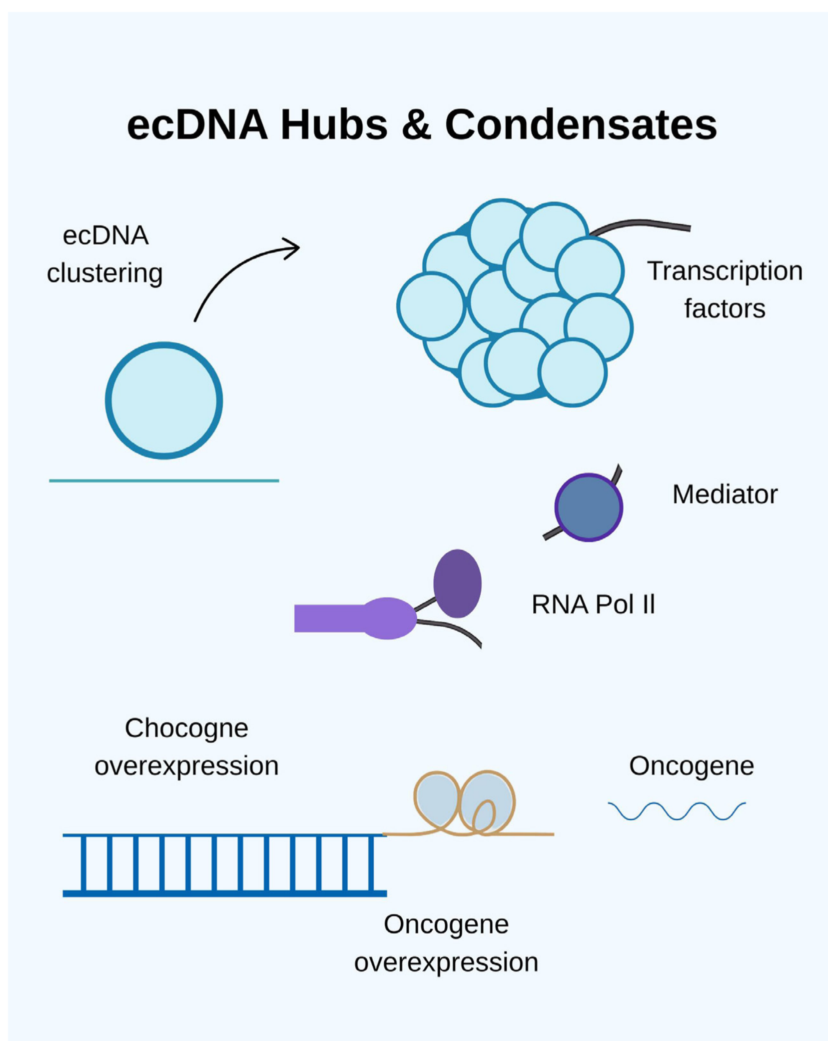

Fig 3 | ecDNA hubs & condensates

DETECTION & QUANTIFICATION

The precise detection and quantification of extrachromosomal DNA in cancer are increasingly at the forefront of precision oncology. A set of experimental, sequencing, and computational approaches has arisen, each with its own set of advantages and limitations, and their integration provides rich insights into ecDNA biology and clinical significance.

Early wet-lab methods remain fundamental to the field. Circle-Seq, for instance, is a common lab technique for the identification of ecDNA, based on exonuclease digestion to remove linear DNA, followed by φ29-polymerase-driven rolling-circle amplification of circular DNA. This enables sensitive recovery of heterogeneous circular DNA components but requires large input amounts and provides low structural resolution for large ecDNAs.[55] CRISPR-CATCH complements these efforts, using CRISPR–Cas9 to selectively excise megabase-sized ecDNA from cancer genomes; subsequent pulsed-field gel electrophoresis and nanopore sequencing deliver high-resolution amplification structure, breakpoint fidelity, and methylation information, exemplified by profiling EGFR and MYC ecDNAs and revealing hypomethylation at key oncogene promoters.[56] Fluorescence in situ hybridization (FISH) continues as a standard for visualizing ecDNAs in metaphase spreads or interphase nuclei, giving critical spatial and cellular context. Machine-learning enhancements like ecSeg are starting to improve ecDNA detection accuracy from imaging.[57]

Long-read sequencing technologies, notably Oxford Nanopore and PacBio, now enable reconstruction of full-length ecDNA structures, including complex rearrangements and epigenetic modifications. When coupled with enrichment methods such as CRISPR-CATCH, they enable direct, single-molecule resolution of ecDNA architecture and its regulatory aspects.[56]

On the computational side, whole-genome sequencing (WGS) can identify ecDNA amplicons by techniques like AmpliconArchitect, which models structural and copy-number variants, albeit with ambiguity in repeats. AmpliconReconstructor is then employed to enhance structural precision by means of optical mapping. While Circle-Map maximizes breakpoint identification from misaligned short reads, it is able to identify smaller eccDNAs.[58,59]

Single-cell and spatial methods, like scEC&T-seq (single-cell extrachromosomal circular DNA and transcriptome sequencing), allow the simultaneous profiling of circular DNA and full-length mRNA in single cancer cells. This demonstrates striking heterogeneity in ecDNA presence, composition, and transcriptional activity, such as the clonal amplification of oncogene-carrying ecDNAs and individual single-cell circular DNA profiles. It also illuminates mechanisms such as circular recombination fueling ecDNA evolution.[60] New technologies such as scCircle-seq extend this resolution even further by unpacking cell-to-cell diversity of eccDNAs in tissues and cancers, demonstrating how eccDNA landscapes can change stochastically with replication stress and cell division.[61]

Each approach offers unique advantages but also comes with constraints. Circle-Seq provides sensitivity with minimal structural context[62]; CRISPR-CATCH in combination with long-read sequencing provides rich information but is technically challenging;[56] FISH delivers spatial information but not molecular sequence, as it lacks sequence details;[57] computational methods are high-throughput but can fail in complex areas[47]; single-cell approaches provide heterogeneity resolution but at lower throughput and greater expense.[60]

In a clinical setting, a tiered strategy is often optimal: begin with computational ecDNA inference from routine WGS (e.g., AmpliconArchitect), follow positive cases with targeted, high-resolution structural mapping via CRISPR-CATCH and long reads, and validate or monitor with imaging such as FISH (enhanced by tools like ecSeg). Where tumor heterogeneity is relevant to treatment resistance or relapse, single-cell tools such as scEC&T-seq can uncover actionable ecDNA dynamics.[58,19]

Lastly, liquid biopsy techniques focused on identifying ecDNA in circulating tumor DNA, while still in their infancy, hold promise. Ultra-sensitive detection, such

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as digital PCR, could potentially allow for noninvasive monitoring of ecDNA-specific breakpoints or oncogene amplifications in plasma, allowing for real-time disease monitoring and adaptive therapy planning.[63,64]

Tumor ecDNA and Circulating eccDNA: Implications for Liquid Biopsy

It is important to distinguish tumor-derived ecDNA from circulating eccDNA present in plasma. While ecDNA originates from tumor cells and often carries oncogenic sequences, eccDNA can arise from normal cellular processes, such as apoptosis and inflammation, and is detectable even in healthy individuals.[16] This overlap creates a major limitation as the detection of circular DNA in plasma does not necessarily indicate tumor-derived ecDNA, thereby reducing specificity.[62,65] Thus, it can be seen that the presence of circular DNA in the circulation does not necessarily reflect the presence of ecDNA tumors. Additionally, pre-analytical variables, such as blood collection, tube type, time to plasma processing, storage condition, and prevention of leucocyte lysis, can significantly influence circulating DNA levels and affect assay accuracy.

This biological distinction poses significant challenges of specificity in cfDNA-based detection methods. The presence of circulating eccDNAs can be detected in healthy subjects, and detecting and quantifying ecDNAs based on circular DNA alone could be misleading, since variable background eccDNAs are also present and are not necessarily related to malignancy.[65] This, in turn, means that different attributes must be considered in addition to the approaches used in liquid biopsy.[58]

In addition to the above biological background, pre-analytical and analytical factors are known to influence the specificity of the eccDNA-based liquid biopsy. Some of these factors include blood tube type, delayed plasma separation, leukocyte lysis during blood handling, and storage conditions. These factors can artificially elevate the level of cell-free circular DNA and decrease the level of tumor cell-derived ecDNA.[16] Apart from these pre-analytical factors, biological factors can also influence the specificity of the eccDNA-based liquid biopsy. Some of these factors include systemic inflammatory responses, apoptosis, and cell turnover. However, these factors may be compounded in cancer patients due to chemotherapy-induced cell death, immune response, and necrosis.[62,65] Therefore, the specificity of eccDNA-based liquid biopsy can be achieved not just through the analysis of circular DNA; rather, some new approaches have been proposed to achieve specificity through the analysis of tumor-informative features of cell-free circular DNA, especially through junction-spanning assays that specifically target unique breakpoint sequences of cell-free circular DNA.[67] Some other approaches that can be used to achieve specificity include cell-free circular DNA methylation profiling, fragmentomics of cell-free circular DNA sizes, and the structure of oncogene-containing amplicons.[16,66,67]

Several approaches have arisen to address the problem of clinical specificity. On the one hand, a breakpoint-spanning assay targeting a unique circular junction formed during ecDNA formation shows high tumor specificity because these junctions are not present in the normal genome.[65] Moreover, elucidation of the DNA methylation signature of ecDNAs in the context of cancers might enable discrimination between tumor ecDNAs and background ecDNAs.[68] Finally, the variables of fragment size and ecDNA structure, including the presence of amplicons carrying oncogenes, might enable greater discrimination of ecDNAs through a combination of genomic and epigenomic analyses.[16,67] Therefore, the detection of tumor-derived ecDNA in plasma remains an evolving area and has not yet been standardized for routine clinical use.[69–72]

PREVALENCE & CLINICAL CORRELATES

During the last decade, extensive genomic investigations have shown that ecDNA is not a singular event but a ubiquitous phenomenon in many cancers. Pan-cancer studies imply that about 15%–20% of cancer cells carry ecDNA, with increased frequencies in aggressive solid tumors compared to hematological malignancies. This frequency highlights ecDNA as a widespread mechanism of oncogene amplification and a characteristic feature of tumor evolution, rather than an isolated exception.[16]

The mass of ecDNA is not equally distributed across cancers. The archetype ecDNA-carrying tumor is glioblastoma multiforme, where circular oncogene amplification has been frequently observed in replicated studies. Similarly, ecDNA is often present in sarcomas, lung cancer, and breast carcinomas, where it promotes intratumoral heterogeneity and clonal evolution. The most amplified oncogenes on ecDNA are MYC, EGFR, ERBB2 (HER2), and MDM2, which mirror their tumorigenic potential when uncoupled from chromosomal regulation.[19]

Clinically, the presence of ecDNA is strongly associated with poor prognosis and adverse outcomes. Amplification of ecDNA in tumors often correlates with a higher risk of relapse, faster tumor progression, and resistance to standard therapies. Mechanistic studies suggest that ecDNA enables tumors to escape the immune system through dynamic reorganization of gene expression, allowing rapid adaptation to selective pressures such as host immune surveillance. In addition, oncogene amplification via ecDNA is associated with enhanced genomic instability, which drives therapy resistance and tumor plasticity.[18]

Evolutionary evidence also outlines a predictive role for ecDNA following therapy. For example, the level of ecDNA harboring EGFR amplifications in glioblastoma has been associated with resistance to targeted inhibitors, whereas loss or reduction of ecDNA copies during treatment can occasionally restore sensitivity. These findings position ecDNA not only as a prognostic biomarker but also as a candidate for predicting and monitoring treatment efficacy in precision oncology (Table 3).[13]

Table 3. ecDNA prevalence and clinical associations by tumor type

Tumor Type	Estimated Prevalence of ecDNA	Frequent Oncogenes on ecDNA	Clinical Associations
Glioblastoma	High (~40%–50%)	EGFR, MYC, CDK4, PDGFRA	Poor prognosis, therapy resistance, high relapse risk
Sarcomas	Moderate to high (~30%–40%)	MDM2, CDK4, MYC	Aggressive biology, relapse risk
Lung cancer (NSCLC, SCLC)	Moderate (~20%–30%)	EGFR, MYC, ERBB2	Resistance to targeted therapy, poor survival
Breast cancer	Moderate (~15%–25%)	ERBB2 (HER2), MYC	Therapy resistance, immune evasion
Other solid tumors	Variable (~10%–20%)	MYC, MDM2, CCND1	Prognostic marker, clonal heterogeneity

THERAPEUTIC STRATEGIES

Target Replication Stress Vulnerabilities CHK1, ATR, and CDK Inhibition

EcDNAs often experience conflicts between transcription and replication, resulting in high levels of replication stress. This makes cancers with ecDNAs particularly reliant on checkpoint kinases. Novel preclinical research has found that blocking CHK1 actually increases replication stress and selectively targets tumors containing ecDNA in cell lines and animal models.[73] One of the preclinical studies lays the groundwork for the first early-phase clinical trial on ecDNA, BBI-355, a Phase 1/2 trial using an oral CHK1 inhibitor (NCT05292408). Additionally, ATR inhibitors such as ceralasertib (NCT03718091) and elimusertib (NCT03188965) are being tested in clinical trials for cancers driven by RS, which may provide a potential approach to treating tumors containing ecDNA.[74] Furthermore, inhibitors of CDK7/9, which increase transcriptional load, can be combined with therapies that induce RS by disrupting the transcriptional machinery.[75] Notably, emerging translational and early clinical data supported this therapeutic rationale. The clinical CHK1 inhibitor BBI-2799 has shown selective efficacy in ecDNA-driven models. These findings, elaborated in Section "Preclinical and Early Clinical Evidence" highlighted the importance of biomarker-guided CHK1 inhibition strategies in ecDNA-associated malignancies.

Disrupt ecDNA Hubs Bet Proteins, Degraders, and Cdk7/9

EcDNAs cluster to form transcriptional hubs, which are stabilized by BRD4 and include enhancers and the RNA polymerase II machinery.[76] Preclinical studies show that inhibiting BET with JQ1 disassembles these hubs and primarily decreases the transcription of oncogenes such as MYC derived from ecDNAs.[77] Several BET inhibitors are undergoing early-phase clinical evaluation, including OTX015 (NCT02259114) and ZEN-3694 (NCT02711956), which aim to disrupt BRD4-mediated transcriptional programs associated with oncogene amplification. Another approach is to block CDK7/9, which disrupts the initiation and elongation of Pol II and transcription driven by super enhancers. The preclinical results for THZ1 and SNS-032 show therapeutic potential in models of tumors driven by MYCN and ecDNAs.[78]

Prevent Reintegration Into Homogenously Staining Regions (HSRs)

One main way ecDNA escapes is by linking into chromosomal arms to form HSRs, which stabilizes oncogene amplification and drug resistance.[13,82] Preclinical evidence suggests that this reintegration may be reduced by inhibiting topoisomerase activity and DNA repair. Topotecan, irinotecan, and etoposide are examples of topoisomerase inhibitors that increase replication-related issues and disruptions. In the meantime, PARP inhibitors, such as talazoparib and olaparib, bind to PARP on DNA, preventing repair and allowing replication to continue.[80]

Induce ecDNA Loss by Targeting Mitotic Tethering

To ensure proper inheritance, ecDNAs assemble and affix to the ends of chromosomes during mitosis. Preclinical studies indicate that this attachment depends on BRD4 and continuous transcription, according to research by Nichols et al. The attachment breaks when BET or CDK7 inhibitors block these processes, leading to mis-segregation and cytoplasmic loss of ecDNAs. This may potentially reduce the number of copies of ecDNA in different cell generations. [81]

Combination Approaches: ecDNA-Directed Plus Oncogene-Targeted Therapy

Since ecDNA often harbors key oncogenes such as EGFR, FGFR2, and MYC, therapies targeting ecDNA can be effectively paired with pathway inhibitors to enhance treatment efficacy. Preclinical testing in gastric and gastroesophageal junction neoplasms demonstrated that BBI-355, an ecDNA-targeted agent, combined with either an EGFR or FGFR inhibitor, produced synergistic effects, yielding substantial, sustained responses.[82]

Preclinical Examples and Rationale

Research by Tang YC et al. indicates that CHK1 inhibition preferentially kills ecDNA-driven tumors in preclinical models.[73] Using JQ1 can disrupt BRD4-tethered hubs, reducing oncogene transcription.[77] Inhibiting BRD4 and CDK7 causes ecDNA to mis-segregate and be lost.[81] Topoisomerase poisons and PARP inhibitors offer a mechanism to disrupt HSR formation, providing a systematic approach (Figure 4).[13,79,80]

PRECLINICAL AND EARLY CLINICAL EVIDENCE

Cancers carrying ecDNA are highly susceptible to CHK1 inhibition due to their dependence on replication stress. Consistent with the mechanistic rationale

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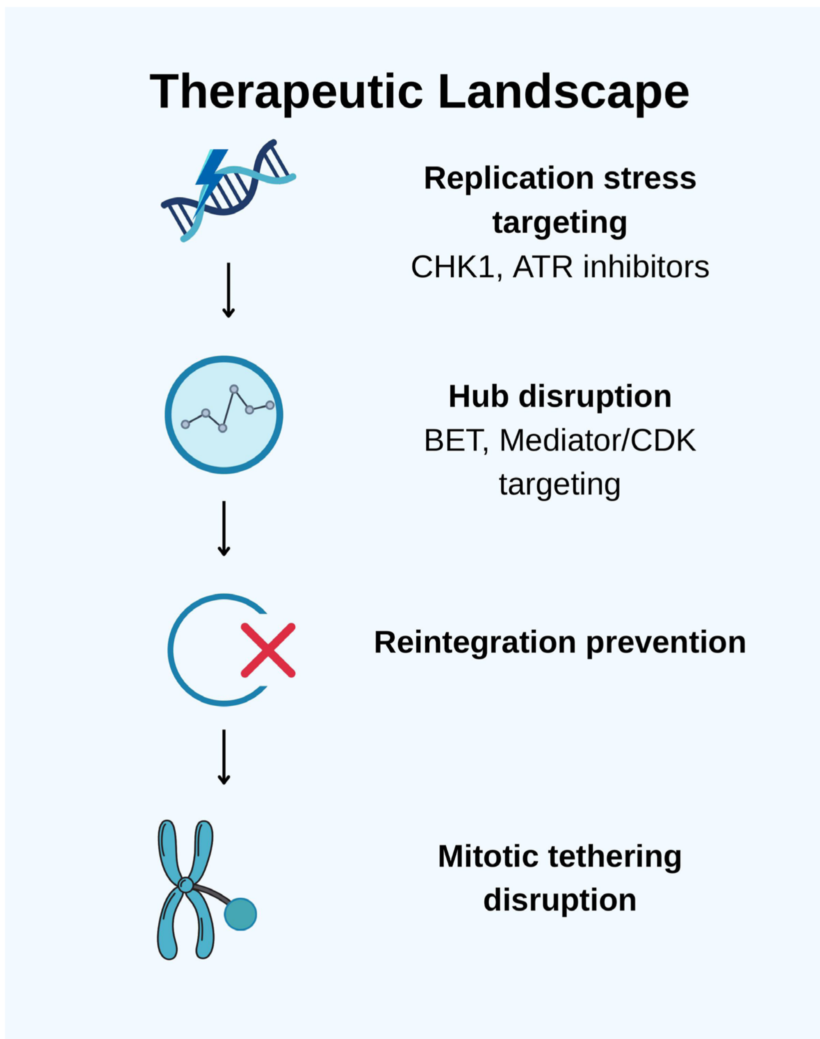

Fig 4 | Therapeutic landscape

outlined in Section "Target Replication Stress Vulnerabilities CHK1, ATR, and CDK Inhibition," pre-clinical indicates that response pathways. In this context, the preclinical CHK1 inhibitor BBI-2799 has demonstrated strong efficacy against cancers harboring ecDNA. Selective tumor cell death is induced by CHK1 inhibition, both genetically and pharmacologically. A selective, bioavailable oral CHK1 inhibitor, BBI-2779, has demonstrated strong efficacy against cancers harboring ecDNA. BBI-2779 inhibited tumor progression and stopped ecDNA-mediated resistance to the pan-FGFR inhibitor infigratinib in gastric cancer models where FGFR2 was amplified on ecDNA, causing continued tumor regression in mice. The results presented here suggest that transcription–replication conflict vulnerabilities may be therapeutically exploited, although clinical validation remains limited. Importantly, this clinical rationale is being translated into early clinical investigation with BBI-355, a clinical-stage CHK1 inhibitor currently under evaluation. Tumor heterogeneity results from differences in ecDNA inheritance among progeny cells, which affects its oncogenic effect. Oncogenic ecDNA is frequently retained during disease progression, as shown by longitudinal patient tumor profiling. This clearly connects ecDNA to tumor growth and helps explain why ecDNA-directed therapies have lagged in clinical translation.[73]

Approximately 20% of breast cancer tumors have HER2 amplification, and double-minute (DM) amplification is seen in approximately 30% of HER2-positive tumors. HER2-targeted treatments commonly induce resistance, even when these malignancies initially respond. Significantly, ecDNA-targeted treatments have shown promising responses in resistant cancers across a limited number of preclinical models, underscoring their potential as a therapeutic option. Using ecDNA detection as a biomarker for patient selection in clinical trials may improve treatment response by identifying patients more likely to benefit based on resistance mechanisms. Remaining challenges include the development of highly specific techniques for ecDNA detection across different cancers and the establishment of robust preclinical models to evaluate therapeutic potency.[84] Observing novel treatment-sensitive pathways requires understanding the processes of ecDNA formation and maintenance. Together, these strategies have the potential to improve the long-term effectiveness of therapy in malignancies resistant to anticancer medications and to accelerate the clinical translation of ecDNA-targeted treatments.[85] However, CHK1inhibition is associated with known safety limitations, including hematological toxicities (e.g., neutropenia and thrombocytopenia), gastrointestinal adverse effects, and potential dose-limiting toxicities.[85]

TRANSLATIONAL CHALLENGES

For extensive genomic testing, next-generation sequencing (NGS) is a very sensitive, high-throughput mechanism. Targeted sequencing (TS), which primarily focuses on a set of medically essential genes, offers more in-depth investigation, lower cost, and a lower data burden than whole-genome (WGS) or whole-exome sequencing (WES). Because of this, TS has proven particularly useful for detecting low-frequency variants in fragmented clinical DNA samples, enabling patient categorization and guiding more effective, customized treatments.[86]

Oncogenes and regulatory elements can be amplified as ecDNA or incorporated into chromosomes as HSRs, according to recent studies of cancer genomes. These alterations foster the overexpression of oncogenes, which leads to aggressive tumor behavior and poor prognosis.[87]

Replication stress, caused by mechanisms including reactive oxygen species (ROS), ribonucleotide misincorporation, secondary DNA structures (hairpins, triplexes, and quadruplexes), transcription–replication collisions, and R-loop formation, is a principal driver of ecDNA instability. These events create genomic instability and impair normal replication, further increasing ecDNA production and contributing to drug resistance.[88]

There remain unresolved issues regarding the functions and clinical implications of ecDNA, despite growing awareness of its role in cancer progression. Precision oncology may find new opportunities as

ecDNA traits are leveraged for diagnosis, prognosis, and treatment, alongside improved detection methods and mechanistic understanding.[58]

FUTURE DIRECTION

Tissue biopsies are used in mainstream cancer diagnosis, but they are also very invasive and not suitable for routine monitoring. A minor invasive approach is liquid biopsy, which uses circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA). ctDNA-based analysis is crucial for directing ecDNA-targeted treatments, facilitating serial sampling, and providing insight into tumor growth.[94] Moreover, other therapeutic methods are also under investigation, such as cell and gene therapies, RNA interference (RNAi), oligonucleotide treatments, CRISPR-based gene editing, and classical small molecules, and antibody-based medications have all proven noteworthy.[58]

Advanced methods are being studied to directly analyze ecDNA. By merging pulsed-field gel electrophoresis with in vitro CRISPR-Cas9 therapy, CRISPR-CATCH enables the differentiation of ecDNA-carrying oncogenes from cancer cells, thereby enabling structural and functional characterization.[58] Nucleases, enzymes that break down nucleic acids, are also being examined as potential cancer markers. Although their enzymatic potential has not yet been altogether utilized for diagnosis, their vast cellular presence and catalytic activity make them an appealing option in effective and highly potent treatment of cancers.[90]

Even after all these advancements, there are still a lot of challenges in the field of oncology. It is still unclear how ecDNA is formed, how it reintegrates into chromosomes as HSRs, and how it causes drug resistance. Moreover, ecDNA has been linked to immune-mediated signaling, particularly activation of the cGAS-STING pathway, and to tumor evolution. EcDNA might soon become a powerful diagnostic and predictive biomarker, enabling targeted precision medicine and advancing ecDNA-targeted therapeutics.[91]

LIMITATIONS

Although tremendous progress has been made in the research on ecDNA, there are still some limitations that have to be addressed. Firstly, there is heterogeneity in the definitions of ecDNA across the research to date. This lack of standardization in the definition of ecDNA makes it hard to compare research across the field. Secondly, there are some limitations in the methods used to detect ecDNA at the moment. The methods used to detect ecDNA are usually based on short reads, which may not be effective at identifying the complex circular structure of ecDNA, leading to incorrect ecDNA identification in the cell. Lastly, although there is some research on the therapeutic potential of ecDNA, most studies are preclinical, with very little prospective research available.

Conclusion

ecDNA has emerged as an important driver of tumor heterogeneity, oncogene amplification, and therapeutic resistance, redefining our knowledge of cancer biology. ecDNA is increasingly recognized as a clinically significant biomarker and therapeutic target. Advances in sequencing, imaging, and computational tools have enabled precise detection of ecDNA, while preclinical studies highlight its potential as a marker of sensitivity to targeted therapies. Major challenges remain in clarifying the mechanisms of ecDNA biogenesis, integration, and immune interactions, as well as in translating preclinical findings into clinical practice, despite advances. Future research integrating liquid biopsy, single-cell profiling, and novel therapeutics promises to accelerate the clinical utility of ecDNA-directed strategies. By bridging mechanistic insights with translational innovation, targeting ecDNA could transform precision oncology and improve outcomes in aggressive, treatment-resistant cancers.

References

1 Cox D, Yuncken C, Spriggs AI. Minute chromatin bodies in malignant tumours of childhood. Lancet. 1965 Jul;1(7402):55–8. https://doi.org/10.1016/S0140-6736(65)90131-5

2 Muleris M, Almeida A, Dutrillaux AM, Pruchon E, Vega F, Delattre JY, et al. Oncogene amplification in human gliomas: a molecular cytogenetic analysis. Oncogene. 1994 Sep;9(9):2717–22.

3 Heim S, Mitelman F. Cancer cytogenetics [Internet]. Hoboken (NJ): John Wiley & Sons, Ltd. 2015 [cited 2025 Jan 20]. Cancer cytogenetics: chromosomal and molecular genetic aberrations of tumor cells.

4 Bridge JA. Advantages and limitations of cytogenetic, molecular cytogenetic, and molecular diagnostic testing in mesenchymal neoplasms. J Orthop Sci. 2008 May;13(3):273–82. https://doi.org/10.1007/s00776-007-1215-1

5 Anguiano A, Wang BT, Wang SR, Boyar FZ, Mahon LW, El Naggar MM, et al. Spectral Karyotyping for identification of constitutional chromosomal abnormalities at a national reference laboratory. Mol Cytogenet. 2012 Jan;5(1):3. https://doi.org/10.1186/1755-8166-5-3

6 Wang S, Wu CY, He MM, Yong JX, Chen YX, Qian LM, et al. Machine learning-based extrachromosomal DNA identification in large-scale cohorts reveals its clinical implications in cancer. Nat Commun. 2024 Feb;15(1):1515. https://doi.org/10.1038/s41467-024-45479-6

7 Chang L, Deng E, Wang J, Zhou W, Ao J, Liu R, et al. Single-cell third-generation sequencing-based multi-omics uncovers gene expression changes governed by ecDNA and structural variants in cancer cells. Clin Transl Med. 2023 Aug;13(8):e1351. https://doi.org/10.1002/ctm2.1351

8 Møller HD. Circle-Seq: isolation and sequencing of chromosome-derived circular DNA elements in cells. Methods Mol Biol. 2020;2119:165–81.

9 Huh YO, Tang G, Talwalkar SS, Khoury JD, Ohanian M, Bueso-Ramos CE, et al. Double minute chromosomes in acute myeloid leukemia, myelodysplastic syndromes, and chronic myelomonocytic leukemia are associated with micronuclei, MYC or MLL amplification, and complex karyotype. Cancer Genet. 2016;209(7–8):313–20. https://doi.org/10.1016/j.cancergen.2016.05.072

10 Sanborn JZ, Salama SR, Grifford M, Brennan CW, Mikkelsen T, Jhanwar S, et al. Double minute chromosomes in glioblastoma multiforme are revealed by precise reconstruction of oncogenic amplicons. Cancer Res. 2013 Oct;73(19):6036–45. https://doi.org/10.1158/0008-5472.CAN-13-0186

11 Koche RP, Rodriguez-Fos E, Helmsauer K, Burkert M, MacArthur IC, Maag J, et al. Extrachromosomal circular DNA drives oncogenic genome remodeling in neuroblastoma [Erratum in: Nat Genet. 2020 Apr;52(4):464. doi: 10.1038/s41588-020-0598-1. PMID: 31844324; PMCID: PMC7008131]. Nat Genet. 2020 Jan;52(1):29–34. https://doi.org/10.1038/s41588-019-0547-z

12 Stöber MC, Chamorro González R, Brückner L, Conrad T, Wittstruck N, Szymansky A, et al. Intercellular extrachromosomal DNA copy-number heterogeneity drives neuroblastoma cell state diversity. Cell Rep. 2024 Sep;43(9):114711. https://doi.org/10.1016/j.celrep.2024.114711

13 Nathanson DA, Gini B, Mottahedeh J, Visnyei K, Koga T, Gomez G, et al. Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA. Science. 2014 Jan;343(6166):72–6. https://doi.org/10.1126/science.1241328

14 Meng X, Qi X, Guo H, Cai M, Li C, Zhu J, et al. Novel role for non-homologous end joining in the formation of double minutes in methotrexate-resistant colon cancer cells. J Med Genet. 2015 Feb;52(2):135–44. https://doi.org/10.1136/jmedgenet-2014-102703

15 Jin Y, Liu Z, Cao W, Ma X, Fan Y, Yu Y, et al. Novel functional MAR elements of double minute chromosomes in human ovarian cells capable of enhancing gene expression. PLoS One. 2012;7(2):e30419. https://doi.org/10.1371/journal.pone.0030419

16 Kim H, Nguyen NP, Turner K, Wu S, Gujar AD, Luebeck J, et al. Extrachromosomal DNA is associated with oncogene amplification and poor outcome across multiple cancers. Nat Genet. 2020 Sep;52(9):891–7. https://doi.org/10.1038/s41588-020-0678-2

17 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 Mar;543(7643):122–5. https://doi.org/10.1038/nature21356

18 Hung KL, Yost KE, Xie L, Shi Q, Helmsauer K, Luebeck J, et al. ecDNA hubs drive cooperative intermolecular oncogene expression. Nature. 2021 Dec;600(7890):731–6. https://doi.org/10.1038/s41586-021-04116-8

19 Behrouzi R, Clipson A, Simpson KL, Blackhall F, Rothwell DG, Dive C, et al. Cell-free and extrachromosomal DNA profiling of small cell lung cancer. Trends Mol Med. 2025 Jan;31(1):64–78. https://doi.org/10.1016/j.molmed.2024.08.004

20 Qiu Q, Ding Y, Guo X, Han J, Zhang J, Liu Y, et al. Extrachromosomal circular DNA as a novel biomarker for the progression of colorectal cancer. Mol Med. 2025 Apr;31(1):123. https://doi.org/10.1186/s10020-025-01164-y

21 Tang J, Weiser NE, Wang G, Chowdhry S, Curtis EJ, Zhao Y, et al. Enhancing transcription-replication conflict targets ecDNA-positive cancers. Nature. 2024 Nov;635(8037):210–8. https://doi.org/10.1038/s41586-024-07802-5

22 Kohl NE, Kanda N, Schreck RR, Bruns G, Latt SA, Gilbert F, et al. Transposition and amplification of oncogene-related sequences in human neuroblastomas. Cell. 1983 Dec;35(2 Pt 1):359–67. https://doi.org/10.1016/0092-8674(83)90169-1

23 Levan A, Levan G. Have double minutes functioning centromeres? Hereditas. 1978;88(1):81–92. https://doi.org/10.1111/j.1601-5223.1978.tb01606.x

24 Gebhart E, Brüderlein S, Tulusan AH, von Maillot K, Birkmann J. Incidence of double minutes, cytogenetic equivalents of gene amplification, in human carcinoma cells. Int J Cancer. 1984 Sep;34(3):369–73. https://doi.org/10.1002/ijc.2910340313

25 Haber DA, Schimke RT. Unstable amplification of an altered dihydrofolate reductase gene associated with double-minute chromosomes. Cell. 1981 Nov;26(3 Pt 1):355–62.

26 Arrey G, Keating ST, Regenberg B. A unifying model for extrachromosomal circular DNA load in eukaryotic cells. Semin Cell Dev Biol. 2022 Aug;128:40–50. https://doi.org/10.1016/j.semcdb.2022.03.002

27 Rajkumar U, Turner K, Luebeck J, Deshpande V, Chandraker M, Mischel P, et al. EcSeg: semantic segmentation of metaphase images containing extrachromosomal DNA. iScience. 2019 Nov;21:428–35. https://doi.org/10.1016/j.isci.2019.10.035

28 Gibaud A, Vogt N, Brison O, Debatisse M, Malfoy B. Characterization at nucleotide resolution of the homogeneously staining region sites of insertion in two cancer cell lines. Nucleic Acids Res. 2013 Sep;41(17):8210–9. https://doi.org/10.1093/nar/gkt566

29 Zhu Y, Gujar AD, Wong CH, Tjong H, Ngan CY, Gong L, et al. Oncogenic extrachromosomal DNA functions as mobile enhancers to globally amplify chromosomal transcription. Cancer Cell. 2021 May;39(5):694–707.e7. https://doi.org/10.1016/j.ccell.2021.03.006.

30 Rausch T, Jones DT, Zapatka M, Stütz AM, Zichner T, Weischenfeldt J, et al. Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. Cell. 2012 Jan;148(1–2):59–71. https://doi.org/10.1016/j.cell.2011.12.013

31 Shorokhova M, Nikolsky N, Grinchuk T. Chromothripsis-explosion in genetic science. Cells. 2021 May;10(5):1102. https://doi.org/10.3390/cells10051102

32 Stevens JB, Abdallah BY, Liu G, Ye CJ, Horne SD, Wang G, et al. Diverse system stresses: common mechanisms of chromosome fragmentation. Cell Death Dis. 2011 Jun;2(6):e178. https://doi.org/10.1038/cddis.2011.60

33 Mardin BR, Drainas AP, Waszak SM, Weischenfeldt J, Isokane M, Stütz AM, et al. A cell-based model system links chromothripsis with hyperploidy. Mol Syst Biol. 2015 Sep;11(9):828. https://doi.org/10.15252/msb.20156505

34 Zavacka K, Plevova K. Chromothripsis in chronic lymphocytic leukemia: a driving force of genome instability. Front Oncol. 2021 Nov;11:771664. https://doi.org/10.3389/fonc.2021.771664

35 Behjati S, Tarpey PS, Haase K, Ye H, Young MD, Alexandrov LB, et al. Recurrent mutation of IGF signalling genes and distinct patterns of genomic rearrangement in osteosarcoma. Nat Commun. 2017 Jun;8(1):15936. https://doi.org/10.1038/ncomms15936

36 Lo AW, Sabatier L, Fouladi B, Pottier G, Ricoul M, Murnane JP. DNA amplification by breakage/fusion/bridge cycles initiated by spontaneous telomere loss in a human cancer cell line. Neoplasia. 2002;4(6):531–8. https://doi.org/10.1038/sj.neo.7900267

37 Murnane JP. Telomere dysfunction and chromosome instability. Mutat Res. 2012 Feb;730(1-2):28–36. https://doi.org/10.1016/j.mrfmmm.2011.04.008

38 Lee JA, Carvalho CM, Lupski JRA. DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. Cell. 2007 Dec;131(7):1235–47. https://doi.org/10.1016/j.cell.2007.11.037

39 Röijer E, Nordkvist A, Ström AK, Ryd W, Behrendt M, Bullerdiek J, et al. Translocation, deletion/amplification, and expression of HMGIC and MDM2 in a carcinoma ex pleomorphic adenoma. Am J Pathol. 2002 Feb;160(2):433–40. https://doi.org/10.1016/S0002-9440(10)64862-6

40 Vogt N, Gibaud A, Lemoine F, de la Grange P, Debatisse M, Malfoy B. Amplicon rearrangements during the extrachromosomal and intrachromosomal amplification process in a glioma. Nucleic Acids Res. 2014 Dec;42(21):13194–205. https://doi.org/10.1093/nar/gku1101

41 Yang L, Luquette LJ, Gehlenborg N, Xi R, Haseley PS, Hsieh CH, et al. Diverse mechanisms of somatic structural variations in human cancer genomes [Erratum in: Cell. 2014 Jun 19;157(7):1736. PMID: 23663786; PMCID: PMC3704973]. Cell. 2013 May;153(4):919–29. https://doi.org/10.1016/j.cell.2013.04.010

42 Chen Y, Qiu Q, She J, Yu J. Extrachromosomal circular DNA in colorectal cancer: biogenesis, function and potential as therapeutic target. Oncogene. 2023 Mar;42(13):941–51. https://doi.org/10.1038/s41388-023-02640-7

43 Ceccaldi R, Rondinelli B, D'Andrea AD. Repair pathway choices and consequences at the double-strand break. Trends Cell Biol. 2016 Jan;26(1):52–64. https://doi.org/10.1016/j.tcb.2015.07.009

44 Dillon LW, Kumar P, Shibata Y, Wang YH, Willcox S, Griffith JD, et al. Production of extrachromosomal microDNAs is linked to mismatch repair pathways and transcriptional activity. Cell Rep. 2015 Jun;11(11):1749–59. https://doi.org/10.1016/j.celrep.2015.05.020

45 Yu M, Ren B. The three-dimensional organization of mammalian genomes. Annu Rev Cell Dev Biol. 2017 Oct;33(1):265–89. https://doi.org/10.1146/annurev-cellbio-100616-060531

46 Spilianakis CG, Lalioti MD, Town T, Lee GR, Flavell RA. Interchromosomal associations between alternatively expressed loci. Nature. 2005 Jun;435(7042):637–45. https://doi.org/10.1038/nature03574

47 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 Nov;575(7784):699–703. https://doi.org/10.1038/s41586-019-1763-5

48 Helmsauer K, Valieva ME, Ali S, Chamorro González R, Schöpflin R, Röefzaad C, et al. Enhancer hijacking determines extrachromosomal circular MYCN amplicon architecture in neuroblastoma. Nat Commun. 2020 Nov;11(1):5823. https://doi.org/10.1038/s41467-020-19452-y

49 Northcott PA, Shih DJ, Peacock J, Garzia L, Morrissy AS, Zichner T, et al. Subgroup-specific structural variation across 1,000

medulloblastoma genomes. Nature. 2012 Aug;488(7409):49–56. https://doi.org/10.1038/nature11327
50 Hung KL, Jones MG, Wong IT, Lange JT, Luebeck J, Scanu E, et al. Coordinated inheritance of extrachromosomal DNA species in human cancer cells. bioRxiv. 2023; Preprint published 2023 Jul 19. https://doi.org/10.1101/2023.07.18.549597.
51 Li J, Hsu A, Hua Y, Wang G, Cheng L, Ochiai H, et al. Single-gene imaging links genome topology, promoter-enhancer communication and transcription control. Nat Struct Mol Biol. 2020 Nov;27(11):1032–40. https://doi.org/10.1038/s41594-020-0493-6
52 Gibson BA, Doolittle LK, Schneider MW, Jensen LE, Gamarra N, Henry L, et al. Organization of chromatin by intrinsic and regulated phase separation. Cell. 2019 Oct;179(2):470–484.e21. https://doi.org/10.1016/j.cell.2019.08.037
53 Deng S, Feng Y, Pauklin S. 3D chromatin architecture and transcription regulation in cancer. J Hematol Oncol. 2022 May;15(1):49. https://doi.org/10.1186/s13045-022-01271-x
54 L'Abbate A, Macchia G, D'Addabbo P, Lonoce A, Tolomeo D, Trombetta D, et al. Genomic organization and evolution of double minutes/homogeneously staining regions with MYC amplification in human cancer. Nucleic Acids Res. 2014 Aug;42(14):9131–45. https://doi.org/10.1093/nar/gku590
55 Yu J, Zhang H, Han P, Jiang X, Li J, Li B, et al. Circle-seq based method for eccDNA synthesis and its application as a canonical promoter independent vector for robust microRNA overexpression. Comput Struct Biotechnol J. 2023 Dec;23:358–68. https://doi.org/10.1016/j.csbj.2023.12.019
56 Hung KL, Luebeck J, Dehkordi SR, Colón CI, Li R, Wong IT, et al. Targeted profiling of human extrachromosomal DNA by CRISPR-CATCH. Nat Genet. 2022 Nov;54(11):1746–54. https://doi.org/10.1038/s41588-022-01190-0
57 Purshouse K, Pollard SM, Bickmore WA. Imaging extrachromosomal DNA (ecDNA) in cancer. Histochem Cell Biol. 2024 Jul;162(1–2):53–64. https://doi.org/10.1007/s00418-024-02280-2
58 Dong Y, He Q, Chen X, Yang F, He L, Zheng Y. Extrachromosomal DNA (ecDNA) in cancer: mechanisms, functions, and clinical implications. Front Oncol. 2023 Jun;13:1194405. https://doi.org/10.3389/fonc.2023.1194405
59 Chapman OS, Luebeck J, Sridhar S, Wong IT, Dixit D, Wang S, et al. Circular extrachromosomal DNA promotes tumor heterogeneity in high-risk medulloblastoma. Nat Genet. 2023 Dec;55(12):2189–99. https://doi.org/10.1038/s41588-023-01551-3
60 Chamorro González R, Conrad T, Stöber MC, Xu R, Giurgiu M, Rodriguez-Fos E, et al. Parallel sequencing of extrachromosomal circular DNAs and transcriptomes in single cancer cells. Nat Genet. 2023 May;55(5):880–90. https://doi.org/10.1038/s41588-023-01386-y
61 Chen JP, Diekmann C, Wu H, Chen C, Della Chiara G, Berrino E, et al. scCircle-seq unveils the diversity and complexity of extrachromosomal circular DNAs in single cells [Erratum in: Nat Commun. 2024 Apr 22;15(1):3390. doi: 10.1038/s41467-024-47535-7. PMID: 38409079; PMCID: PMC10897160]. Nat Commun. 2024 Feb;15(1):1768. https://doi.org/10.1038/s41467-024-45972-y
62 Shibata Y, Kumar P, Layer R, Willcox S, Gagan JR, Griffith JD, et al. Extrachromosomal microDNAs and chromosomal microdeletions in normal tissues [Erratum in: Science. 2012 Jun 22;336(6088):1506. PMID: 22403181; PMCID: PMC3703515]. Science. 2012 Apr;336(6077):82–6. https://doi.org/10.1126/science.1213307
63 Li Q, Zhang RX, Yang JJ, Huang HB, Feng G, Li GR. Characterization of extrachromosomal circular DNAs in plasma of patients with clear cell renal cell carcinoma. World J Urol. 2024 May;42(1):328. https://doi.org/10.1007/s00345-024-05031-z
64 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 Nov;599(7884):308–14. https://doi.org/10.1038/s41586-021-04009-w
65 Grassi T, Harris FR, Smadbeck JB, Murphy SJ, Block MS, Multinu F, et al. Personalized tumor-specific DNA junctions to detect circulating tumor in patients with endometrial cancer. PLoS One. 2021 Jun;16(6):e0252390. https://doi.org/10.1371/journal.pone.0252390
66 Terp SK, Guldbrandsen K, Stoico MP, Mark LR, Frandsen AP, Dybkær K, et al. Genome-wide cfDNA methylation profiling reveals robust hypermethylation signatures in ovarian cancer. Cancers (Basel). 2025 Jun;17(12):2026. https://doi.org/10.3390/cancers17122026
67 Dong W, Hu W, Lu Y, Zheng Q. Cell-free DNA fragmentomics: a universal framework for early cancer detection and monitoring. Am J Clin Exp Immunol. 2025 Aug;14(4):237–40. https://doi.org/10.62347/EBRY4326
68 Tang WWingLunLee, Cario WSLo, Annie TWChu, Karrie Mei-YeeKiang, Wei Ma, Amy HYTong, et al. Integrated genomic and transcriptomic profiling of glioblastoma reveals ecDNA-driven heterogeneity and microenvironmental reprogramming. Cell Rep. 2025;44(10):116426. ISSN 2211-1247. https://doi.org/10.1016/j.celrep.2025.116426
69 Wang LJ, Ye J. Extrachromosomal DNA as a carrier of extra copies of oncogenes for heterogeneity and malignancy of glioma. Acta Pharm Sin B. 2025 May;15(5):2791–4. https://doi.org/10.1016/j.apsb.2025.02.001
70 Khandekar A, Hoang PH, Luebeck J, Díaz-Gay M, Zhao W, McElderry JP, et al. Examining the role of extrachromosomal DNA in 1,216 lung cancers. bioRxiv [Preprint]. 2025 Jul 24:2025.06.03.657117. https://doi.org/10.1101/2025.06.03.657117. PMID: 40501879; PMCID: PMC12157361.
71 Zhu X, Qian Y, Tang Q, Li J, Geng Y, Gong S, et al. Research Advances of Extrachromosomal Circular DNA in Breast Cancer. Cancer Med. 2025 Oct;14(19):e71285. https://doi.org/10.1002/cam4.71285
72 Bailey C, Pich O, Thol K, Watkins TB, Luebeck J, Rowan A, et al. Genomics England consortium. Origins and impact of extrachromosomal DNA. Nature. 2024 Nov;635(8037):193–200. https://doi.org/10.1038/s41586-024-08107-3
73 Tang J, Weiser NE, Wang G, Chowdhry S, Curtis EJ, Zhao Y, et al. Enhancing transcription–replication conflict selectively kills extrachromosomal DNA-containing cancers. Nature. 2024;635:210–218.
74 Dillon MT, et al. Clinical and translational development of ATR inhibitors in cancer therapy. Clin Cancer Res. 2021;27(4):922–34.
75 Kwiatkowski N, Zhang T, Rahl PB, Abraham BJ, Reddy J, Ficarro SB, et al. Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. Nature. 2014 Jul;511(7511):616–20. https://doi.org/10.1038/nature13393
76 Morton AR, Turner KM, Nguyen N, Raviram R, Erb M, Santini J, et al. Functional enhancers shape extrachromosomal oncogene amplifications. Nature. 2019;575(7784):699–703. https://doi.org/10.1038/s41586-019-1763-5
77 Yi E, et al. Disruption of extrachromosomal DNA hubs by BRD4 inhibition suppresses oncogene transcription. Nature. 2021;590:603–8.
78 Chipumuro E, Marco E, Christensen CL, Kwiatkowski N, Zhang T, Hatheway CM, et al. CDK7 inhibition suppresses super-enhancer-linked oncogenic transcription in MYCN-driven cancer. Cell. 2014 Nov;159(5):1126–39. https://doi.org/10.1016/j.cell.2014.10.024
79 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 Mar;591(7848):137–41. https://doi.org/10.1038/s41586-020-03064-z
80 Zandarashvili L, Langelier MF, Velagapudi UK, Hancock MA, Steffen JD, Billur R, et al. Structural basis for allosteric PARP-1 retention on DNA breaks. Science. 2020;368(6486):49–54.
81 Nichols CA, et al. Extrachromosomal DNA clusters form functional hubs in cancer cell mitosis. bioRxiv. 2025. https://doi.org/10.1101/2025.01.05.123456
82 Boundless Bio. First-in-human trial of BBI-355 (CHK1 inhibitor) in patients with tumors harboring extrachromosomal DNA amplifications (POTENTIATE). ClinicalTrials.gov Identifier: NCT05292408.
83 Jaworski JJ, Pfuderer PL, Czyz P, Petris G, Boemo MA, Sale JE. ecDNA replication is disorganized and vulnerable to replication stress. Nucleic Acids Res. 2025 Jul;53(14):gkaf711. https://doi.org/10.1093/nar/gkaf711
84 Gu X, Yu J, Chai P, Ge S, Fan X. Novel insights into extrachromosomal DNA: redefining the onco-drivers of tumor progression. J Exp Clin Cancer Res. 2020 Oct;39(1):215. https://doi.org/10.1186/s13046-020-01726-4
85 Noorani I, Mischel PS, Swanton C. Leveraging extrachromosomal DNA to fine-tune trials of targeted therapy for glioblastoma:

opportunities and challenges. Nat Rev Clin Oncol. 2022 Nov;19(11):733–43. https://doi.org/10.1038/s41571-022-00679-1

86 Bewicke-Copley F, Arjun Kumar E, Palladino G, Korfi K, Wang J. Applications and analysis of targeted genomic sequencing in cancer studies. Comput Struct Biotechnol J. 2019 Nov;17:1348–59.

87 Cheng H, Ma W, Wang K, Chu H, Bao G, Liao Y, et al. ATACAmp: a tool for detecting ecDNA/HSRs from bulk and single-cell ATAC-seq data. BMC Genomics. 2023;24(1):678. https://doi.org/10.1186/s12864-023-09792-6

88 da Costa AA, Chowdhury D, Shapiro GI, D'Andrea AD, Konstantinopoulos PA. Targeting replication stress in cancer therapy. Nat Rev Drug Discov. 2023 Jan;22(1):38–58. https://doi.org/10.1038/s41573-022-00558-5

89 Ge Qian, Zhang ZY, Li SN, Ma JQ, Zhao Z. Liquid biopsy: comprehensive overview of circulating tumor DNA (Review). Oncol Lett. 2024 Sep;28(5):548. https://doi.org/10.3892/ol.2024.14681

90 Balian Alien, Frank JHernandez. Nucleases as molecular targets for cancer diagnosis. Biomark Res. 2021;9(1):86. https://doi.org/10.1186/s40364-021-00342-4

91 Li Z, Wang B, Liang H, Han L. Pioneering insights of extrachromosomal DNA (ecDNA) generation, action and its implications for cancer therapy. Int J Biol Sci. 2022 Jun;18(10):4006–25. https://doi.org/10.7150/ijbs.73479. Available from: https://www.ijbs.com/v18p4006.htm

92 Rajkumar U, Turner K, Luebeck J, Deshpande V, Chandraker M, Mischel P, et al. EcSeg: semantic segmentation of metaphase images containing extrachromosomal DNA. iScience. 2019 Nov;21:428–35. https://doi.org/10.1016/j.isci.2019.10.035

93 Luebeck J, Coruh C, Dehkordi SR, Lange JT, Turner KM, Deshpande V, et al. AmpliconReconstructor integrates NGS and optical mapping to resolve the complex structures of focal amplifications. Nat Commun. 2020 Sep;11(1):4374. https://doi.org/10.1038/s41467-020-18099-z

94 Deshpande V, Luebeck J, Nguyen ND, Bakhtiari M, Turner KM, Schwab R, et al. Exploring the landscape of focal amplifications in cancer using AmpliconArchitect. Nat Commun. 2019 Jan;10(1):392. https://doi.org/10.1038/s41467-018-08200-y

95 Carss K, Halldorsson BV, Hou L, Liu J, Wheeler E, Lo Y, et al.; UK Biobank Whole-Genome Sequencing Consortium. Whole-genome sequencing of 490,640 UK Biobank participants. Nature. 2025 Sep;645(8081):692–701. https://doi.org/10.1038/s41586-025-09272-9. Epub 2025 Aug 6.

96 Møller HD, Bojsen RK, Tachibana C, Parsons L, Botstein D, Regenberg B. Genome-wide purification of extrachromosomal circular DNA from eukaryotic cells. J Vis Exp. 2016 Apr;4110:e54239. https://doi.org/10.3791/54239. PMID: 27077531; PMCID: PMC4841354

97 Evrony GD, Hinch AG, Luo C. Applications of single-cell DNA sequencing. Annu Rev Genomics Hum Genet. 2021 Aug;22(1):171–97. https://doi.org/10.1146/annurev-genom-111320-090436

98 Kumar P, Kiran S, Saha S, Su Z, Paulsen T, Chatrath A, et al. ATAC-seq identifies thousands of extrachromosomal circular DNA in cancer and cell lines. Sci Adv. 2020 May;6(20):eaba2489. https://doi.org/10.1126/sciadv.aba2489

99 Prada-Luengo I, Krogh A, Maretty L, Regenberg B. Sensitive detection of circular DNAs at single-nucleotide resolution using guided realignment of partially aligned reads. BMC Bioinformatics. 2019 Dec;20(1):663. https://doi.org/10.1186/s12859-019-3160-3

100 Mann L, Seibt KM, Weber B, Heitkam T. ECCsplorer: a pipeline to detect extrachromosomal circular DNA (eccDNA) from next-generation sequencing data. BMC Bioinformatics. 2022 Jan;23(1):40. https://doi.org/10.1186/s12859-021-04545-2

101 Zhang P, Peng H, Llauro C, Bucher E, Mirouze M. ecc_finder: A Robust and Accurate Tool for Detecting Extrachromosomal Circular DNA From Sequencing Data. Front Plant Sci. 2021 Dec;12:743742. https://doi.org/10.3389/fpls.2021.743742

102 Henriksen RA, Jenjaroenpun P, Sjøstrøm IB, Jensen KR, Prada-Luengo I, Wongsurawat T, et al. Circular DNA in the human germline and its association with recombination. Mol Cell. 2022 Jan;82(1):209–217.e7. https://doi.org/10.1016/j.molcel.2021.11.027

103 Wanchai V, Jenjaroenpun P, Leangapichart T, Arrey G, Burnham CM, Tümmler MC, et al. CReSIL: accurate identification of extrachromosomal circular DNA from long-read sequences [Erratum in: Brief Bioinform. 2023 Sep 22;24(6):bbad402. doi: 10.1093/bib/bbad402. PMID: 36198068; PMCID: PMC10144670]. Brief Bioinform. 2022 Nov;23(6):bbac422. https://doi.org/10.1093/bib/bbac422.