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You spin me right 'round

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See the article by Liu et al in this issue, pp. 743–756.

Circular RNAs (circRNAs) are a class of RNA molecules that were initially discovered by electron microscopy over 4 decades ago. They obtain their circular structure due to the back-splicing of a splice donor site of an exon with an upstream (5′) splice acceptor site. Since their 5′ and 3′ ends are covalently bound, their transcripts are circular.[1] This closed structure prevents them from degradation by exonucleases, making them more stable in vivo than linear RNAs. CircRNAs lack a poly-A tail and a 5′ cap, which makes common poly(A)$^+$ RNA-seq strategies inappropriate to study them. However, protocols that do not enrich for poly(A)$^+$ are used more and more often for sequencing (eg, when studying degraded RNA from formalin fixed, paraffin embedded tissues), which explains why this class of RNA has recently gained more attention. To increase the circRNA yield, it is common to treat RNA with a specific exonuclease enzyme, RNase R, which specifically degrades linear RNA species.[2]

Different roles have been ascribed to non-coding circRNAs, which include functioning as "sponge" for microRNAs or for RNA-binding proteins.[3] The role for most circRNAs, however, is still not understood. CircRNAs can be dynamically regulated and often are tissue type-specific. Different cancer types, including low- and high-grade glioma, are characterized by differentially expressed circRNAs and dysregulated circRNAs can contribute to tumorigenesis.[4] Interestingly, genomic translocations that result in circRNAs have been reported and these fusion-circRNAs also contribute to tumor aggressiveness.[5] Although circRNAs are believed to be mostly non-protein-coding, some protein-coding circRNAs have been discovered. This opens the option of designing artificial protein-coding circRNAs to drive expression specific proteins.[6] Therefore, besides their role in (patho-) biology, molecular biologists can make use of the higher stability of circRNAs by using them as tool for research, eg, in the field of RNA vaccines.[4,7]

In this issue of *Neuro-Oncology*, Liu et al. provided a comprehensive study on the detection, presence, and functional significance of one specific circRNA in EGFR.[8] They performed RNA-seq on brain tumor-initiating cells (BTIC), neuro stem cells, and normal human astrocytes, using the RNase R trick. They report in particular on one specific "back-spliced" variant in EGFR (exon15 spliced onto exon14) to be one of the strongest upregulated circRNAs in BTIC and confirmed its presence by a variety of independent techniques. What makes this particular circRNA interesting is that it contains an open reading frame with a start- but not a stop codon. As the transcript is circular, it means that when translation initiates, a protein may be produced that contains multiple repeat peptide sequences encoded by exon15-14 transcripts ("Rolling Circle Translation" or "rolling EGFR variants"). To demonstrate the existence of this "infinite protein," the authors neatly made use of an expression vector that contains the exon15 and 14 of EGFR, flanked by two inverted repeats. When transcribed, these repeat regions hybridize and so circularize the nucleotides that lie in-between. This vector indeed resulted in the expression of a "ladder" of larger EGFR protein fragments. These fragments were also found in tumors and their presence was confirmed by mass spectrometry.

Demonstrating the mere presence of a protein, however, does not automatically confer importance or functionality. The authors therefore extensively analyzed the exon15-14 circEGFR and found that knockdown had several major effects: it reduced wild-type EGFR protein levels and downstream pathway activation and reduced colony formation and proliferation. In mice, knockdown and overexpression of this circEGFR, respectively, improved and reduced survival (though for some experiments additional expression of wild-type EGFR was required). Mechanistically, the authors showed that the reduction of EGFR protein levels after knockdown of circEGFR was due to its stabilizing effect on the EGFR protein: circEGFR and wtEGFR appear to interact directly and affect ubiquitinylation of the wild-type protein. The authors end by showing circEGFR can be a target for treatment, at least when knockdown is combined with nimotuzumab.

In brief, this article comprehensively describes the presence of a circRNA derived from the EGFR gene and demonstrates its functional importance. This study adds another chapter to the already complex EGFR signaling in gliomas (see, eg, Gao et al.[9]) and is one of the first to show the role of circRNAs in gliomas. It is likely not to end with this single circRNA: at least one more has recently been described in detail in gliomas (circ-SMO[10]) by the same authors while the presented dataset still harbors many unexplored differentially expressed circRNAs.

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