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Review Article

Extrachromosomal DNA in Adaptive Tumour Evolution: Mechanisms Driving Plasticity, Drug Resistance and Precision Oncology

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ABSTRACT

Extrachromosomal DNA (ecDNA) has been identified as a critical component of tumour evolution, offering a dynamic genetic and regulatory landscape through which cancer cells can alter oncogene dosage, transcriptional states, and cellular phenotypes. Lacking centromeric and telomeric sequences, ecDNA is inherited in a non-Mendelian manner, resulting in substantial cell-to-cell variation in ecDNA copy number and composition. The aim of this review is to synthesize the biogenesis, molecular organization, and functional consequences of ecDNA, focusing on its role in tumour plasticity, adaptive evolution, therapeutic resistance and clinical translation. This narrative review synthesizes literature identified through structured searches of PubMed, Web of Science, Scopus, and Google Scholar covering publications from 2017 to August 2026, with emphasis on mechanistic and translational studies of cancer-associated ecDNA. Evidence indicates that ecDNA acts as a dynamic source of oncogenic amplification and cellular diversity, enabling rapid shifts in tumour states under selective pressure. These properties may help explain its association with disease progression and treatment failure, while emerging technologies are beginning to reveal opportunities for ecDNA-informed therapeutic intervention. Even with significant advances, one of the major problems is the ability to differentiate the biological effects specific to ecDNA from the effects that are due to high level amplification of oncogenes and genomic instability in general. An Integrative multi-dimensional approach will be crucial to uncover causal mechanisms and to advance the field of ecDNA-guided precision oncology.

Keywords: Oncogene Dosage; Intratumoural Heterogeneity; Chromatin Architecture; Enhancer Hijacking; Non-Mendelian Inheritance; Therapeutic Adaptation

Introduction

Cancers are complex and diverse with extrachromosomal DNA (ecDNA) recently becoming a crucial player in driving the evolution of about 20% of all tumours. Although its significance is rapidly gaining recognition, the role of extrachromosomal DNA (ecDNA) DNA constructed from nucleosomal chromatin in cancer is less well characterized. ecDNA has been identified as a major carrier of amplified oncogenes (1), and it is more open than chromosomal DNA, resulting in increased transcriptional activity (2). Owing to its lack of a centromere, ecDNA is inherited in a non-Mendelian pattern during mitosis (3), resulting in some tumour cells carrying high copy numbers of oncogenes that can substantially influence heterogeneity and drug resistance.

Despite its initial identification as a rare genomic anomaly, ecDNA is now understood as a frequent occurrence in many highly aggressive cancers, thanks to the development of whole genome sequencing, long-read sequencing, single-cell multi-omics, and chromatin conformation technologies (4).

However, evidence shows that ecDNA is much more than just an oncogene amplification vehicle. EcDNA can induce intratumoural heterogeneity, rewiring of enhancers and extraordinary levels of expression of oncogenes, thus contributing to a rapid adaptation of the tumour to the therapeutic pressure (5). Their properties enable drug resistance, metastatic competence and immune evasion, and offer novel opportunities for biomarker identification and precision oncology (6).

With so many advances in ecDNA research, a critical synthesis is needed. This review examines ecDNA formation and function, its roles in tumour plasticity, adaptive evolution and therapeutic resistance, emerging detection technologies, and clinical translation. It is guided by an evolutionary framework that views ecDNA as a dynamic genetic and regulatory substrate that generates tumour-cell variation, which can then be shaped by environmental and therapeutic selection. The review further highlights key knowledge gaps and priorities for advancing ecDNA-guided precision oncology.

Methodology

A structured literature search was conducted in PubMed, Web of Science, Scopus, and Google Scholar for publications from 2017 to August 2026, with the final search conducted in August 2026. Search terms included “extrachromosomal DNA,” “ecDNA,” “double minutes,” “oncogene amplification,” “tumour evolution,” “tumour plasticity,” “drug resistance,” “chromatin architecture,” “ecDNA detection,” and “precision oncology,” combined using Boolean operators (AND, OR). Reference lists of key studies and reviews were also screened for additional relevant literature. Eligible publications included original research articles, reviews, landmark studies, methodological and translational studies, and selected preprints addressing cancer-associated ecDNA biology, evolution, chromatin regulation, therapeutic resistance, detection, or clinical relevance. Duplicates and studies lacking substantive relevance to the review objectives were excluded. Selection was guided by relevance, methodological quality, scientific contribution, and mechanistic strength, with priority given to primary studies providing direct experimental evidence. As a narrative review, no formal risk-of-bias assessment or meta-analysis was performed. Evidence was synthesized thematically, distinguishing established findings from associative evidence and emerging hypotheses.

Biology and Biogenesis of Extrachromosomal DNA

ecDNA is a circular, double-stranded DNA. It exists independently of the canonical chromosomal complement within the cell nucleus. ecDNA molecules are typically large and lack centromeres or telomeres. These features distinguish ecDNA from linear chromosomal DNA and contribute to its non-canonical behaviour in tumour cells. ecDNA often contains full-length oncogenes and their associated regulatory sequences, which gives it increased transcriptional potential compared to equivalent genomic loci due to increased chromatin accessibility. This enhances accessibility and contributes to the hyperactive oncogene expression that drives tumour progression and malignant phenotypes (7). The size of ecDNA found in s is 100-to-1000-fold larger than Extrachromosomal circular DNA(eccDNA) and is usually in the range of 1-3 Mb or larger [8]. EcDNA always carries multiple copies of oncogenes that drive tumour growth and survival and plays important roles in promoting tumour occurrence and development. A study by Kim and his colleagues highlights that ecDNA is commonly found in different types of cancers. However, it rarely occurs in healthy tissues, which

emphasizes its association with cancer transformation and disease progression (9).

The circular structure of ecDNA may support independent replication and facilitate the reorganization of regulatory elements. It has been observed to expand the transcriptional repertoire of cancer cells beyond the limitations of chromosomal architecture (10). The formation of ecDNA is a multistep process, directly linked to genomic instability and DNA rearrangements. It involves several distinct but partially overlapping mechanisms. Circular DNA fragments, which later turn into ecDNA, are generated during multiple processes.

Chromothripsis is one of the best-characterized mechanisms of ecDNA formation. It involves a single catastrophic event in which chromosomes undergo extensive fragmentation followed by random rejoining of chromosomal fragments. During this process, some DNA fragments are not integrated into chromosomes but instead circularize to form ecDNA. These circular fragments retain oncogenes and regulatory elements, which contribute to their functional significance in cancer progression (11).

Another mechanism responsible for ecDNA formation is the breakage-fusion-bridge (BFB) cycles. They are initiated by telomere shortening or dysfunction, leading to end-to-end chromosome fusions and the formation of dicentric chromosomes. During mitosis, mechanical stress causes these chromosomes to break unevenly, generating amplified DNA segments that may subsequently be excised and circularized into ecDNA. Repeated BFB cycles further increase genomic rearrangements and amplification levels (12).

Replication-based mechanisms also play a crucial role in ecDNA formation. Replication fork stalling, template switching, and abnormal repair of double-strand breaks can lead to complex genomic rearrangements. These processes generate ecDNA fragments through aberrant replication and misalignment of homologous sequences, facilitating their cyclization of chromosome ends (13).

According to studies ecDNA formation may also occur via the excision of chromosomal regions containing super-enhancers and oncogenes, followed by their circularization. This process enables the creation of highly transcriptionally active ecDNA hubs, which can dynamically regulate oncogene expression and contribute to tumour heterogeneity (14).

These formation mechanisms contribute to tumour evolution by generating ecDNA populations that differ in structure, copy number, and genetic content. Such variation creates distinct tumour-cell states within the same tumour, providing a substrate for selection. Cells carrying ecDNA configurations that confer greater proliferative or survival advantages can expand under changing tumour conditions or therapeutic pressure, enabling rapid adaptation of the tumour population.

Fig 1. illustrates the mechanism contributing to the formation of extrachromosomal DNA (ecDNA). DNA double-strand breaks can undergo repair through non-homologous end joining (NHEJ), homologous recombination (HR), or alternative end joining (a-EJ), resulting in circular DNA structures. Chromothripsis can cause extensive chromosome rearrangements and generate ecDNA fragments. The breakage-fusion-bridge cycle involves telomere loss, chromosome fusion, replication, and subsequent breakage, which can produce circular DNA. Replication fork stalling and associated DNA damage may also contribute to the excision and circularization of genomic fragments, ultimately leading to ecDNA formation.

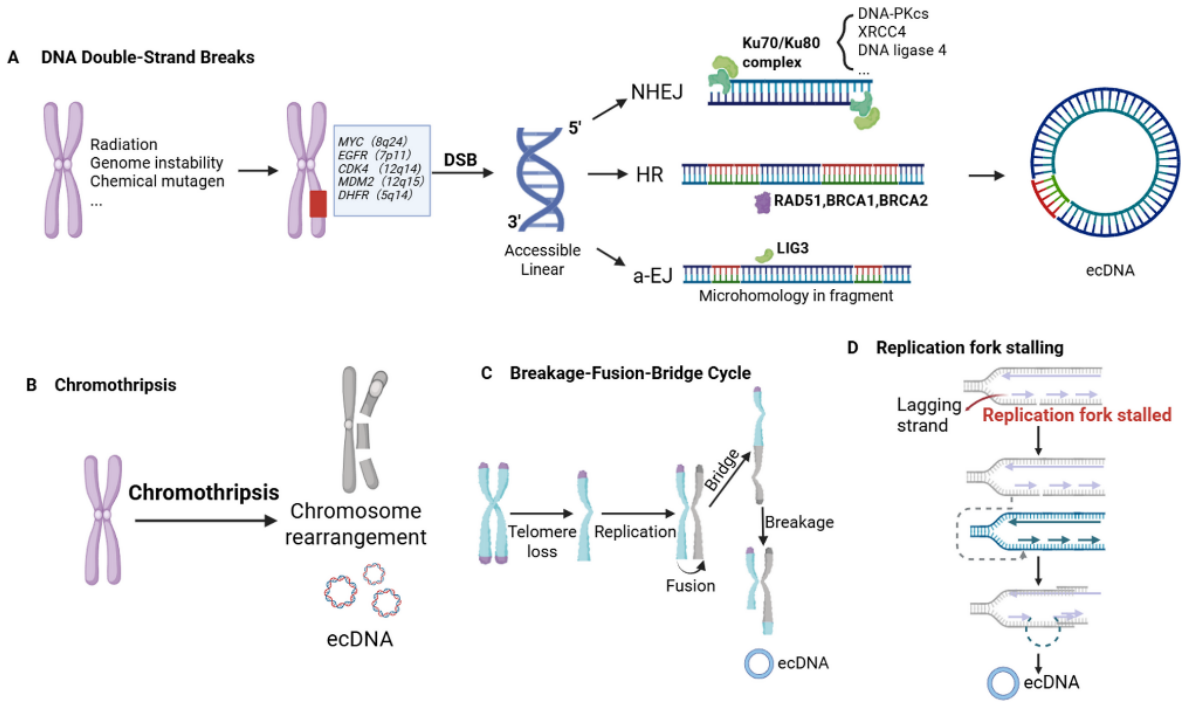

Figure 1. Mechanisms contributing to the formation of extrachromosomal DNA (ecDNA) (15)

Extrachromosomal DNA as an Engine of Adaptive tumour Evolution

Inheritance, variation, and selection are fundamental principles of Darwinian evolution and provide a framework for understanding how tumour populations emerge, adapt, and diversify (16). Within this framework, tumour evolution occurs when genetically and phenotypically diverse cancer-cell populations compete under changing environmental and therapeutic pressures, allowing variants with greater fitness to expand. ecDNA introduces an additional source of variation that differs from conventional chromosomal inheritance because its copy number and distribution can change rapidly between tumour cells. By uncoupling oncogene amplification from the fixed architecture and segregation of chromosomes, ecDNA can increase the diversity of cellular states available for selection and thereby influence tumour evolutionary trajectories (17).

ecDNA generates heritable variation through differences in copy number, composition, and inheritance. This was supported by Live-cell imaging studies showing that ecDNA can segregate unevenly during mitosis, producing daughter cells with different ecDNA burdens and different oncogene dosage (18). Studies have also revealed that tumour cells can harbour multiple ecDNA species carrying distinct oncogenes, which may be differentially inherited between daughter cells. ecDNA molecules were observed to interact within nuclear hubs, enabling cooperation between oncogenes and regulatory elements and altering transcriptional output (9). These findings indicate that ecDNA inheritance does not simply maintain oncogene amplification. It generates variation in oncogene dosage and regulatory configuration continously, providing the phenotypic diversity on which tumour selection can act.

Experimental studies also provided evidence that ecDNA-derived variation can influence tumour adaptation. It was demonstrated using glioblastoma cells carrying epidermal growth factor receptor variant III (EGFRvIII)-amplified ecDNA exhibited substantial cell-to-cell variation in ecDNA copy number and could rapidly alter their copy-number distributions in response to metabolic stress. Similar changes also occurred during erlotinib treatment, indicating that ecDNA-containing populations could adjust oncogene dosage over only a few cell divisions and thereby respond rapidly to environmental and therapeutic pressure (19). Complementing these findings, Hendriksen identified oncogene-carrying ecDNA within distinct tumour subclones and showed that ecDNA-positive populations were associated with different transcriptional states. Supporting the interpretation of ecDNA-associated variation providing cellular states that are subsequently enriched during tumour progression, ecDNA-bearing subclones present at low frequency in primary tumours could become dominant at relapse, (20). These studies have moved beyond the observation that ecDNA correlates with heterogeneity by demonstrating how ecDNA-associated variation can be dynamically redistributed and selected under changing conditions.

These findings imply that ecDNA functions as an evolutionary substrate instead of a product of genomic instability. Its ability to generate variation in oncogene dosage, ecDNA composition, and transcriptional activity expands the range of phenotypic states within a tumour, while dynamic inheritance allows these states to change rapidly across generations. In that way, ecDNA links genomic variation to differential cellular fitness, creating a mechanism through which tumours can rapidly explore, select, and expand adaptive phenotypes. The dynamic evolutionary process through which ecDNA generates, redistributes, and selects phenotypic variation is illustrated in (Fig 2).

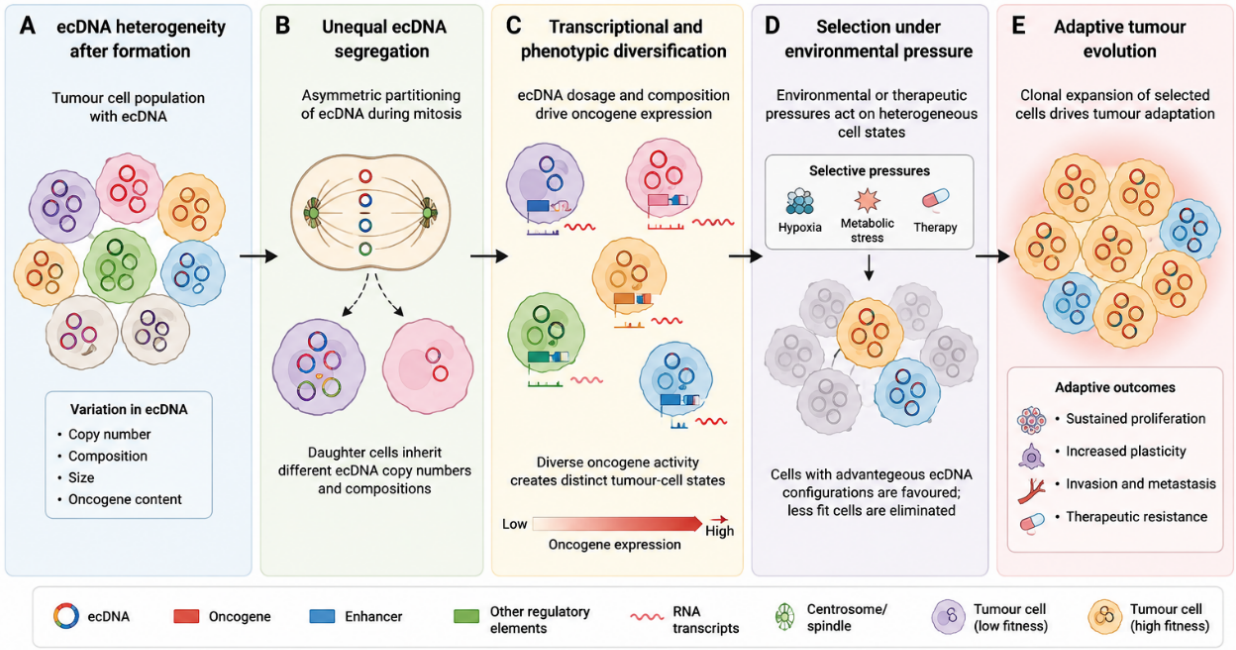

Figure 2. ecDNA as an evolutionary engine driving tumour adaptation.
(A) After ecDNA formation, tumour cells display heterogeneity in ecDNA copy number, composition, size and oncogene content. **(B)** During mitosis, ecDNA molecules are partitioned unequally between daughter cells, generating progeny with different ecDNA loads and compositions. **(C)** These differences lead to variable oncogene expression and the emergence of diverse tumour-cell states with distinct phenotypes. **(D)** Environmental or therapeutic pressures (e.g., hypoxia, metabolic stress, or targeted therapy) act as selective forces, eliminating less fit cells while favouring those with advantageous ecDNA configurations. **(E)** Selected cells expand clonally, producing an adapted tumour population characterized by sustained proliferation, increased plasticity, invasive potential and therapeutic resistance.

Figure 2. Extrachromosomal DNA as an Engine of Adaptive tumour Evolution

Chromatin Architecture and Transcriptional Regulation by ecDNA

The transcriptional consequences of ecDNA cannot be explained by increased oncogene copy number alone. Evidence from structural, chromatin and functional studies indicate that ecDNA simultaneously alters genome architecture, regulatory-element usage and chromatin state, thereby increasing the transcriptional output of amplified genes. It was found that ecDNA lacks the higher-order compaction characteristic of chromosomes, exhibits increased chromatin accessibility and makes more frequent ultra-long-range contacts with active chromatin. These features were seen to be associated with exceptionally high expression of ecDNA-borne oncogenes across cancers (21). As a result, ecDNA amplification appears to couple gene dosages to a distinct regulatory environment instead of simply providing additional copies of an otherwise equivalent chromosomal template.

This altered chromatin configuration creates opportunities for enhancer hijacking, in which oncogenes acquire regulatory input from non-canonical enhancers. Studies of ecDNA-containing tumours have identified enhancer-oncogene interactions extending across genomic regions and, in some cases, different chromosomes (22). In neuroblastoma, enhancer hijacking has been shown to shape the architecture of extrachromosomal MYCN proto-oncogen MYCN amplicons, illustrating that regulatory elements can become co-amplified with oncogenes and contribute directly to their expression (23). More broadly, three-dimensional (3D) genomic analyses have identified enhancer-hijacking events involving oncogenes such as MYC proto-oncogen (MYC), Cyclin D1 (CCND1) and ETS variant transcription factor 1 (ETV1), including complex amplicons containing ecDNA (24). The present suggests that the functional advantage of ecDNA depends not simply on which oncogene is amplified,

but also on which regulatory elements are captured and how they are reorganized within the amplicon.

The regulatory consequences can extend beyond the ecDNA itself. Widespread interactions between ecDNA and chromosomal DNA, with ecDNA-associated regions enriched for H3K27ac and preferentially interacting with actively expressed chromosomal genes. Crucially, experimentally generated enhancer-containing ecDNA was sufficient to increase transcription of chromosomal genes, providing functional evidence that ecDNA can operate as a mobile regulatory element in trans as opposed to acting exclusively on genes carried in cis (21). With this discovery, the interpretation of ecDNA from a passive amplification product to an active component of the transcriptional network. It also suggests that super-enhancer activity on ecDNA may contribute to oncogenesis not merely by intensifying transcription of the amplified oncogene, but by redistributing regulatory activity across the genome.

The high transcriptional capacity of ecDNA is further reinforced by its epigenetic state. Recent studies revealed the enrichment of active chromatin features and reduced higher-order compaction on ecDNA, while subsequent work has confirmed that ecDNA can display highly accessible chromatin and active histone modifications such as H3K27ac and H3K4me3 (21). However, increased accessibility should not be interpreted as an isolated cause of oncogene overexpression. The evidence supports a model in which accessibility acts together with increased copy number and enhancer activity to increase transcriptional output. This distinction is important because it suggests that the regulatory productivity of ecDNA copies also contributes to their oncogenic effect (22). ecDNA hubs can facilitate cooperative interactions among ecDNA molecules, creating localized transcriptionally active environments that enhance oncogene expression. These hubs therefore represent an important spatial mechanism through which ecDNA can increase transcriptional output beyond simple gene-copy amplification (25). The resulting transcriptional plasticity provides a mechanistic link between ecDNA structure and the heterogeneous cellular phenotypes described in tumour evolution. Nevertheless, these mechanisms should not be regarded as universal properties of every ecDNA molecule. Current evidence supports viewing ecDNA not simply as an amplification mechanism, but as a dynamic regulatory architecture in which genetic dosage and epigenetic regulation are functionally coupled.

EcDNA and tumour Plasticity

Tumour plasticity is the ability of cancer cells to change their identity, behavior, and physical traits to survive. IT is driven by Extrachromosomal DNA (ecDNA), which are small circular pieces of DNA found outside normal chromosomes (26).

Direct evidence for this relationship in pancreatic ductal adenocarcinoma (PDAC) was provided by integrating whole-genome sequencing, transcriptomic profiling, patient-derived organoids and functional analyses. They found substantial cell-to-cell variation in MYC ecDNA copy number and expression, with ecDNA-bearing models showing enrichment of aggressive transcriptional programmes, including epithelial–mesenchymal transition and glycolysis. Importantly, MYC expression did not increase proportionally with ecDNA copy number; differences in the regulatory elements retained on individual ecDNAs substantially influenced transcriptional output. Experimentally, altering stromal niche conditions produced reversible changes in MYC dosage, cell morphology and dependence on stromal factors, demonstrating that ecDNA-mediated oncogene dosage can also contribute directly to phenotypic adaptation (27).

Complementary evidence from neuroblastoma further indicates that ecDNA copy-number heterogeneity can translate into distinct cellular states. Using matched single-cell DNA and RNA sequencing, researchers showed that intercellular variation in ecDNA copy number altered MYCN expression and downstream transcriptional programmes, linking ecDNA dosage directly to differences in tumour-cell state (28). Uneven ecDNA segregation can contribute to intratumoural heterogeneity by generating variable oncogene activity among tumour cells, allowing distinct phenotypic states to emerge without permanent chromosomal alterations.

This plasticity has important implications for tumour progression and treatment response. Aws opposed to producing a single fixed phenotype, ecDNA can generate a dynamic range of oncogene dosage and transcriptional states from which cells may respond differently to microenvironmental or therapeutic pressures. Notably, high ecDNA copy number may also impose a fitness cost in the absence of selective pressure, indicating that ecDNA-mediated phenotypes are context dependent (27). Thus, ecDNA can function as a flexible genetic substrate that allows tumour cells to rapidly adjust phenotype and fitness, linking genomic heterogeneity to reversible tumour plasticity and, ultimately, adaptive disease progression.

Intratumoural heterogeneity

Heterogeneity describes the differences among cancer cells within and between tumours. It refers to cancer cells with variations in morphology, transcriptional profiles, metabolism, and metastatic potential. More recently, the field has included the characterization of the tumour immune microenvironment and the depiction of the dynamics underlying the cellular interactions promoting the tumour ecosystem evolution. It has been found in most tumours representing one of the most challenging behaviors in cancer ecosystems (29)

CRISPR-based live-cell imaging has shown that ecDNA can segregate unevenly during mitosis, generating substantial cell-to-cell variation in ecDNA copy number (18). However, ecDNA inheritance may involve more than random partitioning; recent evidence indicates that ecDNA can associate with mitotic chromosomes, suggesting that chromosome tethering contributes to its retention and transmission across cell divisions (30). This provides an additional layer of control over ecDNA inheritance beyond stochastic segregation. Multiple ecDNA species carrying distinct oncogenes can also undergo coordinated but asymmetric inheritance, producing daughter cells with simultaneous changes in ecDNA abundance before selection (31). These studies indicate that ecDNA contributes to intratumoural heterogeneity (ITH) at multiple levels copy number, ecDNA composition and spatial distribution. Crucially, this heterogeneity is dynamic and not just descriptive: ecDNA can generate molecular states before selection and can subsequently be redistributed as conditions change. ecDNA functions as a flexible source of intratumoural diversity that expands evolutionary possibilities, linking genomic heterogeneity to changing tumour states and therapy response.

Mechanisms of ecDNA-Mediated Drug Resistance

Therapeutic resistance in ecDNA-positive tumours can arise from the ability of ecDNA to dynamically alter oncogene expression during treatment. This phenomenon was described in glioblastoma models in which EGFRvIII was carried predominantly on ecDNA. EGFR-targeted treatment reduced EGFRvIII expression through loss of the EGFRvIII-containing ecDNA population, allowing cells with reduced EGFR signalling to survive treatment. Remarkably, following drug withdrawal, EGFRvIII rapidly re-emerged on ecDNA and restored high EGFRvIII expression. This reversible switching indicates that ecDNA-mediated resistance does not necessarily require permanent genetic mutations; instead, tumour cells can dynamically alter the genetic state that determines their drug sensitivity (32).

ecDNA can also facilitate resistance by maintaining heterogeneous oncogene dosage within the tumour. Because ecDNA copy number and composition vary between cells, therapy can selectively eliminate highly drug-sensitive populations while favouring cells carrying ecDNA configurations associated with reduced target dependence (32). In glioblastoma, for example, EGFRvIII-low cells are less sensitive to EGFR inhibition than EGFRvIII-high cells, illustrating how ecDNA-associated variation in oncogene expression can create differential therapeutic vulnerability (19). Thus, the same ecDNA-driven heterogeneity that contributes to tumour evolution can become a mechanism of treatment failure.

Resistance may additionally involve changes beyond the targeted oncogene. Studies of EGFR inhibition have shown that treatment can induce alternative survival programmes, including increased invasion and ALDH1A1-associated cell states, demonstrating that therapeutic pressure can reshape tumour phenotypes alongside changes in oncogenic signalling (33).

This evidence indicates that ecDNA promotes drug resistance through dynamic modulation of oncogene dosage, reversible loss and restoration of amplified oncogenes, and selection of less drug-sensitive cellular states. This plasticity allows ecDNA-positive tumour to alter their dependence on therapeutic targets without necessarily requiring irreversible genomic change, providing a mechanistic basis for rapid and potentially reversible therapeutic adaptation.

ecDNA in the Tumour Microenvironment and Metastatic Evolution

The tumour microenvironment (TME) provides a spatially heterogeneous environment in which cancer cells interact with immune, stromal and vascular components and experience differences in oxygen, nutrients and extracellular signals (34). These conditions should not be treated as passive features of tumour progression; recent evidence suggests that environmental pressures can influence the abundance and persistence of distinct ecDNA-bearing tumour states. The key question is whether ecDNA merely marks aggressive tumours or actively alters tumour-microenvironment interactions.

Evidence for an interaction between ecDNA and immune surveillance shows that ecDNA-positive cancers exhibited reduced immune-cell infiltration and cytotoxic T-cell activity together with lower expression of Major histocompatibility complex (MHC) class I and II antigen-presentation genes, despite comparable tumour mutational burden and neoantigen load (35). This finding suggests that ecDNA-positive tumours may be less effectively recognized by immune cells. However, the study was observational and hence cannot establish that ecDNA directly causes immune suppression. The findings should be interpreted as evidence of an ecDNA-associated immune phenotype and not definitive proof of an ecDNA-driven immune-evasion mechanism.

Evidence concerning other components of the TME remains less developed. ecDNA-containing tumour cells have shown adaptive responses to metabolic stress, indicating that ecDNA can influence cellular fitness under nutrient limitation (19). However, whether hypoxia, fibroblast signalling, angiogenic factors or extracellular-matrix conditions directly select for ecDNA species has not yet been established. This represents an important gap because metastatic niches impose environmental conditions that can differ substantially from those of primary tumours.

Large-scale genomic evidence nevertheless links ecDNA with advanced diseases. 8,060 tumour genomes were analyzed and ecDNA was seen to be more frequent in untreated metastases and heavily pretreated tumours than in newly diagnosed cancers. Longitudinal analysis further showed that ecDNA was more likely to persist than chromosomal amplifications (18). Although these observations do not establish causality, their consistency with persistence during metastatic progression suggests that ecDNA-bearing populations may possess properties favourable to survival in advanced disease.

Overall, current evidence supports ecDNA as a potential mediator of tumour–microenvironment adaptation, with the strongest evidence involving immune interactions and persistence during metastatic progression.

EcDNA Across Human Cancers

EcDNA is not restricted to a single tumour type but represents a recurrent mechanism of focal oncogene amplification across diverse cancers. In a large-scale analysis of 15,832 tumour samples spanning 39 cancer subtypes, researchers identified 4,716 distinct ecDNA in 2,532 ecDNA-positive tumours. The distribution of ecDNA was seen to be uniform across cancers, indicating that although ecDNA is a broadly conserved mechanism of genome alteration, its prevalence and genomic content are influenced by tumour context. The frequent association of ecDNA with amplified oncogenes supports the view that ecDNA provides a distinct route for generating high-level oncogene dosage beyond conventional chromosomal amplification (36).

Evidence from individual tumour types further illustrates this heterogeneity. In a cohort of 1,216 previously untreated lung cancers, ecDNA was detected in 18.9% of cases. EcDNA prevalence was similar between smokers and non-smokers and was largely independent of age, sex and histological subtype, although carcinoid tumours showed lower. Notably, ecDNA carried oncogenes such as MDM2 proto-oncogene (MDM2) suggesting that ecDNA-mediated amplification can occur across lung cancer subtypes and is not restricted to tumours arising in the context of environmental exposures. Patients harbouring ecDNA also had poorer overall survival, linking ecDNA presence with clinically aggressive disease (37). However, this association does not by itself establish that ecDNA causes poor survival; ecDNA may instead mark tumours with greater genomic instability or other aggressive biological features.

Overall, the evidence suggests that the significance of ecDNA lies not simply in its prevalence, but in its capacity to generate and maintain variation within tumour cell populations. Its dynamic copy number, transcriptional activity and unequal inheritance provide a potential basis for rapid changes in oncogene dosage under selective pressure. This raises the possibility that ecDNA contributes to tumour adaptation and treatment resistance by allowing different cell states to emerge and persist as the tumour environment changes. This table depicts contribution of ecDNA to cancer progression across multiple tumour types by amplifying oncogenes and increasing their expression. These effects can promote tumour heterogeneity, plasticity, adaptation, and therapeutic resistance, although the specific oncogenes and biological consequences vary between cancer types.

Table 1. Major ecDNA- oncogene associated oncogenes and their biological effects across human cancers

Cancer type	Common ecDNA-associated genes	Major biological effects
Glioblastoma	EGFR, PDGFRA, CDK4, MDM2	Oncogene amplification, tumour plasticity, therapeutic resistance
Neuroblastoma	MYCN	Increased oncogene expression and tumour progression
Lung cancer	EGFR, MYC, MDM2	Oncogene amplification and therapeutic adaptation
Breast cancer	ERBB2/HER2, MYC	Oncogene amplification and tumour heterogeneity
Colorectal cancer	MYC, EGFR	Tumour evolution and therapeutic resistance
Ovarian cancer	MYC and other amplified oncogenes	Genomic instability and tumour progression
Gastric cancer	ERBB2/HER2, MYC	Enhanced oncogenic signalling and tumour progression

Technologies for Detecting and Characterising ecDNA

Accurate detection and characterization of ecDNA is essential for understanding how these structures contribute to amplification, tumour heterogeneity and adaptive evolution. Current approaches are broadly divided into microscopy-based, sequencing-based and CRISPR-based technologies, each providing complementary information about ecDNA abundance, structure, genomic content and behaviour.

Fluorescence microscopy-based approaches

Fluorescence microscopy provides direct visual evidence of extrachromosomal DNA within individual tumour cells. 4′,6-diamidino-2-phenylindole (DAPI) staining combined with DNA fluorescence in Situ Hybridization DNA-FISH and centromeric probes can distinguish extrachromosomal signals from chromosomal DNA and reveal their spatial distribution. These approaches remain particularly valuable for confirming ecDNA at the cellular level and distinguishing ecDNA from chromosomal amplifications, although they provide limited throughput and require careful image interpretation (38).

To address this limitation, ecdetect was developed as a semi-automated image-analysis approach for quantifying ecDNA from DAPI-stained metaphase cells. Across 2,572 metaphase cells, measurements showed a strong correlation with manual visual assessment ($r = 0.98$), supporting improved reproducibility of ecDNA quantification (39).

Deep-learning approaches such as ecDNA Segmentation (ecSeg) have used DAPI and FISH signals to improve segmentation and distinguish oncogene-containing ecDNA from chromosomal amplifications (40). These approaches reduce some of the subjectivity associated with manual microscopy and allow ecDNA to be assessed across larger numbers of cells.

Sequencing-based approaches

Sequencing approaches provide information that microscopy cannot readily obtain, particularly the genomic composition, breakpoint structure and architecture of ecDNA. Large-scale whole-genome sequencing combined with computational reconstruction has enabled ecDNA to be identified and characterized across thousands of tumours, including the recent pan-cancer analysis of 15,832 tumour samples spanning 39 tumour types (36). Machine-learning approaches have also enabled ecDNA detection from large sequencing cohorts and are primarily focused on identifying ecDNA-associated amplification(41).

Long-read sequencing has ec3D further improved the resolution of ecDNA architecture. Recent long-read whole-genome sequencing of high-grade serous ovarian cancer demonstrated that individual ecDNAs can be reconstructed at sequence level while simultaneously characterizing their genomic instability, methylation and enhancer content (42).

More recently, ec3D has extended structural analysis beyond sequence reconstruction by using High-throughput chromosome conformation capture (Hi-C) data to model the three-dimensional organi of ecDNA, including complex structures with duplicated segments and multi-way interactions (43).

The major contribution of these sequencing approaches is that they move ecDNA analysis beyond detecting the presence of amplification towards determining its genomic and spatial organization. This distinction is important because the arrangement of oncogenes and regulatory elements within ecDNA can

influence its transcriptional activity and evolutionary behaviour.

CRISPR-based approaches

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based approaches extend conventional imaging by enabling specific ecDNA molecules to be labelled and tracked in living cells. The ecDNA tagging |(ecTag) system uses ecDNA-specific breakpoint junctions to design guide RNAs, which are coupled to the Casilio fluorescent-labelling system for live-cell visualization of individual ecDNA molecules (17). The ability to visualise ecDNA in living cells provides information that cannot be obtained from fixed-cell imaging or sequencing alone. ecTag uses ecDNA-specific breakpoint junctions to target individual ecDNA molecules for fluorescent labelling, allowing their behaviour to be followed in living cells. CRISPR-based Capture (CRISPR-CATCH) provides a complementary approach by enabling targeted enrichment of large ecDNA molecules from cancer cell lines and patient-derived samples, facilitating more detailed analysis of their genetic and epigenetic features (14).

All these approaches provide complementary information about ecDNA at different levels. Microscopy can assess its cellular distribution and abundance, sequencing can define its genomic composition and structure, while CRISPR-based methods allow specific ecDNA molecules to be visualised or enriched for further analysis. Current ecDNA technologies offer complementary strengths for resolving its cellular distribution, genomic structure, three-dimensional organization, and dynamic behaviour, with each approach presenting distinct limitations and applications (Table 2). The table summarizes the principal strengths, limitations, and optimal applications of commonly used approaches, including fluorescence-based imaging, genome sequencing and computational reconstruction, long-read sequencing, three-dimensional genome mapping, live-cell ecDNA tracking, and targeted CRISPR-based enrichment. Together, these approaches provide complementary information spanning ecDNA localization, structural architecture, regulatory organization, inheritance, and functional dynamics.

Table 2. Comparative overview of technologies for ecDNA detection and characterization.

Approach	Principal strength	Key limitation	Best suited application
FISH/DAPI	Cellular visualization	Limited structural resolution	Confirmation
WGS/computational reconstruction	Genome-wide architecture	Inference from sequencing data	Discovery
Long-read sequencing	Breakpoint/structure resolution	Cost/availability	Structural characterization
Hi-C/ec3D	3D architecture	Computational complexity	Regulatory architecture
ecTag	Live-cell dynamics	Requires breakpoint knowledge	Inheritance/segregation
CRISPR-CATCH	Targeted enrichment	Requires specific design	Molecular characterization

Therapeutic Targeting of ecDNA

The therapeutic targeting of ecDNA is still challenging because ecDNA is structurally dynamic and can be eliminated, rearranged, or reintegrated into chromosomes under therapeutic pressure. Consequently, current approaches largely target dependencies created by ecDNA instead of ecDNA molecules themselves (44).

DNA damage response (DDR)atm-targeting provides one of the strongest mechanistic opportunities. Studies have shown that ecDNA-bearing cells experience replication-associated stress and depend on DDR signalling to maintain their amplified genomes, providing a rationale for targeting Ataxia telangiectasia mutated/Checkpoint kinase 1(ATM/CHK1) associated pathways. However, DDR inhibition alone may exert selective pressure that favours only ecDNA elimination or chromosomal reintegration (45). This suggests that DDR inhibitors may be more effective when combined with inhibition of the oncogenic pathway maintained by the ecDNA. Considering this, the key therapeutic question is if it

prevents ecDNA-positive cells from recovering through alternative genomic configurations.

Chromatin-directed approaches provide a complementary strategy by targeting the regulatory mechanisms that maintain oncogene expression from ecDNA. Bromodomain and extra terminal |(BET) inhibition with (JQ1 compound/inhibitor) JQ1 was shown to disrupt Bromodomain-Containing Protein 4 (BRD4) associated ecDNA hubs and preferentially reduce transcription from ecDNA-derived oncogenes, providing functional evidence that the transcriptional organization of ecDNA can be therapeutically targeted (14).However, because BRD4 also regulates transcription from chromosomal loci, the observed effect cannot by itself establish an ecDNA-specific therapeutic window. The study supports ecDNA-associated chromatin as a vulnerability, but not yet as a clinically selective target.

The translation of these vulnerabilities into patients is still limited. BBI-355, a selective CHK1 inhibitor developed as an ecDNA-directed therapy, was evaluated in the phase 1/2 POTENTIATE trial in patients with solid tumours harbouring oncogene amplifications. The trial was terminated in June 2026 based on overall clinical experience and market considerations (46). For this reason, the trial could not determine whether CHK1 inhibition is effective specifically against ecDNA-driven tumours. This highlights a broader problem in the field: copy-number amplification is not equivalent to ecDNA, and failure to distinguish the two can obscure the clinical value of ecDNA-specific vulnerabilities

Overall, current evidence supports ecDNA-associated DDR and transcriptional dependencies as promising but incompletely validated therapeutic vulnerabilities. Future studies should determine which vulnerabilities are truly dependent on ecDNA, establish reliable methods for identifying ecDNA-positive tumours, and evaluate rational combination therapies in molecularly selected patients.

EcDNA as a Biomarker for Precision Oncology

The clinical value of ecDNA increasingly extends beyond its biological role, with evidence supporting its potential as a biomarker for diagnosis, prognosis, treatment selection and disease monitoring. However, the strength of evidence differs substantially across these applications.

Large-scale genomic studies showed that ecDNA amplification was linked to multiple tumour types, supporting its potential as a molecular marker of tumour biology. However, detection alone does not establish diagnostic specificity. Stronger evidence for non-invasive detection comes from studies of circulating small eccDNAs. Cancer-associated small eccDNA signatures have been identified in both tissue and plasma samples, with several gene-derived eccDNA signatures achieving area under curve (AUC) above 0.9 in plasma and showing potential for distinguishing cancer from non-cancer samples in independent validation cohorts (47). However, these small eccDNAs are distinct from the large oncogenic ecDNAs that commonly carry amplified oncogenes, limiting their interpretation as direct biomarkers of oncogenic ecDNA amplification.

Evidence is stronger for prognostic associations. Large-scale analysis of more than 13,000 cancers found that ecDNA amplification was associated with poorer survival in several tumour cohorts, although the strength of this association varied between tumour types and genomic contexts. In advanced gastric cancer, ecDNA amplification was also an independent negative predictor of response to anti-programmed cell death protein 1 (anti-PD-)1 therapy after adjustment for Programmed cell death protein ligand (PD-L1) status and tumour mutational burden (48). These findings suggest that ecDNA status may provide prognostic and potentially predictive information beyond amplification status alone, although its clinical value is unlikely to be uniform across cancers.

Early evidence also supports longitudinal monitoring. In a cohort of 32 patients with EGFR-mutant non-small cell lung cancer (NSCLC) receiving osimertinib, higher levels of EGFR-overlapping plasma eccDNA during treatment were associated with shorter progression-free and overall survival (49). These findings suggest that circulating eccDNA may provide a dynamic treatment-response signal, although the small cohort and retrospective analysis limit generalization.

Liquid biopsy is particularly attractive because serial sampling could capture changes in circulating eccDNA without repeated tissue biopsies. However, circulating eccDNA is biologically heterogeneous, and its abundance in plasma does not necessarily reflect the presence or abundance of tumour-derived oncogenic ecDNA. So, establishing tissue-plasma concordance is an important requirement before circulating eccDNA can be considered a reliable surrogate for tumour-associated ecDNA (47).

Current Challenges, Knowledge Gaps, and Future Directions

Despite its significant advances, a major hurdle is identifying which aspects of ecDNA are causal and therapeutically actionable. Association with tumour progression, metastasis and decreased survival has been shown in large studies, but this is not proof of treatment failure due to the presence of ecDNA. Future studies are needed to compare ecDNA-positive tumours with chromosomally amplified tumours that express the same oncogene to separate out the effects of ecDNA from those of high-level amplification (50).

Another is the challenge of unraveling ecDNA as a dynamic and heterogeneous structure. Bulk sequencing may miss rare ecDNA containing clones, structural variations among individual ecDNAs, and there are variations between imaging and sequencing methods in their sensitivity and resolution. Recent progress in single-cell and long-read imaging techniques and automated imaging could be used to improve this, and future research will need to establish these methods in a standard clinical assay and integrate them with longitudinal sampling in order to gain insight into the dynamics of ecDNA structure and abundance during tumour evolution and treatment (38).

A related gap is therapeutic development. In this context, current targets (e.g., DDR and transcriptional regulators) prove that dependencies associated with ecDNA can be targeted, but they have not been shown to be ecDNA-specific, thus restricting translation. Ideally, future drug discovery efforts should target ecDNA-specific mechanisms of maintenance, segregation and mobility, while identifying and targeting dependencies that are less important in chromosomal amplification via CRISPR screening, chemical proteomics and engineered ecDNA models. Such causal relationships can be tested in an experimental system using engineered ecDNA systems that are recently available (51).

Conclusion

ecDNA represents a distinctive form of genomic organization that can reshape how tumours evolve under selective pressure. Its importance lies not in carrying amplified oncogenes, but in creating a flexible genetic context in which oncogenic programmes can change as tumour conditions change. This provides a useful perspective on why some cancers display marked adaptability and treatment failure despite apparently stable genomic drivers. Nevertheless, the clinical significance of ecDNA remains incompletely defined, and its effects must be separated from those of conventional amplification and genomic instability. Continued advances in structural, single-cell, and longitudinal approaches should help resolve these uncertainties and establish which ecDNA-associated features are clinically actionable. Ultimately, understanding how ecDNA influences tumour cell survival may shift precision oncology from identifying static genomic alterations toward anticipating the evolutionary capacity of individual tumours.

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