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Exosomes: a potential biomarker and therapeutic targets in diabetic cardiomyopathy

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Abstract: Diabetic cardiomyopathy (DCM) refers to a complication that arises in diabetic patients and leads to various cardiac dysfunctions. These dysfunctions include oxidative stress, myocardial apoptosis, mitochondrial dysfunction, inflammation, lipotoxicity, fibrosis, impaired Ca^{2+} handling, and increased fatty acid utilization. Despite significant research efforts, the molecular mechanism underlying DCM remains incompletely understood. Recent studies have highlighted the role of exosomes, which are endogenous nanovesicles, in carrying detrimental components capable of initiating and propagating disease-related signaling events. In this review, we summarize the potential underlying mechanisms of DCM and discuss the potential use of exosomes in understanding the cellular mechanisms involved and exploring therapeutic approaches for DCM. These insights and opportunities may pave the way for new advancements in the field.

Keywords: exosomes; diabetic cardiomyopathy; miRNA

1. Introduction

1.1. Diabetes and DCM

Diabetes mellitus (DM) is a global chronic metabolic disorder and as of 2021, approximately 537 million adults worldwide are living with diabetes, and this number is projected to rise to

643 million by 2030 and 783 million by 2045 [1]. DM is primarily classified into two types: type 1 diabetes (T1D) and type 2 diabetes (T2D). Both T1D and T2D, when poorly managed, can lead to severe complications, particularly affecting the cardiovascular system, kidneys, nerves, and eyes [2–4].

Among the complications of diabetes, DCM is a specific cardiac condition that poses significant risks but is often underrecognized. First described by Rubler *et al.* in 1972 [5], DCM is characterized by myocardial dysfunction independent of coronary artery disease or hypertension. The hallmark features of DCM include left ventricular hypertrophy, impaired systolic and diastolic function, and structural alterations such as interstitial fibrosis.

The pathogenesis of DCM involves a complex interplay of metabolic abnormalities, oxidative stress, inflammation, and dysregulated calcium handling in cardiomyocytes. Hyperglycemia and insulin resistance, key features of diabetes, drive these processes by promoting the generation of advanced glycation end products (AGEs), which trigger inflammatory responses and oxidative damage in cardiac tissue [6]. Over time, these metabolic derangements lead to cardiac hypertrophy, fibrosis, and eventual heart failure. Impaired calcium handling further exacerbates cardiac dysfunction, making it critical to address these abnormalities early to prevent the progression of DCM.

1.2. Role of exosomes in DCM

Exosomes, small extracellular vesicles ranging from 30–200 nm in diameter, have emerged as important mediators of cell-to-cell communication in various diseases, including DCM [7]. They are released by multiple cell types, including cardiomyocytes, endothelial cells, and inflammatory cells. Exosomes carry a diverse cargo of bioactive molecules, such as proteins, lipids, and nucleic acids (notably miRNAs), which can be transferred to recipient cells, thereby modulating cellular behavior and disease progression [8].

The biogenesis of exosomes involves the formation of intraluminal vesicles (ILVs) within multivesicular bodies (MVBs), which are subsequently released into the extracellular space upon fusion of MVBs with the plasma membrane (Figure 1). Once released, exosomes can be internalized by recipient cells via endocytosis, direct fusion, or receptor-mediated uptake. Endosomal Sorting Complex Required for Transport (ESCRT) is a key molecular machinery involved in the biogenesis of exosomes, which is responsible for regulating membrane remodeling and trafficking processes within cells [9]. This allows for the transfer of pathogenic signals from diseased cells to healthy cells, thereby perpetuating the progression of DCM. The similarities between exosome uptake and viral entry mechanisms underscore the complexity and efficiency of exosome-mediated intercellular communication [10].

MicroRNAs are secreted by cells through exosomes and have been found to be present in body fluids such as blood, urine, and saliva. Their stability in these fluids makes them excellent biomarkers for some diseases. By detecting the specific patterns of microRNAs in body fluids, researchers can potentially use them for diagnosis, prognosis, and monitoring of various diseases [11].

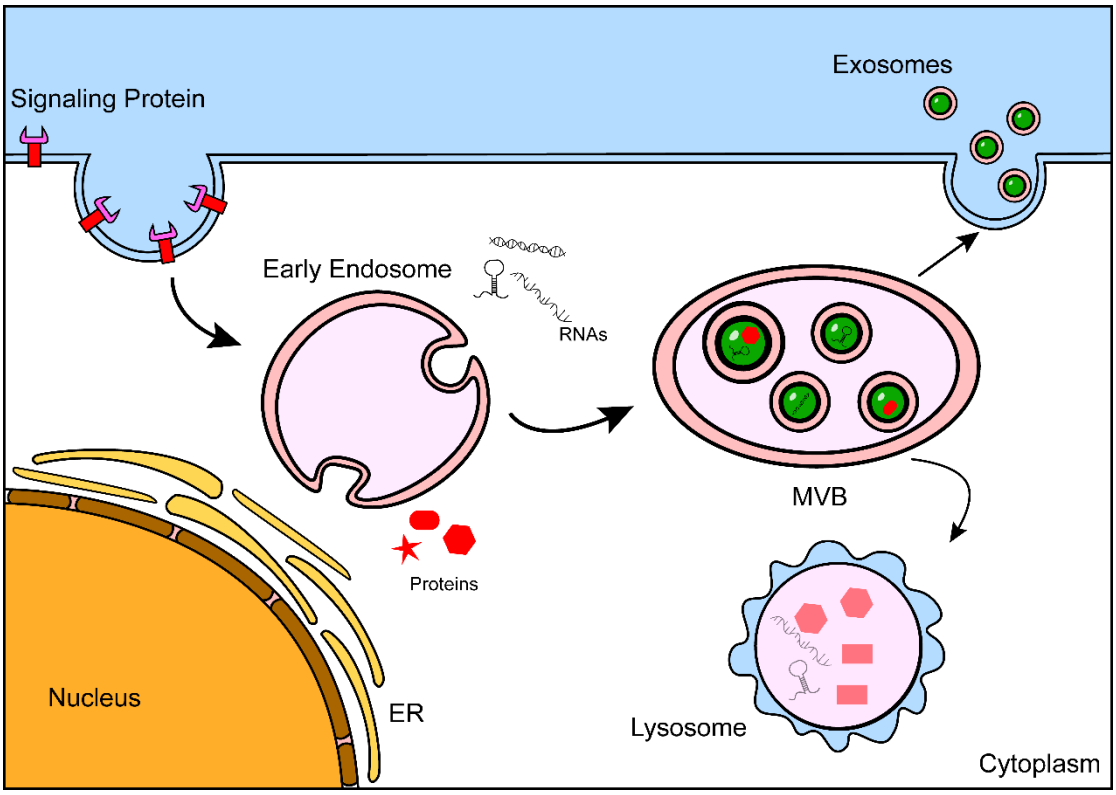

Figure 1. Schematic representation of exosome biogenesis and release.

In the context of DCM, exosomes released from diabetic cardiomyocytes have been found to carry detrimental molecules, such as pro-inflammatory cytokines, oxidative stress-inducing factors, and miRNAs, that exacerbate the disease. Recent research has underscored the potential of targeting exosome production or modulating their cargo content as novel therapeutic strategies for DCM. By manipulating the miRNA composition or inhibiting the release of pathological exosomes, it may be possible to attenuate disease progression and improve clinical outcomes [12,13]. For instance, exosomes containing miR-21 and miR-195 can promote fibrosis and apoptosis in neighboring cells, contributing to the worsening of cardiac function. These findings highlight exosomes as both contributors to disease pathology and potential therapeutic targets [14].

Signaling proteins are internalized into early endosomes, which mature into multivesicular bodies (MVBs) containing intraluminal vesicles. MVBs can fuse with the plasma membrane to release exosomes into the extracellular space, carrying RNAs and proteins for cell communication.

2. Exosome-related mechanisms in DCM

2.1. Oxidative stress and exosome dynamics

In DCM, oxidative stress is a significant contributor to cardiac dysfunction. Hyperglycemia in diabetes leads to an increase in fatty acid β-oxidation, resulting in elevated production of reactive oxygen species (ROS) within mitochondria [15]. ROS are highly reactive molecules that cause cellular damage, including lipid peroxidation and protein modification. In

cardiomyocytes, this oxidative stress can trigger the release of exosomes containing miRNAs such as miR-195, which promote apoptosis and further propagate oxidative damage in neighboring cells [16,17].

Exosomes derived from diabetic cardiomyocytes have been shown to carry detrimental components that exacerbate cardiac dysfunction. For instance, these exosomes can deliver pro-inflammatory factors and ROS-inducing molecules to recipient cells, contributing to myocardial fibrosis and apoptosis. The role of exosomal miRNAs like miR-133a and miR-195 in modulating pathways related to oxidative stress and inflammation highlights their potential as therapeutic targets for mitigating DCM progression [18].

2.2. Calcium homeostasis and exosome signaling

Proper calcium (Ca^{2+}) handling is essential for the contractile function of cardiomyocytes, as it regulates both the contraction and relaxation phases of the cardiac cycle. During an action potential, calcium ions enter cardiomyocytes through L-type calcium channels, triggering a further release of Ca^{2+} from the sarcoplasmic reticulum (SR) via ryanodine receptor 2 (RyR2) channels, a process known as calcium-induced calcium release (CICR) [19]. The reuptake of Ca^{2+} into the SR is facilitated by the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a), which is crucial for diastolic relaxation. In diabetic hearts, oxidative stress, hyperglycemia, and dyslipidemia lead to post-translational modifications of key Ca^{2+}-handling proteins such as SERCA2a and RyR2, impairing their function and contributing to contractile dysfunction and arrhythmogenesis.

Emerging evidence suggests that exosomes play a significant role in regulating calcium signaling pathways in DCM [20]. Exosomes derived from diabetic cardiomyocytes carry specific miRNAs and proteins that modulate the expression and function of Ca^{2+}-handling proteins in recipient cells. For example, exosomal miR-25 has been shown to downregulate SERCA2a expression, impairing Ca^{2+} reuptake into the SR and exacerbating diastolic dysfunction. Similarly, exosomal miR-214 has been implicated in regulating RyR2, promoting abnormal Ca^{2+} release and contributing to arrhythmogenic remodeling [21].

3. Exosomal miRNAs in cardiac remodeling

Exosomal miRNAs are crucial regulators of cardiac remodeling, exerting their effects by modulating gene expression in recipient cells. In DCM, these miRNAs contribute to pathological changes such as fibrosis, hypertrophy, and altered extracellular matrix composition. Among them, miR-208a and miR-21 are particularly significant.

MiR-21 is one of the most studied miRNAs in the context of cardiac fibrosis and hypertrophy. It is abundantly expressed in cardiac fibroblasts and is packaged into exosomes, which can be released under pathological conditions such as hyperglycemia and oxidative stress. Once transferred to neighboring cardiomyocytes and fibroblasts, miR-21 targets the SPRY1 gene, a negative regulator of the MAPK/ERK pathway, which is involved in fibroblast proliferation and collagen production. By inhibiting SPRY1, miR-21 enhances the

proliferation and activation of cardiac fibroblasts, leading to excessive deposition of extracellular matrix components and myocardial fibrosis [14].

MiR-210 plays a pivotal role in mitigating apoptosis and oxidative stress in cardiomyocytes. For instance, research has shown the upregulation of miR-210 by diallyl trisulfide (DATS) leads to the suppression of pro-apoptotic pathways, enhancing cell survival and reducing AGE-induced cardiac injury. This highlights miR-210 as a crucial mediator in the protective mechanism of DATS and suggests its potential as a therapeutic target for combating DCM [22]. This anti-apoptotic effect of miR-210 helps preserve cardiac function after myocardial infarction and suggests that EVs enriched with miR-210 could be a potential therapeutic approach to enhance cardiac repair and function after injury [23].

In addition to miR-21, exosomal miR-208a plays a pivotal role in regulating cardiac hypertrophy and fibrosis. MiR-208a is encoded within the myosin heavy chain gene and is primarily expressed in cardiomyocytes. It regulates the expression of myosin isoforms and is essential for maintaining cardiac muscle integrity. In diabetic hearts, elevated levels of miR-208a are associated with increased fibrosis and hypertrophy. Exosomal transfer of miR-208a to cardiac fibroblasts has been shown to upregulate fibrotic genes, such as collagen type I and III, thereby contributing to pathological cardiac remodeling [24].

Moreover, other exosomal miRNAs, such as miR-29b and miR-133a, also play significant roles in cardiac remodeling [25]. miR-29b is known to regulate genes involved in extracellular matrix degradation, such as matrix metalloproteinases (MMPs). Downregulation of miR-29b in diabetic hearts is linked to reduced MMP activity and increased fibrosis. Conversely, miR-133a is cardioprotective, modulating hypertrophy and apoptosis by targeting genes involved in pro-apoptotic signaling pathways. Its reduced expression in diabetic hearts contributes to increased cardiomyocyte apoptosis and impaired cardiac function.

Inhibiting the release of pathogenic miRNAs like miR-21 and miR-208a, or modifying their content in exosomes, presents a promising therapeutic strategy for mitigating adverse cardiac remodeling in DCM [25]. For instance, antisense oligonucleotides targeting these miRNAs have shown efficacy in reducing fibrosis and hypertrophy in preclinical models of DCM [26]. Additionally, engineered exosomes carrying cardioprotective miRNAs such as miR-133a or miR-30d can be designed to deliver therapeutic cargo specifically to damaged cardiac tissues, potentially reversing pathological remodeling and improving cardiac function [27].

Given their role in disease propagation, targeting exosomal pathways in DCM offers a novel therapeutic approach. Strategies such as inhibiting the release of harmful exosomes or enhancing the delivery of protective miRNAs, like miR-133a, could help restore normal cardiac function and prevent further progression of DCM. Ongoing research is focused on developing exosome-based therapies to modulate the disease course effectively (Table 1).

Table 1. Roles of microRNAs in DCM

MiRNA	Target(s)	Role in DCM
miR-30d [27]	MAP4K4	Potential in regulating insulin production
miR-195 [16]	SIRT-1, Bcl-2	Target for delaying development of dilated cardiomyopathy
miR-21 [21]	Hypertrophy-related genes	Potential therapeutic target for treating cardiac hypertrophy
miR-208a [25]	Fibrosis, mitochondrial dysfunction	Regulatory role in cardiac contractility and remodeling
miR-1, miR-133a [28]	-	Potential diagnostic tools for managing T2DM
miR-141 [29]	-	Possible role in development of cardiac fibrosis
miR-320 [30]	IGF-1, Hsp20 and Ets2	Potential therapeutic target for diabetes-caused aberrant angiogenesis
miR-451 [31]	LKB1/AMPK pathway	Potential target for treating DCM
miR-155 [32]	Nrf2/HO-1 pathway	Potential therapeutic target for cardiac fibrosis in diabetic patients
miR-210 [22]	MAPK-JNK pathway	Potential therapeutic target to suppress apoptosis in diabetic patients

4. Exosomes as therapeutic targets and clinical applications in DCM

Exosomes hold great promise as therapeutic agents in DCM due to their natural ability to transfer bioactive molecules, such as miRNAs, proteins, and lipids, between cells, thereby influencing disease progression. These nanosized vesicles are involved in the regulation of key cellular processes, including apoptosis, fibrosis, and inflammation, which are pivotal in the pathogenesis of DCM [33]. Recent studies have demonstrated that exosomes derived from mesenchymal stem cells (MSCs) and cardiomyocytes can exert cardioprotective effects by modulating the activity of recipient cells. For example, exosomes carrying miR-133a and miR-126 have been shown to reduce oxidative stress and apoptosis in diabetic hearts, thereby improving cardiac function and preventing adverse remodeling [34].

The potential of exosomes in diagnostics is also being actively explored. Exosomal miRNAs, such as miR-29a and miR-30d, have been identified as potential non-invasive biomarkers for the early detection and monitoring of DCM. These miRNAs exhibit altered expression levels in the serum of patients with DCM, reflecting the ongoing pathological

processes in the heart. Their stability in body fluids makes them ideal candidates for diagnostic tests, offering a promising alternative to traditional biopsy or imaging techniques [35].

In addition to diagnostics, exosomes are being investigated as drug delivery vehicles. Their unique properties, such as biocompatibility, low immunogenicity, and the ability to cross biological barriers, enable them to deliver therapeutic agents directly to target tissues [36]. For instance, engineered exosomes loaded with small interfering RNAs (siRNAs) or anti-inflammatory drugs have shown potential in reducing cardiac inflammation and fibrosis in preclinical models of DCM. Moreover, *in vivo* self-assembled small RNAs delivered via exosomes through the circulatory system represents a novel RNAi therapeutic approach for DCM [37]. This targeted delivery approach not only enhances therapeutic efficacy but also minimizes systemic side effects commonly associated with conventional drug therapies.

Such innovations could pave the way for personalized and gene-based therapies in the future. Such innovations could pave the way for personalized and gene-based therapies in the future. As our understanding of the specific roles of miRNAs in DCM deepens, clinical studies are beginning to incorporate miRNA-based approaches for both diagnostic and therapeutic purposes. These include miRNA inhibitors or mimics to precisely target dysregulated pathways and restore normal cardiac function. This perspective opens up possibilities for developing tailored therapies that focus on individual genetic profiles, thereby offering a more precise and effective approach to treating DCM.

5. Current preclinical and clinical studies

Recent studies have expanded the understanding of the diagnostic potential of exosomal miRNAs in DCM. Research has shown that specific exosomal miRNAs, such as miR-30d, miR-126, and miR-320, are significantly altered in the serum of patients with DCM compared to healthy controls [38]. These miRNAs are involved in key pathological processes such as cardiac fibrosis, oxidative stress, and apoptosis, making them valuable non-invasive biomarkers for early detection and disease monitoring.

For instance, miR-320 has been found to be upregulated in exosomes derived from diabetic cardiomyocytes, where it influences endothelial cell function by reducing nitric oxide production and inhibiting angiogenesis [39]. This suggests that monitoring miR-320 levels in exosomes could provide insights into the progression of DCM and its impact on cardiac microvasculature. Similarly, miR-126 is known for its role in promoting angiogenesis and maintaining endothelial integrity. Reduced levels of exosomal miR-126 have been associated with impaired vascular repair mechanisms in DCM, making it a potential marker for evaluating disease severity and therapeutic response [40].

Additionally, exosomal miR-1 and miR-133a have been identified as potential biomarkers for myocardial hypertrophy and fibrosis in DCM. These miRNAs are linked to altered calcium handling and cardiomyocyte apoptosis, key features of DCM pathology [41,42]. The overexpression of these miRNAs in serum exosomes correlates with increased myocardial fibrosis and dysfunction, highlighting their utility in diagnosing and tracking the progression of DCM.

Overall, exosomal miRNAs show great promise as biomarkers for the early detection and monitoring of DCM, with potential to enhance clinical outcomes through timely diagnosis and targeted therapeutic interventions.

Recent research has significantly advanced our understanding of the therapeutic potential of exosomes in treating DCM. Here are some notable clinical applications.

6. Future directions and research gaps

Exosome research has unveiled their pivotal role in the pathogenesis and potential treatment of DCM. However, several critical areas require further investigation to fully exploit their clinical potential.

6.1. Elucidating molecular mechanisms of exosome biogenesis and cargo sorting

To advance exosome-based therapies, it is essential to delve deeper into the molecular mechanisms that regulate exosome biogenesis, cargo sorting, and release. Currently, our understanding of the specific molecular signals and pathways that dictate the selective incorporation of miRNAs, proteins, and other molecules into exosomes is limited. Identifying these regulatory pathways could enable precise manipulation of exosomal cargo, allowing for the engineering of exosomes with tailored therapeutic properties. This would involve exploring key components such as the endosomal sorting complexes required for transport (ESCRT) machinery, lipid raft domains, and various adapter proteins that influence cargo selection. By elucidating these mechanisms, we can develop strategies to enhance the loading of therapeutic molecules into exosomes and control their release, ultimately improving the efficacy of exosome-based treatments.

6.2. Standardizing exosome isolation and characterization techniques

A significant challenge in exosome research lies in the lack of standardized methodologies for their isolation and characterization. The use of varying isolation techniques, such as ultracentrifugation, size-exclusion chromatography, and immunoaffinity capture, often results in discrepancies in exosome purity and yield, which can impact the reproducibility of experimental findings and hinder clinical translation [43]. To ensure consistency and reliability across studies, it is crucial to establish standardized protocols for the isolation, quantification, and molecular profiling of exosomes. These protocols should focus on optimizing isolation methods to achieve high-purity exosome preparations, accurate quantification strategies to measure exosome concentration and size distribution, and comprehensive profiling techniques to characterize the molecular cargo of exosomes. Implementing these standardized procedures will be essential for validating exosome-based diagnostic and therapeutic applications in clinical settings.

6.3. Clinical applications of exosomes in DCM

The therapeutic potential of exosomes in DCM remains vast, yet it has not been fully leveraged. To bridge this gap, future research must prioritize conducting large-scale clinical trials to rigorously assess the safety and therapeutic efficacy of exosome-based interventions. These trials should specifically investigate the use of exosomes as targeted drug delivery systems, given their capacity to modulate critical pathological processes such as cardiac inflammation, fibrosis, and oxidative stress. Moreover, efforts should be directed towards the development of exosomal biomarkers that can serve in the early diagnosis and prognosis of DCM [44]. Such biomarkers would be instrumental in advancing personalized treatment strategies, allowing for more precise monitoring of disease progression and therapeutic responses, thereby improving patient outcomes and optimizing clinical care.

Exosome research in DCM is still emerging compared to fields like oncology and neurology. Comprehensive studies are needed to map the specific miRNA and protein signatures of exosomes derived from diabetic cardiomyocytes. Such profiling will reveal the precise pathways that exosomes influence in DCM, uncovering novel therapeutic targets and biomarkers. This could lead to the development of targeted exosome-based interventions tailored to the unique molecular landscape of DCM.

6.4. Developing targeted exosome-based therapies

An emerging and promising approach in the treatment of DCM involves the development of exosome-based therapies specifically engineered to target damaged cardiac tissues. By bioengineering exosomes to deliver therapeutic agents, such as cardioprotective molecules or pharmacological drugs, directly to diseased cardiac cells, it becomes possible to modulate pathological processes such as fibrosis, inflammation, and apoptosis at the cellular level [45]. However, the effective translation of this strategy into clinical practice faces several challenges, particularly the optimization of targeted delivery systems to ensure specificity and the reduction of off-target effects. Addressing these issues will necessitate innovative research focused on enhancing exosome selectivity for diseased cells, improving the efficiency of therapeutic cargo loading, and advancing imaging technologies to track exosome distribution and bio distribution *in vivo*. Such innovations will be pivotal in refining the therapeutic efficacy of exosome-based interventions and ensuring their successful application in DCM treatment.

6.5. Integrating multi-omics approaches

A comprehensive understanding of exosome functionality in DCM can be greatly enhanced through the application of multi-omics approaches, encompassing genomics, transcriptomics, proteomics, and metabolomics [46]. These methodologies facilitate a holistic analysis of the genetic, transcriptional, protein, and metabolic compositions of exosomal cargo, providing detailed insights into the molecular mechanisms underpinning exosome activity in DCM [47]. By integrating these datasets, novel biomarkers and therapeutic targets can be identified,

allowing for a more nuanced understanding of the disease's pathophysiology. Moreover, such integrative approaches have the potential to unravel complex disease pathways that are otherwise difficult to characterize. To fully leverage these advanced technologies and translate findings into clinical applications, close collaboration among bioinformaticians, molecular biologists, and clinicians is critical. This interdisciplinary synergy will enable the effective interpretation of complex datasets and drive the development of precision therapies for DCM based on exosomal molecular signatures.

By addressing these research gaps and focusing on clinical applications, exosome research could revolutionize the management of DCM, paving the way for novel therapeutic strategies and personalized medicine.

7. Conclusion

The future of exosome research in DCM holds immense potential but also presents several challenges. By addressing these research gaps and focusing on clinical applications, exosomes could become a cornerstone of innovative therapeutic strategies for managing DCM. Continued efforts to understand and manipulate exosome biology will be crucial in realizing their full potential in personalized medicine.

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Conflicts of interests

The authors declare no conflicts of interest.

Authors' contribution

Conceptualization: X.Y.; writing-original draft preparation: A.A. ; writing-review and editing: J.M and A.A.; visualization: A.A.; supervision: X.Y.; project administration: X.Y.; funding acquisition: J.M. All authors have read and agreed to the published version of the manuscript.

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