CircRNAs and Fusion-circRNAs in cancer: new players in an old game

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Abstract

Circular RNAs (circRNAs) are generated from 'back-splicing' events. Their circular structure makes them stable in cells and body fluids. These entities are involved in several human diseases including cancer, as they affect the expression of genes promoting proliferation, invasion, apoptosis, and angiogenesis. Moreover, they are secreted in extracellular vesicles, such as exosomes, having a potential role as messengers in cell-to-cell communications. CircRNAs are also generated by the back-splicing of linear fusion transcripts derived from genomic rearrangements, giving rise to fusion circRNAs (f-circRNAs).

Here we discuss the most relevant results achieved by studying the role of circRNAs in cancer onset and progression, particularly focusing on f-circRNAs in hematological and solid tumors. Moreover, we report recent advances in the application of circRNAs as novel “liquid biopsy” biomarkers for early and non-invasive diagnosis of tumors, and as therapeutic targets in human cancer. Their use as engineered molecules sponging oncogenic miRNAs or stably expressing proteins/drugs is also discussed. All these achievements suggest the crucial importance of circRNAs and f-circRNAs in the future setup of personalized therapies in molecular medicine.

Keywords:

CircRNA; fusion-circRNA; cancer; chimera; biomarker

1. Introduction

Circular RNAs (circRNAs) are a class of endogenous RNAs (Z. Li et al., 2015; Lei et al., 2019) first identified in viroids (Sanger et al., 1976). They result from an alternative splicing process, called “back-splicing” (Jeck et al., 2013) followed by covalent circularization in closed-loop structures (Jiang et al., 2019). Thanks to the development of Next Generation Sequencing (NGS) technologies, many circRNAs have been detected in humans and other species (Jeck et al., 2013; Jiang et al., 2019). In recent years, circRNAs have attracted increasing attention by researchers due to their implication in diverse human diseases, including cancer (Verduci et al., 2019), suggesting their possible use as biomarkers and therapeutic targets (Yong Zhang et al., 2019). Despite the progress made on circRNA identification and characterization, poor information about their biogenesis and function is presently available. In this review, we discuss the most recent discoveries concerning these circular entities, providing a compendium of their role in cancer pathogenesis. In particular, we will focus on a newly discovered class of circRNAs, i.e. fusion-circRNAs (f-circRNAs), originated by the back-splicing of fusion transcripts (Guarnerio et al., 2016). Finally, we highlight the potential application of circRNAs as biomarkers and therapeutic targets/tools in human tumors.

2. Circular RNAs

2.1 CircRNA biogenesis

CircRNAs are produced by juxtaposing a downstream 5' splice site (donor) to an upstream 3′ splice site (acceptor), in a back-splicing process generating circular molecules with covalently linked ends (Fig. 1) (Yang Zhang et al., 2016; Starke et al., 2015; Liang & Wilusz, 2014).

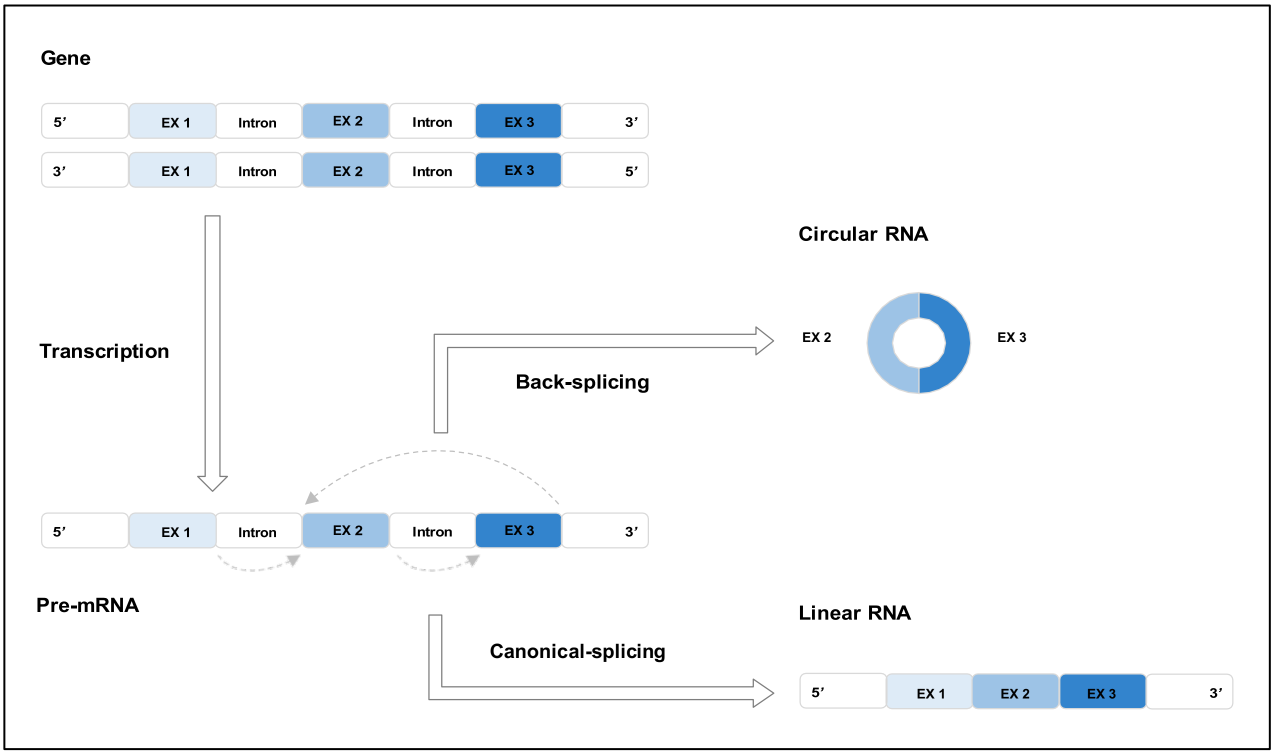

Fig.1: Biogenesis of circular RNAs. The pre-mRNA of a single gene can produce different mature transcripts, based on the type of splicing event it undergoes. Canonical splicing generates linear transcripts, while circRNAs arise from back-splicing events. EX: exon.

This process may occur both co- and post-transcriptionally (Yang Zhang et al., 2016). To date, several mechanisms have been described as prompting back-splicing events. As an example, long introns flanked by highly similar sequence elements, repeated in opposite orientation (e.g. Alu repeats), can facilitate the post-transcriptional circularization of exons (X. O. Zhang et al., 2014; Kramer et al., 2015). These intronic repeats must be base-paired to allow the splice sites to be close to each other and promote back-splicing and circularization (Kramer et al., 2015; Liang & Wilusz, 2014). It is also known that RNA binding proteins (RBPs), such as Muscleblind (MBL/MBNL1) and Quaking (QKI), play an essential role in regulating alternative splicing (Ashwal-Fluss et al., 2014; Conn et al., 2015). Indeed, RBPs can form bridging splice sites that facilitate back-splicing (Newman et al., 2016; Xiang Li et al., 2017).

Moreover, Barrett et al. (2015) showed that skipped exons derived from alternative splicing could generate lariat precursors, promoting back-splicing events (Barrett et al., 2015). There is evidence that some introns are not spliced when mutations of the 3' polyadenylation signal occur (Liang & Wilusz, 2014). This impaired process, causing the accumulation of transcripts in the chromatin fraction, may also promote their back-splicing after a lag period (Kramer et al., 2015; Liang & Wilusz, 2014; Vargas et al., 2011; Yang Zhang et al., 2016).

2.2 Molecular features of circRNAs

CircRNAs may originate from any transcribed region (protein-coding genes, introns, intergenic regions, untranslated regions), being so classified into three categories: i) exon circRNAs (ecircRNAs); ii) intron circRNAs (ciRNAs); iii) exon and intron circRNAs (EIciRNAs) (Panda et al., 2017; Z. Li et al., 2015; Yang Zhang et al., 2013).

The covalently closed structure, lacking 5' CAPs and 3' polyadenylated tails, confers RNase R and exonucleases resistance (Yang Zhang et al., 2016; Suzuki & Tsukahara, 2014; Jeck & Sharpless, 2015; Memczak et al., 2013). This increased stability slows down circRNA turnover (Tan, Gou, et al., 2018; Yang Zhang et al., 2016; Jeck et al., 2013; Memczak et al., 2013), leading to the accumulation and easy detection of such transcripts in body fluids like blood, plasma, saliva, and urine (Jeck et al., 2013; Memczak et al., 2013).

CircRNAs are highly conserved among species: they were firstly identified in viruses (Sanger et al., 1976) and later, thanks to NGS and specifically-designed bioinformatics tools, in a wide spectrum of organisms, from fungi to mammals (Jeck & Sharpless, 2015; Memczak et al., 2013; Salzman et al., 2012; Guo et al., 2014); this allowed to investigate circRNA biogenesis and function in animal models (P. L. Wang et al., 2014).

CircRNAs exhibit a tissue-specific and developmental stage-dependent expression patterns. For instance, some circRNAs are upregulated in fetal development, particularly during neuronal differentiation and synapse formation (Rybak-Wolf et al., 2014; You et al., 2015; Szabo et al., 2015). This finding suggested that they may have a regulatory role in gene expression (Z. Li et al., 2015). Moreover, an altered circRNA expression was also reported in pathological conditions. This is the case of *hsa_circ_0004018,* transcribed from the tumor suppressor *SET and MYND Domain Containing 4 gene* (*SMYD4*) (Hu et al., 2009). This circRNA was found to be downregulated in Hepatocellular carcinoma (HCC), and showed a stage-related expression pattern, suggesting it may be a promising biomarker for HCC diagnosis (Fu et al., 2017).

CircRNAs are enriched in the cytoplasmic fraction (Salzman et al., 2012; Jeck et al., 2013), but can be found also in the nucleus, as well as in extracellular vesicles such as exosomes (exo-circRNAs) (Jost et al., 2018). Recent studies revealed that exo-circRNAs tend to be enriched in exosomes, where they are even more abundant than their linear counterparts (Yan Li et al., 2015), compared to secreting cells. Some authors speculated that cells may accumulate circRNAs in exosomes to eliminate them, or to communicate with other cells (Lasda & Parker, 2016).

Finally, as circRNAs are involved in tumorigenesis (M. Zhang et al., 2018), they represent promising biomarkers for the early diagnosis of tumors, due to their detection in body fluids (Z. Zhang et al., 2018; Yan Li et al., 2015; Lasda & Parker, 2016).

2.3 CircRNA biological functions

CircRNAs can play important biological functions:

2.3.1 CircRNAs can act as miRNA and RBP sponges

It is known that circRNAs can act as miRNA or RBP sponges, modulating their concentration, localization, and binding sites access of target molecules (Jeck & Sharpless, 2015). As an example, circRNA *CDR1as* (also known as *CiRS-7*), interacts with AGO2 (Argonaute-2), a protein involved in the RNA-induced silencing complex (RISC), and also binds *miR-7* in brain tissues, a miRNA playing a crucial role in the functioning of human and mouse neurons (Memczak et al., 2013; Hansen et al., 2011). Moreover, a strong interaction between MBNL1 and *circMbl* has been reported: this circRNA could sponge out the excess of MBNL1 by binding to it, regulating protein levels (Ashwal-Fluss et al., 2014).

2.3.2 CircRNAs can regulate gene expression

CircRNAs may also act as regulators of gene transcription and expression by binding mRNAs or miRNAs (Z. Li et al., 2015; Y. Wang et al., 2018; Rybak-Wolf et al., 2014). For example, the murine *Fmn* circRNA acts as a trap for its cognate linear mRNA, by stacking at the translation start site, and hampering protein translation. Consequently, due to a feedback-regulation process, the linear mRNA expression is reduced, impairing the *Fmn* function (Chao et al., 1998).

Furthermore, circRNAs biogenesis, engaging the splicing machinery, can compete with the linear pre-mRNA maturation process (Salzman et al., 2012), decreasing its production, and thus contributing to alter gene expression. Moreover, several circRNAs localized in the nucleus can modulate the linear product of their parental gene, by regulating the RNA Polymerase II (Pol II) activity (Z. Li et al., 2015; Yang Zhang et al., 2013). As an example, *circPABPN1* modulates the translation of its linear mRNA (*PABPN1*) by preventing HuR binding to *PABPN1* (Abdelmohsen et al., 2017)**.** Another example regards *circHomer1_a*, which competes with *Homer1b/c* biogenesis during synaptogenesis. This mechanism is essential to regulate the synaptic function in the mouse brain (You et al., 2015).

CircRNAs can indirectly regulate gene expression by sponging miRNAs. The latter play a crucial role in post-transcriptional gene expression regulation, mainly by targeting specific mRNAs for translation repression. Hence, by trapping miRNAs and preventing them from binding to mRNA targets, circRNAs indirectly affect gene expression. Several deregulated circRNAs carry out their oncogenic effects through this mechanism (examples will be discussed in paragraph 2.4).

Finally, circRNAs can also act on the overall cell translational activity, controlling the ribosomal RNA maturation by binding proteins with a crucial role in this process. For instance, *circANRIL* binds the Pescadillo Ribosomal Biogenesis Factor 1 (PES1) protein, an essential 60S-preribosomal assembly factor, preventing rRNA maturation. This affects the ribosome biogenesis and activates TP53, inducing apoptosis and inhibiting proliferation in atherosclerosis. As a consequence, *circANRIL* confers atheroprotection (Holdt et al., 2016).

2.3.3 CircRNAs have the ability to encode proteins

As most circRNAs derive from exons, they can have an open reading frame (ORF) and may encode proteins. Legnini et al. (2017) identified *circZNF609*, originated from the second exon of the *ZNF609* gene, encoding the Zinc Finger Protein 609 (151 kDa). They demonstrated that *circZNF609* is expressed in myoblasts and may control their proliferation. Since it contains an ORF and is associated with High Molecular Weight polysomes, the authors hypothesized that this circRNA could have a coding potential. Indeed, they showed that it is translated into a protein in a splicing-dependent/cap-independent manner, driven by an Internal Ribosome Entry Site (IRES). The protein encoded by *circZNF609* lacks the zinc-finger domain, suggesting that it could interfere or modulate the activity of the wild-type isoform (Legnini et al., 2017). Another cap-independent translation mechanism concerns circRNAs containing N6-methyladenosine (m6A) in their 5′ UTR; this modification can directly bind eukaryotic initiation factor 3 (eIF3), recruiting the 43S preinitiation complex and initiating translation (Meyer et al., 2015).

CircRNA translational activity has been reported in a variety of organisms. Pamudurti et al. (2017) showed that a subset of circRNAs was associated with ribosomes in *Drosophila*, suggesting a translational activity. The UTRs of these circRNAs allow cap-independent translation, encoding proteins with specific domains. In particular, the authors identified *circMbl*, generated from the Muscleblind locus. *CircMbl* and the circMbl1-encoded peptide are present in synaptosome fractions, thus suggesting that they may be crucial for the brain (Pamudurti et al., 2017).

2.3.4 CircRNAs play a role in the innate immunity

Recent findings revealed a correlation between circRNAs and innate immunity, involving both exogenous and endogenous circular entities. Chen et al. (2019) showed that cells can recognize exogenous circRNAs since they lack m6A RNA modification, triggering the immune response (Chen et al., 2017; Chen et al., 2019). In particular, exogenous circRNAs stimulate regulators involved in the innate immunity, such as retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated protein 5 (MDA5), 2'-5'-oligoadenylate synthetase 1 (OAS1), OAS-like protein (OASL), and protein kinase R (PKR), protecting against viral infections (Chen et al., 2017).

Moreover, Liu et al. (2019) showed that endogenous circRNAs are natural inhibitors of PKR. PKR activation, indeed, requires long dsRNAs (>33 bp), while circRNAs tend to form short dsRNAs (16–26 bp), thus competing for PKR binding and affecting its activation. Upon viral infection, RNase L degrades circRNAs, allowing the PKR activation within the framework of innate immunity. Particularly, the authors analyzed eight patients with Systemic lupus erythematosus (SLE) autoimmune disease, observing a reduced expression of c*ircCAMSAP1, circPOLR2A, circPVT1, circTBCD,* and *circUIMC1*, compared with normal samples, suggesting an increased PKR activation. This promising result suggests a connection between circRNAs and autoimmune diseases, but it needs to be confirmed in a larger patient cohort (Liu et al., 2019).

Finally, endogenous circRNAs can compete with viral mRNAs for binding to NF90/NF110 immune factors. Li et al., (2017) showed that, when a viral infection occurs, the NF90/NF110 nuclear export promotes circRNAs downregulation. The authors speculated that circRNAs, binding NF90/NF110, may protect from non-specific immune responses (Li et al., 2017).

2.4 CircRNA role in cancer

CircRNAs can modulate gene expression in cancer by sponging miRNAs or RBPs with oncogenic or tumor suppressor roles (Fig. 2).

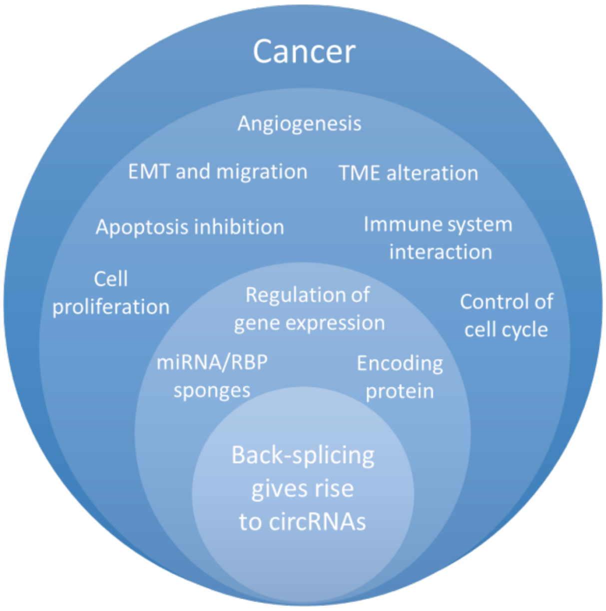

Fig. 2: Role of circRNAs in cancer. CircRNAs can act as miRNA/RBP sponges, regulate gene expression or encode proteins, promote cell proliferation, inhibit apoptosis, promote angiogenesis and other events that lead to cancer initiation and progression. EMT: epithelial-mesenchymal transition; TME: tumor microenvironment.

2.4.1 Hematological malignancies

CircRNAs are involved in tumorigenesis, progression, and drug resistance of hematological malignancies:

i) Acute myeloid leukemia (AML)

Circ-ANAPC7 (*hsa_circ_0005785*) could function as an oncogene in AML, where it is overexpressed, sponging *miR-181* family miRNAs and promoting tumorigenesis (H. Chen et al., 2018).

Similarly, the upregulation of c*irc_0009910* in AML may sponge *miR-20a-5p*, affecting its function as cell proliferation inhibitor and apoptosis enhancer, thus promoting cancer progression (Ping et al., 2019). *circRNA-DLEU2* (*hsa_circ_0000488*) is also upregulated in AML; it stimulates AML cell proliferation by suppressing *miR-496* and, consequently, stimulating the Protein *Kinase CAMP-Activated Catalytic Subunit Beta* (*PRKACB*) transcription (D.M. Wu et al., 2018). Furthermore, *circPAN3* may sponge *miR-153-5p* and *miR-183-5p*, inhibitors of the X-linked inhibitor of apoptosis protein (XIAP), an anti-apoptotic protein resulting in its enhanced expression. Through this mechanism, *circPAN3* mediates drug resistance in AML (Shang et al., 2019)

ii) Acute lymphoblastic leukemia (ALL)

CircPVT1 (*hsa_circ_0001821*) is upregulated in ALL, where it sponges *miR-let-*7 and *miR-125,* having *MYC* and *BCL2* as targets, respectively. By inhibiting these miRNAs, *circPVT1* enhances both MYC transcription factor and BCL2 anti-apoptotic protein expression levels in leukemic cells, thus promoting cell proliferation and apoptosis inhibition (Hu et al., 2018).

iii) Chronic myeloid leukemia (CML)

To the best of our knowledge, two circRNAs have been described as involved in CML pathogenesis: *hsa_circ_0080145* and *circ_100053.* The former acts as a sponge for the miRNA *miR-29b*, which plays a tumor suppressor role by targeting *ABL1* and *BCR/ABL1* in Philadelphia chromosome-positive CML (Liu et al., 2018). The latter is significantly upregulated in CML peripheral blood mononuclear cells and serum samples compared with healthy controls. Its overexpression is correlated with a shorter overall survival of patients, suggesting a potential role as CML biomarker. Moreover, an association between *circ_100053* upregulation and Imatinib resistance has been recently speculated (Ping et al., 2019).

iv) Multiple Myeloma (MM)

Zhou et al. (2020) showed that 122 and 260 circRNAs are upregulated and downregulated in MM, respectively, enabling MM differential diagnosis. Among them, the up-regulated *circPTK2* and the downregulated *circAFF2* are the best characterized circRNA entities. *circPTK2* enhances the expression of the *Protein Tyrosine Kinase 2* (*PTK2*) oncogene*,* its parental gene, increasing MM risk. Moreover, it sponges *miR-1298-5p*, with tumor suppressor activity, promoting tumor progression. *circAFF2* sponges *miR-638*, inhibiting its oncogenic function. Both *circPTK2* and *circAFF2* showed an impact on patient clinical parameters: the former was correlated with poor treatment response and survival, the latter with better prognosis. Therefore, both constitute potential prognostic biomarkers for MM. However, further studies are needed to clarify the molecular mechanisms involving circRNAs in MM (Zhou et al., 2020).

2.4.2 Solid tumors

i) Non-small cell lung cancer (NSCLC)

Among solid tumors, circRNAs have mainly been studied in lung cancer, one of the most devastating forms of tumor in terms of morbidity, mortality, and drug resistance. In detail, in NSCLC, *circFOXM1*, sponging the *miR-1304-5p* tumor suppressor, a regulator of the Pancreatic Progenitor Cell Differentiation And Proliferation Factor (PPDPF) and the Metastasis-Associated In Colon Cancer (MACC1), was found overexpressed. Consequently, it increases proliferation and invasion of NSCLC cells (Y. Wang et al., 2018; G. Liu et al., 2019). *circFOXM1/miR-1304-*5p/*PPDPF/MACC1* signaling was found to be crucial also in papillary thyroid cancer, by promoting cell proliferation, migration and invasion (Yanhui Pan et al., 2019).

Apart from *circFOXM1,* one of the most relevant upregulated circRNA in NSCLC is *circ_0016760* (S. Zhang et al., 2018), which acts as an oncogene by regulating the *miR-1287/GAGE1* axis. By sponging *miR-1287,* it leads to the upregulation of the *G Antigen 1* (*GAGE1*), with an unknown function, but expressed only in tumor tissues. Through this pathway, *circ_0016760* could promote NSCLC cells growth and invasiveness (Yongsheng Li et al., 2018).

A further example regards *circPVT1*, overexpressed not only in NSCLC but also in other cancer types. In NSCLC, *circPVT1* is abundant in the cytoplasm where, by sponging *miR-125b*, it can regulate *E2F2* expression, which controls cell cycle and the epithelial-mesenchymal transition (EMT). Thus, *circPVT1* overexpression increases E2F2 signaling, promoting tumorigenesis (Xiuyuan Li et al., 2018). Moreover, this circular RNA acts as a sponge also for *miR-497*, increasing BCL2 anti-apoptotic activity (Qin et al., 2019).

ii) Colorectal cancer (CRC)

Recent studies revealed that some circRNAs could act as tumor suppressors affecting cell growth and invasion in CRC cells. This is the case of *circCDYL* and *circMTO1*.

Cui et al. (2019) found *circCDYL* downregulation and *miR-150-5p* upregulation in colon cancer, when compared with para-carcinoma tissues. Induced *circCDYL* expression inhibited cell viability and stimulated apoptosis by decreasing *MYC* and *CCND1* expression, while upregulating *TP53*. *miR-150-5p* could be repressed by *circCDYL*, decreasing cell growth and migration (Cui et al., 2019).

circMTO1 was also described as downregulated in CRC tissues and cell lines. Its inhibition activates the Wnt/β-catenin signaling, MYC and CCND1, promoting cell migration and invasion. Additionally, *circMTO1* downregulation is correlated with advanced tumor, node, metastasis (TNM) stages, lymph node metastasis, and poor overall survival (Tang et al., 2017; Ge et al., 2018).

Interestingly, a recent study identified the involvement of the previously described *circPTK2* (see paragraph 2.4.1, sub-section iv) also in CRC, where it interacts with vimentin. *CircPTK2* can bind vimentin Ser38, Ser55 and Ser82 phosphorylation sites, promoting EMT. As a consequence, *circPTK2* may promote tumor growth and metastasis, and correlate with poor prognosis in CRC patients (Yang et al., 2020).

Coding circRNAs can be also deregulated in several cancers and play an important role in their development/progression. For instance, *circPPP1R12A-73aa* encodes a small protein that promotes CRC cell growth *in vitro* and *in vivo*, acting on the Hippo-YAP signaling pathway (Zheng et al., 2019).

iii) Glioblastoma (GBM)

Several coding circRNAs with a tumor-suppressor role have been reported so far in GBM. In detail, Zhang et al. (2018) proved that the *circSHPRH*, deriving from the *SNF2 histone linker PHD RING helicase* (*SHPRH*) gene, encodes the SHPRH-146aa protein in the human brain. Their data show that SHPRH-146aa protects its related full-length SHPRH from degradation. The authors found that both *circSHPRH* and SHPRH-146aa are downregulated in GBM, promoting its progression (Zhang, Huang, et al., 2018).

cPINTexon2 is also downregulated in GBM: it is derived from the exon 2 of the *long intergenic non-protein-coding RNA p53-induced transcript* (*LINC-PINT*), acting as a cell proliferation suppressor in this cancer. *cPINTexon2* encodes an 87-amino-acid peptide that interacts with the polymerase associated factor complex (PAF1c), thus inhibiting the transcriptional elongation of multiple oncogenes. The downregulation of *cPINTexon2* and its peptide has a potential role in GBM tumorigenesis (Zhang, Zhao, et al., 2018).

CircFBXW7 originates from exons 3 and 4 of the *F-box and WD repeat domain containing 7* (*FBXW7*) gene. Its encoded protein, FBXW7-185aa, by binding competitively the de-ubiquitinating enzyme Ubiquitin Specific Peptidase 28 (USP28), inhibits the expression of *MYC*, thus acting as a cell proliferation suppressor. Both *circ-FBXW7* and FBXW7-185aa are downregulated not only in GBM, but also in triple-negative breast cancer clinical samples (TNBC) (Yang et al., 2018; Ye et al., 2019).

iv) Other solid tumors

A coding circRNA with an oncogenic role was reported in liver cancer, where Liang et al. (2019) revealed the overexpression of *circβ-catenin*, deriving from the *CTNNB1* (*Catenin Beta 1*) oncogene. *circβ-catenin* encodes the β-catenin-370aa protein that interacts with the glycogen synthase kinase 3β, preventing the degradation of the full-length protein. This mechanism activates the Wnt/β-catenin pathway, promoting tumor growth in liver cancer (Liang et al., 2019).

In addition, *circFOXK2* is reported as a novel circular RNA with an oncogenic potential. Wong et al. (2020) revealed its upregulation in pancreatic ductal adenocarcinoma (PDAC) cells, where it acts as a sponge for miR-942, enhancing the expression of the *Ankyrin 1* (*ANK1*), *Glial Cell Derived Neurotrophic Factor* (*GDNF*), and *Paired Box 6* (*PAX6*) genes, and promoting cell growth, invasion and metastasis. Notably, *circFOXK2* interacts with proteins involved in cell adhesion, mRNA splicing, and structural molecule activity, such as Y-Box Binding Protein 1 (YBX1) and Heterogeneous Nuclear Ribonucleoprotein K (hnRNPK), enhancing the expression of the *NUF2 Component of NDC80 Kinetochore Complex* (*NUF2*) and *Pyridoxal Kinase* (*PDXK*) oncogenes. These findings demonstrate that *circFOXK2* contributes to PDAC progression (Wong et al., 2020).

circFGD4 and *circ_0000190* are two further examples of circRNAs with a role as tumor-suppressors, both down-regulated in gastric cancer (GC). Dai et al. (2020) showed that a low expression level of *circFGD4* correlates with poor tumor differentiation, lymphatic metastasis, and poor prognosis in GC patients. The authors showed that *circFGD4* plays an anti-cancer effect as it acts as a sponge of miR-532-3p. This interaction results in the increase of the APC Regulator of WNT Signaling Pathway (APC) expression and, hence, leads to the inactivation of the β-catenin signaling. Such data suggest that *circFGD4* may be used as a prognostic biomarker and a therapeutic tool for GC (Dai et al., 2020).

Similarly, a study by Wang et al. (2020) showed the tumor-suppressor role of *circ_0000190* in GC. This circRNA acts as a sponge of miR-1252, which targets P21 (RAC1) Activated Kinase 3 (PAK3), inhibiting cell viability, proliferation and migration, and inducing apoptosis and cell cycle arrest. As a consequence of its downregulation, miR-1252 level increases, while that of PAK3 decreases. Hence, targeting the circ_0000190/miR-1252/PAK3 axis may be a promising therapeutic strategy for the treatment of GC (Wang et al.; 2020).

Furthermore, circRNAs are involved in the modification of the tumor microenvironment, as they interact with immune cells, cancer-associated endothelial cells, cancer-associated fibroblasts, cancer stem cells, growth factors, cytokines, and the extracellular matrix (ECM) (Ma et al., 2020). For instance, *circRNA-MYLK* sponges *miR-29a* in bladder cancer (BC), inhibiting the suppression of the vascular endothelial growth factor A (VEGFA), which induces angiogenesis by activating the VEGFA/VEGFR2 and Ras/ERK signaling pathways. The upregulation of this circRNA in BC promotes proliferation, EMT, angiogenesis, and metastasis (Zhong et al., 2017). Moreover, *CDR1as* was also found as involved in the alteration of the tumor microenvironment, being associated with angiogenesis, ECM organization, integrin binding, collagen-binding, and TGF-β signaling. Additionally, it sponges multiple miRNAs and is overexpressed in several cancer types (Zou et al., 2019).

The data regarding the role in cancer of the described circRNAs are summarized in Table 1.

CircRNA	**Cancer type**	**Role in cancer**	**Reference (DOI)**
circ-ANAPC7 (*hsa_circ_0005785*)	AML	Oncogene: promotion of tumorigenesis	Chen *et al.* 2018 (10.1159/000491468)
c*irc_0009910*	AML	Oncogene: promotion of cancer progression	Ping *et al.*, 2019 (10.1016/j.bcmd.2018.12.006)
circRNA-DLEU2 (*hsa_circ_0000488*)	AML	Oncogene: promotion of cell proliferation	Wu *et al.*, 2018 (10.1128/mcb.00259-18)
circPAN3	AML	Oncogene: mediation of drug resistance	Shang *et al.*, 2019 (10.1016/j.exphem.2018.10.011)

circPVT1 (*hsa_circ_0001821*)	ALL	Oncogene: promotion of cell proliferation and inhibition of apoptosis	Hu *et al.*, 2018 (10.2217/epi-2017-0142)
hsa_circ_0080145	CML	Oncogene: promotion of oncogenesis	Liu *et al.*, 2018 (10.1016/j.bbrc.2018.08.154)
circ_100053	CML	Oncogene: promotion of oncogenesis	Ping *et al.*, 2019 (10.3727/096504018x15412701483326)
circPTK2	MM	Oncogene: promotion of tumor progression	Zhou *et al.*, 2020 (10.1186/s12885-020-6515-2)
circAFF2	MM	Tumor-suppressor: inhibition of *miR-638* oncogenic function	Zhou *et al.*, 2020 (10.1186/s12885-020-6515-2)
circFOXM1	NSCLC and PTC	Oncogene: increase of proliferation and invasion	Wang *et al.*, 2018; (10.3892/or.2018.6733 Liu *et al.*, 2019 (10.1016/j.bbrc.2019.03.213) Pan *et al.*, 2019 (10.1016/j.bbrc.2019.01.108)
circ_0016760	NSCLC	Oncogene: promotion of cell growth and invasiveness	Zhang *et al.,* 2018 (10.1038/s41598-018-21300-5) Li *et al.*, 2018 (10.1016/j.bbrc.2018.07.164)
circPVT1	NSCLC	Oncogene: promotion of tumorigenesis and BCL2 anti-apoptotic activity	Li *et al.*, 2018 (10.1159/000495876) Qin *et al.,* 2019 (10.1016/j.biopha.2018.12.007)
circCDYL	CRC	Tumor suppressor: stimulation of apoptosis and inhibition of cell growth and migration	Cui *et al.*, 2019 (10.1186/s12943-020-01180-y)
circMTO1	CRC	Tumor-suppressor: inhibition of cell migration and invasion	Ge *et al.,* 2018 (10.26355/eurrev_201812_16513)
circPTK2	CRC	Oncogene: promotion of EMT, tumor growth and metastasis	Yang *et al.,* 2020 (10.1186/s12943-020-1139-3)
circPPP1R12A-73aa	CRC	Oncogene: promotion of cell growth	Zheng *et al.*, 2019 (10.1186/s12943-019-1010-6)
circSHPRH	GBM	Tumor-suppressor: its downregulation promotes tumor progression	Zhang, *et al.*, 2018 (10.1038/s41388-017-0019-9)

cPINTexon2	GBM	Tumor-suppressor: its downregulation promotes tumorigenesis	Zhang, *et al.*, 2018 (10.1038/s41467-018-06862-2)
circFBXW7	GBM and TNBC	Tumor-suppressor: suppression of cell proliferation	Yang *et al.,* 2018; (10.1093/jnci/djx166) Ye *et al.,* 2019 (10.1016/j.omtn.2019.07.023)
circβ-catenin	Liver cancer	Oncogene: promotion of tumor growth	Liang *et al.*, 2019 (10.1186/s13059-019-1685-4)
circFOXK2	PDAC	Oncogene: promotion of cell growth, invasion and metastasis.	Wong *et al.*, 2020 (10.1158/0008-5472.CAN-19-3268)
circFGD4	GC	Tumor-suppressor: its downregulation promotes poor tumor differentiation and lymphatic metastasis	Dai *et al.*, 2020 (10.1042/CS20191043)
circ_0000190	GC	Tumor-suppressor: inhibition of cell viability, proliferation and migration, and induction of apoptosis and cell cycle arrest.	Wang *et al.*; 2020 (10.1186/s12935-020-01422-5)
circRNA-MYLK	BC	Oncogene: promotion of proliferation, EMT, angiogenesis, and metastasis.	Zhong *et al.*, 2017 (10.1016/j.canlet.2017.06.027)
CDR1as	Several cancer types	Oncogene: affection of tumor microenvironment being associated with angiogenesis, ECM organization, integrin binding, collagen-binding, and TGF-β signaling.	Zou *et al.,* 2019 (10.3390/biom9090429)

Table 1. List of f-circRNAs described in the literature and reviewed in this work.

1. F-circRNAs derive from linear fusion transcripts

3.1 Linear fusion transcripts

Gene fusions are common driver mutational events in cancer. To date, over 30,000 gene fusions and over 11,000 fusion transcripts associated with cancer have been reported in the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (Mitelman et al., 2020) and in the ChiTaRS database, respectively (Gorohovski et al., 2017). Fusion transcripts are mainly produced by fusion genes arising from structural chromosomal rearrangements, also including genomic amplification (Mertens et al., 2015, Iwakawa et al., 2013; Rudin et al., 2013; L'Abbate et al., 2014; Simon et al., 1997). Additional genomic imbalances, such as dicentric chromosomes, can also result in gene fusions (An et al., 2008; Cazzaniga et al., 2001; Strehl et al., 2003). Intriguingly, extremely complex rearrangements may lead the fusion of three partner genes (Macchia et al., 2018).

Genomic rearrangements play significant roles in cancer development because they can also lead to the juxtaposition of a constitutively active promoter to a downstream proto-oncogene (promoter-swapping). Due to this mechanism, for instance, the *CTNNB1/Pleomorphic adenoma gene 1* (*PLAG1*) fusion in pleomorphic adenomas results in an aberrant activation of *PLAG1*, promoting tumorigenesis (Kas et al., 1997). Another consequence of genomic fusions is the truncation of a gene, resulting in the loss of function of the coded protein (Dai et al., 2018). As an example, the *Runt-Related Transcription Factor 1* (*RUNX1)* is mostly truncated in cancer, causing haploinsufficiency. RUNX1 truncated proteins are able to dominantly repress the wild type function, causing hematopoietic defects and propensity to leukemogenesis (Sood et al., 2017; L'Abbate et al., 2015).

Intriguingly, some genes involved in fusions are named 'promiscuous genes', since they have many fusion partners (Collins e al., 2002). The *Lysine (K)-Specific Methyltransferase 2A*

(*KMT2A,* also known as *MLL*) gene*,* for example, shows more than 80 fusion partners reported in leukemias (Takahashi et al., 2020). *ALK* and *ETS Variant Transcription Factor 6* (*ETV6*) are additional promiscuous genes, involved both in hematological malignancies and solid tumors (Dickson et al., 2018; Strehl et al., 2008).

Moreover, fusions joining the coding sequences of two genes can generate abnormal fusion proteins with aberrant functionality, mainly kinases or transcriptional factors promoting tumorigenesis (e.g. BCR/ABL1) (Lugo et al., 1989).

Several fusion products (both transcripts and proteins) were found in the absence of rearrangements at DNA level, as they may derive from unconventional splicing events, like trans-splicing or long distance cis-splicing of adjacent genes (cis-SAGe) (Jividen & Li, 2014). Trans-splicing, discovered first in trypanosomes (Sutton & Boothroyd, 1986), fuses together exons derived from two separate transcripts, whereas cis-splicing joins exons from adjacent genes, transcribed as a single chimeric pre-mRNA (Jividen & Li, 2014). The literature documented multiple examples of such post-transcriptional mechanisms for the genesis of fusion transcripts: the *JAZF Zinc Finger 1* (*JAZF1)/Polycomb Protein SUZ12* (*SUZ12*) fusion transcript, found both in normal and tumor endometrial stromal cells, is the result of trans-splicing (H. Li et al., 2008); the *Carrier Family 45 Member 3* (*SLC45A3*)/*ETS Transcription Factor ELK4* (*ELK4*) chimeric RNA, detected in prostate cancer, is an example of cis-SAGe product (Y. Zhang et al., 2012). Additional examples have been documented in tumors carrying genomic amplifications (L'Abbate et al., 2018; Macchia et al., 2018).

As many fusion genes/transcripts are pathognomonic, they can be valid biomarkers for cancer diagnosis, as well as valuable targets for therapies. The best-known example is *BCR/ABL1* in hematological malignancies, allowing diagnosis and treatment with Tyrosine Kinase Inhibitors (TKIs), such as Imatinib, one of the first TKIs approved by the Food and Drug Administration and successfully used to treat *BCR/ABL1* positive CML cases (Druker et al., 2001).

Another example is *EML4/ALK* in NSCLC (Soda et al., 2007), targeted by Crizotinib (Shaw et al., 2011) that has been demonstrated to be effective also in patients with *ROS Proto-Oncogene 1, Receptor Tyrosine Kinase* (*ROS1*) fusions (Zhu et al., 2019).

All these evidences underline the crucial role of fusion transcripts with clinical implications for both diagnostic and therapeutic purposes.

3.2 Fusion-circRNAs: a novel class of circRNAs

F-circRNAs are aberrant circRNAs generated after back-splicing of chimeric mRNA transcripts, derived from chromosomal rearrangements (Fig. 3). This event could be triggered by repetitive intronic sequences flanking the breakpoint region in the pre-mRNA transcript (Guarnerio et al., 2016). To date, these molecules have been identified both in hematological and solid tumors (Guarnerio et al., 2016). To the best of our knowledge, f-circRNAs arising from trans-splicing or cis-SAGe have never been reported, despite it cannot be excluded due to the limited number of studies performed so far.

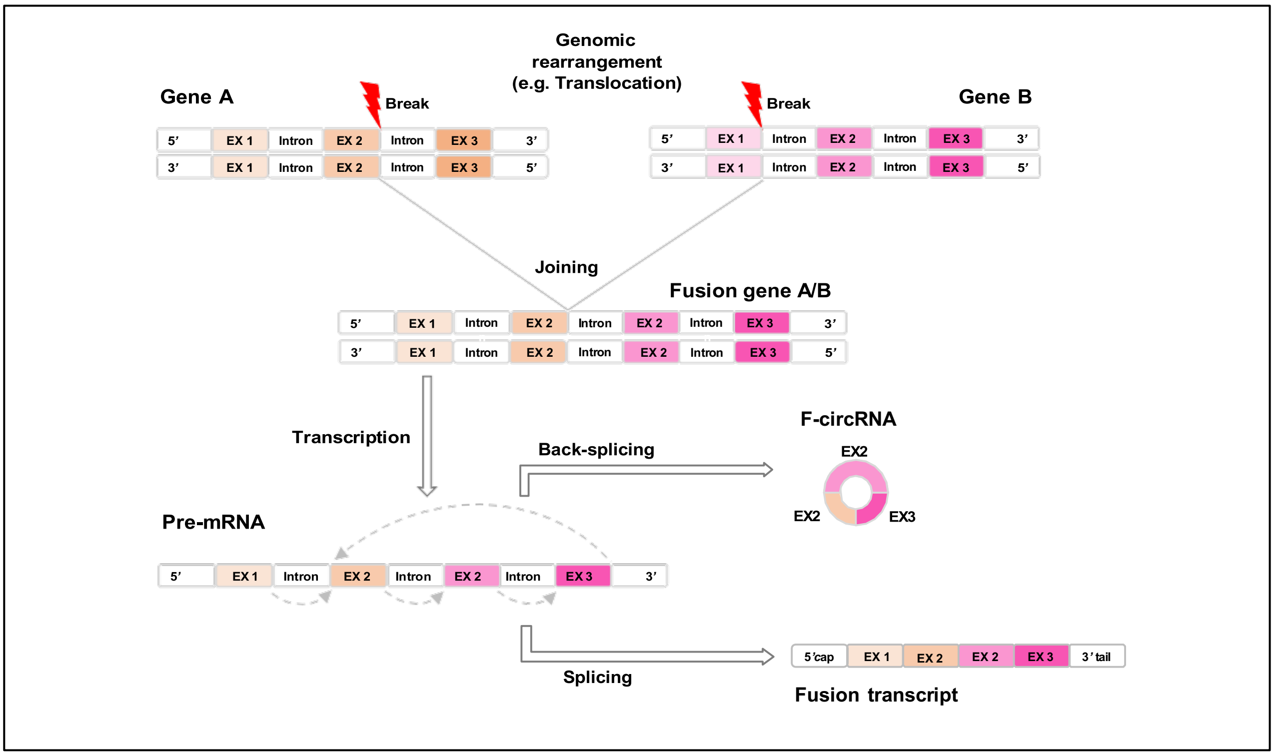

Fig. 3: Biogenesis of f-circRNAs. F-circRNAs are generated after back-splicing of a linear chimeric mRNA, derived from cancer-associated chromosomal rearrangements. EX: exon.

3.2.1 Hematological malignancies

i) AML

The *Promyelocytic Leukemia* (*PML*)/*Retinoic Acid Receptor Alpha* (*RARA*) fusion gene, originated from a t(15;17)(q24;q21) translocation, is the most recurrent chimera in acute promyelocytic leukemia (APL) (Guarnerio et al., 2016). The transcribed chimera encodes for a fusion protein that blocks the myeloid cell differentiation at promyelocytic stage, leading to an accumulation of neoplastic promyelocytes (Dekking et al., 2012). Guarnerio et al. (2016) investigated whether the *PML/RARA* fusion transcript could generate f-circRNAs, called *f-circPR*, both in APL patients and in the NB4 APL-derived leukemic cell line. They found the expression of one or two *f-circPR* isoforms in all patients harboring the *PML/RARA* translocation and in the NB4 cell line. Particularly, one *f-circPR* isoform showed a back-splicing junction joining the *PML* exon 5 and the *RARA* exon 6 (Fig. 4A), in all analyzed patients and in the NB4 cell line. The alternative isoform, harboring the back-splice junction between *PML* exon 4 and *RARA* exon 4 (Fig. 4A), was found in three out of four APL patients. The authors demonstrated that cells expressing *f-circPR* acquire the ability to increase their proliferation rate and form foci *in vitro* (Guarnerio et al., 2016).

Furthermore, in AML, the t(9;11)(p21;q23) translocation generates different isoforms of the recurrent *KMT2A/MLLT3* fusion transcript (Alonso et al., 2008). To investigate the existence of f-circRNAs derived from this chimera, Guarnerio et al. (2016) tested the THP-1 acute monocytic leukemia cell line, harboring the t(9;11)(p21;q23) translocation. They found two f-circRNAs, called *f-circM9*s (*f-circM9_1* and *f-circM9_2*): the former showed its back-splice junction between *KMT2A* exon 7 and *MLLT3* exon 6, while the latter fuses *KMT2A* exon 5 to *MLLT3* exon 6 (Fig. 4

A). The authors focused their attention on *f-circM9_1* and showed that its expression promotes cell proliferation, reduces apoptosis and protects cancer cells from therapy treatment, thus favoring leukemia progression both *in vitro* and *in vivo* (Guarnerio et al., 2016).

ii) CML

In CML, a recent study identified a novel f-circRNA, named *circBA9.3*, juxtaposing *ABL1* exon 3 to *BCR* exon 9 (Fig. 4A). This f-circRNA was detected in cells harboring the t(9;22)(q34;q11) translocation, resulting in the *BCR/ABL1* fusion gene (Pan et al., 2018). Its overexpression in leukemic cells promotes proliferation and apoptosis downregulation, by improving BCR/ABL1 translation or preventing its degradation, and gives TKIs resistance (Pan et al., 2018).

3.2.2 Solid tumors

i) Ewing Sarcoma (ES)

In ES, Guarnerio et al. (2016) identified *f-circEF1,* generated from the *EWS RNA Binding Protein 1* (*EWSR1)/Friend Leukemia Virus Integration 1* (*FLI1*) fusion gene derived from a t(11;22)(q24;q12) translocation. The EWSR1/FLI1 fusion protein acts as an aberrant transcriptional factor and plays a role in RNA splicing (Anderson et al., 2012). *F-circEF1* back-splicing junction joined the *EWSR1* exon 7 to the *FLI1* exon 10 (Fig. 4B); its potential role has not been investigated yet (Guarnerio et al., 2016).

ii) NSCLC

Three f-circRNAs derived from the *EML4*/*ALK* fusion, having a crucial role in NSCLC progression, are reported to date: *f-circEA1* (Guarnerio et al., 2016), *f-circEA-4a* (Tan, Gou, et al., 2018), and *f-circEA-2a* (Tan, Sun, et al., 2018). The *f-circEA1* back-splicing junction occurred between *EML4* exon 12 and *ALK* exon 26 (Guarnerio et al., 2016) (Fig. 4B). The potential role of this circRNA in oncogenesis has not been clarified yet. *F-circEA-4a* harbors the "AAAA" motif at the back-splicing junction, occurring between *EML4* exon 4 and *ALK* exon 22 (Fig. 4B). It was found mainly in the cytoplasm of the H2228 NSCLC cell line, harboring the *EML4/ALK* fusion gene. Notably, *f-circEA-4a* was also detected in the plasma of NSCLC patients with the *EML4/ALK* translocation. This f-circRNA could be used as a biomarker to detect the presence of the translocation and guide the *EML4/ALK*-targeted therapy. *F-circEA-2a*, harboring the "AA" motif at the fusion junction, is also localized in the cytoplasm of the H2228 cell line, but at a lower level, and was not found in the plasma of the NSCLC patients (Fig. 4B). Both *f-circEA-4a* and *f-circEA-2a* do not affect cell proliferation but promote cell migration and invasion in NSCLC cell lines (Tan, Sun, et al., 2018).

Additionally, two novel f-circRNAs have been reported in 2019; they originated from the *SLC34A2/ROS1* fusion transcript in NSCLC. *SLC34A2/ROS1* plays an essential role in NSCLC progression, because it encodes an oncogenic fusion protein that activates ROS1 signaling, promoting cell proliferation (K. Wu et al., 2019). *F-circSR1* and *f-circSR2* were generated from a back-splicing junction joining *SLC34A2* exon 2 to *ROS1* exon 37 or 42, respectively (Fig. 4B). They were identified in the HCC78 NSCLC cell line, where *f-circSR1* expression level was higher than that of *f-circSR2*. Both these f-circRNAs showed a negligible effect on cell proliferation but promoted cell migration in NSCLC. Indeed, the authors demonstrated that *f-circSRs* could increase cell migration sponging miR-150-5p, miR-194-3p, and miR-515-5p, all regulating cell migration. Since circRNAs are stable in body fluids, *f-circSRs* could be potential diagnostic and therapeutic biomarkers for NSCLC diagnosis and treatment (K. Wu et al., 2019).

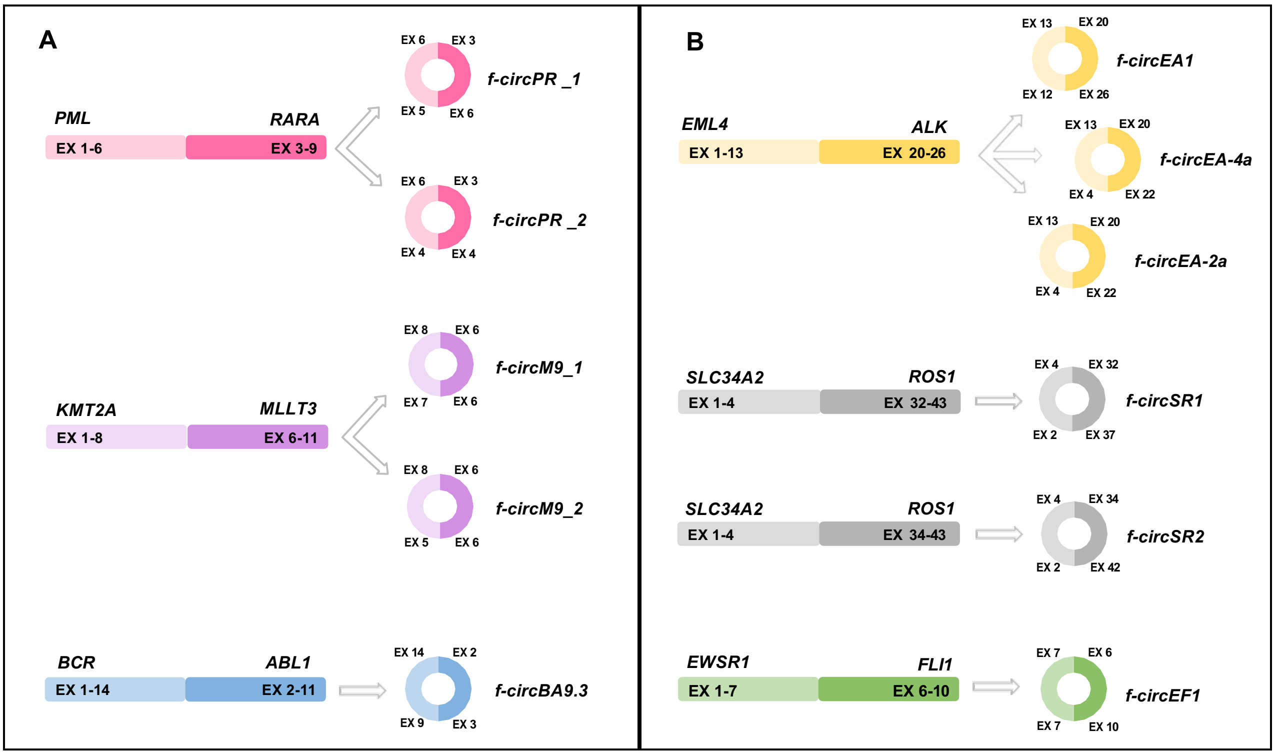

Fig. 4: F-circRNA detected in human cancer. Schematic representation of the genomic fusions and the derived f-circRNAs isolated in A) hematological malignancies and B) solid tumors. EX: exon.

The role in cancer of the f-circRNAs here reviewed is summarized in Table 2.

F-circRNA	**Fusion gene**	**Chromosome rearrangement**	**Cancer type**	**Role in cancer**	**Reference (DOI)**
f-circPR_1	*PML/RARA*	t(15;17)(q24;q21)	APL	Oncogene: increase of proliferation and foci formation	Guarnerio *et al.*, 2016 (10.1016/j.cell.2016.03.020)
f-circPR_2	*PML/RARA*	t(15;17)(q24;q21)	APL	Oncogene: increase of proliferation and foci formation	Guarnerio *et al.*, 2016 (10.1016/j.cell.2016.03.020)
f-circM9_1	*KMT2A/MLLT3*	t(9;11)(p21;q23)	AML	Oncogene: promotion of proliferation, inhibition of apoptosis	Guarnerio *et al.*, 2016 (10.1016/j.cell.2016.03.020)

f-circM9_2	*KMT2A/MLLT3*	t(9;11)(p21;q23)	AML	unknown	Guarnerio *et al.*, 2016 (10.1016/j.cell.2016.03.020)
f-circBA9.3	*BCR/ABL1*	t(9;22)(q34;q11)	CML	Oncogene: promotion of proliferation and downregulation of apoptosis	Pan *et al.*, 2018 (10.1016/j.bcmd.2018.09.002)
f-circEF1	*EWSR1/FLI1*	t(11;22)(q24;q12)	ES	unknown	Guarnerio *et al.*, 2016 (10.1016/j.cell.2016.03.020)
f-circEA1	*EML4/ALK*	inv(2)(p21;p23)	NSCLC	unknown	Guarnerio *et al.*, 2016 (10.1016/j.cell.2016.03.020)
f-circEA-4a	*EML4/ALK*	inv(2)(p21;p23)	NSCLC	Oncogene: increase of cell migration and invasion	Tan, *et al.*, 2018 (10.1038/s41422-018-0033-7)
f-circEA-2a	*EML4/ALK*	inv(2)(p21;p23)	NSCLC	Oncogene: increase of cell migration and invasion	Tan, *et al.*, 2018 (10.1186/s12943-018-0887-9)
f-circSR1	*SLC34A2/ROS1*	t(4;6)(p15;q22)	NSCLC	Oncogene: promotion of cell migration	Wu *et al.*, 2019 (10.1186/s12943-019-1028-9)
f-circSR2	*SLC34A2/ROS1*	t(4;6)(p15;q22)	NSCLC	Oncogene: promotion of cell migration	Wu *et al.*, 2019 (10.1186/s12943-019-1028-9)

Table 2. List of f-circRNAs described in the literature and reviewed in this work.

4. CircRNAs: new biomarkers for cancer diagnosis and therapy

CircRNAs have peculiar features with respect to their linear counterparts, such as relative abundance, high stability, conservation, specificity (tissue-specific and developmental stage-specific expression) (Tan, Gou, et al., 2018; Guo et al., 2014; Rybak-Wolf et al., 2014). They make circRNAs suitable as diagnostic and prognostic biomarkers (Z. Zhang et al., 2018), since they are abundant in body fluids (blood, saliva, urine, cerebrospinal fluid) and can be used for liquid biopsies (Jeck et al., 2013; Memczak et al., 2013).

Several circRNAs have been reported as upregulated in lung cancers and showing a significant correlation with clinical parameters. For example, in NSCLC, *circPVT1* upregulation (see Paragraph 2.4.2, sub-section i) is associated with tumor size, TNM stage, poor prognosis and short overall survival of patients. Thus, it may be a biomarker for the early diagnosis of NSCLC (Qin et al., 2019). Moreover, *hsa_circ_0013958* acts as a sponge of *miR-134* in lung adenocarcinoma, promoting *CCND1* oncogenic activity, and increasing proliferation, invasion and apoptosis inhibition. It was found upregulated in NSCLC cell lines and patients (both in tissues and plasma), and associated with TNM stage, patient pathogenesis and metastasis. *hsa_circ_0013958* expression level is upregulated in the I/II tumor stage of patients if compared to controls. Its specificity suggests a role as a biomarker for the early detection of lung adenocarcinoma (Zhu et al., 2017).

Luo et al. showed *hsa_circ_0000064* upregulation in lung cancer cell lines and patients and a correlation with lymphatic metastasis, TNM stage, and poor prognosis. This may be explained by the fact that *hsa_circ_0000064* increases BCL2 expression and, at the same time, inhibits the expression of pro-apoptotic proteins such as caspase-3, caspase-9, and BAX. Thus, this circRNA promotes cell cycle, proliferation, metastasis, and apoptosis inhibition. These data indicated *hsa_circ_0000064* as a potential biomarker for early diagnosis and prognosis of lung cancer (Luo et al., 2017).

In CRC, *hsa_circRNA_103809* and *hsa_circRNA_104700* showed decreased expression levels compared with normal tissues. *hsa_circRNA_103809* is correlated with lymph node metastasis and TNM stage, and *hsa_circRNA_104700* with distal metastasis. Since their low expression levels are associated with poor prognosis, these circRNAs may be used as diagnostic markers in this cancer type (P. Zhang et al., 2017). Conversely, *circPTK2* was found upregulated in CRC tissues, and associated with tumor growth, metastasis and poor overall survival, indicating that it could be a promising biomarker for CRC early diagnosis and targeted therapy (Yang et al., 2020).

Furthermore, *circ-ANAPC7* was revealed as a promising biomarker in AML, as it was found to be upregulated in patients (see Paragraph 2.4.1, sub-section i). Moreover, due to the pathogenic role of the *circ-ANAPC7–miR-181* axis, this circRNA may be also a potential target for a novel AML therapeutic approach (H. Chen et al., 2018).

Interestingly, in AML samples, *hsa_circ_0004277* showed a dynamic expression according to the progression of the disease. Significantly, patients treated by chemotherapy showed restored levels of *hsa_circ_0004277,* indicating that increasing level of this circRNA was associated with successful treatment. Moreover, the circRNA-miRNA-mRNA interaction network analysis showed that *hsa_circ_0004277* interacts with five miRNA targets (*hsa-miR-138-5p*, *hsa-miR-30c-1-3p*, *hsa-miR-892b*, *hsa-miR-571* and *hsa-miR-328-3p*) and offered several gene-candidates, among which the cancer-related genes *SH3GL2*, *PPARGC1A,* and *SH2B3*. Thus, *hsa_circ_0004277* may be a prognostic and predictive biomarker or therapeutic target in AML (W. Li et al., 2017).

Many circRNAs are reported to be enriched in exosomes (Y. Li et al., 2015). Exosome *circNRIP1* is upregulated and correlated with tumor size and lymphatic invasion in gastric cancer (GC) patients. It acts as a sponge of miR-149-5p, affecting the expression level of *AKT1*. Consequently, *circNRIP1* promotes proliferation and migration in GC (X. Zhang et al., 2019). Moreover, Li et al. compared the exo-circRNAs expression profile of CRC patient serum to that of healthy subjects: they found that exo-circRNAs in CRC samples were more abundant than the control samples. Hence, exo-circRNAs may be novel powerful tools for early non-invasive diagnosis of cancer (Y. Li et al., 2015).

Besides their potential as diagnostic biomarkers, circRNAs could also be exploited as effective therapeutic tools. For example, Han et al. (2020) showed that *circITCH*, with tumor suppressor activity in a variety of tumors, including bladder cancer, breast cancer, and osteosarcoma, represents a potential therapeutic agent for doxorubicin-induced cardiotoxicity (DOXIC). Indeed, the induced overexpression of this circRNA in human-induced pluripotent stem cell-derived cardiomyocytes strongly protects them against DOXIC. Such protective effect could be explained by its action as a sponge for miR-330-5p, upregulating SIRT6, Survivin, and SERCA2a, and resulting in a decrease of cardiotoxicity and an increase of cardiomyocyte survival and function. This result suggests *circITCH* as a potential tool for the prevention and treatment of DOXIC in cancer patients (Han et al., 2020).

Interestingly, engineered circRNAs could be used as molecular medicine tools for personalized therapy (Table 3). For example, Wesselhoeft et al. (2018) generated long engineered circRNAs with translational potential *in vitro,* by means of a ribozymatic method based on permuted intron-exon splicing and addition of homology arms. Their data showed that circRNAs would guarantee the stable production of large amounts of five different proteins, including Gaussia luciferase, Firefly luciferase, eGFP, human erythropoietin, and Cas9 endonuclease, providing an alternative protein source to linear mRNA (Wesselhoeft et al., 2018). Meganck et al. (2018) described the use of recombinant adeno-associated virus (AAV) to deliver an engineered circRNA in multiple cell and tissue types *in vivo*. Two different intron pairs were used to drive the circularization of the sequence containing an IRES and an ORF encoding the GFP transgene. Their results showed the efficient expression and translation of the artificial circRNA in mice cardiac tissue, liver, and astrocytes. This method shows the potential use of engineered circRNAs not only to study their function in animal models, but also to enable their expression *in vivo* (Meganck et al., 2018). Hence it could be hypothesized to exploit this strategy to vehiculate therapeutic proteins *in vivo*.

Artificial circRNAs may be developed to sponge miRNAs, inhibiting their oncogenic properties. For example, Liu et al. synthesized a circRNA that can sponge miR-21, upregulating the *Death Domain Associated Protein* (*DAXX*) tumor suppressor target gene. By inhibiting miR-22 function, the authors observed the suppression of cell proliferation in GC (Liu et al., 2018). Another example of this application of artificial circRNAs is provided by a study by Jost et al (2018) in infectious diseases. The authors designed a synthetic circRNA containing binding sites for the endogenous miR-122, which exerts a protective effect on the Hepatitis C Virus (HCV) genome during liver cell infection. Using the artificial circRNA to sequester miR-122, the inhibition of the HCV life cycle was observed *in vitro* (Jost et al., 2018).

Furthermore, a recent study described the synthesis of circmiR, by flanking the sequence with long inverted complementary introns, thus promoting circularization. circmiR acts as a sponge of miR-132 and miR-212, known to be cardiac pro-hypertrophic miRNAs, inhibiting their activity. The authors used AAVs to deliver circmiR to murine cardiomyocytes *in vivo*, observing the attenuation of the hypertrophic disease characteristics and the protection of the cardiac function, demonstrating the potential of circmiRs as novel therapeutic tools (Lavenniah et al., 2020).

All these findings highlight the wide range of applications of circRNAs in the setting up of novel diagnostic strategies and molecular therapies in cancer and other human diseases.

Exogenous circRNA	**Disease/Tissue**	***In vitro* circularization method**	**Function**	**Reference (DOI)**
Protein coding circRNA	Multiple cell lines (HEK293, HEK293-GFP, HeLa, and A549	Ribozymatic methods using self-splicing introns, followed by RNAse R treatment	Obtaining stable protein expression in eukaryotic cells by IRES-mediated	Wesselhoeft et al., 2018 (10.1038/s41467-018-05096-6)

	cells)		translation	
GFP coding circRNA	Multiple cell and tissue types	Cloning into recombinant adeno-associated viral vectors	Encoding GFP protein by IRES-mediated translation	Meganck et al., 2018 (10.1016/j.omtn.2018.08.008)
Circular miR-21 sponge	Gastric Cancer	Transcription of a linear RNA with multiple miR-21 binding sites, followed by T4 RNA ligase 1 ligation	Sponging *miR-21* to inhibit GC cell proliferation	Liu et al., 2018 (10.1016/j.omtn.2018.09.010)
Circular miR-122 sponge	Hepatitis C	Transcription of a linear RNA with multiple miR-22 binding sites, followed by T4 RNA ligase 1 ligation	Sponging *miR-122* to inhibit HCV propagation	Jost et al., 2018 (10.1080/15476286.2018.1435248)
CircmiR	Cardiovascular disease	Cloning into recombinant adeno-associated viral vectors	Sponging *miR-132* and *miR-212* to attenuate cardiac hypertrophy	Lavenniah et al., 2020 (10.1016/j.ymthe.2020.04.006)

Table 3. List of exogenous circRNAs described in the literature, including genetic engineering methods for circularization and function.

5. Concluding remarks and future perspectives

In the latest years, circRNAs have attracted increasing attention from researchers due to their biological features, functions, and potential application to the clinical management of patients. In this review, we discussed the most recent discoveries on circRNAs functions in cancer, particularly focusing on a novel class of circRNAs, f-circRNAs, and their role in tumorigenesis and tumor progression. Indeed, circRNAs and f-circRNAs have a crucial role in cancer, considering their functions as affecting gene expression and other features of cancer cells, such as proliferation, apoptosis, invasion and metastasis, angiogenesis and microenvironment regulation.

CircRNAs display increased stability in both cells and body fluids, and tumoral specificity that make them perfect candidates as cancer biomarkers. Moreover, due to their pivotal role in several cellular pathways, circRNAs can represent possible targets of novel therapeutic strategies (Yong Zhang et al., 2019). Further, circRNAs are more stable and enriched within exosomes of peripheral blood than their linear counterparts, facilitating their early detection in liquid biopsies. This feature is crucial for the potential use of circRNAs as biomarkers in plasma, serum, and urine for a non-invasive diagnosis of cancer.

Moreover, recent studies described novel approaches for the creation of artificial exogenous circRNAs to be used as new miRNA/RBP sponges or to stably encode therapeutic proteins, representing novel potential tools in molecular medicine for personalized therapies.

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