



# CircCCDC66: the colorectal oncogene

**Katrine Mandrup Nielsen, Thomas Birkballe Hansen**

Department of Molecular Biology and Genetics (MBG), Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus, Denmark

*Correspondence to:* Thomas Birkballe Hansen. Department of Molecular Biology and Genetics (MBG), Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus, Denmark. Email: tbh@mbg.au.dk.

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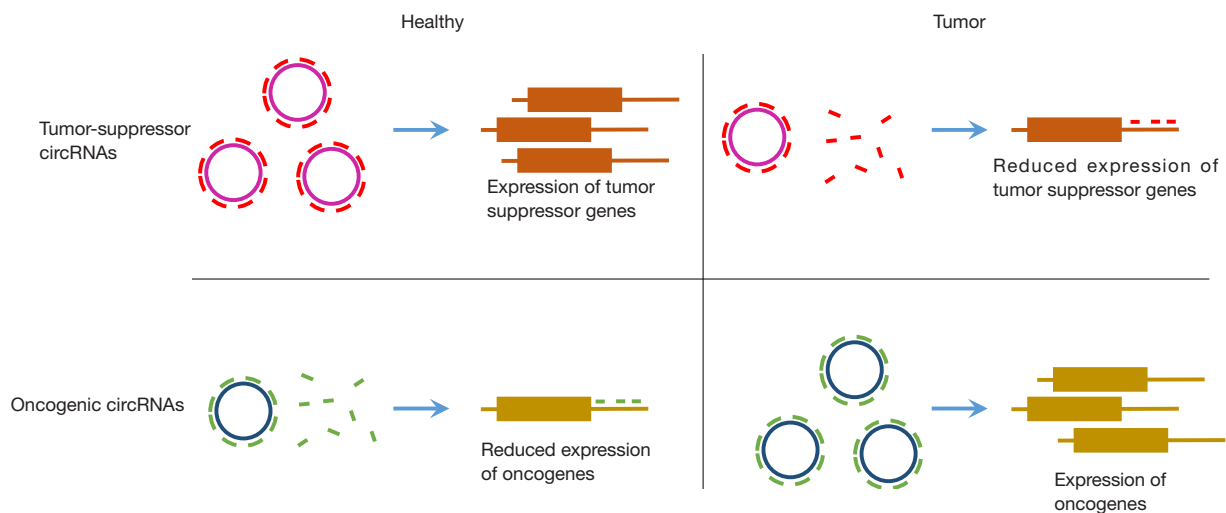
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Circular RNAs (circRNAs) are a large class of long non-coding RNAs produced by backsplicing or “head-to-tail” splicing (1). Due to the lack of free ends, circRNAs escape the conventional exonucleolytic RNA degradation pathways and thus have much higher stability than linear RNAs. The high stability allows the circRNAs to accumulate to high levels and conduct functions for prolonged periods in cells (2-4). Specific circRNAs have been functionally characterized as protein sponges, miRNA sponges, protein scaffolds or regulators of splicing and transcription (5-7). Many circRNAs are differentially expressed in cancerous tissues correlating with cancer progression (8,9). Among these, the circRNAs circFOXO3 and circITHC have been identified as tumor-suppressor circRNAs that are downregulated in tumor tissues. For both circRNAs, it has been suggested that they function as miRNA sponges to protect tumor-suppressor genes from miRNA attack (10,11) (*Figure 1*). However, the function of most circRNAs remains elusive although many of them have a high potential as biomarkers in cancer.

Recently, Hsiao *et al.* published a study revealing a novel oncogenic function of the circRNA circCCDC66 in colorectal cancer progression (12). They developed a pipeline to identify highly expressed circRNAs in RNA sequencing data, and from this, the authors found circCCDC66 as a novel cancer associated circRNA highly upregulated in colorectal cancer. Specifically, they found that 88% of the colorectal cancer patients had elevated levels of circCCDC66 and that the overall survival rate was lower for patients with high circCCDC66 compared to patients with low expression. Hence, circCCDC66 has promising prognostic biomarker potential. The elevated expression of

circCCDC66 in colorectal cancer indicates an important function in tumor development. To validate this hypothesis, Hsiao *et al.* performed knockdown and overexpression of circCCDC66. Knockdown of circCCDC66 in tumor cell cultures was conducted with siRNAs targeting the backsplice junction specifically. This resulted in a decreased tumor cell proliferation, migration and invasion. Furthermore, using xenograft mouse models, depletion of circCCDC66 prior to transplantation showed reduced tumor aggression. These observations were supported by increased tumor cell proliferation, colony formation and DNA synthesis upon circCCDC66 overexpression, strongly implicating circCCDC66 as an important driver in cancer etiology. Consistent with previous studies of circRNAs acting as miRNA sponges in normal and cancerous tissues, the authors performed a miRNA target sequence analysis. They found that circCCDC66 has several target sites for different tumor-suppressor miRNAs. Hence, overexpression of circCCDC66 increased the expression of oncogenes by tethering and inhibiting the activity of miRNAs otherwise involved in oncogene repression. In addition, knockdown of circCCDC66 resulted in decreased expression of oncogenes targeted by miRNAs released from circCCDC66-mediated sponging. As a remarkable good control, simultaneous anti-miR inhibition of the involved miRNAs rescued the effect of circRNA knockdown. Overall, the experiments led to the conclusion that circCCDC66 functions as an oncogenic circRNA by sponging several important tumor-suppressor miRNAs.

The impressive work by Hsiao *et al.* provides important knowledge and insights into the field of circRNAs and identifies a promising biomarker and potential therapeutic target, circCCDC66, in colorectal cancer. While the



**Figure 1** circRNAs as miRNA sponges in cancer. Schematic representation of circRNA sponge expression in healthy and tumor tissue. Onco-circRNAs (blue) and tumor-suppressor circRNAs (purple) sponges are characterized by harboring sponge-sites for tumor-suppressor miRNA (green) and onco-miRs (red), respectively. In cancer compared to healthy tissue, onco-circRNAs are generally upregulated whereas tumor-suppressor circRNAs are repressed in concordance with onco-miR and tumor-suppressor miRNA activity. As a result, oncogene (yellow) expression is elevated and tumor-suppressor mRNA expression (orange) is repressed in tumors.

evidence of circRNAs acting as miRNA sponges is growing almost on a daily basis, it clashes with the stoichiometric modelling of circRNA : miRNA : mRNA levels required for efficient sponge effects (13-15) and the abundancy and conservation of miRNA target sites found in circRNAs in general (2). Clearly, there are aspects of the complex regulatory networks within cells that are well outside our current grasp and knowledge, and hopefully in the years to come, more eminent RNA research will settle the controversy and establish a novel paradigm in which the bioinformatics modelling and the experimental data are aligned with consistent output.

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