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REVIEW ARTICLE

THE MULTIFACETED ROLE OF EXTRACHROMOSOMAL DNA IN CANCER: FUNCTIONS AND CLINICAL IMPLICATIONS

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Abstract:

The Extrachromosomal DNA (ecDNA) in cancer biology has emerged as a critical driver, enhancing the knowledge about tumorigenesis, progression and treatment. The current review article explores the multifaceted role of Extrachromosomal DNA, focusing on its ability to amplify oncogenes, enhance tumor heterogenicity, and support cancer cell survival and understanding therapeutics pressure. Moreover, the unique transcriptional and replication dynamics of ecDNA with linking the uneven segregation during cell division, create a significant environment for the understanding of genetic diversity and adaptive evolution in tumors. In clinics the ecDNA holds significant potential as a biomarker for cancer, diagnosis, treatment, therapeutic monitoring and progenies. The higher level of ecDNA correlates with poor clinical outcomes and resistance to therapies, emphasizing its prognostic value. The current therapeutic techniques targeting ecDNA specific vulnerabilities, such as disrupting replication stress pathways or silencing the transcriptional activity, represents promising ways for cancer treatment This review also highlights the potential of circulating ecDNA in liquid biopsies as a non-invasive diagnostic and monitoring tool. Despite these advances, challenges such as standardizing detection methods and understanding ecDNA diverse roles across cancer types remain. Addressing these gaps will unlock new opportunities in precision oncology, making ecDNA a cornerstone of future cancer diagnostics and therapeutics.

Keywords: *Extrachromosomal DNA, Cancer, diagnosis, biomarker, liquid biopsy and treatment.*

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Introduction:

The extrachromosomal DNA (ecDNA) is a distinct form of DNA found outside the chromosomes in eukaryotic cells. The ecDNA is typically rare in normal cells, and prevalent in various cancers, where it initiates the amplification of oncogenes and enhances malignancy[1,2]. The ecDNA lacks centromere, allowing unequal segregation during cell division which helps in tumor heterogenicity[3]. The current review article was conducted to investigate the biological applications of ecDNA and its growing demands in clinics for cancer diagnosis and therapeutics [4]. The research on ecDNA is not novel, from decades research has been conducted on ecDNA. Although the role of ecDNA in cancer has recently emerged and gained attention[5]. The traditional genetic analysis of cancer was related to the study of chromosomes such as deletion, insertion, mutations, and translocation[6]. However, the current advances in genomic technologies have revolved role of ecDNA in cancer genomics, and provide an additional part in complexity.

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Extrachromosomal DNA is a circular molecule, lacking centromere and telomere which are the characteristics of chromosomal DNA[7]. Due to this structure, the ecDNA has unique replication and segregation dynamics that distinguish ecDNA from chromosomal DNA[2]. Furthermore, the circular nature of ecDNA facilitates the accommodation of amplified oncogenes and regulatory elements, allowing high transcriptional activity without chromosomal context[8]. Additionally, the absence of centromeres contributes to its uneven inheritance during cell division, driving genetic diversity within tumors[9]. The formation of ecDNA is thought to arise from chromosomal DNA through mechanisms such as double-strand breaks and faulty DNA repair pathways, including non-homologous end joining (NHEJ)[10]. These processes can excise segments of the genome, which circularize to form ecDNA. This phenomenon is particularly common in cancers with high genomic instability, where DNA repair mechanisms are frequently compromised (Figure 1).

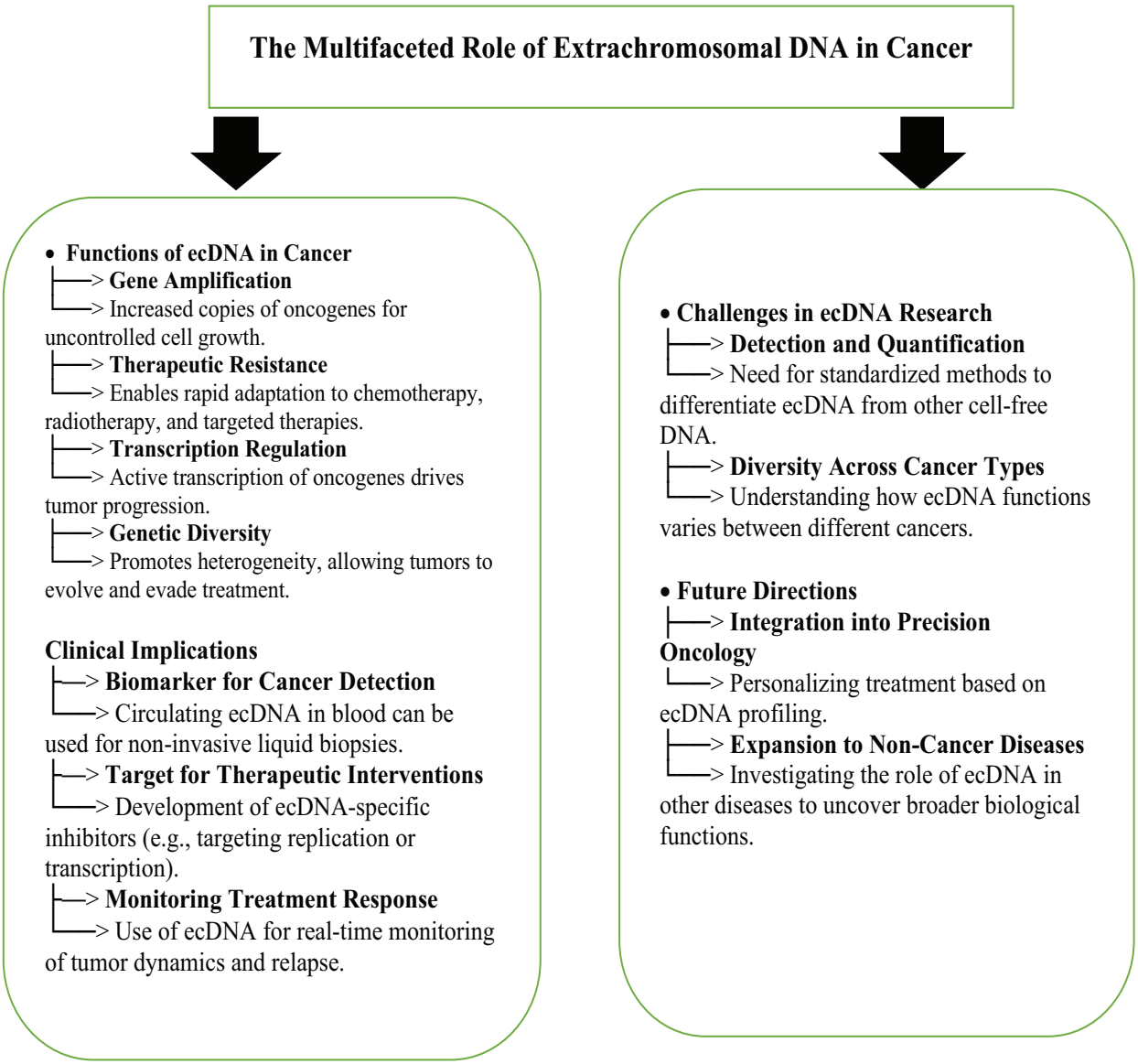

Figure 1. Flowchart provides a clear and concise overview of ecDNA's role in cancer, from its biological functions to its clinical implications and future research directions.

EcDNA is present in a variety of cancers, including glioblastoma, lung cancer, and neuroblastoma, and is often associated with aggressive disease phenotypes[3]. Studies have shown that tumors with ecDNA amplification of oncogenes exhibit higher growth rates, metastatic potential, and resistance to therapy compared to those with chromosomal amplification[11]. These properties underscore the clinical importance of ecDNA in cancer progression. The flexibility of ecDNA allows cancer cells to rapidly evolve and adapt to changing environmental and therapeutic conditions[12]. For instance, ecDNA-mediated amplification of resistance genes can provide a survival advantage under targeted therapy, contributing to relapse nd treatment failure[13]. Furthermore, the presence of ecDNA has been linked to increased tumor heterogeneity, a hallmark of cancer that complicates treatment[14]. Extrachromosomal DNA represents a powerful driver of cancer progression and therapy resistance. Its unique biological functions, coupled with its clinical potential, make it a compelling target for future research. By unraveling the mysteries of ecDNA, we can pave the way for novel diagnostic tools and therapeutic strategies, transforming the landscape of cancer care. In this review we discuss the Multifaceted Role of Extrachromosomal DNA in Cancer: Functions and Clinical Implications

Functions of ecDNA in Cancer:

Extrachromosomal DNA (ecDNA) is circular DNA that plays an important role in the development and heterogeneity of cancer[1,15]. The rapid evolution of methods to detect ecDNA, including microscopic and sequencing approaches, has greatly enhanced our knowledge of the role of ecDNA in cancer development and evolution[16]. Here, we review the molecular characteristics, functions, mechanisms of formation, and detection methods of ecDNA, with a focus on the potential clinical implications of ecDNA in cancer. Specifically, we consider the role of ecDNA in acquired drug resistance, as a diagnostic and prognostic biomarker, and as a therapeutic target in the context of cancer. As the pathological and clinical significance of ecDNA continues to be explored, it is anticipated that ecDNA will have broad applications in the diagnosis, prognosis, and treatment of patients with cancer (Figure 2).

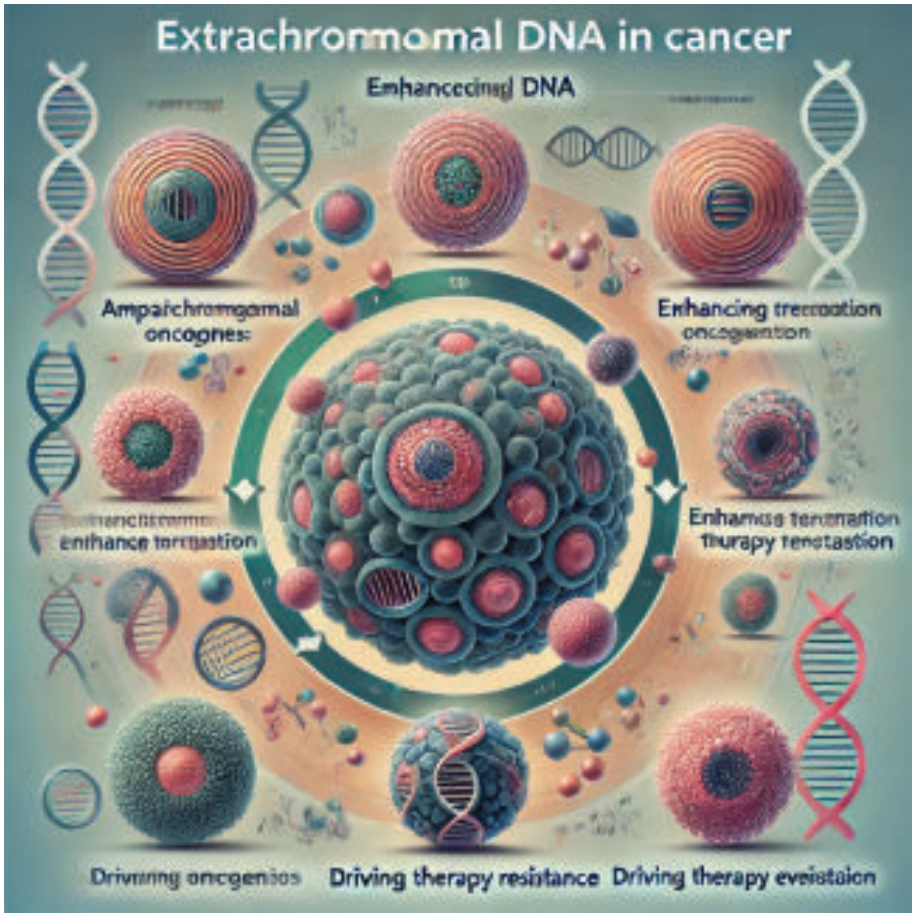

Figure. 2. *Schematic overview of the functions of extrachromosomal DNA (ecDNA) in cancer*

Figure. 2.

Oncogene Amplification:

Extrachromosomal DNA (ecDNA) has recently been recognized as a major contributor to cancer pathogenesis that is identified in most cancer types and is associated with poor[17,18]. When it was discovered over 60 years ago, ecDNA was considered to be rare, and its impact on tumour biology was not well understood[5]. The application of modern imaging and computational techniques has yielded powerful new insights into the importance of ecDNA in cancer[17]. The non-chromosomal inheritance of ecDNA during cell division results in high oncogene copy number, intra-tumoural genetic heterogeneity and rapid tumour evolution that contributes to treatment resistance and shorter patient survival[2] . In addition, the circular architecture of ecDNA results in altered patterns of gene regulation that drive elevated oncogene expression, potentially enabling the remodelling of tumour genomes[19]. The generation of clusters of ecDNAs, termed ecDNA hubs, results in interactions between enhancers and promoters in Trans, yielding a new paradigm in oncogenic transcription[2]. One of the most prominent roles of ecDNA in cancer is the amplification of oncogenes such as **MYC**, **EGFR**, and **CDK4**[3,20]. Unlike chromosomal amplifications, ecDNA-mediated amplification allows rapid and high-level oncogene expression, fueling aggressive tumor growth. For example, in glioblastoma, EGFR amplification via ecDNA correlates with poor prognosis and therapy resistance.

Tumor Heterogeneity and Evolution:

Extrachromosomal DNA (ecDNA) are circular regions of DNA that are found in many cancers. They are an important means of oncogene amplification, and correlate with treatment resistance and poor prognosis[16]. Consequently, there is great interest in exploring and targeting ecDNA vulnerabilities as potential new therapeutic targets for cancer treatment[17]. However, the biological significance of ecDNA and their associated regulatory control remains unclear. Light microscopy has been a central tool in the identification and characterisation of ecDNA[18]. The absence of centromeres in ecDNA facilitates its uneven distribution during cell division[17]. This unique property promotes genetic diversity within the tumor microenvironment, enabling adaptive evolution. Such heterogeneity poses significant challenges for targeted therapies, as subpopulations of ecDNA-enriched cells may evade treatment and drive relapse.

Transcriptional Regulation:

Enhancers are noncoding DNA sequences responsible for orchestrating gene expression programs by interacting with transcription factors and chromatin regulators within complex genome structures[21]. However, their fundamental functions can be disrupted by genetic and epigenetic alterations, leading to aberrant enhancer activation or rewiring that contributes to oncogensis[22]. Analyzing dysregulated enhancer landscapes reveals new subtype-defining genomic features, such as enhancer hijacking, and identifies disease-relevant transcriptional regulators as potential targets for developing enhancer-targeting therapeutic strategies[23]. EcDNA contributes to the transcriptional landscape of cancer cells by forming super-enhancers that boost oncogene expression[24]. This burst-like transcriptional activity is often decoupled from chromosomal regulatory mechanisms, giving cancer cells a growth advantage. Moreover, the epigenetic plasticity of ecDNA enables dynamic responses to environmental and therapeutic pressures.

Replication and Segregation Dynamics:

Extrachromosomal DNA (ecDNA) presents a major challenge for cancer patients. ecDNA renders tumours treatment resistant by facilitating massive oncogene transcription and rapid genome evolution, contributing to poor patient survival[25]. At present, there are no ecDNA-specific treatments[26]. Here we show that enhancing transcription–replication conflict enables targeted elimination of ecDNA-containing cancers. Stepwise analyses of ecDNA transcription reveal pervasive RNA transcription and associated single-stranded DNA, leading to excessive transcription–replication conflicts and replication stress compared with chromosomal loci (Tang et al., 2024). Nucleotide incorporation on ecDNA is markedly slower, and replication stress is significantly higher in ecDNA-containing tumours regardless of cancer type or oncogene cargo. pRPA2-S33, a mediator of DNA damage repair that binds single-stranded DNA, shows elevated localization on ecDNA in a transcription-dependent manner, along with increased DNA double strand breaks, and activation of the S-phase checkpoint kinase, CHK1. Genetic or pharmacological CHK1 inhibition causes extensive and preferential tumour cell death in ecDNA-containing tumours[25]. The replication of ecDNA occurs independently of the cell cycle, leading to rapid proliferation of ecDNA-bearing cells. During mitosis, ecDNA attaches to chromosomal regions or nuclear structures, ensuring its persistence in daughter cells. This mechanism underpins the sustained oncogene amplification observed in ecDNA-driven cancers.

Drug Resistance and Survival:

Recent research has unveiled fascinating insights into the intricate mechanisms governing tumor evolution[27]. These studies have illuminated how tumors adapt and proliferate by exploiting various factors, including immune evasion, resistance to therapeutic drugs, genetic mutations, and their ability to adapt to different environments[12]. Furthermore, investigations into tumor heterogeneity and chromosomal aberrations have revealed the profound complexity that underlies the evolution of cancer[12]. Emerging findings have also underscored the role of viral influences in the development and progression of cancer, introducing an additional layer of complexity to the field of oncology[28]. Tumor evolution is a dynamic and complex process influenced by various factors, including immune evasion, drug resistance, tumor heterogeneity, and viral influences[29]. Understanding these elements is indispensable for developing more effective treatments and advancing cancer therapies[30]. A holistic approach to studying and addressing tumor evolution is crucial in the ongoing battle against cancer[31]. The main goal of this comprehensive review is to explore the intricate relationship between tumor evolution and critical aspects of cancer biology[32]. By delving into this complex interplay, we aim to provide a profound understanding of how tumors evolve, adapt, and respond to treatment strategies. EcDNA is a key contributor to therapeutic resistance. By harboring amplified oncogenes and stress-response elements, ecDNA enables cancer cells to survive targeted therapies. For instance, ecDNA-driven amplification of EGFR has been linked to resistance against tyrosine kinase inhibitors in non-small cell lung cancer (Figure 3).

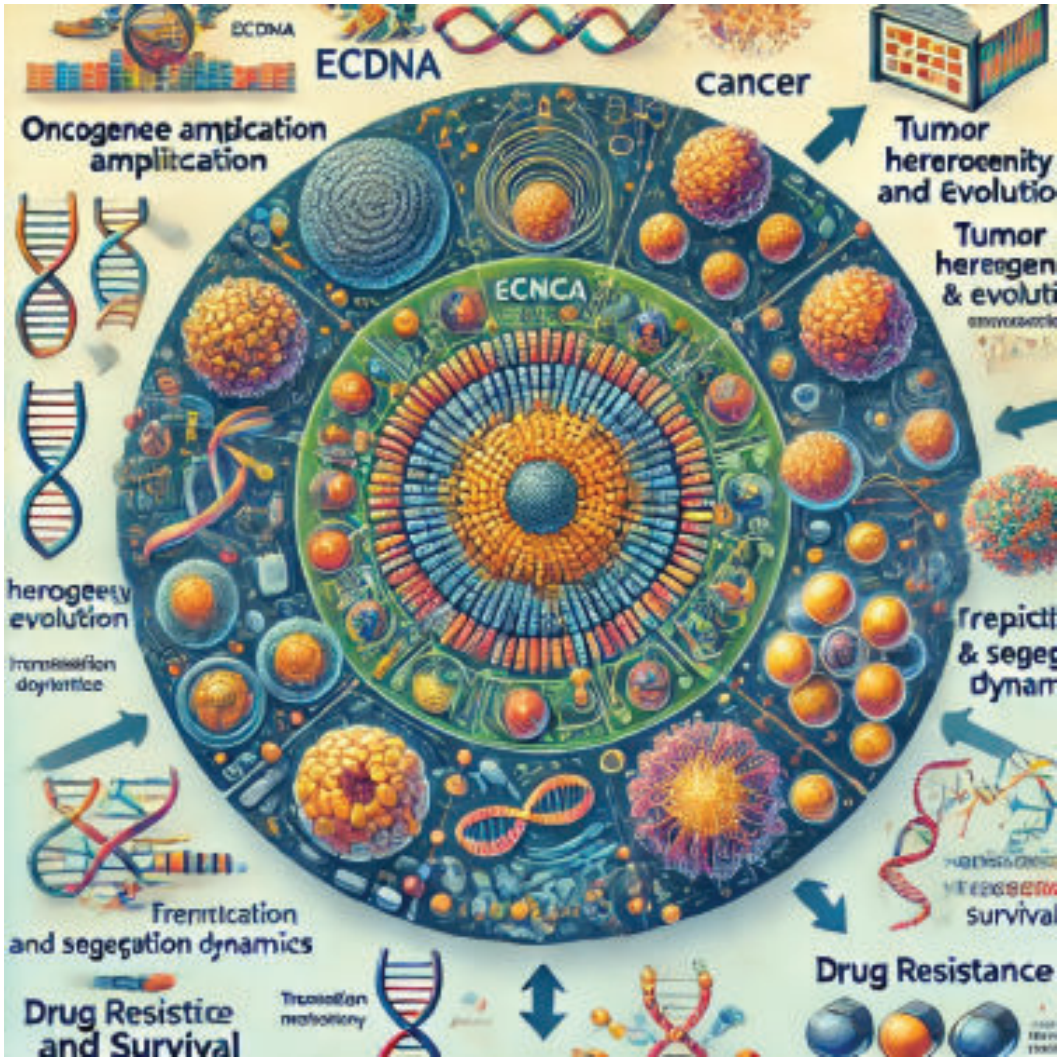

Figure. 3. *Illustration depicting the roles of ecDNA in cancer, highlighting its five key functions.*

Clinical Implications of ecDNA:

Diagnostic Potential

Nowadays, with the rapid development of sequencing and bioinformatics, the accuracy of eccDNAs detection has significantly improved[33]. This advancement has consequently enhanced the feasibility of exploring the biological characteristics and functions of eccDNAs [33]. This review elucidates the potential mechanisms of eccDNA generation, the existing methods for their detection and analysis, and their basic features. Furthermore, it focuses on the biological functions of eccDNAs in regulating gene expression under both physiological and pathological conditions. Additionally, the review summarizes the clinical implications of eccDNAs in human cancers and health. The presence of ecDNA is a hallmark of certain aggressive cancers. Advanced imaging techniques, such as fluorescence in situ hybridization (FISH), and sequencing technologies, including Circle-Seq, allow for the detection of ecDNA in tumor tissues[34]. These methods have the potential to improve early cancer diagnosis and stratify patients based on ecDNA burden (Table 1).

Prognostic Value:

Extrachromosomal circular DNA (eccDNA) is defined as a type of circular DNA that exists widely in nature and is independent of chromosomes. EccDNA has attracted the attention of researchers due to its broad, random distribution, complex biogenesis and tumor-relevant functions. EccDNA can carry complete gene information, especially the oncogenic driver genes that are often carried in tumors, with increased copy number and high transcriptional activity[25]. The high overexpression of oncogenes by eccDNA leads to malignant growth of tumors. Regardless, the exact generation and functional mechanisms of eccDNA in disease progression are not yet clear. There is, however, an emerging body of evidence characterizing that eccDNA can be generated from multiple pathways, including DNA damage repair pathways, breakage-fusion-bridge (BFB) mechanisms, chromothripsis and cell apoptosis, and participates in the regulation of tumor progression with multiplex functions[34]. This up-to-date review summarizes and discusses the origins, biogenesis and functions of eccDNA, including its contribution to the formation of oncogene instability and mutations, the heterogeneity and cellular senescence of tumor cells, and the proinflammatory response of tumors. We highlight the possible cancer-related applications of eccDNA, such as its potential use in the diagnosis, targeted therapy and prognostic assessment of cancer. EcDNA abundance is often associated with poor clinical outcomes. Studies have demonstrated that high levels of ecDNA correlate with increased tumor aggressiveness, metastasis, and resistance to therapy. Monitoring ecDNA dynamics could provide insights into disease progression and recurrence risk (Table 1).

Therapeutic Resistance and Relapse:

Extrachromosomal DNA (ecDNA) plays a crucial role in cancer's ability to resist treatment and relapse after therapy [5]. This form of DNA exists outside the chromosomes, often carrying multiple copies of oncogenes or other genetic elements that drive tumor growth. When tumors are exposed to chemotherapy, radiotherapy, or targeted treatments, the presence of ecDNA allows cancer cells to rapidly adapt by amplifying key genes, mutating them, or altering their expression[17]. This makes it easier for the tumor to circumvent the effects of therapy, resulting in therapeutic resistance. The ability of ecDNA to rapidly evolve and provide genetic diversity within the cancer cell population makes it a major factor in treatment failure and relapse. To address this challenge, strategies that target ecDNA replication and transcription are being explored as potential therapies. By inhibiting the formation or replication of ecDNA, researchers hope to prevent tumors from adapting so quickly to treatment, ultimately improving the effectiveness of conventional therapies. Disrupting ecDNA's role in gene amplification and expression could significantly reduce the likelihood of resistance developing, thereby decreasing the chances of relapse. This approach is seen as a promising avenue for enhancing cancer treatment outcomes, particularly in cases where tumors have become resistant to existing therapies (Table 1).

Targeting ecDNA in Therapy:

Emerging therapeutic strategies are focused on exploiting the unique vulnerabilities of ecDNA to combat its role in therapeutic resistance. One promising approach involves targeting replication stress pathways, which are crucial for the replication of ecDNA. Inhibitors of proteins like ATR (ataxia telangiectasia and Rad3-related) and CHK1 (checkpoint kinase 1) are being investigated for their ability to disrupt the replication of ecDNA [36]. These inhibitors work by inducing replication stress in cancer cells, preventing the efficient replication of ecDNA and reducing the ability of tumors to adapt to treatment. This approach holds promise in enhancing the effectiveness of conventional therapies and overcoming resistance mechanisms driven by ecDNA. Another strategy focuses on the epigenetic regulation of ecDNA[37]. Since ecDNA often contains amplifications of oncogenes that are actively transcribed to promote tumor growth, silencing this transcription could inhibit the tumor's ability to survive and proliferate. Epigenetic modulators are being studied for their ability to alter the chromatin state of ecDNA, effectively silencing its transcription and reducing its contribution to cancer progression[38]. By targeting the mechanisms that control ecDNA expression, these treatments aim to weaken the tumor's adaptive capabilities and improve the overall response to therapies, potentially reducing the likelihood of relapse (Table 1).

Liquid Biopsy and Non-Invasive Monitoring:

Circulating ecDNA in bodily fluids, such as blood, has emerged as a potential non-invasive biomarker for cancer detection and monitoring. Unlike traditional tissue biopsies, which can be invasive and difficult to perform regularly, liquid biopsies using blood samples allow for easier tracking of cancer's presence and progression[39]. EcDNA, released into the bloodstream as a result of tumor cell death or secretion, can contain genetic material that mirrors the tumor's characteristics. By analyzing this circulating ecDNA, clinicians can gain valuable insights into the genetic makeup of a tumor, monitor its response to treatment, and detect early signs of relapse[40]. However, distinguishing ecDNA from other forms of cell-free DNA (cfDNA), such as fragments from healthy cells, remains a significant challenge. The detection and isolation of ecDNA require highly sensitive and specific methods to avoid contamination from cfDNA that is not tumor-derived[41]. Advances in sequencing technologies are expected to overcome this hurdle, enabling more accurate and routine use of liquid biopsies for cancer detection. With improved sequencing techniques, it may become possible to differentiate ecDNA from other DNA fragments, making liquid biopsies a powerful tool in cancer diagnostics and treatment monitoring in the near future (Table 1).

Current Gaps and Challenges:

Despite the promising potential of ecDNA as a biomarker and therapeutic target, there are several hurdles that need to be overcome to fully realize its clinical applications. One major challenge is the lack of standardized methods for detecting and quantifying ecDNA. The sensitivity and specificity required to isolate ecDNA from other forms of cell-free DNA (cfDNA) are not yet fully established, making it difficult to use this biomarker in routine clinical practice[3]. The development of reliable, widely accepted protocols for ecDNA detection will be critical for its integration into cancer diagnostics and treatment monitoring. Additionally, ecDNA functions can vary significantly across different cancer types, adding another layer of complexity to its therapeutic targeting[3]. While ecDNA plays a role in promoting therapeutic resistance and tumor progression, its exact mechanisms and contributions can differ depending on the genetic landscape of the tumor. A deeper understanding of how ecDNA operates in various cancers is essential for developing targeted therapies that can effectively disrupt its function. Addressing these challenges will require interdisciplinary collaboration between researchers, clinicians, and technology developers, as well as innovative advances in sequencing, bioinformatics, and therapeutic approaches to unlock the full potential of ecDNA in cancer treatment.

Table 1

Summarizing the clinical implications of ecDNA in cancer, highlighting its role in tumor progression, resistance, and potential as a diagnostic and therapeutic target

Aspect	Implications	Description
Oncogene Amplification	Enhanced Tumor Progression	ecDNA enables rapid and high-level amplification of oncogenes, driving aggressive tumor growth and proliferation.
Tumor Heterogeneity	Increased Complexity of Tumors	ecDNA promotes genetic diversity within tumor cells, contributing to tumor heterogeneity and resistance to targeted therapies.
Therapy Resistance	Adaptation to Anti-Cancer Treatments	Tumors with ecDNA can bypass therapeutic stress by amplifying resistance genes or altering transcriptional programs.
Prognostic Marker	Association with Poor Prognosis	High levels of ecDNA correlate with worse clinical outcomes, including increased recurrence rates and lower overall survival.
Dynamic Evolution	Rapid Adaptation and Evolution	ecDNA allows tumors to adapt quickly to environmental pressures, including immune responses and therapeutic interventions.
Diagnostic Potential	Biomarker for Liquid Biopsies	Circulating ecDNA in blood or other bodily fluids can serve as a non-invasive biomarker for cancer detection and monitoring.
Target for Novel Therapies	Opportunities for Targeted Treatments	Inhibiting ecDNA replication, segregation, or transcription may provide novel approaches to limit tumor growth and resistance.
Metastatic Potential	Facilitated Spread of Cancer	ecDNA-related oncogene amplification can enhance cellular invasion and migration, increasing the likelihood of metastasis.
Epigenetic Regulation	Alteration of Gene Expression	The presence of ecDNA can modify the epigenetic landscape of cancer cells, influencing gene expression without changing the underlying DNA sequence.
Patient Stratification	Personalized Treatment Strategies	Profiling ecDNA content can help stratify patients for therapies tailored to their specific genetic and epigenetic tumor profiles.

Future Directions:

The future of ecDNA research holds great promise, particularly in the realm of precision oncology. As our understanding of ecDNA deepens, it is becoming clear that targeting this form of DNA could play a pivotal role in revolutionizing cancer treatment. Developing ecDNA-specific inhibitors to prevent its replication or transcription could provide a new class of therapies aimed at overcoming therapeutic resistance, while improving detection techniques will allow for more precise and non-invasive cancer monitoring through liquid biopsies[3]. By leveraging ecDNA as a biomarker, clinicians could gain real-time insights into the genetic evolution of tumors, enabling more personalized and timely treatment adjustments. This would not only improve cancer diagnostics but also optimize therapeutic strategies based on the tumor's specific genetic profile. Moreover, expanding ecDNA research beyond cancer could uncover broader biological roles for this enigmatic DNA form. While much of the focus has been on its involvement in cancer progression and resistance, ecDNA may play a role in other diseases as well, offering potential insights into its involvement in various pathological processes. This broader scope of study could reveal new therapeutic avenues for non-cancer diseases, expanding the potential impact of ecDNA research across the medical field. As research progresses, interdisciplinary collaboration and technological advancements will likely lead to significant breakthroughs that shape the future of both cancer treatment and our understanding of ecDNA's biological functions.

Conclusion:

Extrachromosomal DNA (ecDNA) has emerged as a powerful driver of cancer progression and therapy resistance, offering both challenges and opportunities for cancer treatment. Its ability to harbor and amplify oncogenes, as well as its role in enabling rapid genetic evolution within tumors, makes ecDNA a central player in the development of resistance to chemotherapy, radiotherapy, and targeted treatments. The unique biological functions of ecDNA—such as its involvement in gene amplification, transcription regulation, and rapid adaptation to environmental pressures—highlight its critical role in cancer survival and relapse. As a result, understanding and targeting ecDNA has the potential to significantly alter how we approach cancer therapy. Given its clinical significance, ecDNA presents an exciting frontier for future research, with the potential to lead to novel diagnostic tools and therapeutic strategies. Advances in detection methods, such as liquid biopsies, could enable non-invasive monitoring of cancer progression and response to treatment by analyzing ecDNA in bodily fluids. Additionally, developing targeted therapies that disrupt ecDNA replication or transcription could overcome the challenge of therapy resistance, offering more effective and personalized treatment options for patients. By unraveling the mysteries of ecDNA, researchers and clinicians can transform the landscape of cancer care, offering new avenues for early detection, precision medicine, and the fight against resistant and relapsed cancers.

Author Contribution:

Shafee Ur Rehman design the idea, collect the information and wrote the manuscript.

Conflict of Interest:

The author declare that they have no conflict of Interest

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