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Commentary

Forging the future of circRNA therapeutics: Unleashing synthetic potential and conquering challenges

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Circular RNAs (circRNAs) are a unique class of RNAs formed from back-splicing or alternative splicing of exons from pre-messenger RNAs or transfer RNAs (tRNAs).[1,2] They can be categorized into several types depending on how they are processed: exonic circRNAs, intronic circRNAs, exon-intron circRNAs, readthrough circRNAs, fusion circRNAs, and tRNA-derived circRNAs.[1,2] The discovery of circRNAs can be traced back to the 1970s,[3] and their functions gained significant attention in the 2010s owing to high-throughput RNA sequencing and bioinformatic analysis, particularly as microRNA (miRNA) sponges.[2] The extensive identification of circRNAs has sparked great interest among researchers, leading to investigation into their diverse functions.[4] circRNAs have been reported to play crucial roles in various biological processes. They can regulate gene expression at the transcriptional level by interacting with gene promoters, serving as macromolecule recruiters through structure-based scaffolding, acting as miRNA decoys through complementary duplex formation, and even facilitating the translation of circRNA-specific peptides.[1,2] Particularly in recent years, circRNAs have garnered attention in cancer research, as they have been identified in numerous cancer types. The tissue-specific expression patterns of circRNAs make them promising candidates for the development of disease-specific biomarkers and targeted therapeutics. Several studies have identified upregulated circRNAs in cancer and explored methods to target them, such as RNA interference, the RNA-targeting CRISPR-Cas13 system, and antisense oligonucleotides.[2] While clinical trials in this field are currently lacking due to its relative novelty, continuous efforts are being made to advance the understanding and application of circRNAs in cancer research. The cellular functions, regulatory mechanisms, and emerging roles of circRNAs in cancer and oncology have been comprehensively reviewed by Li et al.[1] and Kristensen et al.,[2] in which more intriguing details are described and discussed.

According to the above-mentioned reviews and other literature, current research on circRNAs is advancing across several pivotal directions, each contributing to our understanding and application of these unique RNA molecules. These encompass the following areas of investigation.

(1) characterizing circRNAs in terms of their structure, abundance, and biogenesis mechanisms,
(2) investigating the regulation of circRNA abundance, nuclear export, and turnover,
(3) exploring the functions of circRNAs in normal cellular processes and pathways,
(4) understanding how the functions of circRNAs vary depending on the cellular context and specific conditions,
(5) studying the dysregulation of circRNAs in diseases, particularly cancer, to identify potential biomarkers and therapeutic targets,
(6) synthesizing artificially designed and engineered circRNAs to enhance their functionality or to apply them as tools for diagnostic and therapeutic purposes.[1,2,5,6].

Accumulated knowledge regarding the expression, cellular localization, and biological functions of circRNAs has paved the way for deliberate design, development, and engineering for cancer treatment. A recent study published by Bayat et al. in *Molecular Therapy – Nucleic Acids* sheds light on the promising field of circRNA engineering.[7] This research represents a significant contribution to our understanding of circRNAs and their potential applications. In their study, Bayat et al. designed a specific circRNA, CM21D, to target the miRNA miR-21 by utilizing the function of circRNAs as "miRNA decoys," which can bind miRNAs. CM21D was generated using the tRNA-splicing mechanism and tested in preclinical glioblastoma models. The study demonstrated the affinity of CM21D for miR-21 and its effectiveness in reducing cell growth and oncogenic signaling pathways in glioblastoma cells. In a rat model, CM21D-expressing cells developed smaller tumors than controls. Although further research is needed to understand CM21D's mechanism of action, half-life, degradation and its efficacy *in vivo*, the study has enhanced our ability to investigate and translate our knowledge of circRNA from bench to bedside.

Other research groups are also actively working on modifying, designing, and synthesizing circRNAs to enhance their original function, target antigens and stabilize mRNAs by circularization. Therefore, designing circRNA therapeutics has become a prominent focus for both academic spinouts and drug discovery companies. Indeed, designing circRNA therapeutics became one of six academic

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spinouts of Nature Biotechnology 2021, according to a 2022 survey, and one of the major focuses of drug discovery companies.[8] For example, Orna Therapeutics has formed a circRNA collaboration with Merck worth up to $3.5 billion, as circRNAs possess remarkable stability that can overcome the current challenging issues in mRNA therapeutics, such as instability and short lifespan, which limit protein production in human cells.[9] Synthetic circRNAs targeting miRNAs have also been reported previously. For instance, a study designed a synthetic circRNA switch to control protein expression in mammalian cells.[10] The current finding from Bayat et al. is quite impactful in this regard, as it provides another option to target miR-21, a prevalent oncogenic miRNA in many types of cancer, not limited to glioblastoma. Beyond this study, several other studies have designed synthetic circRNAs to target miRNAs, including miR-21, in different cancer types. A study published in 2020 by Muller et al. in *NAR Cancer* reported another synthetic circRNA as a miR-21-5p decoy designed to target it in lung adenocarcinoma and tested the therapeutic effect using nanoparticle-carried delivery in xenograft mice.[5]

Moving forward, in order to facilitate the clinical translation of findings such as those presented by Bayat et al., it is crucial to obtain a comprehensive understanding of the mechanism of action and the precise binding kinetics of CM21D. Unraveling how CM21D interacts with its target molecule, miR-21, and identifying the specific molecular pathways involved will greatly enhance our knowledge of its therapeutic potential. Moreover, conducting thorough testing of CM21D in preclinical models is essential to evaluate its toxicity, stability, and potential off-target effects. These comprehensive assessments will enable us to assess the safety and efficacy of CM21D, ensuring its suitability for further development as a therapeutic agent. It is crucial to uncover any potential risks or limitations associated with CM21D and refine its design accordingly. In addition to assessing the biological properties of CM21D, addressing the practical challenges of its clinical application is equally important. One of the key hurdles in RNA therapeutics lies in determining the optimal dosage and developing effective delivery methods. Therefore, it is vital to explore diverse delivery strategies and evaluate their efficiency in successfully transporting CM21D to target cells or tissues. By addressing these critical aspects, we can advance the development of reliable and efficient delivery systems, ultimately maximizing the clinical utility of CM21D and similar RNA therapeutics.

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DECLARATION OF INTERESTS

The authors declare that there are no conflicts of interest.

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