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Review Article

The contributions of extrachromosomal DNA elements in neoplasm progression

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Abstract: Extrachromosomal DNA (ecDNA) is a small, circular structure of DNA found outside chromosomes, in the cytoplasm and outside cells. Since the discovery of ecDNA in 1964, more studies have verified the significant prospect and application potential of its use in oncology. The presence of ecDNA is associated with a series of tumor activities such as the increasing or decreasing of oncogene copies, carcinogenic transmission, and activation of related signaling pathways. This review focuses on discussing the structure of ecDNA and its relevance in carcinogenesis, angiogenesis, drug resistance and metastasis.

Keywords: Extrachromosomal DNA, cancer, oncogene, carcinogenesis, target therapy

Introduction

Malignant cancer is a significant factor that leads to death and affects human health globally. The acquisition of these malignant characteristics in cells is closely related to genes and environmental elements. Changes to chromosomes at the microscopically level are currently recognized as one of the leading causes of cell malignancy [1, 2]. Extrachromosomal DNA (ecDNA, such abbreviations in main text are listed in **Table 1**) that was first detected as circular DNA structure by Yasuo Hoota and his colleagues in 1964 [3, 4], plays a specific role in tumorigenesis in mammal cancer cells [2, 5, 6]. In 1988, Susan M. Carroll and her colleagues confirmed that ecDNA is a component of exosomes [7]. Two subtypes of interest are the large extrachromosomal DNA circles which are referred to as double minutes (DMs, there are overlaps and differences between the concepts of DMS and ecDNA while ecDNA was first discovered as paired small chromatin bodies in neuroblastoma cells called DMs) [8-10], can autonomously replicate extrachromosomal genetic elements of genomic origin, and reintegrate themselves into chromosomes; and the small extrachromosomal DNA circle also called extrachromosomal circular DNA (eccDNA) [4, 8, 11, 12]. EccDNA contains Small polydispersed circular DNA (spcDNA), telomeric circles, microDNA and ecDNA [8]. EcDNA, which is generally 1-3 Mb in size, 100-1,000 times larger in kilobase compared to other circular DNA found in normal human tissues [6, 13-15], demonstrated to be provided oncogene amplification and drug resistance when it was studied in the developing fetus as well as in the noninvasive diagnosis and management of tumors [6, 12]. SpcDNA, about 100 bp to 10 kb in size, could enhance genomic instability [16]. Telomeric circles (738 bp) is involved in the alternative-lengthening of telomeres (ALT) pathway in ALT+ tumors [17]. MicroDNA (100-400 bp) can mediate the biogenesis of microRNA [18]. While the rest of the eccDNA has not shown significant transcription function [13]. Consequently, the ecDNA discussed in this article generally refers to the DMs and functional eccDNA. AmpliconArchitect, a new gene technology, is often used to study nucleic acid structure and function. Other functions include integration of ultra-

Table 1. English abbreviation

Abbreviation	Full Name
ALT	alternative-lengthening of telomeres
DHFR	dihydrofolate reductase
DMs	double minutes
eccDNA	extrachromosomal circular DNA
ecDNA	extrachromosomal DNA
EGFR	epidermal growth factor receptor
EVs	extracellular vesicles
FOXE1	forkhead box E1
HR	hormone receptor
HSR	homogeneously staining region
IR	initiation region
JAK-STAT	Janus kinase-Signal Transducer and Activator of Transcription
MAR	matrix attachment region
MDM2	murine double minute 2
MDR1	multidrug resistance 1
MET	mesenchymal-epithelial transition factor
miRNA	microRNA
MTX	methotrexate
MVs	membrane microvesicles
NMIIA	non-myosin heavy chain IIA
PDGFRA	platelet derived growth factor receptor
P-gp	P-glycolprotein
PTC	papillary nodal cancer
SOCS5	suppressor of cytokine signaling 5
THFA	tetrahydrofolic acid
TKIs	tyrosine kinase inhibitors

structural imaging, long-range optical mapping, computational analysis of whole-genome sequencing, circular chromosome conformation capture combined with high-throughput sequencing (4C-seq), fluorescence in situ hybridisation (FISH) and high-throughput sequencing on extrachromosomal cellular DNA [2, 4, 5, 19].

Over the past 4 decades, ecDNA as intermediary of gene amplification has been studied extensively [6, 20]. Oncogene amplification on ecDNA is considered a frequent event in cancer cells which gives them selective growth advantages by overexpressing oncogenes and pivotal functional elements [6]. Oncogene amplification on ecDNA provides a mechanism by which cancer cellules promptly adapt to changes in tumor microenvironment [20]. EcDNA has an effect on the pathogenesis, metastasis and drug resistance of tumor cellules in the last thirty years [2, 5, 13, 15]. This article aims to review the role of ecDNA in tumorigenesis and drug resistance through existing literature.

The structure and genetics of ecDNA

The mechanism of how ecDNA is generated is poorly understood. It is widely recognized that ecDNA is a structurally circular DNA formed through the non-homologous recombination among chromosomal or DNA segments due to genomic instability (Chromothripsis model) [5, 6, 8, 21, 22] (**Figure 1A**). There are also hypotheses that considered ecDNA originated in breakage-fusion-bridge (BFB) cycle or translocation-deletion-amplification model. BFB cycle involved intelomere loss, replication, fusion, breakage and looping out of oncogene [8, 23] (**Figure 1B**) while the translocation-deletion-amplification model made by oncogene near the chromosome translocation breakpoints which amplified, retained or deleted and therefore form ecDNA [24] (**Figure 1C**). In addition to that, the formation of ecDNA can be mediated by small circular extrachromosomal molecules ("Episome" model) [7, 8] (**Figure 1D**), Wahl and his colleagues disclosed that episomes are produced by a recombination of adjacent genes and then episomes can enlarge to form ecDNA.

EcDNA closely relates to chromosome and exosomal micronucleus. Micronucleus is a chromosomal reactive element of cell cancelation [25]. Micronucleus is considered a novel biomarker and its' appearance can aid in identifying cancer patients [19, 26]. At present, micronucleus is thought to be filled with abundant ecDNA. Shimizu's research confirmed that microscopically, ecDNA is generated by the recombination between microscopically invisible episomes [27]. This formation of ecDNA may also be related to the inaccurate transcription and replication of nuclear DNA [2, 5, 11, 13, 15].

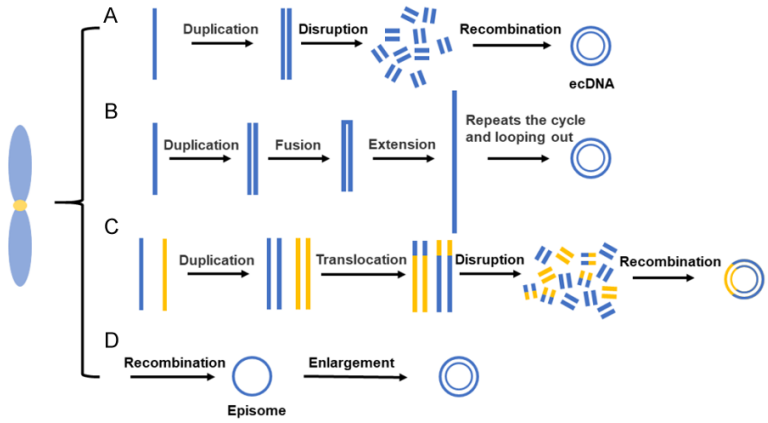

Figure 1. The probable production of ecDNA. The main production models of ecDNA in tumor cells. A. The oncogenic instability causes chromosomal breakage in cell nucleus and thus creates fractured DNA segments. The DNA segments travels through the nuclear membrane and form circular DNA structures through non-homologous recombination in the cytoplasm. B. The breakage-fusion-bridge (BFB) cycle model of ecDNA including the fusion of duplicated gene and the same repeats of the cycle. C. The translocation-deletion-amplification model of ecDNA which involves translocation, rupture and recombination of multiple oncogenes. D. The Episome model of ecDNA based on the enlargement of episome.

Cytogenetically, ecDNA in tumor cells can be assigned to daughter cells stochastically. However, the specific form of ecDNA transmission and proliferation in cancer cells is still unclear [28]. The genetic behavior of ecDNA is closely related to chromosomes. Noriaki Shimizu's research group identified that after ecDNAs replicate in the early S phase, they migrate into the nucleus and participate in the mitotic process [29]. Lamin-B-rich micronucleus are abundant in the S phase of cell cycle [29-31]. Furthermore, evidence has demonstrated that the expression of ecDNA type micronuclei relates to lamin-B binding protein, which suggested that the expression of ecDNA in the cell micronucleus changes with in different phases of cell cycle [32]. EcDNA is thought to be inherited by the random distribution and uneven segregation between two daughter cells at the end of mitosis [2, 28, 33]. While Kanda's and Tsubasa Tanaka's confirmed that, coreless ecDNA are steadily separated into daughter cells by binding to chromosomes during mitosis [34, 35] (**Figure 2**).

The existence of ecDNA extends beyond the non-chromosomal DNA structure, which is widely present in tumors and can effectively promote the amplification of oncogenes [5]. This hypothesis is supported by the distribution of ecDNA. Kristen M Turner's team found-ed that ecDNA is rarely present in normal human cells [2]. However, Teressa Paulsen's team research achievement also demonstrated that ecDNA (mainly non-functional eccDNA) is widely found in the normal cells of various organisms from yeast to human [36], and their overlap is consistent with the generation of tumor formation and drug resistance cells. The authentic distribution of ecDNA in nature needs further investigate.

While ecDNA contains activated histone markers and is associated suppressed histones, the basic base components of ecDNA and nuclear DNA are the similar [5]. But at the same time, there are also several differences between those two DNA structures. EcDNA contains highly activated chromatin, with less compression of structure and greater transcriptional activity than nuclear DNA [5]. EcDNA also has the same complete domain as chromatin, although it lacks the higher-order compression state of chromosomes, thus enhancing chromatin accessibility. Generally, chromosomes are high-order substructures formed by high-order compression of chromatin [37, 38], this limits DNA accessibility and thus regulates the level of gene transcription. So, there are significant changes to the ecDNA structure occur in the tumor cells [39, 40]. As a result, ecDNA formation becomes one of the way oncogenes increase their malignant copies [41].

EcDNA is highly autonomous in the expression of oncogenes and has RNA polymerase activity, suggesting that genes in ecDNA may be expressed automatically [42]. And Koh-ichi Utani and colleagues established that highly amplified genes in cancer are mainly located in DMs homogeneously staining region (HSR) [32] which testified to the phenotypical effect micronucleus and ecDNA has on tumor cells. The ability of the micronucleus to persist in the cytoplasm, in turn, suggests their ability to significantly disrupt the cellular phenotype when expressed differently from that of their nuclear

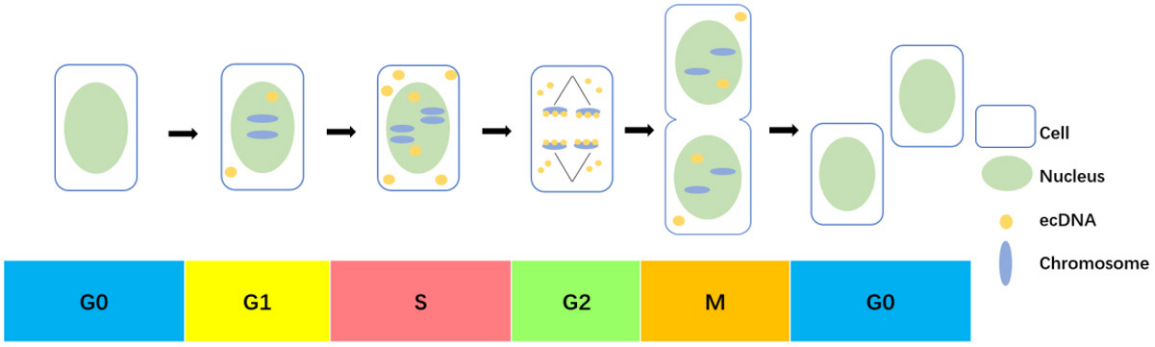

Figure 2. The activity of ecDNA in cell cycle. After ecDNA completes self-replication in S phase, some of them enters the nucleus and binds to chromosomes, which move towards the poles during G2 phase. While the remaining ecDNAs were randomly distributed between the two progeny cells.

copy. Therefore, the tumorous transcription activity of micronucleus DMs are higher compared to intracellular chromosome because gene amplification in DMs can be regulated by micronucleus affecting the phenotype of tumor cells [32].

The function of ecDNA

EcDNA is a significant mediator of oncogene amplification and concertation. Sihan Wu and colleagues noted that ecDNA may be the conceptual equivalent similar to bacterial plasmids, which presumably has an impact on tumor pathogenesis and drug resistance [5]. The potential of tumor cells is stimulated by ecDNA. Sihan Wu described that ecDNA exists "ultra-long-range chromatin contacts" with transcriptional active chromatin [5]. They also considered ecDNA as a plasmid in the eukaryotic nucleus. Close to the plasmid, ecDNA is extremely malleable. Noriaki Shimizu's team has shown that plasmids containing mammalian replication initiation region (IR) and nuclear matrix attachment region (MAR) can effectively initiate gene amplification in mammalian cells and generate structures in primary cancer cells that are hard to distinguish from DMs or HSR [27] which may partly explain the effect of ecDNA. Other explorations have also confirmed that plasmids can incorporate both a mammalian replication origin and a nuclear MAR into DMs to enhance the expression level [27].

While the function of ecDNA in normal cells is poorly understood, ecDNA is known to contain a large number of known exon oncogenes in malignant cell which has a direct impact on tumorigenesis. Traces of ecDNA activity and mutation can be found in a variety of tumor cells, including thyroid cancer, ovarian cancer, hepatic carcinoma, gastric carcinoma, neuroblastoma, neuroepithelioma, colon cancer and prostate carcinoma [41, 43-47]. In a sort of sense, ecDNA remodels the epigenomic landscape phenotype of chromosomal genome and affects chromosomal gene expression and tumorigenesis [9, 20, 48]. Oncogenes on ecDNA include epidermal growth factor receptor (EGFR), MYC, c-MYC, HER2, platelet derived growth factor receptor (PDGFRA), mesenchymal-epithelial transition factor (MET), MECOM/PIK3CA/SOX2 gene cluster and CDK4/Murine Double Minute 2 (MDM2) gene cluster [5, 9, 49, 50] (**Table 2**). The improved chromatin accessibility of ecDNA brings a higher amplification level to oncogenes. And the presence of these oncogenes creates the necessary conditions for malignant progression. For instance, EGFR signal pathway can activate the RAS/MAPK/ERK, PI3K/AKT, p38 and STATS pathways to promote tumorigenesis [51, 52]. Furthermore, the over-expression of MYC can affect many cells functions including cell cycle, self-renewal, survival, growth, metabolism, protein and ribosomal biogenesis, differentiation and canceration [53-55]. Tumorigenesis, tumor progression and cancer immunosuppression in various carcinoma types can be promoted by an over-activation of the MET axis [55-57]. The highly expressive nature of oncogenes encoded in ecDNA are also identified by the relative high copies of oncogenes on ecDNA compared to any other gene expression [5]. As shown in **Figure 3A**, taking EGFR/p38 pathway as example, the presence of ecDNA structure drove the amplification of oncogenes

Table 2. The roles of known ecDNA oncogenes in tumorigenesis

Oncogenes in ecDNA	The role in tumorigenesis via ecDNA	Reference
EGFR	activate the RAS/MAPK/ERK, PI3K/AKT, p38 and STATS pathways in cancer pathogenesis and progression	[51, 52]
MYC	affect cell cycle, cellular energy metabolism and protein metabolism	[53, 54]
c-MYC	induce carcinoma genomic instability and inhibit apoptosis	[100]
HER2	activate EGFR family to regulate cellular proliferation and induce cell transformation	[9, 50, 101]
PDGFRA	activate mutations in the KIT receptor tyrosine kinase and promote the cancer angiogenesis	[75, 102]
MET	encode receptor tyrosine kinase and thus trigger cell migration, proliferation, and angiogenesis	[55-57]
MDM2	negative regulation of p53	[103]

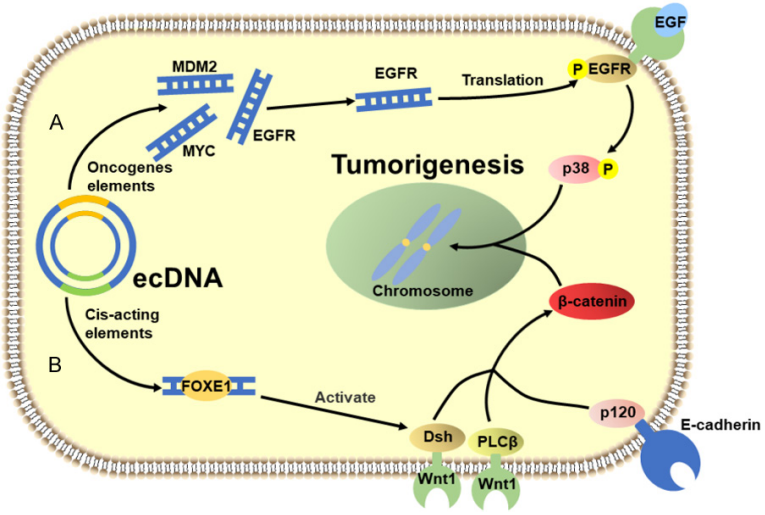

Figure 3. The effects of ecDNA in tumorigenesis. A. The direct effects of ecDNA to encode tumorigenesis through the amplification of multiple ecDNA oncogenes elements, such as oncogene EGFR and EGFR/EGF/p38 signal pathways; B. The indirect effect of ecDNA in tumorigenesis which carries cis-acting elements (FOXE1, e.g.) to impact the activity of other signal pathways like Wnt/β-catenin pathways to activate tumorigenesis.

and thus to elevate the tumorigenesis transcription level directly [2, 49].

Moreover, there are many functional cis-acting elements in ecDNA that can mediate oncogenic activity indirectly. For instance, confirmation of the extrachromosomal origin and fine structure of the forkhead box E1 [FOXE1, and thyroid transcription factors (TTF)]-containing hybrid amplicon via AmpliconArchitect reconstruction [58]. FOXE1 modulates thyroid cell migration which suggests a role in epithelial-to-mesenchymal transition (EMT) [59]. Current studies on FOXE1 transcription factors have found its significant value in oncology. FOXE1 gene has increased expression level in papillary nodal cancer (PTC) cells, which significantly correlates with extra-capsular invasion of tumor cells, lymph node metastasis and tumor stage, and serve as a potential biomarker for prognosis as well as a new therapeutic target [60, 61]. FOXE1 can promote PTC proliferation, migration, and invasion by activating the Wnt/β-catenin pathway [62] (**Figure 3B**). Another cancer suppressor, mir-524-5P, targets multiple genes approved in several types of cancer cells. It effectively inhibits the activity, migration and invasion of PTC cells and promote the apoptosis of tumor cells by inhabiting FOXE1 [63]. Recently, FOXE1 has been found to be highly expressed in pericytes of burn eschar, Alexander Evdokiou has demonstrated that angiogenesis can be promoted by FOXE1 transcription factor [64]. Regardless, it is worth mentioning that the high expression of FOXE1 plays an anticancer role in PTC and other tumors. In addition, Ding Zheng showed that FOXE1 can inhibit the proliferation, migration and invasion of PTC by negatively regulating the expression of target gene PDGFA [65]. The regulation of FOXE1 in tumorigenesis is considered bidirectional. FOXE1 may inhibit the growth, invasion and migration of certain tumor (PTC, e.g.) [65], but further investigations are needed to confirm this suppression. Similarly, ecDNA expresses functional small regulatory RNA including microRNA and novel siRNA which have various functions including indirect modulation of gene expression [36].

The expression difference of ecDNA between normal mammalian cell and tumor cells and the various factors mentioned above that ecDNA is directly or indirectly involved in tumor growth all indicate that it plays a significant role

in tumor behaviors. We will describe the role of ecDNA in different views detailly.

EcDNA in tumorigenesis

Gene amplification in ecDNA participates in tumorigenesis. Gene amplification is considered one of the major mechanisms of oncogene activation and cells with amplified oncogenes may gain a growth advantage through the overproduction of protein products [11]. As described previously, a large number of oncogenes are carried by ecDNA which can be seen as a hotbed of oncogene amplification. In particular, ecDNA is found to carry a double amplification of the N-MYC oncogene in neuroblastoma [49]. Malignant gliomas also have large amounts of ecDNA with oncogenic activity via [2]. Investigations have also shown that the deletion of MYC oncogene amplified on DMs in human tumor cells can reverse the malignant phenotype of cells and induce cell differentiation [66-68]. Since gene amplification is responsible for the malignant transformation of some cancer cells, the reduction of the amplified gene copy leads to the reversal of tumor cell phenotypes [68]. This amplification mechanism of ecDNA oncogene leads to increased consistency and variability in tumors [2]. More precise, ecDNA amplification increases oncogene copy number and intratumoral heterogeneity much more effectively than chromosome amplification [2]. Also, the ecDNA contained in the micronucleus has transcriptional activity that may alter the phenotype of cancer cells [32].

Besides, oncogenesis may be also influenced by genetic mutations in the ecDNA. Florence Le Page and his colleagues testified that G-T transcriptional mutations can be present in ecDNA to mediate spontaneous tumorigenesis [69]. There is transcript fusion phenomenon in ecDNA in malignant cell. The clonal selection of malignant glioma cells with competitive advantage in xenograft experiment can produce the CAPZA-MET fusion gene and transcript, thus increasing the tumor variability and promoting tumor progression [49]. Taken together, the results obtained from current studies suggest that ecDNA plays a crucial role in tumor progression [5, 6, 32].

EcDNA in tumor angiogenesis

The main regulation form of angiogenesis in tumor relies on paracrine signaling. ecDNA has been shown to play a specific role in this process [5, 70, 71]. EcDNA, in the form of intercellular vesicles are stored in extracellular vesicles (EVs). It can increase paracrine signaling between cancer cells, increased tumor cell aggressiveness, proliferation, angiogenesis, and chemotherapeutic resistance [71]. EGFR, vascular endothelial growth factor (VEGF) and VEGR receptor (VEGFR) are major cytokines involved in tumor angiogenesis [72, 73]. Oncogenic EFGR can promote the accumulation and proliferation of endothelial cells and fibroblasts ultimately leading to the formation of vessels. One of the significant mechanisms by which oncogenic EGFR contributes to tumor angiogenesis is via the up-regulation of VEGF in tumor cells [70]. Khalid Al-Nedawi and colleagues demonstrated that oncogene-containing tumor cell-derived membrane microvesicles (MVs) with EFGR has been proven to act as a unique form of angiogenesis-modulating stimuli and function in an autocrine manner [74]. Furthermore, Alicia M. Viloria Petit testified that the usage of anti-EGFR/VEGF neutralizing antibody can cause a dose-dependent inhibition of VEGF protein expression and lead to significant reduction in tumor blood vessel *in vivo* [70]. In addition to EGFR, PDGFRA could be another affecting oncogene for angiogenesis, which impact the angiogenesis of ovarian tumor [75].

On the other hand, ecDNA can express functional small regulatory RNA including microRNA (miRNA) [36]. These miRNAs have favorable intra- and extracellular regulatory features. For example, miR-9 effectively reduces the suppression of cytokine signaling 5 (SOCS5), leading to activated Janus kinase-Signal Transducer and Activator of Transcription (JAK-STAT) pathways [76]. This signaling cascade promotes endothelial cell migration and tumor angiogenesis.

EcDNA in tumor drug resistance

Tumor's resistance stems from the changes in metabolic pathways, production of efflux P-glycolprotein (P-gp) pumps to chemotherapy drugs, and changes in membrane permeability mostly due to acquired or spontaneous gene mutations [77-79]. Cells that acquire adaptive mutations are more likely to pass those mutations on to daughter cells, driving tumor progression and chemotherapeutic resistance [33, 80]. EcDNA oncogene amplification may maximize proliferation and survival by increasing the likelihood of oncogenic expression in

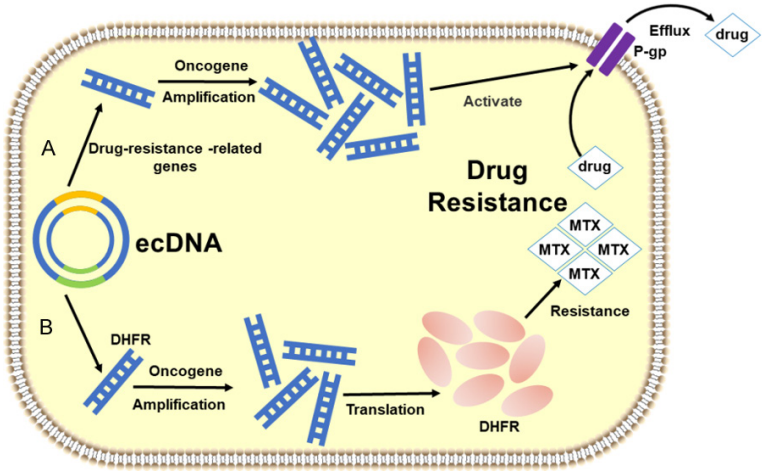

Figure 4. The effects of ecDNA in drug resistance. A. ecDNA drug-resistance-related oncogene amplification improves the activity of P-gp and thus increases the drug efflux; B. ecDNA increases the amplification of the dihydrofolate reductase (DHFR) gene to promote tumor resistance to methotrexate (MTX) when the tumor was exposed to high levels of MTX.

subsets of cells or improve the expression and activity of P-gp (**Figure 4A**), thus enabling tumors to adapt effectively to the changing micro environment, which contributes to drug resistance and difficult to cure cancers [81-83].

Gene amplification in ecDNA is highly sensitive to its growing environment. It has been verified that of cytotoxic regimes may result in drug resistance in tumors with a high copy number of gene amplification in ecDNA while the absence of cytotoxic drugs may lead to the loss of unstable gene [84, 85]. Moreover, Frederick Alt and his teammates found ecDNA promotes tumor resistance to methotrexate (MTX) by increasing the amplification of the dihydrofolate reductase (DHFR) gene [85] (**Figure 4B**). MTX, as a methylenetetrahydrofolate reductase inhibitor, can inhibit DHFR and block the production of tetrahydrofolic acid (THFA) from dihydrofolate, which then obstructs the transfer of one carbon unit in the biosynthesis of purine nucleotide and pyrimidine nucleotide and thus inhibit DNA synthesis. Cells with DHFR in ecDNA remarkably lose the amplified DHFR gene over time as they grow in the absence of MTX. This phenomenon is also called as drug-mediated loss of unstable genes. At some point this also reflects the characteristics of ecDNA in tumor resistance. The loss of unstable gene also occurs when cells are cultured with hydroxyurea in higher proportions. Interestingly, hydroxyurea can effectively reduce gene loss in ecDNA at low concentrations. Treatment of several human tumor cell lines with low concentrations of hydroxyurea accelerated the loss of oncogenes represented by MYC in ecDNA amplification, thereby reducing tumorigenicity [67, 68]. Hydroxyurea mediates EGFR gene loss, though the process is reversible and the EGFR gene recovers after withdrawal of hydroxyurea [86]. DNA replication inhibitors represented by low-dose hydroxyurea (50-150 μm) can induce the loss of amplified genes in ecDNA [11]. This treatment resulted in a reduction in the number of DHFR copies amplified in the hamster CHO cells [87, 88]. This indicates that, hydroxyurea can be used as a potential chemotherapy drug to interfere with ecDNA. By the way, hormone receptor (HR) pathway may be a new target to improve chemotherapeutic outcome by decreasing extrachromosomal amplification in cancer [46].

Gene amplification of ecDNA is also affected by radiation. Radiation-mediated loss of extrachromosomal amplified multidrug resistance 1 (MDR1) genes is accompanied by a reduction in P-gp levels and function [89]. Furthermore, ionizing radiation accelerates the loss of amplified MDR1 on DMs in multi-drug resistant KB cell [89]. The elimination of MDR1 gene amplification in DMs led to the reversal of more sensitive phenotypes [89, 90]. This phenomenon implies that ecDNA mutation plays a crucial role in the selective loss of amplified unstable genes involved in cell resistance [87, 88].

Conversely, mutations in ecDNA could also be a source of tumor resistance. Mutations in the function of EGFRvIII in ecDNA make glioblastoma resistant to EGFR inhibitors and tyrosine kinase inhibitors (TKIs) [6, 91]. The absence of EGFRvIII in ecDNA promotes tumor resistance. And the loss of the EGFR gene in ecDNA allows glioblastoma to develop resistance to the EGFR TKIs Erotinib [91]. Resistance to EGFR TKIs has proven to occur by elimination of mutant EGFR from EGFR clone mutations in ecDNA and reappears after the drug is discontinued. The intermittent EGFR TKI administration allows

glioblastoma to regain drug sensitivity with rapidly elevated levels of EGFRvIII DNA outside the chromosome [91]. In addition, treatment of glioblastoma with Erotinib results leads to an increase in the MDM2 DM copies [91].

These results suggest that cancer can evade treatment by targeting oncogenes that maintain DNA outside of chromosomes in a highly specific, dynamic and adaptive way [91]. In conclusion, ecDNA participates in tumor resistance and may become a potentially new target for therapy in the future.

EcDNA in tumor metastasis, prognosis and diagnosis

A review of the cause of tumor metastasis and the types of oncogenes revealed the involvement of diverse genes, including the S100 protein family, MYC, RAS, c-SIS, MYB, ERBA and other genes. Tumor microenvironment plays a central role in promoting tumor metastasis [92]. S100A4 for example, is recognized as a protein that promotes metastasis. S100A4 can alter cell adhesion, stimulate angiogenesis, attract immune cells to growing tumor lesions, and promote secretion of various cytokines and growth factors into tumor microenvironment [93]. Intracellular S100A4 interacts covalently with its targets, including actin, non-myosin heavy chain IIA (NMIIA) and tropomyosin, and is thus related to cell migration [94, 95]. Also, S100A4 has been shown to be involved in the metastasis of various tumors [96].

Studies have demonstrated that c-MYC can promote the expression of S100A4 by influencing downstream signaling molecules in prostate carcinoma cells [97]. The mutated p53 gene is also related to c-MYC and S100A4, which indirectly regulates the invasiveness of tumor cells [98]. Although EcDNA is currently thought to play a certain role in tumor metastasis [5], but the definitive mechanism is still unclear. It is thought to be related to the presence of c-MYC and other genes in the circular structure of ecDNA. However, whether or not ecDNA contains genes of the S100 protein family or directly affects metastasis remains to be explored further.

The purpose of studying the molecular mechanism of ecDNA's is to be able to understand and implement its use in clinical oncology. At present, there's little evidence of the clinical significance of ecDNA in cancer treatment. Notably, evidence of ecDNA in blood has been reported raising interest in its potential as a diagnosis and prognostic tool to improve of tumors detection and treatment [41]. EcDNA containing MET has been investigated as a marker to identify subclonal cell populations of malignant glioma [49]. Also, micronucleus containing ecDNA has been detected outside the cell [99]. Blood ecDNA levels has also been used to guide the prognosis of tumors such as ovarian cancer [43]. However, at present, there is no clinical application of ecDNA although it can be detected by liquid biopsy in blood [41]. We have reason to believe that in the future, ecDNA can become a central as an indicator for the diagnosis of tumors and the prognosis of malignant neoplastic diseases.

Research deficiency and conclusion

Until now, studies on ecDNA mainly focus on the structure of ecDNA and the genes it contains, in contrast, there are very few studies that explore the production mechanism, type and normal physiological function of ecDNA. Whether ecDNA itself has a unique regulatory mechanism for downstream signaling pathways, and the transmission mechanism of ecDNA between cellules is also unknown. In addition, there is a lack of targeted therapeutic drugs for ecDNA. This series of limitations has become a deficiency and bias in current ecDNA studies.

So, taken together, ecDNA is a significant extracellular gene carrier structure containing highly accessible chromatin, which plays a vital role in tumor genesis, angiogenesis, drug resistance formation and metastasis. EcDNA as a tumor diagnostic and prognostic indicator has recently become a subject of interest to researchers. In the future, drugs targeting ecDNA as a whole or some of its genes can become new target for cancer therapy. We believe focus in new cancer therapies should start to shift from nucleus centric studies to investigate other contributors outside the chromosome, outside the nucleus, and even outside the cellule.

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Disclosure of conflict of interest

None.

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