**Potential functions of mammalian circRNA**

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**Circular RNAs(circRNA) were described and modeled decades ago but considered rather rare RNAs. However, recent researches discovered that these special RNAs are widely produced across species by so-called back-splicing mechanics. It has been reported that circRNAs play an important role in mammalian neural tissues, protein synthesis and protein interaction. Some evidence suggest circRNA are relevant to different human diseases including cancer. However, more hidden functions of circRNAs are still to be explored. Legnini et al. reported circRNAs expression is highly relevant to in muscle differentiation and diseases. They identified circ-ZNF609, a highly conserved circular RNA expressed in both murine and human myoblasts, which encodes a protein.**

**Introduction**

Circular RNA (circRNA) is a new class of non-coding RNAs with regulatory functions, which have a closed loop structure and are abundantly present in the eukaryotic transcriptome. Most of the circular RNA is composed of exon sequences, is conserved in different species, and has the expression specificity of tissues and different developmental stages.

Circular RNAs(circRNA) were described and modeled decades ago but considered rather rare RNAs. However, recent researches discovered that these special RNAs are widely produced across species by so-called back-splicing mechanics. Ashwal et.al studied the biogenesis of cricRNA and proposed a adjacent-assisted mechanism of exons and introns. They also demonstrated that circularization and splicing compete against each other.[1]

However, the exact functions of circular RNAs remain unknown for a long time. CircRNA was initially categorized as a kind of long non-coding RNA(LncRNA). LncRNA was once thought to be the "noise" of genomic transcription, a by-product of RNA polymerase II transcription, and has no biological functions.[2] So, few researches had ever explored the functions of circRNA before.

In 2013, Hanse et.al firstly reported on Nature that circRNA functions as efficient microRNA sponges, which promoted researchers to explore hidden functions of circRNA. They identified ciRS-7, a circular RNA sponge for miR-7, which contains more than 70 selectively conserved miRNA target sites. The circRNA binds to the corresponding microRNA, and the "sponge" adsorbs the miRNA, so that the miRNA cannot bind to the target gene, and thus participates in the regulation of the expression of the target gene. This mechanism of action is known as the competitive endogenous RNA (ceRNA) mechanism. Through the interaction of miRNAs associated with diseases, circRNA plays an important regulatory role in disease, which is the most important research idea of circRNA.[3]

Afterwards, several possible functions of circRNA have been reported by different researchers but more functions are to be explored and details are still unclear.

**Recent Progress**

As mentioned before, circRNA was recognized as a member of LncRNA. However, Legnini et.al reported circRNA is not only crucial for the regulation in muscle differentiation but can also encode proteins.[4]

Firstly, they collected total RNA from two replicates of human and mouse (C2C12) myoblasts and applied FindCirc computational pipeline to detect circRNA among them. Compared with the expression of known specific markers of muscle differentiation, they found about 10% of circRNAs was expressed at similar or higher levels with respect to the linear ones. Further criterion filters out 137 circRNAs in human related to muscle differentiation, 90 of which are shared by the mouse. CircRNAs are transcribed from exons highly conserved in different species due to evolutionary pressure, which indicates the significance of circRNA in organisms.

RNA-seq data human myoblasts derived from Duchenne muscular dystrophy (DMD) patients were also analyzed as evidence. Hierarchical clustering analysis of normal and dystrophic cells showed that DMD cells have obviously different characteristics in terms of circRNA expression levels, which support the formal results. This reminds us the possible application of circRNA as signals to diagnose specific diseases.

Then, specific criteria including conservation, expression level, circular/linear ratio were applied and 31 circRNAs were selected for further characterization. All except one have conserved expression in both human and mouse. They used RNase R exonuclease to check whether the selected RNAs are truly circular and 29 of them shows resistance to exonuclease.

Among the 29 circRNAs, circ-QKI were finally proven to directly regulate myoblast differentiation. They designed two siRNAs target at circ-QKI and the siRNAs both inhibit myoblast differentiation. Since the two siRNAs have effects on QKI mRNA as well, circ-QKI and QKI mRNA may have similar functions.

Lastly, Circ-ZNF609, one of the 29 circRNAs, was proven to encode a protein. Circ-ZNF609 originates from the circularization of the second exon of its host gene and the residual intron part gives it unique characteristics compared with its linear counterpart. The association of circ-ZNF609 with heavy polysomes was both confirmed in human and mouse, which provides solid evidence that circRNA does encode functional peptides or proteins. Mutants experiments show that two initiation codons (p-circD1 and p-circD2) play a crucial role in circRNA translation. They possibly served as positioning codons indicating two different translating path on RNA bands. They did several other experiments to confirm the conclusion but got few hints on the detailed function related to differentiation of the protein derived from Circ-ZNF609.

**Discussion**

Legnini et.al reported the evidence that supports encoding of circRNA and propose the significance of encoding circRNA. They found that the linear RNA and circular RNA transcribed from the same exon can encode different proteins because circRNA contains extra intron. Thus, circRNA can form peptides with additional amino acids compared with linear products. This mechanism may help simplify genome by increasing the complexity of output. There are thousands of circRNAs in human body and the accumulation of their encoding diversity can have considerable impacts on us.

However, Legnini et. al have not investigated the functions of the protein derived from circ-ZNF609. Since circularization and splicing are competitive process, it is a reasonable prediction that there are regulation mechanisms between peptides derived from linear and those from circular RNAs.

Most of the circular RNA is enriched in the cytoplasm, and its abundance is sometimes even higher than the corresponding linear mRNA, which may be due to the fact that the circular RNA is more stable than the linear RNA. Nucleases often act by recognizing the ends of linear RNA molecules, which are a closed structure and therefore highly resistant to nucleases. Because circular RNA is insensitive to nucleases, it is more stable than linear RNA, which makes circular RNA a distinct advantage in the development and application as a new clinical diagnostic marker.

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