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Circular RNAs in the pathogenesis of cancer

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Chapter 7

Summary, discussion, and future perspectives

1. Summary

Circular RNAs (circRNAs) belong to the family of non-coding RNAs. They are characterized by their circular shape that is formed by back-splicing. The covalently closed loops lack 5' caps and 3' polyadenylated tails and therefore are resistant to cleavage by RNases. CircRNAs play critical roles in almost all physiological processes, and a mounting body of evidence indicates that aberrant expression significantly impacts cancer development, including B-cell lymphoma and breast cancer [1-7]. Nevertheless, the genome-wide involvement, as well as the molecular mechanisms underlying their role, have not been comprehensively investigated. In this study, we aimed to characterize the circRNA landscape and function in B-cell lymphoma and breast cancer.

In **chapter 2**, we outlined current knowledge on circRNA biogenesis, with a particular focus on the role of RNA-binding proteins. Additionally, we reviewed recent studies investigating the molecular mechanisms and functions of circRNAs in cancer pathogenesis. There are four main circRNA subtypes, i.e., exonic circRNAs (ecircRNAs), circular intronic RNAs (ciRNAs), exonic-intronic circRNAs (EIciRNAs), and tRNA intronic circRNAs (tricRNAs). These circRNAs can be formed through various mechanisms, such as lariat-driven circularization, back-splicing, base-pairing-driven circularization, or tRNA splicing enzymes. The flanking regions of circRNAs often contain repetitive sequences, such as ALU repeats, which can facilitate circular RNA formation. RNA-binding proteins (RBPs) are thought to play crucial roles in circRNA biogenesis by binding to the circRNA flanking regions. Different RBPs can have different effects on the back-splicing process. Some are described as promoting (for example QKI), and others are indicated to inhibit (for example ADAR1) circRNA formation. CiRNAs and EIciRNAs are predominantly localized in the nucleus, while ecircRNAs are mainly located in the cytoplasm. CircRNAs play crucial roles in cancer pathogenesis by serving as molecular sponges for microRNAs and proteins, binding partners for DNA, RNA, and proteins, and alternative splicing regulators. A small subset of the circRNAs encodes for novel proteins, crossing the back-splice junction region. Overall, current knowledge of biogenesis and the diversity in functions make circRNAs an interesting area of research. This knowledge will contribute to a more comprehensive understanding of their role in disease pathology.

In **chapter 3**, we characterized the circRNA landscape in a panel of Hodgkin lymphoma (HL), Burkitt lymphoma (BL), diffuse large B cell lymphoma (DLBCL) cell lines, and sorted germinal center (GC) B cells as controls. To enrich circRNAs, we used ribosomal RNA depleted, RNase R treated RNA samples as input. In total, we identified 116,612

unique circRNAs confirmed by at least two back-splice junction (BSJ) reads. Characterization of the most abundant 13,952 circRNAs (at least 5 BSJ reads in the majority of samples of each group) based on predictions indicated that most circRNAs are exonic and contain 1-5 exons. Moreover, most host genes produced a single circRNA, although we also identified host genes that produced >10 circRNAs. Nanopore long-read sequencing for 30 circRNA loci yielded a total of 177 distinct circRNA isoforms, with 60 being categorized as novel circRNAs. Twenty circRNAs had sufficient reads to determine the exon compositions. Five of the twenty had BSJ that did match with the BSJ coordinates as defined by the RNAseq analysis. The composition of 14 circRNAs was consistent with the circBase annotation, while one had an exon composition that was different from the circBase annotation. Analysis of differential expression (DE) circRNAs revealed a total of 3,239 circRNAs, with 1,940 being up and 1,322 being downregulated. The circular nature of eight selected DE circRNA candidates was validated on RNase R treated RNA samples, and DE patterns of these circRNAs were verified in an expanded cell line cohort by RT-qPCR. Analysis of the RNA-seq data obtained from Ribosomal RNA-depleted RNA samples not treated with RNase R revealed differential expression of 5,521 linear counterparts of the circRNA host genes. Expression of around 70% of the linear counterparts of the DE circRNAs remained unchanged, indicating distinct regulatory mechanisms for the circRNA and the linear transcripts. For the 30% with DE patterns for both the circRNA and the linear counterpart, we observed a strong positive correlation, which suggested a potential shared regulatory mechanism between the host gene and the circRNA for this subgroup. In summary, our study provides the first comprehensive landscape of circRNAs in B-cell lymphoma, providing a valuable source for future studies. The extensive characterization, together with the confirmed DE patterns of selected circRNAs, can be used as a starting point for future research to elucidate the functional roles and regulatory mechanisms of circRNAs.

In **chapter 4**, we focused on a circRNA derived from the PVT1 locus. This circRNA was significantly differentially expressed in BL and HL in our circRNA sequencing data (generated in Chapter 3). PVT1 has been extensively studied in several malignancies, but current knowledge of this circRNA in B-cell lymphoma is scarce. Besides encoding for a circRNA, the PVT1 locus also encodes for various linear transcripts, making it a complicated locus. We investigated the expression pattern of circPVT1 and its linear counterparts (lncPVT1, focusing on transcripts derived from the 5' and 3' region of the PVT1 locus) in a variety of B-cell lymphoma cell lines and normal B-cell subsets. LncPVT1 transcripts were downregulated, while circPVT1 transcripts were upregulated in B-cell lymphoma. Both transcripts exhibited a moderate correlation with MYC, which

was significant only for the linear transcripts, mainly in MYC translocation-negative cell lines. We observed a homozygous deletion of the 5' PVT1 region, including the region encoding for circPVT1, in KM-H2 and SUPHD1. Knockdown of lncPVT1 transcripts decreased cell growth in all BL and the SUDHL5 DLBCL cell lines. Knockdown of circPVT1 transcripts resulted in a growth decrease in all BL and the L1236 HL cell line. Overexpression of circPVT1 led to a slight increase in growth in cell lines lacking endogenous or moderate circPVT1 expression levels. LncPVT1 transcripts were consistently located in the nucleus, while circPVT1 transcripts were mainly detected in the cytoplasm, suggesting different mechanisms of action. Despite its predominant cytoplasmic localization, AGO2-RIP analysis did not show enrichment of circPVT1 transcripts in the AGO2-IP fraction. Consistent with its nuclear localization, lncPVT1 transcripts were also not enriched in the AGO2-IP fraction. These data indicated that the PVT1 transcripts most likely do not interact with miRNAs in B-cell lymphoma. CircPVT1 pull-down experiments are ongoing to unravel potential other regulatory mechanisms of PVT1 transcripts in B-cell lymphoma. Altogether, our study has demonstrated that linear and circular PVT1 transcripts show opposite differential expression patterns and that their roles in promoting proliferation are independent of their ability to bind miRNAs in B-cell lymphoma. These findings underscore the need for further mechanistic studies to fully understand the molecular mechanisms underlying their functions in lymphomagenesis.

A second circRNA identified as being relevant for BL is circZDHHC11. This circRNA and its linear counterpart have 18 miR-150 binding sites and are strongly bound to miR-150 suggesting a miR-150 sponge function. The ZDHHC11 gene was shown to be part of an oncogenic network involving MYC, MYB, and miR-150. The linear and especially the circular ZDHHC11 were highly enriched in the AGO2-IP fraction upon miR-150 overexpression and were proposed to ensure effective depletion of miR-150 to ensure high MYB levels. In **chapter 5**, we further investigated the relevance of circZDHHC11 and the miR-150 binding site region in regulating growth of BL cells. CircZDHHC11 and miR-150 transcripts were primarily localized in the cytosol, whereas the linear circZDHHC11 counterparts were predominantly localized in the nucleus. Overexpression of miR-150 did not affect the subcellular localization of individual ZDHHC11 transcripts. Knockdown of circZDHHC11, using shRNAs specifically targeting the BSJ, inhibited growth of BL without affecting expression of other members of the oncogenic MYB, MYC, miR-150, and ZDHHC11 network. Remarkably, overexpression of circZDHHC11 did not affect BL cell growth and could not reverse the strong inhibitory effect of miR-150 overexpression on BL cell growth. To confirm the effective binding of the ectopically expressed circZDHHC11, we performed AGO2-IP and this revealed that

ectopic circZDHHC11 was able to interact with miR-150. Despite this effective interaction, suggesting a miR-150 sponge-like function, it did not affect MYB levels, which are also regulated by miR-150. Removing the miR-150 binding site region from the endogenous ZDHHC11 locus using CRISPR/Cas9 technology did not impact circZDHHC11 formation. Knockdown of circZDHHC11 lacking the miR-150 binding site region also inhibited BL growth, similar to the effect of full-length circZDHHC11 knockdown. Overall, our study showed that circZDHHC11 supports proliferation of BL and this effect is independent of its ability to bind miR-150. Further studies are ongoing focusing on the identification of binding partners of circZDHHC11. Identification of these binding partners is required to obtain further insights into the mechanisms by which circZDHHC11 supports the growth of BL.

In chapter 6, we studied the role of circ-NOL10 in breast cancer. Our previously reported profiling study showed that circ-NOL10 was the most downregulated circRNA in triple-negative breast cancer (TNBC). We now examined potential associations between circ-NOL10 expression and clinical characteristics of breast cancer patients. Furthermore, we investigated the function of circ-NOL10 in breast cancer carcinogenesis, aiming to characterize the molecular mechanisms governing breast cancer progression. The expression of circ-NOL10 was decreased in breast cancer samples and cell lines compared to control samples. Receiver operating characteristic (ROC) curves were generated for circ-NOL10, and AUC values of 0.9212, 0.9275, 0.7619, and 0.9154 for TNBC, LA, LB, and Her-2 subtypes, respectively, indicated the diagnostic potential as a marker for breast cancer. The decreased expression of circ-NOL10 was significantly correlated with advanced clinical stage, increased lymphatic metastasis, shorter disease-free and overall survival time in patients with TNBC. Increased circ-NOL10 expression reduced cell proliferation, invasion, and migration while promoting early but not late apoptosis. Treatment with circ-NOL10 siRNA resulted in an opposite cellular response. Furthermore, circ-NOL10 overexpression inhibited tumor growth *in vivo*. Circ-NOL10 was localized in both the cytoplasm and nucleus of breast cancer cells. We showed an interaction with miR-149-5p, miR-330-3p, and miR-452-5p of circ-NOL10, which prevented an inhibitory effect of these miRNAs on PDCD4. Thus, circ-NOL10 indirectly regulates the expression of PDCD4. Moreover, we showed the binding of two RBPs (MTDH and CASC3) to circ-NOL10 by RNA immunoprecipitation (IP) and luciferase reporter assays. This binding was shown to be associated with decreased circ-NOL10 levels. In conclusion, we discovered a novel regulator of PDCD4 in TNBC. The decreased expression of circ-NOL10 in TNBC resulted in enhanced targeting of PDCD4 by three miRNAs (miR-149-5p, miR-330-3p, and miR-452-5p). This indicates that circRNAs can exert their regulatory effects through the binding of

different miRNAs that target the same gene. This property is quite novel since most circRNAs acting through binding miRNAs were shown to do this via binding one specific miRNA. The levels of circ-NOL10 can be decreased by binding to MTDH and CASC3, which results in a release of three miRNAs that can subsequently regulate PDCD4, thereby promoting the development of breast cancer.

2. Discussion and future perspectives

2.1 Identification of circRNA full-length sequence

The function of a circRNA is defined by its sequence, including the presence of binding sites for RNA binding proteins (RBP) and miRNA response elements (MREs). Therefore, it is essential to obtain circRNA full-length sequences for comprehensive functional analysis. RNA sequencing to identify BSJ reads is the standard approach for genome-wide circRNA identification. In comparison to a microarray-based approach, it has the advantage of more accurately detecting low-abundance and novel circRNAs, as it directly measures the abundance of RNA molecules through sequencing without being limited by the specificity and sensitivity of probes on the array. To enrich circRNAs, the standard RNA sequencing protocols include steps to deplete ribosomal RNA and linear transcripts. The identification of circRNAs involves identifying BSJ reads using bioinformatic tools [8]. Various computational tools, such as circseq_cup [9], CIRI-full [10], CircAST [11], psirc [12], FUCHS [13], FcircSEC [14], circRNA-full [15], and CYCLeR [16], have been developed to not only identify BSJ reads but also predict the circRNA full-length sequences. It is important to realize that tools only predict full-length sequence of circRNAs based on the annotation of exons of linear host transcripts. As the RNA sequencing reads are too short to detect the actual full-length sequence accurately, little is known about the actual sequence of many circRNAs. Exons in the middle of a circRNA may be skipped, introns may be retained, and potentially different isoforms containing the same BSJ may exist.

Recent advances in long-read sequencing technologies, such as Nanopore sequencing, made it possible to reliably identify the full-length sequence of specific circRNAs [17]. Full-length sequencing technologies have led to the discovery of numerous novel introns retained within circRNAs, circRNAs with novel exon usage, and the existence of micro-exon-derived circRNAs [18-21]. Currently applied circRNA long-read sequencing methods (CIRI-long [20], isoCirc [18], circFL-seq [19], circNick-LRS [21], and circPanel-LRS [21]) utilize splice sites from known exon annotations and canonical de novo GT/AG splice signals to align junction sites with customized computational pipelines, which may introduce intrinsic biases. As such, caution should be exercised when

interpreting the results of circRNA full-length sequencing studies, and the limitations of the sequencing methods employed should be considered.

After defining the circRNA landscape of circRNAs in B-cell lymphoma, we conducted Nanopore long-read sequencing using circPanel-LRS for 39 specific circRNAs and obtained sufficient reads to determine exon composition for 20 circRNAs. This point to a limitation of Nanopore long-read sequencing in terms of sensitivity, especially for low abundant circRNAs. For the circRNAs that matched the initially observed BSJ, we defined multiple isoforms, which did not always match the circBase annotation, indicating identification of novel isoforms. Our findings highlight the importance of full-length sequencing for functional studies of selected circRNA candidates. Taken together, it is critical to select samples with high endogenous expression and validate the sequence of the circRNAs using alternative methods such as RT-qPCR or Northern blot with strategically placed primers or probes.

2.2 Functional characterization of circRNAs in B-cell lymphoma

CircRNAs are often derived from exons of protein-coding genes. Consequently, there is a complex and variable relationship and a potential regulatory mode of action between them. At the transcriptional level, circRNAs can control their linear counterparts by recruiting proteins that regulate their transcription or splicing, which may produce protein isoforms with different biological properties [22-24]. At the post-transcriptional level, circRNAs can compete with their linear counterparts for binding of miRNAs with shared MREs to regulate the translation or functionality of their linear counterparts [25, 26] or influence the stability of their host gene mRNA by recruiting or sponging RBPs that bind to the mRNA, thereby enhancing or reducing mRNA stability [27-29]. We showed a significant change in expression for the host gene in the same direction as the DE circRNA for 30% of the linear host genes, which indicates a potential guilt-by-association relationship between them. This might imply that only one of them is functionally relevant. In contrast, we observed no change in expression for 70% of linear transcripts of the DE circRNAs, which indicates a specific regulation of the circRNA and could point to a specific functionality of the circRNA.

We verified the differential expression of eight circRNAs and studied the current knowledge of these candidates and their host genes in more detail (**Table 1)**. Among the eight circRNAs studied, four show differential expression of their linear counterparts. Notably, three of these (ANKRD36BP2, FHAD1, and TNFRSF11A) have the same direction of expression change as their corresponding circRNA, suggesting a shared regulatory mechanism. In contrast, ZNF609 shows an opposing expression

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change compared to its circular counterpart, indicating a potential negative regulatory mechanism between them or independent regulation of both RNA molecules. Of the four linear counterparts that are differentially expressed, only TNFRSF11A has been previously reported in B-cell lymphoma. This gene encodes the protein RANK, associated with the activation of Akt or cytokine and chemokine secretion in HL [30, 31]. Among the four linear counterparts that are not differentially expressed, ZNF385B has been reported in B-cell lymphoma and is specifically expressed in BL and GC B cells [32]. Two of the eight circRNAs, i.e., circZNF609 and circZKSCAN1, were studied previously. CircZNF609 is upregulated in multiple tumor tissues and cell lines and promotes tumor proliferation, migration, invasion, and other malignant phenotypes mainly by acting as a molecular sponge for microRNAs [33]. Expression of circZNF609 was elevated in DLBCL compared to control tissues [34]. In our study, the expression of circZNF609 was significantly increased in HL but not in BL and DLBCL. CircZKSCAN1 is downregulated in hepatocellular carcinoma and bladder cancer but upregulated in non-small cell lung cancer [35-38]. No research has been conducted on its expression and role in B-cell lymphoma. In our sequencing results, circZKSCAN1 was upregulated in BL and DLBCL. Thus there are already indications that some of these eight circRNAs may have relevance for B-cell lymphoma biology. Nonetheless, further investigation regarding their functions and molecular regulatory mechanisms in B-cell lymphoma is needed for all eight candidates, regardless of whether their host gene expression is altered.

Table 1. The number of publications related to circRNA candidates and their host genes.

circRNA		Host gene		# of linear publications		# of circular publications	
Location (hg38)	**Expression**	**Name**	**Expression**	**Total**	**B-cell lymphoma**	**Total**	**B-cell lymphoma**
chr1:15367462-15369502	up	FHAD1	up	5	0	0	0
chr2:88801098-88804880	down	ANKRD36BP2	down	2	0	0	0
chr2:179769502-179770672	up	ZNF385B	-	14	1	0	0
chr7:23611170-23611553	down	CCDC126	-	4	0	0	0
chr7:100023418-100024307	up	ZKSCAN1	-	44	0	13	0
chr12:8713810-8754093	up	RIMKLB	-	14	0	0	0
chr15:64499292-64500166	up	ZNF609	down	2	0	48	1
chr18:62348167-62349937	up	TNFRSF11A	up	2064	10	0	0

2.3 Possibility of circRNA as miRNA sponges

The idea that different RNA molecules with the same miRNA binding site can compete for miRNA binding was originally named the competing endogenous or ceRNA hypothesis [39]. The concept of circRNA acting as a miRNA sponge was initially proposed following the discovery of 63 putative miR-7 binding sites in circRNA CDR1as

and 16 putative miR-138 binding sites in Sry [40]. Since then, many circRNAs have been identified as miRNA sponges in a variety of biological processes. However, it is important to realize that a proportion of these circRNAs only have a low number of miRNA binding sites and are expressed at relatively low levels compared to the miRNAs and the miRNA-target mRNAs. This has led to some controversy over the importance of circRNAs as miRNA sponges [41], with a potentially more limited role in post-transcriptional gene regulation. As miRNA target predictions are still known to have high false-positive predictions, predictions alone are not sufficient to pinpoint miRNA-circRNA interactions. Moreover, the prediction of miRNA binding sites on circRNAs through bioinformatics may be hampered by the complex structure of circRNAs [42], which can affect the actual binding efficiency. Therefore, careful experimental validation is required to confirm the functionality of circRNAs as miRNA sponges, which can be achieved using various approaches.

First, AGO2-IP experiments can be performed, followed by circRNA sequencing to allow a genome-wide identification of circRNAs with miRNA sponge activities. However, relying solely on AGO2-IP experiments might fail to detect potential circRNA-miRNA interactions if miRNAs are loaded into RISC containing other AGO family members [43]. To overcome this, IP experiments can be performed using a peptide that binds all AGO proteins and thus allows pull-down of all AGO-containing complexes [44]. Interactions can be further confirmed by performing circRNA pull-downs and analyzing the subsequent enrichment of the AGO and miRNAs. Once interaction between the circRNA of interest and miRNA has been established, luciferase reporter assays can be used to test specific putative miRNA binding sites for functionality and pinpoint the exact binding site(s). Further insights may be obtained by measuring the ratio of the circRNA copy number relative to the binding miRNA copy number in the cell type of interest. A high ratio supports the sponging potential of the circRNA, as miRNA binding efficiency will increase with higher circRNA copy numbers and more miRNA binding sites [45]. However, as the number of potential miRNA targets can be high and their expression levels and binding affinities might differ, this ratio does not prove the existence of an interaction. Co-localization studies and analysis of the expression correlations between circRNA and miRNA may offer some hints on whether a circRNA can function as a sponge but cannot be regarded as conclusive proof [41]. Functional experiments such as determining the effect of deleting or mutating the miRNA binding site(s) in the circRNA on the expression of other targets of the miRNA should be done to further support a miRNA sponge function for a circRNA.

The regulatory function of circPVT1 by serving as a miRNA decoy has been extensively investigated in various cancers (**Table 2**). However, most studies have relied on

luciferase reporter assays to confirm the interaction between circPVT1 and miRNAs, which supports the binding of a miRNA to the MRE of interest. However, these assays do not consider the secondary structure of circPVT1 or the potential impact of the endogenous lncPVT1. Some studies have provided direct evidence demonstrating the binding of circPVT1 to miRNAs through AGO2-IP and pull-down experiments, making them strong candidates for further validation. Unlike previous studies, we did not observe an enrichment of circPVT1 in the AGO2-IP fraction of B-cell lymphoma, while control mRNAs and miRNAs showed a clear enrichment. In addition, we also could not detect enrichment of lncPVT1 in the AGO2-IP fraction. Based on these findings, we concluded that circPVT1 does not act as a miRNA sponge in B-cell lymphoma. Subsequently, we attempted circPVT1 pull-down using a previously published protocol [46]. We obtained a ~4-fold enrichment of circPVT1 in the pull-down fraction compared to controls but with a very low efficiency (data not shown), which we regarded to be insufficient for subsequent steps to identify circPVT1 interacting partners. We are currently optimizing the pull-down protocol for further validation using a commercial kit, including a crosslinking step and a sonification step to effectively release the cellular content. After confirming pull-down efficiency, it will be interesting to determine whether miR-26b-5p, miR-24-3p, miR-30d/e, and miR-339-3p, which are identified as circPVT1 binding partners by pull-down in other studies and abundant in B-cell lymphoma, are enriched in the pull-down fraction or not. Next, the global identification of interacting molecules can be achieved using high-throughput methods for identifying proteins (e.g., mass spectrometry or antibody array analysis) or other RNA molecules (e.g., RNA-sequencing).

Besides the papers published by our group, no studies are available focusing on the role of ZDHHC11 or circZDHHC11 in B-cell lymphoma. Although we showed effective binding of miR-150 to circZDHHC11, its role in promoting BL proliferation seems independent of its miR-150 sponging ability. This might be caused by the presence of the linear ZDHHC11 transcripts, which also contain the miR-150 binding sites. However, we showed that knockdown of the circZDHHC11 without the miR-150 binding site region also affects the growth of BL. Together, these data indicate that circZDHHC11 might interact with other molecules to support the growth of BL. These data indicate that caution should be taken before concluding that the circRNA sponging capacity is the only functionally relevant mechanism of action. Similar to circPVT1, potential interactions of circZDHHC11 will be investigated through pull-down to explore the regulatory mechanism of circZDHHC11 affecting BL growth.

Table 2. Overview of the experimental approaches used to proof a sponge function for circPVT1.

Disease (cancer)	Target miRNA	Downstream effector	Methods used to verify miRNA binding	Ref.
Gastric cancer	miR-124-3p	ZEB1	luciferase reporter assay	[47]
	miR-30a-5p	YAP1	luciferase reporter assay	[48]
	miR-152-3p	HDGF	luciferase reporter assay	[49]
Osteosarcoma	miR-423-5p	Smad3	luciferase reporter assay	[50]
	miR-205-5p	c-FLIP	luciferase reporter assay	[51]
	miR-526b	FOXC2	luciferase reporter assay, circPVT1 pull-down	[52]
	miR-137	TRIAP1	luciferase reporter assay, AGO2-IP	[53]
	miR-423-5p	Wnt5a/Ror2, c-FLIP	luciferase reporter assay, AGO2-IP	[54]
	miR-26b-5p	CCNB1	luciferase reporter assay, circPVT1 pull-down	[55]
	miR-24-3p	KLF8	luciferase reporter assay, circPVT1 pull-down	[56]
Head and neck squamous cell carcinoma	miR-497-5p	Aurka, mki67, bub1	RT-qPCR	[57]
Non-small cell lung cancer	miR-125b	E2F2	luciferase reporter assay	[58]
	miR-497	Bcl-2	luciferase reporter assay	[59]
	miR-30d/e	CCNF	luciferase reporter assay, circPVT1 pull-down	[60]
	Let-7	NRAS	luciferase reporter assay	[61]
	miR-429	FOXK1	luciferase reporter assay	[62]
	miR-145-5p	ABCC1	miRNA pull-down	[63]
Acute lymphoblastic leukemia	miR-30e	DDL4	luciferase reporter assay	[64]
Colorectal carcinoma	miR-145	PAK4	luciferase reporter assay	[65]
Hepatocellular carcinoma	miR-203	HOXD3	luciferase reporter assay	[66]
	miR-3666	SIRT7	luciferase reporter assay, miRNA pull-down	[67]
	miR-377	TRIM23	luciferase reporter assay, AGO2-IP	[68]
Esophageal carcinoma	miR-4663	Pax-4, Pax-6	RT-qPCR	[69]
Breast cancer	miR-204-5p	-	luciferase reporter assay	[70]
	miR-29a-3p	AGR2	luciferase reporter assay	[71]
Renal cell carcinoma	miR-145-5p	TBX15	luciferase reporter assay, miRNA pull-down	[72]
Thyroid cancer	miR-455-5p	CXCL12/CXCR4	luciferase reporter assay, AGO2-IP	[73]
	miR-384	-	luciferase reporter assay, miRNA pull-down	[74]
Gallbladder cancer	miR-339-3p	MCL-1	luciferase reporter assay, AGO2-IP, miRNA pull-down	[75]
oral squamous cell carcinoma	miR-143-3p	SLC7A11	luciferase reporter assay, AGO2-IP, miRNA pull-down	[76]

2.4 Other molecular mechanisms of a circRNA

Our findings indicate that the proliferation promoting effect by circPVT1 in B-cell lymphoma is not dependent on miRNA binding, and circZDHHC11 promotes B-cell lymphoma proliferation independently of miR-150 binding. These observations suggest the involvement of additional mechanisms through which circPVT1 and circZDHHC11 regulate B-cell lymphoma proliferation. Future research should focus on identifying additional binding partners of circPVT1 and circZDHHC11 to define which signaling pathways these circRNAs influence to regulate B-cell lymphoma growth.

CircPVT1 was reported to also interact with proteins. A first interaction was shown with β-TrCP, thereby inhibiting β-TrCP-induced c-Myc ubiquitination and degradation. This inhibition enhanced the migration and invasion of nasopharyngeal carcinoma cells [77]. A second interaction was shown with YAP, leading to an increased circPVT1 expression. Mechanistically, mutant p53 and TEAD are recruited to the same genomic

site as YAP, forming a complex which subsequently initiates the transcription of circPVT1 [57]. CircPVT1 might potentially play a role in this interaction. Nan et al. reported decreased expression levels of circNOL10 upon inhibition of the splicing factor ESRP1 in lung cancer. In addition, circNOL10 was observed to bind directly and inhibit the ubiquitination of the transcription factor SCML1, thereby promoting its expression [78].

Several databases have been developed to predict circRNA-protein interactions based on crosslinking immunoprecipitation (CLIP) sequencing data, including CSCD2 [79], CircInteractome [80] and ENCORI [81]. Two proteins (DGCR8 and EIF4A3) were consistently predicted to bind circPVT1 by all three tools. 17 proteins, including AGO2, were predicted by two tools (**Figure 1A**). The identified proteins suggest a role of circPVT1 in miRNA binding and processing based on the interactions with DGCR8 and AGO2 and RNA secondary structure, including translation initiation, nuclear and mitochondrial splicing, and ribosome and spliceosome assembly, based on EIF4A3 and DDX54. Moreover, NOP58 and FBL are involved in the processing of ribosomal RNA molecules, and ELAVL1, CNBP, and IGF2BP2 regulate translation. RBM10, CSTF2T, RBFOX2, SRSF9, DDX54, and SRSF1 are involved in pre-mRNA processing and splicing. YTHDC1 and YTHDF1 are involved in binding RNA that contains N6-methyladenosine. Finally, MOV10 and UPF1 are involved in RNA molecule decay and interference.

Prediction of circ-NOL10 interactions revealed two proteins (IGF2BP3 and EIF4A3) that were consistently identified by all three tools, while 30 proteins were common between two tools (**Figure 1B**). IGF2BP3 plays a role in regulating mRNA stability, translation, and localization. EIF4A3 is involved in the initiation of translation or pre-mRNA splicing. The majority of the proteins that overlapped are related to pre-mRNA processing and splicing. We showed direct binding of circ-NOL10 to MTDH and CASC3 in breast cancer. However, The prediction tools were unable to identify these two proteins as the CLIP-seq data used did not contain any information regarding these two specific proteins. MTDH is known to be involved in RNA-induced silencing complex (RISC) and plays a crucial role in RISC and miRNA functions, whereas CASC3 is deposited on spliced mRNAs at exon-exon junctions and functions in nonsense-mediated mRNA decay (NMD). These functions of MTDH and CASC3 may explain the inhibition of circ-NOL10 expression to some extent. However, the exact mechanism of how MTDH and CASC3 regulate the expression of circ-NOL10 needs further investigation.

The translational potential of circRNAs can be evaluated by examining the presence of open reading frames spanning back-splicing junctions. According to predictions from

circRNAdb [82], circPVT1 potentially encodes for a single peptide consisting of 104 amino acids (aa) **(Figure 1C)**. However, Tolomeo et al. evaluated the protein-coding potential of circPVT1 using polysome fractionation and did not observe the protein-coding activity of circPVT1 in small cell lung cancer [83]. Despite these negative findings in lung cancer, further experiments are required to support or exclude the potential protein-coding property of circPVT1 in other cell types. circ-NOL10 was predicted to have an open reading frame of 200 amino acids, however no proof exists that this protein indeed can be generated **(Figure 1D)**.

Due to the fact that most databases have only annotated the known circRNAs, there are currently no predictions available for circZDHHC11 for both binding to proteins nor translational potential.

Figure 1. RBP interaction and translation potential of circPVT1 and circ-NOL10 predicted by databases. (A) CircPVT1 binding proteins predicted by circInteractom (n=4), CSCD2 (n=41), and ENCORI (n=19). **(B)** Circ-NOL10 binding proteins predicted by circInteractom (n=5), CSCD2 (n=52), and ENCORI (n=54). **(C)** circPVT1 and **(D)** circ-NOL10 translation potential predicted by circRNAdb.

2.5 The potential value of circRNAs in clinical application

Circular RNAs (circRNAs) are a class of non-coding RNA molecules that exhibit high stability compared to their linear counterparts due to their closed-loop structure without 5' and 3' ends. This unique feature makes circRNAs resistant to exonucleases

and other RNA degradation mechanisms, which can lead to their accumulation in cells, tissues and body fluids. A study has shown that circRNAs are more abundant than linear forms in biofluids in cases where genes produce both circRNAs and linear mRNAs [84], further highlighting their potential as biomarkers. Further functional characterization is required to establish the potential applications of circRNA as therapeutic targets. Although cancer and SARS-CoV-2 vaccines based on circRNA structure have recently been developed [85, 86], no circRNA-based medical applications have received approval. Our understanding of circRNAs remains in its infancy and is far from reaching the stage of translational medicine. To move circRNA-based diagnostic and therapeutic approaches toward clinical practice, it is important to standardize quantification in biological samples and further characterize circRNAs potentially relevance as therapeutic targets.

CircPVT1 has been identified as upregulated in various cancers and was suggested as a promising diagnostic marker for lung cancer and oral squamous cell carcinoma. Moreover, its upregulation has been linked to unfavorable outcomes such as shorter survival time, advanced TNM stage, larger tumor size, and lymph node metastasis across diverse cancer types [87]. Recent investigations have also highlighted the potential role of circPVT1 in modulating the response of cancer patients to chemotherapeutic agents, such as cisplatin, doxorubicin, and paclitaxel [87]. Collectively, these findings underscore the possible utility of circPVT1 as a diagnostic and prognostic marker for various cancers. Work from others within our group showed an association of lncPVT1 levels with overall survival in DLBCL (paper submitted). We also showed a significant elevation of circPVT1 levels in B-cell lymphoma cell lines relative to GC B cells. However, its potential as a prognostic biomarker needs to be established by correlating circPVT1 levels to diverse clinical characteristics. It will also be very interesting to determine to what extent circPVT1 levels in the circulation correlate with disease load or response to therapy, which will establish the value of circPVT1 as a diagnostic marker. The same methodology could be utilized to evaluate the diagnostic and prognostic potential of circZHHC11 levels in B-cell lymphoma. Circ-NOL10 is downregulated in both colorectal and breast cancer, and this reduced expression has been associated with advanced clinical stage, lymph node metastasis, and poor prognosis [88, 89]. Our study also observed a significant correlation between decreased circ-NOL10 expression and advanced clinical stage, increased lymphatic metastasis, and shorter disease-free and overall survival times in TNBC. These data indicate that circ-NOL10 may hold promise as a diagnostic and prognostic biomarker for breast cancer, particularly in TNBC. However, further validation in larger cohorts and investigation of its expression in patients' biofluids are necessary to confirm its

potential clinical utility. Furthermore, in-depth *in vivo* studies are recommended to establish the proliferation-inhibiting effects of circ-NOL10 in breast cancer.

References

1. Wang, X. and L. Fang, *Advances in circular RNAs and their roles in breast Cancer.* J Exp Clin Cancer Res, 2018. **37**(1): p. 206.
2. Tian, T., et al., *Circular RNA: A potential diagnostic, prognostic, and therapeutic biomarker for human triple-negative breast cancer.* Mol Ther Nucleic Acids, 2021. **26**: p. 63-80.
3. Lyu, L., et al., *Regulatory mechanisms, functions, and clinical significance of CircRNAs in triple-negative breast cancer.* J Hematol Oncol, 2021. **14**(1): p. 41.
4. Zhou, X., et al., *The functions and clinical significance of circRNAs in hematological malignancies.* J Hematol Oncol, 2020. **13**(1): p. 138.
5. Yang, L., J.E. Wilusz, and L.L. Chen, *Biogenesis and Regulatory Roles of Circular RNAs.* Annu Rev Cell Dev Biol, 2022. **38**: p. 263-289.
6. Liu, C.X. and L.L. Chen, *Circular RNAs: Characterization, cellular roles, and applications.* Cell, 2022. **185**(12): p. 2016-2034.
7. Kristensen, L.S., et al., *The emerging roles of circRNAs in cancer and oncology.* Nat Rev Clin Oncol, 2022. **19**(3): p. 188-206.
8. Vromman, M., J. Vandesompele, and P.J. Volders, *Closing the circle: current state and perspectives of circular RNA databases.* Brief Bioinform, 2021. **22**(1): p. 288-297.
9. Ye, C.Y., et al., *Full-length sequence assembly reveals circular RNAs with diverse non-GT/AG splicing signals in rice.* RNA Biol, 2017. **14**(8): p. 1055-1063.
10. Zheng, Y., et al., *Reconstruction of full-length circular RNAs enables isoform-level quantification.* Genome Med, 2019. **11**(1): p. 2.
11. Wu, J., et al., *CircAST: Full-length Assembly and Quantification of Alternatively Spliced Isoforms in Circular RNAs.* Genomics Proteomics Bioinformatics, 2019. **17**(5): p. 522-534.
12. Yu, K.H., et al., *Quantifying full-length circular RNAs in cancer.* Genome Res, 2021. **31**(12): p. 2340-53.
13. Metge, F., et al., *FUCHS-towards full circular RNA characterization using RNAseq.* PeerJ, 2017. **5**: p. e2934.
14. Hossain, M.T., et al., *FcircSEC: An R Package for Full Length circRNA Sequence Extraction and Classification.* Int J Genomics, 2020. **2020**: p. 9084901.
15. Hossain, M.T., et al., *Reconstruction of Full-Length circRNA Sequences Using Chimeric Alignment Information.* Int J Mol Sci, 2022. **23**(12).
16. Stefanov, S.R. and I.M. Meyer, *CYCLeR-a novel tool for the full isoform assembly and quantification of circRNAs.* Nucleic Acids Res, 2023. **51**(2): p. e10.
17. Wang, Y., et al., *Nanopore sequencing technology, bioinformatics and applications.* Nat Biotechnol, 2021. **39**(11): p. 1348-1365.
18. Xin, R., et al., *isoCirc catalogs full-length circular RNA isoforms in human transcriptomes.* Nat Commun, 2021. **12**(1): p. 266.
19. Liu, Z., et al., *circFL-seq reveals full-length circular RNAs with rolling circular reverse transcription and nanopore sequencing.* Elife, 2021. **10**.
20. Zhang, J., et al., *Comprehensive profiling of circular RNAs with nanopore sequencing and CIRI-long.* Nat Biotechnol, 2021. **39**(7): p. 836-845.
21. Rahimi, K., et al., *Nanopore sequencing of brain-derived full-length circRNAs reveals circRNA-specific exon usage, intron retention and microexons.* Nat Commun, 2021. **12**(1): p. 4825.
22. Ashwal-Fluss, R., et al., *circRNA biogenesis competes with pre-mRNA splicing.* Mol Cell, 2014. **56**(1): p. 55-66.
23. Conn, V.M., et al., *A circRNA from SEPALLATA3 regulates splicing of its cognate mRNA through R-loop formation.* Nat Plants, 2017. **3**: p. 17053.
24. Yang, F., et al., *Circ-HuR suppresses HuR expression and gastric cancer progression by inhibiting CNBP transactivation.* Mol Cancer, 2019. **18**(1): p. 158.
25. Chen, J., et al., *Androgen receptor-regulated circFNTA activates KRAS signaling to promote bladder cancer invasion.* EMBO Rep, 2020. **21**(4): p. e48467.
26. He, R., et al., *circGFRA1 and GFRA1 act as ceRNAs in triple negative breast cancer by regulating miR-34a.* J Exp Clin Cancer Res, 2017. **36**(1): p. 145.
27. Abdelmohsen, K., et al., *Identification of HuR target circular RNAs uncovers suppression of PABPN1 translation by CircPABPN1.* RNA Biol, 2017. **14**(3): p. 361-369.
28. Du, W.W., et al., *A circular RNA circ-DNMT1 enhances breast cancer progression by activating autophagy.* Oncogene, 2018. **37**(44): p. 5829-5842.
29. Zhao, W., et al., *Circular RNA (circ-0075804) promotes the proliferation of retinoblastoma via combining heterogeneous nuclear ribonucleoprotein K (HNRNPK) to improve the stability of E2F transcription factor 3 E2F3.* J Cell Biochem, 2020. **121**(7): p. 3516-3525.
30. Georgakis, G.V., et al., *Inhibition of the phosphatidylinositol-3 kinase/Akt promotes G1 cell cycle arrest and apoptosis in Hodgkin lymphoma.* Br J Haematol, 2006. **132**(4): p. 503-11.
31. Fiumara, P., et al., *Functional expression of receptor activator of nuclear factor kappaB in Hodgkin disease cell lines.* Blood, 2001. **98**(9): p. 2784-90.

32. Iijima, K., et al., *ZNF385B is characteristically expressed in germinal center B cells and involved in B-cell apoptosis.* Eur J Immunol, 2012. **42**(12): p. 3405-15.
33. Wang, S., et al., *Emerging Roles of Circ-ZNF609 in Multiple Human Diseases.* Front Genet, 2022. **13**: p. 837343.
34. Yang, C.S., et al., *[Mechanism of circZNF609 targeting miR-153 to regulate the proliferation and apoptosis of diffuse large B-cell lymphoma].* Zhonghua Zhong Liu Za Zhi, 2022. **44**(3): p. 238-245.
35. Zhu, Y.J., et al., *Circular RNAs negatively regulate cancer stem cells by physically binding FMRP against CCAR1 complex in hepatocellular carcinoma.* Theranostics, 2019. **9**(12): p. 3526-3540.
36. Wang, Y., et al., *Circ-ZKSCAN1 regulates FAM83A expression and inactivates MAPK signaling by targeting miR-330-5p to promote non-small cell lung cancer progression.* Transl Lung Cancer Res, 2019. **8**(6): p. 862-875.
37. Li, J., et al., *CircZKSCAN1 Suppresses Hepatocellular Carcinoma Tumorigenesis by Regulating miR-873-5p/Downregulation of Deleted in Liver Cancer 1.* Dig Dis Sci, 2021. **66**(12): p. 4374-4383.
38. Bi, J., et al., *Circular RNA circ-ZKSCAN1 inhibits bladder cancer progression through miR-1178-3p/p21 axis and acts as a prognostic factor of recurrence.* Mol Cancer, 2019. **18**(1): p. 133.
39. Salmena, L., et al., *A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?* Cell, 2011. **146**(3): p. 353-8.
40. Hansen, T.B., et al., *Natural RNA circles function as efficient microRNA sponges.* Nature, 2013. **495**(7441): p. 384-8.
41. Jarlstad Olesen, M.T. and S.K. L, *Circular RNAs as microRNA sponges: evidence and controversies.* Essays Biochem, 2021. **65**(4): p. 685-696.
42. Liu, C.X., et al., *Structure and Degradation of Circular RNAs Regulate PKR Activation in Innate Immunity.* Cell, 2019. **177**(4): p. 865-880.e21.
43. Abe, M., et al., *Impact of age-associated increase in 2'-O-methylation of miRNAs on aging and neurodegeneration in Drosophila.* Genes Dev, 2014. **28**(1): p. 44-57.
44. Hauptmann, J., et al., *Biochemical isolation of Argonaute protein complexes by Ago-APP.* Proc Natl Acad Sci U S A, 2015. **112**(38): p. 11841-5.
45. Li, F., et al., *Circular RNAs in cancer: Limitations in functional studies and diagnostic potential.* Semin Cancer Biol, 2021. **75**: p. 49-61.
46. Panda, A.C., et al., *Identification of senescence-associated circular RNAs (SAC-RNAs) reveals senescence suppressor CircPVT1.* Nucleic Acids Res, 2017. **45**(7): p. 4021-4035.
47. Liu, Y.Y., L.Y. Zhang, and W.Z. Du, *Circular RNA circ-PVT1 contributes to paclitaxel resistance of gastric cancer cells through the regulation of ZEB1 expression by sponging miR-124-3p.* Biosci Rep, 2019. **39**(12).
48. Yao, W., et al., *Exosome-Derived Circ-PVT1 Contributes to Cisplatin Resistance by Regulating Autophagy, Invasion, and Apoptosis Via miR-30a-5p/YAP1 Axis in Gastric Cancer Cells.* Cancer Biother Radiopharm, 2021. **36**(4): p. 347-359.
49. Wang, X., et al., *Knockdown of cir_RNA PVT1 Elevates Gastric Cancer Cisplatin Sensitivity via Sponging miR-152-3p.* J Surg Res, 2021. **261**: p. 185-195.
50. Li, H., et al., *CircRNA PVT1 modulated cell migration and invasion through Epithelial-Mesenchymal Transition (EMT) mediation in gastric cancer through miR-423-5p/Smad3 pathway.* Regen Ther, 2022. **21**: p. 25-33.
51. Liu, Y.P., et al., *circPVT1 Facilitates Invasion and Metastasis by Regulating miR-205-5p/c-FLIP Axis in Osteosarcoma.* Cancer Manag Res, 2020. **12**: p. 1229-1240.
52. Yan, M., et al., *Circular RNA PVT1 promotes metastasis via regulating of miR-526b/FOXC2 signals in OS cells.* J Cell Mol Med, 2020. **24**(10): p. 5593-5604.
53. Li, D., Y. Huang, and G. Wang, *Circular RNA circPVT1 Contributes to Doxorubicin (DXR) Resistance of Osteosarcoma Cells by Regulating TRIAP1 via miR-137.* Biomed Res Int, 2021. **2021**: p. 7463867.
54. Wan, J., et al., *circPVT1 promotes osteosarcoma glycolysis and metastasis by sponging miR-423-5p to activate Wnt5a/Ror2 signaling.* Cancer Sci, 2021. **112**(5): p. 1707-1722.
55. Huang, S.X., et al., *CircPVT1 promotes the tumorigenesis and metastasis of osteosarcoma via mediation of miR-26b-5p/CCNB1 axis.* J Bone Miner Metab, 2022. **40**(4): p. 581-593.
56. Wang, B., et al., *CircRNA PVT1 promotes proliferation and chemoresistance of osteosarcoma cells via the miR-24-3p/KLF8 axis.* Int J Clin Oncol, 2022. **27**(4): p. 811-822.
57. Verduci, L., et al., *The oncogenic role of circPVT1 in head and neck squamous cell carcinoma is mediated through the mutant p53/YAP/TEAD transcription-competent complex.* Genome Biol, 2017. **18**(1): p. 237.
58. Li, X., et al., *Circular RNA circPVT1 Promotes Proliferation and Invasion Through Sponging miR-125b and Activating E2F2 Signaling in Non-Small Cell Lung Cancer.* Cell Physiol Biochem, 2018. **51**(5): p. 2324-2340.
59. Qin, S., et al., *Circular RNA PVT1 acts as a competing endogenous RNA for miR-497 in promoting non-small cell lung cancer progression.* Biomed Pharmacother, 2019. **111**: p. 244-250.
60. Shi, J., et al., *CircPVT1 promotes proliferation of lung squamous cell carcinoma by binding to miR-30d/e.* J Exp Clin Cancer Res, 2021. **40**(1): p. 193.
61. Danac, J.M.C. and R.L. Garcia, *CircPVT1 attenuates negative regulation of NRAS by let-7 and drives cancer cells towards oncogenicity.* Sci Rep, 2021. **11**(1): p. 9021.

62. Cao, L., et al., *Knockdown of circ-PVT1 inhibits the progression of lung adenocarcinoma and enhances the sensitivity to cisplatin via the miR-429/FOXK1 signaling axis.* Mol Med Rep, 2021. **24**(4).
63. Zheng, F. and R. Xu, *CircPVT1 contributes to chemotherapy resistance of lung adenocarcinoma through miR-145-5p/ABCC1 axis.* Biomed Pharmacother, 2020. **124**: p. 109828.
64. Jia, Y. and W. Gu, *Up-regulation of circPVT1 in T cell acute lymphoblastic leukemia promoted cell proliferation via miR-30e/DLL4 induced activating NOTCH signaling.* Pathol Res Pract, 2021. **224**: p. 153536.
65. Wang, Z., et al., *Circular RNA PVT1 promotes metastasis via miR-145 sponging in CRC.* Biochem Biophys Res Commun, 2019. **512**(4): p. 716-722.
66. Zhu, Y., et al., *The circular RNA PVT1/miR-203/HOXD3 pathway promotes the progression of human hepatocellular carcinoma.* Biol Open, 2019. **8**(9).
67. Li, Y., et al., *Downregulation of circular RNA circPVT1 restricts cell growth of hepatocellular carcinoma through downregulation of Sirtuin 7 via microRNA-3666.* Clin Exp Pharmacol Physiol, 2020. **47**(7): p. 1291-1300.
68. Bu, N., et al., *CircPVT1 Regulates Cell Proliferation, Apoptosis and Glycolysis in Hepatocellular Carcinoma via miR-377/TRIM23 Axis.* Cancer Manag Res, 2020. **12**: p. 12945-12956.
69. Zhong, R., et al., *Potential Role of circPVT1 as a proliferative factor and treatment target in esophageal carcinoma.* Cancer Cell Int, 2019. **19**: p. 267.
70. Bian, Q., *Circular RNA PVT1 promotes the invasion and epithelial-mesenchymal transition of breast cancer cells through serving as a competing endogenous RNA for miR-204-5p.* Onco Targets Ther, 2019. **12**: p. 11817-11826.
71. Wang, J., et al., *CircPVT1 Promoted the Progression of Breast Cancer by Regulating MiR-29a-3p-Mediated AGR2-HIF-1α Pathway.* Cancer Manag Res, 2020. **12**: p. 11477-11490.
72. Zheng, Z., et al., *CircPVT1 promotes progression in clear cell renal cell carcinoma by sponging miR-145-5p and regulating TBX15 expression.* Cancer Sci, 2021. **112**(4): p. 1443-1456.
73. Zheng, X., et al., *circPVT1 regulates medullary thyroid cancer growth and metastasis by targeting miR-455-5p to activate CXCL12/CXCR4 signaling.* J Exp Clin Cancer Res, 2021. **40**(1): p. 157.
74. Hua, T. and Y. Luo, *Circular RNA PVT1 promotes progression of thyroid cancer by competitively binding miR-384.* Exp Ther Med, 2022. **24**(4): p. 629.
75. Wang, S., et al., *CircPVT1 promotes gallbladder cancer growth by sponging miR-339-3p and regulates MCL-1 expression.* Cell Death Discov, 2021. **7**(1): p. 191.
76. Wang, S., et al., *CircPVT1 facilitates the progression of oral squamous cell carcinoma by regulating miR-143-3p/SLC7A11 axis through MAPK signaling pathway.* Funct Integr Genomics, 2022. **22**(5): p. 891-903.
77. Mo, Y., et al., *Circular RNA circPVT1 promotes nasopharyngeal carcinoma metastasis via the β-TrCP/c-Myc/SRSF1 positive feedback loop.* Mol Cancer, 2022. **21**(1): p. 192.
78. Nan, A., et al., *Circular RNA circNOL10 Inhibits Lung Cancer Development by Promoting SCLM1-Mediated Transcriptional Regulation of the Humanin Polypeptide Family.* Adv Sci (Weinh), 2019. **6**(2): p. 1800654.
79. Feng, J., et al., *CSCD2: an integrated interactional database of cancer-specific circular RNAs.* Nucleic Acids Res, 2022. **50**(D1): p. D1179-d1183.
80. Dudekula, D.B., et al., *CircInteractome: A web tool for exploring circular RNAs and their interacting proteins and microRNAs.* RNA Biol, 2016. **13**(1): p. 34-42.
81. Zhou KR, L.S., Cai L, Bin L, et al., *ENCORI: The Encyclopedia of RNA Interactomes.*
82. Chen, X., et al., *circRNADb: A comprehensive database for human circular RNAs with protein-coding annotations.* Sci Rep, 2016. **6**: p. 34985.
83. Tolomeo, D., et al., *circPVT1 and PVT1/AKT3 show a role in cell proliferation, apoptosis, and tumor subtype-definition in small cell lung cancer.* Genes Chromosomes Cancer, 2022.
84. Hulstaert, E., et al., *Charting Extracellular Transcriptomes in The Human Biofluid RNA Atlas.* Cell Rep, 2020. **33**(13): p. 108552.
85. Qu, L., et al., *Circular RNA vaccines against SARS-CoV-2 and emerging variants.* Cell, 2022. **185**(10): p. 1728-1744.e16.
86. Li, H., et al., *Circular RNA cancer vaccines drive immunity in hard-to-treat malignancies.* Theranostics, 2022. **12**(14): p. 6422-6436.
87. Ghafouri-Fard, S., et al., *A Concise Review on the Role of CircPVT1 in Tumorigenesis, Drug Sensitivity, and Cancer Prognosis.* Front Oncol, 2021. **11**: p. 762960.
88. Zhang, Y., et al., *CircNOL10 Acts as a Sponge of miR-135a/b-5p in Suppressing Colorectal Cancer Progression via Regulating KLF9.* Onco Targets Ther, 2020. **13**: p. 5165-5176.
89. Wang, F., et al., *CircNOL10 suppresses breast cancer progression by sponging miR-767-5p to regulate SOCS2/JAK/STAT signaling.* J Biomed Sci, 2021. **28**(1): p. 4.