CircRNAs in Human Cancer

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In human cancer, circular RNAs (circRNAs) were implicated in the control of oncogenic activities such as tumor cell proliferation, epithelial-mesenchymal transition, invasion, metastasis and chemoresistance. The most widely described mechanism of action of circRNAs is their ability to act as competing endogenous RNAs (ceRNAs) for miRNAs, lncRNAs and mRNAs, thus impacting along their axis, despite the fact that a variety of additional mechanisms of action are emerging, representing an open and expanding field of study.

Keywords: circRNA ; cancer ; backsplicing ; host gene ; parental gene ; translation of ncRNA ; miRNA

1. Overview

Circular RNAs (circRNAs) belong to a new class of non-coding RNAs implicated in cellular physiological functions but also in the evolution of various human pathologies. Due to their circular shape, circRNAs are resistant to degradation by exonuclease activity, making them more stable than linear RNAs. Several findings reported that circRNAs are aberrantly modulated in human cancer tissues, thus affecting carcinogenesis and metastatization. We aim to report the most recent and relevant results about novel circRNA functions and molecular regulation, to dissert about their role as reliable cancer biomarkers, and to hypothesize their contribution to multiple hallmarks of cancer.

Next generation RNA sequencing techniques, implemented in the recent years, have allowed us to identify circular RNAs (circRNAs), covalently closed loop structures resulting in RNA molecules that are more stable than linear RNAs. This class of non-coding RNA is emerging to be involved in a variety of cell functions during development, differentiation, and in many diseases, including cancer. Among the described biological activities, circRNAs have been implicated in microRNA (miRNA) sequestration, modulation of protein–protein interactions and regulation of mRNA transcription. In human cancer, circRNAs were implicated in the control of oncogenic activities such as tumor cell proliferation, epithelial-mesenchymal transition, invasion, metastasis and chemoresistance. The most widely described mechanism of action of circRNAs is their ability to act as competing endogenous RNAs (ceRNAs) for miRNAs, lncRNAs and mRNAs, thus impacting along their axis, despite the fact that a variety of additional mechanisms of action are emerging, representing an open and expanding field of study. Furthermore, research is currently focusing on understanding the possible implications of circRNAs in diagnostics, prognosis prediction, effectiveness of therapies and, eventually, therapeutic intervention in human cancer. The purpose of this review is to discuss new knowledge on the mechanisms of circRNA action, beyond ceRNA, their impact on human cancer and to dissect their potential value as biomarkers and therapeutic targets.

2. circRNAs

Many in vivo and in vitro studies and, more recently, analysis of liquid biopsy from cancer patients have shown that non-coding RNA (ncRNA), such as microRNA (miRNA) and long ncRNA (lncRNA), can be considered as good biomarkers for the diagnosis, prognosis and treatment of various human cancers [1][2]. Circular RNAs (circRNAs) were first detected by electron microscopy nearly 45 years ago and later confirmed to be present in the cytoplasm of eukaryotic cells [3][4]. Interestingly, through new RNA sequencing methodologies and new bioinformatics approaches, circRNAs have forcefully emerged in the clinical and basic research landscape [1][5][6]. These technologies highlighted that human transcriptome counts more than 180,000 circRNAs [7] and that their expression pattern varies among cell types, diseases and the developmental stages of living beings, including plants and invertebrates [8][9][10][11].

circRNAs are formed by a peculiar pre-mRNA that circularizes forming a covalently closed continuous loop through a process called “back-splicing”, in which a downstream 3′ splice donor site is joined with an upstream 5′ splice acceptor site. They can derive from spliced introns or from one or more exons, and sometimes they can have retained introns. Furthermore, they are poorly subjected to degradation by exonucleases as they lack 5′- and 3′- ends [12]. The splice event that generates a linear mRNA can compete with the back-splice mechanism [13]. circRNAs can be divided into three main categories: exonic circRNA (ecircRNA) consisting of one or more exons and representing 85% of all circRNAs, exon-

intronic circRNA (EicirRNA) and circularized intronic RNA (ciRNA). The biogenesis mechanisms are briefly illustrated in the **Figure 1**. The characterization of circRNA groups is fully reviewed in [14].

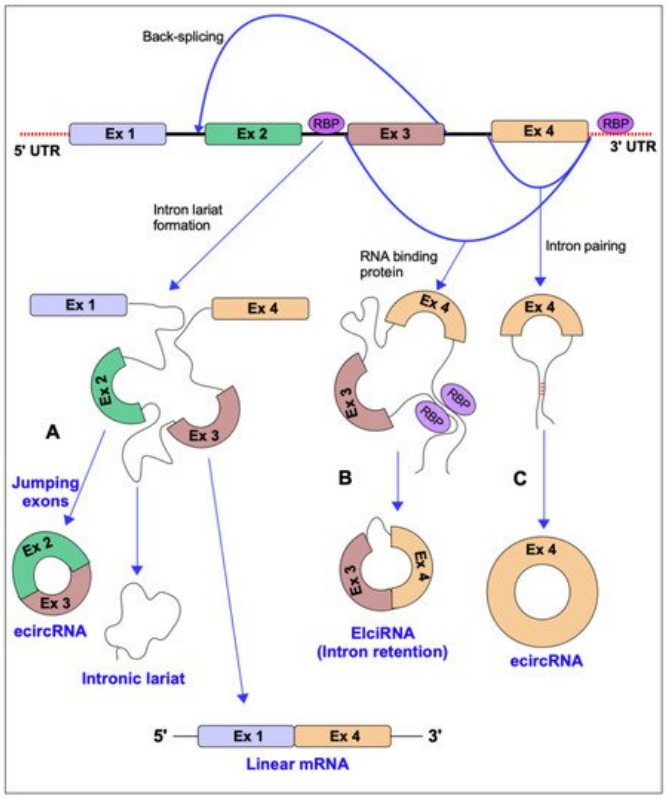

Figure 1. The circular RNA (circRNAs) biogenesis. Three mechanisms that lead to circRNA biogenesis are briefly described in the picture. (**A**) The first mechanism causes exon skipping and intron lariat production. “Jumping exons” consist of bringing together two distant exons with the formation of intron circular lariat. The lariat formation allows for the circularization of the “skipped” exons. Three different types of RNA molecules are produced: circRNA, intron lariat, and linear mRNA containing skipping exons. (**B**) The second mechanism is regulated by RNA-binding proteins, which bind the neighboring introns of the exon that should circularize and create an RNA loop when RBPs dimerize. The link between 3′ and 5′ ends of the circularized exons can facilitate splicing. (**C**) In the third mechanism, the circularization of the exon/exons is promoted by the complementary pairing of the flanking introns. Pairing with a complementary inverted sequence, such as ALU repeats, could increase back-splicing.

Most circRNAs are exported to the cytoplasm, while intron-containing circRNAs are usually kept in the nucleus and affect the regulation of host-gene expression. Both EIciRNAs and ciRNAs can act as cis-regulatory molecules in the regulation of host-genes. EIciRNAs bind to the small nuclear ribonucleoprotein U1 (snRNP) to form the EIciRNAs-U1 snRNP complex, which combines with polymerase II (Pol II) to regulate the transcription promoter region of the host genes [15].

It has been reported that circRNA expression is globally downregulated in diverse human tumors such as colorectal and gastric adenocarcinoma, osteosarcoma, renal cell carcinoma, lung adenocarcinoma, hepatocellular carcinoma, and prostate cancer [16]. An explanation can be given by the fact that circRNAs are more stable than linear RNAs and that they can therefore accumulate in slow growing cells or in non-proliferating cells, while in the proliferating cells they are distributed and diluted in the progeny cells [7][17]. However, over-expressed circRNAs are also observed in human tumors and support the maintenance of the tumor phenotype. Interestingly, the distinct evaluation of expression and function between circRNAs and their linear RNAs showed that circRNAs are not mere by-products of splicing, but they play an important role in carcinogenesis independently of their linear transcripts [7][9][16].

Diverse biological functions of circRNAs have been reported. In the scientific literature, the most reported function is that in which the circRNAs act as “sponges” for miRNAs, lncRNAs and some mRNAs, affecting the functions of their target genes [5][9]. This review aims to discuss other circRNA functions that are emerging. circRNAs can bind to specific RNA binding proteins (RBPs), sequester specific protein factors, and encode proteins/peptides that are involved in carcinogenesis and metastatization [16][17]. Recently, it has been shown that circRNAs are localized in exosomes, and therefore they are very stable in biological fluids.

New studies have revealed the roles of exosomal circRNAs in the onset of cancer [18]. From this point of view, circRNAs are considered novel and promising biomarkers for the diagnosis and progression of cancer, for the evaluation of

chemotherapy treatments and perhaps as new tools for targeted molecular therapies.

3. Conclusions

Due to the higher stability of circRNAs with respect to miRNAs and lncRNAs, shown by the covalently closed ring structure, circRNAs cannot be degraded by most ribonucleases compared with linear RNAs [19]. Furthermore, deregulated circRNA expression is significantly associated with cancer and could have clinical significance as a diagnostic and prognostic biomarker, as well as an evaluator of the therapeutic efficacy of cancer treatments. Some intron-containing circRNAs remain in the nucleus and can act as regulators of parental genes, whereas others are transferred to the cytoplasm and may play a role as miRNA sponges and protein sponges or can be translated into proteins or peptides [20]. However, there are still a discrete number of unknown circRNAs with unexplored functions that could have interesting clinical applications. Interestingly, the majority of circRNAs are also incorporated into exosomes, strongly enriching their presence in plasma compared with cancer tissues to perform functions away from the primary tumor.

In many cancer patients, early symptoms are not easily identifiable, as happens in ovarian carcinoma or gastric cancer, and without robust and specific biomarkers for early diagnosis, patients experience disease progression and further development of metastases. To date, a variety of ncRNAs, as miRNAs and circRNAs, are emerging as powerful biomarkers for early diagnosis and prognosis prediction, especially due to their presence and easy detection in body fluids [21][22][23]. The extensive study of these ncRNAs in large cohorts of patients and in various biological samples will hopefully strongly contribute to the development of novel diagnostic/prognostic strategies that will help cancer management.

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