



Review

circFOXO3: Going around the mechanistic networks in cancer by interfering with miRNAs regulatory networks

Rares Drula^a, Radu Pirlog^a, Monica Trif^b, Ondrej Slaby^{c,d}, Cornelia Braicu^{a,*}, Ioana Berindan-Neagoe^{a,e}

^a Research Center for Functional Genomics Biomedicine and Translational Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

^b Centiv DE, Bremen, Germany

^c Central European Institute of Technology, Masaryk University, Brno, Czech Republic

^d Department of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute, Brno, Czech Republic

^e Department of Functional Genomics and Experimental Pathology, "Prof. Dr. Ion Chiricuta" Oncology Institute, Cluj-Napoca, Romania



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ABSTRACT

Circular RNAs (circRNA) have gained recent interest due to their functional versatility due to their interactions with other RNA species and proteins, all of which underline complex regulatory networks involved in pathogenic mechanisms. As a result, recent insights in circRNA biology are investigating their biomarker and therapeutic potential. One such circRNA is CircFOXO3, which consists of the circularized second exon of the FOXO3 mRNA, a member of the forkhead box transcription factor family involved in the regulation of developmental programs. Recent research focused on the role of circFOXO3 in the context of cancer has highlighted several implications in key tumorigenesis mechanisms, thus consolidating its relevance among other identified circRNAs. In this paper, we will focus on the currently identified case-specific implications of circFOXO3 in cancer, with a focus on the circFOXO3-miRNA-mRNA regulatory networks, its interactions with different proteins, and their cumulated biological effects upon tumor development. Therefore, we aim to provide an integrated perspective of the mechanistic implications of circFOXO3 in different cancers while also highlighting its biomarker or therapeutic potential based on the current evidence.

1. Introduction - circular RNAs, and the circFOXO3 transcript

The ongoing interest on circular RNAs (circRNAs) has emerged based on their functional versatility as gene expression regulators. The overall focus regarding circRNAs study of function is based on the ability to sponge free circulating miRNAs by competitively binding, thus diminishing their regulatory effect upon mRNA translation. Additional functions have been attributed to these species of circular transcripts, such as the formation of RNA-protein complexes that can also impact gene expression [1–3]. Circular RNAs result from the canonical splicing machinery as 3' to 5' circularized products, most of them originating from a parental gene that mainly codifies mRNA that is further subjected to translation [1]. While the exact biogenesis of circRNAs has not been elucidated up to date, studies consider that impairment of splicing factors by their partial depletion can facilitate RNA circularization [1,2,4].

One of the main identified mechanisms of RNA circularization is

backsplicing, which consists of the joining of a downstream splicing donor and upstream acceptor sites, consisting of reverse-complementary regions that can hybridize. Therefore, the biogenesis of circRNAs is dependent on the presence and localization of looping-promoting factors [5]. Inverted sequence repeats, such as *Alu* elements and RNA-binding proteins that dimerize following RNA binding promote looping of transcripts [6].

CircRNAs can be either composed of the parental gene's introns, exons, or a combination between the two, this being dependent on the distance of the flanking complementary regions. The presence of exons in the construction of a circRNA can provide a limited protein-coding function in the case of several circRNAs, yet this function requires further investigations. In some cases, the copy number of circRNAs can be up to 10 times greater than that of related linear RNAs, indicating that these circRNAs hold important biologic roles rather than accidental errors during splicing. Even so, the role of circRNAs remained unclear

* Corresponding author.

E-mail addresses: drula.rares@gmail.com (R. Drula), pirlog.radu@yahoo.com (R. Pirlog), mt@centiv.de (M. Trif), on.slaby@gmail.com (O. Slaby), cornelia.braicu@umfcluj.ro (C. Braicu), ioana.neagoe@umfcluj.ro (I. Berindan-Neagoe).

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until recently [7,8]. Current analyses denote that alterations of circRNAs are prevalent in human cancers.

A circular RNA, which has gained recent attention, is circFOXO3, the circularized transcript of the FOXO3 forkhead family box transcription factor, encoding the linear FOXO3 mRNA. Therefore, in this paper, we will attempt to outline the main identified functions of circFOXO3 in cancer and to discuss its relationship with the parenteral genes.

2. Overview of FOXO3, roles and post-transcriptional regulation

The FOXO3 protein belongs to a larger class of transcription factors characterized by their specific forkhead DNA binding domain, functionally involved in the transduction of environmental stimuli related to growth and metabolic signaling that cumulate in the activation of different developmental processes [9]. As a transcription factor, the cellular localization of FOXO3 is functionally dependent on the presence on the mentioned stimuli and its interactions with regulating tyrosine kinases, such as AKT, PARP1, and ERK [10,11] that modify its phosphorylation status which dictates it is shuttling between the nucleus and cytoplasm [12–14]. Therefore, it is heavily implied that the mutational status of the upstream kinases [15] can have a pivotal role upon either the tumor-promoting or suppressor activity of FOXO3, based on the pathological context. Based on this, it is hard to attribute FOXO3 to a clear status of either tumor suppressor or promoter. FOXO3 and the forkhead box transcription protein family members have been continuously explored in the context of cancer and other pathologies [16], leading to the identification of several important roles, ranging from involvement in the development of drug resistance [17] to cell cycle control [18] and apoptosis regulation [9,19,20]. Emphasizing its tight regulation with kinase activity, FOXO3 downregulation via alterations in kinase activity has been linked with increased tumorigenicity and disease progression in several cancers [21,22]. Additionally, there are accumulating mechanistic examples of occurring alterations in the AKT signaling pathway, such as PTEN loss and other MAPK pathway components becoming constitutively active can have a direct effect on the cellular localization [23,24] and thus, the functionality of FOXO3 in different pathological contexts.

Although the functional aspects of FOXO3 have been canonically attributed to the response from environmental stimuli and subjected to tyrosine kinase regulation, post-transcriptional regulation of FOXO3 via microRNA(miRNA) targeting has gained recent interest due to its extensive implication in many pathological mechanisms [25]. MiRNAs are short non-coding RNA transcripts considered as master post-transcriptional regulators which promote mRNA degradation and/or hinder translation based on the base-pair complementarity with specific regions of a target mRNA in conjunction with the RISC complex [1,4,26,27]. Their regulatory action also extends to the FOXO3 mRNA, where the 3'-UTR (3'-untranslated region) harbors several validated miRNA target sequences, generally referred to as miRNA response elements (MREs). Several examples include miR-155 in the case of several solid tumors [21,28,29]. The extent of miR-155 regulation varies from modulating the PIK3R1-FOXO3a-cMYC [30] to implications in FOXO3a-mediated gefitinib sensitivity and the development of CSC characteristics [31]. Additional miRNAs validated to target FOXO3 are miR-223 [32], miR-122 [33], miR-182 [34]. The transposition of miRNA-FOXO3 interactions in the case of cancer has not been limited to the miRNA-FOXO3 mRNA canonical regulatory actions, but also considered the different transcript forms that arise from the paternal FOXO3 gene. Such events will be furtherly described in the following chapters.

3. CircFOXO3 expression level in cancer and biomarker potential

CircFOXO3 is the circular transcript variant derived from the FOXO3 pre-mRNA. Specifically, it is composed of the second exon of the FOXO3 gene containing 1435 nucleotides. The main CircFOXO3 transcript

variant is identified in the circRNAs databases as hsa_circ_0006404 (according to CircBase) [35] and originates from chromosomal position chr6:108984657–108,986,092. The sequence displays high conservation levels between humans and mice [14], making it a suitable candidate for further in vivo studies and indicating the important functional role of the FOXO3 gene and produced transcript. The 1437 nucleotide sequence of circFOXO3 is highly maintained between the FOXO3 pseudogene (FOXO3p) and the FOXO3 mRNA, from which there is an 11-nucleotide deviation. As a mention, the pseudogene (Foxo3p) and circFOXO3 have been previously indicated to be involved in tumor growth, by promoting cell proliferation and pro-survival mechanisms [36]. Referring to the functional importance of this conservation is reflected in the high number of MREs common to the transcript variants, as 25 identical MRE is shared between the 3 transcripts. This adds a layer of complexity in the miRNA regulatory mechanisms of the FOXO3 transcripts [36].

One of the hallmarks of circRNAs is their specific expression pattern, which is the generally reflective expression level of their parental gene. More so, cumulating evidence in the case of many circRNA indicates that expression levels are dependent on pathological status and cellular type, indicating a potential utility as biomarkers [37,38]. Referring to circFOXO3, its expression levels have been investigated in several types of cancers (Table 1), where a general cancer-wide expression trend has not been observed.

Studies that evaluated expression levels of circFOXO3 in solid tumors indicated a downregulated status in tumor tissues originating from lung [39], breast [7,40], and bladder samples [41,42], while expression in glioblastoma was higher when compared to normal adjacent tissues [43]. In the last case, overexpression of circFOXO3 in glioblastoma tissue samples was correlated with a later disease stage [43], more so, in situ hybridization indicated an agglomeration of circFOXO3 in the cytoplasm of the glioma cells, implying an enhanced regulatory activity [43].

Expression levels of circFOXO3 have been previously correlated with disease stage in several cases. One study evaluated the correlation between the expression of the FOXO3 gene and circFOXO3 abundance in AML patients' samples [22]. The study identified significantly lowered expression for both the parental gene and the circular transcripts in de novo patients' samples. Additionally, the study indicated that higher circFOXO3 expression was correlated with a better prognostic [22]. A correlation between circFOXO3 overexpression and metastasis status was observed in the case of esophageal squamous carcinoma. While circFOXO3 expression was associated with advanced tumor node metastasis stage (TNM) in this case, other clinical parameters did not show any significant correlation [44]. While the expression rate of circFOXO3 has been validated in several cases of solid tumors or hematological pathologies to be correlated with pathological status and cell type [22,39,42,44,45], further proof is required to reach a consensus regarding expression levels in some types of pathologies and tissues. For example, in the case of prostate cancer, one group identified circFOXO3 as being overexpressed in prostate tumor tissue and plasma samples [45], while a different group assigned its expression level as downregulated in their investigated tumor tissue samples [46]. Several factors could explain the variations, mostly in the area of technical artifacts resulted from differences in sample types, cohort selection, stratification, and differences in quantification and normalization methods. Thus, additional investigations are required for establishing a consensus in the case of all cancer types. Nonetheless, the group investigating the implications of overexpressed circFOXO3 in prostate cancer indicated that the expression levels were correlated with the Gleason score, but not with age and PSA levels [45]. Meanwhile, the second study on prostate cancer reveals the downregulation of circFOXO3 in high-grade versus low-grade or normal prostate tissues and estrogen-independent cell lines [46].

Table 1
Altered circFOXO3 and their related implications in key cellular processes.

Pathology	Evaluation method	The expression level of circFOXO3	Biological role	References
Acute myeloid leukaemia	qRT-PCR 116 AML patients and 30 bone marrow controls (normalization to ABL)	↓ AML versus controls	Prognostic marker	[22]
Esophageal squamous cell cancer	qRT-PCR in 94 pairs (healthy-adjacent) of tissue samples	↓ tumor versus adjacent normal tissue	Prognostic marker and therapeutic target	[44]
Lung cancer	qRT-PCR in 45 NSCLC paired samples (normalization to GAPDH and U6)	↓ tumor versus adjacent normal tissue	Prognostic marker and therapeutic target	[39]
Breast cancer	14 matched breast and adjacent cancer samples	↓ tumor versus adjacent normal tissue	Therapeutic target	[40]
Bladder cancer	30 tumor tissues and adjacent normal bladder tissue samples	↓ tumor versus adjacent normal tissue	Therapeutic target	[42]
Prostate cancer	qRT-PCR from 18 normal prostate tissue samples; 22 low-grade and 76 high-grade prostate cancer tissue samples	↓ high-grade versus low-grade and normal prostate tissues	Prognostic marker, downregulation promotes EMT and drug resistance	[46]
	qRT-PCR in 53 matched samples; 26 serum samples from patients with PCa and 19 serum samples from healthy controls (normalized to the β -actin.)	↑ tumor tissue and plasma	Prognostic marker correlates with Gleason score	[45]
Glioblastoma	qRT-PCR from 48 glioma tissue samples and 10 normal brain tissue samples	↑ TT versus TN; significantly higher in high-grade versus low-grade glioma	Prognostic marker	[43]

4. Functions of circFOXO3

Up to this point, the main highlighted implications of circFOXO3 in oncological mechanisms are based on its competing endogenous RNAs (ceRNAs) effect for either tumor-promoter or suppressor miRNAs. The descriptive term for this function is “miRNA sponging”, a process that has been increasingly investigated in tandem with the accumulating evidence regarding the roles of miRNA in tumorigenesis and cancer progression. As circRNAs generally harbor multiple MREs [47], circFOXO3 as well contains multiple interaction sites that have been functionally investigated (Table 2) and will be furtherly described. It is worth mentioning that the enrichment status is a relevant variable in the sponging capacity (and all associated downstream effects) [48], and current evidence indicating that CircFoxo3 is highly expressed in the cytosol [36], yet this is highly dependent on cell type and also the status of its parental gene.

As previously mentioned, the observed biological effect of an either overexpressed or downregulated circFOXO3 is dependent on the nature and downstream effect of the sponged miRNA. For example, in the case of glioblastoma, circFOXO3 displays a pro-tumorigenic effect by acting a ceRNA for miR-138-5p and miR-432-5p [43]. The two sponged miRNAs are regulators of the nuclear factor of activated T cells 5 (NFAT5), a transcription factor often involved in promoting cell motility in cancer. CircFOXO3 can thus increase the expression of NFAT5 by inhibiting the activity of miR-138-5p and miR-432-5p, considered tumor suppressor miRNAs in the particular case [43]. More so, the study confirmed that the interaction between circFOXO3 and the miRNAs is AGO2-dependent, as confirmed via anti-AGO2 RIP assay [43].

Similarly, upregulated circFOXO3 in prostate tissues can regulate the expression of *SLC25A15*, a mitochondrial metabolite transporter, by sponging miR-29a-3p, reversing its presumed tumor-suppressing roles [45].

A regulatory loop involving circFOXO3, miR-155, and the FOXO3 gene in the case of non-small cell lung cancer (NSCLC) has been described [39]. The initial investigation confirmed the sequestering of miR-155 by circFOXO3 via immunoprecipitation assays. Further investigation observed that ectopic expression of circFOXO3 also promoted FOXO3 expression, by sequestering miR-155, a post-transcriptional regulator of the FOXO3 mRNA. Restoration of FOXO3 expression via circFOXO3 ceRNA activity promoted cell death, and reduced motility and invasive potential of the cells in vitro by hindering the regulatory effects miR-155. The study contributed to assessing the potential of both circFOXO3 as potential therapeutic use and FOXO3s tumor-suppressor activity in NSCLC [39]. This is a relevant example of a regulatory axis

with promising therapeutic potential, as miR-155 is one of the most prevalent miRNAs with an altered expression pattern in lung cancer, often associated with a poor prognostic [49].

Another sponging interaction has been validated in the case of hepatocellular carcinoma, where the interaction between circFOXO3 and miR-199-5p has been indicated to regulate the expression of TP Binding Cassette Subfamily C Member 1 (ABCC1) in tissues from Adriamycin resistant tumors and developed ADM-resistant cells. Based on gain- and loss-function, the study suggested that circFOXO3 promotes Adriamycin resistance along with the development of EMT characteristics by indirectly upregulating ABCC1 [50].

The emphasis on the functional connection between circFOXO3, the parental gene, or its related pseudogene of FOXO3 has been investigated in a limited number of studies [36]. CircFOXO3 is expected to promote Foxo3 expression by two different mechanisms: namely, reducing the Foxo3 protein degradation or indirectly by increasing the enhancing Foxo3 translation via sponging effect for some specific miRNAs that target this gene [48]. This was the main interest of a group that focused on the expression levels of the before mentioned transcripts and their implications in breast cancer, all while taking into account the putative MRE present in all three transcripts. The group outlined several miRNAs that could interact with all transcript variants (circFOXO3, the FOXO3 pseudogene, and the FOXO3 mRNA), thus confirming the existence of overlapping MRE. The study validated that the transcripts could interact with several miRNAs that target the FOXO3 mRNA and thus hinder its translation. Ectopic overexpression of circFOXO3 and the pseudogene were noticed to effectively promote FOXO3 translation by sponging 8 different miRNAs thus mitigating their effect on the proper mRNA. More so, the cumulative sponging of the 8 miRNAs and the upregulation of FOXO3 inhibited tumor growth and angiogenesis in vivo [36].

A functional indirect relation between circFOXO3 and the transforming growth factor-beta (TGF β) receptor type 2 via miR-9 sponging has been highlighted in a study focused on bladder cancer. The transcript was initially validated as significantly downregulated in patient tumor samples ($n = 49$), the event also correlated with poor survival rates for the BC patients. Furtherly, the group investigated whether the common putative miR-9 MREs between circFOXO3 and the TGFBR2 mRNA could have a mechanistic implication. The in vitro validation of the circFOXO3-miR-9-5p-TGFBR2 regulatory axis confirmed that the ectopic expression of circFOXO3 downregulated miR-9-5p levels, thus promoting TGFBR2 translation in BC cell lines. Cells transfected with the circFOXO3 expression vector displayed hindered proliferative and metastatic abilities, highlighting a potential tumor suppressor effect of circFOXO3 in bladder cancer [41].

Table 2
CircFoxo3 biological functions identified in preclinical studies.

Nr.	Pathology	CircFoxo3 expression level	Function of circFOXO3	Cell lines	Biological effect	Reference
1	Esophageal squamous cell carcinoma	↓	Sponging miR-23a	KYSE510, TE-13, TE-1, and CA109 versus HEEPIC	circFOXO3 restoration promote upregulation of PTEN via miR-23a sponging	[44]
		↓	sponging miR-22, miR-136*, miR-138, miR-149*, miR-433, miR-762, miR-3614-5p and miR-3622b-5p	MDA-MB-231	Increased sponging via ectopic expression of circFOXO3 containing constructs promoted expression of FOXO3 and its related pseudogene and inhibited growth	[36]
2	Breast cancer	↓	Interaction with MDM2 and p53	MDA-MB-468, MDA-MB-231, 67NR, 66C14, 4 T07, 4 T1, and B16 versus normal cells (Hek293T, BEAS2B, HaCaT, NIH3T3, MEF, and MCF-10A)	Regulated apoptosis via ↑PUMA, ↑FOXO3, ↓p53	[40]
4	Non-small cell lung carcinoma	↓	Sponging miR-155	Cell lines A549, SPC-A1, NCI-H1299, NCI-H1650, SK-MES-1, and 16HBE	Sponging for miR-155; promoting linear FOXO3 expression; controlling apoptosis, cell proliferation, and chemoresistance	[39]
		↓	Sponging miR-191-5p	T24, UM-UC-3, and J82, versus normal cells SV-HUC-1	Upregulation through vector both in vivo and in vitro construct promoted apoptosis via sponging miR-191	[42]
5	Bladder cancer	↓	Sponging miR-9-5p	EJ and T24	Upregulation displayed tumor suppressor activity via sponging miR-9 and upregulating TGFBR2	[41]
		↓	–	↓ VCaP, LNCaP, DU145, PC3 versus PWPEI	enhanced chemosensitivity to docetaxel via circFOXO3/FOXO3/ EMT after restoring circFOX3	[46]
6	Prostate cancer	↑	sponging miR-29a-3p	↑ LNCaP, LNCaP-AI versus WPMY-1	Sponging promoted aggressive phenotype, presumed via SLC25A15 upregulation and miR-29a-3p down-regulation	[45]
7	Glioblastoma	↑	Sponging miR-138-5p/miR-432-5p	↑U87-MG, U251-MG, A172, T98G versus HEBs HL-7702 (normal)	ceRNA for NFAT5 by sponging miR-138-5p/miR-432-5p	[43]
8	Hepatocellular carcinoma	↑	Sponging miR-199-5p	SK-HEP-1, HepG2, SK-HEP-1/ADM and HepG2/ADM as resistant	CircFOXO3 expression increased invasion and growth via miR-199-5p sponging by upregulating ABCC1	[50]

Besides the sponging activity identified in the case of many other circular transcripts, circFOXO3 has been proven to have important roles in key cellular processes by interacting with several RNA binding proteins [40,51]. One of the highlighted cases of such interaction, circFOXO3 interacts directly with the MDM2 ubiquitin and creates a regulatory axis with p53 and the FOXO3 protein. CircFOXO3 can interact with both p53 and MDM2 and induce p53 ubiquitination and subsequent degradation. Furthermore, the ectopic expression of circFOXO3 prevented MDM2 from targeting FOXO3 and therefore increased its levels. Upregulation of FOXO3 and cumulative degradation of p53 promoted apoptosis in tumor cells both in vitro and in vivo via the intrinsic apoptotic pathway due to the FOXO3-PUMA interaction [40].

CircFOXO3 forms an RNA-protein ternary complex with CDK2 and p21. This prevents CDK2 from interacting with cyclin A and E, which arrests cell cycle progression [33]. The identification of the circFOXO3–p21–CDK2 interacting complex was one of the first evidence that circRNAs can bind and possibly sequester proteins, the biological effect being arguably more complex in conjunction with the miRNA sponging effect. Worth mentioning is the previously described, circFOXO3's ability to form ternary complexes with cell cycle and apoptosis-associated factors like NFAT5 (in conjunction with the sponging miR-138-5p and miR-432-5p), consolidating circFOXO3s physical interaction with transcription factors involved in oncological processes [43].

Although not a direct oncological mechanism, a study focused on the modulation of doxorubicin-induced cardiomyopathy highlighted an implication of circFOXO3 in cellular senescence. Ectopic expression of circFOXO3 aggravated the senescent phenotype induced by doxorubicin, presumably as a result of circFOXO3's interaction with anti-

senescent protein ID-1 and the transcription factor E2F1. Additionally, anti-stress factors FAK and HIF1 α were also pointed out as possible target of circFOXO3 in this case [51]. An interesting particularity regarding circFOXO3 has been outlined by the same group, which implicated circFOXO3 in the p53-dependent apoptotic pathway. Specifically, the group noticed a significant increase in the expression rate of circFOXO3 in the case of apoptotic tumor cells, hinting towards a mechanistic implication, yet additional investigations are required in this case [36].

While not a direct oncological mechanism, a study provided evidence regarding circFOXO3's role in cardiac senescence and aging-related mechanism evaluated on a mouse model of Doxo-induced cardiomyopathy. The study observed that by restoring circFOXO3, Doxo-induced cardiomyopathy was aggravating. On the other hand, siRNA inhibition resulted in a protective effect. The regulation and underlying mechanism of cellular senescence by circFOXO3 is believed to rely on the anti-senescence proteins (ID1 and E2F1), and anti-stress proteins (FAK and HIF1 α) [51].

A study focused on the neuroprotective properties of circFOXO3, yet with possible implications in tumoral metabolism, has indicated that circFOXO3 regulates apoptosis in neurons by promoting the mitochondrial apoptotic pathway as a result of glutamate-induced oxidative stress [52]. CircFOXO3 and the FOXO3 protein appeared to be upregulated in glutamate-treated neurons. More so, FOXO3 displayed increased nuclear shuttling as a result of the glutamate treatment, indicating increased functionality. This was later confirmed by the pro-apoptotic protein BimEL, a target gene of FOXO3. The study concluded that silencing circFOXO3 reduced the generated ROS and downregulated the levels of

the BimEL, both cumulating in anti-apoptotic effects. Glutamate is the direct product of glutamine, and the possible relevance of this metabolic implication of circFOXO3 is the shift towards a glutamine-based metabolism observed in several cancer types [53,54]. Overall, future research in the implications of circFOXO3 and FOXO3 in the glutamine metabolism could provide useful insight regarding cellular ROS balance in conjunction with tumor metabolic alterations (Fig. 1).

5. CircFOXO3 as a therapeutic target

Recent investigations explore the possibility of using circRNA as therapeutic agents and molecular targets [55]. To the current date, there is a limited amount of studies focused on restoring expression level and inhibiting the sponging capabilities of circFOXO3, all dependent on pathological context and type of interaction. While the expression of a specific circRNA cannot be directly controlled at genome and spliceosome level, studies have simulated circRNA overexpression via inducing expression ectopically with the use of plasmids that contain the circularized FOXO3 exon. The process of restoring the expression level of a particular circRNA implies the use of mini-gene constructs on RNA/DNA vectors or disturbing the endogenous circRNA-generating locus. The general architecture of these construct contains RNA polymerase II transcription start and terminator sites, splice acceptor and splice donor sites; as length have over 300 nucleotides and are generally flanked by intronic inverted repeats regions using lentiviral or adenoviral vectors [56]. The main limitations of transfection consist of the stability and possible technical artifacts that could arise from the use of these vectors

[57]. Specifically, the artificially expressed vector should be distinguishable from the endogenous circFOXO3 for accurate quantification of their respective expression levels. More so, in some cases the RNA polymerase can bypass the transcriptional termination signals, thus generating rolling circle transcriptional concatemer products.

The potential use of the ectopic expression of circFOXO3 would be the counteraction of the pro-tumorigenic activity of several overexpressed miRNAs, such as miR-155, miR-23a, miR-195p, and many others [1,58]. This kind of activity has been repeatedly confirmed in a set of studies (Table 3) and might constitute a viable therapeutic option against the miRNA for which circFOXO3 has validated MRE. On the other hand, targeted inhibition of the circFOXO3 sponging ability is another method of experimentally validating the interaction between circFOXO3 and a specific miRNA. Short siRNA sequences that can bind specific MRE are useful in hindering a miRNA-circRNA sponging interaction, which can be useful both for validation of biological effect and furtherly as a therapeutic method, as there is a multitude of tumor-suppressor miRNA that might be sponged by circFOXO3. One such example is miR-29a-3p in the case of prostate cancer, where its anti-tumor activity is nullified based on its sponging by circFOXO3. siRNA transfection rescued miR-29a-3p levels and promoted apoptosis in cancer cells. CircFOXO3 has been indicated to be involved in the development of therapeutic resistance in the case of prostate cancer. Specifically, siRNA silencing of circFOXO3 promoted positive effects on the cancer cells, such as reduced apoptosis and increased migration and tolerance to Docetaxel. Delivery of circFOXO3 reversed the observed effects and increased the sensitivity to the treatment.

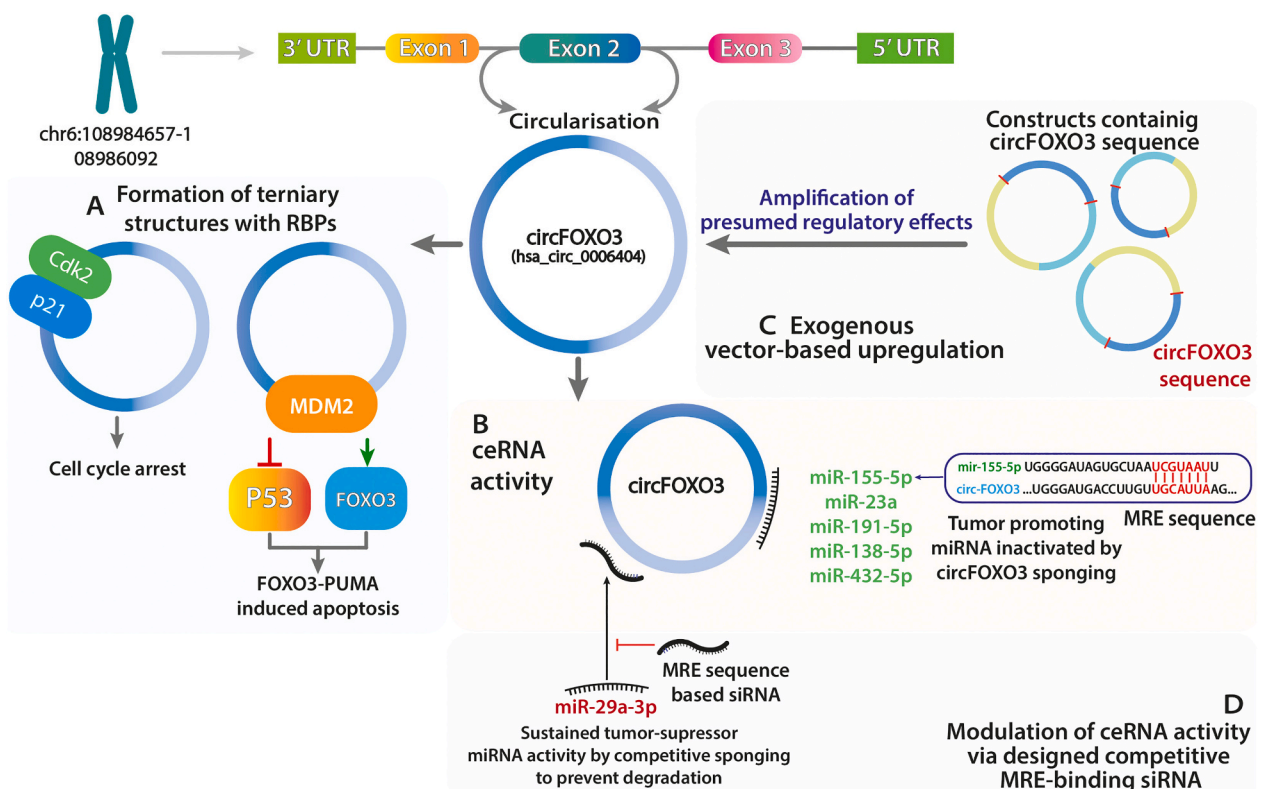


Fig. 1. Origin, main identified functions, and exogenous-based presumptive therapeutical applications for circFOXO3. CircFOXO3 originates from the parental FOXO3 gene and consists of the circularized second exon of the FOXO3 pre-mRNA. Attributed functions consist of interactions with RBPs (A) that coalesce in cell cycle arrest (interaction with cdk2 and p21) and apoptosis promoting effects (via interaction with MDM2). MiRNA sponging (B) represent the complementary based interaction between different miRNA and circFOXO3 based on an existent seed sequence. The effects of miRNA sponging are dependent on their regulated targets and nature (tumor promoter/suppressor) and abundance (upregulated/downregulated) of the specific miRNA. Ectopic expression of circFOXO3 (C) is the most used method in upregulating circFOXO3 to enhance its presumed antitumor effects based on the identified interactions. CircFOXO3 silencing (D) using siRNA is a utilized tactic in the case when the sponging activity has a tumor-promoting effect, due to inhibition of a tumor-suppressing miRNA. Therefore, a short sequence that competitively binds the specific MRE can be used to silence circFOXO3's sponging properties against that miRNA, such as the exemplified miR-29a-3p in the case of prostate cancer.

Table 3
Studies that manipulated the expression level of circFOXO3 as a therapeutic target in cancer.

Nr.	Pathology	Therapeutic strategy	Method	Biological effect	Reference
1	Esophageal squamous cell cancer (KYSE510, TE-13, TE-1, and CA109)	Restoring	pcDNA3.1 plasmid/lipofectamine	Cancer progression via miR-23a/PTEN axis	[44]
2	Non-small cell lung cancer (A549, SPC-A1, NCI-H1299, NCI-H1650, SK-MES-1, and 16HBE)	Restoring	pCMV vector transfection	Tumor suppressor effect	[39]
3	Urothelial carcinoma (T24, UM-UC-3 and J82)	Restoring	pCD-ciR vector transfection	Tumor suppressor effect, promotion of apoptosis	[42]
4	Breast cancer (MDA-MB-231, 67NR, 66C14, 4 T07, 4 T1, and B16)	Restoring	plasmid -PEG-Au NP (PG1-TH-2 k, Nanocs+10 nm gold nanoparticles)	Tumor suppressor effect, regulation of apoptosis via formation of circFOXO3-p53-MDM2 complex	[40]
		Inhibition	siRNA FOXO3	cell viability, decreasing apoptosis rate	[40]
		Restoring	HindIII-BamHIdigested circ-Fox-GFP/ pUC57	Inhibits tumor growth and angiogenesis	[36]
5	Triple-negative breast cancer (MDA-MB-231)	Inhibition	siRNA for circFOXO3	no effects Foxo3 mRNA and its related pseudogene	[36]
		Inhibition	circFOXO3 siRNA	↓FOXO3 and circFOXO3	[46]
6	Prostate cancer (VCaP, LNCaP, DU145, PC3 versus PWPEI)	Inhibition	plasmids, a basic sequence (flanked by HxoI and AgeI; spacer sequence for HindIII and SalI restriction enzymes)	enhanced cell proliferation, colony formation, invasion, and migration	[43]

Yet, these can act as foundations for further therapeutic applications of circRNA, and specifically circFOXO3. A series of studies that have applied and investigated the biological effects of circFOXO3 upregulation and silencing are presented in Table 3.

6. Conclusions and perspectives

Current research has only started to include circular RNAs as valuable agents in the interplay between different species of ncRNA (especially miRNA) and other dysregulated cancer-associated factors. In this review, we emphasized how the circular transcript circFOXO3 displays an extensive functional versatility in different pathologies based on its interaction with protein and other ncRNA factors. While we cannot ignore the promising evidence regarding the contextual implications of circFOXO3 in either tumor-promoting or suppressor programs, the key aspects to be considered is that most of the interactions are display a high degree of specificity for the type of cancer and cellular context (i.e. expression level of specific proteins and miRNAs), events that contribute to its applicability as a biomarker and/or therapeutic targets. Thus, as noted from all the regulatory networks identified and listed in this review, there is still a lack of consensus regarding the role of circFOXO3. Promising results are inferring the role of circFOXO3 in a wide area of mechanisms, ranging from cell cycle control, EMT, drug resistance, and even metabolic regulation, each worth further development. All in all, the main tasks for further research focused on circFOXO3 are to establish biological consensus regarding enrichment status in different pathologies and the in vivo validation of the proposed mechanism, all to assess the clinical utility of circFOXO3 either as a reliable disease indicator or even therapeutic target.

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CRediT authorship contribution statement

Rares Drula: writing-original draft and figures. Radu Parlog: data curation; writing-review and editing. Monica Trif: data curation, visualization, writing-review, and editing. Ondrej Slaby: writing-review and editing. Cornelia Braicu: supervision, visualization, writing-original draft; writing-review, and editing. Ioana Berindan-Neagoe: conceptualization, writing-review, and editing.

Declaration of competing interest

The authors declare no conflict of interest.

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