frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 30 January 2023
DOI 10.3389/fonc.2022.1092705

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Oncology

RECEIVED 08 November 2022
ACCEPTED 28 December 2022
PUBLISHED 30 January 2023

CITATION
Luo J, Li Y, Zhang T, Xv T, Chen C, Li M, Qiu Q, Song Y and Wan S (2023) Extrachromosomal circular DNA in cancer drug resistance and its potential clinical implications.
Front. Oncol. 12:1092705.
doi: 10.3389/fonc.2022.1092705

Extrachromosomal circular DNA in cancer drug resistance and its potential clinical implications

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Chemotherapy is widely used to treat patients with cancer. However, resistance to chemotherapeutic drugs remains a major clinical concern. The mechanisms of cancer drug resistance are extremely complex and involve such factors such as genomic instability, DNA repair, and chromothripsis. A recently emerging area of interest is extrachromosomal circular DNA (eccDNA), which forms owing to genomic instability and chromothripsis. eccDNA exists widely in physiologically healthy individuals but also arises during tumorigenesis and/or treatment as a drug resistance mechanism. In this review, we summarize the recent progress in research regarding the role of eccDNA in the development of cancer drug resistance as well as the mechanisms thereof. Furthermore, we discuss the clinical applications of eccDNA and propose some novel strategies for characterizing drug-resistant biomarkers and developing potential targeted cancer therapies.

KEYWORDS

cancer genetics, extrachromosomal circular DNA, drug resistance, chromothripsis, genomic instability

1 Introduction

Cancer is the leading cause of death in China, and chemotherapy is one of the main treatments for cancer patients (1, 2). However, cancer patients frequently develop resistance to chemotherapy during treatment. Drug resistance as one of the main reasons for clinical chemotherapy failure is defined as the decline of drug effects during treatment (3). It can be categorized as intrinsic resistance and acquired resistance (4). Intrinsic resistance predates treatment and refers to the ability of a population of cells within a treatment-naive cancer to survive initial therapy due to a preexisting genetic alteration or cell state, whereas acquired

resistance develops by the acquisition of new mutations, metabolic adaptations, and epigenetic changes in the original cancer (5). The drug resistance in most cancer cells belongs to acquired drug resistance, which is gradually produced in the process of chemotherapy. As a result, the curative effect becomes worse and most cancer patients have no available chemotherapeutics in their late-stage treatments. Therefore, it is one of the most urgent problems to be solved in treating malignant tumors. There are many mechanisms that are involved in the drug resistance of cancer cells, including genomic instability, DNA repair, chromothripsis, and drug-target mutations (3, 6, 7). These factors involve a series of genetic changes, and the formation of extrachromosomal circular DNA (eccDNA) is closely related to these genetic factors (5, 8). Recently, more and more studies have suggested that eccDNA is involved in the resistance to cancer treatment (9, 10).

eccDNA refers to a type of single-stranded or double-stranded circular DNA that originates from but is likely independent of chromosomes, and its size varies from hundreds of base pairs (bp) to several megabases (Mb) (11). According to different sizes and sequences, eccDNA is further categorized into microDNA (100–400 bp), small poly-dispersed DNA (spcDNA) (100 bp–10 kb), telomeric circles (t-circles) (multiples of 738 bp), and the largest extrachromosomal DNA (ecDNA) (millions of bp). These DNA molecules can carry oncogenic driver genes or increase the copy number of genes to regulate cancer growth and drug resistance (11). Some microDNA may be transcribed into some functional small regulatory RNAs, including microRNAs and novel small interfering RNAs, which can mediate cancer development or cancer drug resistance by regulating gene expression patterns (12, 13). The generation of spcDNA is closely related to genomic instability, which can cause genetic variation on a genome-wide scale (14). These variations will give cancer cells the advantage of clonal growth and genetic evolution and could ultimately cause tumorigenesis. The t-circle can effectively lengthen telomeres through rolling circle amplification and play an important role in alternative lengthening of telomeres (ALT), affecting cancer cell proliferation and cell-cycle progression (15, 16). The large ecDNA, also known as the double minute chromosome (DM), can directly encode some drug-resistant genes or increase the copy number of genes to regulate drug resistance. Moreover, most studies suggested that the large-size eccDNA, termed as ecDNA, is involved in drug resistance, instead of the small-size eccDNA. Therefore, in this review article, we will summarize the role of ecDNA in cancer drug resistance and its molecular mechanism. Furthermore, we also propose its clinical applications, which will provide new strategies for screening drug-resistant biomarkers to improve targeted therapy effects.

2 The role of eccDNA in cancer drug resistance

In recent years, with the rise of high-throughput sequencing technology, the structure and genetic characteristics of eccDNA have been gradually revealed. eccDNAs have been found in most cancers, and their role in the drug resistance of many cancers has been widely explored (17). The roles and identification methods of eccDNA in the drug-resistant process of various cancers are summarized in Table 1 by cancer types.

TABLE 1 Summary of eccDNA in chemotherapy drug resistance by cancer types.

Cancer	Host genes	Drugs	Samples	Detection methods	Reference
Glioblastoma	EGFRvIII	Erlotinib	GBM39 cell	Single-cell analyses	(18)
Glioblastoma	MDM2	Erlotinib	GBM cell	FISH, PCR, and Southern blot	(18)
Glioblastoma	ABCG2	Mitoxantrone	SF295 cells	FISH	(19)
Neuroblastoma	MYCN	–	NB tumor biopsy tissue	FISH and qPCR	(20)
Cervical carcinoma	DHFR	Methotrexate	HeLa S3 cell	FISH and *in situ* Hi-C sequencing	(7)
Cervical carcinoma	DHFR	Methotrexate	HeLa cell	FISH	(21)
Colon cancer	DHFR	Methotrexate	HT29 cell	RT-PCR and FISH	(22)
Human epidermal carcinoma	MDR1	Vinblastine	KB-V1 cell	Electrophoresis of DNAs and gamma-irradiation	(23)
Human epidermal carcinoma	MDR1, MDR2	Colchicine	KB carcinoma cell	Southern blot, Giemsa staining, and pulsed-field gel electrophoresis	(24)
Hypopharyngeal squamous cell carcinoma	RAB3B	Cisplatin	FaDu cell	Circle-seq	(25)
Oral squamous cell carcinoma	MDR1	Vinblastine	KB cell	DNA electrophoresis and DNA probe	(26)
Small-cell lung carcinoma	DHFR	Methotrexate	NCI-H249P,NCI-H187 cell	Dot blot hybridization	(27)
Choriocarcinoma	DHFR	Methotrexate	HCCM and CC1 cell	Giemsa staining and Southern blot	(28)
Breast cancer	DHFR	Methotrexate	EMT-6 cell	Pulsed-field gel electrophoresis	(29)
chronic myelogenous leukemia	DHFR	Methotrexate	HAP1 cell	CRISPR-C; ddPCR	(30, 31)

2.1 The eccDNA in glioblastoma

Glioblastoma (GBM) is the most common and malignant primary brain cancer in adults with a poor prognosis and high risk of chemotherapy resistance (32). So far, the epidermal growth factor receptor (EGFR) and ATP-binding cassette subfamily G member 2 (ABCG2) have been reported frequently as drug resistance-related genes that are carried by ecDNA in GBM.

EGFR deletions and point mutations are often found in GBM, of which 50% have EGFR gene amplification in ecDNA, but 30%–60% of EGFR genes are mutated, and the most common mutation is EGFRvIII (33). EGFRvIII activates the NF-κB (nuclear factor κB) pathway and increases the aggressiveness of GBM, and cancer cells expressing EGFRvIII are more sensitive to EGFR tyrosine kinase inhibitors (TKIs) (34, 35). Nathanson et al. (18) used erlotinib to treat GBM-loading mice; 80% of the mice had a reduction in cancers, but cancer cells changed from predominantly high EGFRvIII expression to low EGFRvIII expression, accompanied by a decrease in drug sensitivity. The loss of ecDNA with EGFRvIII in erlotinib resistance was specific, as these cells still contained abundant ecDNA that carry other genes, such as murine double minute 2 (MDM2). In addition, they also found that MDM2 gene amplification was also associated with drug resistance during the study through fluorescence *in situ* hybridization (FISH) and polymerase chain reaction (PCR). After erlotinib treatment, the copy number of ecDNA with the MDM2 gene was increased and remained elevated, even after drug withdrawal.

The ABCG2 gene is located on chromosome 4, and the protein it encodes can efficiently transport a variety of chemotherapeutic drugs (36). Rao et al. (19) detected DM carrying ABCG2 gene amplification in the SF295 MX50 and MX100 sublines. They generated these sublines by exposing SF295 cells belonging to GBM to mitoxantrone. Interestingly, with the increase in mitoxantrone concentration, fewer DMs were observed, but homogeneously staining regions (HSR) that carried ABCG2 gene amplicons were visible through FISH. Obviously, amplification of ABCG2 occurred initially in the form of DM, followed by chromosomal reintegration of the amplicon at multiple sites and producing stable genotypes associated with drug resistance.

2.2 The eccDNA in neuroblastoma

Neuroblastoma is the most common solid extracranial neoplasm in children, showing an appreciable heterogeneity in clinical evolution. Amplification of the MYCN oncogene in this cancer is detected in 20–30% of cases and is associated with non-effective chemotherapy (37, 38). Through FISH and quantitative PCR analyses, Valent et al. (20) found that the MYCN oncogene can be amplified by ecDNA, especially in patients with advanced neuroblastoma who were resistant to chemotherapy. The MRP gene encodes special transmembrane glycoproteins, which can act as plasma membrane drug-efflux pumps, discharge drugs from the cells, eliminate the accumulation of drugs in cells, and make cancer cells acquire tolerance to varieties of drugs (39, 40). In neuroblastoma, the MYCN gene can be amplified with the help of ecDNA, which increased the expression of this gene and then upregulated the expression of multidrug resistance genes, resulting in enhanced resistance. Therefore, reducing the expression of MYCN may avoid the occurrence of chemotherapy resistance. It was reported that hydroxyurea induced the overexpression of MDR1 in cells to reduce the expression of extrachromosomal MYCN (41, 42), which provides a treatment target for the high-risk neuroblastoma in clinical settings.

2.3 The eccDNA in cervical carcinoma

Cervical carcinoma is the most common female reproductive system cancer in developing countries (43). Chemotherapy is considered as the standard treatment for patients with advanced or recurrent cervical cancer. Resistance to chemotherapy substantially affects the efficacy of cervical cancer treatment. Michael et al. (44) used FISH to analyze cervical cancer cells with methotrexate (MTX) resistance and found that all cells could amplify the dihydrofolate reductase (DHFR) gene *via* DMs. In addition, they found that chromosome 5 fragmentation events could form HSR with the DHFR gene. HSR could break again and produce fragments because of its instability, then produce DMs. Then, in HeLa cells, it was also confirmed that the defects in homologous recombination (HR) could play a role in the amplification of extrachromosomal DNA elements. HR-deficient cell lines had a significantly higher frequency of gene amplification, and the clone frequency of all MTX-resistant cells was higher than that of HeLa parental cells (21). Recently, Shoshani et al. (7) also performed whole-genome sequencing of clonal isolates developing MTX resistance, and the results further identified chromothripsis as a major driver of DM amplification in DMs and proved the amplification of DHFR genes in DMs, which enabled HeLa cells to rapidly acquire tolerance to altered growth conditions.

2.4 The eccDNA in colon cancer

Morales et al. (45) studied the resistance of colon cancer HT29 cells to MTX and the dynamic process of DHFR amplification. They characterized the DHFR genome region at the cytogenetic and molecular levels in HT29 cells treated with increasing doses of MTX. HSRs were the main form of DHFR amplification in the process of increasing the dose of MTX. DMs with the DHFR gene appear in large numbers only after the cells have been exposed to higher doses of drugs for 3 months, and cancer cell resistance to MTX also increases. HT29 cells' resistance was reduced after withdrawal of the drug, and the sensitivity of these cells to MTX was restored. At the same time, the DM carrying the DHFR gene also disappeared. Some studies showed that the homologous recombination activity of MTX-resistant cells containing DM was increased compared with MTX-sensitive cells. With the silence of the key player BRCA1 in the HR pathway, the attenuation of HR activity decreased the number of DMs and DM-form-amplified gene copies (such as DHFR, ZFYVE16, and MSH3) and increased the exclusion of micronuclei and nuclear buds that contained DM-form amplification, which were accompanied by increased MTX sensitivity (46). Similar studies had also reported that non-homologous end joining (NHEJ) decreased MTX resistance and

cell proliferation in MTX-resistant colon cancer cells, which was related to blocking of the generation of DM and the exclusion of DHFR. Therefore, the DNA repair pathway might represent a novel target to reverse drug resistance and improve therapeutic outcome by eliminating extrachromosomal amplification in cancer (22).

2.5 The eccDNA in human epidermal carcinoma

In KB cells from human epidermoid carcinoma, the amplified multidrug resistance (MDR) genes were contained in DM molecules; cells were then treated with colchicine to analyze MDR amplification events. As the concentration of colchicine increased, circular DNAs (890 kb) harboring MDR dimerized to large DM structures (1,780 kb) by intramolecular homologous recombination, which then dimerized to form the larger DMs (3,560 kb). Their studies revealed that the dimerization of circular amplicons was the crucial mechanism for DM generation and MDR gene amplification. Colchicine exposure also induced the mutation of the MDR1 gene. The mutated MDR gene residing on an extrachromosomal DNA element underwent random segregation at mitosis and then enhanced drug resistance to cancer cells in KB cells (24). Joseph et al. (23) also revealed that DM molecules contained the amplified MDR1 genes. Although there were few DMs in each cell, there was a >100-fold amplification of the MDR1 gene. MDR1 overexpression results in cross resistance to a variety of lipophilic compounds, including anthracene-clines (e.g., doxorubicin) and vinca alkaloids (e.g., vinblastine). In addition, Sanchez et al. (47) emphasized that fractionated ionizing radiation obviously reduced the extrachromosomal copy number of MDR1 in KB cancer cells, and this decrease was accompanied by a reduction in multidrug resistance and in P-glycoprotein levels, which might help to improve the efficacy of anticancer therapies.

2.6 The eccDNA in hypopharyngeal and oral squamous cell carcinoma

Hypopharyngeal squamous cell carcinoma (HSCC) was an aggressive form of head and neck squamous cell carcinoma (HNSCC) that had a poor prognosis and was rapidly rising in incidence (48). Cisplatin (DDP)-based chemotherapy was an important factor impairing the effectiveness of chemotherapy for HSCC (49, 50). Lin et al. (25) recently identified more than 10,000 eccDNA in DDP-resistant FaDu cell samples from HSCC and amplified encoding genes (such as RAB3B and RAD54L) from eccDNA ($chr1^{circle\ 46219–52682\ kb}$) that carried different gene fragments. Furthermore, research found that RAB3B could promote DDP resistance in hypopharyngeal squamous cell carcinoma by inducing autophagy. However, loss of MDR1-carrying ecDNA induced by hydroxyurea increased the sensitivity of vinblastine in oral squamous cell carcinoma (OSCC), and the specific mechanism by which hydroxyurea induced to accelerate loss of extrachromosomal amplified genes is still unclear, one of which may involve the formation of micronuclei. Hydroxyurea did not deeply affect the synthesis of cell DNA and preferentially inhibited the replication of DNA outside chromosomes (26). These studies suggest that eccDNA might play a significant role in cancer drug resistance by amplifying related functional genes, and we need to explore further the novel mechanisms of eccDNA in drug resistance.

2.7 The eccDNA in other cancers treated with MTX

In cancer cells from a patient with small-cell lung carcinoma (SCLC) who received MTX treatment, a large number of DMs were discovered, and the DHFR gene was amplified and overexpressed. During serial passages of this cell line in drug-free medium, the number of DMs and the expression level of DHFR declined. The results showed that cancer cells were more sensitive to MTX and the prevalence of DMs in metaphase cells correlated with the concentrations of resistant MTX (27). Amplification of the DHFR gene on DMs led to an increase in mRNA and protein and provided the MTX resistance of human choriocarcinoma cells (28). A similar phenomenon was also observed in mouse EMT-6 cells from breast cancer. The cells that had been irradiated and subjected to stepwise increases in MTX concentration were detected as having numerous DMs. These studies showed that ecDNA-mediated gene amplification played an important role in the MTX resistance of cancer cells. The DHFR genes on DMs were also amplified in a dose-dependent manner (29). Recent studies used CRISPR-C, which is a technology that uses Clustered Regularly Interspaced Short Palindromic Repeats to generate extrachromosomal circular DNA (30), to generate ecDNA containing the dihydrofolate reductase (DHFR) gene in the HAP1 cell line of chronic myelogenous leukemia in humans. In the absence of methotrexate, the cells maintain their initial ecDNA copies. Then, the ecDNA copy number of DHFR ecDNA rose in a strong, dose-dependent pattern in response to MTX treatment (31).

3 Mechanism of eccDNA driving cancer drug resistance

eccDNA can promote cancer drug resistance development in various ways, and most of these ways are related to gene amplification. It is a common manifestation of genomic instability and plays an important role in cancer progression and drug resistance (10).

3.1 eccDNA increases tumor heterogeneity

Cancers are not static entities: they start from a genetically normal cell and end with billions of malignant cells that have accumulated a large number of mutations in the process. Most of those occur during chromosome replication in mitosis (51). Due to the accumulation of these mutations, tumor heterogeneity is promoted, which is characteristic of malignancies (52). The existence of eccDNA is an important factor driving genetic heterogeneity in cancer. It can

further enhance the heterogeneity of cancer cells, depending on its unique genetic mechanism.

In cytogenetics, the inheritance of eccDNA is not the same as that of chromosomes, which does not follow Mendel's law of inheritance (Figure 1). It is randomly distributed to daughter cells during mitosis, making the number of eccDNA in daughter cells unequal (17). Consequently, one of these daughter cells may have multiple oncogene copies of eccDNA during each division, thereby gaining a proliferation advantage. This approach enhances genomic diversity and promotes tumor heterogeneity, which helps cancer adapt to different environmental changes. It enables different cell subsets to have different sensitivities to therapeutic agents.

3.2 eccDNA is involved in gene expression associated with drug resistance

Early studies have proved that eccDNA is an important form of oncogene amplification, and its contribution to oncogene expression is mainly caused by the increase in gene copy number (10). eccDNA has the same complete domain as chromatin, although it lacks the higher-order compression state of chromosomes (53). Therefore, genes on eccDNA are more easily transcribed than those on chromosomes. The enhancers carried by eccDNA molecules can drive the transcription of their own genes and promote the transcription of other eccDNA molecules and even genomic genes (Figure 2) (54). We can speculate that both the increase in copy number and the high transcriptional activity of eccDNA itself can enable the overexpression of oncogenes. On the premise that ecDNA may carry a variety of genes, including oncogenes and drug resistance genes, ecDNA can make cancer cells resistant through gene amplification. Andrew et al. detected copy number alterations in 4,577 human cancer samples representing nine different solid cancers and discovered that cell-derived enhancers were co-amplified with oncogenes in multiple solid tumors, including MYCN, which bore a compact relationship with drug resistance in medulloblastoma (55). Another significant study on ecDNA in GBM revealed the molecules' function as mobile transcriptional enhancers, which were features of widespread intra-ecDNA and genome-wide chromosomal interactions. It was co-located in the same chromatin structure region to regulate the transcriptional activity of specific genes. These genes also included MYC and EGFR, which were closely related to drug resistance, and there was mutual regulation between ecDNA molecules derived from these genes (56). Therefore, ecDNA

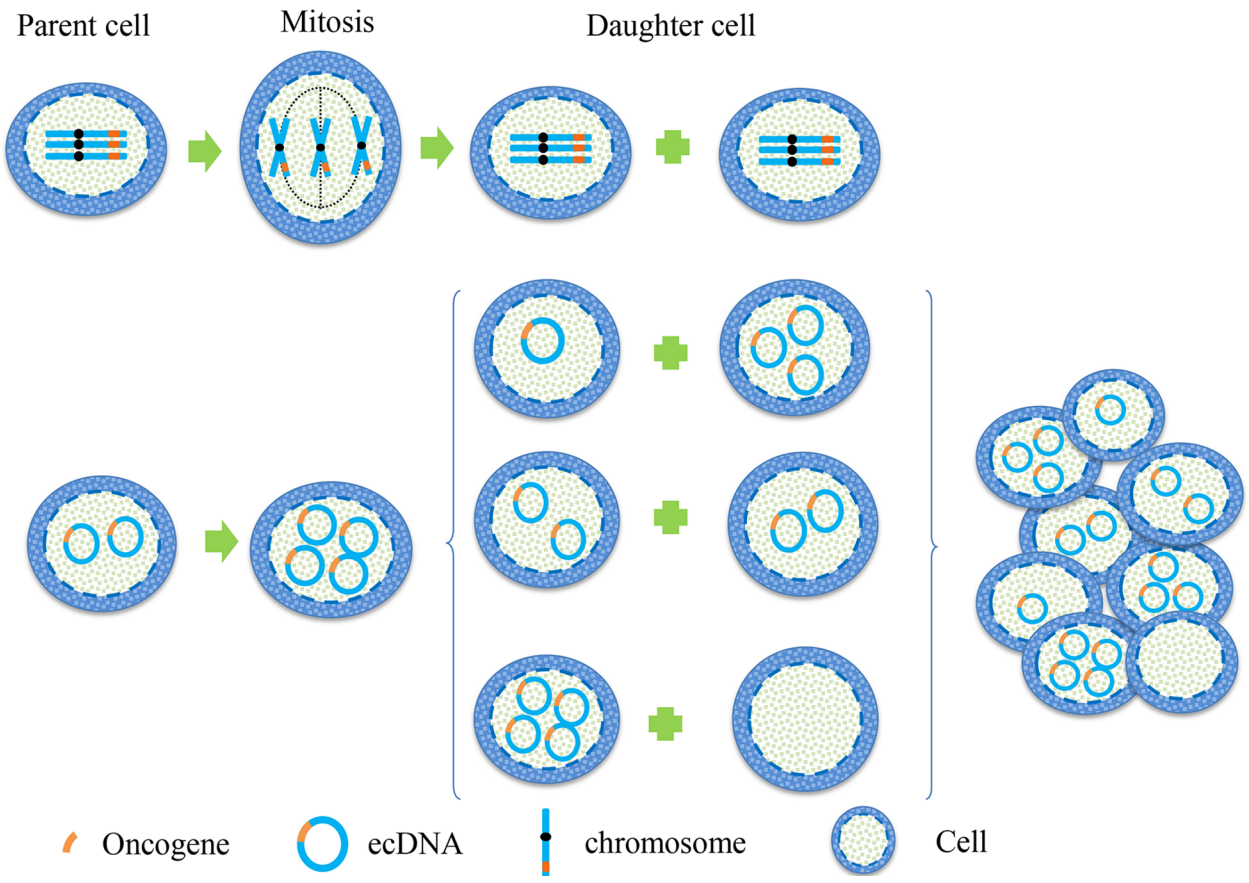

FIGURE 1
Hereditary difference between chromosome and eccDNA. When the oncogene is on a chromosome, the two sister chromatids are separated by pulling the centromere by the spindle during mitosis, and then they were equally distributed between the two daughter cells. This is a classic example of Mendel's Law. When the oncogene is located on eccDNA, eccDNA replicates with the chromosome during mitosis. Because eccDNA has no centromere, the separation of eccDNA is not controlled by the spindle, resulting in the copied eccDNA being randomly assigned to daughter cells during cell division. This unique genetic mode makes the number of eccDNA in each cell unequal, leading to heterogeneity of eccDNA in each cell subgroup.

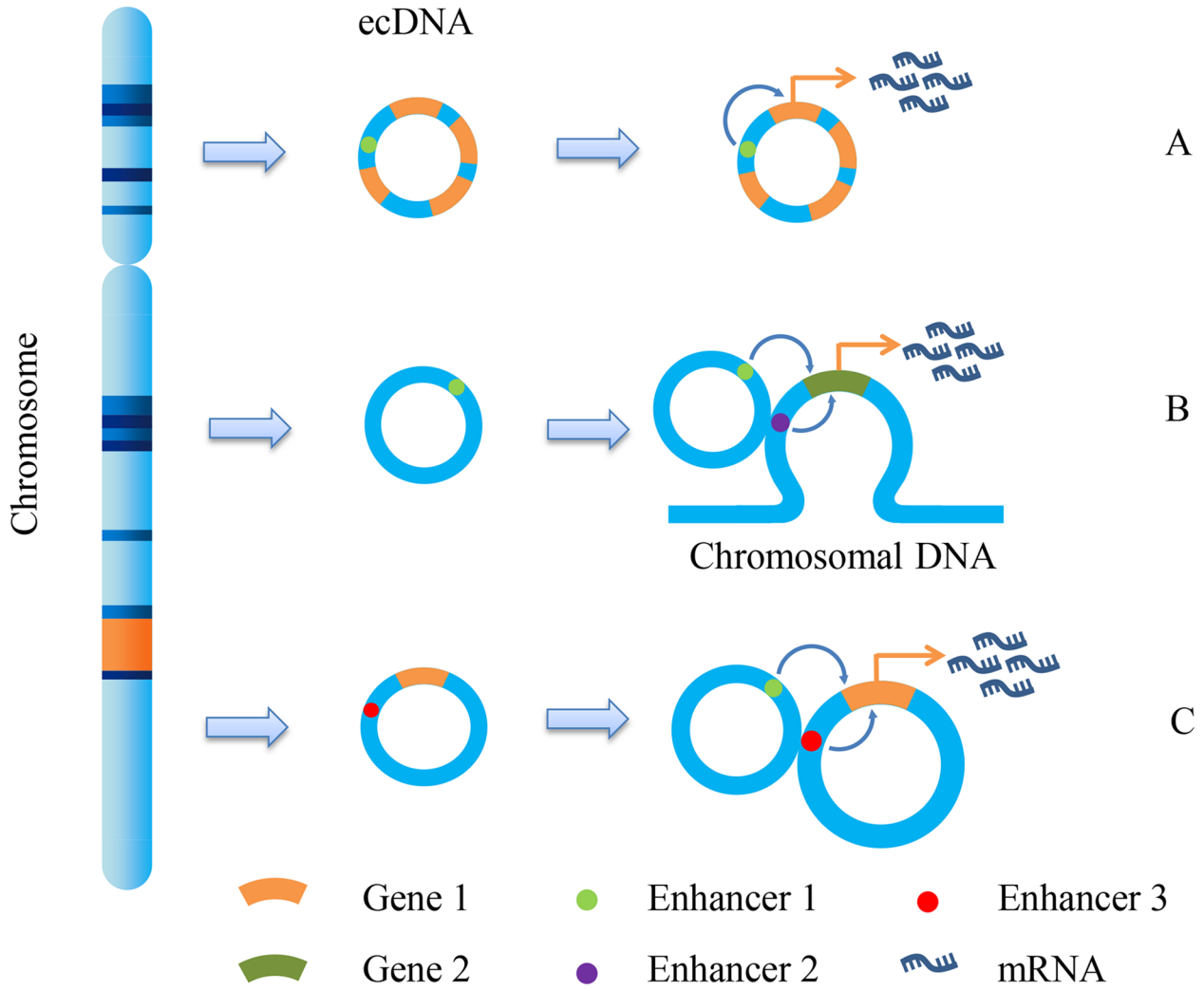

FIGURE 2
ecDNA promotes gene transcription. Due to the different fragments carried by ecDNA, it promotes gene transcription and expression in different ways. There are mainly three methods shown in the figure: **(A)** Enhancer1, located on ecDNA, can promote the expression of gene 1; **(B)** Enhancer1, located on ecDNA, can promote the expression of gene 2 on chromosomes; **(C)** the enhancer 1 on the ecDNA can promote the expression of gene 1 on another ecDNA, and the enhancer 3 on this ecDNA can also promote the expression of gene 1.

can make cancer cells acquire drug resistance through high expression of drug resistance-related genes.

3.3 eccDNA is integrated into chromosome to form HSR

The amplification of oncogenes or drug-resistant genes, which plays a pivotal role in human cell malignant transformation, confers a growth advantage to the cells through the overproduction of the amplified gene product. In cytogenetic research, the amplified gene is located in ecDNA or HSR (57).

HSR was initially detected in Chinese hamster cells resistant to MTX in 1976. Moreover, HSR are longer segments of chromosome than any single band in the karyotype. The staining intensity of HSRs in G-band staining was medium, rather than the normal pattern of alternating dark and light bands in the rest of the chromosome (58). ecDNA and HSR could be converted to each other and are homologous sequences (59). The structure of ecDNA is unstable. ecDNA can be integrated into the chromosome arm, where it efficiently initiated the breakage-fusion-bridge cycle (BFB) that generated HSR (10). As described above, the ABCG2 gene carried by ecDNA in GBM (19) and the DHFR gene carried by ecDNA in HeLa cells resistant to MTX (44) were integrated into a certain site of the chromosome to form HSR and produce a stable condition, so that the drug resistance of cancer cells was more stable. There is evidence that, compared with oncogenes or drug resistance genes amplified on ecDNA, HSR-amplified oncogenes or drug resistance genes located in chromosomes are not easy to eliminate from cells (60).

3.4 Dynamic regulation of gene expression by eccDNA

Previously, it was reported that EGFR derived from adult GBM was often mutated to produce a constitutively active oncogenic variant, EGFRvIII (18, 61). EGFRvIII amplification on ecDNA can provide growth advantages for cancer cells and make cancer cells

more sensitive to TKI treatment (34, 62). After stopping TKI treatment, the amount of ecDNA carrying EGFRvIII may increase again, suggesting that ecDNA deletion of EGFRvIII leads to TKI resistance, allowing cancer to adapt to its growth environment and evade treatment against oncogenes maintained on ecDNA (18). This suggests a highly specific dynamic mechanism, in contrast to cancer cells amplifying resistance genes through ecDNA to improve drug resistance, highlighting the diversity and complexity of ecDNA-promoting resistance mechanisms.

4 Clinical application prospect for eccDNA

The relationship between eccDNA and cancer has been studied for decades. With the development of next-generation sequencing (NGS) and the completion of human genome sequencing, the clinical application of eccDNA became an area of intense research interest (Figure 3). However, in the same patients, studies had identified that the average length of human ovarian cancer eccDNA was slightly longer than that of normal tissue, and the circulating eccDNA of patients after tumor resection was usually shorter than before operation (63). This phenomenon has also been confirmed in lung cancer (63). In acute myeloid leukemia (AML), the amount of eccDNA in cancer cells far exceeds that in normal cells. The average number of eccDNA gradually increased as primitive cells differentiated into terminal cells, and multiple recurrent and specific eccDNA were also identified in abnormal and normal cells (64). If the enrichment of eccDNA, especially the change of sequence, can predict the development of benign diseases into malignant cancers (such as hepatitis into liver cancer), eccDNA should be exhibited as a promising biomarker for cancer monitoring and prognosis.

Amplification of oncogenes and drug resistance genes in ecDNA promotes tumorigenesis and drug resistance. Many studies in recent years have confirmed that ecDNA is highly opened and contains enhancer sequences. These characteristics have improved its transcriptional activity (53, 54). These findings revealed a new understanding of eccDNA. Furthermore, when used as an immune stimulus, the circular nature of eccDNA can endow immune cells, such as dendritic cells and macrophages, with super immune capacity. The level of cytokines induced by eccDNA is far higher than that of linear DNA, which will help us better understand the pathogenesis of some inflammation-related tumors (65). As a result, combining gene therapy with immunotherapy can improve the efficacy of traditional anticancer drug therapy while also providing a new idea for the development of new anticancer drugs.

Kristen et al. performed whole-genome sequencing on 17 different cancer types, revealing that the frequency of ecDNA varies with cancer type (66). This phenomenon has almost never been found in normal cells, indicating that eccDNA in the blood of cancer patients may be used as a promising tool to track and determine the source and type of cancer. Although there are few studies on the

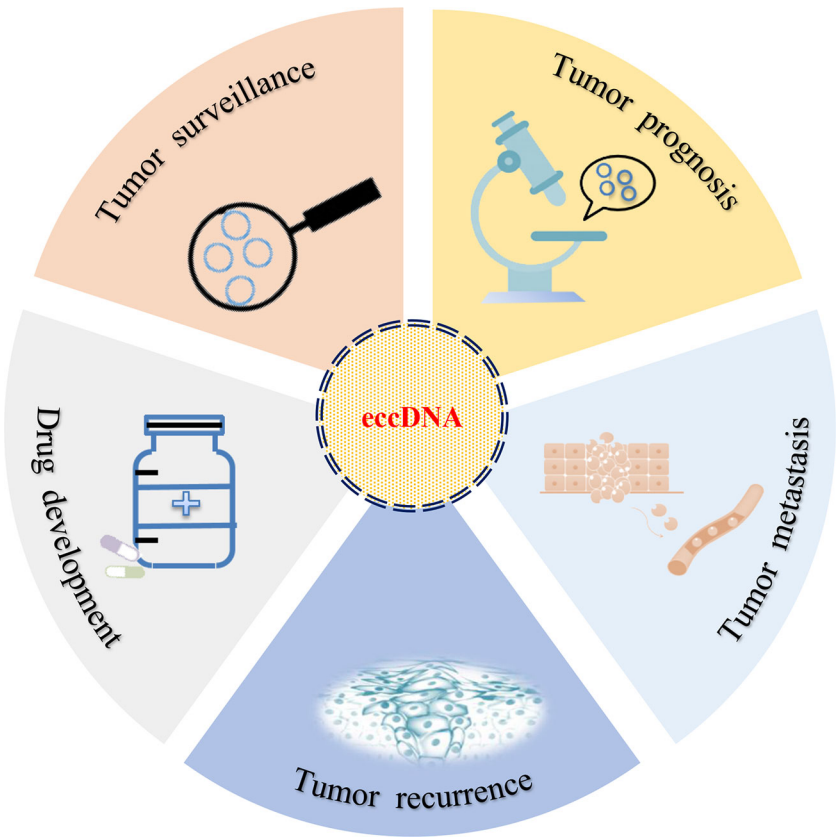

FIGURE 3
Clinical implications of eccDNA. eccDNA may be used as a biomarker for tumor surveillance, prognosis, metastasis, and recurrence. In addition, the development of target drugs may also provide a new strategy for the treatment of cancer in clinical setting.

role of eccDNA in tumor recurrence at present, a study on neuroblastoma demonstrated that cancer cells with an invasive phenotype of DM might be the source of tumor recurrence, which largely depends on the internal heterogeneity of tumors (67). The latest study by Cen et al. also reported the important role of eccDNA in tumor metastasis. They explored the eccDNA profile in high-grade serous ovarian cancer (HGSOC) by circle-sequencing analysis and found that the expression of DNMT1$^{circle10302690\text{-}10302961}$ (identified a novel eccDNA) was significantly downregulated in metastatic HGSOC tumor tissues and its reduction was associated with poor prognosis in HGSOC patients (68). Moreover, eccDNA can express functional small regulatory RNAs, including microRNA (miRNA). These miRNAs would regulate the downstream signal pathway (12), such as miR-145 (69), miR-191 (70), and miR-126 (71), by promoting tumor angiogenesis through related kinase signal transduction and transcriptional activation. In addition, the coding of oncogenes by eccDNA has been widely confirmed, such as c-myc (cellular-myelocytomatosis viral oncogene). C-myc promoted the expression of S100A4 (S100 Calcium Binding Protein A4) in prostate cancer cells by affecting downstream signaling molecules, which played an important role in tumor metastasis (72). Another example is EGFR, which promotes the invasion and metastasis of GBM by regulating the expression of matrix metalloproteinase-9 (MMP-9) (73). However, the exact mechanism of eccDNA directly mediating tumor metastasis remains to be studied.

5 Conclusion

EccDNA is widely found in various tumor tissues. Its unique genetic characteristics allow the number of oncogenes or drug-resistance genes in cells to increase sharply, resulting in a higher level of gene expression in cancer cells and providing tumor heterogeneity, which will contribute to cancer progression and resistance to chemotherapy. In addition, the diversity of somatic mutations in human cancer genomes also promoted the evolution of eccDNA. Some scholars attributed these mutations to the activity of the APOBEC3 (Apolipoprotein B mRNA Editing Catalytic Polypeptide-like) enzyme, which is a cytosine deaminase in cells. APOBEC3 can treat circular ecDNA as foreign viruses and try to limit or cut them. In this process, APOBEC3 induces the formation of mutation clusters within a single ecDNA molecule, which in turn plays a key role in accelerating cancer evolution and possibly leading to drug resistance (74). Here, we propose the following prospects regarding the regulation of eccDNA in drug resistance: (1) The particular molecular mechanism of resistance mediated by eccDNA in cancers needs to be investigated further. (2) According to the synthesis of microDNA mimics and its transcription *in vitro* and *in vivo*, microDNA can be transcribed into the functional, small regulatory microRNAs, which can regulate the expression of drug resistance genes (12). This synthesis might be a potential method to investigate the relationship between eccDNA and the regulation of gene expression. (3) The interactions between the multiple drug resistance genes on eccDNA and multitarget drugs can be deeply explored. (4) Most studies of eccDNA in tumor drug resistance are currently limited to the cell and animal level, and further population study should be conducted to improve the clinical significance of eccDNA as a biomarker. In a word, research on many scientific issues about eccDNA has revealed a new mechanism of cancer progression and regulation of chemotherapy resistance. Targeting specific genes and regulatory elements of eccDNA will hopefully become a therapeutic strategy for clinical cancer treatment.

Author contributions

JL contributed to the data collection and manuscript writing. YL contributed to the data collection and manuscript writing. TZ contributed to the manuscript revision. TX contributed to the manuscript revision. CC contributed to the manuscript revision. ML contributed to the manuscript revision. QQ contributed to the manuscript revision. YS contributed to the study design and manuscript revision. SW contributed to the study design and manuscript writing and revision. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (grant no. 82060524), the Youth Jinggang Scholars Program in Jiangxi Province, the Provincial Program of Gannan Medical University (YC2022-S965), and the National College Student's Innovation Entrepreneurial Training Plan Program (202210413018).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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