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Review

Extrachromosomal DNA: Redefining the pathogenesis of glioblastoma

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ABSTRACT

Glioblastoma is an incurable most prevalent primary malignant brain tumor in adults. Surgery followed by radiotherapy with concomitant chemotherapy is the standard of care in patients with glioblastoma. Although, prognosis remains poor with a median survival in the range of 12–15 months. Over the decades of research has identified the gene mutation, angiogenesis, cell signaling for the development novel therapeutics. However, recent understanding on extrachromosomal DNA (ecDNA) put extra-layer of complexity in glioblastoma pathogenesis. These ecDNAs are present in significantly higher copy number in the nucleus of the cancer cells and contains several oncogenes which are instrumental for intra-tumoral genetic heterogeneity, accelerated tumor evolution and therapy resistance. In this review, we will discuss the current understanding on biogenesis, disease progression and potential therapeutic implications of ecDNAs in glioblastoma.

1. Introduction

Glioblastoma is a highly malignant and lethal primary brain tumor of the Central Nervous System (CNS). The tumors are marked with a high level of inter and intra tumoral heterogeneity, thereby making them less amenable to therapy. The heterogeneity of glioblastoma stems from multiple factors like- presence of tumor-initiating stem cells, genetic and molecular disparities. Recently, extrachromosomal DNA (ecDNA) is considered as a one of major player for glioblastoma heterogeneity and pathogenesis. Although, the karyotyping data obtained from neuroblastoma and pediatric brain tumor cells revealed evidence of small extrachromosomal DNA (ecDNA) fragments in long back [1,2]. At the time of discovery, the function and biogenesis of those ecDNA was not clear. The recent advancement of molecular biology and bioinformatic techniques has enabled us to locate several commonly expressed oncogenes in ecDNA of the cancer cells. The ecDNAs are self-replicating structures of varied sizes and are broadly categorized into microDNAs of <1Kb size or large circularized DNA of >100 Kb to 5 Mb size is known as double-minute chromosomes or ecDNAs [3,4]. They are mainly formed during the process of re-circularization and annealing that occurs during DNA replication, recombination and repair. EcDNAs contain one or multiple full genes and regulatory regions and are visible by light microscopy [5]. These ecDNA plays a significant role in tumor evolution and progression of pathogenesis. In this review, we will explore the biogenesis and oncogenesis of ecDNAs, their localization and possible link with extracellular vesicles and applications of ecDNAs to diagnosis and therapy in glioblastoma.

2. Biogenesis, localization and transport of ecDNA

The molecular mechanism of the biogenesis of ecDNA is still ambiguous. Lack of significant evidences for the formation of ecDNA leads to development of controversy around researchers. For example, earlier report claimed that levels of ecDNA increase when ongoing replication is blocked by inhibitors [6]. On the contrary, other report also suggested that ecDNA formation does not require DNA replication. However, replication slippage is considered as one of the mechanisms of ecDNA formation and it was described by a model called Origin-Dependent Inverted-Repeat Amplification (ODIRA) [7,8]. The ODIRA model suggested that a replication error can occurs at pre-existing short, interrupted, inverted repeats (Fig. 1) [7,8]. As a result of replication interruption, newly synthesized template DNA is expelled by the adjacent replication forks to generate single stranded DNA with closed loops (Fig. 1). Further, the single stranded DNA acts as template to generate double stranded ecDNA.

On the other hand, most widely accepted finding indicated that DNA repair machinery plays significant role in the formation of ecDNA [9]. During biogenesis of ecDNA, the circularization paths that are likely mediated by the repair machinery for DNA damage, such as nonhomologous end joining (NHEJ) and homologous recombination (HR). The recent findings indicated that not the HR but NHEJ DNA repair pathway

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Abbreviations

ecDNA	Extrachromosomal DNA
ODIRA	Origin-Dependent Inverted-Repeat Amplification
HR	Homologous recombination
NHEJ	Non-homologous end Joining
MYC	Myelocytomatosis
EGFR	Epidermal growth factor receptor
PDGFRA	Platelet derived growth factor receptor alpha
MET	Mesenchymal to epithelial transition
CDKs	cyclin-dependent kinases
MDM2	Mouse double minute 2 homolog
ERBB2	Erb-B2 Receptor Tyrosine Kinase 2
EVs	Extracellular vesicles
ATAC-seq	Assay for transposase-accessible chromatin using sequencing
PCR	Polymerase Chain Reaction
PDX	Patient-derived xenograft
WGS	Whole-genome sequencing

with ligase IV is responsible for sealing the DNA double-strand breaks and is a major player in ecDNA formation in glioblastoma [3,10,11]. Additionally, catastrophic events like chromothripsis shatters the chromosome and generates multiple fragments which can be re-arrange within chromosome or the release of DNA fragments can be circularized to form ecDNA (Fig. 1) [5].

Once the ecDNAs are formed, they self-replicate during the S phase of the cell cycle and generate more copies of ecDNAs [10]. The lack of centromeres on the ecDNAs causes their skewed and disproportionate distribution to daughter cells. The ecDNAs may be localized either to the nucleus or cytoplasm of tumor cells, yet in case of GBM, they mainly partition to the nucleus [10,12]. Studies indicate that ecDNAs localize to the nuclear periphery during G1 and then relocate to the nuclear interior and are amplified during early S phase [10]. It would be exciting to determine as to how ecDNAs transport back to the nucleus at the end of cell division as they lack centromeres (Fig. 1)? Do the ecDNAs contain any nuclear localization signals (NLS)? Or are there any nuclear importer molecules which facilitate their transportation back into the nucleus? Therefore, gaps remain regarding our understanding about ecDNA localization raising fundamental questions that need to be answered.

Interestingly, ecDNAs carrying EGFR or c-MYC genes have been detected in large extracellular vesicles of glioblastoma cells [13]. Hence, it is likely that the ecDNAs that are localized to the cytoplasm are transferred to other neighboring cells via extracellular vesicles or are released due to necrosis (Fig. 1). Additionally, the extracellular vesicles carrying ecDNAs may enter into blood vessels by a process called transcytosis (Fig. 1). Thus, extracellular vesicle mediated transportation of ecDNAs plays a significant role in glioblastoma pathogenesis.

3. Oncogenic role of ecDNA in glioblastoma

Recent development of AmpliconArchitect algorithm has enabled the researcher to identify the ecDNAs on basis of sequencing reads connecting amplified DNA segments [4]. The identified ecDNA elements from glioblastoma patients contained oncogenes including Myelocytomatosis (MYC) gene family, Epidermal growth factor receptor (EGFR), Platelet derived growth factor receptor alpha (PDGFRA), Mesenchymal to epithelial transition (MET) gene, cyclin-dependent kinase (CDK; CDK4 and CDK6) and Mouse double minute 2 homolog (MDM2), Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2) etc. [10,14–18]. The presence of those oncogenes were further confirmed by fluorescent in-situ hybridization (FISH) with oncogene specific probe in tumor sample, patient-derived xenograft (PDX) model as well as metaphase neurosphere of glioblastoma [10,14,15]. Additionally, ecDNA exhibits in higher copy number which refers to a marked increase in the number of oncogenes in glioblastoma. Recently, Morton el al. reported that the enhancer architecture of ecDNA mediates oncogenic transcriptional regulation. They also suggested that local enhancer elements are almost always included in the ecDNA structures along with oncogenes during the formation of ecDNA [19]. This particular finding explained how oncogenes associated with ecDNA are so highly transcribed.

There are evidences that secondary somatic mutation including point mutations, insertion and deletions (INDELs) can occur on the ecDNAs which further suggests a constantly evolving trajectory in primary tumors, upon treatment and also upon relapse in glioblastoma [10,18]. Further, uneven inheritance of ecDNA in offspring cells allows rapid increase of genomic heterogeneity during gliomagenesis, independently of chromosomal DNA alterations [10]. Additionally, oncogene amplification on ecDNA may be a driving force in tumor evolution and the development of genetic heterogeneity in glioblastoma. The extrachromosomal oncogene amplification may enable tumor to acclimatize more efficiently under variable environmental conditions that also accelerate tumor cell proliferation, survival, aggressiveness and therapy resistance [4,18].The transferring of oncogenic ecDNA via EVs to neighboring cells may orchestrate tumor microenvironment for its growth and invasion.

4. Use of EcDNA for diagnostic and therapeutic purpose

Circulating nucleic acids are being investigated as minimally invasive biomarkers for the diagnosis and prognosis of various cancer which is also called as liquid biopsy [20]. Recent study depicted that the changes in the levels of circulating nucleic acids have been associated with tumor burden and malignant progression. Therefore, current research is developing novel tool for the utilization of circulating nucleic acids for screening, prognosis of cancer. However, cell free circulating nucleic acids have very short half-life (15 min to few hours) due to presence of nucleases in blood and further they are cleared from the blood by liver and kidney [21]. On the contrary, circular DNA such as ecDNA is resistant to digestion from exonucleases, thus making it more stable and likely to retain longer than linear DNA after release from cells into the blood [22]. The presence of ecDNA in blood provides evidence that the cell free ecDNAs may also serve as potential biomarkers for early detection and outcome prediction [23]. Interestingly, circular DNAs are reported to be secreted into blood from both healthy tissues as well as tumor tissues and which are the major challenging factor for liquid biopsy.

However, Kumar et al. showed that ecDNA from the tumor is significantly longer in size as compared to the normal tissue-derived ecDNA [24]. They also observed that shortening the length of circulating ecDNA after surgery may indicate a good outcome, and subsequent lengthening of the circulating ecDNA may indicate recurrence of the disease. Further, using an assay for transposase-accessible chromatin using sequencing (ATAC-seq), Kumar et al. identified more than 18,000 shorter microDNAs and longer ecDNAs in glioma cell lines and many of longer ecDNAs carrying known cancer driver genes including EGFR gene that were also validated by inverse PCR and by metaphase FISH [25]. They also claimed that ATAC-seq can be used to detect ecDNA present in a tumor at the pre-amplification stage and to predict therapeutic resistance [25]. Recent report also shown that the presence of ecDNA based oncogene amplification had significantly shortened survival of patients as compared to the patients whose cancers were not driven by ecDNA [26]. However, the rigorous study is necessary for the unleashing potential of ecDNA for future use as prognostic markers.

The detailed understanding the role of ecDNA in tumor evolution, oncogene amplification, may help to identify more effective treatments that will prevent cancer progression and better survival [4]. Modern research is developing innovative new therapies targeted to ecDNA in various cancers [27]. Consequently, medicines capable of targeting

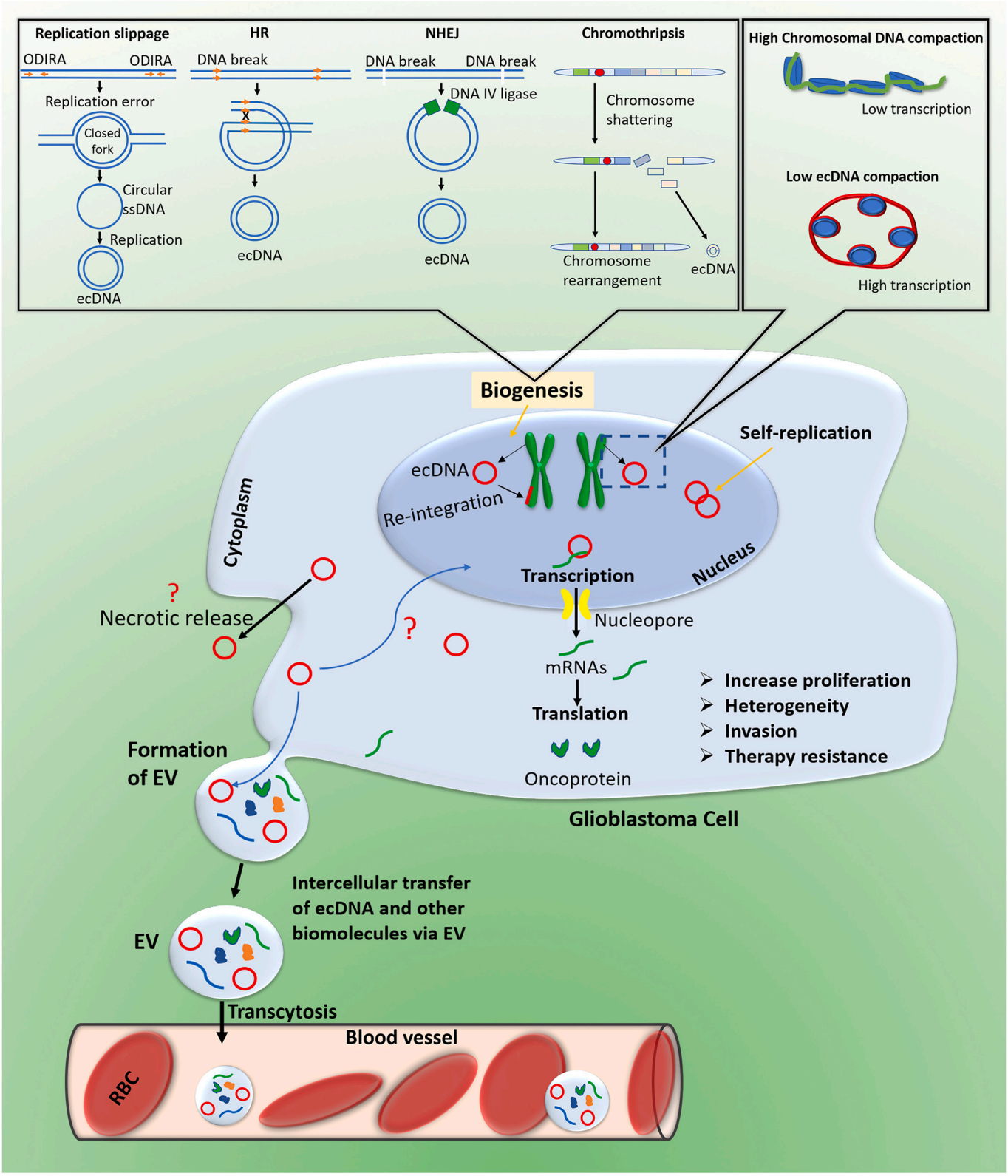

Fig. 1. Overview of ecDNA biogenesis and its role in glioblastoma progression. The formation of ecDNA is mediated by replication slippage, HR, NHEJ mechanism and chromothripsis. EcDNA can be re-integrated into the chromosome under various environmental condition. EcDNA shows low compaction and high transcription as compared to chromosomal DNA. Further, newly formed ecDNA can self-replicate to increase the copy number and also able to produce mRNAs for several oncogenes. Newly synthesized mRNA exported to cytoplasm via exportin for the production of oncoproteins. However, it is unclear how ecDNA enter into the nucleus at the end of cell division. Possibly, a small number of ecDNA resides in the cytoplasm and transfer to other cells (locally or distally) through extracellular vesicles (EVs). On the other hand, EVs can enter into blood vessels by the process called transcytosis. EcDNA can be released by necrotic glioblastoma cells.

ecDNA will be new avenue in glioblastoma treatment.

5. Conclusion and future perspective

Since the discovery, we have gained significant knowledge about tumor-associated ecDNA and its contribution to malignancy. However, the molecular mechanism of cellular secretion of ecDNA has not been studied. Additionally, the source of the ecDNAs in blood of healthy individual is unclear. Therefore, the analysis of the post biogenesis modification on the ecDNA will help to identify epigenetic marks that may indicate their tissue of origin in future. Further, studies on extracellular vesicle-mediated transfer of ecDNA are crucial to understanding pathogenic signaling in glioblastoma.

Recent studies also observed several interesting phenomena which are still ambiguous in ecDNA mediated pathogenesis. For an instance, Decarvalho et al. showed that oncogenic ecDNA amplifications dramatically decreased in the neurosphere cultures and recurred at high frequency after intracranial implantation [10]. This particular characteristic of ecDNA also suggests that the ecDNA could be formed from chromosome on an as-needed basis or a specific microenvironment triggers it biogenesis and consequent re-integration. However, the molecular switch between the appearance and disappearance of ecDNA in glioma cells under a specific environment are yet to be elucidated.

Whole-genome sequencing (WGS) datasets combined with sequencing of neuroblastoma cells derived ecDNA structures identified non-coding regions along with oncogenes [16]. This particular finding is in line with another observation related to the presence of the enhancer element in ecDNA by Scacheri group [19]. Additionally, we can speculate that the noncoding nucleotide sequences of ecDNA may transcribe into non-coding RNA which may play a critical role in transcriptional regulation in ecDNA as well as chromosomal genes. Moreover, ecDNA gains significant attention of researcher worldwide and further study is indispensable to decipher the molecular details of ecDNA mediated pathogenesis in glioblastoma.

Author contribution

AM and AS: Conceptualize the topic; AM: Writing-original draft and graphical image preparation; AS: Writing-manuscript and editing.

Declaration of Competing Interest

The authors declare no competing interests.

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