APJAI
Asian Pacific Journal of
Allergy and Immunology

REVIEW ARTICLE

Extrachromosomal circular DNA (eccDNA) in immune regulation and autoimmunity: From mechanisms to clinical applications

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Abstract

Extrachromosomal circular DNA (eccDNA) has transitioned from genomic curiosity to a pivotal regulator at the intersection of genomics and immunology. This review synthesizes recent advances in eccDNA biology, elucidating its integral roles in both innate and adaptive immunity and its pathogenic contributions to autoimmune diseases. eccDNA arises from diverse genomic events, including DNA damage repair, replication stress, and chromothripsis. In innate immunity, it functions as a potent damage-associated molecular pattern (DAMP), activating cytosolic sensors like cGAS-STING and AIM2 to drive type I interferon and pro-inflammatory cytokine responses. In the adaptive immune system, eccDNA is not merely a byproduct of processes such as V(D)J recombination (e.g., TRECs, KRECs); it also acts as an active modulator regulating immune gene expression via enhancer-like activity, influencing antigen presentation, and shaping T and B cell development. Critically, aberrant eccDNA accumulation is implicated in the pathogenesis of autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis. Here, plasma eccDNA levels not only correlate with clinical disease activity indices but also track with therapeutic response, positioning eccDNA as a powerful non-invasive biomarker. Targeting eccDNA biogenesis or its sensing pathways thus represents a promising therapeutic frontier for restoring immune homeostasis. Future research integrating single-cell omics and longitudinal profiling is poised to dissect cell-specific functions and unlock the full clinical potential of eccDNA. Collectively, these findings establish eccDNA as both a functional regulator and a diagnostic tool, offering novel insights into the fundamental interplay between genomic integrity and immune function.

Key words: eccDNA, extrachromosomal circular DNA, innate immunity, adaptive immunity, biomarker

Citation:
Le, H. B. C., Somsri, K., Bhunyakarnjanarat, T., Suksamai, C., Wimon, S., Do, H. T., Pedcharat, S., Hirankarn, N., Leelahavanichkul, A. (2026). Extrachromosomal circular DNA (eccDNA) in immune regulation and autoimmunity: From mechanisms to clinical applications.
Asian Pac J Allergy Immunol, 44(1), 1-16.
https://doi.org/10.12932/ap-180725-2123

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Introduction

Autoimmune disorders comprise a diverse group of medical conditions arising from immune system dysregulation, in which B and T lymphocytes aberrantly target and destroy healthy cells.[1] Advances in autoimmunity have principally relied on the utilization of antibodies as pivotal tools for pathogenesis investigation, as well as for informing diagnostic and prognostic assessments. The multidimensional nature of autoimmune conditions raises significant challenges across the healthcare spectrum. Clinicians are often confronted with the intricacies of establishing accurate diagnoses, as patients frequently present with generalized symptoms possibly attributed to various underlying conditions requiring distinct treatment regimens. Researchers simultaneously contend with discerning whether autoimmune responses are directly caused by disease pathogenesis or are due to a secondary reaction. From a public health standpoint, healthcare infrastructures are squeezed on one side by demands to implement cost-effective and efficient methods for early detection and on the other the obligation to ensure effective disease management.[1]

Extrachromosomal circular DNA (eccDNA) is broadly defined as any double-stranded circular DNA molecule, from 0.017 kb – 9.9 Mb, located internally within the nuclei or externally outside the linear chromosomal genome. eccDNAs are not confined to centromeres but in fact circulate throughout the body in both normal and malignant tissues, thus enabling them to independently replicate. eccDNA characteristics contrast with those of linear chromosomal DNA which consists of multiple pole-shaped chromosomes with dissimilar ends.[2]

eccDNAs originate from various genomic regions, including introns, exons, and both repetitive and regulatory sequences. eccDNA detection positively correlates to the presence of malignancies or stressors, which in turn promote genomic instability, altered gene expression, and gene amplification. In the context of malignancies, eccDNA modulates oncogene expression and immune cell expression. Telomeric circle (t-circles), a type of eccDNA, has been utilized as a biomarker for alternative lengthening of telomeres (ALT) pathway activity, especially in suspected osteosarcoma and glioblastoma. Traditional research in eccDNA has thus focused on cancer for these reasons.[3]

Recent discoveries suggest that eccDNA has broader immunological relevance as a molecular effector rather than just a mere niche in oncology-related aspects as it activates immune responses in scenarios relevant to aging and genetics, in neurological and autoimmune diseases, and in drug resistance.[4] eccDNA additionally serves as a biomarker for immune-associated diseases as evident through cyclic GMP-AMP synthase (cGAS) innate immune sensor and Stimulator of Interferon Genes (STING) pathway activations.[5,6] Indeed, this uncharted area of research may be more relevant than previously thought for understanding fundamental immune responses, immune associated disease progression, and directions for clinical treatment. Therefore, this review article seeks to provide current insight and perspective in this field intersecting eccDNA and immunology.

1. eccDNA Biogenesis and Molecular Characteristics

1.1 Brief History of eccDNA

Nascent extrachromosomal circular DNA (eccDNA) studies since the 1960s were profoundly influenced by molecular technology advancements and electron microscopy, a technique which first enabled eccDNA imaging.[7] Subsequent methods including Southern blotting, density gradient purification, and gel electrophoresis enabled researchers to isolate and characterize eccDNA, shedding light on its size and genomic origin.[7,8] The advent of next-generation sequencing (NGS) and bioinformatics, enabled systematic identification of eccDNA across diverse cellular contexts, uncovering its roles in gene regulation, chromatin dynamics, and disease progression.[9-11]

1.2 Classification of eccDNA

eccDNA can be classified based on sequence characteristics, but more commonly by base pair size, genomic origin, or biological function. Small polydispersed DNA (spcDNA), ranging from 100 bp to 10 kb, is associated with genomic instability and arises from repetitive genomic regions.[12]

Telomeric circles (t-circles and c-circles) are extrachromosomal circular DNA structures composed of telomeric repeats, typically integral multiples of 738 bp. Existing as double-stranded (t-circles) or single-stranded (c-circles) DNA, their sizes range from 100 bp to 30,000 bp.[13] Their production is influenced by DNA damage-associated proteins; for example, knockdown of the Ku70/80 heterodimer reduces t-circles and significantly impairs cell growth in SaOS2 osteosarcoma cells.[14,15]

microDNA, ranging from 100 to 400 bp, is derived from non-repetitive genomic regions, often enriched in CpG islands, and contributes to gene regulation.[16] As tumor cells release specific microDNAs into the bloodstream, microDNAs have potential as biomarkers for monitoring cancer progression and evaluating therapeutic efficacy.[17]

Larger eccDNAs ranging from 1 to 3 Mb classified as extrachromosomal DNA (ecDNA), were first identified as paired small chromatin bodies called Double Minutes (DMs). These structures are predominantly found in tumor cells and actively contribute to oncogene amplification and tumor evolution.[2,11,18]

When considering genomic origins and genetic composition, eccDNAs can be classified into eight categories: full-gene eccDNA, exon eccDNA, intron eccDNA, repeat eccDNA, intergenic eccDNA, repeat-intergenic eccDNA, TE (transposable element) eccDNA, and promoter/enhancer eccDNA. This classification allows researchers to prioritize functional studies, develop targeted detection tools, and identify specific eccDNA subtypes linked to biological or pathological processes.[2] Some studies classify circularized mitochondrial DNA (mtDNA), virus-derived eccDNA, extrachromosomal ribosomal DNA circles (ERCs), and large non-repetitive eccDNAs as distinct categories (**Table 1**).[19]

Table 1. eccDNA categorized according to base pair size, origin, and function.

Type	Size	Origin	Function
Small polydisperse circular DNAs (spcDNA)[2,20]	100 pb-kb	Repetitive sequences (satellite DNA and transposable elements)	Genomic instability, cellular senescence, aging
microDNA[20,21]	100-400 bp	Exons, untranslated regions (UTRs), short genomic sequences associated with gene-rich regions, CpG islands, and transcription start sites	Gene regulation, decoys for transcription factors or microRNAs, and immune activation
Telomeric circles (t-circles and c-circles)[20,22,23]	Variable	Telomeric repeats and alternative lengthening of telomeres (ALT) pathway	Telomere maintenance (ALT) and recombination-based telomere elongation
Double minutes (DMs)[24,25]	> 100 kb to Mb	Chromosomal fragments, extrachromosomal DNA elements	Oncogene amplification, oncogene carrier (MYC, EGFR, MDM2), cancer progression
Extrachromosomal DNA (ecDNA)[26]	> 1 Mb	Often cancer-derived	Tumor evolution, therapy resistance

1.3 eccDNA Formation Mechanisms

eccDNAs can arise through diverse mechanisms, including replication stress, DNA damage repair, and chromatin remodeling.[2,25,27,28] Biogenesis of eccDNA originates from fundamental molecular mechanisms which directly impact chromosomal DNA. These processes include DNA damage repair pathways such as homologous recombination (HR) and nonhomologous end-joining (NHEJ), DNA replication errors, and the formation of R-loops.[5,19,25,29,30]

In addition to localized molecular mechanisms, eccDNA biogenesis (**Figure 1**) is influenced by overarching genomic models that account for large-scale structural rearrangements. These models, including the Breakage-Fusion-Bridge (BFB) cycle, chromothripsis, the episome model, and the translocation-deletion-amplification mechanism, highlight how complex structural rearrangements within the genome lead to the generation of eccDNA.[19,31-33]

1.3.1 Mechanisms of eccDNA Formation:

1. Replication Stress:

DNA replication stress can lead to DNA damage and successive restoration mechanisms, which generate eccDNA. For instance, stalled or collapsed replication forks can cause double-strand breaks (DSBs), and strand repair can involve pathways which form eccDNA.[4]

2. DNA Damage Repair:

DNA damage, including DSBs, can trigger DNA damage response (DDR) signaling, which may initiate multiple repair pathways. These mechanisms, including homologous recombination (HR) and non-homologous end-joining (NHEJ), can contribute to eccDNA formation. For instance, NHEJ can repair DSBs, but if repaired fragment ends self-ligate, it can generate eccDNA and chromosomes with deletions.[34]

3. Chromatin Remodeling:

Chromatin remodeling, which entails modifying chromatin structures to regulate gene expression and DNA accessibility, contributes to eccDNA formation. The ATP-dependent chromatin remodeling complexes involved displaces histones, thus exposing DNA and facilitating DNA damage, including DSBs, leading to potential eccDNA formation.[2]

4. Replication Slippage and R-loops (Episome Model):

Polymerase slippage during DNA replication may result in DNA loop excision and circularization which form eccDNAs. R-loops, formed during transcription when the unpaired strand of a DNA sequence forms a loop, can also be excised and ligated into circles, contributing to eccDNA formation. The episome model proposes that eccDNA forms from errors in DNA replication, specifically DNA polymerase "slippage" during synthesis or R-loop formation, leading to looped-out DNA regions. These loops undergo excision and ligation to form circular episomes, which subsequently self-replicate and incorporate additional genomic elements. Episome-derived eccDNA are circular, self-replicating, and vary in size. They can be amplified, dynamically change content through the incorporation of genomic elements, and possess enhancer/promoter regions.[2,35]

5. Breakage-Fusion-Bridge (BFB) Cycle:

The BFB cycle, involving DNA breakage, fusion, and bridging, is a well-established genomic mechanism which promotes the generation of eccDNA. The BFB cycle arises from the loss of telomeres—the protective caps at the ends of chromosomes—which leads to end-to-end chromosome fusion and subsequent genomic instability. The BFB cycle consists of three key steps: telomere loss and end-to-end fusion, anaphase bridge formation and breakage, and cycle initiation repeats.[36-38] During cell division, dicentric chromosome with two centromeres form a bridge between daughter cells, and as the centromeres are pulled in opposite directions, the bridge breaks randomly, generating fragmented DNA segments. These broken fragments, now telomere-deficient, can re-enter the BFB cycle, initiating iterative rounds of breakage, fusion, and further fragmentation.[2]

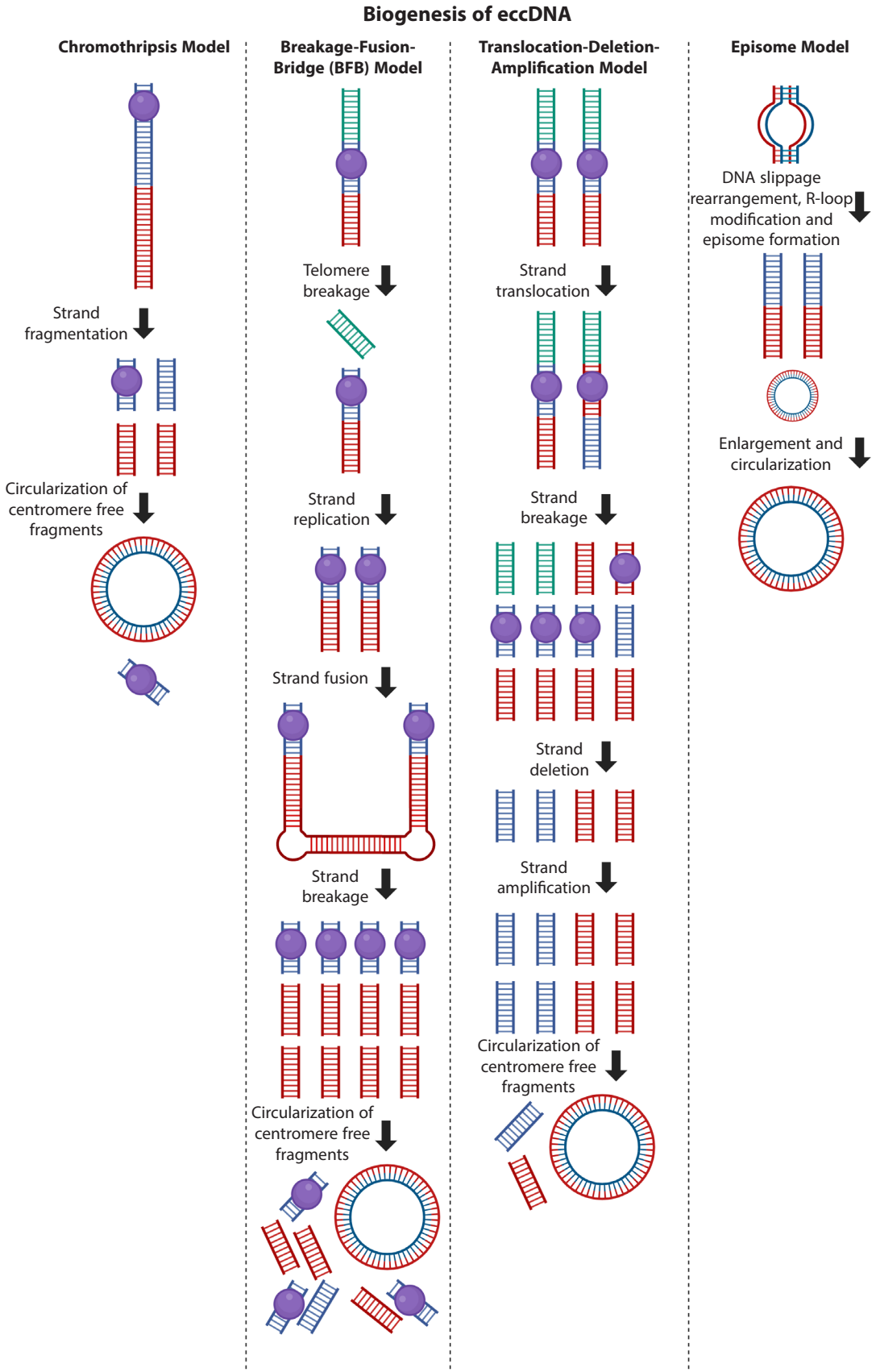

Figure 1. The biogenesis of eccDNA by genomic instability, cellular stress, or routine DNA maintenance is indicated by i) the chromothripsis model, a chromosomal shatter with haphazard reassembly; ii) the Breakage-Fusion-Bridge (BFB) cycle, the interconnected mechanisms of focal gene amplification and genomic instability; iii) the translocation-deletion-amplification, chromosomal rearrangement errors in DNA repair; and iv) the episome model (deletion-plus-episome), small submicroscopic circular DNA precursors (episome) originating from a circularization of segmental linear DNA after excision.

6. Chromothripsis:

Chromothripsis, a catastrophic genomic event potentially leading to eccDNA formation, involves chromosome fragmentation typically by extreme cellular stress or mitotic failure. Some of the fragments randomly ligate to form eccDNA rather than reassemble into their original linear configuration.[38-40] Chromothripsis is normally initiated by widespread double-strand breaks (DSBs), which can be triggered by exogenous stressors such as radiation and chemotherapy, or intrinsic factors like defective mitotic machinery.[10,41,42] The recognization of chromothripsis as a source of eccDNA enabled further comprehension of genomic malignancies and potential targets for therapeutic intervention.[25,43,44]

7. Microhomology-Mediated End Joining (MMEJ):

MMEJ, a DNA repair pathway contributing to eccDNA formation, especially upon conventional NHEJ (c-NHEJ) compromise,[45] produces eccDNA quantities dependent on resection after double-strand DNA breakage (DSB) and microhomology mediated end joining (MMEJ) repair.[46,47] While microhomology is found around eccDNA breakpoints, eccDNA breakpoints detected using short-read sequencing technologies could be mis-annotated if microhomologous sequences are present due to interference to precise break-point detection.[48,49]

8. Translocation-Deletion-Amplification Mechanism:

The translocation-deletion-amplification mechanism, a genomic rearrangement process, contributes to eccDNA formation as external and intrinsic cellular stressors cause chromosomal translocations, localized deletions, and subsequent amplification of DNA segments.[2,50] The process initiates with chromosomal translocation, where external stimuli or genomic instability drives DNA segment exchange between nonhomologous chromosomes, resulting in translocation breakpoints at specific loci. Subsequently, localized deletion occurs as DNA near these breakpoints is excised during repair, producing various fragments. The excised DNA fragments successively undergo amplification prior to circularization to form eccDNA. eccDNA formed via this mechanism often contains oncogenes or regulatory elements, including MYC, MDM2, or HMGIC, which contribute to tumor growth and progression.[51]

1.4 Purification, Detection, and Analytical Methods of eccDNA

eccDNA detection and characterization (**Figure 2**) entail analytical methods to sequence, enrich, and amplify eccDNA molecules.

Rolling Circle Amplification (RCA) isothermally amplifies eccDNA using phi29 DNA polymerase and creates high-molecular-weight concatemeric DNA from eccDNA circular templates. RCA has been utilized in conjunction with hybrid methods and downstream sequencing to further profile eccDNA molecules.[49]

Circle-Seq, a high-throughput sequencing-based technique, relies on exonuclease treatment to selectively degrade linear DNA and enriching circular DNA preceding random fragmentation and library fragmentation.[16]

Long-read sequencing methods have proven to be formidable tools in characterizing eccDNA due to the capabilities of long-read platforms to cover entire eccDNA molecules, a limitation short-read sequencing methods inherently possess. Long-read sequencing enabled mapping of complex sequences and arrangements, especially those of megabase-sized eccDNAs and extrachromosomal DNA (ecDNA) found in cancer tissue.[52]

The above-mentioned sequencing and detection methods are supplemented with bioinformatics computation to enhance detection of circular junctions, rapidly process eccDNA quantities, and determine genomic origins. These bioinformatics tools comprise Circle-Map and ecc_finder which integrates read alignment, split-read, and perform discordant-pair analysis to enhance sensitivity and specificity of eccDNA detection from sequencing data.[42]

Experimental methods for eccDNA detection focus on operating configurations or functionalities within eccDNA bioinformatics software tools which process data from high throughput sequencing. Most recent eccDNA detection methods involve an integrated approach which combines advanced experimental enrichment techniques (e.g., Circle-seq, 3SEP) with sophisticated bioinformatics analysis pipelines tailored to different sequencing platforms (short-read and long-read). Enrichment techniques may employ 3SEP (Solution A for selective circular DNA recovery),[53] Circle- Seq[54] or CIDER-Seq[30] with subsequent verification using fluorescence in situ hybridization (FISH)[55] and qPCR.[56] Relevant pipelines include WGS-SR, WGS-LR, ATAC-Seq-SR, 3SEP-SR, 3SEP-LR,[53] Circle-Seq-SR, Circle-Seq-LR.[57] SR methods commonly used comprise Circle-Map,[58] ECCsplorer,[59] ecc_finder (map-sr mode), Circle_finder[60] while LR methods include CReSIL,[58,61] NanoCircle,[61] ECCFP,[62] and ecc_finder.[60] Emerging analytical methods include deep learning approaches like HyenaCircle[63] and ECCNET.[64]

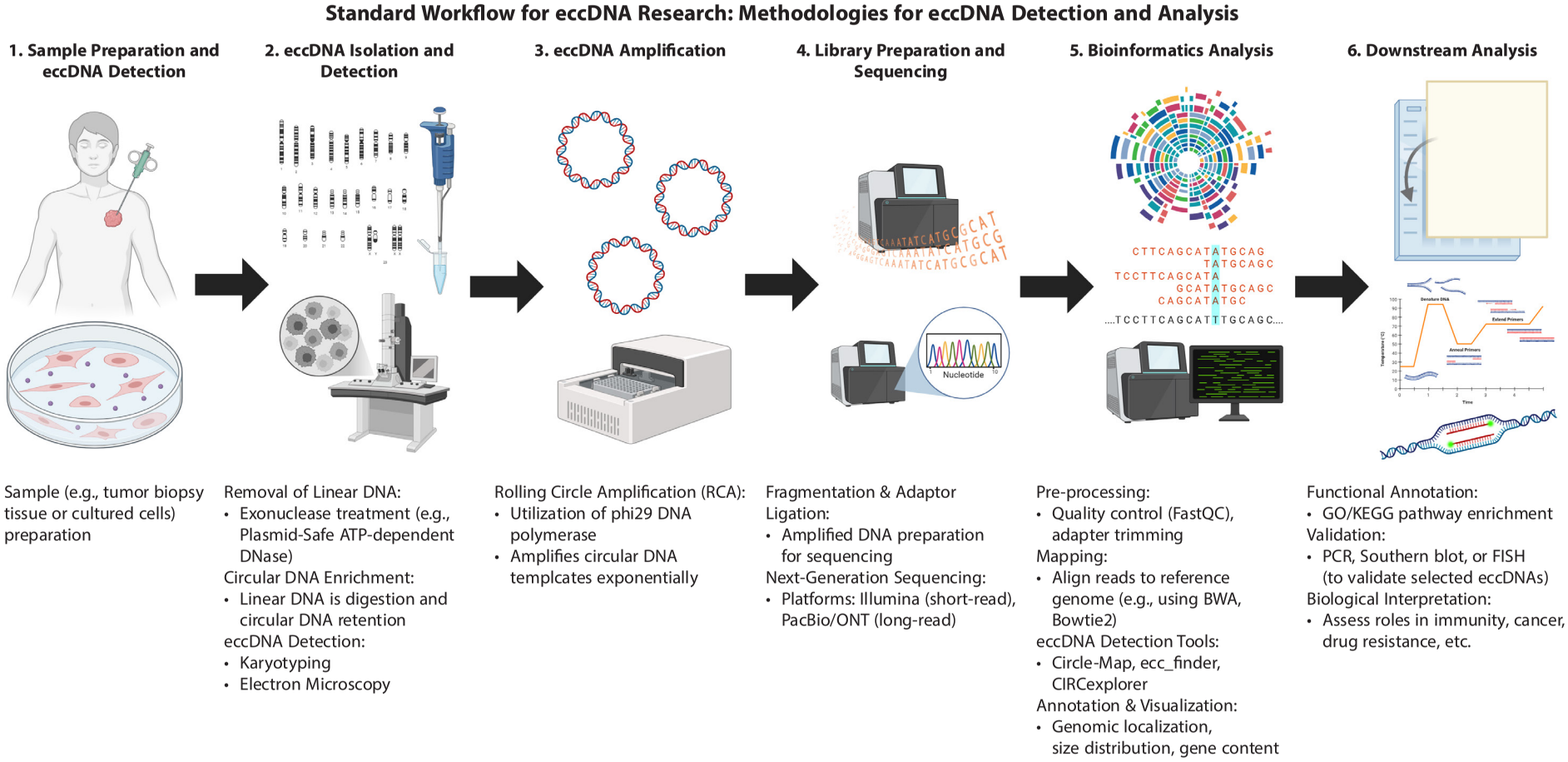

Figure 2. A basic flow of the standard research on eccDNA is sample Preparation, enrichment, sequencing, bioinformatics, and orthogonal validation. Due to a low abundance of eccDNA compared to the linear genome, specialized enrichment and computational tools are frequently required.

Table 2. Characteristic and immunogenic comparison between eccDNA and linear cfDN.

	eccDNA	Linear cfDNA
Structure[2,19,21,29,65-67]	Circular and relatively stable	Linear and more degradable
Immune Pathways[1,68-74]	Strong activation of cGAS-STING, AIM2	Variable activation of cGAS, TLR9, AIM2
Potency[41,43,68,75,76]	High; Sustained immune activation	Moderate; Immune status and context-dependent
Role in Disease[20,26,77]	Autoimmunity, Cancer immunogenicity	Biomarker, Potential immunogen
Therapeutic Use[57-59,61,64]	Emerging as adjuvant or target	Standard utilization in liquid biopsy diagnostics

2. eccDNA in Innate Immunity

With linear cell-free DNA (cfDNA) often utilized as the standard biomarker structure and diagnostic entity and eccDNA emerging as an adjunct or alternative, **Table 2** provides an excerpt of how the two structures compare and contrast in both characteristics and immunogenic roles.

2.1 Detection of eccDNA by Cytosolic DNA Sensors

eccDNA can act as an immunostimulatory molecule by engaging several cytosolic DNA sensors, including cGAS-STING, AIM2, and DDX41.

Among these, the cGAS-STING pathway is the most extensively characterized in the context of eccDNA sensing (**Figure 3**). Due to its high GC content and stable cyclic structure, eccDNA robustly activates cyclic GMP-AMP synthase (cGAS) upon entry into the cytoplasm. This activation leads to the production of pro-inflammatory cytokines including IFN-α, IFN-β, IL-6, and TNF-α.[70,78] Notably, eccDNA loses its immunostimulatory capacity when linearized, underscoring the functional significance of its circular conformation. This unique structural stability makes eccDNA a more potent regulator of immune activation compared to cfDNA. In autoimmune diseases, eccDNA's ability to amplify inflammatory responses through both cGAS-STING and TLR9 pathways positions it as a novel contributor to immune dysregulation.

2.2 eccDNA as a Damage-Associated Molecular Pattern (DAMP)

DAMPs are endogenous molecules which are typically located within cells but uphold immunostimulatory potential when release, thus activating pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) or cytosolic DNA sensors. eccDNA can function as a DAMP and contribute to inflammatory responses when exposed to cellular stress. Further immune responses initiate through eccDNA binding to PRRs on immune cells, including macrophages and dendritic cells.[79]

2.3 Inflammatory responses triggered by eccDNA (e.g., IFN-I, IL-6, TNF-α)

Cytosolic accumulation of eccDNA—resulting from DNA damage, chromatin instability, or cellular stress—is recognized by the cyclic GMP-AMP synthase (cGAS). This recognition activates the STING pathway, leading to phosphorylation of TBK1 and IRF3 and culminating in the transcription of type I interferons (IFN-I), particularly IFN-β, which enhance pro-inflammatory gene expression and antiviral defense.[80]

eccDNA additionally upregulates other key pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), via activation of the NF-κB pathway.[73,81] These cytokines are central mediators of inflammation and play key roles in both tumor immunity and chronic inflammatory conditions. Macrophages and dendritic cells exposed to eccDNAs derived from tumor or senescent cells exhibit a robust induction of IL-6 and TNF-α, suggesting that eccDNAs may act as DAMPs in sterile inflammation.[5]

Notably, the immunogenic potential of eccDNA is influenced by its size and sequence composition. Smaller eccDNAs (< 1 kb) are more readily internalized by immune cells and are more efficient at activating cGAS-STING signaling compared to larger counterparts. These findings establish eccDNA as a significant endogenous trigger of inflammation and highlight its potential involvement in the pathogenesis of autoimmune and age-related inflammatory diseases.[73]

2.4 Relevance in diseases such as IBD and psoriasis

In inflammatory bowel disease (IBD), a chronic relapsing inflammatory condition of the gastrointestinal tract, epithelial damage and microbial dysbiosis promote persistent immune activation. eccDNAs derived from apoptotic epithelial cells or translocated bacteria can act as DAMPs, triggering innate immune receptors including cGAS-STING and TLR9 within intestinal macrophages and dendritic cells.[68] This results in production of IFN-I, IL-6, and TNF-α, all of which are key contributors to the IBD cytokine environment. Additionally, microbial-derived eccDNA from gut bacteria exacerbate mucosal inflammation by activating plasmacytoid dendritic cells, further linking eccDNA to intestinal immune homeostasis.[76]

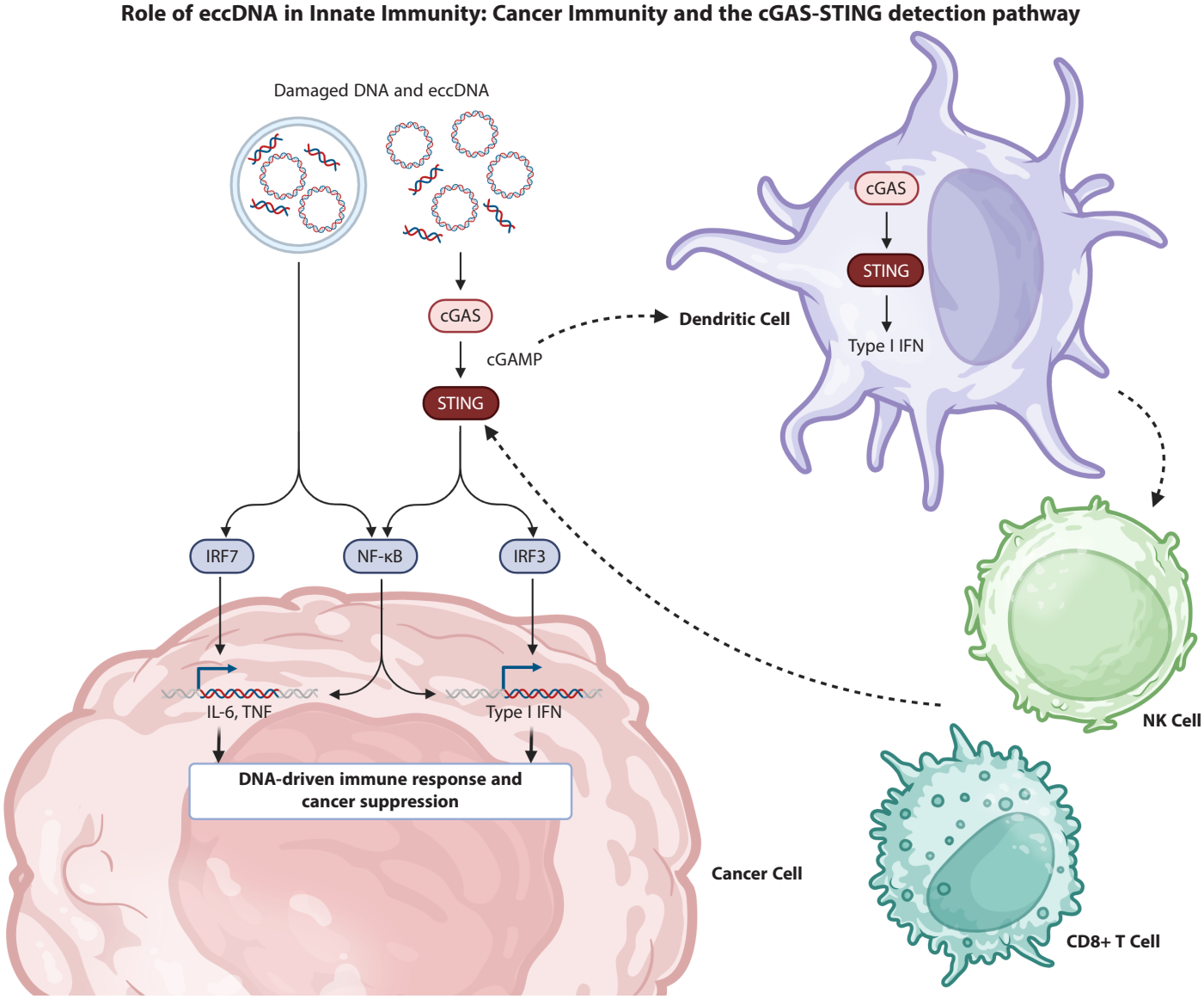

Figure 3. An innate immune activation of eccDNA by the cGAS-STING (cyclic GMP-AMP synthase-stimulator of interferon genes) pathway is demonstrated as an example of eccDNA recognition as a danger signal from damaged or abnormal genetic material. The damaged DNA can be recognized by interferon regulatory factor (IRF)3 and IRF7, recognizing the core 5′-GAAA-3′ tetranucleotide sequence within interferon-stimulated response elements), or NF-κB that might alter activities of the cancer cells. The cyclic GMP-AMP (cGAMP; the circular DNA initiated by cGAS) might induce denfritic cell functions, while the cGAS-STING pathway is a critical bridge between innate sensing and natural killer (NK) cell-mediated immunity with the intrinsic pathway (NK cells recognize eccDNA) or extrinsic pathways. In the extrinsic pathway, the STING activation in other cells (e.g., tumor cells, macrophages, or endothelial cells) leads to the secretion of chemokines (CCL5 and CCL10) that indirectly activated NK cells.

In psoriasis, a chronic autoimmune skin disorder characterized by keratinocyte hyperproliferation and dysregulated immune responses, eccDNAs have been implicated in promoting local inflammation. Psoriatic skin lesions show increased cell turnover and apoptosis, conditions that favor eccDNA generation. Elevated eccDNA levels in lesional skin have been associated with activation of the cGAS-STING pathway in both keratinocytes and infiltrating immune cells, resulting in enhanced expression of IFN-β, IL-23, and IL-17, cytokines with major contributions to psoriasis pathogenesis. Moreover, eccDNA may contribute to the formation of autoantigens or promote neoantigen presentation, thereby sustaining chronic immune activation in genetically susceptible individuals.[82] Together, these findings highlight eccDNA as a novel immunostimulatory component in chronic inflammatory diseases.

3. eccDNA in Adaptive Immunity

Adaptive immunity relies on highly specific and dynamic mechanisms to detect and eliminate pathogens. Key to this system is the generation of antigen receptor diversity, the precise regulation of gene expression in lymphocytes, and the effective presentation of antigens—processes that enable targeted immune responses while maintaining self-tolerance.

3.1 Potential influence on V(D)J recombination and TCR/BCR diversity

A hallmark of adaptive immunity is the vast diversity of T-cell receptors (TCRs) and B-cell receptors (BCRs), achieved through somatic recombination of variable (V), diversity (D), and joining (J) gene segments. This recombination process generates circular DNA byproducts, notably T-cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs), which serve as molecular markers of recent lymphocyte maturation.[83,84]

TRECs are specifically formed during T-cell receptor alpha (TRA) gene rearrangement, when the excised DNA containing delta-coding segments is circularized through ligation of blunt-ended DNA signal sequences. This process creates a signal joint within stable circular excision products that persist as markers of recent thymic output. Similarly, KRECs emerging during B-cell development as products of recombination events determine the allelic and isotypic exclusion of the immunoglobulin kappa locus formed when the intron RSS-IGKDEL signal joint is circularized.[83,84]

Interestingly, recent studies have hinted at a more active role for eccDNA beyond its status as a mere byproduct. High-throughput mapping in glioblastoma multiforme identified eccDNAs located near immunoglobulin VDJ loci, suggesting a potential regulatory interface between eccDNA and the genomic architecture of antigen receptor genes. Such findings suggest that eccDNA may influence recombination or chromatin accessibility in lymphocytes, particularly under pathological or stressed conditions.[85]

3.2 Regulation of immune cell gene expression through eccDNA-derived enhancers

Beyond V(D)J recombination, eccDNAs may act as epigenetic regulators by functioning as mobile enhancer elements. Chromatin interaction studies, including ChIA-PET analyses, have demonstrated that eccDNAs can physically associate with chromatin and RNA polymerase II complexes, influencing the transcriptional landscape.[86] This enhancer-like activity is facilitated by the formation of eccDNA "hubs"—nuclear foci where multiple eccDNAs cluster and collectively enhance gene expression through long-range chromatin interactions.[87]

eccDNA can also interact with specific sites within chromosomes, with these interaction sites associated with increased transcriptional activity and active histone marks. This suggests that eccDNA may act as "mobile enhancer carriers" modulating chromosomal gene expression in immune cells.[87] The unique structural properties of eccDNA, including its circularity and accessibility, potentially enable distinctive modes of gene regulation different from conventional chromosomal mechanisms.

3.3 eccDNA and its possible role in modulating antigen presentation

Antigen presentation is central to adaptive immunity, and recent evidence implicates eccDNA in regulating this process, particularly in the context of immune evasion by tumors. Tumor-derived eccDNAs are associated with downregulation of major histocompatibility complex (MHC) class I and II genes, which potentially impairs antigen presentation and immune recognition.[86] Thus, eccDNA could potentially influence adaptive immune response by modulating the expression of genes involved in antigen processing and presentation.

Additionally, the high GC content and structural stability of many eccDNAs may render them potent activators of cytosolic DNA sensing pathways, such as cGAS-STING. This innate immune activation could indirectly shape the microenvironment in which antigen-presenting cells operate, influencing the efficiency and context of T-cell priming.[88]

3.4 Effects on T and B cell development, memory, and activation

eccDNAs also appear to influence broader aspects of lymphocyte biology. Quantification of TRECs and KRECs has been widely adopted in clinical immunology to assess thymic and bone marrow output, particularly in the diagnosis and monitoring of primary immunodeficiencies. Decreased levels of these eccDNAs in peripheral blood often reflect impaired lymphopoiesis or lymphocyte exhaustion.[83]

Beyond clinical utility, eccDNAs possess intrinsic immunostimulatory properties. Compared to linear DNA, both synthetic and purified eccDNAs more robustly activate dendritic cells and macrophages, promoting the secretion of type I interferons (IFN-α/β), IL-6, and TNF-α in a STING1-dependent manner. This innate immune activation can subsequently modulate adaptive responses by enhancing antigen presentation and promoting T-cell activation and differentiation.[5]

In the context of cancer immunity, eccDNA has been implicated in aberrant B-cell regulation. For instance, in neuroblastoma, genes such as *SOX11* are frequently amplified and circularized as eccDNA, originating from focal genomic amplifications. The resulting high *SOX11* expression sustains *PAX5* and suppresses *Blimp1*, thereby blocking terminal B-cell differentiation. In *SOX11*-positive mantle cell lymphoma (**Figure 4**), this dysregulation is associated with reduced expression of tumor suppressor genes and pronounced immune infiltration.[89] Additionally, eccDNA has been shown to trigger primary B-cell responses and promote T-helper 2 (Th2) polarization.[5,88] These findings position eccDNA as not only a clinical biomarker but also a functional modulator of both innate and adaptive immune responses.

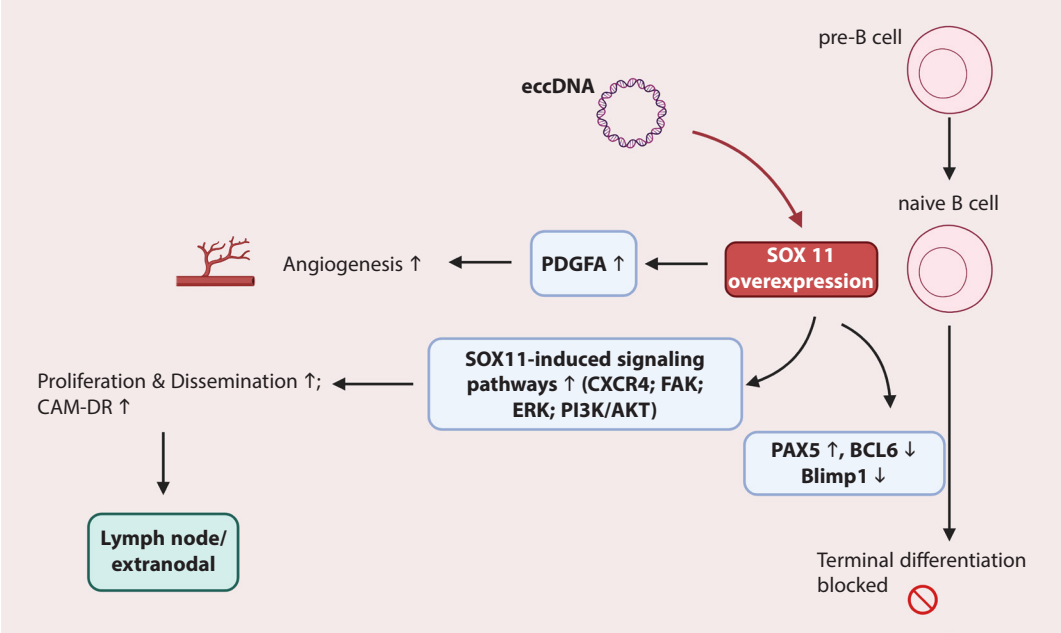

Figure 4. An example role of eccDNA in cancer is demonstrated. As such, eccDNAs amplify the SOX11 (SRY-box transcription factor 11) oncogene that induces cancer aggressiveness through i) enhanced angiogenesis through increased PDGFA (Platelet-Derived Growth Factor Subunit A); ii) elevated cell activities using chemokines (CXCR4), focal adhesion kinase (FAK), extracellular signal-regulated kinase (ERK), and phosphoinositide 3-kinase/protein Kinase B/ mammalian target of rapamycin (PI3K/Akt/mTOR) pathways that lead to drug resistance using cell adhesion-mediated drug resistance (CAM-DR) and extranodal cancer dissemination; and iii) regulated B cells through increased PAX5 (Paired Box 5) with a decrease in B-cell lymphoma 6 (BCL6) and B lymphocyte-induced maturation protein 1 (Blimp1) that increased tumor growth.

4. eccDNA in Autoimmunity and Inflammatory Disorders

While eccDNA contributes to maintaining immune homeostasis under physiological conditions, its dysregulation is increasingly implicated in driving aberrant immune activation in autoimmune and chronic inflammatory diseases. Emerging evidence suggests that eccDNA may not only play a pathogenic role but also serve as a sensitive biomarker (**Figure 5**) for monitoring disease activity and therapeutic response.

4.1 Aberrant eccDNA accumulation and self-DNA sensing

The inappropriate accumulation of eccDNA can disrupt immune tolerance by activating cytosolic DNA-sensing pathways. Owing to its circular topology and high GC content, eccDNA is a potent activator of the cGAS–STING axis, promoting robust type I interferon and pro-inflammatory cytokine production in dendritic cells and macrophages. Notably, this immunostimulatory activity is dependent on DNA circularity; linear forms fail to elicit similar responses.[5,88] Under normal conditions, mechanisms such as autophagy help eliminate cytosolic DNA, including eccDNA, to prevent excessive immune activation. However, impairments in these clearance pathways—observed in autoimmune conditions—can result in chronic activation of innate sensors and persistent inflammation.[90]

4.2 Involvement in autoimmune diseases pathogenesis

Dysregulated eccDNA dynamics have been implicated in multiple autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and type 1 diabetes (T1D).

In SLE, multiple studies have demonstrated that patients-both with active (ASLE) and inactive (ISLE) disease-exhibit significantly higher plasma eccDNA levels compared to healthy controls.[88,91,92] Differential expression analysis of exon-derived eccGenes in SLE revealed that genes such as TNFSF14, TRIM21, SLAMF7, SOS1, BCL11B, PPT1, and GCNT3 are significantly altered in abundance between disease states, and their levels correlate with the SLEDAI-2K disease activity index. SLE patients with DNASE1L3 deficiency display a distinctive eccDNA profile, further supporting the link between eccDNA metabolism and disease.[88]

In rheumatoid arthritis (RA), plasma eccDNA levels are significantly elevated.[88,93] eccDNA in RA can stimulate the TLR9-MyD88-NF-κB pathway, which promotes pro-inflammatory cytokines secretion and disease progression. Treatment with biological disease-modifying antirheumatic drugs (bDMARDs) substantially reduces plasma eccDNA levels, highlighting its potential as a disease activity biomarker and therapeutic response monitoring tool.[88]

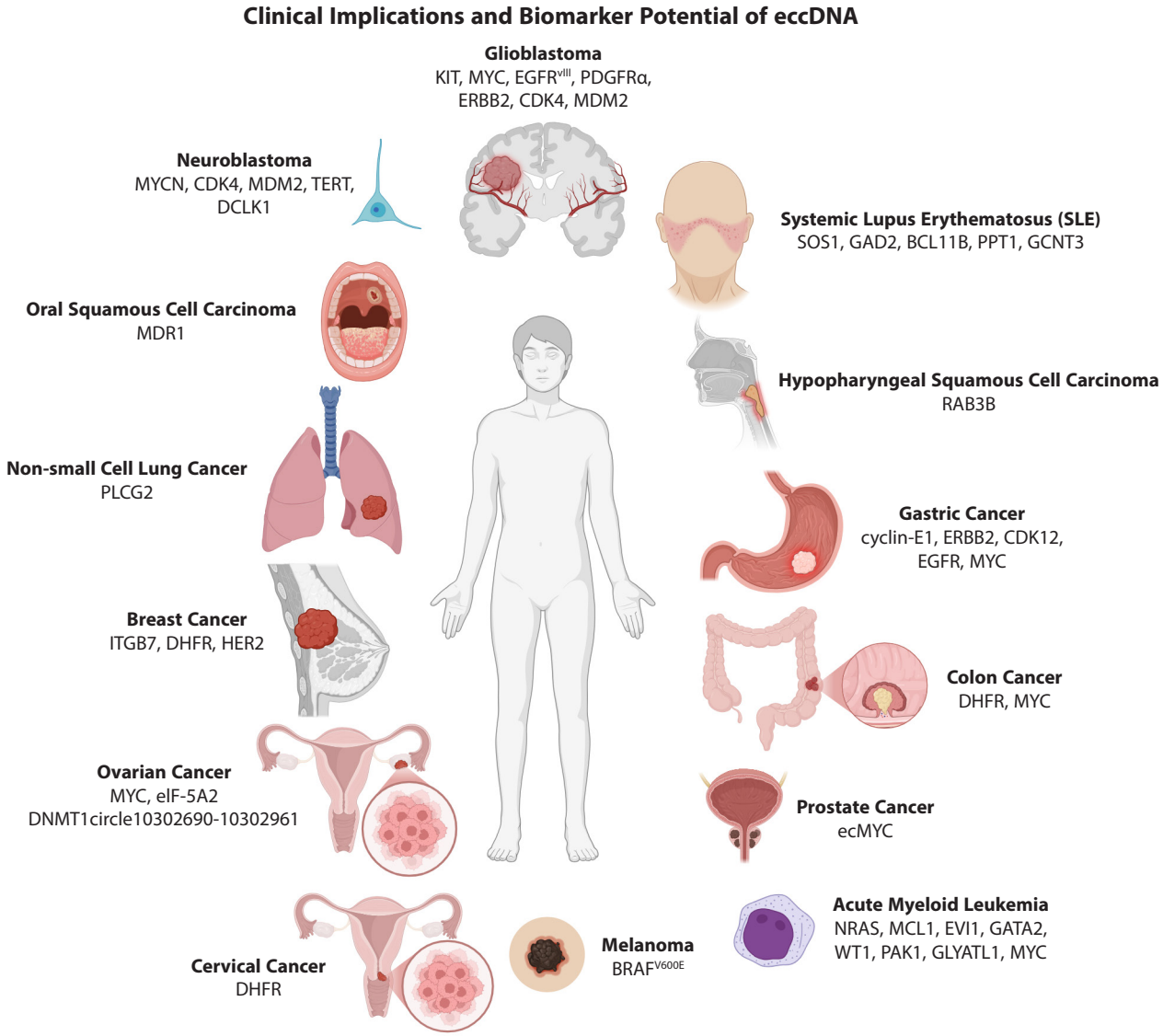

Figure 5. The potential of eccDNA used for a cancer biomarker through the identification of several genes in various cancers is demonstrated.

While direct evidence in T1D remains limited, eccDNA is presumed to contribute to shared autoimmune mechanisms, particularly via chronic immune activation and interferon signaling. Common genetic and transcriptomic overlaps between SLE, RA, and T1D[94] suggest that eccDNA might play a convergent role across autoimmune phenotypes.[88,94]

Given its potent immunostimulatory properties, targeting eccDNA or its associated sensing pathways presents a novel therapeutic strategy. Enhancing DNA clearance via autophagy induction or DNase activity (e.g., restoring DNASE1L3 function) has shown promise in experimental lupus models, reducing eccDNA burden and ameliorating disease features.[91] Alternatively, pharmacological inhibition of cGAS-STING signaling may dampen eccDNA-mediated inflammation.[88,93] Modulating eccDNA interactions with pattern recognition receptors could also attenuate downstream cytokine production, thereby restoring immune tolerance and limiting tissue damage.[88,91]

4.3 Correlation between eccDNA levels and disease activity

eccDNA as a biomarker in monitoring both active (ASLE) and inactive (ISLE) SLE disease activity pivots on the premise that SLE patients exhibit significantly elevated plasma eccDNA levels. Notably, SLE-associated eccDNA tends to have a lower GC content, which negatively correlates with established disease markers such as anti-dsDNA antibodies and complement C3/C4 levels. Beyond overall abundance, specific exon-derived eccDNAs (eccGenes) have been identified as differentially abundant in SLE, including genes like SOS1, GAD2, BCL11B, PPT1, and GCNT3. These eccGenes not only distinguish between active and inactive disease but also correlate with the SLE Disease Activity Index (SLEDAI-2K). Functional enrichment analyses reveal that these eccGenes are involved in immune-related pathways central to SLE pathogenesis.[88,92]

Although the characterization of specific eccDNA sequences or genes in RA is less advanced than in SLE, there is evidence that eccDNA can stimulate the TLR9-MyD88-NF-κB signaling pathway, promoting the secretion of pro-inflammatory cytokines that drive RA pathogenesis.[88]

5. Therapeutic and Diagnostic Implications

5.1 eccDNA as biomarkers in immune-related diseases

The structural features of eccDNA, including its covalently closed circular shape, diverse genomic origins, and resistance to exonucleases, provides relative stability when compared to linear cfDNA in both biofluids and tissues. This improved stability, along with its immunogenic properties, suggests that eccDNA could serve as a valuable biomarker for non-invasive diagnosis and disease monitoring in immune-related conditions. Although cfDNA is already widely used, eccDNA may offer added benefits due to its unique structure and stronger ability to activate immune responses.[81]

In immune-mediated diseases such as systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), and rheumatoid arthritis (RA), eccDNA has been detected at elevated levels in serum, tissue biopsies, and even extracellular vesicles. The abundance and sequence composition of eccDNA appear to correlate with disease activity and tissue inflammation. For instance, increased levels of eccDNAs derived from immunologically relevant genomic loci encoding cytokines, MHC molecules, and pattern recognition receptors have been observed in autoimmune disease models, suggesting disease-specific patterns that may serve as diagnostic signatures.[5] eccDNA profiles have shown potential in distinguishing disease subtypes and predicting disease treatment responses. In IBD, specific eccDNA signatures distinguish Crohn's disease from ulcerative colitis, and their abundance correlates with the severity of mucosal inflammation. In psoriasis, eccDNA content in lesional skin biopsies has been linked to responsiveness to biologic therapies targeting IL-17 or TNF-α pathways. These findings highlight the feasibility of eccDNA-based liquid biopsies for personalized medicine approaches in inflammatory disorders.[68,93]

Importantly, eccDNA can be isolated from blood and other body fluids, offering a minimally invasive and repeatable alternative to tissue biopsies. Recent advances in sequencing methods and enrichment techniques have made it easier to detect and analyze eccDNA from clinical samples. When combined with data from immune transcriptomics and proteomics, eccDNA profiling could offer deeper insights into immune dysregulation and disease mechanisms at the epigenomic level.[10]

5.2 Therapeutic targeting of eccDNA generation or sensing pathways

The emerging role of eccDNA as a potent activator of innate immune responses has opened new therapeutic avenues in the management of inflammatory, autoimmune, and neoplastic diseases. Strategies aimed at modulating either eccDNA biogenesis or its cellular sensing hold promise for mitigating pathological inflammation while preserving host defense.

eccDNA formation is tightly linked to genomic stress, DNA damage, and chromatin remodeling. Key molecular events supporting eccDNA biogenesis include DNA double-strand break (DSB) repair via non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ) pathways.[10,46] Specific targeting of these DNA repair pathways with small-molecule inhibitors—namely DNA-PKcs and PARP inhibitors—has been proposed to reduce eccDNA production, particularly in cancers where eccDNA contributes to oncogene amplification and therapy resistance.[9]

In chronic inflammatory diseases, limiting eccDNA release from dying or senescent cells may also curb innate immune activation. Pharmacological senolytics and apoptosis-modulating agents, including BCL-2 inhibitors, may indirectly reduce eccDNA burden by promoting clearance of damaged cells and limiting necrosis-driven DAMP release.

Once eccDNA is present in the cytoplasm, it is sensed primarily by the cGAS-STING pathway, triggering type I interferon (IFN-I) and pro-inflammatory cytokine production. Inhibiting this pathway represents a promising therapeutic approach for conditions characterized by aberrant innate immune activation. Small molecule STING antagonists, including H-151 and C-176, have demonstrated efficacy in preclinical models of autoimmune diseases, including Aicardi–Goutières syndrome and lupus.[71,72] Similarly, targeting cGAS directly with small-molecule inhibitors like RU.521 has demonstrated anti-inflammatory potential by suppressing eccDNA-induced IFN-I production.

Beyond direct inhibition, modulation of DNA clearance mechanisms—such as enhancing DNase II or TREX1 activity—can help prevent eccDNA accumulation in the cytoplasm. For example, TREX1-deficient mice develop a lupus-like phenotype due to cytosolic DNA accumulation, including eccDNA, highlighting the critical role of nuclease activity in maintaining immune homeostasis.

5.3 Challenges and technical limitations

eccDNA as a non-invasive biomarker is compelling due to its stability, abundance in circulation, and disease-specific signatures. However, one major obstacle is the lack of standardized protocols for eccDNA isolation, enrichment, and quantification from biofluids such as plasma, urine, or cerebrospinal fluid. Current methods, including Circle-Seq, CIDER-Seq, and eccDNA-RCA, vary in sensitivity, length bias, and their ability to distinguish between endogenous and exogenous circular DNA, leading to inconsistencies across comparative studies.[81]

Therapeutically, strategies targeting eccDNA biogenesis or its immune sensing pathways—such as cGAS-STING inhibition—have shown promise in preclinical models. However, systemic administration of STING antagonists or DNA repair inhibitors can result in off-target effects, given the ubiquitous roles of these pathways in tissue homeostasis, DNA repair, and antiviral defense.[71,72]

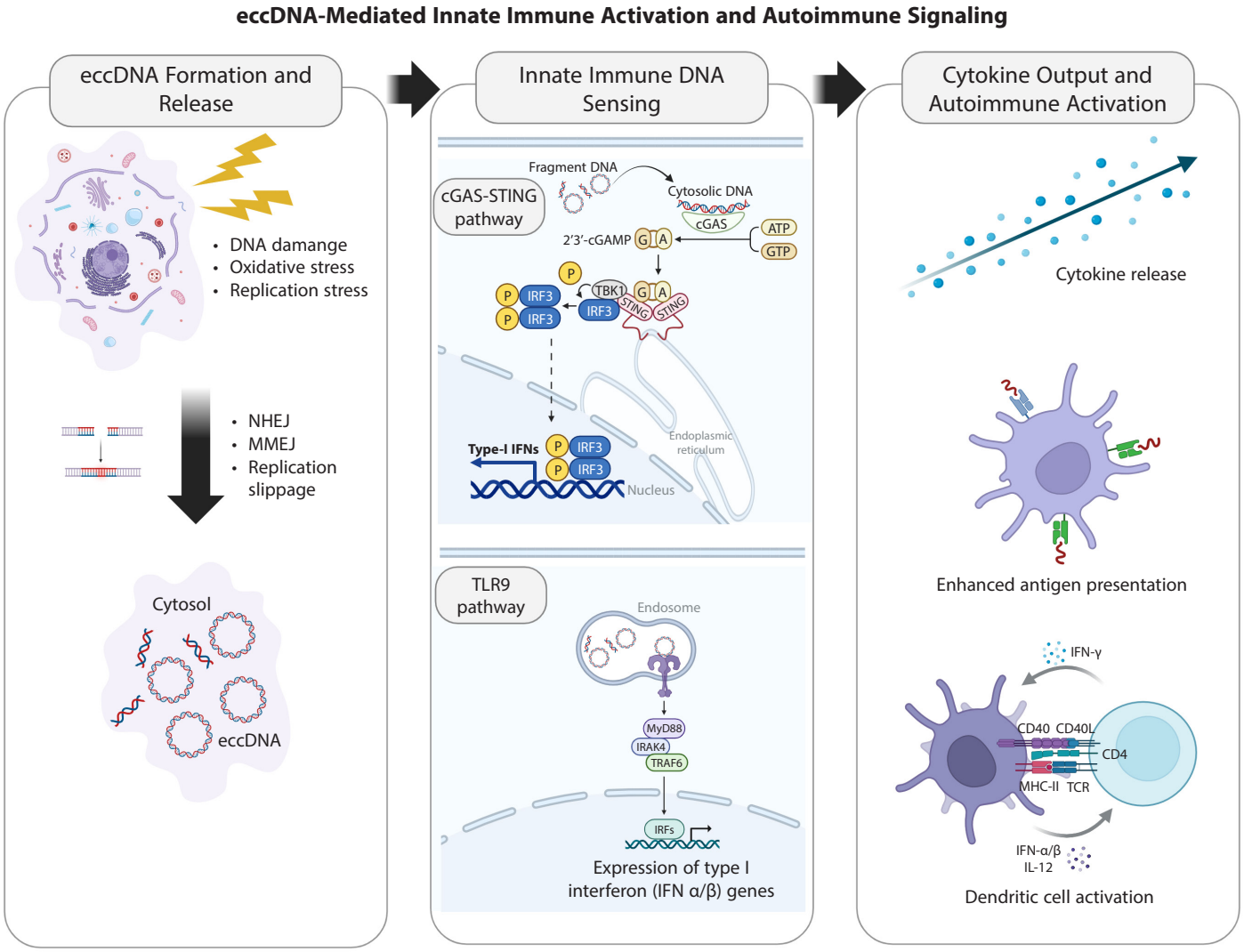

Figure 6. A schema of the conclusion of the immune activation of eccDNA through innate and adaptive immunity is demonstrated. First, eccDNAs are formulated from cell stress through non-homologous end joining (NHEJ); microhomology-mediated end joining (MMEJ: the error-prone mechanism circularizes short direct repeat sequences after double-strand breaks (during a DNA repair process); and replication slippage (DNA polymerase pauses and dissociates, causing misalignment of strands at direct repeats, leading to a loop formation). Second, these eccDNAs stimulate innate immunity through cGAS-STING or TLR-9 recognition receptors. Third, the innate-immune-mediated cytokines enhanced functions of antigen presenting cells, especially dandritic cells, driving adaptive immune responses, and might induce autoimmune-like features or paraneoplastic symdrome with some immune modifications (e.g., cancer neoantigens and the loss of tolerance from the cancer microenvironment).

Since eccDNA activates innate immune sensors (**Figure 6**) including cGAS, TLR9, and AIM2, therapies modulating eccDNA responses carry risks of broad immunosuppression or unintended immune activation. For instance, inhibiting cGAS-STING to reduce inflammation in autoimmune disease could impair host defense against viruses and tumors. Conversely, therapeutic administration of synthetic eccDNA mimics designed to stimulate anti-tumor immunity may provoke systemic cytokine storms or trigger autoimmune flares if not carefully controlled.[5]

Conclusion and Future Perspectives

eccDNA has emerged as a pivotal player in immunology, influencing both innate and adaptive immune responses and contributing to the pathogenesis of autoimmune and inflammatory diseases. From its role as a DAMP activating cGAS-STING signaling, to its involvement in shaping antigen receptor diversity and modulating antigen presentation, eccDNA is increasingly recognized as a bridge between genomic instability and immune regulation.

Importantly, eccDNA's distinct structural properties—its circularity, genomic origin diversity, and resistance to degradation—render it a promising biomarker for non-invasive monitoring of immune-related diseases including systemic lupus erythematosus and rheumatoid arthritis. Furthermore, eccDNA's immunostimulatory potential underscores its relevance as a therapeutic target, particularly through modulation of DNA sensing pathways or enhancement of DNA clearance mechanisms.

Looking ahead, eccDNA holds significant promise for advancing our understanding of immune regulation and disease. One particularly exciting avenue is its potential role in trained immunity, where eccDNA may contribute to the epigenetic reprogramming of innate immune cells—a concept that could redefine how we view immunological memory beyond the adaptive system. Longitudinal profiling of eccDNA dynamics alongside immune biomarkers may enable earlier prediction of disease flares and more precise monitoring of therapeutic responses. Furthermore, coupling eccDNA analysis with single-cell transcriptomic and epigenomic technologies could uncover previously unrecognized, cell type–specific functions of eccDNA, particularly within the complex landscape of immune cell populations.

As technological advances continue to expand our understanding, eccDNA is emerging not merely as a biomarker or a byproduct of genomic instability, but as a potential regulatory element within the immune system itself. This evolving perspective places eccDNA research at the forefront of immunological discovery and translational medicine.

Acknowledgments

The figures were created in https://BioRender.com

Author contributions

Contributor roles according to CRediT Taxonomy is as follows:

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- Data curation - HBCL, KS
- Formal analysis - HBCL, KS
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- Investigation - HBCL, KS, TB, CS, HTD, SP
- Methodology - HBCL, KS, NH, AL
- Project administration - HBCL, KS, TB, CS
- Resources - HBCL, KS, TB, NH, AL
- Software - HBCL, KS, TB, NH, AL
- Supervision - NH, AL
- Validation - TB, CS, SW, HTD, SP
- Visualization - HBCL, KS
- Writing – original draft - HBCL, KS
- Writing – review & editing - HBCL, KS, NH, AL

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