Heliyon 10 (2024) e27733

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Review article

Insight on ecDNA-mediated tumorigenesis and drug resistance

Qing Huang [1], Su Zhang [1], Guosong Wang, Junhong Han [*]

Department of Biotherapy, Cancer Center and State Laboratory of Biotherapy and Frontiers Science Center for Disease-related Molecular Network, West China Hospital, Sichuan University, Chengdu, 610041, China

ARTICLE INFO

Keywords:
ecDNA
Tumor evolution
Oncogene amplification
Intratumoral heterogeneity
Cancer therapy

ABSTRACT

Extrachromosomal DNAs (ecDNAs) are a pervasive feature found in cancer and contain oncogenes and their corresponding regulatory elements. Their unique structural properties allow a rapid amplification of oncogenes and alter chromatin accessibility, leading to tumorigenesis and malignant development. The uneven segregation of ecDNA during cell division enhances intercellular genetic heterogeneity, which contributes to tumor evolution that might trigger drug resistance and chemotherapy tolerance. In addition, ecDNA has the ability to integrate into or detach from chromosomal DNA, such progress results into structural alterations and genomic rearrangements within cancer cells. Recent advances in multi-omics analysis revealing the genomic and epigenetic characteristics of ecDNA are anticipated to make valuable contributions to the development of precision cancer therapy. Herein, we conclud the mechanisms of ecDNA generation and the homeostasis of its dynamic structure. In addition to the latest techniques in ecDNA research including multi-omics analysis and biochemical validation methods, we also discuss the role of ecDNA in tumor development and treatment, especially in drug resistance, and future challenges of ecDNA in cancer therapy.

1. Introduction

Circular DNAs are abundantly present, along with different sizes, sources and functions [1–5]. The genomic DNA and plasmids of microorganisms such as bacteria or yeast exist are in circular form. Certain special transmission elements carrying genetic information are also existing and function as circular DNAs, such as transposon DNA and integrons [6]. Extrachromosomal circular DNA (eccDNA) is prevalent in humans in various types, including mitochondrial DNA (mtDNA) [7], small polydispersed circular DNA (spcDNA) [8], telomeric circle (t-circle/c-circle) [9,10] and others [11,12]. These eccDNAs are widely distributed across different types of human cells and play crucial functions in physiological processes. In addition, they have been implicated in pathological conditions such as senescence and cancer.

Extrachromosomal DNAs (ecDNAs), a type of circular DNA element as well, were originally discovered in tumor cells. These circular DNAs were initially termed "minute chromatin bodies" and later referred to as "double minutes (DMs)" [13], as they frequently come in pairs [14]. They predominantly exist with a mean size of 1.3 Mb [15] with the majority length ranging from 168 Kb to 5 Mb [16], which is substantially larger than the commonly-seen eccDNAs. Certain cancers have developed an especially effective way to "trick" the system by dropping growth-accelerating oncogenes onto circular extrachromosomal DNA elements. This mechanism allows

* Corresponding author. Department of Biotherapy, Cancer Center and State Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, 610041, China.
E-mail address: hjunhong@scu.edu.cn (J. Han).
[1] Qing Huang and Su Zhang contributed to this work equally.

https://doi.org/10.1016/j.heliyon.2024.e27733
Received 21 December 2023; Received in revised form 5 March 2024; Accepted 6 March 2024
Available online 11 March 2024

oncogenes to evade the typical constraints imposed by normal chromosomes [17,18], resulting in replication at an uncontrolled rate and a non-Mendelian inheritance pattern [19]. Oncogene-enriched ecDNA or transcriptional enhancers-carrying ecDNA can drive gene co-amplification [20–22], intrigue enhancer proximity effects [23] and the formation of specialized nuclear cluster particles (aka. ecDNA hubs) [24–27] leading to intra-tumoral genetic heterogeneity [28–30] and causing drug resistance [31–33]. In addition, ecDNA can be reintegrated into the genome, potentially disrupting gene regulation network in tumor cells [34,35]. Previous researches have revealed that ecDNA is extensively present in almost half of human cancers [36], particularly in advanced tumors, but it is rarely detected in healthy cells. However, a recent study conducted by Luebeck J et al. first proposed that ecDNA even exist in precancerous cells and its presence may serve as a triggering event in the initiation of cellular carcinogenesis [37]. Comparison with other focal amplifications, ecDNA is correlated with poorer patient prognosis [36,38–40]. ecDNA has emerged as a compelling focus in cancer research, following the debut of chemotherapy, targeted therapy and immunotherapy, with the potential to trigger the fourth revolution in the field of cancer treatment.

In this review, we summarise the process of ecDNA formation and the stabilization of its structure through cancer development and the emerging property of the crosstalk between epigenetics and ecDNA. Additionally, we provide an in-depth overview of the advancements made in technologies for detecting and characterizing oncogenic ecDNA, including both sequencing and experimental validation strategies, and the mechanisms of how ecDNA drives malignant tumor progression and its role in promoting therapeutic resistance. Furthermore, we discuss the future of the latest development of ecDNA-targeting drugs and how these innovations in clinical trials can improve the molecular stratification of tumor patients, ultimately augmenting the translational potential of novel therapeutic targets.

2. The generation and dynamic structural evolution of ecDNA

Persistent strong selection pressure applied to genomic instability is considered the major source which cause the formation of ecDNA [31,41]. The formation and fusion of broken chromosomal segments following chromothripsis are considered as the major source of ecDNA formation [42–47] (Fig. 1). We have summarized a variety of situations where ecDNA can form.

2.1. Breakage-fusion-bridge (BFB) cycles

The BFB cycle results in the production of anaphase bridges fusing sister chromatids or connecting the ends of different chromosomes, causing rearrangement of genomic segments [48]. During mitosis, newly formed chromosomes are pulled in the opposite

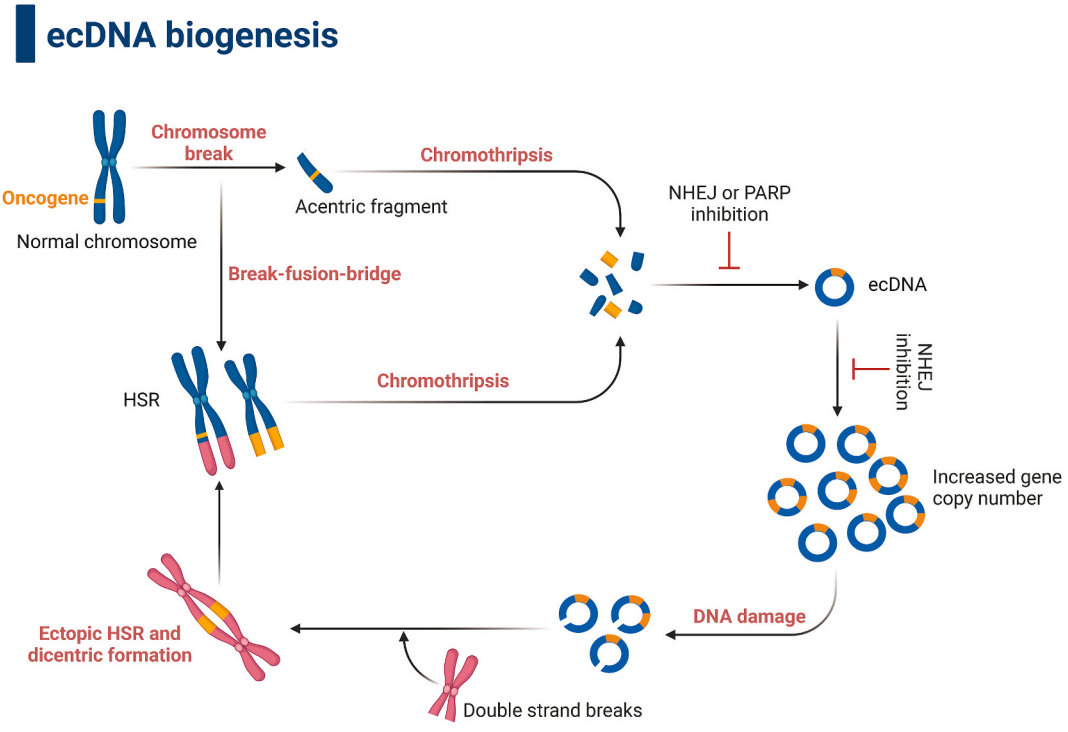

Fig. 1. Schematic illustration of ecDNA biogenesis. The origin of ecDNA in cells includes simple cyclization of broken segments after chromosome breakage and cojoining of multiple fragments after chromothripsis in nonhomologous end joining (NHEJ). DNA damage motivates ecDNA integration into the HSRs near the end of the chromosome while the initial HSR (through several BFB cycles) is broken during selection, generating highly rearranged ecDNA.

directions, causing breaks of the chromosome and then invoking the next BFB cycle. Due to the stochastic nature of the breakage, repetitive BFB cycles can produce a variety of genomic aberrations intriguing chromosomal and extrachromosomal gene amplifications, according to the location of the breakages.

2.2. Chromothripsis

Another consolidated mechanism of ecDNA production known as "seismic amplification" was proposed by Rosswog et al. This intricate pattern of copy number amplification resembles seismic waves, which provides new insights into the origin of ecDNA in cancer cells [49]. They suggested that chromothripsis may be one of the initiators driving seismic amplification. Chromothripsis facilitates a large number of gene rearrangement events, but this process alone does not lead to the expression of genes on the resulting fragments. Therefore, they proposed the circular recombination model, in which ecDNA was generated through recombination of different DNA elements after chromothripsis, which activated the abnormal expression of genes. This model primarily includes four processes: (1) Chromothripsis occurs on one or more chromosome regions to produce DNA fragments; (2) one or more chromosome segments are integrated into a form of circular DNA structures; (3) circular recombination of several cycles proceeds until stable; (4) the ecDNA-carrying DNA elements may be amplified or re-integrated into the chromosomes to form HSRs (Fig. 1). One study revealed that a 1.4 Mb complex ecDNA identified consisted of 14 nonadjacent and individually fragmented HSRs with variable copy numbers. This ecDNA formation was acquired by chromothripsis and respliced under continuous strong selection pressure. Moreover, Shoshani et al. [50] proposed that multiple selective pressure-induced BFB cycles lead to chromothripsis and eventually form highly rearranged ecDNAs.

2.3. Nonhomologous end joining (NHEJ)

Previous studies have documented that ecDNA can be incorporated into HSRs, subsequently leading to the generation of more ecDNAs [32,48,51]. Researchers found that DNA damage motivates the integration of ecDNA into HSRs located near the terminal end of the chromosome (Fig. 1). In the case of random double-strand breaks induced by ionizing radiation or doxorubicin, as well as specific double-strand breaks caused by site-specific nucleases, nearly 1/3 of ecDNAs were observed to form ectopic HSRs (eHSRs). In addition, in the presence of double-strand breaks caused by Cas9 at some specific targets on the chromosome, ecDNA was directly detected at these sites. Moreover, Shimizu et al. [52] confirmed that poly-ADP-ribose polymerase (PARP) inhibition following random DNA damage in Colo320DM-GFP cells increased the frequency of conversion of ecDNA to HSRs. Notably, Shoshani et al. [50] highlighted the crucial role of nonhomologous end joining (NHEJ) during ecDNA formation and observed that both DNA-PKcs and PARP inhibitors, as NHEJ inhibitors, could both effectively reduce the production of ecDNA (Fig. 1). Collectively, the generation of ecDNA occurred *via* BFB-chromothripsis is highly dependent on HSR and NHEJ, and the presence of PARP inhibitors greatly reduced the frequency of ecDNA formation (Fig. 1).

2.4. CCCTC binding factor (CTCF)-mediated loop extrusion

Recent studies have shown that apoptosis results in genomic instability which gives the forming of some small circular elements [53]. DNA damage susceptibility is affected by chromatin topology and accessibility which is largely maintained by CCCTC binding factor (CTCF) [54]. Koche RP et al. found that the enrichment of CTCF binding factor in chromatin using ChIP-seq is comparable to regions where ecDNA formed [55]. Whether in single cells or bulk cell populations, prominently enrichment of circular DNA breakpoints were found at CTCF-binding sites. Whereas at loci with high ATAC-seq signal, regions originating from small circular DNA origin were clearly depleted. This finding indicates that CTCF binding sites in chromatin and the abundant inaccessible sites on them [56] may predispose to the breakage and formation of circular DNA. The researchers also found a very low frequency of enrichment of histone modifications (H3K27me3, H3K4me1, and H3K27ac) within small circular DNA-forming sites, suggesting that the H3K27me3-, H3K4me1-, and H3K27ac -labeled sites were possibly shielded from disruption and circularization. CTCF regulates chromatin topology through intermediating chromatin ring formation, which indicates that CTCF-mediated DNA breakage resulting from the loop extrusion process may contribute to the formation of small circular DNAs, suggesting a new way to investigate the formation of ecDNA.

2.5. Alternative end-joining (alt-EJ)

As research progresses, more and more ways of ecDNA formation are being discovered. Retrotransposons are extremely abundant and present in almost all eukaryotic genomes. In human genome, retrotransposons occupy up to 38% of genomic regions [57–59]. Upon activation, the retrotransposon can utilize its mRNA as a template to synthesize double-stranded DNA, which can be inserted into the host genome [57–59]. The latest research has found that 90% of the retrotransposons form circular DNA during replication, while only 10% of them are integrated into the genome [60]. The retrotransposons encode the reverse transcriptase that synthesizes first-strand DNA, and Zhang et al. reported that the retrotransposons exploit the alternative end-joining (alt-EJ) of the host during DNA repair to produce their second-strand DNA in a circularization step [60]. This repair mechanism allows the ends of the retrotransposon DNA strand to be sutured and alt-EJ promotes the long terminal repeat fragments generation of the second strand and guides the second circular strand, resulting in the formation of double-stranded circular DNA.

Studies on cancer genomes have described the structural complexity of ecDNA [16,61–66]. Evaluation of the structural

heterogeneity of ecDNA between cells from a patient contributes to inferences about the dynamic structure of ecDNA. Through the analysis of neuroblastomas containing structurally complicated and large *MYCN*-containing ecDNA, it was observed that the ecDNA of one patient was consisted of five separate genomic segments, all of which were derived from chromosome 2, connected to each other by several structural variants (SVs) in a way that was simple enough to be reconstructed reliably in a single cell [67] (Fig. 2). However, in some patients, the ecDNA structure is exceedingly intricate, making it challenging to fully reconstruct computationally. This finding suggests that ecDNA exhibits considerable structural variations within a subset of single cells.

3. Approaches for ecDNA detection

3.1. Sequencing technology

3.1.1. Whole genome sequencing (WGS)

Increasing popularity of routine whole genome sequencing (WGS) will enhance the detection of the existence of ecDNA during clinical cancer diagnosis [36,68]. WGS enables characterization of ecDNA that the structure of ecDNA can be inferred from WGS data using software tools such as AmpliconArchitect [69], AmpliconReconstructor [70], Circle_finder and Circle-Map [71]. These methods identify genomic regions with elevated copy numbers as seeds to construct circular diagrams in combination with junctional reads at the break points of circular DNA. Successful construction of the ecDNA can facilitate the determination of its structural characteristics and the content of the gene or regulatory element it carries. AmpliconArchitect is able to analyze low-coverage (down to 10 ×) WGS data with short reads to identify breakpoints and infer circles from chimeric sequences. This tool can be used to characterize the structure of ecDNA amplicons, e.g., oncogenes carried by the ecDNA, the detection of human-viral hybridized ecDNA signatures in viral-associated tumor samples [69]. With the ability of ecDNA to induce amplification, a sequencing depth of 10 × is generally sufficient to cover a high number of ecDNA sites, allowing even lower coverage could be adequate for detecting ecDNA through WGS [69]. The analysis tool AmpliconReconstructor combines short-read WGS and optical mapping data for fluorescence-based localization of the approximate position of short DNA sequences within longer DNA fragments (>150 kbp), allowing for a clearer resolution of ecDNA structure [70]. As the cost of next-generation sequencing (NGS) declines, WGS data can be utilized for a routine clinical ecDNA characterization thanks to the automated, newer computational tools which are more easier to use in clinical laboratories and require less expertise from bioinformaticians (Table 1) (Fig. 3A).

3.1.2. Circle-Seq

For more precise and specific identification of ecDNA, protocols such as Circle-seq has been developed (Fig. 3A). By removing linear DNA using exonuclease cleavage, followed by circular DNA enrichment through rolling circle amplification in combination of short- or long-read sequencing, Circle-Seq gives strong technical support for comprehensive identification of ecDNA [34,72]. This method can also be integrated with nanopore sequencing to enable full-length characterization of ecDNA in cell lines [53] (Table 1).

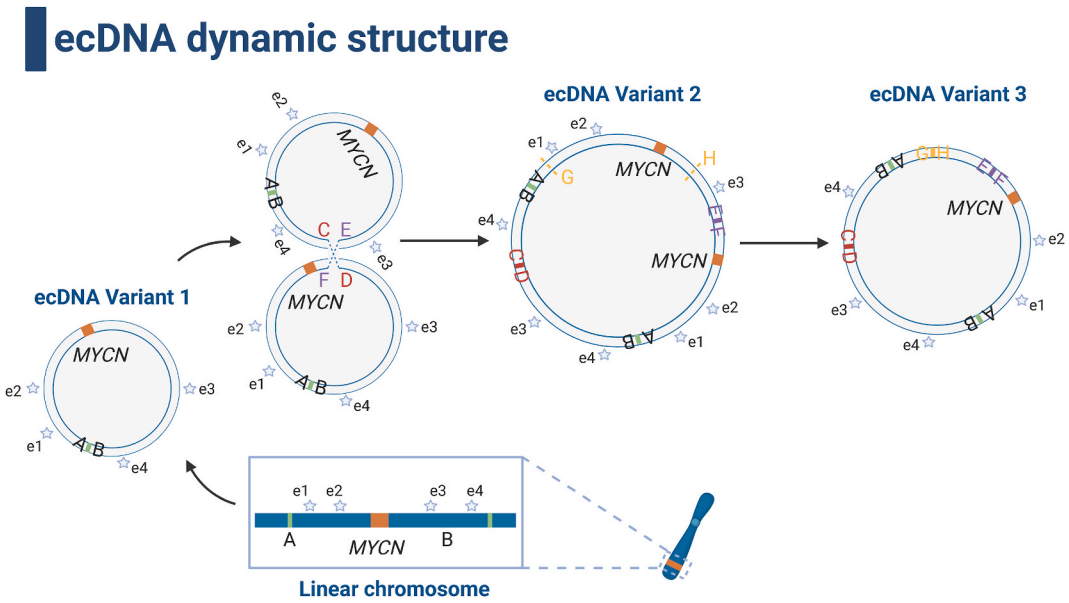

Fig. 2. Representation of the evolution of ecDNA structure. The simplest sequence of ecDNA begins with a region containing MYCN and neighbouring chromosomes, termed ecDNA variant no. 1. The position of the MYCN oncogene and its local enhancer elements (e1-e4) in each ecDNA variant is indicated by a single asterisk. Two ecDNA variant no. 1 forming a more complex rearranged ecDNA variant no. 2. A large deletion upon ecDNA variant no. 2 would generate ecDNA variant no. 3. The dominance of ecDNA variant no. 3 in tumour cells suggests that it may have a positive selection advantage.

Table 1
Overview of ecDNA detection methods.

Methods	Description	Advantage or Limitation	Refs
Whole genome sequencing (WGS)	bulk short- or long-read sequencing, provides much higher sequence resolution.	High throughput, high resolution, sensitive, but the ambiguous origin of sequencing reads and the sequencing reads are far too short to span the entire length of an ecDNA molecule.	[36,68, 69,71]
Circle-Seq	Enrich circular DNA and eliminate linear DNA interference.	High throughput, high resolution, sensitive, expensive.	[61,72]
CRISPR-CATCH	Target identification of predetermined genes	Targeting analysis of both the genetic sequence and epigenomic landscape of isolated ecDNA.	[165]
Single-cell extrachromosomal circular DNA and transcriptome sequencing (scEC&T-seq)	Parallel sequencing of all circular DNAs and full-length mRNA in a single cell.	Distinguish the transcriptional consequences arising from ecDNA-driven intercellular oncogene copy number heterogeneity, low throughput.	[67]
Sequencing enzyme-accessible chromatin in circular DNA (CCDA-seq)	Based on methylase labeling of unbroken open chromatin and exonucleases to enrich the depth of ecDNA sequencing, followed by long-read nanopore sequencing.	Investigate ecDNA chromatin accessibility at a single-molecule resolution, loss of mega ecDNA, the insufficient sequencing depth.	[166]
Fluorescence in situ hybridization (FISH)	Localization, detection, quantification.	Provides excellent separation of ecDNA and chromosomal DNA signals and has been used to confirm the presence of oncogenes and drug resistance genes on ecDNA, but little sequence information	[65,80, 147]
Electron microscopy	Localization, detection, quantification.	No sequence information	[65]

3.1.3. CRISPR-CATCH

A further powerful technique for targeted ecDNA analysis is CRISPR-CATCH (Fig. 3A), a new method to isolate ecDNA containing different oncogenes from cancer cells using *in vitro* CRISPR-Cas9 treatment and pulsed-field gel electrophoresis of agarose-encapsulated genomic DNA. Hung, King L et al. [73]enriched *NRAS* ecDNA in human metastatic melanoma with acquired treatment resistance and ecDNA containing *FGFR2*, *MYC* and *EGFR* in several different cancer cells. Targeted enrichment of ecDNA can phasing of genetic variants compared to chromosomal DNA. They also identified that EGFRvIII mutations present only on ecDNA, and supported a model for excision of ecDNA occurrence in glioblastoma models. Followed by nanopore sequencing, CRISPR-CATCH allowed for single-molecule methylation analysis of ecDNA and revealed hypomethylation of the EGFR promoter on ecDNA. Thus, this approach allows not only targeted analyses of ecDNA genetic sequences but reveals epigenomic landscapes. In summary, CRISPR-CATCH is a supervised method (used to identify targets for predetermined genes) and Circle-Seq is unsupervised (Table 1).

3.1.4. Single-cell extrachromosomal circular DNA and transcriptome sequencing (scEC&T-seq)

The non-mendelian inheritance of ecDNA was demonstrated to be a substantial reason for causing tumor heterogeneity [74]. Thus, investigating ecDNA and its impact at a single-cell resolution can be a powerful tool for decoding tumor heterogeneity. scEC&T-seq (Fig. 3A) enables simultaneous sequencing of full-length mRNA and all circular DNAs in a single cell, regardless of their sizes, contents, and copy numbers [55]. It can easily distinguish the transcriptional consequences resulted from copy number alterations induced by ecDNA. And the intercellular structural differences in ecDNA suggest that circular recombination is a way for ecDNA to evolve (Table 1).

3.1.5. Sequencing enzyme-accessible chromatin in circular DNA (CCDA-seq)

Currently, novel technologies such as nanopore sequencing of Nucleosome Occupancy and Methylome (NanoNOMe-seq) [75], single-molecule long-read accessible chromatin mapping sequencing assay (SMAC-seq) [76] and chromatin fiber sequencing (Fiber-seq) [77] have been employed to simultaneously obtain base information and methylation information of a single DNA molecule. These approaches can also been applied to examine distal interactions of regulatory elements and to explore single-molecule epigenetic heterogeneity. A new technique, known as CCDA-seq, offers the capability to investigate ecDNA chromatin accessibility at a single-molecule resolution (Fig. 3A). This technology is based on methylase labeling of unbroken open chromatin and exonucleases to enrich the depth of ecDNA sequencing, followed by long-read nanopore sequencing. Moreover, CCDA-seq is able to investigate the chromatin state of ecDNA by mapping the distribution of nucleosomes, which enables us to explore the relationship between the ecDNA-carrying regulatory elements and oncogenes. CCDA-seq provides comprehensive understandings of ecDNA epigenomic regulation [78] (Table 1).

3.2. Biochemical validation methods

3.2.1. Fluorescence in situ hybridization (FISH)

Cytogenetic methods including 4′,6-diamidino-2-phenylindole (DAPI) staining and fluorescent specific DNA probes to reveal the presence of ecDNAs. The use of FISH probes and complementary quantitative PCR (qPCR) to specifically detect and quantify ecDNA of interest have now become a well-established and highly targeted tool [79–81]. Particularly *in vitro*, FISH is effective in distinguishing ecDNA oncogenes and their corresponding genomic loci for cells at metaphase stage, as ecDNA-associated fluorescence signals will be

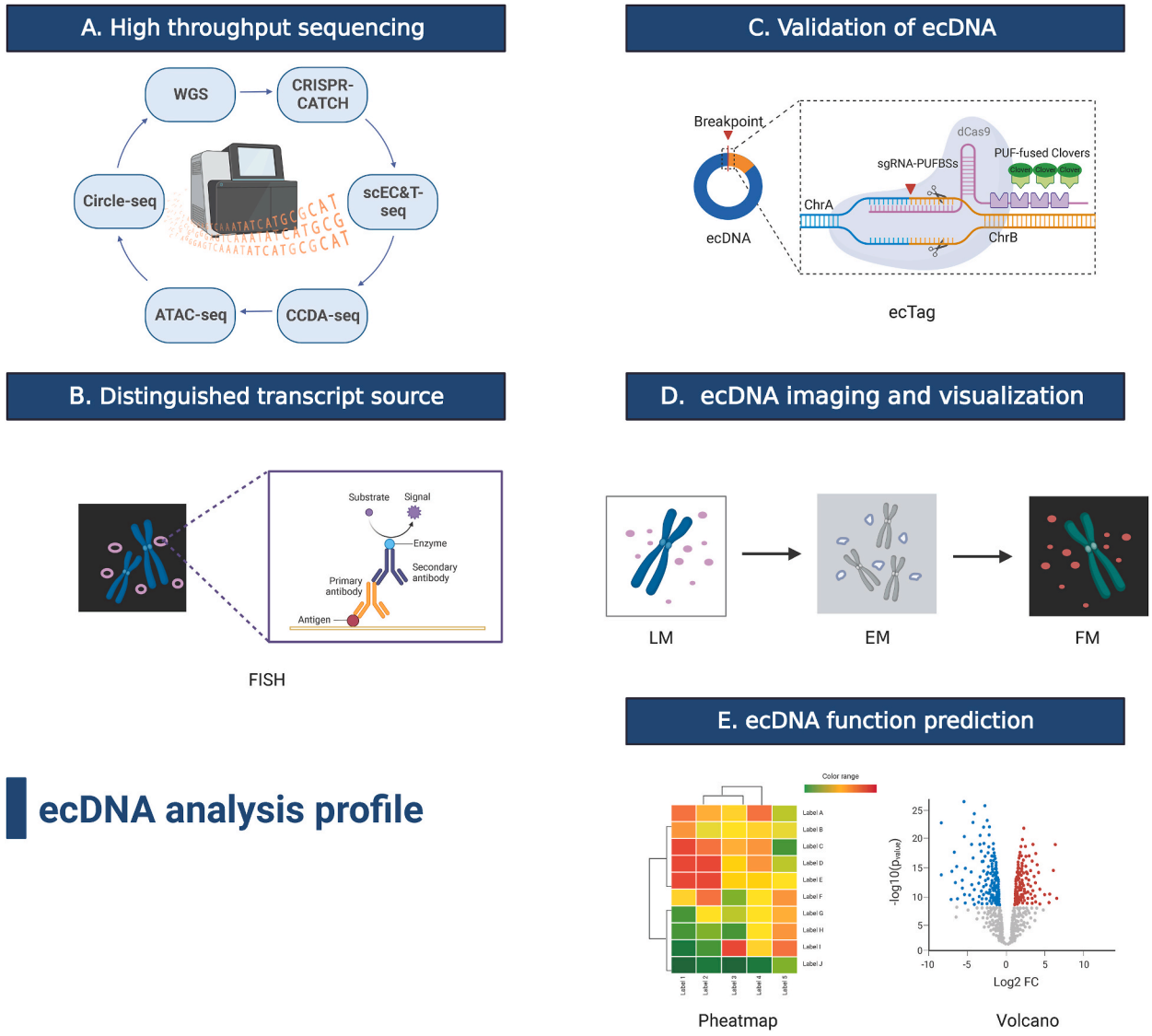

Fig. 3. A scheme for ecDNA research. (A) Computational tools from bulk and single-cell sequencing approaches to analyze regulatory elements, reconstruct chromatin interactions, and elucidate cellular heterogeneity in the context of ecDNA. (B) In situ hybridization (FISH) detects oncogenes on ecDNA by hybridizing sequence-specific probes. (C) ecTag was utilized for the validation of ecDNA. (D) Imaging of ecDNA through light microscopy (LM), electron microscopy (EM), and fluorescence microscopy (FM). (E) The prediction of ecDNA functions.

outside of dividing chromosomes. Due to the high accuracy of metaphase FISH in identifying known oncogenes, it has served as the golden standard for ecDNA detection in cancer cells. However, when dealing with primary tumor samples, metaphase FISH is usually not applicable because of the limited number of cells in metaphase. Therefore, the analysis of primary tumor samples is generally restricted to interphase FISH. In cell culture, cells can be induced to enter the metaphase stage, which would be impossible in intact primary tissues. The utilization of the corresponding image analysis tools in conjunction with metaphase FISH allows for more accurate and efficient detection and quantification of ecDNA. Further studies incorporating long-read sequencing and FISH-based image analysis for the detection of ecDNA in interphase cells of primary tissue would significantly contribute to routine clinical ecDNA detection, eliminating the necessity of using metaphase FISH for primary cell cultures (Table 1 and Fig. 3B).

3.2.2. Semantic segmentation of metaphase images containing extrachromosomal DNA(ecSeg)

ecSeg is a method based on metaphase FISH that combines traditional microscopy and deep neural networks to quantify ecDNA and oncogene amplification in single cells [82]. Furthermore, a CRISPR-based tagging technique called ecTag can be applied to tag ecDNA in living cells. This technique marks the loop-forming sites of ecDNA, enabling researchers to directly observe the true distribution of ecDNA. Researchers utilized live-cell imaging to investigate whether ecDNA is indeed asymmetrically distributed to offspring cells during cell division [83]. The development of 'ecTag' removed a barrier to the discovery of the temporal dimension of ecDNA (Fig. 3C).

Precise and sensitive ecDNA identification methods are essential for understanding ecDNA biology. In addition to the aforementioned new technologies, other epigenetic techniques, such as circular chromosome conformation capture sequencing (4C-seq), assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) and chromatin immunoprecipitation sequencing (ChIP-seq), can be used to probe ecDNA topology and chromatin accessibility (Fig. 3A). Visualization of ecDNA has been achieved using various microscopy techniques (Table 1), including light microscopy (LM), electron microscopy (EM) and fluorescence microscopy (FM) (Fig. 3D). Moreover, a number of bioinformatics analysis can be used to predict the consequences of genes amplified on ecDNA (Fig. 3E). WGS-based approaches have greatly accelerated our understanding of ecDNA, yet more cellular and animal models are needed for in-depth functional studies.

4. The functions and mechanisms of ecDNA in cancer

Progression from normal cells to cancer is characterised by a progressive accumulation of genetic and epigenetic changes [84]. The emergence of ecDNA and the role it plays in tumorigenesis have attracted considerable attentions. ecDNA was demonstrated to play a key role in altering chromatin accessibility and oncogene amplifications, driving genetic heterogeneity and malignant transformation and facilitating cancer cell evasion of immune surveillance. Moreover, ecDNA has been associated with poor prognosis in multiple cancers, presenting a significant challenge that urges us to reconsider our fundamental understandings about cancer.

4.1. Enhancing oncogene amplification and evolution

Oncogene amplification generally falls into two categories depending on the scale of the region: high-level copy gains and low-level amplification. The latter involves large-scale DNA recombination *via* a phenomenon known as chromothripsis [85]. Turner et al. performed a comprehensive analysis of 2572 cells in metaphase from 17 different types of cancer and revealed that ecDNAs were widely present in more than half of these cancers, with different frequencies of emergence in different cancers [51]. In addition to being identified in glioblastoma, head and neck, oesophageal, lung, kidney, colon, and melanoma cancers, ecDNA was also detected in males with prostate cancer and females with breast or ovarian cancer [34,36,86–89], among which *MYC*, *PDGFRα*, *EGFR*, *MDM2*, *FGFR1*, *CDK4* and *ERBB2* were the most frequent oncogenes being carried by ecDNA [62,79] (Table 2).

Unequal mitotic segregation of ecDNA results in significant variation in the copy number of oncogenes among individual cells [74,83], introducing expression of oncogene heterogeneity. The relative copy number of ecDNA is accordance with the differences in gene expression driven by copy number, with a positive correlation between the number of mRNA reads for genes contained in ecDNA.

Table 2
Functions of ecDNA in multiple cancers.

Oncogenes on ecDNA	Located on chromosome	Cancer types	Functions	Refs
MYC	Chr 8: 8q24.21	Colon cancer Glioblastoma Ovarian cancer Lung cancer Medulloblastoma prostate cancer Leukemia	copy number alteration, reintegration into chromosomes, distal DNA interaction, intratumoral heterogeneity	[16,65,167]
MYCN	Chr 2: 2p24.3	Neuroblastoma Wilms tumor Medulloblastoma	copy number alterations, reintegration into chromosomes, distal DNA interaction	[65,168]
EGFR	Chr 7: 7p11.2	Glioblastoma Lung cancer Head and neck cancer Bladder cancer Breast cancer	copy number alteration, distal DNA interaction, regulated to evade therapy, intratumoral heterogeneity	[16,32,65,83,169]
DHFR	Chr 5: 5q14.1	Colon cancer Cervical cancer Breast Cancer	contributes to MTX resistance	[144,145,170–172]
CCND1	Chr 11: 11q13.3	Lung cancer Liver cancer Head and neck cancer Breast cancer Bladder cancer	coamplified with the enhancers in the upstream of the noncoding region of ecDNA	[65]
MDM2	Chr 12: 12q15	Glioblastoma Bladder cancer Breast cancer Leukemia	copy number alterations, distal DNA interaction, intratumoral heterogeneity	[173]
FGFR1	Chr 8: 8p11.23	Lung cancer Breast cancer	copy number alteration, intratumoral heterogeneity	[65]
ERBB2	Chr 17: 17q12	Breast cancer Bladder cancer Head and neck cancer	copy number alteration, distal DNA interaction	[65]

Although enhancer interactions within ecDNA hubs potentially contribute to the differences in gene expression, Koche RP et al. found that copy number of ecDNA gains the key determinant of cell-to-cell differences in terms of oncogene expression.

Moreover, amplification of regulatory elements on ecDNA is also important in the transcriptional regulation of oncogenes and is thought to be under strong positive selection [65,66]. Enhancers are small DNA sequences that are recognized by specific proteins called transcription factors, distinguished by different chromatin modifications [90], serving as key regulatory elements in the temporal and spatial control of gene expression [91]. Using ChIP-seq and immunofluorescence techniques, Mischel et al. confirmed that ecDNA has a chromatin structure consisting of nucleosome units [16]. Meanwhile, histone modifications (H3K4me1/3, H3K27ac) around the enhancers and promoters of ecDNAs, not inhibitory histone modifications such as H3K9me3 and H3K27me3 [16,92], further illustrates the reason why oncogenes on ecDNA can be expressed in large amounts. Jeremy et al. found that the *EGFR* gene is co-amplified with its upstream noncoding sequence of approximately 130 Kb in glioblastoma. Using multiomics analysis between this sequence and ChIP-seq-captured peaks revealed two strong H3K27ac binding peaks, indicating the presence of enhancers in this region. They also found a significant co-amplification of oncogenes with enhancers in five different s olid tumor types, suggesting a common mechanism of oncogene regulation through ecDNA-mediated enhancer activation in cancer [23]. Tian, Rui et al. recently reported that HPV integration resulted in the formation of super-enhancers in the integration site region, which further formed ecDNA that regulated transcription at the genome-wide level [93]. Recent reports have shown the existence of ecDNAs that do not contain oncogenes but only enhancer elements can act in *trans* to augment transcriptional output of linear chromosomes or other ecDNAs as part of ecDNA hubs [27,83]. However, no enhancer-only ecDNA was found in single neuroblastoma cells, and all recurrences of detected ecDNA harboured at least one oncogene [55].

The high expression levels of amplified genes on ecDNA have been revealed to be related with the (RNA polymerase II) RNAPII complex [94]. Pulling down the RNAPII-associated chromatin complex by ChIA-PET allows identification and characterization of the ecDNA-associated chromatin interaction network group. RNAPII-binding chromatin was found to be significantly enriched in the related regions of ecDNA. To quantitatively assess the extent of chromosomal region interactions, researchers introduced the metric of genome-wide *trans*-chromosomal interaction frequency (nTIF) and found a significant enrichment of nTIFs in the ecDNA amplified region. After copy number normalization, nTIFs were still significantly higher in ecDNA than in autosomal DNA. Furthermore, the RNAPII binding sites and H3K27ac modification sites were analyzed by ChIP-seq to explore the relationship between RNAPII-mediated ecDNA-chromosome interactions and gene transcription. The results demonstrated a higher frequency of *cis*-interactions in the ecDNA region, and the interaction sites were mainly located in the promoter region. In the transcriptionally active regions of high-frequency ecDNA-chromosome interactions, there was a significant enrichment of H3K27ac signals, which have a large signal peak range and are characteristics of super enhancers (SEs) [95]. The researchers found that the superenhancer function of ecDNA leads to coaggregation of oncogenes, suggesting that ecDNA and its superenhancer structure may act as a flexible transcriptional enhancer to promote the occurrence of neoplasia.

In addition to enhancing the expression of oncogenes [96,97], the latest studies have confirmed the ability of ecDNAs to drive genomic reshuffling of oncogenes [34,49,50,93]. Researchers have discovered that the ecDNA exists in neuroblastoma serves as a primary source of somatic genomic rearrangements [34]. They found that ecDNAs disrupt the integrity of the genome sequence through detachment from the genome and/or integration into chromosomes at different loci. In addition, not only *EGFR*-containing ecDNA but also many ecDNA promoted neuroblastoma development. Strikingly, some of these sequences were not originally located around the *EGFR* gene. It seems that they were targeted "pulled" from the different regions of the genome and were specifically employed to enhance the expression of oncogenes. Monitoring this process in the relatively stable pediatric tumor genome could help to elucidate similar mechanisms that may be overlooked in more complex adult cancers. This approach provides information on clinical outcomes and identifies potential therapeutic targets for both pediatric and advanced cancers.

4.2. Driving tumor heterogeneity

Tumors consist of a large number of cells dividing uncontrollably. Somatic cell division, or mitosis is dependent on spindle fibers that pull the replicated chromosomes toward the centromeres at the poles. Such process ensures that the nuclear genetic materials are distributed equally to the two daughter cells [98]. In the absence of the centromere structure, the inheritance of ecDNA is non-mendelian [74,99–101]. Such characteristics may result in an unequal allocation of ecDNA in the offspring cells. Researchers simulated the transmission of proto-oncogenes in ecDNAs and HSRs, respectively. It was found that higher copy numbers could be quickly acquired when the proto-oncogenes were located on ecDNAs. It is predicted that the diversity within the tumor tissue will increase more rapidly than when it is located in HSRs, indicating that ecDNAs can rapidly drive genomic chaos in tumors [51]. The Shannon diversity index [102] was introduced to measure the heterogeneity of tumor tissue (equivalent to a biological community) as well.

4.3. Involvement in drug resistance

Acquired resistance to cancer therapies is a widespread event. Acquired resistance in malignancies are typically initiated by the amplification of resistance driver genes. The mechanisms associating acquired resistance to cancer to genomic instability signaling pathways remain unclear. The selective pressure of different cancer interventions causes differences in the level of amplification of core genes, which leads to a certain plasticity in the degree of cellular carcinogenesis.

Intrachromosomal complex genomic rearrangements (CGRs) and ecDNAs can be referred to as SV-associated amplicons, which have a series of transiently correlated structures of genomic signaling plasticity and are factors of genomic instability [103]. The

asymmetric distribution of ecDNA leads to cancer cells respond to changing environments, including cancer treatment in a more resistant way due to the over-expression of oncogenes [104] (Table 3). Mischel et al. suggested that ecDNAs were associated with drug resistance in cancer therapy and may affect the effectiveness of *EGFR* gene-related targeted therapy [32]. They reported that erlotinib, a drug targeting the EGFR protein kinase domain, was able to remove the EGFR gene on ecDNA; upon discontinuation of the drug, the amplified *EGFR* located on ecDNA reappeared. This dynamic regulation of oncogene copy number created a 'hide-and-seek' mechanism that allows tumors to evade the drug and relapse when it is withdrawn [105,106], suggesting that high-dose pulsed intermittent treatment may provide better targeted inhibition than continuous administration while restoring drug sensitivity of the tumor (Fig. 4A).

Methotrexate (MTX) is a class of antitumor folate metabolizing agents that inhibit tumor growth by competitively binding to *DHF,* which is a substrate of the *DHFR,* and blocking DNA anabolism. Resistance to MTX can be reflected in the three main aspects. First, the amount of MTX entering the cell decreases (reduced expression of drug transport proteins); then, the affinity between MTX and *DHFR* is reduced (e.g., mutations in the *DHFR* gene); and finally, the overall *DHFR* copy number is increased [107,108]. Researchers observed that HT29 colon cancer cells treated with MTX led to an increased copy number of the *DHFR* genes, which were found to be contributed from ecDNA by FISH (Table 3). This leads to the abundant expression of *DHFR* gene, which overcomes the MTX inhibition of folate metabolism, maintaining DNA anabolic activity and rendering drug resistance. Many independent studies have also shown that an up-to-10-copies increase in the copy number of the *DHFR* gene can be found in MTX-resistant tumor samples. These increased copy numbers can be found not only on chromosomes but also in ecDNA [109–111](Fig. 4B).

There is hyperactivated RAS-RAF-MEK-ERK signaling in >30–40% of human cancers [112] comprising ~70% of advanced melanomas driven by non-*BRAF*^{V600} (atypical *BRAF*), *BRAF*^{V600}, *NF1* mutants and *NRAS*. Treatment of *BRAF*^{V600MUT} metastatic cutaneous melanoma with BRAF inhibitors (BRAFi) targeting the MAPK signaling pathway leads to rapid development of acquired resistance [113]. BRAFi induces reactivation of the MAPK signaling pathway for the result in resistance, and this activation can be inhibited by the intervention with MEK inhibitors (MEKi) [114–116]. However, the 5-year survival rate is <20% for the patients in the BRAFi combined with MEKi treatment [117]. There are still no viable MAPKi therapies for patients with melanoma caused by *NRAS* mutations, and MEKi monotherapy has limited efficacy [118]. Certainly, adding drugs to inhibit resistance in MEKi is of some positive clinical significance, and genomic events such as focal amplification of *BRAF*^{WT}, *CRAF*^{WT} and *NRAS*^{MUT} genes are drivers of acquired resistance [119]. Cutaneous melanoma is a class of tumor types with a high degree of chromothripsis burden even before targeted therapy. Moreover, chromothripsis has been implicated as a central mechanism for the rapid generation and acquisition of large numbers of dynamic SVs, leading to the production and accumulation of CGRs and ecDNAs [120]. CGR and ecDNA amplicons bearing MAPKi resistance-specific or driver genes coamplify with their enhancers and possibly contain extra enhancer or superenhancer activity in *cis* or *trans*, accompanied by reprogramming of the acquired resistance transcriptome [31] (Table 3) (Fig. 4A).

Table 3
Cancer therapy and ecDNA-related drug resistance.

Cancer types	Oncogenes on ecDNA	Targeted therapy drugs	Drug effects	Potential mechanisms of drug resistance	Refs
Glioblastoma	*EGFR*	Erlotinib	Erlotinib initially resulted in 80% tumor shrinkage (response), converting EGFRvIII ^{high} to EGFRvIII ^{low} in tumor cells.	EGFRvIII on ecDNA in response to Erlotinib, coupled with intercellular signaling between EGFRvIII ^{high}/EGFRvIII ^{low} tumor cells, enables GBMs to achieve an optimal growth and survival ratio yet be resistant to treatment.	[16,65,94, 105,106]
Colon cancer	*DHFR* c-Myc	Methotrexate Hydroxyurea	Methotrexate and its polyglutamate forms competitively inhibit DHFR. Hydroxyurea acts as an inhibitor of ribonucleoside diphosphate reductase, targeting proliferating cells and inhibiting DNA replication.	Deletion of *DHFR* or *c-Myc* on ecDNA increases drug sensitivity	[144,145, 174–177]
Breast cancer	*HER2* *DHFR*	Trastuzumab Methotrexate	Trastuzumab specifically binds to its epitope on the extracellular portion of the HER2 receptor and releases its toxic load directly into cancer cells *via* lysosomal cleavage.	*HER2* mutations impair the inhibitory effect of trastuzumab on the PI3K/AKT pathway. X-rays cause MTX resistance due to DHFR amplification	[172,178, 179]
Oral squamous cell carcinoma	*MDR1*	Hydroxyurea	Hydroxyurea (HU), a ribonucleotide diphosphate reductase inhibitor, interferes with the S phase resulting in inhibition of DNA synthesis.	Removal of *MDR1* on ecDNA improve drug sensitivity	[180]
Melanoma	*BRAF*^{V600E}	Vemurafenib Cobimetinib Selumetinib Dabrafenib Trametinib	These selective BRAF inhibitors (BRAFi) were developed with a structure-guided approach to block the proliferation of melanoma cells harboring the BRAF^{V600} mutation at the nanomolar level, which can be used alone or in combination with others.	Increasing and/or decreasing the dose of these kinase inhibitors are reproducible modulators of the number of ecDNAs, the length of HSRs, the mode switching between ecDNA and HSR, and the coupling of additional genomic rearrangements.	[31,103, 113,181]

The role of ecDNA in drug resistance

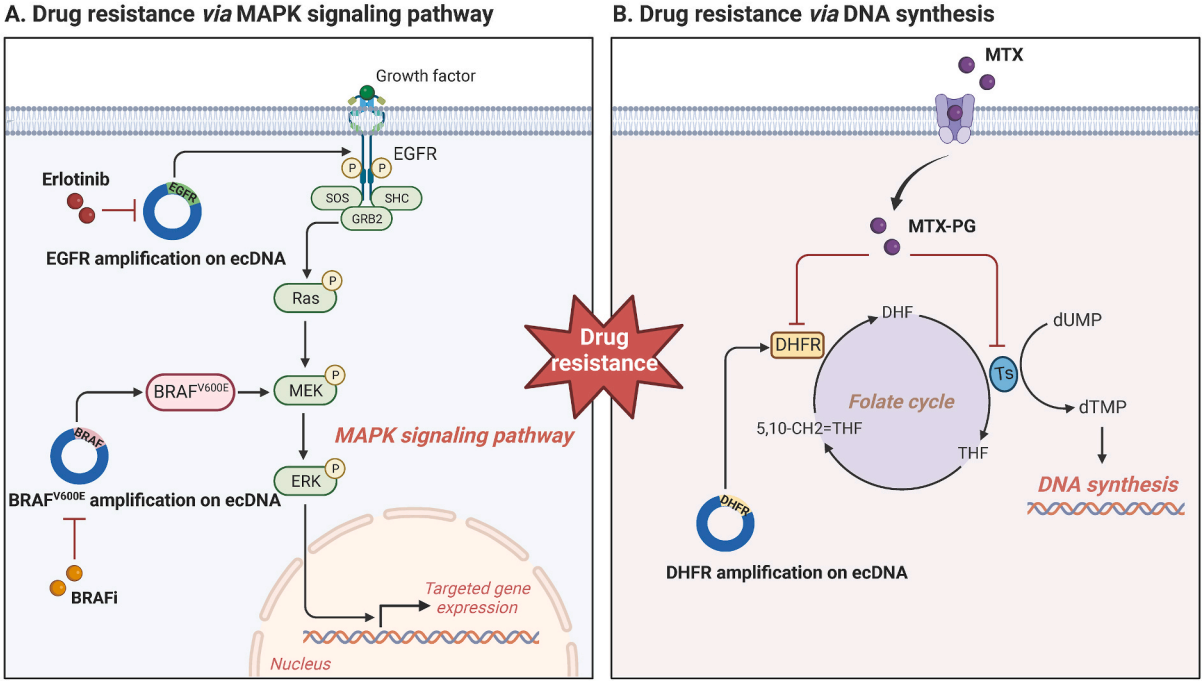

Fig. 4. The role of ecDNA in drug resistance. (A) Erlotinib targets the EGFR protein kinase structural domain and is able to remove the EGFR gene from ecDNA. Once discontinuation of erlotinib, the clonal *EGFR* mutation located on ecDNA reappeared. BRAF inhibitor (BRAFi) therapy targeting the MAPK signaling pathway for the treatment of BRAFV600MUT on ecDNA tumors results in the rapid development of acquired resistance. (B) Cancer cells treated with MTX led to an increased copy number of the *DHFR* genes, which leads to abundant expression of the *DHFR* gene, overcoming the inhibitory effect of MTX on folate metabolism, maintaining DNA anabolic activity and rendering the drug resistant.

4.4. Driving malignant transformation

Although the role of ecDNA in a variety of tumors has been identified, it remains unclear whether ecDNA is formed as a result of genomic instability following tumor formation, or whether it is involved in the pre-processing of the organism from the onset of the associated lesion to cancer. Luebeck et al. [121] analyzed WGS data from patients with Barrett's oesophagus or oesophageal adenocarcinoma (EAC), and WGS and histology data from biopsies, found the presence of ecDNA in non-cancerous oesophageal tissues that are susceptible to cancer development, and suggest that this DNA may confer a selective advantage for cells that will progress to cancer and may be involved in other processes that promote tumor formation. This study indicated that ecDNA may be available in precancerous lesions and that high levels of ecDNA are directly related to cancer progression in patients. This indicates that ecDNA can be used as a pre-cancerous diagnostic marker for early intervention in Barrett's oesophagus patients by performing ecDNA testing in the early stages of the disease; and points to future directions for more in-depth studies to develop clinical testing and corresponding therapeutic approaches.

4.5. Altering chromatin accessibility and 3D structure

Compared with the average chromatin accessibility of homologous linear DNA, Chen, W. et al. [78] conducted CCDA-seq and found that the chromatin state of ecDNA is as twice accessible as linear DNA chromatin. The chromatin state at ecDNA junction is significantly more accessible than at distal regions of the junction. Nucleosome depletion regions (NDRs) are evenly distributed in ecDNA, while those of linear DNA are restricted to 200 bp prior to the transcription start site (TSS). The patterns of nucleosome/regulatory factor elements binding to ecDNA observed at single-nucleotide resolution is significantly different from those in linear DNA.

Our current understandings of the 3D structure of genome are primarily limited to the 23 pairs of chromosomes [122]. Variations of the 3D structure of genome can impact gene transcription by facilitating interactions between DNA elements [123–125]. Typically, the frequency of chromatin interactions between DNA elements decreases as the spatial distance increases [126]. For example, one of higher-level chromatin structures is the formation of chromatin loops, which mediate interactions between promoters and enhancers to promote gene expression [127]. Various chromatin conformation capture and sequencing techniques have been employed to confirm the 3D structures of chromatin and the organization of topologically associated domains (TADs) in ecDNA, including

proximity ligation-assisted ChIP-seq (PLAC-seq), in situ Hi-C followed by chromatin immunoprecipitation (Hi-ChIP) [127], MNase-seq, ATAC-seq and 4C-seq [16]. This 3D circular structure enables ultradistant interactions of distant DNA elements in ecDNA, which in turn may form new gene regulation mechanisms.

5. Potential therapeutic application to ecDNA in cancer

In addition to traditional treatments such as small molecules and antibody drugs [128], recent research has focused on developing advanced therapies such as the cell and gene therapy [129], RNA interference (RNAi) and other oligonucleotide therapies [130]. Additionally, gene editing therapies that utilize the CRISPR system [131] along with other novel types of molecules have emerged as promising approaches in the field of medicine. These treatments will become the focus of the biomedical industry and improve the future treatment for patients. ecDNA is a potential target for clinical intervention (Fig. 5). By blocking the life cycle of ecDNA or precisely targeting initiation of oncogene amplification. In the near future, small molecular inhibitors that disrupt the formation and function of ecDNA to eliminate cancer cells would be a hotspot.

5.1. As a new biomarker

As a crucial driver of the most invasive and refractory cancers, ecDNAs are present in many solid tumors [62] and are generally not present in normal cells. ecDNAs have stable biological activities and unique molecular structures, which provide a new direction for a rapid development of noninvasive biopsy. Several studies have revealed the prospective utilization of ecDNA in body fluids as a potential biomarker for the surveillance and diagnosis of numerous diseases [132–134]. All these advances in research are conducive to promoting the application of ecDNA in liquid biopsy as well as biomarkers.

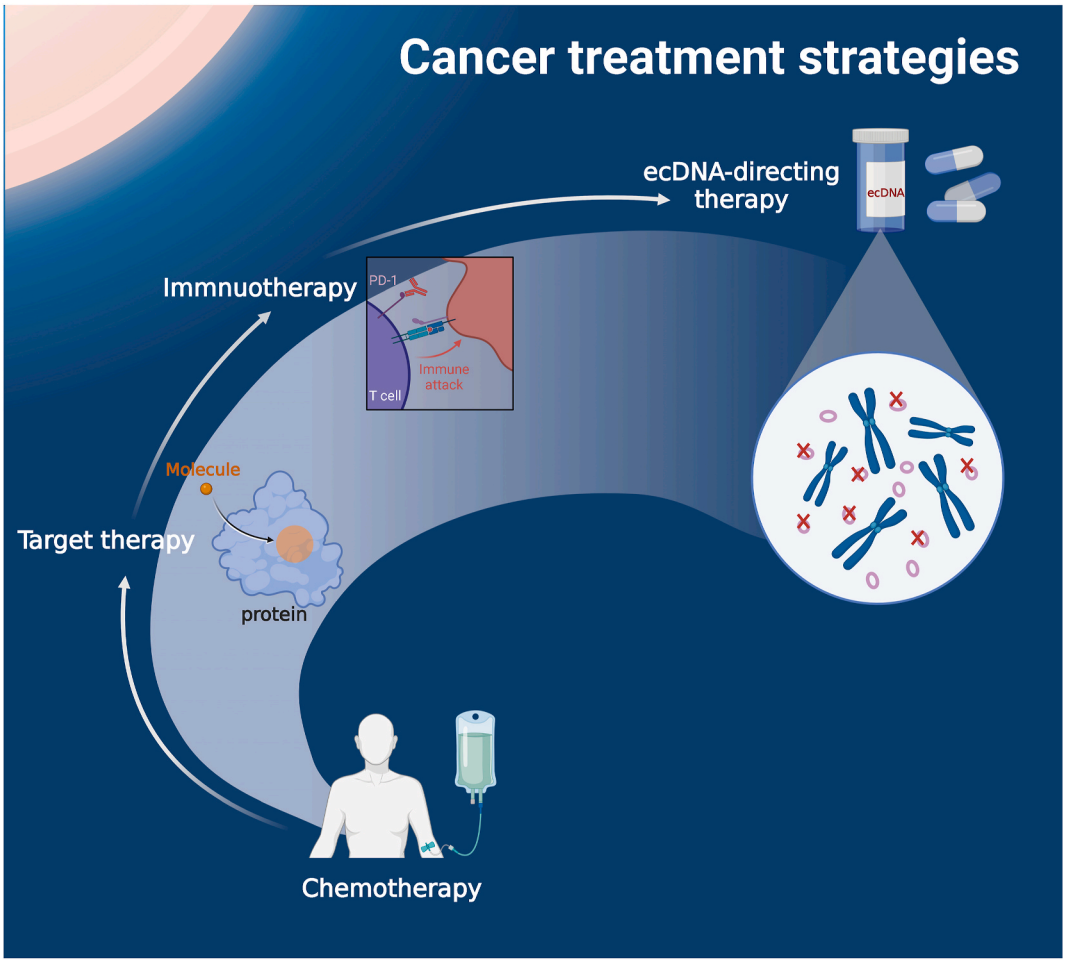

Fig. 5. Cancer treatment milestones. After three revolutions in cancer treatment: chemotherapy, targeted therapy and immunotherapy. ecDNA-directed therapy may become the focus of the biomedical industry and promote the future treatment landscape for patients.

5.2. Treatment strategies

Different stages of the ecDNA formation and maintanince have been used for development of targeted therapies: (1) ecDNA replication pressure, (2) ecDNA assembly and repair, (3) ecDNA inheritance and (4) ecDNA elimination. More identification and preclinical validation of additional ecDNA-essential targets spanning multiple distinct ecDNA synthesis lethal nodes in oncogene-amplified cancers. These targets form the basis of our broad range of ecDNA-targeted therapies to address oncogene-amplified cancers.

Replication pressure (RP) results in uncoupling of replication helicase and DNA polymerase [135] which further expose long segments of vulnerable single-stranded DNA (ssDNA). RP is a major source of genomic instability, tumorigenesis, and cancer progression [136,137]. Hyperactive RP leads to replication disaster and cell deaths, which in turn could be utilized as a therapeutic strategy to treat high RP cancers [138,139]. Excessive transcription-replication of ecDNAs would increase RP and activate certain cellular RP response factors, suggesting that RP inducers could serve as a synthetic lethality to destroy ecDNA-driven cancer cells, providing a new molecular strategy for high-RP tumors. Cells carrying ecDNAs showed higher basal levels of phosphorylated replication protein A (RPA) [140] and replication fork speed. Compared with normal cells and chromosomal genetically amplified cells, ecDNA-amplified cells showed greater sensitivity to the deoxyribonucleotide inhibitor BBI-355 which inhibits checkpoint kinase 1 (CHK1), one of the major regulators of DNA RP [141]. ecDNA-activated oncogene-amplified cancers is synthetically lethal using inhibition of CHK1 by BBI-355, as they are heavily reliant on CHK1 to mediate their intrinsically elevated RP. BBI-355 is now in a phase 1/2 clinical trial [142].

For maintenance and perpetuation of ecDNA, ecDNA-activated cancer cells exhibit an increased demand for materials used for replication to sustain ecDNAs. Thus, increased sensitivity to the supply of raw materials required for ecDNA assembly and repair could lead to the death of ecDNA-dependent cancer cells. BBI-825 [141], a novel and effective oral drug, could inhibit ecDNA assembly and repair. It is a ribonucleotide reductase (RNR) selective inhibitor. RNR is the rate-limiting enzyme necessary for the production of deoxyribonucleotide triphosphates (dNTPs), which are the building blocks of DNA essential for ecDNA assembly and repair. It was demonstrated by previous models of gene amplification formation that DSB repair pathways involves gene amplification [46,143], in which homologous recombination (HR) is the classical mechanism consisting of several interconnected pathways [144]. Of interest, silencing the core HR repair gene *BRCA1* to attenuate HR activity reduced the amount of MTX-driven *DHFR* ecDNAs, caused the acceleration of the cell cycle and the increase in MTX sensitivity [144]. In addition, PARP inhibitors disrupt DNA damage repair process and can suppress the frequency of ecDNA. Thus, the HR pathway may serve as a new target by reducing extrachromosomal amplification of tumors to improve the efficacy of chemotherapy. Meanwhile, NHEJ has been demonstrated to participate in the formation of ecDNA during DNA damage repair in tumor cells, which can be disrupted by DNA-dependent protein kinase inhibitors [145]. These strategies may be more effective in combination with radiation therapy, as well as other available tumor chemotherapeutic agents.

ecDNA lacks centromeres, the structural component necessary for the correct segregation of chromosomes during cell division. As a consequence, targeting inhibition of the motor proteins, which are essential for ecDNA inheritance, has been synthetically lethal to ecDNA-carrying cancer cells. Kanda et al. has shown that chromosomes act as "cargo ships" on which ecDNA is loaded, enabling it to be efficiently delivered to daughter nuclei [146]. The mechanisms and possible molecular cues behind the ecDNA attachment to chromosomes remain obscure.

Cancer cells are hooked on ecDNA which is the vector for maintaining oncogene amplification [64,65,147]. The dynamic equilibrium with respect to the gene amplification between the generation and elimination determines gene dosage. The export of nuclear material involving surplus chromosomes, amplified DNA, and DNA repair complexes is accomplished primarily by micronuclei (MN) and nuclear buds (NBUDs) [148,149]. It was reported that ecDNA is more easily discharged and eliminated by MN compared to linear chromosomal DNA, and the generation of MN is attributed to the elimination of ecDNA. Therefore, elimination of ecDNA may be an effective treatment to limit the survival of cancer cells [150–154]. Hydroxyurea (HU), gemcitabine and other inhibitors applied to DNA synthesis or repair have been found to be able to eliminate gene amplification through the formation of MN/NBUDs [145,155,156]. Shimizu et al. found that HU as an antimetabolite that inhibits DNA replication in early S-phase, induced the aggregation of ecDNA, resulted in its detachment from anaphase chromosomes and the formation of MN followed by their elimination [157]. However, the mechanisms behind this damage are unclear, such as the aggregation occurs in this context needs to be further investigated, and whether it is associated with ecDNA related to homologous repair templates. Moreover, ecDNA aggregates under natural conditions and participates as mobile enhancers for cooperative transcriptional regulation [27], so what is the difference between these two aggregations. The relative specificity of HU against ecDNA and its underlying differential potency towards cancers driven by ecDNA remains a pending question. Although HU has not been clinically effective in tumor therapy and has not been used to treat ecDNA-positive tumors [158], existing observations provide a theoretical foundation for later drug screening. Similarly, except for chemotherapy [159–162], radiation exposure can also capture ecDNA in radiation-induced MN carrying the drug resistance genes *MYCC* and *MDR1* [33,163]. MN can suppress DNA repair of captured ecDNA. Further insights into the molecular signaling pathways of ecDNA expulsion by MN are likely to yield novel therapeutic targets. And it is believed that ecTag could reveal the mechanisms that facilitate ecDNA elimination through tracking the spatiotemporal dynamics of ecDNA entry into the MN.

6. Perspectives

A rapidly evolving field of ecDNA research is underway. Several innovative tools have been developed in recent years to facilitate comprehensive ecDNA studies. Further investigation is necessary to clarify the dynamics of ecDNAs, particularly the mechanism involved in their detachment from and reintegration into the chromosomes. Elucidation of the mechanisms of chromosomal instability

during ecDNA formation is also necessary, including the effects of double-strand breaks and NHEJ in this process. Capsules are therefore used for the treatment of cancers that are likely to be resistance to drugs by producing double-stranded DNA breaks. Researchers can hinder the construction of ecDNA by combining chemotherapy drugs with molecules that block DNA elements generated by chromosome fragmentation. In addition, the consequences of ecDNA reintegration into chromosomes on genomic rearrangement and repositioning as HSR remains incompletely understood. The reintegration of HSR has been shown to be related to drug resistance, but the exact mechanisms underlying this phenomenon is not clear yet.

To shed light on the role of ecDNA in the malignant development of tumors, molecular pathogenesis, and its potential influence on the tumor immune microenvironment, it is essential to investigate how ecDNA influences the genetic diversity, heterogeneity of tumors and the extent to how it contributes to tumor progression and metastasis. Additionally, understanding the interactions between ecDNA and the tumor immune microenvironment can provide insights into potential therapeutic targets and strategies for cancer immunotherapy. This requires comprehensive molecular and cellular characterization of ecDNA in tumor samples and careful evaluation of its functional significance in cancer biology.

As ecDNA is specifically present in tumor cells with high oncogene copy numbers and corresponding expression levels, it plays an important role in the field of tumor drug resistance. It can primarily drive tumorigenesis and progression, as well as progression of malignant phenotypes such as multidrug resistance. Accordingly, it is expected that the function and mechanism of ecDNA in tumor drug resistance will open up new directions for future research in tumor therapy [164]. Tumor cells containing ecDNA expand drug resistance through taking advantage of the higher copy number of oncogenes, such as *DHFR*.

Kim et al. noted that only 40% of the established cell lines were successful in detecting ecDNA, compared to 90% in the patient-derived xenograft (PDX) models [36]. This result suggests that the 'innate' deficiencies of the subjects may also lead to the neglect of ecDNAs. In recent years, the application of PDX or patient-derived cells (PDCs) has been gradually accepted by experts in the field of oncology, and various drawbacks of the established cell lines have been noted. Another possible limitation is the methodological technology. ecDNA must be observed in metaphase. The preparation of metaphase requires the use of cells in mitosis and therefore cannot be directly studied in tissue context, so primary cell isolation and culture must be performed first. In addition, ecDNA might be lost in an established cell line constructed by a traditional protocol instead of the serum-free sphere culture protocol advocated in recent years. Advances in sequencing and analytical technology will boost the construction of ring amplicons and help us study the genome with increased precision at a single-base resolution. However, the existence of ecDNA poses a challenge for current genetic sequencing and analysis. To overcome these challenges, there is a need to develop more efficient and reliable approaches for ecDNA enrichment and sequencing. This could involve improving the sensitivity and specificity of current enrichment methods, developing new approaches for circular amplicon sequencing, or combining different sequencing technologies to achieve more comprehensive ecDNA detection and analysis.

Yeast and many other organisms contain circular DNAs as well, which resemble ecDNA in some characteristics in terms of replication, maintenance and chromatin regulation. This observation indicates that several salient problems regarding ecDNA in cancer will probably have to be addressed in multiple model systems through an interdisciplinary research approach. Therefore, to fully utilize the characteristics of ecDNA and advance the diagnosis and treatment of ecDNA-driven cancer patients, it is required that continuous academic and industrial efforts be made in ecDNA-related research.

Funding

This study was supported by grants from the National Natural Science Foundation of China (31972884), the Foundation for Innovative Research Groups of the National Natural Science Foundation of China (81821002), the Natural Science Foundation of Sichuan Province (2023NSFSC0719) and the 1·3·5 Project for Disciplines of Excellence, West China Hospital (ZYJC21021).

Data availability statement

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have given their approval for publication of this manuscript.

CRediT authorship contribution statement

Qing Huang: Writing – review & editing, Writing – original draft. **Su Zhang:** Writing – review & editing, Writing – original draft. **Guosong Wang:** Writing – review & editing. **Junhong Han:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Not applicable.

Abbreviations

ecDNA Extrachromosomal DNA
DM Double minutes
mtDNA Mitochondrial DNA
spcDNA Small polydispersed circular DNA
t-circle telomeric circle
DHFR Dihydrofolate reductase
MTX Methotrexate
ChIP Chromatin immunoprecipitation
5′UTR 5′ untranslated regions
EGFR Epidermal growth factor receptor
SNP Single nucleotide polymorphism
scEC&T-seq Single-cell extrachromosomal circular DNA and transcriptome sequencing
ATAC-seq Accessible chromatin using sequencing
MNase-seq Micrococcal nuclease digestion and sequencing
ChIA-PET Chromatin interaction analysis by paired-end tag
ChIA-Drop: Chromatin-interaction analysis *via* droplet-based
Hi-C: High-throughput chromosome conformation capture
nTIFs *trans*-chromosomal interaction frequencies
NanoNOMe-seq Nanopore sequencing of Nucleosome Occupancy and Methylome
SMAC-seq Single-molecule long-read accessible chromatin mapping sequencing
Fiber-seq Chromatin fiber sequencing
c-BFB Circular breakage-fusion-bridge
HSRs Homogenously staining regions
NHEJ Non-homologous end joining
CTCF CCCTC binding factor
alt-EJ Alternative end-joining
eHSRs Ectopic homogenously staining regions
CCDA-seq Sequencing of enzyme-accessible chromatin in circular DNA
CNV Copy number variation
H3K4me1 Histone 3 lysine 4 monomethylation
H3K27ac Histone 3 lysine 27 acetylation
H3K27me3 Trimethylation of histone H3 at lysine 27
SEs Super enhancers
TADs Topologically associated domains
PLAC-seq Proximity Ligation-Assisted ChIP-seq
Hi-ChIP Hi-C followed by chromatin immunoprecipitation
4C-seq Circular Chromosome Conformation Capture combined with high-throughput sequencing
PVT1 Plasmacytoma variant transcript 1
RNAi RNA interference
RS Replication stress
ssDNA Single stranded DNA
RPA Replication protein A
PDX Patient-derived xenografts
PDCs Patient-derived cells

References

[1] R. Dahm, Discovering DNA: friedrich Miescher and the early years of nucleic acid research, Hum. Genet. 122 (2008) 565–581.
[2] W.T. Molin, A. Yaguchi, M. Blenner, C.A. Saski, Autonomous replication sequences from the Amaranthus palmeri eccDNA replicon enable replication in yeast, BMC Res. Notes 13 (2020) 330.
[3] S. Cohen, A. Houben, D. Segal, Extrachromosomal circular DNA derived from tandemly repeated genomic sequences in plants, Plant J. 53 (2008) 1027–1034.
[4] M.J. Shoura, I. Gabdank, L. Hansen, J. Merker, J. Gotlib, S.D. Levene, A.Z. Fire, Intricate and cell type-specific populations of endogenous circular DNA (eccDNA) in Caenorhabditis elegans and Homo sapiens, G3 (Bethesda) 7 (2017) 3295–3303.
[5] Z. Cohen, E. Bacharach, S. Lavi, Mouse major satellite DNA is prone to eccDNA formation via DNA Ligase IV-dependent pathway, Oncogene 25 (2006) 4515–4524.
[6] S.C. Barreto, M. Uppalapati, A. Ray, Small circular DNAs in human pathology, Malays. J. Med. Sci. 21 (2014) 4–18.
[7] S. Anderson, A.T. Bankier, B.G. Barrell, M.H. de Bruijn, A.R. Coulson, J. Drouin, I.C. Eperon, D.P. Nierlich, B.A. Roe, F. Sanger, P.H. Schreier, A.J. Smith, R. Staden, I.G. Young, Sequence and organization of the human mitochondrial genome, Nature 290 (1981) 457–465.
[8] S. Cohen, A. Regev, S. Lavi, Small polydispersed circular DNA (spcDNA) in human cells: association with genomic instability, Oncogene 14 (1997) 977–985.
[9] H.A. Pickett, J.D. Henson, A.Y.M. Au, A.A. Neumann, R.R. Reddel, Normal mammalian cells negatively regulate telomere length by telomere trimming, Hum. Mol. Genet. 20 (2011) 4684–4692.
[10] A.J. Cesare, C. Groff-Vindman, S.A. Compton, M.J. McEachern, J.D. Griffith, Telomere loops and homologous recombination-dependent telomeric circles in a Kluyveromyces lactis telomere mutant strain, Mol. Cell Biol. 28 (2008) 20–29.
[11] T. Paulsen, Y. Shibata, P. Kumar, L. Dillon, A. Dutta, Small extrachromosomal circular DNAs, microDNA, produce short regulatory RNAs that suppress gene expression independent of canonical promoters, Nucleic Acids Res. 47 (2019) 4586–4596.
[12] R.M. Hull, J. Houseley, The adaptive potential of circular DNA accumulation in ageing cells, Curr. Genet. 66 (2020) 889–894.
[13] D. Cox, C. Yuncken, A.I. Spriggs, Minute chromatin bodies in malignant tumours of childhood, Lancet 1 (1965) 55–58.
[14] G. Balaban-Malenbaum, F. Gilbert, Double minute chromosomes and the homogeneously staining regions in chromosomes of a human neuroblastoma cell line, Science 198 (1977) 739–741.
[15] D.A. Chiu Rwk, A.G. Hensson, Y.M.D. Lo, P. Mischel, B. Regenberg, What Is Extrachromosomal Circular DNA and what Does it Do? Clinical Chemistry, 2020.
[16] S. Wu, K.M. Turner, N. Nguyen, R. Raviram, M. Erb, J. Santini, J. Luebeck, U. Rajkumar, Y. Diao, B. Li, W. Zhang, N. Jameson, M.R. Corces, J.M. Granja, X. Chen, C. Coruh, A. Abnousi, J. Houston, Z. Ye, R. Hu, M. Yu, H. Kim, J.A. Law, R.G.W. Verhaak, M. Hu, F.B. Furnari, H.Y. Chang, B. Ren, V. Bafna, P. S. Mischel, Circular ecDNA promotes accessible chromatin and high oncogene expression, Nature 575 (2019) 699–703.
[17] T. Paulsen, P. Kumar, M.M. Koseoglu, A. Dutta, Discoveries of extrachromosomal circles of DNA in normal and tumor cells, Trends Genet. 34 (2018) 270–278.
[18] X. Ling, Y. Han, J. Meng, B. Zhong, J. Chen, H. Zhang, J. Qin, J. Pang, L. Liu, Small extrachromosomal circular DNA (eccDNA): major functions in evolution and cancer, Mol. Cancer 20 (2021) 113.
[19] S. Wu, V. Bafna, P.S. Mischel, Extrachromosomal DNA (ecDNA) in cancer pathogenesis, Curr. Opin. Genet. Dev. 66 (2021) 78–82.
[20] D.A. Haber, R.T. Schimke, Unstable amplification of an altered dihydrofolate reductase gene associated with double-minute chromosomes, Cell 26 (1981) 355–362.
[21] F.W. Alt, R.E. Kellems, J.R. Bertino, R.T. Schimke, Selective multiplication of dihydrofolate reductase genes in methotrexate-resistant variants of cultured murine cells, J. Biol. Chem. 253 (1978) 1357–1370.
[22] G.R. Stark, Regulation and mechanisms of mammalian gene amplification, Adv. Cancer Res. 61 (1993) 87–113.
[23] A.R. Morton, N. Dogan-Artun, Z.J. Faber, G. MacLeod, C.F. Bartels, M.S. Piazza, K.C. Allan, S.C. Mack, X. Wang, R.C. Gimple, Q. Wu, B.P. Rubin, S. Shetty, S. Angers, P.B. Dirks, R.C. Sallari, M. Lupien, J.N. Rich, P.C. Scacheri, Functional enhancers shape extrachromosomal oncogene amplifications, Cell 179 (2019) 1330–1341.
[24] N. Shimizu, N. Itoh, H. Utiyama, G.M. Wahl, Selective entrapment of extrachromosomally amplified DNA by nuclear budding and micronucleation during S phase, J. Cell Biol. 140 (1998) 1307–1320.
[25] N. Itoh, N. Shimizu, DNA replication-dependent intranuclear relocation of double minute chromatin, J. Cell Sci. 111 (Pt 22) (1998) 3275–3285.
[26] T. Kanda, M. Otter, G.M. Wahl, Mitotic segregation of viral and cellular acentric extrachromosomal molecules by chromosome tethering, J. Cell Sci. 114 (2001) 49–58.
[27] K.L. Hung, K.E. Yost, L. Xie, Q. Shi, K. Helmsauer, J. Luebeck, R. Schopflin, J.T. Lange, R. Chamorro Gonzalez, N.E. Weiser, C. Chen, M.E. Valieva, I.T. Wong, S. Wu, S.R. Dehkordi, C.V. Duffy, K. Kraft, J. Tang, J.A. Belk, J.C. Rose, M.R. Corces, J.M. Granja, R. Li, U. Rajkumar, J. Friedlein, A. Bagchi, A.T. Satpathy, R. Tjian, S. Mundlos, V. Bafna, A.G. Henssen, P.S. Mischel, Z. Liu, H.Y. Chang, ecDNA hubs drive cooperative intermolecular oncogene expression, Nature 600 (2021) 731–736.
[28] A.C. deCarvalho, H. Kim, L.M. Poisson, M.E. Winn, C. Mueller, D. Cherba, J. Koeman, S. Seth, A. Protopopov, M. Felicella, S. Zheng, A. Multani, Y. Jiang, J. Zhang, D.H. Nam, E.F. Petricoin, L. Chin, T. Mikkelsen, R.G.W. Verhaak, Discordant inheritance of chromosomal and extrachromosomal DNA elements contributes to dynamic disease evolution in glioblastoma, Nat. Genet. 50 (2018) 708–717.
[29] E. Yi, A.D. Gujar, M. Guthrie, H. Kim, D. Zhao, K.C. Johnson, S.B. Amin, M.L. Costa, Q. Yu, S. Das, N. Jillette, P.A. Clow, A.W. Cheng, R.G.W. Verhaak, Live-cell imaging shows uneven segregation of extrachromosomal DNA elements and transcriptionally active extrachromosomal DNA hubs in cancer, Cancer Discov. 12 (2022) 468–483.
[30] M. Weller, N. Butowski, D.D. Tran, L.D. Recht, M. Lim, H. Hirte, L. Ashby, L. Mechtler, S.A. Goldlust, F. Iwamoto, J. Drappatz, D.M. O'Rourke, M. Wong, M. G. Hamilton, G. Finocchiaro, J. Perry, W. Wick, J. Green, Y. He, C.D. Turner, M.J. Yellin, T. Keler, T.A. Davis, R. Stupp, J.H. Sampson, A.I.t. investigators, Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial, Lancet Oncol. 18 (2017) 1373–1385.
[31] P. Dharanipragada, X. Zhang, S.X. Liu, S.H. Lomeli, A.Y. Hong, Y. Wang, Z.T. Yang, K.Z. Lo, A. Vega-Crespo, A. Ribas, S.J. Moschos, G. Moriceau, R.S. Lo, Blocking genomic instability prevents acquired resistance to MAPK inhibitor therapy in melanoma, Cancer Discov. 13 (2023) 880–909.
[32] D.A. Nathanson, B. Gini, J. Mottahedeh, K. Visnyei, T. Koga, G. Gomez, A. Eskin, K. Hwang, J. Wang, K. Masui, A. Paucar, H. Yang, M. Ohashi, S. Zhu, J. Wykosky, R. Reed, S.F. Nelson, T.F. Cloughesy, C.D. James, P.N. Rao, H.I. Kornblum, J.R. Heath, W.K. Cavenee, F.B. Furnari, P.S. Mischel, Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA, Science 343 (2014) 72–76.
[33] P.V. Schoenlein, J.T. Barrett, A. Kulharya, M.R. Dohn, A. Sanchez, D.Y. Hou, J. McCoy, Radiation therapy depletes extrachromosomally amplified drug resistance genes and oncogenes from tumor cells via micronuclear capture of episomes and double minute chromosomes, Int J Radiat Oncol 55 (2003) 1051–1065.
[34] R.P. Koche, E. Rodriguez-Fos, K. Helmsauer, M. Burkert, I.C. MacArthur, J. Maag, R. Chamorro, N. Munoz-Perez, M. Puiggros, H. Dorado Garcia, Y. Bei, C. Roefzaad, V. Bardinet, A. Szymansky, A. Winkler, T. Thole, N. Timme, K. Kasack, S. Fuchs, F. Klironomos, N. Thiessen, E. Blanc, K. Schmelz, A. Kunkele, P. Hundsdorfer, C. Rosswog, J. Theissen, D. Beule, H. Deubzer, S. Sauer, J. Toedling, M. Fischer, F. Hertwig, R.F. Schwarz, A. Eggert, D. Torrents, J.H. Schulte, A.G. Henssen, Extrachromosomal circular DNA drives oncogenic genome remodeling in neuroblastoma, Nat. Genet. 52 (2020) 29–34.
[35] O. Shoshani, S.F. Brunner, R. Yaeger, P. Ly, Y. Nechemia-Arbely, D.H. Kim, R.X. Fang, G.A. Castillon, M. Yu, J.S.Z. Li, Y. Sun, M.H. Ellisman, B. Ren, P. J. Campbell, D.W. Cleveland, Chromothripsis drives the evolution of gene amplification in cancer, Nature 591 (2021) 137–141.
[36] H. Kim, N.P. Nguyen, K. Turner, S.H. Wu, A.D. Gujar, J. Luebeck, J.H. Liu, V. Deshpande, U. Rajkumar, S. Namburi, S.B. Amin, E. Yi, F. Menghi, J.H. Schulte, A. G. Henssen, H.Y. Chang, C.R. Beck, P.S. Mischel, V. Bafna, R.G.W. Verhaak, Extrachromosomal DNA is associated with oncogene amplification and poor outcome across multiple cancers, Nat. Genet. 52 (2020) 891–897.
[37] J. Luebeck, A.W.T. Ng, P.C. Galipeau, X. Li, C.A. Sanchez, A.C. Katz-Summercorn, H. Kim, S. Jammula, Y. He, S.M. Lippman, R.G.W. Verhaak, C.C. Maley, L. B. Alexandrov, B.J. Reid, R.C. Fitzgerald, T.G. Paulson, H.Y. Chang, S. Wu, V. Bafna, P.S. Mischel, Extrachromosomal DNA in the cancerous transformation of Barrett's oesophagus, Nature 616 (2023) 798–805.

[38] A. Sakhdari, Z.Y. Tang, C.Y. Ok, C.E. Bueso-Ramos, L.J. Medeiros, Y.O. Huh, Homogeneously staining region (hsr) on chromosome 11 is highly specific for KMT2A amplification in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), Cancer Genet-Ny 238 (2019) 18–22.

[39] J.H. Christiansen, N.P. Nguyen, R. Verhaak, H. Kim, V. Bafna, P. Mischel, C. Hassig, Extrachromosomal DNA (ecDNA) carrying amplified oncogenes as a biomarker for insensitivity to pembrolizumab treatment in gastric cancer patients, J. Clin. Oncol. 38 (2020).

[40] L.A. Moreau, P. McGrady, W.B. London, H. Shimada, S.L. Cohn, J.M. Maris, L. Diller, A.T. Look, R.E. George, Does MYCN amplification manifested as homogeneously staining regions at diagnosis predict a worse outcome in children with neuroblastoma? A Children's Oncology Group study, Clin. Cancer Res. 12 (2006) 5693–5697.

[41] I. Cortés-Ciriano, J.J.K. Lee, R.B. Xi, D. Jain, Y.L. Jung, L.X. Yang, D. Gordenin, L.J. Klimczak, C.Z. Zhang, D.S. Pellman, P.J. Park, K.C. Akdemir, E.G. Alvarez, A. Baez-Ortega, R. Beroukhim, P.C. Boutros, D.D.L. Bowtell, B. Brors, K.H. Burns, P.J. Campbell, K. Chan, K. Chen, A. Dueso-Barroso, A.J. Dunford, P. A. Edwards, X. Estivill, D. Etemadmoghadam, L. Feuerbach, J.L. Fink, M. Frenkel-Morgenstern, D.W. Garsed, M. Gerstein, D.A. Gordenin, D. Haan, J.E. Haber, J.M. Hess, B. Hutter, M. Imielinski, D.T.W. Jones, Y.S. Ju, M.D. Kazanov, Y. Koh, J.O. Korbel, K. Kumar, E.A. Lee, Y.L. Li, A.G. Lynch, G. Macintyre, F. Markowetz, I. Martincorena, A. Martinez-Fundichely, S. Miyano, H. Nakagawa, F.C.P. Navarro, S. Ossowski, J.V. Pearson, M. Puiggròs, K. Rippe, N. D. Roberts, S.A. Roberts, B. Rodriguez-Martin, S.E. Schumacher, R. Scully, M. Shackleton, N. Sidiropoulos, L. Sieverling, C. Stewart, D. Torrents, J.M.C. Tubio, I. Villasante, N. Waddell, J.A. Wala, J. Weischenfeldt, X.T. Yao, S.S. Yoon, J. Zamora, P.S.V.W. Grp, P. Consortium, Comprehensive analysis of chromothripsis in 2,658 human cancers using whole-genome sequencing, Nat. Genet. 52 (2020) 331–341.

[42] P.J. Stephens, C.D. Greenman, B. Fu, F. Yang, G.R. Bignell, L.J. Mudie, E.D. Pleasance, K.W. Lau, D. Beare, L.A. Stebbings, S. McLaren, M.L. Lin, D.J. McBride, I. Varela, S. Nik-Zainal, C. Leroy, M. Jia, A. Menzies, A.P. Butler, J.W. Teague, M.A. Quail, J. Burton, H. Swerdlow, N.P. Carter, L.A. Morsberger, C. Iacobuzio-Donahue, G.A. Follows, A.R. Green, A.M. Flanagan, M.R. Stratton, P.A. Futreal, P.J. Campbell, Massive genomic rearrangement acquired in a single catastrophic event during cancer development, Cell 144 (2011) 27–40.

[43] T. Rausch, D.T. Jones, M. Zapatka, A.M. Stutz, T. Zichner, J. Weischenfeldt, N. Jager, M. Remke, D. Shih, P.A. Northcott, E. Pfaff, J. Tica, Q. Wang, L. Massimi, H. Witt, S. Bender, S. Pleier, H. Cin, C. Hawkins, C. Beck, A. von Deimling, V. Hans, B. Brors, R. Eils, W. Scheurlen, J. Blake, V. Benes, A.E. Kulozik, O. Witt, D. Martin, C. Zhang, R. Porat, D.M. Merino, J. Wasserman, N. Jabado, A. Fontebasso, L. Bullinger, F.G. Rucker, K. Dohner, H. Dohner, J. Koster, J.J. Molenaar, R. Versteeg, M. Kool, U. Tabori, D. Malkin, A. Korshunov, M.D. Taylor, P. Lichter, S.M. Pfister, J.O. Korbel, Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations, Cell 148 (2012) 59–71.

[44] K. Nones, N. Waddell, N. Wayte, A.M. Patch, P. Bailey, F. Newell, O. Holmes, J.L. Fink, M.C.J. Quinn, Y.H. Tang, G. Lampe, K. Quek, K.A. Loffler, S. Manning, S. Idrisoglu, D. Miller, Q. Xu, N. Waddell, P.J. Wilson, T.J.C. Bruxner, A.N. Christ, I. Harliwong, C. Nourse, E. Nourbakhsh, M. Anderson, S. Kazakoff, C. Leonard, S. Wood, P.T. Simpson, L.E. Reid, L. Krause, D.J. Hussey, D.I. Watson, R.V. Lord, D. Nancarrow, W.A. Phillips, D. Gotley, B.M. Smithers, D. C. Whiteman, N.K. Hayward, P.J. Campbell, J.V. Pearson, S.M. Grimmond, A.P. Barbour, Genomic catastrophes frequently arise in esophageal adenocarcinoma and drive tumorigenesis, Nat. Commun. 5 (2014) 5224.

[45] P. Ly, S.F. Brunner, O. Shoshani, D.H. Kim, W. Lan, T. Pyntikova, A.M. Flanagan, S. Behjati, D.C. Page, P.J. Campbell, D.W. Cleveland, Chromosome segregation errors generate a diverse spectrum of simple and complex genomic rearrangements, Nat. Genet. 51 (2019) 705–715.

[46] M.J. Singer, L.D. Mesner, C.L. Friedman, B.J. Trask, J.L. Hamlin, Amplification of the human dihydrofolate reductase gene via double minutes is initiated by chromosome breaks, P Natl Acad Sci USA 97 (2000) 7921–7926.

[47] B. Windle, B.W. Draper, Y.X. Yin, S. O'Gorman, G.M. Wahl, A central role for chromosome breakage in gene amplification, deletion formation, and amplicon integration, Genes Dev. 5 (1991) 160–174.

[48] A. Coquelle, L. Rozier, B. Dutrillaux, M. Debatisse, Induction of multiple double-strand breaks within an hsr by meganuclease I-SceI expression or fragile site activation leads to formation of double minutes and other chromosomal rearrangements, Oncogene 21 (2002) 7671–7679.

[49] C. Rosswog, C. Bartenhagen, A. Welte, Y. Kahlert, N. Hemstedt, W. Lorenz, M. Cartolano, S. Ackermann, S. Perner, W. Vogel, J. Altmuller, P. Nurnberg, F. Hertwig, G. Gohring, E. Lilienweiss, A.M. Stutz, J.O. Korbel, R.K. Thomas, M. Peifer, M. Fischer, Chromothripsis followed by circular recombination drives oncogene amplification in human cancer, Nat. Genet. 53 (2021) 1673–1685.

[50] O. Shoshani, S.F. Brunner, R. Yaeger, P. Ly, Y. Nechemia-Arbely, D.H. Kim, R. Fang, G.A. Castillon, M. Yu, J.S.Z. Li, Y. Sun, M.H. Ellisman, B. Ren, P. J. Campbell, D.W. Cleveland, Chromothripsis drives the evolution of gene amplification in cancer, Nature 591 (2021) 137–141.

[51] K.M. Turner, V. Deshpande, D. Beyter, T. Koga, J. Rusert, C. Lee, B. Li, K. Arden, B. Ren, D.A. Nathanson, H.I. Kornblum, M.D. Taylor, S. Kaushal, W. K. Cavenee, R. Wechsler-Reya, F.B. Furnari, S.R. Vandenberg, P.N. Rao, G.M. Wahl, V. Bafna, P.S. Mischel, Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity, Nature 543 (2017) 122–125.

[52] N. Shimizu, T. Hashizume, K. Shingaki, J.K. Kawamoto, Amplification of plasmids containing a mammalian replication initiation region is mediated by controllable conflict between replication and transcription, Cancer Res. 63 (2003) 5281–5290.

[53] Y.G. Wang, M. Wang, M.N. Djekidel, H. Chen, D. Liu, F.W. Alt, Y. Zhang, eccDNAs are apoptotic products with high innate immunostimulatory activity, Nature 599 (2021) 308–314.

[54] M. Downey, D. Durocher, Chromatin and DNA repair: the benefits of relaxation, Nat. Cell Biol. 8 (2006) 9–10.

[55] R.C. Gonzalez, T. Conrad, M.C. Stober, R.B. Xu, M. Giurgiu, E. Rodriguez-Fos, K. Kasack, L. Bruckner, E. van Leen, K. Helmsauer, H.D. Garcia, M.E. Stefanova, K.L. Hung, Y. Bei, K. Schmelz, M. Lodrini, S. Mundlos, H.Y. Chang, H.E. Deubzer, S. Sauer, A. Eggert, J.H. Schulte, R.F. Schwarz, K. Haase, R.P. Koche, A. G. Henssen, Parallel sequencing of extrachromosomal circular DNAs and transcriptomes in single cancer cells, Nat. Genet. 55 (2023) 880–890.

[56] J.E. Phillips, V.G. Corces, CTCF: master weaver of the genome, Cell 137 (2009) 1194–1211.

[57] J.N. Wells, C. Feschotte, A field guide to eukaryotic transposable elements, Annu. Rev. Genet. 54 (2020) 539–561.

[58] H.H. Kazazian, J.V. Moran, Mobile DNA in health and disease, N. Engl. J. Med. 377 (2017) 361–370.

[59] R. Fueyo, J. Judd, C. Feschotte, J. Wysocka, Roles of transposable elements in the regulation of mammalian transcription, Nat. Rev. Mol. Cell Biol. 23 (2022) 481–497.

[60] F. Yang, W.J. Su, O.W. Chung, L. Tracy, L. Wang, D.A. Ramsden, Z.Z. Zhang, Retrotransposons hijack alt-EJ for DNA replication and eccDNA biogenesis, Nature 620 (2023) 218–225.

[61] R.P. Koche, E. Rodriguez-Fos, K. Helmsauer, M. Burkert, I.C. MacArthur, J. Maag, R. Chamorro, N. Munoz-Perez, M. Puiggros, H. Dorado Garcia, Y. Bei, C. Roeefzaad, V. Bardinet, A. Szymansky, A. Winkler, T. Thole, N. Timme, K. Kasack, S. Fuchs, F. Klironomos, N. Thiessen, E. Blanc, K. Schmelz, A. Kuenkele, P. Hundsdoerfer, C. Rosswog, J. Theissen, D. Beule, H. Deubzer, S. Sauer, J. Toedling, M. Fischer, F. Hertwig, R.F. Schwarz, A. Eggert, D. Torrents, J.H. Schulte, A.G. Henssen, Extrachromosomal circular DNA drives oncogenic genome remodeling in neuroblastoma, Nat. Genet. 52 (2020) 29–34.

[62] H. Kim, N.P. Nguyen, K. Turner, S. Wu, A.D. Gujar, J. Luebeck, J. Liu, V. Deshpande, U. Rajkumar, S. Namburi, S.B. Amin, E. Yi, F. Menghi, J.H. Schulte, A. G. Henssen, H.Y. Chang, C.R. Beck, P.S. Mischel, V. Bafna, R.G.W. Verhaak, Extrachromosomal DNA is associated with oncogene amplification and poor outcome across multiple cancers, Nat. Genet. 52 (2020) 891–897.

[63] E. Yi, R.C. Gonzalez, A.G. Henssen, R.G.W. Verhaak, Extrachromosomal DNA amplifications in cancer, Nat. Rev. Genet. 23 (2022) 760–771.

[64] E. van Leen, L. Bruckner, A.G. Henssen, The genomic and spatial mobility of extrachromosomal DNA and its implications for cancer therapy, Nat. Genet. 54 (2022) 107–114.

[65] A.R. Morton, N. Dogan-Artun, Z.J. Faber, G. MacLeod, C.F. Bartels, M.S. Piazza, K.C. Allan, S.C. Mack, X.X. Wang, R.C. Gimple, Q.L. Wu, B.P. Rubin, S. Shetty, S. Angers, P.B. Dirks, R.C. Sallari, M. Lupien, J.N. Rich, P.C. Scacheri, Functional enhancers shape extrachromosomal oncogene amplifications, Cell 179 (2019) 1330–1341.

[66] K. Helmsauer, M.E. Valieva, S. Ali, R. Chamorro Gonzalez, R. Schopflin, C. Roefzaad, Y. Bei, H. Dorado Garcia, E. Rodriguez-Fos, M. Puiggros, K. Kasack, K. Haase, C. Keskeny, C.Y. Chen, L.P. Kuschel, P. Euskirchen, V. Heinrich, M.I. Robson, C. Rosswog, J. Toedling, A. Szymansky, F. Hertwig, M. Fischer, D. Torrents, A. Eggert, J.H. Schulte, S. Mundlos, A.G. Henssen, R.P. Koche, Enhancer hijacking determines extrachromosomal circular MYCN amplicon architecture in neuroblastoma, Nat. Commun. 11 (2020) 5823.

[67] R.C. González, T. Conrad, M.C. Stöber, R.B. Xu, M. Giurgiu, E. Rodriguez-Fos, K. Kasack, L. Brückner, E. van Leen, K. Helmsauer, H.D. Garcia, M.E. Stefanova, K.L. Hung, Y. Bei, K. Schmelz, M. Lodrini, S. Mundlos, H.Y. Chang, H.E. Deubzer, S. Sauer, A. Eggert, J.H. Schulte, R.F. Schwarz, K. Haase, R.P. Koche, A. G. Henssen, Parallel sequencing of extrachromosomal circular DNAs and transcriptomes in single cancer cells, Nat. Genet. 55 (2023) 880–890.
[68] K. Hadi, X.T. Yao, J.M. Behr, A. Deshpande, C. Xanthopoulakis, H.S. Tian, S. Kudman, J. Rosiene, M. Darmofal, J. DeRose, R. Mortensen, E.M. Adney, A. Shaiber, Z. Gajic, M. Sigouros, K. Eng, J.A. Wala, K.O. Wrzeszczynski, K. Arora, M. Shah, A.K. Emde, V. Felice, M.O. Frank, R.B. Darnell, M. Ghandi, F. Huang, S. Dewhurst, J. Maciejowski, T. de Lange, J. Setton, N. Riaz, J.S. Reis, S. Powell, D.A. Knowles, E. Reznik, B. Mishra, R. Beroukhim, M.C. Zody, N. Robine, K.M. Oman, C.A. Sanchez, M.K. Kuhner, L.P. Smith, P.C. Galipeau, T.G. Paulson, B.J. Reid, X.H. Li, D. Wilkes, A. Sboner, J.M. Mosquera, O. Elemento, M. Imielinski, Distinct classes of complex structural variation uncovered across thousands of cancer genome graphs, Cell 183 (2020) 197–210.
[69] V. Deshpande, J. Luebeck, N.P.D. Nguyen, M. Bakhtiari, K.M. Turner, R. Schwab, H. Carter, P.S. Mischel, V. Bafna, Exploring the landscape of focal amplifications in cancer using AmpliconArchitect, Nat. Commun. 10 (2019) 392.
[70] J. Luebeck, C. Coruh, S.R. Dehkordi, J.T. Lange, K.M. Turner, V. Deshpande, D.A. Pai, C. Zhang, U. Rajkumar, J.A. Law, P.S. Mischel, V. Bafna, AmpliconReconstructor integrates NGS and optical mapping to resolve the complex structures of focal amplifications, Nat. Commun. 11 (2020) 4374.
[71] I. Prada-Luengo, A. Krogh, L. Maretty, B. Regenberg, Sensitive detection of circular DNAs at single-nucleotide resolution using guided realignment of partially aligned reads, BMC Bioinf. 20 (2019) 663.
[72] H.D. Moller, M. Mohiyuddin, I. Prada-Luengo, M.R. Sailani, J.F. Halling, P. Plomgaard, L. Maretty, A.J. Hansen, M.P. Snyder, H. Pilegaard, H.Y.K. Lam, B. Regenberg, Circular DNA elements of chromosomal origin are common in healthy human somatic tissue, Nat. Commun. 9 (2018) 1069.
[73] K.L. Hung, J. Luebeck, S.R. Dehkordi, C.I. Colon, R. Li, I.T. Wong, C. Coruh, P. Dharanipragada, S.H. Lomeli, N.E. Weiser, G. Moriceau, X. Zhang, C. Bailey, K. E. Houlahan, W. Yang, R.C. Gonzalez, C. Swanton, C. Curtis, M. Jamal-Hanjani, A.G. Henssen, J.A. Law, W.J. Greenleaf, R.S. Lo, P.S. Mischel, V. Bafna, H. Y. Chang, Targeted profiling of human extrachromosomal DNA by CRISPR-CATCH, Nat. Genet. 54 (2022) 1746–1754.
[74] J.T. Lange, J.C. Rose, C.Y. Chen, Y. Pichugin, L. Xie, J. Tang, K.L. Hung, K.E. Yost, Q. Shi, M.L. Erb, U. Rajkumar, S. Wu, S. Taschner-Mandl, M. Bernkopf, C. Swanton, Z. Liu, W. Huang, H.Y. Chang, V. Bafna, A.G. Henssen, B. Werner, P.S. Mischel, The evolutionary dynamics of extrachromosomal DNA in human cancers, Nat. Genet. 54 (2022) 1527–1533.
[75] I. Lee, R. Razaghi, T. Gilpatrick, M. Molnar, A. Gershman, N. Sadowski, F.J. Sedlazeck, K.D. Hansen, J.T. Simpson, W. Timp, Simultaneous profiling of chromatin accessibility and methylation on human cell lines with nanopore sequencing, Nat. Methods 17 (2020) 1191–1199.
[76] Z. Shipony, G.K. Marinov, M.P. Swaffer, N.A. Sinnott-Armstrong, J.M. Skotheim, A. Kundaje, W.J. Greenleaf, Long-range single-molecule mapping of chromatin accessibility in eukaryotes, Nat. Methods 17 (2020) 319–327.
[77] A.B. Stergachis, B.M. Debo, E. Haugen, L.S. Churchman, J.A. Stamatoyannopoulos, Single-molecule regulatory architectures captured by chromatin fiber sequencing, Science 368 (2020) 1449–1454.
[78] W. Chen, Z. Weng, Z. Xie, Y. Xie, C. Zhang, Z. Chen, F. Ruan, J. Wang, Y. Sun, Y. Fang, M. Guo, Y. Tong, Y. Li, C. Tang, Sequencing of methylase-accessible regions in integral circular extrachromosomal DNA reveals differences in chromatin structure, Epigenet. Chromatin 14 (2021) 40.
[79] R.G.W. Verhaak, V. Bafna, P.S. Mischel, Extrachromosomal oncogene amplification in tumour pathogenesis and evolution, Nat. Rev. Cancer 19 (2019) 283–288.
[80] A.C. deCarvalho, H. Kim, L.M. Poisson, M.E. Winn, C. Mueller, D. Cherba, J. Koeman, S. Seth, A. Protopopov, M. Felicella, S.Y. Zheng, A. Multani, Y.Y. Jiang, J. H. Zhang, D.H. Nam, E.F. Petricoin, L. Chin, T. Mikkelsen, R.G.W. Verhaak, Discordant inheritance of chromosomal and extrachromosomal DNA elements contributes to dynamic disease evolution in glioblastoma, Nat. Genet. 50 (2018) 708–717.
[81] K.M. Turner, V. Deshpande, D. Beyter, T. Koga, J. Rusert, C. Lee, B. Li, K. Arden, B. Ren, D.A. Nathanson, H.I. Kornblum, M.D. Taylor, S. Kaushal, W. K. Cavenee, R. Wechsler-Reya, F.B. Furnari, S.R. Vandenberg, P.N. Rao, G.M. Wahl, V. Bafna, P.S. Mischel, Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity, Nature 543 (2017) 122–125.
[82] U. Rajkumar, K. Turner, J. Luebeck, V. Deshpande, M. Chandraker, P. Mischel, V. Bafna, EcSeg: semantic segmentation of metaphase images containing extrachromosomal DNA, iScience 21 (2019) 428–435.
[83] E. Yi, A.D. Gujar, M. Guthrie, H. Kim, D. Zhao, K.C. Johnson, S.B. Amin, M.L. Costa, Q.R. Yu, S. Das, N. Jillette, P.A. Clow, A.W. Cheng, R.G.W. Verhaak, Live-cell imaging shows uneven segregation of extrachromosomal DNA elements and transcriptionally active extrachromosomal DNA hubs in cancer, Cancer Discov. 12 (2022) 468–483.
[84] I. Martincorena, P.J. Campbell, Somatic mutation in cancer and normal cells, Science 349 (2015) 1483–1489.
[85] J.O. Korbel, P.J. Campbell, Criteria for inference of chromothripsis in cancer genomes, Cell 152 (2013) 1226–1236.
[86] X. Cao, S. Wang, L. Ge, W. Zhang, J. Huang, W. Sun, Extrachromosomal circular DNA: category, biogenesis, recognition, and functions, Front. Vet. Sci. 8 (2021) 693641.
[87] N. Vogt, A. Gibaud, F. Lemoine, P. de la Grange, M. Debatisse, B. Malfoy, Amplicon rearrangements during the extrachromosomal and intrachromosomal amplification process in a glioma, Nucleic Acids Res. 42 (2014) 13194–13205.
[88] A. L'Abbate, G. Macchia, P. D'Addabbo, A. Lonoce, D. Tolomeo, D. Trombetta, K. Kok, C. Bartenhagen, C.W. Whelan, O. Palumbo, M. Severgnini, I. Cifola, M. Dugas, M. Carella, G. De Bellis, M. Rocchi, L. Carbone, C.T. Storlazzi, Genomic organization and evolution of double minutes/homogeneously staining regions with MYC amplification in human cancer, Nucleic Acids Res. 42 (2014) 9131–9145.
[89] K. Xu, L. Ding, T.C. Chang, Y. Shao, J. Chiang, H. Mulder, S. Wang, T.I. Shaw, J. Wen, L. Hover, C. McLeod, Y.D. Wang, J. Easton, M. Rusch, J. Dalton, J. R. Downing, D.W. Ellison, J. Zhang, S.J. Baker, G. Wu, Structure and evolution of double minutes in diagnosis and relapse brain tumors, Acta Neuropathol. 137 (2019) 123–137.
[90] E. Calo, J. Wysocka, Modification of enhancer chromatin: what, how, and why? Mol. Cell 49 (2013) 825–837.
[91] W. Schaffner, Enhancers, enhancers - from their discovery to today's universe of transcription enhancers, Biol. Chem. 396 (2015) 311–327.
[92] G. Smith, C. Taylor-Kashton, L. Dushnicky, S. Symons, J. Wright, S. Mai, c-Myc-induced extrachromosomal elements carry active chromatin, Neoplasia 5 (2003) 110–120.
[93] R. Tian, Z.Y. Huang, L.F. Li, J.P. Yuan, Q.H. Zhang, L.R. Meng, B. Lang, Y.F. Hong, C.Y. Zhong, X. Tian, Z.F. Cui, Z. Jin, J.S. Liu, Z.Y. Huang, Y.Y. Wang, Y. Chen, Z. Hu, HPV integration generates a cellular super-enhancer which functions as ecDNA to regulate genome-wide transcription, Nucleic Acids Res. 51 (2023) 4237–4251.
[94] Y.F. Zhu, A.D. Gujar, C.H. Wong, H. Tjong, C.Y. Ngan, L. Gong, Y.A. Chen, H. Kim, J.H. Liu, M.H. Li, A. Mil-Homens, R. Maurya, C. Kuhlberg, F.Y. Sun, E. Yi, A. C. DeCarvalho, Y.J. Ruan, R.G.W. Verhaak, C.L. Wei, Oncogenic extrachromosomal DNA functions as mobile enhancers to globally amplify chromosomal transcription, Cancer Cell 39 (2021) 694–707.
[95] J. Loven, H.A. Hoke, C.Y. Lin, A. Lau, D.A. Orlando, C.R. Vakoc, J.E. Bradner, T.I. Lee, R.A. Young, Selective inhibition of tumor oncogenes by disruption of super-enhancers, Cell 153 (2013) 320–334.
[96] B.C. Carlton, D.R. Helinski, Heterogeneous circular DNA elements in vegetative cultures of Bacillus megaterium, Proc. Natl. Acad. Sci. U. S. A. 64 (1969) 592–599.
[97] J. George, J.S. Lim, S.J. Jang, Y. Cun, L. Ozretic, G. Kong, F. Leenders, X. Lu, L. Fernandez-Cuesta, G. Bosco, C. Muller, I. Dahmen, N.S. Jahchan, K.S. Park, D. Yang, A.N. Karnezis, D. Vaka, A. Torres, M.S. Wang, J.O. Korbel, R. Menon, S.M. Chun, D. Kim, M. Wilkerson, N. Hayes, D. Engelmann, B. Putzer, M. Bos, S. Michels, I. Vlasic, D. Seidel, B. Pinther, P. Schaub, C. Becker, J. Altmuller, J. Yokota, T. Kohno, R. Iwakawa, K. Tsuta, M. Noguchi, T. Muley, H. Hoffmann, P. A. Schnabel, I. Petersen, Y. Chen, A. Soltermann, V. Tischler, C.M. Choi, Y.H. Kim, P.P. Massion, Y. Zou, D. Jovanovic, M. Kontic, G.M. Wright, P.A. Russell, B. Solomon, I. Koch, M. Lindner, L.A. Muscarella, A. la Torre, J.K. Field, M. Jakopovic, J. Knezevic, E. Castanos-Velez, L. Roz, U. Pastorino, O.T. Brustugun, M. Lund-Iversen, E. Thunnissen, J. Kohler, M. Schuler, J. Botling, M. Sandelin, M. Sanchez-Cespedes, H.B. Salvesen, V. Achter, U. Lang, M. Bogus, P. M. Schneider, T. Zander, S. Ansen, M. Hallek, J. Wolf, M. Vingron, Y. Yatabe, W.D. Travis, P. Nurnberg, C. Reinhardt, S. Perner, L. Heukamp, R. Buttner, S. A. Haas, E. Brambilla, M. Peifer, J. Sage, R.K. Thomas, Comprehensive genomic profiles of small cell lung cancer, Nature 524 (2015) 47–53.
[98] A.N. Blackford, M. Stucki, How cells respond to DNA breaks in mitosis, Trends Biochem. Sci. 45 (2020) 321–331.

[99] Z. Liao, W. Jiang, L. Ye, T. Li, X. Yu, L. Liu, Classification of extrachromosomal circular DNA with a focus on the role of extrachromosomal DNA (ecDNA) in tumor heterogeneity and progression, Biochim. Biophys. Acta Rev. Canc 2020 (1874) 188392.
[100] C. Bailey, M.J. Shoura, P.S. Mischel, C. Swanton, Extrachromosomal DNA-relieving heredity constraints, accelerating tumour evolution, Ann. Oncol. 31 (2020) 884–893.
[101] D.W. Cleveland, Y. Mao, K.F. Sullivan, Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling, Cell 112 (2003) 407–421.
[102] H. Sun, Q. Chen, W. Chen, C. Qu, J. Mo, J. Song, J. Guo, Y. Tian, Assessment of biological community in riparian zone contaminated by PAHs: linking source apportionment to biodiversity, Sci. Total Environ. 851 (2022) 158121.
[103] K. Song, J.K. Minami, A. Huang, S.R. Dehkordi, S.H. Lomeli, J. Luebeck, M.H. Goodman, G. Moriceau, O. Krijgsman, P. Dharanipragada, T. Ridgley, W. P. Crosson, J. Salazar, E. Pazol, G. Karin, R. Jayaraman, N.G. Balanis, S. Alhani, K. Sheu, J. Ten Hoeve, A. Palermo, S.E. Motika, T.N. Senaratne, K.H. Paraiso, P. J. Hergenrother, P.N. Rao, A.S. Multani, D.S. Peeper, V. Bafna, R.S. Lo, T.G. Graeber, Plasticity of extrachromosomal and intrachromosomal BRAF amplifications in overcoming targeted therapy dosage challenges, Cancer Discov. 12 (2022) 1046–1069.
[104] D.D. Von Hoff, T. Waddelow, B. Forseth, K. Davidson, J. Scott, G. Wahl, Hydroxyurea accelerates loss of extrachromosomally amplified genes from tumor cells, Cancer Res. 51 (1991) 6273–6279.
[105] D.A. Nathanson, B. Gini, J. Mottahedeh, K. Visnyei, T. Koga, G. Gomez, A. Eskin, K. Hwang, J. Wang, K. Masui, A. Paucar, H.J. Yang, M. Ohashi, S.J. Zhu, J. Wykosky, R. Reed, S.F. Nelson, T.F. Cloughesy, C.D. James, P.N. Rao, H.I. Kornblum, J.R. Heath, W.K. Cavenee, F.B. Furnari, P.S. Mischel, Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA, Science 343 (2014) 72–76.
[106] Y.H. Zhou, Y.M. Chen, Y.J. Hu, L.P. Yu, K. Tran, E. Giedzinski, N. Ru, A. Gau, F. Pan, J. Qiao, N. Atkin, K.C. Ly, N. Lee, E.R. Siegel, M.E. Linskey, P. Wang, C. Limoli, The role of EGFR double minutes in modulating the response of malignant gliomas to radiotherapy, Oncotarget 8 (2017) 80853–80868.
[107] R. Gubner, S. August, V. Ginsberg, Therapeutic suppression of tissue reactivity. II. Effect of aminopterin in rheumatoid arthritis and psoriasis, Am. J. Med. Sci. 221 (1951) 176–182.
[108] S. Farber, L.K. Diamond, Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid, N. Engl. J. Med. 238 (1948) 787–793.
[109] S.E. Benner, G.M. Wahl, D.D. Von Hoff, Double minute chromosomes and homogeneously staining regions in tumors taken directly from patients versus in human tumor cell lines, Anti Cancer Drugs 2 (1991) 11–25.
[110] G. Pauletti, E. Lai, G. Attardi, Early appearance and long-term persistence of the submicroscopic extrachromosomal elements (amplisomes) containing the amplified DHFR genes in human cell lines, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 2955–2959.
[111] M.J. Singer, L.D. Mesner, C.L. Friedman, B.J. Trask, J.L. Hamlin, Amplification of the human dihydrofolate reductase gene via double minutes is initiated by chromosome breaks, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 7921–7926.
[112] A.A. Samatar, P.I. Poulikakos, Targeting RAS-ERK signalling in cancer: promises and challenges, Nat. Rev. Drug Discov. 13 (2014) 928–942.
[113] K.T. Flaherty, I. Puzanov, K.B. Kim, A. Ribas, G.A. McArthur, J.A. Sosman, P.J. O'Dwyer, R.J. Lee, J.F. Grippo, K. Nolop, P.B. Chapman, Inhibition of mutated, activated BRAF in metastatic melanoma, N. Engl. J. Med. 363 (2010) 809–819.
[114] K.T. Flaherty, J.R. Infante, A. Daud, R. Gonzalez, R.F. Kefford, J. Sosman, O. Hamid, L. Schuchter, J. Cebon, N. Ibrahim, R. Kudchadkar, H.A. Burris, G. Falchook, A. Algazi, K. Lewis, G.V. Long, I. Puzanov, P. Lebowitz, A. Singh, S. Little, P. Sun, A. Allred, D. Ouellet, K.B. Kim, K. Patel, J. Weber, Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations, N. Engl. J. Med. 367 (2012) 1694–1703.
[115] H. Shi, G. Moriceau, X. Kong, M.K. Lee, H. Lee, R.C. Koya, C. Ng, T. Chodon, R.A. Scolyer, K.B. Dahlman, J.A. Sosman, R.F. Kefford, G.V. Long, S.F. Nelson, A. Ribas, R.S. Lo, Melanoma whole-exome sequencing identifies (V600E)B-RAF amplification-mediated acquired B-RAF inhibitor resistance, Nat. Commun. 3 (2012) 724.
[116] R. Nazarian, H.B. Shi, Q. Wang, X.J. Kong, R.C. Koya, H. Lee, Z.G. Chen, M.K. Lee, N. Attar, H. Sazegar, T. Chodon, S.F. Nelson, G. McArthur, J.A. Sosman, A. Ribas, R.S. Lo, Melanomas acquire resistance toB-RAF(V600E) inhibition by RTK or N-RAS upregulation, Nature 468 (2010) 973–977.
[117] G.V. Long, Z. Eroglu, J. Infante, S. Patel, A. Daud, D.B. Johnson, R. Gonzalez, R. Kefford, O. Hamid, L. Schuchter, J. Cebon, W. Sharfman, R. McWilliams, M. Sznol, S. Redhu, E. Gasal, B. Mookerjee, J. Weber, K.T. Flaherty, Long-Term outcomes in patients with BRAF V600-mutant metastatic melanoma who received dabrafenib combined with trametinib, J. Clin. Oncol. 36 (2018) 667–673.
[118] R. Dummer, D. Schadendorf, P.A. Ascierto, A. Arance, C. Dutriaux, A.M. Di Giacomo, P. Rutkowski, M. Del Vecchio, R. Gutzmer, M. Mandala, L. Thomas, L. Demidov, C. Garbe, D. Hogg, G. Liszkay, P. Queirolo, E. Wasserman, J. Ford, M. Weill, L.A. Sirulnik, V. Jehl, V. Bozon, G.V. Long, K. Flaherty, Binimetinib versus dacarbazine in patients with advanced NRAS-mutant melanoma (NEMO): a multicentre, open-label, randomised, phase 3 trial, Lancet Oncol. 18 (2017) 435–445.
[119] A. Hong, M. Piva, S.X. Liu, W. Hugo, S.H. Lomeli, V. Zoete, C.E. Randolph, Z.T. Yang, Y. Wang, J.J. Lee, S.J. Lo, L. Sun, A. Vega-Crespo, A.J. Garcia, D. B. Shackelford, S.M. Dubinett, P.O. Scumpia, S.D. Byrum, A.J. Tackett, T.R. Donahue, O. Michielin, S.L. Holmen, A. Ribas, G. Moriceau, R.S. Lo, Durable suppression of acquired MEK inhibitor resistance in cancer by sequestering MEK from ERK and promoting antitumor T-cell immunity, Cancer Discov. 11 (2021) 714–735.
[120] I. Cortes-Ciriano, J.J.K. Lee, R.B. Xi, D. Jain, Y.L. Jung, L.X. Yang, D. Gordenin, L.J. Klimczak, C.Z. Zhang, D.S. Pellman, P.J. Park, K.C. Akdemir, E.G. Alvarez, A. Baez-Ortega, R. Beroukhim, P.C. Boutros, D.D.L. Bowtell, B. Brors, K.H. Burns, P.J. Campbell, K. Chan, K. Chen, A. Dueso-Barroso, A.J. Dunford, P. A. Edwards, X. Estivill, D. Etemadmoghadam, L. Feuerbach, J.L. Fink, M. Frenkel-Morgenstern, D.W. Garsed, M. Gerstein, D.A. Gordenin, D. Haan, J.E. Haber, J.M. Hess, B. Hutter, M. Imielinski, D.T.W. Jones, Y.S. Ju, M.D. Kazanov, Y. Koh, J.O. Korbel, K. Kumar, E.A. Lee, Y.L. Li, A.G. Lynch, G. Macintyre, F. Markowetz, I. Martincorena, A. Martinez-Fundichely, S. Miyano, H. Nakagawa, F.C.P. Navarro, S. Ossowski, J.V. Pearson, M. Puiggros, K. Rippe, N. D. Roberts, S.A. Roberts, B. Rodriguez-Martin, S.E. Schumacher, R. Scully, M. Shackleton, N. Sidiropoulos, L. Sieverling, C. Stewart, D. Torrents, J.M.C. Tubio, I. Villasante, N. Waddell, J.A. Wala, J. Weischenfeldt, X.T. Yao, S.S. Yoon, J. Zamora, P.S.V.W. Grp, P. Consortium, Comprehensive analysis of chromothripsis in 2,658 human cancers using whole-genome sequencing, Nat. Genet. 52 (2020) 331–341.
[121] J. Luebeck, A.W.T. Ng, P.C. Galipeau, X.H. Li, C.A. Sanchez, A.C. Katz-Summercorn, H. Kim, S. Jammula, Y.D. He, S.M. Lippman, R.G.W. Verhaak, C.C. Maley, L.B. Alexandrov, B.J. Reid, R.C. Fitzgerald, T.G. Paulson, H.W.Y. Chang, S.H. Wu, V. Bafna, P.S. Mischel, Extrachromosomal DNA in the cancerous transformation of Barrett's oesophagus, Nature 616 (2023) 798–805.
[122] H. Wang, X. Xu, C.M. Nguyen, Y. Liu, Y. Gao, X. Lin, T. Daley, N.H. Kipniss, M. La Russa, L.S. Qi, CRISPR-mediated programmable 3D genome positioning and nuclear organization, Cell 175 (2018) 1405–1417.
[123] T. Sexton, G. Cavalli, The role of chromosome domains in shaping the functional genome, Cell 160 (2015) 1049–1059.
[124] J.A. Bertolini, R. Favaro, Y. Zhu, M. Pagin, C.Y. Ngan, C.H. Wong, H. Tjong, M.W. Vermunt, B. Martynoga, C. Barone, J. Mariani, M.J. Cardozo, N. Tabanera, F. Zambelli, S. Mercurio, S. Ottolenghi, P. Robson, M.P. Creyghton, P. Bovolenta, G. Pavesi, F. Guillemot, S.K. Nicolis, C.L. Wei, Mapping the global chromatin connectivity network for Sox2 function in neural stem cell maintenance, Cell Stem Cell 24 (2019) 462–476.
[125] C.Y. Ngan, C.H. Wong, H. Tjong, W. Wang, R.L. Goldfeder, C. Choi, H. He, L. Gong, J. Lin, B. Urban, J. Chow, M. Li, J. Lim, V. Philip, S.A. Murray, H. Wang, C. L. Wei, Chromatin interaction analyses elucidate the roles of PRC2-bound silencers in mouse development, Nat. Genet. 52 (2020) 264–272.
[126] E.P. Nora, B.R. Lajoie, E.G. Schulz, L. Giorgetti, I. Okamoto, N. Servant, T. Piolot, N.L. van Berkum, J. Meisig, J. Sedat, J. Gribnau, E. Barillot, N. Bluthgen, J. Dekker, E. Heard, Spatial partitioning of the regulatory landscape of the X-inactivation centre, Nature 485 (2012) 381–385.
[127] J.R. Dixon, S. Selvaraj, F. Yue, A. Kim, Y. Li, Y. Shen, M. Hu, J.S. Liu, B. Ren, Topological domains in mammalian genomes identified by analysis of chromatin interactions, Nature 485 (2012) 376–380.
[128] N.D. Brownstone, J. Hong, M. Mosca, E. Hadeler, W. Liao, T. Bhutani, J. Koo, Biologic treatments of psoriasis: an update for the clinician, Biologics 15 (2021) 39–51.
[129] E.Y.X. Loh, P.S. Goh, A.M.M. Mannan, N. Mohd Sani, A. Ab Ghani, Cell and gene therapy products in Malaysia: a snapshot of the industry's current regulation preparedness, Cytotherapy 23 (2021) 1108–1113.
[130] K. Bakowski, S. Vogel, Evolution of complexity in non-viral oligonucleotide delivery systems: from gymnotic delivery through bioconjugates to biomimetic nanoparticles, RNA Biol. 19 (2022) 1256–1275.

[131] S.W. Wang, C. Gao, Y.M. Zheng, L. Yi, J.C. Lu, X.Y. Huang, J.B. Cai, P.F. Zhang, Y.H. Cui, A.W. Ke, Current applications and future perspective of CRISPR/Cas9 gene editing in cancer, Mol. Cancer 21 (2022) 57.
[132] S.T.K. Sin, L. Ji, J.E. Deng, P.Y. Jiang, S.H. Cheng, M.M.S. Heung, C.S.L. Lau, T.Y. Leung, K.C.A. Chan, R.W.K. Chiu, Y.M.D. Lo, Characteristics of fetal extrachromosomal circular DNA in maternal plasma: methylation status and clearance, Clin. Chem. 67 (2021) 788–796.
[133] A.J. Bronkhorst, V. Ungerer, S. Holdenrieder, The emerging role of cell-free DNA as a molecular marker for cancer management, Biomol Detect Quantif 17 (2019) 100087.
[134] Y. Cen, Y. Fang, Y. Ren, S. Hong, W. Lu, J. Xu, Global characterization of extrachromosomal circular DNAs in advanced high grade serous ovarian cancer, Cell Death Dis. 13 (2022) 342.
[135] T. Wilhelm, M. Said, V. Naim, DNA replication stress and chromosomal instability: dangerous liaisons, Genes 11 (2020) 642.
[136] M.K. Zeman, K.A. Cimprich, Causes and consequences of replication stress, Nat. Cell Biol. 16 (2014) 2–9.
[137] M.J. O'Connor, Targeting the DNA damage response in cancer, Mol. Cell 60 (2015) 547–560.
[138] M. Dobbelstein, C.S. Sorensen, Exploiting replicative stress to treat cancer, Nat. Rev. Drug Discov. 14 (2015) 405–423.
[139] C. Gelot, I. Magdalou, B.S. Lopez, Replication stress in Mammalian cells and its consequences for mitosis, Genes 6 (2015) 267–298.
[140] Y. Zou, Y. Liu, X. Wu, S.M. Shell, Functions of human replication protein A (RPA): from DNA replication to DNA damage and stress responses, J. Cell. Physiol. 208 (2006) 267–273.
[141] B. Bio, A New Approach to Cancer Treatment, 2023.
[142] ClinicalTrials.gov, Study of the CHK1 inhibitor BBI-355, an ecDNA-directed therapy, in: Subjects with Tumors with Oncogene Amplifications, POTENTIATE), 2023.
[143] A.B. Reams, J.R. Roth, Mechanisms of gene duplication and amplification, Csh Perspect Biol. 7 (2015) a016592.
[144] M.D. Cai, H.S. Zhang, L.Q. Hou, W. Gao, Y. Song, X.B. Cui, C.X. Li, R.W. Guan, J.F. Ma, X. Wang, Y. Han, Y.F. Lv, F. Chen, P. Wang, X.N. Meng, S.B. Fu, Inhibiting homologous recombination decreases extrachromosomal amplification but has no effect on intrachromosomal amplification in methotrexate-resistant colon cancer cells, Int. J. Cancer 144 (2019) 1037–1048.
[145] X.N. Meng, X.Y. Qi, H.H. Guo, M.D. Cai, C.X. Li, J. Zhu, F. Chen, H. Guo, J. Li, Y.Z. Zhao, P. Liu, X.Y. Jia, J.C. Yu, C.Y. Zhang, W.J. Sun, Y. Yu, Y. Jin, J. Bai, M. R. Wang, J. Rosales, K.Y. Lee, S.B. Fu, Novel role for non-homologous end joining in the formation of double minutes in methotrexate-resistant colon cancer cells, J. Med. Genet. 52 (2015) 135–144.
[146] T. Kanda, M. Otter, G.M. Wahl, Mitotic segregation of viral and cellular acentric extrachromosomal molecules by chromosome tethering, J. Cell Sci. 114 (2001) 49–58.
[147] S.H. Wu, K.M. Turner, N. Nguyen, R. Raviram, M. Erb, J. Santini, J. Luebeck, U. Rajkumar, Y.R. Diao, B. Li, W.J. Zhang, N. Jameson, M.R. Corces, J.M. Granja, X.Q. Chen, C. Coruh, A. Abnousi, J. Houston, Z. Ye, R. Hu, M. Yu, H. Kim, J.A. Law, R.G.W. Verhaak, M. Hu, F.B. Furnari, H.Y. Chang, B. Ren, V. Bafna, P. S. Mischel, Circular ecDNA promotes accessible chromatin and high oncogene expression, Nature 575 (2019) 699–703.
[148] M. Fenech, M. Kirsch-Volders, A.T. Natarajan, J. Surralles, J.W. Crott, J. Parry, H. Norppa, D.A. Eastmond, J.D. Tucker, P. Thomas, Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells, Mutagenesis 26 (2011) 125–132.
[149] M. Fenech, J.W. Crott, Micronuclei, nucleoplasmic bridges and nuclear buds induced in folic acid deficient human lymphocytes - evidence for breakage-fusion-bridge cycles in the cytokinesis-block micronucleus assay, Mutat Res-Fund Mol M 504 (2002) 131–136.
[150] Y. Oobatake, N. Shimizu, Double-strand breakage in the extrachromosomal double minutes triggers their aggregation in the nucleus, micronucleation, and morphological transformation, Gene Chromosome Cancer 59 (2020) 133–143.
[151] N. Shimizu, N. Itoh, H. Utiyama, G.M. Wahl, Selective entrapment of extrachromosomally amplified DNA by nuclear budding and micronucleation during S phase, JCB (J. Cell Biol.) 140 (1998) 1307–1320.
[152] B.H. Nevaldine, P. Rizwana, P.J. Hahn, Differential sensitivity of double minute chromosomes to hydroxyurea treatment in cultured methotrexate-resistant mouse cells, Mutat Res-Genomics 406 (1999) 55–62.
[153] P. Prochazka, J. Hrabeta, A. Vicha, T. Eckschlager, Expulsion of amplified MYCN from homogenously staining chromosomal regions in neuroblastoma cell lines after cultivation with cisplatin, doxorubicin, hydroxyurea, and vincristine, Cancer Genet. Cytogenet. 196 (2010) 96–104.
[154] R. Narath, I.M. Ambros, A. Kowalska, E. Bozsaky, P. Boukamp, P.F. Ambros, Induction of senescence in MYCN amplified neuroblastoma cell lines by hydroxyurea, Genes Chromosomes Cancer 46 (2007) 130–142.
[155] L.S. Yu, Y. Zhao, C. Quan, W. Ji, J. Zhu, Y. Huang, R.W. Guan, D.L. Sun, Y. Jin, X.N. Meng, C.Y. Zhang, Y. Yu, J. Bai, W.J. Sun, S.B. Fu, Gemcitabine eliminates double minute chromosomes from human ovarian cancer cells, PLoS One 8 (2013) e71988.
[156] A. Valent, J. Benard, B. Clausse, M. Barrois, D. Valteau-Couanet, M.J. Terrier-Lacombe, B. Spengler, A. Bernheim, In vivo elimination of acentric double minutes containing amplified MYCN from neuroblastoma tumor cells through the formation of micronuclei, Am. J. Pathol. 158 (2001) 1579–1584.
[157] N. Shimizu, N. Misaka, K.I. Utani, Nonselective DNA damage induced by a replication inhibitor results in the selective elimination of extrachromosomal double minutes from human cancer cells, Gene Chromosome Cancer 46 (2007) 865–874.
[158] E. Raymond, S. Faivre, G. Weiss, J. McGill, K. Davidson, E. Izbicka, J.G. Kuhn, C. Allred, G.M. Clark, D.D. Von Hoff, Effects of hydroxyurea on extrachromosomal DNA in patients with advanced ovarian carcinomas, Clin. Cancer Res. 7 (2001) 1171–1180.
[159] D.D. Vonhoff, J. Mcgill, K. Davidson, B. Forseth, A.A.E. Elzayat, H. Burris, Preclinical leads for innovative uses for etoposide, Semin. Oncol. 19 (1992) 10–13.
[160] M.A. Wani, R.M. Snapka, Drug-induced loss of unstably amplified genes, Cancer Invest. 8 (1990) 587–593.
[161] H. Shima, M. Nakayasu, S. Aonuma, T. Sugimura, M. Nagao, Loss of the myc gene amplified in human hl-60 cells after treatment with inhibitors of poly(adp-ribose) polymerase or with dimethyl-sulfoxide, P Natl Acad Sci USA 86 (1989) 7442–7445.
[162] R.M. Snapka, Gene amplification as a target for cancer-chemotherapy, Oncology Research 4 (1992) 145–150.
[163] A.M. Sanchez, J.T. Barrett, P.V. Schoenlein, Fractionated ionizing radiation accelerates loss of amplified MDR1 genes harbored by extrachromosomal DNA in tumor cells, Cancer Res. 58 (1998) 3845–3854.
[164] D.S. Neel, T.G. Bivona, Resistance is futile: overcoming resistance to targeted therapies in lung adenocarcinoma, npj Precis. Oncol. 1 (2017).
[165] K.L. Hung, J. Luebeck, S.R. Dehkordi, C. Colón, R. Li, I. Tsz-Lo Wong, C. Coruh, P. Dharanipragada, S.H. Lomeli, N.E. Weiser, G. Moriceau, X. Zhang, C. Bailey, K.E. Houlahan, W.T. Yang, R.C. González, C. Swanton, C. Curtis, M. Jamal-Hanjani, A.G. Henssen, J.A. Law, W.J. Greenleaf, R.S. Lo, P.S. Mischel, V. Bafna, H. Y. Chang, Targeted profiling of human extrachromosomal DNA by CRISPR-CATCH, Nat. Genet. 54 (2022) 1746–1754.
[166] W.T. Chen, Z. Weng, Z. Xie, Y.M. Xie, C. Zhang, Z.C. Chen, F.Y. Ruan, J. Wang, Y.X. Sun, Y.T. Fang, M. Guo, Y.Q. Tong, Y.N. Li, C. Tang, Sequencing of methylase-accessible regions in integral circular extrachromosomal DNA reveals differences in chromatin structure, Epigenet. Chromatin 14 (2021) 40.
[167] V.I. Minina, M.Y. Sinitsky, V.G. Druzhinin, A. Fucic, M.L. Bakanova, A.V. Ryzhkova, Y.A. Savchenko, A.A. Timofeeva, R.A. Titov, E.N. Voronina, V.P. Volobaev, V.A. Titov, Chromosome aberrations in peripheral blood lymphocytes of lung cancer patients exposed to radon and air pollution, Eur. J. Cancer Prev. 27 (2018) 6–12.
[168] M.K. Fath, N. Karimfar, A.F. Naghibi, S. Shafa, M.G. Shiran, M. Ataei, H. Dehghanzadeh, M.N. Afjadi, T. Ghadiri, Z. Payandeh, V. Tarhriz, Revisiting characteristics of oncogenic extrachromosomal DNA as mobile enhancers on neuroblastoma and glioma cancers, Cancer Cell Int. 22 (2022) 200.
[169] S. Nikolaev, F. Santoni, M. Garieri, P. Makrythanasis, E. Falconnet, M. Guipponi, A. Vannier, I. Radovanovic, F. Bena, F. Forestier, K. Schaller, V. Dutoit, V. Clement-Schatlo, P.Y. Dietrich, S.E. Antonarakis, Extrachromosomal driver mutations in glioblastoma and low-grade glioma, Nat. Commun. 5 (2014) 5690.
[170] C. Morales, M.J. Garcia, M. Ribas, R. Miro, M. Munoz, C. Caldas, M.A. Peinado, Dihydrofolate reductase amplification and sensitization to methotrexate of methotrexate-resistant colon cancer cells, Mol. Cancer Therapeut. 8 (2009) 424–432.
[171] P. Hahn, B. Nevaldine, W.F. Morgan, X-ray induction of methotrexate resistance due to dhfr gene amplification, Somat. Cell Mol. Genet. 16 (1990) 413–423.
[172] D. Furrer, S. Jacob, C. Caron, F. Sanschagrin, L. Provencher, C. Diorio, Validation of a new classifier for the automated analysis of the human epidermal growth factor receptor 2 (HER2) gene amplification in breast cancer specimens, Diagn. Pathol. 8 (2013) 17.
[173] J. Liu, Z.J. Zhu, Y.Y. Liu, L.L. Wei, B. Li, F.X. Mao, J. Zhang, Y.C. Wang, Y.F. Liu, MDM2 inhibition-mediated autophagy contributes to the pro-apoptotic effect of berberine in p53-null leukemic cells, Life Sci. (2020) 242.

[174] D.A. Haber, R.T. Schimke, Unstable amplification of an altered dihydrofolate-reductase gene associated with double-minute chromosomes, Cell 26 (1981) 355–362.
[175] R.J. Kaufman, P.C. Brown, R.T. Schimke, Loss and stabilization of amplified dihydrofolate-reductase genes in mouse sarcoma S-180 cell-lines, Mol. Cell Biol. 1 (1981) 1084–1093.
[176] D.D. Vonhoff, J.R. Mcgill, B.J. Forseth, K.K. Davidson, T.P. Bradley, D.R. Vandevanter, G.M. Wahl, Elimination of extrachromosomally amplified myc genes from human tumor-cells reduces their tumorigenicity, P Natl Acad Sci USA 89 (1992) 8165–8169.
[177] M.J. Chen, T. Shimada, A.D. Moulton, A. Cline, R.K. Humphries, J. Maizel, A.W. Nienhuis, The functional human dihydrofolate-reductase gene, J. Biol. Chem. 259 (1984) 3933–3943.
[178] K. Takezawa, V. Pirazzoli, M.E. Arcila, C.A. Nebhan, X.L. Song, E. de Stanchina, K. Ohashi, Y.Y. Janjigian, P.J. Spitzler, M.A. Melnick, G.J. Riely, M.G. Kris, V. A. Miller, M. Ladanyi, K. Politi, W. Pao, HER2 amplification: a potential mechanism of acquired resistance to EGFR inhibition in EGFR-mutant lung cancers that lack the second-site EGFR(T790M) mutation, Cancer Discov. 2 (2012) 922–933.
[179] L. Gianni, T. Pienkowski, Y.H. Im, L. Roman, L.M. Tseng, M.C. Liu, A. Lluch, E. Staroslawska, J. de la Haba-Rodriguez, S.A. Im, J.L. Pedrini, B. Poirier, P. Morandi, V. Semiglazov, V. Srimuninnimit, G. Bianchi, T. Szado, J. Ratnayake, G. Ross, P. Valagussa, Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial, Lancet Oncol. 13 (2012) 25–32.
[180] D.D. Vonhoff, T. Waddelow, B. Forseth, K. Davidson, J. Scott, G. Wahl, Hydroxyurea accelerates loss of extrachromosomally amplified genes from tumor-cells, Cancer Res. 51 (1991) 6273–6279.
[181] Y.H. Xue, L. Martelotto, T. Baslan, A. Vides, M. Solomon, T.T. Mai, N. Chaudhary, G.J. Riely, B.T. Li, K. Scott, F. Cechhi, U. Stierner, K. Chadalavada, E. de Stanchina, S. Schwartz, T. Hembrough, G. Nanjangud, M.F. Berger, J. Nilsson, S.W. Lowe, J. Reis, N. Rosen, P. Lito, An approach to suppress the evolution of resistance in BRAF(V600E)-mutant cancer, Nat. Med. 23 (2017) 929–937.