Chapter

Circular DNA: How Circular DNA Assists Cancer Roll with Therapeutic Punches

Parvaiz Yousuf

Abstract

DNA within cells is either present in the form of long strands as in eukaryotes or circular shapes in Yeast plasmids, mitochondrial DNA, and double minutes in tumor cells. Apart from them, ribosomal or telomeric DNA has been found to produce specialized forms of extrachromosomal circular DNA (eccDNA). eccDNA was discovered in both normal and cancer cells in recent times, indicating a much more significant role. The eccDNA has been found to promote tumor proliferation, survival, and aggressiveness in almost half of all cancers by increasing oncogene copy numbers. This chapter will discuss the biogenesis and function of eccDNA and how it promotes tumor adaption under changing microtumour environmental conditions, as in the case of drugs.

Keywords: DNA, DNA, circular, neoplasms, antineoplastic protocols, oncogenes

1. Introduction

DNA is the basic genetic material present in most living species. It was first discovered by Friedrich Miescher in 1869 [1]. This genetic material exists in the form of chromosomes that consist of linearized double-stranded DNA fused with histone proteins within the nucleus and store most of the genetic information of an organism [2]. However, DNA or genes are not confined to the nucleus only as they can also be found in other extra-nuclear structures. Eukaryotes have circular DNA present within the mitochondria and chloroplast and are structurally similar to the bacterial genome [3, 4]. In addition, other forms of circular DNA also exist within the cytoplasm and nucleus [5]. This DNA has been named eccDNA, and its size varies from a few base pairs to millions of base pairs. On the basis of size and sequence, this form of DNA has further been categorized into 100 bp to 10 kb long small polydispersed DNA (spcDNA), 100 to 400 base pair long micro-DNA, millions of base pairs long extrachromosomal DNA, and telomeric circles or t-circles consisting of base pairs that are multiplies of 738. In addition to eukaryotes, viruses and bacteria also possess circular DNA in different forms with varying lengths [6–15], which are summarized in **Table 1**. In recent years, a lot of studies have been done on this form of DNA that has improved our knowledge. Their biological, physiological properties, as well as

Type of circular DNA	Size	Functions	Refs
Viruses			
ssDNAs	2–6 kb	Role in replication	[6]
Retroviral DNA	7–12 kb	Role in replication	[7]
dsDNA	6–375 kb	Role in replication	[8]
Bacteria			
Plasmids	30–2430 kb	Role in reproduction, drug resistance etc.	[9]
Eukaryotes			
Mitochondrial DNA	16 kb	Maintains mitochondrial function	[10]
MicroDNA	100–400 kb	Produces miRNAs	[11]
Double minute	100 kb–3 Mb	Extrachromosomal gene amplification	[12, 13]
Telomeric circle	Integral multiplies	Restore telomere length of 738 bp	[14]
S small polydispersed circular DNA	100 bp–10 kb	Enhances genomic stability	[15]

Table 1.
Circular DNA is present in several forms among viruses, bacteria and eukaryotes with different size ranges and functions.

their functions, are becoming more noticed. This has led to the discovery of more previously unknown forms of circular DNA [16]. Some researchers believed that DNA exists in rings within the cytoplasm of higher organisms, which was later verified with the eccDNA discovery [17]. However, scientists observed this circular DNA in many other organisms before they were seen present in many heterogeneous cancer cells. For instance, Alix and Yasuo had observed the different lengths of eecDNA in sperm of boar observed under an electron microscope [5]. In many other eukaryotic species, such as humans, Yeast, hamsters, mice, and Drosophila, this form of DNA was observed [18–23]. At the same time, the size of eccDNA present in normal eukaryotic cells has been found to be very small and is usually less than 500 base pairs [20, 22–25]. However, eccDNA of a much bigger size has also been discovered in certain types of tumor cells. After the tumors were surgically removed and mitotic abnormalities were investigated after being stained by Cox, Spriggs, and acetic orcein, small chromatin bodies of the size of an intact chromosome were observed [26]. The name given to these chromosomes was double minutes (DMs) as they were present in pairs. Studies by Sprigg later concluded that such chromatin bodies were present more in malignant childhood brain tumors [27]. These DMs were also spotted in HeLa cells using the buoyant density method [28]. Thus, it is clear that the eccDNA was more commonly found in genetically unstable cells, such as tumor cells, and was not as common in normal cells [29, 30]. Although this DNA has been found to be homologous to the genomic DNA, it is clear that the presence of eccDNA means genome instability. This is why most of the tumors have exceptionally high levels of eccDNA, and the oncogenes usually occur in this genome only. Much research has been conducted to find out how this DNA carries driver oncogenes and contributes to tumor resistance and heterogeneity. The levels of eccDNA are much more common in tumor cells than was earlier thought. It makes tumors highly resistant to the

therapeutic drugs by increasing the oncogene copy number and heterogeneity. This chapter explains in detail the eccDNA discovery in tumors and how it will shape the therapeutic operations for the treatment of varied cancer types.

2. eccDNA: from hypothetical existence to role in cancer in eukaryotes

The eccDNA was once thought to play some secondary roles in both prokaryotes and eukaryotes. However, with the advent of technology, it became clear that eccDNA plays more pivotal functions in eukaryotes than ever thought. It is estimated that over half of all tumor types have eccDNA, enabling them to develop resistance against different therapeutic drugs.

2.1 Historical perspective

The eccDNA was discovered by Yasuo Hoota and Alix Bassel [5]. They were actually investigating a theory proposed by Franklin Stahl in 1964 that higher organisms' chromosomes consist of DNA rings [5]. Their experiments lead to the discovery of DNA circles of varying sizes that range from 100s to 1000s of bp's resembling DNA of higher organisms. Double minutes (DM'S) was the name given to these large circles of DNA. Later on, scientists began investigating the presence of eecDNA in other cell types. For instance, another group of researchers discovered the DMs in human tumor cells by preparing karyotypes and utilizing purification by CsCl gradience [26, 28]. The DNA from many other organisms was studied using EM imaging techniques and CsCl gradient purification [22, 31–36]. The researcher used the technique of Southern Blotting to determine the homology between genomic DNA and eccDNA. Most of the eccDNA observed was less than 500 bps in size and is called poly-disperse circular DNA (spcDNA) [24, 37]. Moreover, most of the DNA obtained came from repetitive sequences of DNA, while some of the spcDNA molecules fused with particular sequences. Even some researchers observed that 9–11 base pair long direct repeats flank few non-repetitive spcDNA sequences on both sides [19–21]. This indicated that DNA circles were formed by certain DNA repair pathways, such as microhomology-mediated end joining or homologous recombination, which mediate the joining of ends in between small base repeats. However, many later studies proved that no repetitive sequences are needed to mediate this end-to-end joining. This occurred when eccDNA with specific sequences was isolated and sequenced with no repetitive areas within or flank the DNA [38]. Around the same time period, another group of researchers utilized exonuclease III for quantification of eccDNA and concluded that there occur varying levels of eccDNA among mice tissues [39]. As far as the formation of eccDNA is concerned, many groups used techniques studying eccDNA from repetitive sequences to determine their formation. 70 times increase in eccDNA formation occurred in murine cells when Cycloheximide (a protein synthesis inhibitor) was used. The same results were obtained with many other chemicals, such as hydroxyurea (DNA replication inhibitor, and 7,1-dimethylbenz[a]anthracene (a carcinogen) [20]. Similarly, a higher concentration of longer eccDNA molecules was observed in cells obtained from patients suffering from Fanconi Anemia (wherein particular DNA repair pathway errors occur) [30]. Afterwards, smaller eccDNA molecules were explored upon the usage of 2-dimensional gel electrophoresis, which showed that eccDNA levels are increased by carcinogens, and different stages of development show varied eecDNA formation in flies and frogs. It has been

found that even the foreign DNA can result in the formation of eccDNA and DNA sequence organization within tandem repeats prepared eccDNA preparation from the DNA [40–42]. In a nutshell, it suggests that the formation of eccDNA depends on DNA organization, DNA damage repair, and the sequence of DNA. All this indicates how eecDNA has the tendency to code for driver oncogenes as eccDNA are amplified in terms of oncogenes leading to drug resistance in most cancers [43–45].

2.2 Recent advances in eccDNA

The eecDNA is not well understood, and researchers have been trying to understand it better. Human cancer and mouse tissue cells were lyzed, and eccDNA was isolated to recently sequence the entire length of an eccDNA compliment. This was done using a paired-end high sequencing technology that allowed the characterization of this form of DNA [46]. The junctional sequences of this eccDNA were identified through high-end sequencing technology on exonuclease-resistant eecDNA that was amplified using a rolling circle mechanism [46]. After these studies, it was found that all human cancer and mouse tissue cells showed consistent patterns with specific eccDNA sizes peaking around 180 and 380 base pairs. Around 5% of these molecules extended in the range of 2–3 kilobase pairs. The term given to this DNA was microDNA. However, the study may have resulted in the under-representation of long eccDNA molecules as small DNA circles are amplified more efficiently than large-sized circles. Moreover, most of the eccDNA circles were smaller, as represented by electron microscopy [47]. After mapping this eccDNA, many bases extended to over 100–1000 specific sites with the genome. It also showed enrichment in some particular regions, transcriptionally active chromatin, hotspots that include CpG regions and UTR regions, and DNA patches with high GC content (around 60%) [46]. Most microDNA is flanked by the genomic DNA containing 2–15 base pair repeats, suggesting a micro-homology mode generates DNA circles [46]. The sites where eccDNA is formed might be associated with cell lineage, which is indicated by weak clustering of eccDNA in the ovarian and prostate cell lines. Other studies claimed that if MSH3, which encodes a peptide in the DNA mismatch repair pathway, is deleted, it results in an 80% decrease in the eccDNA levels [47]. Moreover, there is no clear proof of whether this small eccDNA replicates or not. However, there have been rough estimates of electron microscopy about the eccDNA abundance prepared from a specific number of cells, indicating that 125–200 circles of eccDNA exist per DT40 cell [47]. Similarly, another study was performed in *Saccharomyces cerevisiae,* where researchers found 1/4th of the Saccharomyces being covered with about 2000 DNA circles. Moreover, this study ignored smaller eccDNA (less than 1-kilo base pair in size), and there was no dependency on the junctional sequence identification to label a particular DNA sequence as eccDNA. Therefore, the eccDNA was 1–38 kilobase pairs long, which were significantly enriched with circles from repeated genome parts, such as gene duplications, ribosomal DNA circles, and transposons. This suggests that circles were formed in a homologous recombination form. Furthermore, specific sequences were the precursors of around 60% eccDNA, and seven base pair direct repeats were revealed by over 90% of the genomic sites. This means that DNA circles are in a microhomology-directed mechanism.

The mechanism responsible for eccDNA formation is yet to be understood clearly. However, researchers have found that tandemly repeated genomic sequences are present in eccDNA [30, 48, 49]. It indicates that eccDNA formation primarily occurs from such tandemly repetitive DNA sequences [48]. At the same time, nonrepetitive

DNA also results in eccDNA formation. For instance, a group of researchers isolated eccDNA from HeLa S3 cells and found the presence of eccDNA [28]. May it be coding or non-coding regions, eccDNAs originate from both. Many studies concluded DMs bear drug-resistant oncogenes and another oncogene [50]. Different researchers have proposed four distinct models of how eccDNA formation occurs. The first one is the translocation-deletion-amplification model, in which genes' rearrangements occur near the chromosome's translocation site [51]. The fragments closer to the translocation breakpoints can be deleted, amplified, and circularized, which results in eccDNA formation [52, 53]. The second model-Chromothripsis model, explains that multiple acentric DNA fragments can form due to chromosome shattering, with some of the fragments self-ligating to form eccDNAs [54, 55]. The third model is the breakage-fusion-bridge (BFB) model [56]. Losing a telomere by a chromosome is what initiates the BFB cycle. Two chromatids are formed during anaphase after chromosome duplicates. Afterwards, the fusion between broken chromosome ends (chromatids) occurs, which results in the formation of a dicentric chromosome [57]. A bridge is formed between these two chromatids during anaphase due to the presence of two centromeres that are disrupted when two centromeres are directed to opposite poles of the spindle: The chromosome breakage and uneven distribution of genetic material results in the centromeres being segregated into daughter cells [58]. Thus, one of the daughter cells has a chromosome with an inverted repetitive base sequence on its ends, while the other has a chromosome with deletion at the ends. When DNA replication continues in the next cycle, the fusion of sister chromatids occurs once again, resulting in the repetition of the BFB cycle. All the events result in DNA sequence amplification at the telomeres that eventually loops out to form eccDNAs [59]. The fourth model is the episome model, which explains that excision of small circular DNA results in episome formation, which eventually results in recombination or over-replication, resulting in eccDNA formation [34, 60]. It is well known that eccDNAs can lead to mutations, amplifications, translocations, deletions, etc. In human somatic cells, certain loci that include HLA, DAZ4, and KIR have been found to be more prone to circular product formation, resulting in chromosomal deletions [61]. Similarly, in a study performed on Yeast, identification of 1756 eccDNAs was made, which covered 23% of the total Yeast genome [62]. This means it is highly likely for an oncogene to be present on eccDNA. Similarly, the presence or absence of DMs is a vital factor to consider as it relates to the clinical outcomes of the patients. For example, some oncogenes, such as oncogene Sei-1, induce DM formation [63]. Similarly, another study found that oncogene Met's higher amplification and expression occurs when present on DMs. The promotion of Sei-1 induced DM generation results because of this Met signaling cascade pathway. Moreover, in the patients' ovarian cancer (OC) cells, a greater copy number amplification of eukaryotic initiation factor 5A2 (EIF5A2) was found by way of DMs, indicating the role of DMs in carrying and sustaining oncogenes [64].

Recently, a group of researchers studied human cell lines and *Caenorhabditis elegans* by using a mechanism that relied on Cesium Chloride- Ethidium Bromide (density gradient centrifugation) and high-throughput sequencing and tagmentation [65]. They reported circles that mapped on non-coding and coding genomic regions. The protein-coding regions of the DNA encoding titin and mucin lead to the appearance of eccDNA in these cases. The study concluded that the eccDNA promotes and interferes with the particular exon transcription, thereby leading to the expression of various isoforms of a genetic code [46]. In cancerous cells, the eccDNA has been found to play complex roles to promote tumourigenesis. The changes in DMs,

eccDNAs between normal and cancerous cells have been quantified by fluorescent microscopy [12, 66]. The amplification of myc and EGFR genes in tumor cells occurred through a few passages by eccDNA formation [66]. Similarly, another group of researchers combined fluorescence imaging with software analyzing images for quantification of certain oncogene copies. There was a 40% amplification of EGFR and MYC gene in the examined human cancer cells, whereas normal cells showed no enrichment [12]. What surprised researchers were that more oncogene amplification occurred through a mechanism involving eccDNA formation rather than a mechanism involving chromosomal amplification [12]. The detection of eccDNA within the normal tissues may be primarily because of having no procedures helping circle enrichment and not enough dye binds to small microDNA (eccDNA) for microscopic detection. All of these studies prove solid evidence that tumor heterogeneity is a result of eccDNA, wherein they also assist cancer cells in evolving through an increase in oncogene copy number [47]. The validation of these studies was done by another group of researchers that found MET (an oncogene) in glioblastoma cells showed amplification on eccDNA as was indicated by FISH [67].

3. Extrachromosomal oncogene amplification drives tumor evolution and genetic heterogeneity

Human tumors are highly heterogeneous, and they evolve and adapt in quickly changing microenvironments from individual cells to a mass of genetically heterogeneous cells. Only those cells are selected by the Darwinian selection, which adapts quickly to their environments. Tumor heterogeneity offers a mutation pool from which tumor-friendly mutations are selected [68–72]. The progression of neoplasm and resistance to therapeutic drugs is driven when mutations are passed on to daughter cells after cells have acquired the mutations enhancing fitness. For instance, one of the usual mutations of cancer cells is oncogene amplification, which is either present on chromosomal DNA or eccDNA parts that include DMs too [60, 73, 74]. Compared to the chromosomal DNA, the eccDNA does not have high stability and segregates to daughter cells in an unequal fashion (**Figure 1**). As per the Mitelman database, 1.4% of cancers possess DMs, with neuroblastoma showing a maximum of 31.7% DMs [75]. However, no accurate quantification of the eccDNA has been done in the cancer cells, as there has been no systemic examination of oncogenes present in this eccDNA. At the same time, how eccDNA impacts tumor cell evolution is yet to be understood well. Although we can sequence DNA in an unbiased manner to analyze the cancer genes, the spatial resolution of amplicons is not possible yet to determine the specific regions of chromosomes of eccDNA. Moreover, DNA circularity can potentially be inferred by using bioinformatic analysis [76], but eccDNA amplicons may show variations from cell to cell. This means there has been a great underestimation of oncogene amplification related to eccDNA. Although by cytogenetically analyzing the cancer cell metaphases, the localization of amplicons can be done, there is always some bias associated with this technique. Recently, a group of researchers quantified the eccDNA spectrum in human tumor cells and systemically interrogated the contents by integrating the Whole Genome Sequencing (WGS) of 117 human tumor cell lines, varied tumor tissues from various cancer types, and cancer cell cultures derived from patients [12]. The researchers analyzed 2049 metaphases from around 72 samples of cancer cells bioinformatically and cytogenetically. In addition, they analyzed a total of 233 metaphases from eight normal tissue cultures and 290 metaphases from 10 immortal

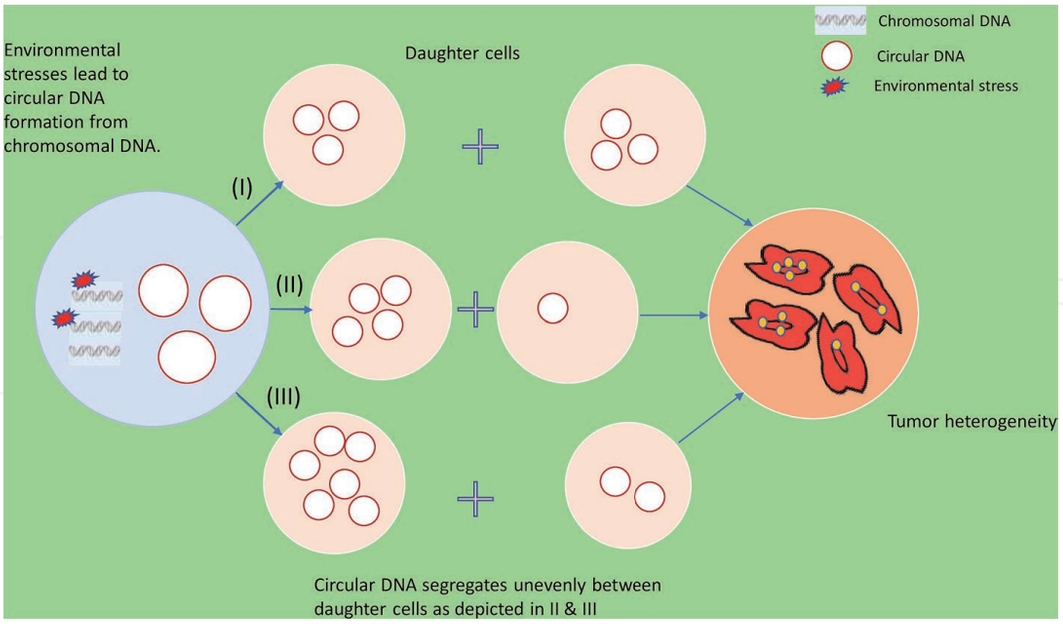

Figure 1.
Environmental stresses lead to the formation of circular DNA from chromosomal DNA, which can be (I) equally distributed or (II and III) unequally distributed as in most of the cases, thereby increasing heterogeneity of oncogenes when present.

cancer cell cultures with a sum of 2572 metaphases. The eccDNA is detected by the DAPI, 4′, 6-diamidino-2-phenylindole (a fluorescent dye), which FISH and genomic DNA probes confirm. As expected, they found that eccDNA is present abundantly in cancer cell samples and was seldom present in normal cells. This shows eccDNA may have a closer association with the tumor cells than earlier thought. Moreover, almost 30% of the eccDNAs were found to be paired DMs [12]. However, it is necessary to mention that different tumors showed different levels of eccDNA, and the levels were much higher in the cultures obtained from patients. In two of 20 metaphases, the conservative metric of two eccDNAs was used, and approximately 40% of cancer cell lines and 90% of brain tumor models from patients were found to possess eccDNA [12]. There is no association between levels of eccDNA and either treated versus untreated samples, metastatic versus primary status, tumors not irradiated versus tumors that were irradiated. Moreover, with respect to the size of samples taken, it is tougher to determine the effect of different therapies on eccDNA levels undertaking the various treatment options available. There is a great variation of eccDNA number within this tumor cell culture between cells. Thus, it is confirmed that the levels of eccDNA in cancer cells are very common; however, there may be variations from cell to cell. At the same time, the eccDNA levels in normal tissues are quite rare. Using Whole Genome Sequencing, focal amplifications were revealed from cancer cell lines of different types that included amplified oncogenes defined earlier from 13 varied types of tumors [77]. Surprisingly, most cancer oncogenes are present on eccDNA only and other Homologous Staining regions of the chromosomes. Moreover, mRNA transcripts are high expression levels within the oncogenes present on eccDNA. At the same time, the diversity in the copy number of oncogenes present on eccDNA is much greater than the diversity of the copy number of genes presents on other chromosomes (**Figure 2**) [12].

Researchers have studied the origin of both intra- and extra-chromosomal structures and try to determine whether they originate from the same or different

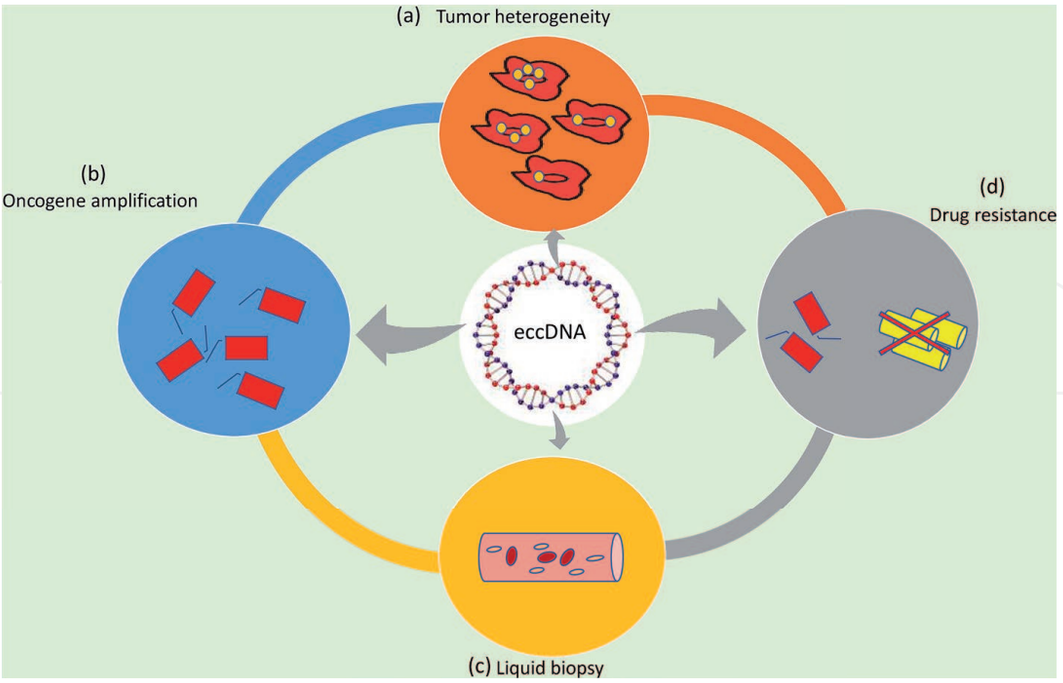

Figure 2.
EccDNA plays complex roles in a TME which include (a) tumor heterogeneity: Tumor cells shown in red receive an unequal number of circular DNA molecules shown as yellow dots. (b) Oncogene amplification: Increase in a number of a particular oncogene as depicted by red-tailed blocks (c) liquid biopsy: A test used to detect cancer by measuring circulating eccDNA levels as shown by dark-red ovals (d) inducing drug resistance: Oncogenes develop resistance to drugs depicted as yellow cylinders.

precursors. The relationship that exists between amplicon and sub-nuclear regions was understood by taking advantage of GBM39 subclone cells, which occur spontaneously and in which EGFRvIIIs high copies shift from eccDNA to Homogeneous Staining Regions [12]. The eccDNA amplicon on separate GBM39 replicates showed a circle-shaped structure with about 1.29 Megabases that contained 1 EGFRvIII copy. Surprisingly, the GBM39 subclone, which harbored EGFRvIII on homogeneous staining regions, showed a similar structure with tandem duplications containing multiple EGFRvIII copies. This indicates that EGFRvIII, which contained eeCDNA structures, integrated to form Heterogeneous Staining Regions. On this eeCDNA, the reversible loss of EGFRvIII leads to resistance in GBM39 cells to the inhibitors of EGFR tyrosine kinase [78]. The EGFRvIII amplicon that contains eccDNA is conserved in the naïve cells as indicated by the structural analysis of these structures, which indicates that eccDNA maintains its primary structural properties by relocating to HSRs on chromosomes [13, 60].

Now, whether is the localization of eccDNA is beneficial or not is yet to be studied. However, a hypothesis about the same exists, which postulates that eccDNA amplification may be responsible for helping an oncogene get a high copy number. This way, tumor genes are able to replicate and start forming products necessary for the overall survival and growth of a tumor. This hypothesis may be true as eccDNA containing oncogenes is unequally segregated to the tumor cells due to intrachromosomal amplification [79]. Moreover, experiments have been carried out using a branching process (Simplified Galton-Watson) to explain how tumors evolve. In these experiments, either replication or death of a cell in the current generation occurs to build the upcoming generation. Considering some assumptions, they found that independent segregation of the eccDNA copies into the two daughter cells occurs during the process of cell

division. Moreover, the same study confirmed that oncogene favors the development and replication of an oncogene in a better way. To strengthen and prove this point, the researchers have found a greater copy number of oncogenes c-MYC and EGFR in the eccDNA rather than in the chromosomes. Moreover, the intra-chromosomal amplification of an oncogene results in the stabilization of heterogeneity of a tumor at a lower level. In comparison, the eccDNA is unequally segregated, resulting in greater tumor heterogeneity and maintaining it [80, 81]. Also, the copy number of an oncogene may increase the tumor heterogeneity but is likely to do so quite slowly if present on a chromosome than if the same oncogene is present on an eccDNA molecule. Moreover, there is strong evidence that it is quite difficult to cure genetically heterogeneous tumors [80]. The tumor cells have the ability to maintain the oncogene transcriptional level and copy number variability from cell to cell, which results in resistance to drugs and the progression of tumors. The worst thing about eccDNA is that the oncogene amplification in eccDNA enables the tumor adaptability more effectively, helping it survive tough microenvironment conditions. This is further done because of an increase in the chances that a particular cell population expresses a specific oncogene at such a level that considerably increases the survival and proliferation of a tumor [60, 78, 82–85]. This worsens the situation even more as it becomes immensely difficult to treat such tumors after they become progressively aggressive. Thus, the extra and intrachromosomal amplification mechanism varies greatly, leading to high copy number heterogeneity and greater amplicons copy number. It means that even if oncogenes present on eccDNA confer a little selection advantage increase, it will still lead to a greater advantage of fitness for a tumor. Thus, as far as the evolution of a tumor is concerned, there is certainly a great role for oncogene present on eccDNA to play as they are linked to tumor heterogeneity and greater survival. This does not happen when the same gene (even if) is present on a chromosome. Thus, it is of immense importance and vitality to understand how a tumor evolves on a molecular level and how oncogene amplification occurs in eccDNA. This will help the scientific community to effectively treat tumors by either preventing their progression or successfully eradicating them.

When it comes to detecting eccDNA, the circulating levels of this DNA aid in the noninvasive diagnosis of tumors, thereby helping manage them. This becomes possible because tumors and normal tissues have the property of releasing eccDNA into circulation. The eccDNA has been observed in the mammalian tissue nuclei and other cell lines. However, researchers are demonstrating that eccDNA with unique mapping regions are detectable in serum and plasma obtained from both humans and mice [85]. Moreover, the eccDNA obtained from the serum and plasma has longer lengths with over 250 base pairs than the one found in cells (around 150 base pairs). Researchers have detected human microDNA in mouse circulation after transferring human cancer lines. Moreover, when microDNA from normal cells and tumor cells was compared, it revealed that longer micro-DNA occurred in the tumor cells. Moreover, researchers even collected the micrDNA samples from cancer patients before and after the surgery and found that longer and higher concentrations of microDNA are released into circulation when the tumor is there and shorter when the tumor is excised. This indicates that circular DNA is not confined to cells (cytoplasm) only but can be thought of as everywhere in the presence of a tumor [86].

3.1 Functional importance of eccDNA with emphasis on tumors

The eccDNA performs a varied number of functions within and around the cells. In tumor presence, a person shows a higher concentration of circular DNA

in and around the cells, indicating an ever-thought greater role. Although insufficient research has been performed on the eccDNA and its functions in tumors, they certainly have been found to contribute to genetic heterogeneity in tumor cells. They are responsible for oncogene amplification, a hallmark of many cancer types, thereby inducing drug resistance. Thus, a particular tumor is more likely to express an oncogene on the eccDNA, providing it with a suitable environment for the proliferation, progression, and metastasis of a tumor. Apart from this, numerous theoretical functions of eccDNA have been reported, including the gene dosage compensation, heterogeneity among cells, transcription factor sponging. Moreover, they have a predominant role in producing a mutational pool of DNA that helps tumors evolve, a role in intercellular communication, aging, stimulating certain innate immune pathways, and uses in the technique of liquid biopsy. Numerous researchers have studied the role of eccDNA in tumors and unequivocally revealed the role of eccDNA in tumors and inducing drug resistance. For instance, several drug-resistant genes and oncogenes are carried on DMs, which leads to the advancement of cancer by the phenomenon of amplification of genes [13, 87–89]. With more advanced research being done in the field, it was revealed that it is much more common for eccDNA to mediate amplification of genes that thought earlier. Thus, the presence or absence of eccDNA is vital for the evolution and heterogeneity of tumors. For instance, around 40% of the cancer cell lines and around 90% of brain tumor cell lines from patients showed eccDNA presence [41]. Apart from this, researchers have provided experimental and mathematical evidence suggesting that heterogeneity among tumors and amplification of driver oncogenes are much greater upon amplification on eccDNA than when the same happens on chromosomal loci. Furthermore, an immense proliferation advantage is acquired when eccDNA is distributed randomly to the daughter cells. A greater eccDNA copy number with an oncogene can be inherited [12]. Tumor cells engage in great adaptive mechanisms that help cancer cells survive in whatsoever conditions. For example, with respect to environmental conditions, the particular eccDNA number in tumors is changed, thereby helping tumor cells adapt with a different mechanism. This has been found to occur in glioblastomas, wherein mutation of EGFR is common, which results in the development of an oncogenic EGFRvIII variant. Tumor cell proliferation and growth are promoted by EGFRvIII, which also increases the sensitization of cancer cells to tyrosine kinase inhibitors [78]. Moreover, when cells lose DMs that carry mutated EGFR, they become resistant to the EGFR tyrosine kinase inhibitors [78]. It is also believed that extrachromosomal driver mutations are possible that occur when eccDNAs amplify, which proves vital for helping tumors evolve [81]. Thus, it can be said that the presence of eccDNA occurs more commonly in tumors resulting in adaption, heterogeneity of tumors, and thus their evolution (**Table 2**).

Apart from these functions, eccDNA has a role in aging, which is directly or indirectly linked to the tumors. In Yeast, the accumulation of eccDNAs containing ribosomal RNA genes occurs, thereby contributing to Yeast cell aging [88]. These ribosomal DNAs can replicate because of the presence of an Autonomously replicating sequence (ARS) sequence [88]. Furthermore, they show a tendency to transfer to mother cells during each cell division that leads to a higher accumulation of eccDNAs in aging mother cells. The lifespan of the daughter cell is prolonged with this lesser eccDNA concentration. However, researchers do not know the exact mechanism of how senescence is triggered by the eccDNAs, eventually leading to the mortality of aging cells. But it is presumed that it affects the replication and transcription mechanism by titrating different components involved [90]. The phenomenon that

Properties	Chromosomal DNA	Extrachromosomal Circular DNA	References
Size	Much larger in size	Smaller	[24, 26, 37]
Stability	Highly stable	Not stable	[75]
Segregation	Segregates equally to daughter cells	Segregates unequally to daughter cells	[12]
Oncogene presence rate	Less number of oncogenes/ base pair	High number of oncogenes/ base pair	[12, 80]
Heterogeneity	Lesser	Higher	[80]
Oncogene amplification	Lesser	Higher	[12]
Effect on heterogeneity	Overcomes heterogeneity	Maintains heterogeneity	[80]
Oncogene survival	Lesser	Higher	[81]
Mutations	Tumour unfriendly	Tumour unfriendly	[81]
Occurrence	No significant difference between normal and cancer tissues	Occurs more commonly in cancer tissues	[12]
Harm	Oncogenes are less harmful, if present	Oncogenes are much harmful when present on eccDNA	[12]
Drug resistance	Lesser	Higher	[12]
Tumour cell diversity	Lesser	Higher	[12]
Tumour growth	Lower growth rate	Higher growth rate	[12]

Table 2.
Difference between chromosomal DNA and extrachromosomal circular DNA in promoting tumour survival and aggressiveness.

eccDNAs occur abundantly in these cells may be responsible for senescence and mortality of aging cells. Considering this hypothesis, expressing ARS plasmid at an abnormal place is enough to arrest aging cells, which eventually leads to their death [90]. Since eccDNAs have been found to accumulate in normal cells, too, the discoveries have led scientists to scratch their heads whether accumulating levels of eccDNA cause aging in other higher eukaryotes or not. Besides relating to aging in Yeast, eccDNA has a similar role in mammals [90–92]. The concentrations of eccDNA in cells serve as an index of aging, as was found in senescence-resistant SAM-R mice, where they showed a higher amplification [93]. Another study supported this finding in which a stronger ERC load resulted due to sgs1 gene mutation, which associates with reduced life span and premature aging [90]. Another study studied the eccDNA replication in the cell cycle, where the concentrations increased in an exponential manner. This may be a sort of clock determining the yeast life span. In Yeast, the rise in the mortality rates also correlates with the increase in ERC numbers [90]. Some other studies focused on the relationship between the formation of eccDNA and transcriptional activity. It was found that certain genes that are sensitive to environmental stimuli are transcriptionally stimulated, which triggers protein-coding eccDNA generation in aging budding yeasts [94]. Similarly, under aging conditions, the structural characteristics of eccDNA were studied in cultured human lung fibroblasts and

rat lymphocytes. The results showed a greater size of eccDNA, and high dispersion with the eccDNA almost doubles in number [91]. This means that the accumulation of eccDNA in tumors is primarily because of the upregulation of the synthesis rather than the downregulation of degradation.

In addition, other roles of eccDNA may also exist, such as gene compensation [95]. For instance, in Yeast two pairs of genes-HTA1-HTB1 and HTA2-HTB2 encode histones H2A and H2B, respectively. Upon deletion of HTA1-HTAB1, HTA2-HTB2 genes are amplified through dosage compensation by forming a naïve eccDNA. This eccDNA contains 39 kilobase pairs of the 2nd chromosome, including a centromere, HTA2-HTB2, H3-H4 locus of histones, and several origins of replication [95]. The creation of this naïve eccDNA occurs when two Ty1 retrotransposon elements recombine, flanking this portion [95]. Thus, the compensation for the H2A and H2B decrease occurs through elevation in HTA2-HTB2 eccDNA formation in these deleted strains of HTA1-HTB1. In comparison to DMs, the smaller types of eccDNAs occur more commonly, but we do not know much about them yet [46, 84]. Their size is little to have genes encoding proteins but larger enough to encode parts of genes or short RNAs with regulatory functions. Moreover, microDNAs have the property of acting as molecular sponges; indirect gene expression occurs when they sponge different transcription factors. More recently, it became clear that the microDNAs may occur in plasma and serum of both human beings and mice as circulating DNA [84]. In the technique or liquid biopsy, they may act as potential biomarkers. A novel cell communication mechanism may exist if microDNAs are present in other cells. This may be just a theoretical assumption right now but may open new fields of investigation in the future. Another important query to solve is how this eccDNA survives autoimmune pathways in the cytoplasm. If the naked DNA is present in the cell cytoplasm, then the cGAS pathway is activated, which stimulates the immune system through interferon expression [96, 97]. This is how our bodies respond to any foreign antigen entering our bodies and vital parts of the immune system. EccDNAs release may be stimulated during mitosis, which either leads to their degradation by TREX1 like enzymes or activation of cGAS pathways. Thus, it can be said that the eccDNAs are usual endogenous antigens if not having chromatin protection, which leads to activation of several autoimmune pathways.

3.2 Clinical utility

Tumors possess specific characteristics that help in their prognosis and identification. One of them is the presence of eccDNA, which may be an essential tool for tumor prognosis. However, having said that, normal tissues also release eccDNAs; thus, it becomes pivotal to identify these differences between normal and cancerous tissues. One of the ways to do so may be identifying the eccDNA length. For instance, research indicates that tumor cells of human origin show longer eccDNA molecules than those found in the normal mouse cell lines [46]. Similarly, both normal and cancerous lung tissues showed the presence of microDNA, with both the types showing the same known properties of eccDNA [84]. However, the eccDNA was removed and analyzed from human patients who have lung cancer, and the length of eccDNA was measured. It was found that eccDNA from the same patient shows variations in length, with tumor eccDNA larger in size than the normal cell eccDNA [84]. This property will certainly help researchers to differentiate cancerous and normal tissue and might prove a good biomarker. This will be true if eccDNA from normal and tumor cells show a predictable behavior. At the same time, many researchers have focused on

liquid biopsy, which used high-throughput sequencing technology for the identification of linearized DNA fragments specific to tumors in the plasma or serum [78]. This discovery has rather been more recent [84]. Moreover, the microDNA obtained from mice, mice tissues, human tumors, and chicken showed the same characteristics as that of the microDNA obtained from circulation. These properties include distribution in genome, higher GC content, direct repeats that are 2–15 bases long and flank the source genomic sites, etc. Moreover, it is an intergenic and genic region that gives rise to eccDNA. As mentioned, the microDNA obtained from lung cancers was of greater length than microDNA from normal tissue of the same person. The surgery also affects the eccDNA length, as observed in some studies [84]. Researchers found longer circular DNA levels in patients prior to surgery and smaller lengths post-surgery (6 weeks after surgical resection) [84]. This indicates the stability of eccDNAs in comparison to linear DNA and thus may be advantageous to use eccDNA for the purpose of liquid biopsy.

4. Conclusion

The eccDNA has a great role in the evolution of cancer as driver oncogene amplification contributes to heterogeneity of tumors and resistance to drugs. What makes the condition worse is that driver oncogenes present on eccDNA prove to be more harmful than in chromosomal DNA. The frequency of eccDNA has been much more common than was earlier thought, helping tumors evolve and become resistant to various drugs. The latest research conducted on the topic also found that the presence of eccDNA in nearly all tumors is much more common, providing immunity against therapeutic drugs [12]. The team explored the eccDNA presence after analyzing cell lines from 17 different tumor types. This was done by taking metaphase chromosomes from 2572 dividing cells whose ECdetect (a software package for conducting unbiased analysis and detecting eccDNA) was developed to study structural and functional properties. They concluded that almost half of the human cancers showed eccDNA presence. Moreover, eccDNA aids in better driver oncogene amplification than the chromosomal DNA, thereby accelerating transcript level [12]. Importantly, it is clear that eccDNA has a much greater role in promoting drug resistance, diversity, and growth of the tumor cells than the same genes located on chromosomal loci. This explains how the evolution and diversification of cancers occur. Moreover, around 90% of the tumors (patient-derived) models show eccDNA presence, meaning that eccDNA is more likely to harbor cancer oncogenes than chromosomes. The copy number of an oncogene and intratumoural heterogeneity is increased more effectively by the eccDNA amplification as predicted by mathematical models [12]. Moreover, a growing number of studies are showing the role of eccDNAs in harboring proto-oncogenes. The eccDNA is not only offering them a suitable environment to sustain themselves but also making them resistant to numerous drugs. Different genes show a link with different cancers, such as DHFR gene with colon, cervical and breast cancer, CA125 gene with ovarian cancer, MDR1 gene with oral squamous cell carcinoma (OSCC), a c-Myc gene with colon cancer and leukemia, HER2 gene with breast cancer, MDM2 and EGFRvIII with glioblastoma, as depicted in **Table 3** [78, 98–106]. Thus, tumors maintain their high gene number and heterogeneity more easily with the help of eccDNA. Moreover, the distribution of eccDNA to the daughter cells occurs randomly, meaning that a tumor may have either whole or no eccDNA. This increases the variation in the copy number of oncogenes, which ultimately makes the tumor more heterogeneous in terms of

Genes	Cancer type	References
DHFR	Colon cancer, cervical cancer, breast cancer	[98–101]
CA125	Ovarian cancer	[102]
MDR1	OSCC	[103]
c-Myc	Colon cancer, leukaemia	[104, 105]
HER2	Breast cancer	[106]
MDM2	Glioblastoma	[78]
EGFRvIII	Glioblastoma	[78]

Table 3.
Some common oncogenes that are contained on eccDNAs.

resistance to any kind of environmental changes, like due to drugs. The more common discovery of eccDNA in different cancer types is surprising as researchers have been focusing more on which genes cause cancer rather than where these genes occur. Although some cancer biologists reported the eccDNA presence as early as the 1960s, the tools to quantify eccDNA were lacking. More studies are demanding to know the exact mechanisms of eccDNA formation and maintenance and how the Tumor Microenvironment changes eccDNA levels by altering its composition.

Conflict of interest

The authors declare no conflict of interest.

Other declarations/Notes

None.

Abbreviations

ARS	autonomously replicating sequence
DM	double minutes
dsDNA	double-stranded deoxyribonucleic acid
eccDNA	extrachromosomal circular DNA
EGFR	epidermal growth factor receptor
EIF5A2	eukaryotic translation initiation factor 5A2
FISH	fluorescence in situ hybridization
HER2	human epidermal growth factor receptor 2
MDM2	mouse double minute 2 homolog
MDR1	multidrug resistance 1
OC	ovarian cancer
OSCC	oral squamous cell carcinoma
SB	Southern Blotting
ssDNA	single-stranded deoxyribonucleic acid
UTR	untranslated regions
WGS	whole-genome sequencing

Circular DNA: How Circular DNA Assists Cancer Roll with Therapeutic Punches
DOI: http://dx.doi.org/10.5772/intechopen.102687

Author details

Parvaiz Yousuf
Department of Zoology, Central University of Kashmir, Ganderbal,
Jammu and Kashmir, India

*Address all correspondence to: saleemparvaiz444@gmail.com

References

[1] Dahm R. Discovering DNA: Friedrich Miescher and the early years of nucleic acid research. Human Genetics. 2008;**122**(6):565-581. DOI: 10.1007/s00439-007-0433-0

[2] WATSON JD, CRICK FH. Genetical implications of the structure of deoxyribonucleic acid. Nature. 1953;**171**(4361):964-967. DOI: 10.1038/171964b0

[3] Saccone C, Gissi C, Lanave C, Larizza A, Pesole G, Reyes A. Evolution of the mitochondrial genetic system: An overview. Gene. 2000;**261**(1):153-159. DOI: 10.1016/s0378-1119(00)00484-4

[4] Sakamoto W, Takami T. Chloroplast DNA dynamics: Copy number, quality control and degradation. Plant & Cell Physiology. 2018;**59**(6):1120-1127. DOI: 10.1093/pcp/pcy084

[5] Hotta Y, Bassel A. Molecular size and circularity of dna in cells of mammals and higher plants. Proceedings of the National Academy of Sciences of the United States of America. 1965;**53**(2):356-362. DOI: 10.1073/pnas.53.2.356

[6] Martin DP, Biagini P, Lefeuvre P, Golden M, Roumagnac P, Varsani A. Recombination in eukaryotic single stranded DNA viruses. Viruses. 2011;**3**(9):1699-1738. DOI: 10.3390/v3091699 Epub 2011 Sep 13

[7] Hindmarsh P, Leis J. Retroviral DNA integration. Microbiology and Molecular Biology Reviews . 1999;63(4):836-843, table of contents. DOI: 10.1128/MMBR.63.4.836-843.1999

[8] Smith GL, Vaccinia virus, Brian WJ Mahy, Marc HV Van Regenmortel. Encyclopedia of Virology (3rd). USA Academic Press. 2008, 243-250. 9780123744104. 10.1016/B978-012374410-4.00525-2

[9] Drlica K, Gennaro ML, Plasmids. Sydney Brenner, Jefferey H. Miller, Encyclopedia of Genetics, Academic Press, USA 2001. 1485-1490. 9780122270802, 10.1006/rwgn.2001.1000

[10] Taanman JW. The mitochondrial genome: Structure, transcription, translation and replication. Biochimica et Biophysica Acta. 1999;**1410**:103-123

[11] Paulsen T, Shibata Y, Kumar P, Dillon L, Dutta A. Small extrachromosomal circular DNAs, microDNA, produce short regulatory RNAs that suppress gene expression independent of canonical promoters. Nucleic Acids Research. 2019;**47**:4586-4596

[12] Turner KM, Deshpande V, Beyter D, Koga T, Rusert J, Lee C, et al. Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. Nature. 2017;**543**(7643):122-125. DOI: 10.1038/nature21356

[13] Storlazzi CT et al. Gene amplification as DMs or homogeneously staining regions in solid tumours: Origin and structure. Genome Research. 2010;**20**:1198-1206

[14] Tomaska L, Nosek J, Kramara J, Griffith JD. Telomeric circles: Universal players in telomere maintenance? Nature Structural & Molecular Biology. 2009;**16**:1010-1015

[15] Cohen S, Regev A, Lavi S. Small polydispersed circular DNA (spcDNA) in

human cells: Association with genomic instability. Oncogene. 1997;**14**:977-985

[16] Koche RP, Rodriguez-Fos E, Helmsauer K, Burkert M, MacArthur IC, Maag J, et al. Extrachromosomal circular DNA drives oncogenic genome remodeling in neuroblastoma. Nature Genetics. 2020;**52**(1):29-34. DOI: 10.1038/s41588-019-0547-z

[17] Stahl F. A chain model for chromosomes. Journal de Chimie Physique. 1961;**58**:1072-1077

[18] Møller HD, Parsons L, Jørgensen TS, Botstein D, Regenberg B. Extrachromosomal circular DNA is common in yeast. Proceedings of the National Academy of Sciences of the United States of America. 2015 Jun;**112**(24):E3114-E3122. DOI: 10.1073/pnas.1508825112

[19] Stanfield SW, Lengyel JA. Small circular DNA of Drosophila melanogaster: chromosomal homology and kinetic complexity. Proceedings of the National Academy of Sciences of the USA. 1979;**76**(12):6142-6146. doi: 10.1073/pnas.76.12.6142

[20] Sunnerhagen P, Sjöberg RM, Karlsson AL, Lundh L, Bjursell G. Molecular cloning and characterization of small polydisperse circular DNA from mouse 3T6 cells. Nucleic Acids Research. 1986;**14**(20):7823-7838. DOI: 10.1093/nar/14.20.7823

[21] Stanfield SW, Helinski DR. Cloning and characterization of small circular DNA from Chinese hamster ovary cells. Molecular and Cellular Biology. 1984;**4**(1):173-180. DOI: 10.1128/mcb.4.1.173-180

[22] Smith CA, Vinograd J. Small polydisperse circular DNA of HeLa cells. Journal of Molecular Biology. 1972 Aug;**69**(2):163-178. DOI: 10.1016/0022-2836(72)90222-7

[23] Motejlek K, Assum G, Krone W, Kleinschmidt AK. The size of small polydisperse circular DNA (spcDNA) in angiofibroma-derived cell cultures from patients with tuberous sclerosis (TSC) differs from that in fibroblasts. Human Genetics. 1991;**87**(1):6-10. DOI: 10.1007/BF01213083

[24] Motejlek K, Schindler D, Assum G, Krone W. Increased amount and contour length distribution of small polydisperse circular DNA (spcDNA) in Fanconi anemia. Mutation Research. 1993;**293**(3):205-214. DOI: 10.1016/0921-8777(93)90071-n

[25] Neidlinger C, Assum G, Krone W, Dietrich C, Hochsattel R, Klotz G. Increased amounts of small polydisperse circular DNA (spcDNA) in angiofibroma-derived cell cultures from patients with tuberous sclerosis (TS). Human Genetics. 1988;**79**(3):286-288. DOI: 10.1007/BF00366254

[26] Cox D, Yuncken C, AI S. Minute chromatin bodies in malignant tumours of childhood. Lancet. 1965;**1**(7402):55-58. DOI: 10.1016/s0140-6736(65)90131-5

[27] Spriggs AI, Boddington MM, Clarke CM. Chromosomes of human cancer cells. British Medical Journal. 1962;**2**(5317):1431-1435. DOI: 10.1136/bmj.2.5317.1431

[28] Radloff R, Bauer W, Vinograd J. A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA: The closed circular DNA in HeLa cells. Proceedings of the National Academy of Sciences of the United States of America. 1967;**57**(5):1514-1521. DOI: 10.1073/pnas.57.5.1514

[29] Paulsen T, Kumar R, Koseoglu M.M, Dutta A, Discoveries

of extrachromosomal circles of DNA in Normal and tumour cells, Trends in Genetics. 2018;**34**(4);270-278

[30] Cohen S, Lavi S. Induction of circles of heterogeneous sizes in carcinogen-treated cells: Two-dimensional gel analysis of circular DNA molecules. Molecular and Cellular Biology. 1996;**16**(5):2002-2014. DOI: 10.1128/MCB.16.5.2002

[31] Agsteribbe E, Kroon AM, van Bruggen EF. Circular DNA from mitochondria of neurospora crassa. Biochimica et Biophysica Acta. 1972;**269**(2):299-303. DOI: 10.1016/0005-2787(72)90439-x

[32] Billheimer FE, Avers CJ. Nuclear and mitochondrial DNA from wild-type and petite yeast: Circularity, length, and buoyant density. Proceedings of the National Academy of Sciences of the United States of America. 1969;**64**(2):739-746. DOI: 10.1073/pnas.64.2.739

[33] Buongiorno-Nardelli M, Amaldi F, Lava-Sanchez PA. Electron microscope analysis of amplifying ribosomal DNA from xenopus laevis. Experimental Cell Research. 1976;**98**(1):95-103. DOI: 10.1016/0014-4827(76)90467-5

[34] Ono T, Ozeki Y, Okubo S, Inoki S. Characterization of nuclear and satellite DNA from trypanosomes. Biken Journal. 1971;**14**(3):203-215

[35] Stanfield S, Helinski DR. Small circular DNA in Drosophila melanogaster. Cell. Oct 1976;**9**(2):333-345. DOI: 10.1016/0092-8674(76)90123-9

[36] Wong FY, Wildman SG. Simple procedure for isolation of satellite DNAs from tobacco leaves in high yield and demonstration of minicircles. Biochimica et Biophysica Acta. 1972;**259**(1):5-12. DOI: 10.1016/0005-2787(72)90468-6

[37] Bertelsen AH, Humayun MZ, Karfopoulos SG, Rush MG. Molecular characterization of small polydisperse circular deoxyribonucleic acid from an African green monkey cell line. Biochemistry. 1982;**21**(9):2076-2085. DOI: 10.1021/bi00538a015

[38] Van Loon N, Miller D, Murnane JP. Formation of extrachromosomal circular DNA in HeLa cells by nonhomologous recombination. Nucleic Acids Research. 1994;**22**(13):2447-2452. DOI: 10.1093/nar/22.13.2447

[39] Gaubatz JW, Flores SC. Purification of eucaryotic extrachromosomal circular DNAs using exonuclease III. Analytical Biochemistry. 1 Feb 1990;**184**(2):305-310. DOI: 10.1016/0003-2697(90)90685-3

[40] Cohen S, Mechali M. A novel cell-free system reveals a mechanism of circular DNA formation from tandem repeats. Nucleic Acids Research. 2001;**29**(12):2542-2548. DOI: 10.1093/nar/29.12.2542

[41] Cohen S, Agmon N, Sobol O, Segal D. Extrachromosomal circles of satellite repeats and 5S ribosomal DNA in human cells. Mobile DNA. 2010;**1**(1):11. DOI: 10.1186/1759-8753-1-11

[42] Navrátilová A, Koblízková A, Macas J. Survey of extrachromosomal circular DNA derived from plant satellite repeats. BMC Plant Biology. 2008;**8**:90. DOI: 10.1186/1471-2229-8-90

[43] Beland JL, Longo JA, Hahn PJ. CpG island mapping of a mouse double-minute chromosome. Molecular and Cellular Biology. 1993;**13**(8):4459-4464. DOI: 10.1128/mcb.13.8.4459-4464.1993

[44] Carroll SM, Gaudray P, De Rose ML, Emery JF, Meinkoth JL, Nakkim E, et al. Characterization of an episome produced in hamster cells that amplify a

transfected CAD gene at high frequency: Functional evidence for a mammalian replication origin. Molecular and Cellular Biology. 1987;**7**(5):1740-1750. DOI: 10.1128/mcb.7.5.1740-1750.1987

[45] Ståhl F, Wettergren Y, Levan G. Amplicon structure in multidrug-resistant murine cells: A nonrearranged region of genomic DNA corresponding to large circular DNA. Molecular and Cellular Biology. 1992;**12**(3):1179-1187. DOI: 10.1128/mcb.12.3.1179-1187.1992

[46] Shibata Y, Kumar P, Layer R, Willcox S, Gagan JR, Griffith JD, et al. Extrachromosomal microDNAs and chromosomal microdeletions in normal tissues. Science. 2012;**336**(6077):82-86. DOI: 10.1126/science.1213307 Epub 2012 Mar 8. Erratum in: Science. 2012 Jun 22;336(6088):1506

[47] Dillon LW, Kumar P, Shibata Y, Wang YH, Willcox S, Griffith JD, et al. Production of extrachromosomal MicroDNAs is linked to mismatch repair pathways and transcriptional activity. Cell Reports. 2015;**11**(11):1749-1759. DOI: 10.1016/j.celrep.2015.05.020

[48] Cohen S, Yacobi K, Segal D. Extrachromosomal circular DNA of tandemly repeated genomic sequences in drosophila. Genome Research. 2003;**13**:1133-1145

[49] Cohen S, Houben A, Segal D. Extrachromosomal circular DNA derived from tandemly repeated genomic sequences in plants. The Plant Journal. 2008;**53**:1027-1034

[50] Zhu J, Yu Y, Meng X, Fan Y, Zhang Y, Zhou C, et al. De novo-generated small palindromes are characteristic of amplicon boundary junction of double minutes. International Journal of Cancer. 2013;**133**:797-806

[51] Barr FG, Nauta LE, Davis RJ, Schafer BW, Nycum LM, Biegel JA. In vivo amplification of the PAX3-FKHR and PAX7-FKHR fusion genes in alveolar rhabdomyosarcoma. Human Molecular Genetics. 1996;**5**:15-21

[52] Rodley P, McDonald M, Price B, Fright R, Morris C. Comparative genomic hybridization reveals previously undescribed amplifications and deletions in the chronic myeloid leukemia-derived K-562 cell line. Genes, Chromosomes & Cancer. 1997;**19**:36-42

[53] Van Roy N, Vandesompele J, Menten B, Nilsson H, De Smet E, Rocchi M, et al. Translocation-excision-deletion-amplification mechanism leading to nonsyntenic coamplification of MYC and ATBF1. Genes, Chromosomes & Cancer. 2006;**45**:107-117

[54] Korbel JO, Campbell PJ. Criteria for inference of chromothripsis in cancer genomes. Cell. 2013;**152**:1226-1236

[55] Zhang CZ, Spektor A, Cornils H, Francis JM, Jackson EK, Liu S, et al. Chromothripsis from DNA damage in micronuclei. Nature. 2015;**522**:179-184

[56] Mc CB. Chromosome organization and genic expression. Cold Spring Harbor Symposia on Quantitative Biology. 1951;**16**:13-47

[57] Vukovic B, Beheshti B, Park P, Lim G, Bayani J, Zielenska M, et al. Correlating breakage-fusion-bridge events with the overall chromosomal instability and in vitro karyotype evolution in prostate cancer. Cytogenetic and Genome Research. 2007;**116**:1-11

[58] Zakov S, Kinsella M, Bafna V. An algorithmic approach for breakage-fusionbridge detection in tumor genomes. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**:5546-5551

[59] Murnane JP, Sabatier L. Chromosome rearrangements resulting from telomere dysfunction and their role in cancer. BioEssays. 2004;**26**:1164-1174

[60] Carroll SM, DeRose ML, Gaudray P, Moore CM, Needham-Vandevanter DR, Von Hoff DD, et al. Double minute chromosomes can be produced from precursors derived from a chromosomal deletion. Molecular and Cellular Biology. 1988;**8**(4):1525-1533. DOI: 10.1128/mcb.8.4.1525-1533.1988

[61] Moller HD, Mohiyuddin M, Prada-Luengo I, Sailani MR, Halling JF, Plomgaard P, et al. Circular DNA elements of chromosomal origin are common in healthy human somatic tissue. Nature Communications. 2018;**9**:1069

[62] Moller HD, Parsons L, Jorgensen TS, Botstein D, Regenberg B. Extrachromosomal circular DNA is common in yeast. Proceedings of the National Academy of Sciences of the United States of America. 2015;**112**:E3114-E3122

[63] Bao Y, Liu J, You J, Wu D, Yu Y, Liu C, et al. Met promotes the formation of double minute chromosomes induced by Sei-1 in NIH-3T3 murine fibroblasts. Oncotarget. 2016;**7**:56664-56675

[64] Guan XY, Fung JM, Ma NF, Lau SH, Tai LS, Xie D, et al. Oncogenic role of eIF-5A2 in the development of ovarian cancer. Cancer Research. 2004;**64**:4197-4200

[65] Shoura MJ, Gabdank I, Hansen L, Merker J, Gotlib J, Levene SD, et al. Intricate and Cell Type-Specific Populations of Endogenous Circular DNA (eccDNA) in Caenorhabditis elegans and Homo sapiens. G3 (Bethesda). 2017;**7**(10):3295-3303. DOI: 10.1534/g3.117.300141

[66] Vogt N, Gibaud A, Lemoine F, de la Grange P, Debatisse M, Malfoy B. Amplicon rearrangements during the extrachromosomal and intrachromosomal amplification process in a glioma. Nucleic Acids Research. 2014;**42**(21):13194-13205. DOI: 10.1093/nar/gku1101

[67] deCarvalho AC, Kim H, Poisson LM, et al. Discordant inheritance of chromosomal and extrachromosomal DNA elements contributes to dynamic disease evolution in glioblastoma. Nat Genet. 2018;**50**:708-717. DOI: 10.1038/s41588-018-0105-0

[68] Nowell PC. The clonal evolution of tumour cell populations. Science. 1976;**194**(4260):23-28. DOI: 10.1126/science.959840

[69] McGranahan N, Swanton C. Biological and therapeutic impact of intratumour heterogeneity in cancer evolution. Cancer Cell. 2015;**27**:15-26

[70] Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: A looking glass for cancer? Nature Reviews. Cancer. 2012;**12**:323-334

[71] Yates LR, Campbell PJ. Evolution of the cancer genome. Nature Reviews. Genetics. 2012;**13**:795-806

[72] Greaves M, Maley CC. Clonal evolution in cancer. Nature. 2012;**481**:306-313

[73] Von Hoff DD, Needham-VanDevanter DR, Yucel J, Windle BE, Wahl GM. Amplified human MYC oncogenes localized to replicating submicroscopic circular DNA molecules. Proceedings of the National Academy of Sciences of the United States of America. 1988;**85**:4804-4808

[74] Garsed DW, Marshall OJ, Corbin VD, Hsu A, Di Stefano L, Schröder J,

et al. The architecture and evolution of cancer neochromosomes. Cancer Cell. 2014;**26**(5):653-667. DOI: 10.1016/j.ccell.2014.09.010

[75] Mitelman F, Johansson B, Mertens F. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer. The Lund University; 2016. Available from: https://www.clinicalgenetics.lu.se/division-clinical-genetics/database-chromosome-aberrations-and-gene-fusions-cancer

[76] Sanborn JZ et al. Double minute chromosomes in glioblastoma multiforme are revealed by precise reconstruction of oncogenic amplicons. Cancer Research. 2013;**73**:6036-6045

[77] Zack TI et al. Pan-cancer patterns of somatic copy number alteration. Nature Genetics. 2013;**45**:1134-1140

[78] Nathanson DA, Gini B, Mottahedeh J, Visnyei K, Koga T, Gomez G, et al. Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA. Science. 2014 Jan 3;**343**(6166):72-76. DOI: 10.1126/science.1241328

[79] Windle B, Draper BW, Yin YX, O'Gorman S, Wahl GM. A central role for chromosome breakage in gene amplification, deletion formation, and amplicon integration. Genes & Development. 1991;**5**:160-174

[80] Andor N et al. Pan-cancer analysis of the extent and consequences of intratumour heterogeneity. Nature Medicine. 2016;**22**:105-113

[81] Li X et al. Temporal and spatial evolution of somatic chromosomal alterations: A case-cohort study of Barrett's esophagus. Cancer Prevention Research (Philadelphia, Pa.). 2014;**7**:114-127

[82] Mishra S, Whetstine JR. Different facets of copy number changes: Permanent, transient, and adaptive. Molecular and Cellular Biology. 2016;**36**:1050-1063

[83] Schimke RT, Kaufman RJ, Alt FW, Kellems RF. Gene amplification and drug resistance in cultured murine cells. Science. 1978;**202**:1051-1055

[84] Nikolaev S et al. Extrachromosomal driver mutations in glioblastoma and low-grade glioma. Nature Communications. 2014;**5**:5690

[85] Biedler JL, Schrecker AW, Hutchison DJ. Selection of chromosomal variant in amethopterinresistant sublines of leukemia L1210 with increased levels of dihydrofolate reductase. Journal of the National Cancer Institute. 1963;**31**:575-601

[86] Kumar P, Dillon LW, Shibata Y, Jazaeri AA, Jones DR, Dutta A. Normal and cancerous tissues release extrachromosomal circular DNA (eccDNA) into the circulation. Molecular Cancer Research: MCR. 2017;**15**(9):1197-1205

[87] Vogt N et al. Molecular structure of double-minute chromosomes bearing amplified copies of the epidermal growth factor receptor gene in gliomas. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(31):11368-11373

[88] Zuberi L et al. Rapid response to induction in a case of acute promyelocytic leukemia with MYC amplification on double minutes at diagnosis. Cancer Genetics and Cytogenetics. 2010;**198**(2):170-172

[89] Del Rey J, Prat E, Ponsa I, Lloreta J, Gelabert A, Algaba F, et al. Centrosome clustering and cyclin D1 gene amplification in double minutes are common events in chromosomal unstable bladder tumours. BMC Cancer. 2010;**10**:280. DOI: 10.1186/1471-2407-10-280

[90] Sinclair DA, Guarente L. Extrachromosomal rDNA circles--a cause of aging in yeast. Cell. 1997;**91**(7):1033-1042

[91] Kunisada T, Yamagishi H, Ogita Z, Kirakawa T, Mitsui Y. Appearance of extrachromosomal circular DNAs during in vivo and in vitro ageing of mammalian cells. Mechanisms of Ageing and Development. 1985;**29**:89-99

[92] Gaubatz JW, Flores SC. Tissue-specific and age-related variations in repetitive sequences of mouse extrachromosomal circular DNAs. Mutation Research. 1990;**237**:29-36

[93] Yamagishi H, Kunisada T, Takeda T. Amplification of extrachromosomal small circular DNAs in a murine model of accelerated senescence. A brief note. Mechanisms of Ageing and Development. 1985;**29**:101-103

[94] Hull RM, King M, Pizza G, Krueger F, Vergara X, Houseley J. Transcription-induced formation of extrachromosomal DNA during yeast ageing. PLoS Biology. 2019;**17**:e3000471

[95] Libuda DE, Winston F. Amplification of histone genes by circular chromosome formation in Saccharomyces cerevisiae. Nature. 2006;**443**(7114):1003-1007

[96] Mackenzie KJ, Carroll P, Martin CA, Murina O, Fluteau A, Simpson DJ, et al. cGAS surveillance of micronuclei links genome instability to innate immunity. Nature. 2017 Aug 24;**548**(7668):461-465. DOI: 10.1038/nature23449

[97] de Oliveira Mann CC, Kranzusch PJ. cGAS conducts micronuclei DNA surveillance. Trends in Cell Biology. 2017

[98] Meng X, Qi X, Guo H, Cai M, Li C, Zhu J, et al. Novel role for nonhomologous end joining in the formation of double minutes in methotrexate-resistant colon cancer cells. Journal of Medical Genetics. 2015;**52**(2):135-144

[99] Cai M, Zhang H, Hou L, Gao W, Song Y, Cui X, et al. Inhibiting homologous recombination decreases extrachromosomal amplification but has no effect on intrachromosomal amplification in methotrexate-resistant colon cancer cells. International Journal of Cancer. 2019;**144**(5):1037-1048

[100] Ruiz-Herrera A, Smirnova A, Khoriauli L, Nergadze SG, Mondello C, Giulotto E. Gene amplification in human cells knocked down for RAD54. Genome Integrity. 2011;**2**(1):5

[101] Hahn P, Nevaldine B, Morgan WF. X-ray induction of methotrexate resistance due to dhfr gene amplification. Somatic Cell and Molecular Genetics. 1990;**16**(5):413-423

[102] Zhang CY, Feng YX, Yu Y, Sun WJ, Bai J, Chen F, et al. The molecular mechanism of resistance to methotrexate in mouse methotrexate-resistant cells by cancer drug resistance and metabolism SuperArray. Basic & Clinical Pharmacology & Toxicology. 2006;**99**(2):141-145

[103] Von Hoff DD, Waddelow T, Forseth B, Davidson K, Scott J, Wahl G. Hydroxyurea accelerates loss of extrachromosomally amplified genes from tumor cells. Cancer Research. 1991;**51**(23 Pt 1):6273-6279

[104] Shimizu N, Itoh N, Utiyama H, Wahl GM. Selective entrapment of

extrachromosomally amplified DNA by nuclear budding and micronucleation during S phase. The Journal of Cell Biology. 1998;**140**(6):1307-1320

[105] Eckhardt SG, Dai A, Davidson KK, Forseth BJ, Wahl GM, Von Hoff DD. Induction of differentiation in HL60 cells by the reduction of extrachromosomally amplified c-myc. Proceedings of the National Academy of Sciences of the United States of America. 1994;**91**(14):6674-6678

[106] Vicario R, Peg V, Morancho B, Zacarias-Fluck M, Zhang J, MartinezBarriocanal A, et al. Patterns of HER2 gene amplification and response to anti-HER2 therapies. PLoS One. 2015;**10**(6):e0129876