Commentary: The therapeutic potential of circular RNA

Drug developers have been paying increasing attention to nucleic-based medicines ever since messenger RNA (mRNA) vaccines showed the ability to prevent the spread of the SARS-CoV-2 virus and save millions of lives around the world. Less well known is a subunit of RNA called circular RNA (circRNA) which is still in preclinical development. CircRNA is a sequence of single stranded RNA that forms a covalently closed continuous loop. Its unusual configuration gives it a long half-life and unusual promising therapeutic potential. This is my observation after years of academic work on the subject, and my current leadership role at Circio, based in Norway and Sweden.

The natural expression of circRNA was established in the early 1990s. However, it was not until the 2010s that this type of RNA gained significant interest as a common, highly conserved, non-coding RNA species with structural and regulatory functions in human cells. Cirs-7 was the first thoroughly studied endogenous human circRNA. This was initially reported in 2011 by myself, Thomas Hansen, and colleagues in The EMBO Journal.[1] In the subsequent 15 years to date, over 100,000 human circRNA species have been annotated. Considering that the human genome contains around 20,000 protein-coding genes, this demonstrates the abundance and importance of this RNA class.

The most interesting characteristic of circRNA from a therapeutic perspective is its substantially increased durability compared with linear RNA. Linear RNA is subject to exonucleolytic decay within a cell, whereby ribonucleotides are chopped off one by one from the end of the RNA. A circular RNA is a closed loop lacking a free end and is therefore intrinsically resistant to this process. This leads to a dramatic increase in stability. With short durability being one of the major drawbacks of mRNA therapeutics, this characteristic has therefore triggered substantial interest in engineered circRNAs for therapeutic applications.

Following the discovery and characterisation of human non-coding circRNA, substantial efforts went into designing circRNAs that could express proteins. This created the circular mRNA concept. In 2019, Wesselhoeft et al. showed successful delivery and protein expression from an LNP-packaged circular mRNA in mouse models. The discovery led to the creation of oRNA Therapeutics of Cambridge, USA, in 2021. From this, several high-profile biotechs have now focused on capturing this opportunity, including Orbital, Renegade, Sail Biomedicines and our company, Circio.

It has now been established that LNP-packaged circRNA can achieve robust expression *in vivo* for seven to 10 days, a substantial improvement over current gold-standard modified mRNA designs that typically have an expression window of only two to three days. Other advantages of the circular format include the cap-independent mechanism to drive protein expression. When well-designed and optimised, cap-independent translation can achieve three to five times higher expression rates versus linear mRNA. From a patient delivery perspective, circRNA is expected to have 80-90% reduced manufacturing costs compared to mRNA by omitting expensive processing steps.

These advantages have led to strong interest in this emerging RNA class from both industry and investors. In the past three years, circRNA companies attracted approximately 40% of all venture capital that went into RNA-related concepts. Proponents of circRNA believe that as the technology makes its way into the clinic and products are approved, circRNA will rapidly make linear mRNA redundant as a therapeutic format.

Replacing linear with circular mRNA as a novel platform for infectious disease vaccines is the logical place to start. However, new entrants are finding ingenious ways to leverage the advantage of circular RNA in novel settings. Circio saw an interesting opportunity to use circular mRNA as a way to enhance the durability and level of protein expression from viral and DNA vectors. This could bring the advantages of circRNA to improve genetic medicine. Currently, synthetic RNA approaches, both circular and linear, fall far short of the durability required to replace a mutated or missing protein on a permanent basis.

By engineering a DNA-based genetic cassette driving circRNA-based protein expression inside cells, Circio has shown substantially improved protein output *in vitro* and *in vivo* compared to classic mRNA-based vector expression. Current gene therapy approaches, which are mainly based on the AAV vector, face major limitations due to low expression, leading to high dosing levels and toxicity in patients. By switching to circRNA-based expression, the potency of AAV gene therapy could be substantially improved and consequently result in both reduced dosing and cost. In addition, new disease targets where AAV gene therapy has not yet achieved sufficient expression level could potentially be opened up, bringing new therapeutic options to patients with no good treatment alternatives. Therefore, as synthetic circRNA is expected to replace linear mRNA for vaccines, circRNA-based expression could replace traditional mRNA vector systems in future therapies.

In addition, circRNA extends beyond vaccines and gene therapy. It can also be used for non-coding related modes of action, by sequestering and regulating molecules within cells, such as microRNAs, involved in many disease processes.

Circular RNA is still early stage, and no circRNA therapeutic candidates have yet entered clinical trials. The most advanced programme is the development of circRNA-based CAR-T therapy by oRNA tx enabling *in situ* T-cell transduction directly in patients. oRNA intends to bring its lead CD19 circRNA candidate into clinical trials within 12 months, and is expected to be the first circRNA-based therapeutic to enter human studies.

The biochemical advantages and applications bode well. CircRNA is poised to play an important role in shaping the nucleic acid medicines to come.

Reference. The EMBO Journal (2011), 30, 4414-4422.

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