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Original Article

Circular RNAs: the next level of gene regulation

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Abstract: Gene regulation is a highly complex process involving the presence and participation of many molecules and complexes that regulate gene expression in the genome, which occurs in a precise and coordinated way. Among all these regulatory molecules, the circular RNAs (circRNAs) are the most novel and peculiar family of noncoding RNAs (ncRNAs) as they have a circular structure, are very specific on their expression, highly conserved, and highly resistant to degradation. These molecules have been described in recent years as excellent disease markers and as potential therapeutic targets. In this review, we focused on general characteristics and on the evolution of the circRNAs, as well as on their biological functions, emphasizing on their participation in the formation of brain tumors.

Keywords: Noncoding RNAs, circular RNAs, circulating RNAs, biomarkers, evolution, cancer, brain tumor

Introduction

The complexity of the eukaryotic genome

The complexity involved in the regulation of gene expression has shown in recent years to be totally intriguing. Thus opening a path to a universe of molecules with known and many unknown, canonical, and non-canonical functions. Which has revealed that gene regulation is a fine and precise orchestra that performs its function in the instant required by the complex machinery of the cells.

The immense repertoire of ncRNAs involved in expression regulation is undoubtedly poorly understood, although in recent years there has been a meaningful increase in their research, yet the types and roles they may play has not been fully described. An immense proportion of non-coding sequences that are transcribed in the human genome are characterized by having been, until very recently, poorly represented in the currently available databases, either because they do not have characteristic or key elements to determine whether the sequence generates a protein product: (open reading frames (ORFs)), splicing sites, transcription factor binding sites, regulatory signals, or because it is a highly repeated sequence, which is difficult to sequence. This has contributed to the poor understanding we still have of most noncoding sequences. Interestingly, this complexity is at the core of the cellular processes that have evolutionarily generated adaptation and change in organisms [1]. Therefore, the study of ncRNAs allows us to better understand their organization, regulation, and the fundamental role they play during the organism's development and disease.

General information about circRNAs

As previously shown, ncRNAs are generated from any genomic region under specific cellular circumstances, even from regions that were never thought to be transcribed [1-5], to control gene regulation and genome stability [6-8].

One of the most unknown families of ncRNAs until recently are circRNAs. Their expression has been reported for 30 years; however, as they are generated during the mRNAs splicing, they were initially considered to be errors of the splicing machinery [9, 10]. CircRNAs have very peculiar characteristics not only in their struc-

ture, since they are more resistant to degradation and have a longer high-life than mRNAs and linear lncRNAs (long noncoding RNAs) [6-11]. They are known to have a higher resistance to exonuclease digestion when compared to linear sequences and can remain at room temperature for a whole day without changing their concentration [6-10]. In addition, circRNA concentrations, isoform expressions, and the circRNA type is cell-type specific [6, 7, 9-11]. Another feature is that an exon can be shared by multiple circRNAs, originating multiple isoforms that are the result of splicing; however, so far it is not known whether their functions are similar, redundant, or distinct [12, 13]. In metazoans, it has been described that circRNAs can be generated from: *i)* exon-exon sequences (ecircRNAs); *ii)* exon-intron sequences (EIciRNAs); *iii)* only intronic sequences (ciRNAs); or *iv)* intronic circular tRNAs (tricRNAs). CircRNAs carry out diverse regulatory functions and they have been reported to be critical in processes such as human development and pathologies [7, 9, 12, 13]. These peculiar molecules were poorly represented until a few years ago; however, the development of more specific techniques, such as deep sequencing, has led to circRNAs identification and recognition as indispensable elements in gene regulation. In addition, the development of more specific bioinformatic approaches has allowed us to understand their biogenesis and some of their functions (**Figure 1A-D**) [14-21]. CircRNAs have been associated with several diseases and, so far, their characteristics make them the best candidates to function as biomarkers of diseases and as therapeutic tools [7, 11, 22-28].

CircRNAs discovery

Before it became clear that circRNAs are present in all known organisms and that they have essential cellular functions, "simple" discoveries laid the groundwork for a glimpse into the world of RNAs [29-34].

In the early 1970s, researchers such as Sanger [31] and Diner [32-34] described viroids. Diner [32] described that the infection generated in common potatoes was not being produced by a virus, but by a viroid, a 250-400 nt circRNA without a nuclear envelope and significantly smaller than the genome size of the smallest viruses, with ribozyme activity, and capable of infecting plants. This discovery not only showed this peculiar family of ncRNAs as determinants in controlling specific biological processes, but also evidenced their existence as RNAs with circular structures, supporting the theories of the "RNA World", in which it is hypothesized that all forms of life come from RNA molecules [29, 35-37].

The identification of circRNAs has not been an easy task; however, with the availability of better bioinformatic and sequencing tools, it has been possible to confirm their presence in Archaea - *Sulfolobus solfataricus P2*, where circRNAs from tRNA introns have been identified. In addition, the circRNA expression from C/D box RNAs and from RNase P was demonstrated [35-37]. Interestingly, tRNA genes can generate a class of highly conserved and abundant circRNAs, called tricRNAs, which are produced in all metazoans during splicing. These transcripts possess highly conserved sequence motifs that are recognized by specific enzymes, important for their biogenesis and processing; these type of circRNAs seem to be fundamental in the control of specific biological processes in metazoans [35]. Meanwhile, Rybak et al. [11] demonstrated that thousands of circRNAs are conserved from nematode (*Caenorhabditis elegans*) to mammal (Homo sapiens) genomes [12, 38]. Between mice and humans, the circularization of transcribed exons is conserved and correlated with the presence of inverted repeats within long flanking introns [36, 38]. Taken together, these findings suggest that circRNAs are not the product of mere splicing accidents but they regulate gene expression in a highly specific manner in all organisms [36].

Types of circRNAs and their biogenesis

The biogenesis of these molecules is mediated by different mechanisms, depending on the organism. It has been shown that most eukaryotic circRNAs are generated by the spliceosome machinery by back splicing [39-41], whereas in plants this process is mediated by extensive introns, without high complementarity to be circularized [42-44]. In archaea, for example, some introns can cleave on their own - ribozyme activity - and generate circRNAs with regulatory functions [42-44]. This evidence demonstrates the great variability of mechanisms and sequences that can originate circRNAs in all the known kingdoms [30].

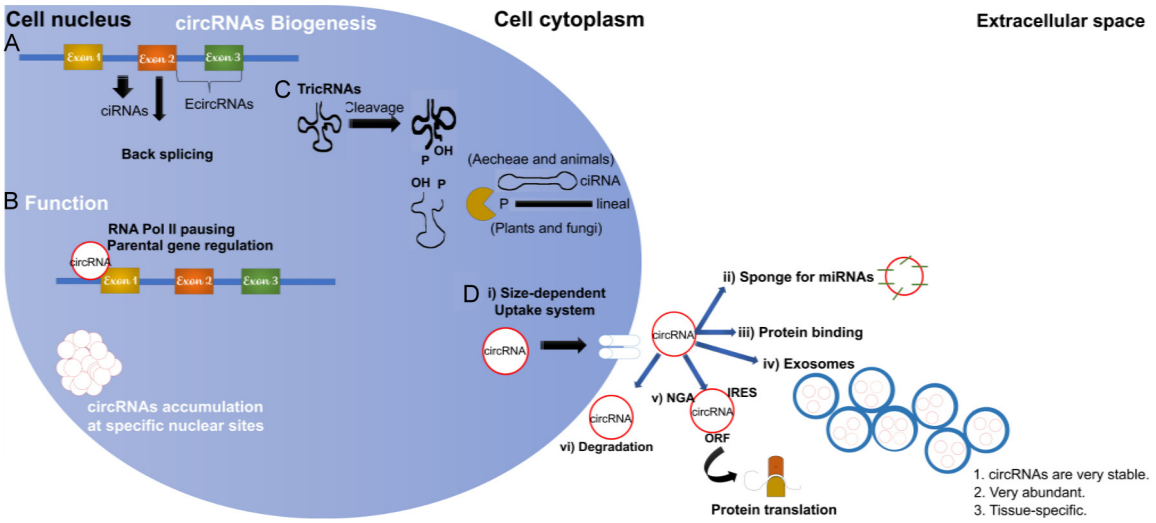

Figure 1. CircRNAs biogenesis and known functions. A. In eukaryotes, circRNAs are generated by back splicing. B. CircRNAs can regulate the expression of their parental gene and one of the known mechanisms is the control of POL II activity. It has been observed that circRNAs accumulate at specific sites within the nucleus, without the physiological impact of this being known so far. C. The formation of circRNAs from the processing of tRNAs has been observed in mammals, but in humans it has not yet been demonstrated. In organisms such as archaea, circRNAs are generated by autocatalysis of introns. D. CircRNAs are transported from the nucleus into the cytoplasm by mechanisms that are both size-dependent and size-independent *(i)*. Once in the cytoplasm, circRNAs can function as miRNA sponges *(ii)*; they can bind to proteins *(iii)*; they can be packaged into exosomes for secreted into different body fluids *(iv)*; they can be translated into proteins *(v)*; or they can be degraded *(vi)*.

In general, four mechanisms describe the circRNAs biosynthesis: *i)* The donor (GU-50 of the intron) and acceptor sites (AG-30) covalently bind and generate a lariat with an exon, which will be internally spliced to form an ecircRNAs [45, 46] (**Figure 1A**). *ii)* Alu elements into the pre-mRNA introns can be coupled with complementary sequences and then direct cycling occurs, eliminating the retained introns [36, 46-58]. *iii)* Involvement of only introns, where the element closest to the abundant branch in C and the element close to the GU-rich splice site 50 bind. Subsequent removal of other exons and introns gives rise to cirRNAs [47-51] (**Figure 1A**). *iv)* Involvement of QKI (Quaking), MBL (Muscle blind), and FUS (FUS RNA binding protein) proteins activating circRNAs biogenesis [47, 48].

EcircRNAs: In eukaryotes, most circRNAs are generated from protein-coding genes, so most circRNAs are composed exclusively of exons with a different order with respect to the parental mRNA, and they are produced by the same spliceosome machinery that control de production of mRNAs. EcircRNAs are the best identified circRNAs [40-43]. It has been suggested that they are transcribed by the RNA polymerase II (RNA pol II) and that their biogenesis must be mediated by the spliceosome machinery. Recent research on their biogenesis has shown that back splicing is regulated by the canonical splicing machinery [41]. Back splicing occurs after alternative splicing, where the donor site joins with the acceptor site through a phosphodiester bond, originating a circRNA [43]. EcircRNAs can also originate from mRNA isoforms; however, they are less abundant compared to mRNA isoforms, but they have a longer half-life [43]. Depending on the tissue under study, it has been observed that ecircRNAs regulate the expression of their parental mRNA, by selectively modulating other circRNAs or the mRNA production [44].

There are two important aspects that increase the efficiency of circRNAs biosynthesis. The first is based on the complementarity of flanking introns; the second is mediated by the formation of homodimers with the QKI, MBL, and FUS proteins, which regulate them in trans and can guide donor and acceptor sites. Both mechanisms result in the ecircRNAs formation with one or more than ten exons [36, 45]. Dubin

et al. [49] showed that inverted repeats within introns were necessary for circRNAs formation and described the presence of ALU-SINE sequences in humans and ALU elements in mice [47-50]. ALU sequences not only induce new splicing sites to generate new mRNA isoforms, but they are also complementary to each other, forming Inverted Repeated Alu elements (IRAlus) [48-50]. For this reason, the regulation of circRNAs expression is very different from their linear counterpart, suggesting that circRNAs have a dynamic regulation and function which is independent to that of lincRNAs (long intergenic non-coding RNAs) [49].

EIcircRNAs: EIciRNAs are a circRNA subclass characterized by their nuclear location and by having intronic retention sequences [36, 51]. EIciRNAs regulate their parental genes through RNA-RNA interactions and, to date, no miRNAs sponge activity has been described for this class of molecules. It is estimated that about 20% correspond to this class of circRNAs [36] and they are associated with factors such as the U1 snRNP (Small Nuclear Ribonucleoprotein U1). The binding between U1 snRNA and EIciRNA forms a complex interacting with the Pol II transcription complex in the promoters of the parental genes. Once the transcription of a gene is activated, the EIciRNAs synthesis, from that gene, will further promote the transcription of its own gene, thus generating positive feedback [52]. The canonical function of U1 snRNA is splicing; however, other cellular functions have been described for it, such as the activation of early transcriptional events, the prevention of premature polyadenylation, and the determination of promoter directionality [53, 54]. Experiments in which the knockdown of a specific EIciRNA was carried out, a decrease in the levels of the parental gen transcript was observed. Therefore, gene transcription regulation by EIciRNAs-U1 snRNP opens a new perspective on the mechanisms by which circRNAs act [53].

CiRNAs: It has been demonstrated that a great variety of ncRNAs, such as miRNAs (microRNAs), snoRNAs (small nucleolar RNAs), lncRNAs, among others, are inside intronic sequences of the genome [6, 55]. Qian et al. [55] described the presence of a spliced intron with the ability to circularize itself or to form a lariat with different lengths within the nucleus. In the same way, Zhang 2013 [6] systematically identified from intronic sequences hundreds of ciRNAs expressed in human cells. In bacteria and in some eukaryotic organelles, we find group II introns; these introns have the capacity to produce introns that are circularized through the 2'-5' linkage [55-58]. Similarly, they can generate full-length circles and, in both cases, can contribute to gene mobility by inserting both, DNA and RNAs [59-61]. CiRNAs are not subject to debranching and depend on consensus RNA elements near the splice site 50 and branch point (nucleotide 100) for proper processing [60]. It is not yet clear how these intronic elements can avoid the debranching process. In this sense, there are three sequences that are important for the splicing machinery within the intron sequence to carry out its function; among them is the branch point near nucleotide 100, starting from the 3' splice site, which is a fundamental site for cutting and splicing. This site is characterized by an adenine followed by a pyrimidine chain [60-63].

There are two main steps in splicing. The first step is the nucleophilic attack by the 2'OH of branch point A at the 5' splice site (the junction of exon 5' and the intron). As a result of a transesterification reaction, 5' exon is released and a loop, composed by the 3' end of the exon and the intron, is generated. In the second stage, the OH 3' of the exon 5', attacks the 3' cleavage and splice sites; then, the two exons are joined together and the looped intron is released [59-61].

How do these introns escape this process during the formation of a ciRNAs? This is still not clear, since we do not know how these consensus RNA elements resist debranching nor what associated proteins are involved in this process. One proposed mechanism is that RNA Pol II activity is paused in many promoters, as part of gene regulation, and that this "pausing" is mediated and regulated by ncRNAs [59-61]. Consistently, Zhang et al. [6] observed that ciRNAs partially accumulate in their synthesis sites and colocalize with the Pol II elongation activity (**Figure 1B**). To date, it is known that all ciRNAs are located into the cellular nucleus and that their main action mechanism is to regulate their parental genes; however, unlike EIciRNAs, ciRNAs are arranged in dispersed nuclear sites [6, 61], suggesting their involve-

ment in cellular functions other than that of EIcircRNAs.

TricRNAs: The tricRNAs expression has been observed in archaea and eukaryotes and although the presence of the tricRNAs-generating machinery has been demonstrated in humans, the expression of these RNAs has not been evaluated [43, 62, 63]. Cellular processing and localization of the tRNAs varies among species and it is a complex process that involves many steps [62]. The presence of intronic elements in the sequence of some tRNAs has been demonstrated and these regions must be removed from the main sequence for the correct tRNAs processing [62]. The mechanisms by which introns are removed from tRNAs depend on the domain in question [62, 63]. In bacteria, the intronic region has a ribozyme function, so it can be cleaved autonomously [62]. By contrast, an endonuclease is required for processing in archaea and eukaryotes [62, 63]. There is currently little evidence regarding the mechanisms governing the elimination of intronic sequences in tRNAs; however, the intron cleaved in archaea can be circularized; this has also been observed in eukaryotes (**Figure 1C**) [62, 63].

Summarizing, the mechanisms and combinations of sequences: Exon; exon-intron originating circRNAs offers clues about their importance in cell regulation. To date, it has not been corroborated whether circRNAs isoforms biosynthesis, sharing exons or introns, perform redundant functions such as compensatory mechanisms or distinct functions. However, genomes have demonstrated to "use" all their sequences to generate regulatory transcripts to maintain cellular homeostasis.

CircRNAs regulation

It is widely known that chemical modifications are necessary for RNA maturation, activating or repressing their function; so far, more than one hundred-sixty modifications have been described to regulate the function of these molecules [64-66]. The most frequent and best studied is N6-methyladenosine (m6A), at least in eukaryotes. This reversible modification can regulate RNA transcription, splicing, degradation, and transcription [64, 65]. In miRNAs and lncRNAs, this modification allows the acquisition of crucial functions in processes such as T cell homeostasis, sex determination in Drosophila, spermatogenesis, heat shock responses, reprogramming, and pluripotency, among other [66-68]. As m6A is so important for the cell, it is not surprising that its deregulation is involved in several diseases, such as cancer [67]. In this regard, both alterations in the m6A modification and in proteins that regulate it are frequently observed in various types of cancer [68]. Although the mechanisms by which the m6A modification regulates circRNAs activity has not been fully elucidated, it has been reported that m6A regulates their translation and degradation [67, 68].

CircRNAs functions

Although several studies have shown that circRNAs synthesis is finely regulated to control very specific cellular processes, to date, very few studies have elucidated their functions [69]. CircRNAs expression is highly specific and they are vastly expressed in brain, heart, intestine, and testes, while in other tissues, such as muscle, they have been reported with a relative low expression [46, 70]. The main functions described for circRNAs include: *i)* binding to miRNAs - miRNAs sponges; *ii)* parental gene regulation, and, in some cases; *iii)* their translation into proteins, thus regulating various signaling pathways (**Figure 1D**) [16, 70].

Both, the regulation and general functions of ncRNAs are carried out by very fine processes dependent on cellular needs, and circRNAs are no exception. These molecules exhibit unique characteristics and exert their functions by diverse regulatory mechanisms, placing them as potent regulators of gene expression, during organism development, cellular homeostasis, disease, and as potential therapeutic targets [16].

CircRNAs as sponges: Some lincRNAs and pseudogenes bind to miRNAs and, thus, regulate their function [12]. Therefore, it is not surprising that circRNAs derive mainly from exonic sequences and bind miRNAs, thus controlling miRNAs disposal, both in the nucleus and in the cytoplasm [12, 71].

Memczak et al. [12] described the first circRNA with a "super" sponge function. CDR1as contains approximately seventy binding sites for mir-7 and binds to the miR-7/Argonaute com-

plex. Additionally, this circRNA has a very particular regulation since its sequence also has binding sites for miR-138. The miR-138 binding promotes the endonuclease activity of the RISC complex, cleaving CDR1as and releasing the miR-7/Argonaute complex. Meanwhile, more recent studies have shown that CDR1as binds in trans, as a single-stranded RNA, to the 3'UTR regions of target mRNAs, controlling their expression. Therefore, it is not known whether miR-7 binds to CDR1as to modulate its activity or whether CDR1 as binds miR-7 to regulate its activity. It is known that miR-7 is involved in many signaling pathways related to both oncogenesis and neurodegenerative diseases, such as Parkinson disease [71]. This regulatory mechanism was confirmed for other circRNAs as one of the main regulatory mechanisms exerted by them.

Liu et al. [72] described that circGRAMD1B binds to miR-4428 and this leads to SOX4 (SRY-Box Transcription Factor 4) regulation. This results in an increased SOX4 expression and in the MEX34 (Mex-34 RNA Binding Family Member C) activation, which in turn modulates the PI3K/AKT pathway activity and promotes Lung Adenocarcinoma (LUAD) leukemia cell malignancy. Then, GRAMD1B modulates the miR-4428/SOX4/MEX3A axis to over activate the PI3K/AKT pathway.

As mentioned above, circRNAs do not have binding sites for a single miRNA. Circ-HIPK3 bind nine miRNAs; the most studied one is miR-124. The binding of this miRNA to circ-HIPK3 results in the promotion of cell proliferation. Additional circRNAs functions could be related to the silencing of mRNAs [9, 71, 73].

As demonstrated for other ncRNAs, such as pseudogenes, one of the most fascinating mechanisms recently described is the ability of certain ncRNAs to bind miRNAs. miRNAs are highly efficient regulatory molecules; therefore, cells developed mechanisms controlling the disposal of these sncRNAs. EcircRNAs and EIcircRNAs bind miRNAs and regulate their activity, and normally regulate the expression of their parental gene exerting a sponge function, which is a level of regulation that was not contemplated until recently.

Interaction and modulation of protein activity: CircRNAs modulate cell fate as they participate in different signaling pathways [16, 74]. RNA-binding proteins (RBP), which interact with circRNAs, may promote either protein sequestering or loss of protein function, or the circRNA action as RBP sponge. An example of this is HuR, a translation activator that binds to the PABPN1 mRNA, making protein translation more efficient. However, it has been described that HuR interacts with circPABPN1, reducing the binding of HuR to the PABPN1 mRNA, down-regulating its translation [74, 75].

The circRNAs-RBPs interactions occur frequently with protein kinases involved in cell survival and cell cycle arrest [74]. One of these circRNAs is derived from the FOXO3 gene sequence - circ-FOXO3 - an essential gene during embryonic development for apoptosis control and cell cycle progression [74]. FOXO3 (Forkhead Box O3) silencing leads to cell cycle progression induction and its ectopic expression leads to the opposite effect, by binding to CDK2 (cyclin-dependent kinase 2) and p21 (cyclin-dependent kinase inhibitor 1) [74]. This dual interaction results in the formation of a ternary RNA-protein complex (circ-Foxo3-p21-CDK2), repressing proliferation and cell cycle progression in tumor cells [74, 75].

Generating peptides or proteins: Recent studies have shown that specific circRNAs are translated into proteins [76-78]. Due to their structure, circRNAs lack a 5' cap and translation cannot be initiated in a regular manner by recruiting cap-dependent regulatory elements [76, 77]; therefore, the existence of internal ribosome entry recognition sites (IRES) or other elements or sequences activating an alternate mechanism could be regulating circRNAs translation. The presence of ORFs, m6A modifications and internal IRES in the circRNAs sequence makes evident the potential for coding by a cap-independent translation initiation mechanism [77-79].

Wang et al. [77] performed experiments in cell lines in which they introduced IREs into circRNA sequences and detected the corresponding protein products [76]. However, in addition to IRES sites, it has been shown that the m6A modification was extremely abundant in circRNAs structures and this modification directed the circRNAs translation in a similar way to IRES, but studies demonstrating circRNAs translation are still in their early stages and

Table 1. Number of circRNAs in human tissues

Sample	Number of circRNAs detected in tissue by sequencing
Plasma	57
Serum	39
Neutrophils	274
Platelets	3,324
Right atrium	340
Vena cava	702
Umbilical cord	85
Chondrocytes	148
Osteocytes	104
Placenta	63
Muscle	184
Cerebellum	21,071
Diencephalon	24,632
Frontal cortex	38,983
Occipital lobe	31,085
Parental lobe	23,303
Temporal lobe	21,835

Taken and modified from CircBase [16].

there is still much to learn. The majority of the studied circRNAs have been rarely reported to be bound to ribosomes, indicating that many circRNAs do not encode for proteins [76-78]. It is still unclear whether protein synthesis involves unknown mechanisms or occurs at very specific cellular times. In this regard, it is also worth noting that many circRNAs contain a start codon and sometimes the AUG codon (e.g., HIPK2/3) [78]. Whether circRNAs translate or only do so under certain cellular signals is an area of active study.

The biological functions of most of these molecules has not yet been demonstrated; however, there is sufficient evidence of their participation in gene regulation. These molecules form regulatory loops in various signaling pathways essential for cells and surely, we will be able to have a much broader vision of the importance of circRNAs in coming years.

Subcellular localization

The subcellular localization of these molecules is extremely interesting. They have been mostly observed in the cytoplasm, but as previously mentioned, this depends on the composition of each circRNA, conferring or assigning their location [39]. The vast majority of circRNAs are composed by exons, and these are in the cytoplasm, but EIcircRNAs and ciRNAs are retained in the nucleus [39]. Therefore, the subcellular localization of these molecules tells us about the functions they may be performing in the cell [39, 80]. In *D. melanogaster*, it has been suggested that the mechanism by which circRNAs are exported from the nucleus to the cytoplasm is transcript size dependent, with helicase Hel25E being responsible for exporting circRNAs larger than eight hundred nucleotides into the cytoplasm. Meanwhile, the DX39B (DexD-Box Helicase 39B) and DX39A (DExD-Box Helicase 39A) proteins transport circRNAs in a size-independent manner [80, 81].

Degradation

In animals, GW182 proteins are a key component of the miRNA-dependent posttranscriptional silencing pathway. They function as scaffold proteins to mediate the interaction of Argonaute-forming complexes (AGO) with cytoplasmic poly(A)-binding proteins (PABP) and deadenylases, inducing translational repression and promoting P-body formation [82, 83]. Interestingly, it was shown that GW182 is essential for circRNAs degradation and has a strong potential to affect the stability of mature circRNAs, but not that of nascent circRNAs, so it would not affect their biogenesis. Subsequent experiments using siRNAs showed an increase of twelve circRNAs in the absence of these proteins, which could be involved in the degradation of certain circRNAs [82].

CircRNAs as disease biomarkers

The discovery and structural and functional characterization of circRNAs have demonstrated that these molecules are critical in the development and progression of diseases [84-87]. It has been described that circRNAs are tissue specific (**Table 1**) and thanks to their structural stability, conservation, and abundance in body fluids, these molecules are strong candidates as biomarkers and therapeutic targets [26, 84].

CircRNAs have been strongly associated with the onset of various pathologies such as cancer, neurodegenerative diseases, cardiovascular diseases, and diabetes [26, 84-85]. It has been reported that the global expression of cir-

cRNAs is high during development; however, a negative correlation between circRNAs and cell proliferation has been described in cancer [84-87]. It has also been shown that during development and in pathologies ecircRNAs are favored over that of mRNAs, a process that promotes cell proliferation in most cases [85].

CircRNAs associated with exosomes in body fluids are very abundant. Exosomes are vesicles 30 to 100 nm in diameter, capable of transporting various molecules. These vesicles deliver their cargo to the recipient cells and mediate intercellular communication, which helps to form the tumor microenvironment, facilitating proliferation, invasion, and immune escape [88-90]. Interestingly, circRNAs have been shown to be the most abundant component contained within exosomes [88], although the mechanisms that favor the packaging of these molecules in exosomes are still unclear. It has been proposed that some of the functions of circRNAs in exosomes include control of cell proliferation, EMT and invasion, metastasis, chemoresistance, and adipocyte browning (cachexia) derived from their regulatory functions as miRNA, protein sponges and translational regulators [88-91]. Given the structure and regulation characteristics that circRNAs can exert, it has been proposed that they can be used as therapeutic molecules and, favorably, the in vitro synthesis of these molecules does not seem to be so complicated [92, 93]. Liu et al. [93] demonstrated that the synthesis and therapeutic application of a circRNA with sponge function is effective in suppressing gastric cancer cell proliferation. This demonstrates that circRNAs are an extremely useful group of molecules as potential biomarkers to develop new treatments and also as therapeutic targets.

CircRNAs in the brain

The diversification and appearance of non-coding sequences in the brain represents one of the most intriguing processes on different levels: evolutionary, physiological, cognitive, and others [94, 95]. Although the existence and expression of circRNAs with important functions has been shown in the brains of many organisms, the diversity and abundance of ncRNA sequences is extremely high in mammals, especially in primates [11]. The complex transcriptome of the primate brain is characterized by expressing a greater number of mRNA [96] and ncRNAs [97] isoforms than in other tissues. Although the precise functions of many of the transcripts produced in the brain are unknown, it is becoming increasingly clear that the diversity of the brain transcriptome is necessary for the production and regulation of proteins that mediate specific processes.

CircRNAs are highly expressed molecules during cell differentiation in the mammalian brain. Their expression and abundance are finely regulated and restricted to the time of development of the individual, the cell type, and the region in which they should be expressed at that time under specific circumstances. The expression of circRNAs is extremely high during the development and neuronal differentiation of an organism and it is highly regulated in the adult organism. In a study by Rybak-Wolf et al. [11], in which the abundance of circRNAs in the human and mouse brain was compared by sequencing, a total of 15,849 candidate circRNAs sequences were found in the mouse and 65,731 in humans. Most of these sequences are not yet annotated in current databases and most of these sequences are from exonic regions with annotations (CDS and 5'UTR). It was also shown that there are typically splicing sites for three exons within these sequences. In the same way, it was detected that even though the formation of circRNA isoforms is typically restricted to three, in this study a non-negligible number - approximately 2,338 sequences - was detected, originating more than ten circular isoforms, being this more frequent in humans than in mice.

In the brain, in contrast to other tissues, circular isoforms are produced much more abundantly than their linear counterpart, but each isoform is expressed specifically in particular regions of the brain. According to Rybak-Wolf et al. [11], the longer isoforms containing exons two through five were highly expressed in the adult brain, while short isoforms, two exons in length, were predominantly expressed in stem cells, embryonic tissue, and lungs. Based on the above, the deregulation of circRNAs was associated with various pathologies [11, 98], including cancer [99-101].

Brain tumors

As it is well known, adult and pediatric brain tumors are different molecular entities. That is,

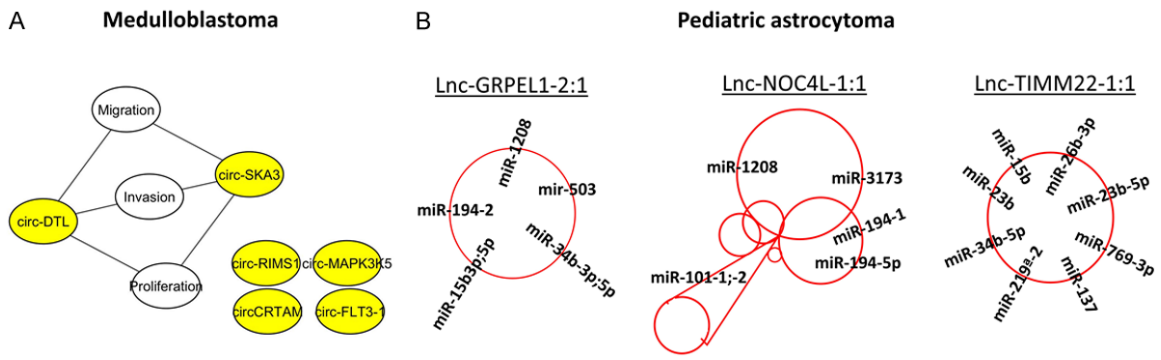

Figure 2. CircRNAs differentially expressed in pediatric astrocytoma and medulloblastoma. A. Medulloblastoma. Differentially expressed circRNAs were identified in pediatric medulloblastoma. This circRNAs were postulated as potential biomarkers for this tumor type. B. Pediatric astrocytoma. Diverse differentially expressed lncRNAs, including three predicted circRNAs, were identified in p-Ast. This analysis also predicted their interaction with miRNAs to regulate specific signaling pathways.

although they are histologically classified within the same subtype, the molecular features in one group are not necessarily the same as in the other [102].

Pediatric tumors: Pediatric brain tumors are the leading cause of cancer-associated death in the pediatric age group and efforts are being made to improve the classification and find effective treatments. The study of ncRNAs in these tumors has yielded a myriad of biomolecules with potential to function as biomarkers for classification and treatment monitoring, and circRNAs are no exception. In medulloblastoma [103], these types of lncRNAs can either be overexpressed or underexpressed, driving hallmarks of the cancer (**Figure 2A**). In a study performed by our working group, we demonstrated the differential expression of three bioinformatically predicted circRNAs in pediatric astrocytoma compared to the control condition. Their interaction with miRNAs was predicted, which indicates their probable involvement as miRNA sponges in this type of cancer and control of cell signaling pathways (**Figure 2B**) [101].

Adult tumors: The study of adult brain tumors is more arduous than that of pediatric brain tumors. The study is focused mainly on GBM (glioblastoma multiforme) and this is because these tumors are the most common brain tumors in adults and, to date, there are no effective treatments against this type of tumors, resulting in a high rate of deaths [104]. Regarding this, diverse circRNAs have been identified with expression changes in glioma, mainly GBM, functioning primarily as sponges for miRNAs [105]. Reducing the availability of miRNAs in the cytoplasm leads to a decrease in the control of their target mRNAs, which in turn induces the activation of pathways that would normally be down-regulated. This activation induces various hallmarks of cancer, such as migration, invasion, angiogenesis, proliferation, among others (**Figure 3**). A very interesting case is that of Circ-E-Cad, which codes for a protein that activates the EGFR-regulated pathway [106].

Conclusions

CircRNAs are a type of ncRNAs with surprising molecular characteristics and their physicochemical properties point them out as the best biomarkers known so far. There is still a long way to go to identify all the functions carried out by these circRNAs and to establish them as useful biomarkers in the clinic.

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None.

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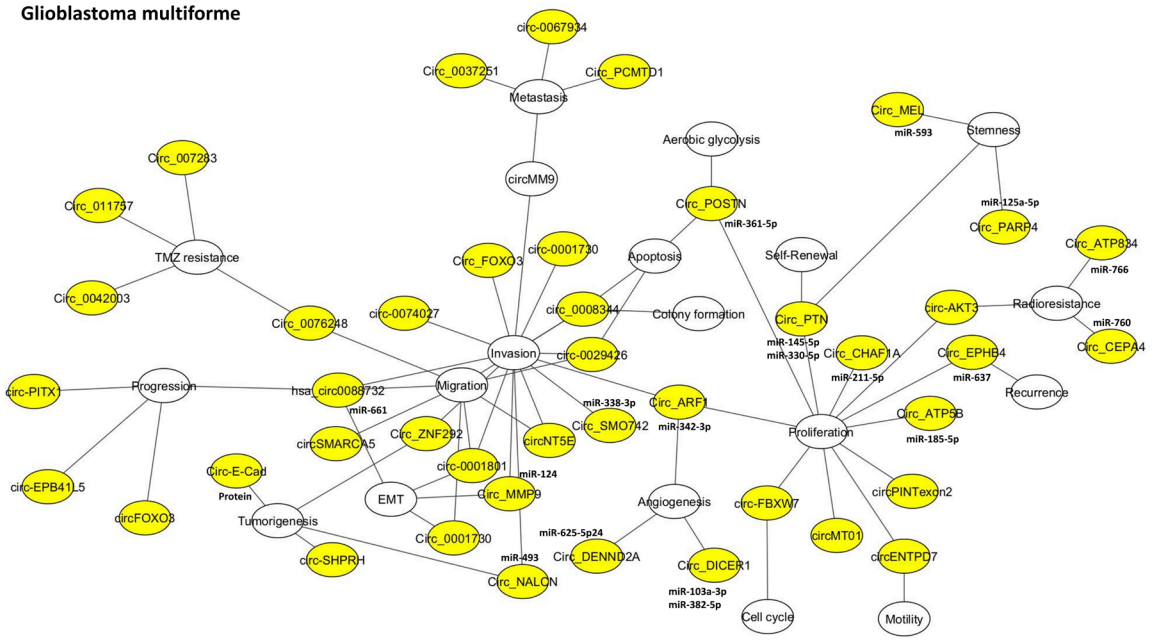

Figure 3. CircRNAs differentially expressed in adult gliomas. Differentially expressed circRNAs function as miRNAs sponges, resulting in an increased translation of the target mRNAs and on their respective signaling pathways. This aberrant activation led to cell proliferation, migration, TMZ resistance, among others.

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