Chronic Diseases Prevention Review

25 (2023) 1-6

ISSN 2158-0820

Available at http:// www.cancercellresearch.org

Advances in glioma ecDNA research

Zhongyou Que[1], Sheng Liu[1], Bingxi Lei[2*]

[1] Department of Neurosurgery, Shenzhen Samii Medical Center, Shenzhen, 518118, China

[2] Department of Neurosurgery, Sun Yat-sen Mmemorial Hospital of Sun Yat-Sen University, Guangzhou, 510120, China

Abstract: Glioma is the most common primary tumor of the central nervous system and the tumor genetics of glioma has been a hot topic. In recent years, the role of ecDNA in tumors, especially glioma, has been gradually recognized. ecDNA can accelerate tumor evolution and mediate glioma drug resistance activity by randomly distributing in the interphase due to the lack of mitotic structure. The circle structure of ecDNA also has the characteristics of high openness, strong transcriptional activity and diverse regulatory modes. The study of glioma ecDNA has gone through three stages: classical karyotype analysis, basic function study and regulatory molecular mechanism study. In the era of karyotype analysis, early studies focused mostly on the description of cell karyotypes, but later merged with research on oncogenes. In the era of functional study of ecDNA, not only have many oncogenes located on ecDNA had been discovered, but also the mechanism of ecDNA involvement in glioma drug resistance had been revealed. In the past five years, the finer molecular regulatory mechanisms have become essential in further revealing the unique functions of ecDNA, mainly due to the advancement of biology experiments research tools and bioinformatics technologies.

Keywords: glioma; double minutes; ecDNA.

Received 5 February 2023, Accepted 21 March 2023

*Corresponding Author: Leibing Xi, leibingxi@mail.sysu.edu.cn

1. Introduction

Glioma is the most common primary tumor of the central nervous system, with an annual incidence of 6 per 100,000 [1]. Currently, the main treatment modalities for gliomas are comprehensive therapy, including surgery, radiotherapy, and chemotherapy [2]. New technologies such as targeted drugs [3], immunotherapy [4], and electric field therapy [5] are also being used as adjuncts to these therapies. However, the five-year survival rate of high-grade glioma, especially glioblastoma (GBM), is only 6.8% due to its malignant biological characteristics, such as high aggressiveness and drug resistance [6]. As a genetic disease, glioma has long been studied at the genetic level [7]. However, current genetic studies on glioma have mainly focused on the function of specific oncogenes or tumor suppressor genes, without paying attention to the specific location of the genes, and with more focus on the study of chromosomal linear DNA in the nucleus. In recent years, the function of extrachromosomal DNA has been gradually recognized, providing a new perspective for better understanding of oncogene amplification, tumor microevolution, and other phenomena, and further expanding the research content of tumor genomics [8].

2.The basic biological properties of ecDNA

Extrachromosomal circular DNA (eccDNA) was first discovered in the 1960s by Alix Bassel and Yasuo Hoota, who found a large amount of eccDNA in boar sperm cells in 1964, ranging in size from a few hundred to several thousand base pairs [9]. Almost simultaneously, Spriggs et al. found a large number of small extrachromatin granules in neuroblastoma, which they named double minutes (DM) because they were often found in pairs [10]. Since then, eccDNA has been found in eukaryotic yeast [11], nematodes [12], and Drosophila [13].eccDNA can be broadly classified into four categories based on molecular size and generation mechanism: small polydispersed DNA (spcDNA), telomeric loops, tiny DNA, and extrachromosomal DNA (ecDNA). spcDNA is usually a few hundred to several thousand base pairs in size, derived from repetitive sequences of the genome, and is associated with genomic stability [14]. Telomeric loops containing only telomere-related repetitive sequences immortalize tumor cells through the mechanism of selective lengthening of telomeres (ALT) [15]. Micro DNA is the smallest class of eccDNA, with a size of only 100-400 bp, first discovered in mouse embryonic brain tissue [16]. It has been shown that micro DNA can regulate the tumor genome by producing micro RNA [17], and it has important potential for liquid biopsy [18].ecDNA is the most functionally rich of all eccDNAs. Since its size spans approximately 1-3 MBp, it can carry encoded genes to perform biological functions. ecDNA research began with the discovery of DM, but subsequent studies have shown that only about thirty percent of ecDNA exhibits DM [19]. However, 46% of tumor cell lines were detected with ecDNA and expressed at significantly higher levels in primary tumor cells than in vitro cell lines, suggesting that the in vivo environment in which tumor cells are located has a significant effect on ecDNA [20].Four main models have been proposed to explain the mechanism of ecDNA formation, including the breakage-fusion-bridge cycle (BFB cycle) [21], ectopic deletion amplification [22], additional bodies [23], and the chromosome fragmentation model [24]. ecDNA degradation is thought to be associated with micronuclei

[25]. However, the details of the mechanism have not been studied in depth.

3.History of ecDNA research in glioma

The study of extrachromosomal DNA (ecDNA) in gliomas dates back to the 1960s, following the discovery of DM. As experimental techniques and bioinformatics tools evolved, ecDNA research in glioma can be broadly divided into three stages: classical karyotype analysis, basic functional descriptive studies, and studies of molecular regulatory mechanisms. During the karyotype analysis era, limited molecular biology technology restricted cell nuclear chromosome study to qualitative analysis and classification of chromosome morphology. The focus was on identifying the presence of DM in different cases with minimal exploration of its specific gene functions. Later, basic functional descriptive studies emerged, and the landmark achievement during this period was the discovery of ecDNA's specific role in drug resistance in glioma. In the last five years, ecDNA's molecular regulatory mechanisms have been identified due to the development of relevant high-throughput technologies and bioinformatics tools. These revealed ecDNA's genomic diverse regulatory patterns and initially recognized the complex functions of ecDNA in glioma. Importantly, the above three research stages are not absolute but represent different levels of ecDNA research, with each level representing a research paradigm that will persist in the basic and clinical research of ecDNA in glioma.

3.1 The era of classical karyotyping

From the 1960s to the 1990s, research on ecDNA in gliomas primarily involved karyotype analysis to identify double minutes (DM) and extrachromosomal oncogenes. In 1968, Kucheria Kiran reported the first description of DM structure in gliomas. A child with subventricular glioma showed DM in six out of eleven glioma cells by karyotype analysis. DM was found in both normal and abnormal karyotypes, and the child had not received radiotherapy, indicating that the presence of DM was not related to karyotypic changes in the cell nucleus or to chromosome fragmentation[26]. In 1970, Joachim Mark analyzed karyotypes of 40 gliomas and found that three of them contained DM in almost every cell, while five showed scattered DM. The study showed that DM was not associated with specific variants in glioma cell chromosomes[27]. Joachim Mark reported similar findings in 1971 by analyzing karyotypes of a larger number of glioma cases, where DM was widespread in four out of 54 gliomas and scattered in nine. Sandra H. Bigner et al. identified seven cases containing DM out of 15 gliomas in their study[28].

The first oncogenes identified in glioma DM were EGFR and MYC. J Trent et al. found c-myc amplified and rearranged in DM of the glioma cell line SF-188[29]. By karyotyping 33 gliomas, Sandra H. Bigner et al. found that 13 out of 15 cases containing EGFR amplification contained DM. Only four of the cases without EGFR amplification contained DM, indicating that DM may be a common locus for amplified EGFR[30]. Further research found that glioma cell lines containing DM were able to maintain EGFR gene amplification in a nude mouse transplantation tumor model[31]. In 2010, Concha Lopez-Gines et al. studied and analyzed EGFR status in 40 newly diagnosed glioblastomas using fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR), and found that the EGFR copy number was significantly higher in cells with DM[32].

The early studies on DM not only identified their presence morphologically through karyotype analysis but also investigated oncogenes in DM, suggesting that DM may play an important role in promoting glioma development.

3.2 Basic functional descriptive studies of ecDNA

After the 2000s, research on the function of ecDNA intensified and progressed from the study of ecDNA oncogenomic structure, drug resistance function, and methodology. Nicolas Vogt et al. used PCR to investigate the EGFR amplification structure and repeat sequence of DM in seven gliomas. They found that all of these amplified structures were derived from simple ecDNA, that the EGFR gene-containing chromosomal fragments circularized eventually accumulated to generate ecDNA, and that the fusion of the ends of the initial amplicon was derived from a non-homologous end-joining mechanism (NHEJ) [33]. This group further identified different mechanisms involved in the formation of ecDNA in another study on the structure of glioma ecDNA oncogene. Anne Gibaud et al. studied glioma cells containing four extrachromosomal amplification motifs (7p11, 1q32.1, 5p15, and 9p2). They found that 1q32.1 was formed by linear chromosomal fragment cyclization, while 7p11, 5p15, and 9p2 were derived from interchromosomal fusions [34].

Promoting glioma drug resistance is an important function of ecDNA. On the one hand, this is due to studies that found that ecDNA carries drug resistance genes, and on the other hand, it is due to the structural features of ecDNA itself, which enhance glioma cell heterogeneity. V. Koneti Rao et al. first reported in 2005 that ecDNA carries drug

resistance genes. In mitoxantrone-treated SF-295 glioma cell lines, the drug resistance gene ABCG2 was found to be amplified by FISH through DM in the replication interval. At higher concentrations of the drug, DM decreased, and the homogeneous staining region (HSR) increased. The amplification of ABCG2 initially occurred as DM and was subsequently able to reintegrate into the chromosome to form the HSR, which functioned as a more stable form of drug resistance [35].

David A. Nathanson et al. first reported in 2013 that mutant EGFR genes in glioma cells mediate resistance to EGFR tyrosine kinase inhibitors (EGFR TKIs) through the dynamic regulation of ecDNA itself. Single-cell analysis of GBM patients revealed that GBM cells were able to reversibly regulate EGFRvIII expression for optimal growth after treatment with erlotinib. Inhibition against EGFR TKIs is achieved by removing EGFRvIII from ecDNA, and mutant EGFRvIII on ecDNA can reappear after drug withdrawal [36]. Further studies have shown that DM regulates drug resistance in GBM through a mechanism known as Amplification-Linked Extrachromosomal Mutations (ALEMs). Since ecDNA can be randomly distributed among progeny cells, mutations that favor cell proliferation can be continuously selected by the environment and accumulate rapidly, eventually allowing the cell population to reach an optimal state of adaptation to the environment [37]. The ability of ecDNA to accelerate the microevolution of primary/relapsed GBM and to participate in drug resistance regulation has been demonstrated in clinical cases. F. Favero, through a whole-genome sequencing study of a GBM patient before and after relapse, found that PI3k-related mutations occurring on DM in relapsed GBM replaced the original IDH1 and led to the failure of imatinib treatment [38]. Ke Xu et al. in another study involving children and adults with five cases of pre- and post-relapsed GBM also found that DM was involved in resistance to erlotinib and increased cellular heterogeneity in GBM [39].In 2018, Ana C. deCarvalho and colleagues conducted a systematic study to investigate the heterogeneity of glioblastoma (GBM) and its association with extrachromosomal DNA (ecDNA). Through genomic sequencing of 12 new GBM and 1 recurrent GBM samples, they found that GBM cells exhibited high heterogeneity within in situ GBM tissue, extracted neurosphere tumor cells, and cultured intracranial graft tumor models. Furthermore, they observed that cancer-promoting ecDNA can be preserved longitudinally in different models, indicating that the rapid evolution of GBM cells during treatment and recurrence is facilitated by the drive of ecDNA [40].

The emergence of novel research tools has enabled the exploration of ecDNA structure and function, and in 2017, Kristen M. Turner and colleagues developed a software tool called ECdetect to analyze whole-genome sequencing data from up to 17 species of tumor cells totaling 2572. Their analysis revealed that ecDNA was present in nearly half of the tumors and that ecDNA amplification was more effective than linear chromosome amplification in increasing oncogene copy number and intra-tumor heterogeneity, thereby contributing to accelerated tumor evolution [19].

3.3 Molecular regulatory mechanisms of ecDNA

Over the past five years, there have been significant advancements in the study of extrachromosomal DNA (ecDNA), particularly in terms of understanding its regulatory mechanisms. The internal gene regulation characteristics, gene regulation between different ecDNAs, and regulation mechanisms between ecDNA and linear chromosomes have been gradually revealed. Additionally, the necessary chromatin imaging techniques and bioinformatics tools required for ecDNA research have also gradually emerged.

A significant milestone in ecDNA research was marked in 2019 by Sihan Wu et al., who conducted a systematic study of ecDNA from structure to function in a variety of tumors containing ecDNA within the GBM of the human brain. This study utilized the AmpliconArchitect tool to analyze sequencing data to infer the loop structure of ecDNA, and combined it with scanning electron microscopy and other methods to obtain the first visual image of ecDNA and clarify its loop structure. RNA-seq and other methods were subsequently used to find that more transcripts in oncogenes are derived from ecDNA, confirming the direct cancer-promoting role of ecDNA. The study also confirmed that ecDNA has the same chromatin structure as linear DNA, but differs in histone modifications, lacking the repressive histone modification H3K9me3/H3K27me3. ATAC-seq, MNase-seq, and ATAC-see techniques further confirmed that ecDNA has higher openness. The study also analyzed the pro-oncogenic function of ecDNA from the perspective of three-dimensional chromosome morphology using Hi-C and 4C-seq techniques, concluding that the loop-like structure of ecDNA facilitates the addition of new gene regulatory loops[41].

In a separate study, Andrew R. Morton et al. conducted an in-depth study targeting EGFR and its enhancers in GBM. They found that EGFR in ecDNA interacts not only with its intrinsic enhancer but also with other related enhancers, and a "positive selection effect" of GBM oncogenes and their regulatory elements was found at the ecDNA level, which was also verified in neuroblastoma and nephroblastoma [42]. Additionally, in neuroblastoma, Konstantin Helmsauer et al. showed that MYCN amplicons on ecDNA can function on ecDNA in the absence of

local enhancers by using TAD to carry distal enhancer fragments, a phenomenon known as "enhancer hijacking." [43]

In 2021, King L et al. conducted the first investigation of the population nature of extrachromosomal DNA (ecDNA) in various tumors, including glioblastoma multiforme (GBM). They discovered that ecDNA molecules in the intercellular phase were often clustered together into dozens of "ecDNA centers" to function collectively. The trans-regulatory relationships between different oncogenes and enhancers within the centers were much more extensive and efficient than the regulation within a single ecDNA molecule [44]. Furthermore, they identified a mechanism called "ecDNA-associated phase separation" in GBM, which suggests that ecDNA can recruit RNA polymerase and transcription factors and act as mobile enhancers to participate in oncogene transcription. This reveals a direct regulatory relationship between ecDNA and chromosomal DNA for the first time [45]. Recent studies have revealed that ecDNA can play a pro-oncogenic role not only as a regulatory element but also by being modified by other molecules in tumor cells. In 2022, Erik N. Bergstrom et al. investigated the phenomenon of somatic mutations in 30 tumor cells, including GBM. They found that 31% of the ecDNA samples were associated with the APOBEC3 deaminase family Kataegis mutations. This phenomenon, known as "kyklonas," is thought to be due to the mutation of ecDNA after an attack by APOBEC3, resulting in the promotion of tumor evolution [46].

4.Concluding remarks and future perspectives

Since the earliest discovery of DM or ecDNA, ecDNA has been studied for over 60 years, advancing from basic karyotype analysis to the study of ecDNA gene regulation mechanisms. However, only in the last 5 years has ecDNA received significant attention, mainly due to recent technological advances in chromosome morphology and bioinformatics analysis, which have led to the maturation of research tools in this field. The significance of ecDNA research in glioma is primarily due to several studies demonstrating that glioma is one of the tumors with the highest ecDNA content. Moreover, several breakthrough studies on ecDNA in recent years have utilized PDX models of GBM or GBM origin, supporting the notion that ecDNA plays a unique role in glioma development. The current discovery of molecular mechanisms mediated by ecDNA has enriched the theory of tumor genetics, yet more novel questions have also arisen. For instance, while there are several theories on ecDNA production and degradation, the specific regulatory mechanisms remain unknown. Although ecDNA has a unique role in the microevolution of GBM, further investigation is needed to determine whether other mechanisms beyond passive natural selection explain the phenomenon of "positive selection." Currently, functional gene research on ecDNA primarily focuses on oncogenes, while the role of anti-oncogenes in ecDNA has been neglected. Whether ecDNA can provide more precise targets for personalized treatment of glioma requires further exploration.

Acknowledgments:

This review is supported by the funding of Shenzhen Science and Technology Innovation Commission: JCYJ20180307155043326

References

[1] Weller M, Van den Bent M, Preusser M, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of Adulthood[J]. Nat Rev Clin Oncol, 2021,18(3):170-186.
[2] Mair M J, Geurts M, Van den Bent M J, et al. A basic review on systemic treatment options in WHO grade II-III Gliomas[J]. Cancer Treat Rev, 2021, 92: 102124.
[3] Yang K, Wu Z, Zhang H, et al. Glioma targeted therapy: Insight into future of molecular Approaches[J]. Mol Cancer, 2022, 21(1):39.
[4] Xu S, Tang L, Li X, et al. Immunotherapy for glioma: Current management and future Application[J]. Cancer Lett, 2020, 476:1–12.
[5] Chen D, Le S B, Hutchinson T E, et al. Tumor Treating Fields dually activate STING and AIM2 inflammasomes to induce adjuvant immunity in Glioblastoma[J]. J Clin Invest, 2022, 132(8): e149258.
[6] Kang J H, Desjardins A. Convection-enhanced delivery for high-grade Glioma[J]. Neurooncol Pract, 2021, 9(1): 24–34.
[7] Beroukhim R, Mermel C H, Porter D, et al. The landscape of somatic Copy-number alteration across human Cancers[J]. Nature, 2010, 463(7283): 899–905.
[8] Wu S, Bafna V, Chang H Y, et al. Extrachromosomal DNA: An Emerging Hallmark in Human Cancer[J]. Annu Rev Pathol, 2022, 17(1): 367–386.

[9] HOTTA Y, A BASSEL. MOLECULAR SIZE AND CIRCULARITY OF DNA IN CELLS OF MAMMALS AND HIGHER PLANTS[J]. Proc Natl Acad Sci U S A, 1965, 53 (2): 356-362.
[10] Cox D, Yuncken C, Spriggs A. MINUTE CHROMATIN BODIES IN MALIGNANT TUMOURS OF CHILDHOOD[J]. Lancet, 1965, 1(7402):55-58.
[11] Møller H D, Parsons L, Jørgensen T S, et al. Extrachromosomal circular DNA is common in Yeast[J]. Proc Natl Acad Sci U S A, 2015, 112(24): E3114–E3122.
[12] Shoura M J, Gabdank I, Hansen L, et al. Intricate and Cell Type-Specific Populations of Endogenous Circular DNA (eccDNA) in Caenorhabditis elegans and Homo Sapiens[J]. G3 (Bethesda), 2017, 7(10): 3295–3303.
[13] Anonymous. Small circular DNA of Drosophila melanogaster: Chromosomal homology and kinetic complexity[J]. Proc Natl Acad Sci U S A,1979 76 (12): 6142-6146.
[14] Cohen S, Regev A, Lavi S. Small polydispersed circular DNA (spcDNA) in human cells: Association with genomic Instability[J]. Oncogene, 1997, 14(8): 977–985.
[15] Reddel R R. Alternative lengthening of telomeres, telomerase, and Cancer[J]. Cancer Lett, 2003, 194(2): 155–162.
[16] Shibata Y, Kumar P, Layer R, et al. Extrachromosomal MicroDNAs and Chromosomal Microdeletions in Normal Tissues[J]. Science, 2012, 336(6077): 82–86.
[17] Paulsen T, Shibata Y, Kumar P, et al. Small extrachromosomal circular DNAs, microDNA, produce short regulatory RNAs that suppress gene expression independent of canonical Promoters[J]. Nucleic Acids Res, 2019, 47(9): 4586–4596.
[18] Kumar P, Dillon L W, Shibata Y, et al. Normal and Cancerous Tissues Release Extrachromosomal Circular DNA (eccDNA) into the Circulation[J]. Mol Cancer Res, 2017, 15(9): 1197–1205.
[19] Turner K M, Deshpande V, Beyter D, et al. Extrachromosomal oncogene amplification drives tumour evolution and genetic Heterogeneity[J]. Nature, 2017, 543(7643): 122–125.
[20] Tandon I, Pal R, Pal J K, et al. Extrachromosomal circular DNAs: An extra piece of evidence to depict tumor Heterogeneity[J]. Future Sci OA, 2019, 5(6): FSO390.
[21] McClintock B. Chromosome organization and genic expression[J]. Cold Spring Harb Symp Quant Biol, 1951, 16(0): 13–47.
[22] Barr F G, Nauta L E, Davis R J, et al. In Vivo Amplification of the PAX3-FKHR and PAX7-FKHR Fusion Genes in Alveolar Rhabdomyosarcoma[J]. Hum Mol Genet, 1996, 5(1): 15–21.
[23] Carroll S M, Gaudray P, De Rose M L, 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[J]. Mol Cell Biol, 1987, 7(5): 1740–1750.
[24] Stephens P J, Greenman C D, Fu B, et al. Massive Genomic Rearrangement Acquired in a Single Catastrophic Event during Cancer Development[J]. Cell, 2011, 144(1): 27–40.
[25] Valent A, Bénard J, Clausse B, et al. In Vivo Elimination of Acentric Double Minutes Containing Amplified MYCN from Neuroblastoma Tumor Cells Through the Formation of Micronuclei[J]. Am J Pathol, 2001, 158(5): 1579–1584.
[26] Kucheria K. Double minute chromatin bodies in a Sub-ependymal Glioma[J]. Br J Cancer, 1968, 22(4): 696–697.
[27] Mark J, Granberg I. The chromosomal aberraation of Double-minutes in three Gliomas[J]. Acta Neuropathol, 1970, 16(3): 194–204.
[28] Bigner S H, Mark J, Bullard D E, et al. Chromosomal evolution in malignant human gliomas starts with specific and usually numerical Deviations[J]. Cancer Genet Cytogenet, 1986, 22(2): 121–135.
[29] Trent J, Meltzer P, Rosenblum M, et al. Evidence for rearrangement, amplification, and expression of c-myc in a human Glioblastoma[J]. Proc Natl Acad Sci U S A, 1986, 83(2): 470–473.
[30] Bigner S H, Wong A J, Mark J, et al. Relationship between gene amplification and chromosomal deviations in malignant human Gliomas[J]. Cancer Genet Cytogenet, 1987, 29(1): 165–170.
[31] Bigner S H, Schold S C, Friedman H S, et al. Chromosomal composition of malignant human gliomas through serial subcutaneous transplantation in athymic Mice[J]. Cancer Genet Cytogenet, 1989, 40(1): 111–120.
[32] Lopez-Gines C, Gil-Benso R, Ferrer-Luna R, et al. New pattern of EGFR amplification in glioblastoma and the relationship of gene copy number with gene expression Profile[J]. Mod Pathol, 2010, 23(6): 856–865.
[33] Vogt N, Lefèvre S-H, Apiou F, et al. Molecular structure of Double-minute chromosomes bearing amplified copies of the epidermal growth factor receptor gene in Gliomas[J]. Proc Natl Acad Sci U S A, 2004, 101(31): 11368–11373.

[34] Gibaud A, Vogt N, Hadj-Hamou N-S, et al. Extrachromosomal amplification mechanisms in a glioma with amplified sequences from multiple chromosome Loci[J]. Hum Mol Genet, 2010, 19(7): 1276–1285.
[35] Rao V K, Wangsa D, Robey R W, et al. Characterization of ABCG2 gene amplification manifesting as extrachromosomal DNA in Mitoxantrone-selected SF295 human glioblastoma Cells[J]. Cancer Genet Cytogenet, 2005, 160(2): 126–133.
[36] Nathanson D A, Gini B, Mottahedeh J, et al. Targeted Therapy Resistance Mediated by Dynamic Regulation of Extrachromosomal Mutant EGFR DNA[J]. Science, 2013, 343(6166): 72–76.
[37] Nikolaev S, Santoni F, Garieri M, et al. Extrachromosomal driver mutations in glioblastoma and Low-grade Glioma[J]. Nat Commun, 2014, 5(1)□5690.
[38] Favero F, McGranahan N, Salm M, et al. Glioblastoma adaptation traced through decline of an IDH1 clonal driver and Macro-evolution of a double-minute Chromosome[J]. Ann Oncol, 2015, 26(5): 880–887.
[39] Xu K, Ding L, Chang T-C, et al. Structure and evolution of double minutes in diagnosis and relapse brain Tumors[J]. Acta Neuropathol, 2018, 137(1): 123–137.
[40] deCarvalho A C, Kim H, Poisson L M, et al. Discordant inheritance of chromosomal and extrachromosomal DNA elements contributes to dynamic disease evolution in Glioblastoma[J]. Nat Genet, 2018, 50(5): 708–717.
[41] Wu S, Turner K M, Nguyen N, et al. Circular ecDNA promotes accessible chromatin and high oncogene Expression[J]. Nature, 2019, 575(7784): 699–703.
[42] Morton A R, Dogan-Artun N, Faber Z J, et al. Functional Enhancers Shape Extrachromosomal Oncogene Amplifications[J]. Cell, 2019, 179(6): 1330-1341.e13.
[43] Helmsauer K, Valieva M E, Ali S, et al. Enhancer hijacking determines extrachromosomal circular MYCN amplicon architecture in Neuroblastoma[J]. Nat Commun, 2020, 11(1):5823.
[44] Hung K L, Yost K E, Xie L, et al. ecDNA hubs drive cooperative intermolecular oncogene Expression[J]. Nature, 2021,600(7890):731-736.
[45] Zhu Y, Gujar A D, Wong C-H, et al. Oncogenic extrachromosomal DNA functions as mobile enhancers to globally amplify chromosomal Transcription[J]. Cancer Cell, 2021, 39(5): 694-707.e7.
[46] Bergstrom E N, Luebeck J, Petljak M, et al. Mapping clustered mutations in cancer reveals APOBEC3 mutagenesis of EcDNA[J]. Nature, 2022, 602(7897): 510–517.