The Potential of Circular RNAs as Cancer Biomarkers

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Running Title: CircRNAs as Cancer Biomarkers

Key words: Circular RNA, Cancer, Biomarker, Early Detection

Financial Support: N/A

Word Count: 5999

# Figures/Tables: 5

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Conflict of Interest Disclosure: JRB has no conflicts of interest to disclose. AMC has stock/ownership interest in Oncopia, Tempus, Esanik, OncoFusion Therapeutics, and Medsyn and a consulting/advisory role with Tempus.
Abstract

Circular RNA (circRNA) is a covalently closed RNA structure that has several proposed functions related to cancer development. Recently, cancer-specific and tissue-specific circRNAs have been identified by high throughput sequencing and are curated in publicly available databases. CircRNAs have features that are ideal properties of biomarkers, including conservation, abundance, and stability in plasma, saliva, and urine. Many circRNAs with predictive and prognostic significance in cancer have been described, and functional mechanisms for some circRNAs have been suggested. CircRNA also has great potential as a non-invasive biomarker for early cancer detection, although further investigation is necessary before clinical application is feasible.
Introduction

RNA molecules with a circular structure were first reported in plant viroids in 1976[1] and in eukaryotic cells in 1979[2]. Initially deemed aberrant splicing artifacts[3], circular RNA (circRNA) was identified in mammalian cells in the 1990s[4-6]. Abundance and potential circRNA functions were further characterized by high-throughput sequencing in the 2010s[7-10]. Large-scale studies have implicated circRNA in disease pathogenesis, including in cardiovascular disease[11, 12], neurodegenerative diseases[13, 14], and diabetes[15].

Hematologic malignancies and solid tumors also have associated circRNAs. The first reported was Cdr1as (ciRS-7), a circRNA regulator of miR-7, a tumor suppressor in breast, lung, and brain cancers[16]. Subsequently, multiple circRNAs have been reported with oncogenic and tumor suppressive potential[17]. These circRNAs have reported effects on important cancer hallmarks, including sustained proliferation, resisting cell death, angiogenesis, and metastasis[17-19]. CircRNA in several known cancer signaling pathways have been identified[20-22].

With proposed roles in oncogenesis, circRNA has emerged as a novel cancer biomarker. In this review, we discuss the circRNA features that are advantageous as a biomarker and its potential clinical utility in determining prognosis, predicting treatment response, and detecting cancer non-invasively.

CircRNA Biogenesis

CircRNA consists of a single-stranded RNA molecule covalently closed in a loop structure. Unlike linear RNA, circRNA lacks 5’-3’ polarity and polyadenylated (poly(A)) tails[23]. Whereas linear RNA is generated by alternative exon splicing, circRNA is often formed by backsplicing the 3’ end of an exon to the 5’ end of either an upstream exon or itself[8, 24] (Figure 1). In order to facilitate backsplicing, flanking introns are typically longer than ten thousand base pairs[7, 8], although not always[25]. CircRNAs typically contain one to five exons[9].

Three mechanisms for circRNA structure biogenesis have been proposed. The first is intron-pairing, in which reverse complementary sequences, often repetitive ALU elements, are paired between flanking introns to facilitate backsplicing[8, 26, 27]. The second mechanism involves RNA binding proteins (RBPs) attaching to flanking introns, stabilizing intron pairs to promote circularization [28, 29]. The third mechanism is exon skipping, in which an exon-containing lariat precursor formed during canonical RNA splicing is subsequently backspliced into a circular structure[30]. The final circRNA product can be characterized based on its composition as exonic, exon-intron, or intronic[10, 31] (Figure 1).

CircRNA Properties and Biologic Functions

CircRNA has several unique qualities that facilitate its biomarker potential. CircRNA is abundantly expressed, with reported increased abundance compared to parental mRNA in eukaryotic cells[8]. Millions of circRNAs have been detected by bioinformatics analyses on human tissue[32]. CircRNA has also been conserved among multiple species[33]. CircRNAs are predominantly
cytoplasmic[4, 7, 8], although occasionally nuclear[31]. Because circRNA lacks 5’ and 3’ ends, including poly(A) tails, it resists exoribonuclease mediated degradation[34, 35] (Figure 1). Therefore, circRNA is more stable than its linear counterpart, which remains susceptible to RNase degradation[8]. Finally, several tissue and disease specific circRNAs have been identified[25, 32, 36].

Multiple potential biological functions for circRNA have been proposed (Figure 2). The most frequently reported function is as a competing endogenous RNA. By sponging microRNA (miRNA), circRNAs suppress interaction between miRNA and mRNA, and affected circRNA/miRNA/mRNA axes underlie oncogenic and tumor suppressive properties[16, 36, 37]. Some circRNAs sponge multiple miRNAs, for example circITCH sponges miR-216b, miR-17, miR-214, miR-7, and miR-128 in esophageal squamous cell carcinoma[20], miR-7 and miR-214 in lung cancer[38], and miR-17 and miR-224 in bladder cancer[39].

CircRNAs also interact directly with proteins, preventing target binding and scaffolding to form larger protein complexes. One example observed in HeLa cells is circPABN1 suppresses PABN1 binding to HuR, an RBP, to inhibit translation[40]. CircFOXO3 complexes with CDK2 and p21 to form a scaffold and induce cell cycle arrest[41]. CircFOXO3, unlike linear FOXO3, binds MDM2 and p53, inducing MDM2-mediated p53 ubiquitination to promote apoptosis in breast cancer[42].

Direct circRNA translation has also been described. Some circRNAs contain an open reading frame (ORF) and have an internal ribosomal entry site (IRES) to mediate translation and compensate for lack of 5’ cap and 3’ end[43, 44]. Some CircRNAs undergo multiple consecutive rounds of translation when the stop codon is not recognized within the ORF on the first read[45]. Examples of reported directly translated circRNAs in cancer include circβ-catenin in hepatocellular carcinoma[46], circFBXW7 in glioma[47], and circPPP1R12A in colon cancer[48]. Additionally, some nuclear localized circRNAs can promote parental gene transcription[31].
Methods

A literature search was performed on PubMed using terminology related to the subject of interest, including “circular RNA” and “cancer.” References of the articles reviewed were also evaluated so that relevant studies missed by the keyword search were not excluded. All articles reviewed were published prior to May 1, 2020. Studies included in this review were selected based on relevance to the topic, methodology utilized, and clinical applicability of proposed circular RNA biomarkers.
Results

CircRNA Discovery and Quantification

Many circRNAs have been discovered by high throughput sequencing. One technique utilizes RNA-seq to reliably identify backspliced junctions. Alternative strategies are necessary to identify circRNA to compensate for inability to enrich polyadenylated transcripts\cite{49}. One method uses an exoribonuclease, RNase R, to enrich for circRNA\cite{8}. A downside is linear mRNA degradation, preventing mRNA quantification for further downstream analysis. One reported method to improve circRNA purification uses a lithium-based reaction buffer to prevent RNase R stalling in guanine base rich regions\cite{50}. Another method to improve circRNA detection efficiency is RiboZero, which uses microsphere beads to deplete ribosomal RNA, therefore enriching circRNA but also allowing further linear mRNA analysis\cite{7, 51}. Five milligrams total RNA are necessary for this method. These challenges are addressed by exome capture RNA sequencing. In this protocol, complementary capture RNA probes targeting exons of interest are hybridized with cDNA fragments\cite{52}. This method consistently detected more circRNA in cell lines and cancer tissues\cite{53}.

Another approach to circRNA discovery is microarray, which involves RNase-R based circRNA enrichment prior to labelling and hybridization\cite{54}. This targeted method allows for increased certainty in circRNA annotation, for example putative miRNA binding sites, and requires less bioinformatics expertise and computing power than RNA-seq\cite{55}. Disadvantages compared to RNA-seq include lower sensitivity and specificity and decreased novel transcript detection. A commercially available circRNA microarray (Arraystar, Inc.) has profiled circRNA expression in multiple cancers\cite{56-58}.

Quantitative circRNA expression reported by RNA-seq or microarray allows for characterization of cancer-specific circRNAs by differential analysis between circRNA and adjacent normal tissues. CircRNAs may be upregulated or downregulated in cancer compared to normal tissue\cite{53, 59-73}. Individual cancer-specific circRNA biomarkers identified by this analysis require further validation to ascertain biologic and clinical relevance. Quantitative RT-PCR (qPCR) is commonly used to validate circRNA expression with fluorescence-based detection of amplified primers surrounding each circRNA-specific backspliced junction. Another sensitive and accurate method is droplet-digital PCR, which determines circRNA concentration by quantifying the ratio of positive to negative droplets. This technique avoids a potential issue of multiple rolling cDNA pCR products that potentially overestimates circRNA expression by qPCR\cite{74}. Droplet-digital PCR accurately detected circRNA in gastric cancer plasma and tissue\cite{75}. Another technology that measures circRNA expression is the NanoString nCounter, which hybridizes a biotinylated capture probe and uniquely color-coded reporter probe. This technique avoids enzymatic reactions and can multiplex multiple targets\cite{76}. One study utilizing nCounter specifically detected 52 circRNAs in B-cell malignancies\cite{77}.

CircRNA Expression Databases

Several publicly available databases catalogue and characterize circRNAs discovered by high throughput sequencing. These databases provide a valuable resource for further investigation into circRNA biomarkers. Overlap between databases is minimal, likely due to variability in specimens...
analyzed and detection platforms used[78]. Table 1 summarizes each database’s unique qualities and circRNA detection tools.

CircBase compiles circRNA identified in initial landmark circRNA studies on mouse and human cell lines and lists over 90,000 unique human circRNAs[79]. TCSD, a database of tissue-specific circRNAs identified in adult human, fetal human, and mouse tissues, contains more than one million human circRNAs[32]. CircAtlas 2.0 and CIRCpedia databases detail circRNA diversity and conservation across multiple species[80, 81].

Other databases explore potential circRNA functions and downstream effects, for example miRNA binding sites for competing endogenous RNA. CircBank utilizes the same dataset as circBase to predict miRNA binding[82]. CircInteractome[83] and circNet[84] both establish networks between circRNA, miRNA, and associated genes. To further understand circRNA translation, circRNAdb reports important translation elements, including ORF and IRES for 32,914 circRNAs[85]. CircPro reports circRNA coding potential[86]. CircFunBase explores potential circRNA functions conserved between species[87]. Other circRNA databases, including Circ2Disease[88], Circ2Traits[89], and CircR2Disease[90], explore reported phenotypic associations and physiologic relevance.

Some expression databases focus on cancer-specific circRNAs (Table 2). CSCD reports more than one million circRNA expressed in cancer-specific cell lines[91]. CircRic also reports circRNAs across 935 cancer cell lines along with integrative analysis and potential drug response[92]. Other databases report circRNA expression analyzed on human specimens. MiOncoCirc is a compendium of 160,120 circRNAs discovered by exome capture sequencing on cancer tissues. This database reports multiple isoforms obtained by alternative backsplicing ranked by expression[53]. BBCancer details expression of six RNA types in plasma, including potential circRNA biomarkers of liver, pancreatic, and colorectal cancer [93]. Although not cancer-specific, exoRbase characterizes exosomal RNA, including potential non-invasive circRNA biomarkers[94].

**CircRNA Clinical Relevance as Cancer Biomarkers**

CircRNA demonstrates emerging potential as a clinically useful biomarker in cancer. Tissue specificity, stability, abundant expression, and documented importance in pathways and processes that underscore cancer development are circRNA characteristics essential to its promise as a biomarker. Therefore, circRNA could improve upon classical protein-based cancer biomarkers, which are often nonspecific. Potential uses for circRNA biomarkers include prognosis, predicting treatment response, early detection, and non-invasive disease monitoring.

CircRNA has independent potential as a biomarker from its parental linear mRNA, as evidenced by differences in abundance and expression[25]. In one analysis of 348 primary breast cancer specimens, correlation between circRNA and linear mRNA expression ranged between $\rho$ -0.34 to 0.97, where 210 of 1624 (12.9%) pairs were negatively correlated. This correlation often varied between different backspliced isoforms, for example circESR1, with one isoform positively correlated (chr6:151842597-151880771) and another isoform negatively correlated (chr6:151880655-151944508)[95].
CircRNA can also effectively differentiate between cancer subtypes. In breast cancer, circRNA specific to triple negative, ER-positive, and HER2-positive breast cancer have been identified. Within ER-positive breast cancer, differences between circRNA expressed in luminal A and luminal B subtypes were described[96]. In lung cancer, circACVR2A and circCCNB1 expression effectively differentiated between squamous cell carcinoma and adenocarcinoma[97].

CircRNA Prognostic Biomarkers

CircRNAs with favorable and unfavorable prognostic significance have been described in multiple cancers. Numerous studies reporting prognostic circRNAs have been published recently, some of which are discussed in this review. Interestingly, circRNAs may have variable prognostic significance between cancers, for example circZKSCAN1 which portends poor prognosis in lung cancer[98] and good prognosis in bladder cancer[99]. Many prognostic circRNAs have been reported as competing endogenous RNAs, effectively functioning as oncogenes and tumor suppressors to regulate processes that drive tumorigenesis and metastasis through signaling pathways. Therefore, these biomarkers may provide eventual therapeutic targets.

Breast Cancer

CircUBAP2 expression was associated with increased tumor size, advanced stage, lymph node metastasis, and comparatively worse overall survival in triple negative breast cancer. CircUBAP2 was shown to interact with miR-661, a regulator of MTA1 which has been implicated in metastasis[100]. CircKIF4A expression was also upregulated in triple negative breast cancer, correlating with worse disease-free and overall survival. Functionally, circKIF4A induces proliferation and metastasis by sponging miR-375[101]. Elevated CircAGFG1 expression indicated triple negative breast cancer and worse overall survival. CircAGFG1 was found to increase cell migration, invasion, tumorigenesis, metastasis, and angiogenesis by sponging miR-195-5p and indirectly regulating CCNE1[102]. Elevated CircUBE2D2 expression also correlated with worse overall and disease-free breast cancer survival as a miR-1236 and miR-1287 sponge[103].

Conversely, high circVRK1 expression significantly correlated with improved overall survival and negatively correlated with tumor size and stage. CircVRK1 was not associated with any breast cancer subtype. Increased circVRK1 expression induced apoptosis in vitro[104]. CircLARP4 is similarly favorably prognostic, with improved disease-free and overall survival as well as decreased tumor size and stage. Expression was comparatively decreased in breast cancer compared to normal tissue but not correlated with any subtype[105]. CircLARP4 has also been identified as favorably prognostic and potential tumor suppressor in gastric cancer[106], hepatocellular carcinoma[107], ovarian cancer[108], and osteosarcoma[109].

Lung Cancer

In non-small cell lung cancer (NSCLC), circSNAP47 expression resulted in decreased overall survival and significantly correlated with metastasis through the miR-1287/GAGE axis[110]. Similarly,
circZKSCAN1 exhibited decreased overall survival and higher stage (II vs. I) but not lymph node metastasis with increased expression in NSCLC. This circRNA sponged miR-330-5p, thus increasing FAM83A expression and regulating MAPK/ERK signal transduction[98]. CircDDX42[111] and circARHGAP10[71] also correlated with worse NSCLC overall survival.

CircSHPRH is associated with favorable NSCLC prognosis, including improved overall survival and downregulated metastasis. MicroRNA targets include miR-331-3p and miR-338-5p[112]. In lung adenocarcinoma, high circBCAR3 expression correlated with indicated improved overall survival, whereas low expression correlated with advanced stage and lymph node metastasis. CircBCAR3 sponged miR-6783-3p to increase DKK1 expression and inhibit Wnt/β-catenin signaling[113].

In small cell lung cancer, high expression of two circularized FLI1 isoforms correlated with metastasis. Patients with exosomal isoform (FECR1) had higher rates of extensive stage disease and experienced worse disease-free survival after remission. This circRNA regulated the miR-584/ROCK1 pathway, associated with metastasis[114].

**Gastric Cancer**

CircAGO2 expression significantly correlated with decreased gastric cancer survival. CircAGO2 interacted with HuR to diminish miRNA gene silencing and promote tumorigenesis[115]. In gastric cancer, circPRMT5 functions as an oncogene, sponging miR-145 and miR-1304, thereby upregulating myc and decreasing overall survival when highly expressed. CircPRMT5 knockdown reduced invasion in vitro, which partially reversed with myc overexpression[116]. Expression of CircLMTK2, a miR-150-5p sponge, significantly correlated with worse overall survival, higher stage, and lymph node metastasis[117]. CircHIPK3 was also significantly associated with worse overall gastric cancer survival by inhibiting Wnt/β-catenin signaling[118].

PVT1 is a long-noncoding RNA often coexpressed with myc[119]. In gastric cancer, circPVT1 was upregulated with favorable disease-free and overall survival following resection. Higher stage tumors and those with perineural invasion expressed less circPVT1. Stratified survival analysis revealed that the subset with high circPVT1 and low linear PVT1 expression exhibited the most favorable overall and disease-free survival[120]. CircYAP1, which sponged miR-367-5p to upregulate p27kip1, associated with improved overall survival in early and late stage gastric cancers[121].

**Colorectal Cancer**

Two miR-7 sponges, Cdr1as and CircHIPK3 were prognostic of poor colorectal cancer overall survival[122, 123]. Moreover, Cdr1as expression is prognostic for advanced tumor stage, metastatic disease, and overexpression resulted in increased EGFR and RAF1[122]. CircCCDC66 expression was higher in tumor compared to precancerous polyps and indicated poor overall survival, whereas linear CCDC66 was not prognostic. CircCCDC66 overexpression increased myc expression[124]. CircPVT1 expression significantly correlated with liver and lymph node metastases and worse overall colorectal cancer survival, unlike in gastric cancer[125]. CircSLC30A7 also significantly correlated with reduced overall survival via the miR-516b/FZD4 axis upstream of Wnt/β-catenin, a colorectal cancer
developmental pathway[126]. CircPPP1R12A expression also signified poor overall colon cancer survival. CircPPP1R12A reportedly encoded a protein that promoted colon cancer cell proliferation, migration, and invasion via the Hippo-Yap pathway[48].

High circCCT3 expression resulted in significantly improved overall survival following surgery and was suggested to impact p16 downstream[127]. CircACVRL1 expression significantly correlated to lower stage, decreased lymph node metastasis, and improved overall survival. Potential targets include miR-21 and miR-31[128]. CircMTO1 also carried favorable prognosis with downstream effects on Wnt/β-catenin signaling[129].

**Hepatocellular Carcinoma**

Elevated circRHO1T expression indicated advanced disease and worse overall and recurrence-free survival following hepatectomy. CircRHO1T had a reported direct interaction with TIP60 to regulate NR2F6 expression and the NOTCH2 pathway downstream[130]. High circSNX27 expression significantly correlated with poor survival[68]. CircSNX27 sponged miR-141-3p with downstream effects on the mTOR pathway in hepatitis B-associated HCC[131]. CircZFR expression also correlated with poor survival via Wnt/β-catenin signaling[132]. A circSCD isoform demonstrated significantly decreased recurrence-free and overall survival. This isoform interacts directly with RBM3, an RBP upregulated by hypoxia and chronic inflammation[133]. Three circPTGR1 isoforms correlated with inferior HCC survival and with MET expression to mediate metastasis[134]. Upregulated circCul2 expression also correlated with poor overall survival in conjunction with Twist1, a key mediator of epithelial mesenchymal transition (EMT)[135].

CircSMARCA5 was significantly associated with decreased tumor size, grade, stage, and improved overall and recurrence-free survival after hepatectomy. This circRNA sponged miR-17-3p and miR-181b-5p to impact tumor suppressor TIMP3 downstream[136]. CircADAMTS13 was downregulated in HCC and resulted in improved recurrence-free survival. CircADAMTS13 sponged miR-484, a mediator of hepatocyte malignant transformation[137]. CircMTO1 expression also correlated with improved overall HCC survival via miR-9/p21[138]. CircITCH, which impacts Wnt/β-catenin signaling, was also associated with improved HCC survival[139]. High CircTRIM33-12 expression also significantly resulted in improved overall survival and recurrence-free survival following surgery via miR-191/TET1[140].

**Bladder Cancer**

Elevated circMYLK expression indicated worse bladder survival. CircMYLK directly bound miR-29a, thus regulating VEGFA expression, EMT, and RAS/ERK signaling[141]. CircBPTF expression also significantly correlated with worse overall survival, and expression was higher in muscle invasive than non-muscle invasive bladder cancer via miR-31-5p/RAB27B[142]. CircTFRC was more expressed in higher grade bladder cancer and associated with reduced survival. This circRNA sponged for miR-107 and induced EMT through TGFβ downstream[143].

Thirteen circRNAs predicted risk of progression from non-muscle invasive to muscle invasive bladder cancer. Four of these circRNAs exhibited higher expression than corresponding linear
transcripts. CircHIPK3 and circCDYL significantly demonstrated decreased progression risk, which was independent of parental linear transcript expression[144]. CircITCH expression is associated with improved bladder cancer survival. Reported miRNA targets are miR-17 and miR-224, which regulate p21 and PTEN to drive tumor progression[39]. CircSLC8A1 also reportedly regulated PTEN by sponging miR-130b and miR-494 to reduce bladder cancer progression[145]. CircMTO1 expression significantly correlated with improved overall and disease-free bladder cancer survival. In vitro, circMTO1 reduced bladder cell invasion by sponging miR-221 and inhibiting EMT[146]. CircUBXN7 expression also results in significantly improved overall survival, whereas linear UBXN7 mRNA is not prognostic. CircUBXN7 suppressed miR-1247-3p, thus promoting B4GALT3 expression[147]. A poor prognostic marker in lung cancer, high circZKSCAN1 expression correlated with improved overall and disease-free bladder cancer survival via miR-1178-3p/p21[99]. In muscle-invasive bladder cancer circLPAR1 expression correlated to improved disease-specific survival and targeted four unique miRNAs[148].

Prostate Cancer

Compared with castration resistant prostate cancer, circAURKA was upregulated and circAMACR was downregulated in neuroendocrine prostate cancer, a rare and aggressive subtype[53]. High circHIPK3 expression also indicated worse prostate cancer prognosis and advanced tumor stage through interaction with the miR-193a-3p/MCL1 axis[149]. In one study abundance or paucity of overexpressed circRNAs was a poor prognostic factor. Here, a circRNA index (CRI) was calculated to reflect the number of overexpressed circRNAs. In an intermediate risk localized prostate cancer cohort, the combined subset of patients with the lowest and highest CRI quartiles had worse biochemical recurrence-free interval than patients with intermediate CRI[87].

Higher CircITCH expression was associated with lower stage, decreased lymph node metastasis, and improved disease-free and overall survival[150]. CircMTO1 expression also correlated to favorable prostate cancer disease-free and overall survival and targeted miR-17-5p[151].

Ovarian Cancer

Elevated CircHIPK3 expression signified worse disease-free and overall ovarian cancer survival, higher FIGO stage, and lymph node invasion[152]. CircPIP5K1A expression also significantly correlated with worse overall ovarian cancer survival via miR-661/IGFBP5[153]. Elevated circABCB10 expression indicated higher grade, larger tumor size, and worse overall survival. Potential miRNA targets include miR-1271, miR-1252, and miR-203[154]. High circFAM53B also resulted in decreased overall survival. This circRNA sponged miR-646 and miR-647 to increase VAMP2 and MDM2 expression, respectively[155].

CircPLEKH3 sponged miR-9 to increase wild-type BRCA1, resulting in improved overall and recurrence-free survival. Other miR-9 targets included KLF4 and DNAJ8B6, with β-catenin and AKT1 downstream[156]. CircITCH is favorably prognostic in ovarian cancer via miR-145/RASA1[157].

CircRNA Predictive Biomarkers
CircRNAs have also been reported that predict response and resistance to multiple cancer treatment modalities. Therefore, they may prove clinically valuable for personalizing treatment to achieve optimal outcomes with fewer toxicities.

**Radiation**

Analysis of esophageal squamous cell carcinoma radioresistant and radiosensitive cell lines revealed 74 differentially expressed circRNAs, of which nine were validated by qPCR. Several circRNA downregulated in resistant cells affected Wnt signaling downstream[158]. Further evidence of circRNA conferring radiation resistance through this pathway includes circDCAF8 sponging miR-217 to regulate Wnt3 in radioresistant esophageal cancer cells[159]. In cervical cancer HeLa cells, RNA-seq on irradiated cells identified 153 differentially expressed circRNA targets, most commonly affecting MAP kinase signaling[160]. In NSCLC, Cdr1as was shown to inhibit radioresistant effects of miR-1246[161], and circMTDH4 promoted radiation resistance in lung cancer cell lines through the miR-630/AEG-1 axis[162].

Predictive circRNA have also been identified in irradiated cancer tissues. In radiosensitive and radioresistant colon cancer tissues, circCCDC66 expression correlated with resistance whereas its target, miR-338-3p associated with radiosensitivity. In vitro, circCCDC66 expression increased with higher radiation doses, whereas CCDC66 knockdown induced apoptosis through caspase-3[163]. In nasopharyngeal cancer, circHIPK3 expression was increased in radioresistant tissues[164]. CircMALAT1 expression was also increased in radioresistant nasopharyngeal tissue and cell lines via miR-9/PDGFRA[165]. Also in nasopharyngeal cancer, curcumin generated radiosensitization through circRNA regulation[166]. CircAKT3 has been associated with protein translation to decrease glioblastoma radioresistance[167].

**Chemotherapy**

Platinum-based chemotherapies induce DNA damage to kill tumor cells. CircPVT1 is implicated in cisplatin resistance in gastric cancer, NSCLC, and osteosarcoma. In lung adenocarcinoma, circPVT1 was upregulated in cisplatin and pemetrexed resistant cancer cells through the miR-145-5p/ABCC1 axis, and higher ABCC1 expression resulted in worse prognosis[168]. CircPVT1 was also associated with doxorubicin and cisplatin resistance in osteosarcoma by upregulating ABCB1, previously implicated in drug efflux and multidrug resistance[169]. Cdr1as has been associated with cisplatin resistance and sensitivity. In lung cancer cells, Cdr1as was associated with cisplatin and pemetrexed resistance, which was reversed with EGFR overexpression[170]. Alternatively in bladder cancer, Cdr1as expression demonstrated improved cisplatin response and induced apoptosis via miR-1270/APAF1[171]. In ovarian cancer, Cdr1as was downregulated in cisplatin-resistant tissue and directly interacted with miR-1270 to increase SCAI[172]. CircAKT3 has also been associated with cisplatin resistance in lung cancer via miR-516b-5p/STAT3[173] and in gastric cancer via miR-198/PIK3R1[174]. CircPGC also increases cisplatin resistance via the STAT3 pathway in NSCLC by sponging miR-296-5p[175]. CircFNTA activates KRAS signaling through interaction with miR-370-3p to inhibit apoptosis and cisplatin response and is regulated by androgen receptor[176]. Other circRNA mediating cisplatin resistance include circHIPK3 and circELP3 in bladder cancer[177, 178], circZFR in NSCLC [179], circEIF6 in anaplastic thyroid...
cancer[180], and circFN1 in gastric cancer[181]. In microarray expression analysis of colon cancer cells exposed to 5-FU and oxaliplatin, resistant cells displayed 773 upregulated and 732 downregulated circRNAs. CircSATB1 was the most upregulated circRNA[182]. CircCCDC66 was more highly expressed in oxaliplatin-resistant colorectal cancer cells, with expression induced by DHX9 phosphorylation after oxaliplatin treatment[183]. CIRS-122 generated oxaliplatin resistance in colorectal cancer cells by exosomal delivery via the miR-122/PKM2 axis[184]. In HCC, circFBXO11 induced oxaliplatin resistance by targeting miR-605/FOXO3 to promote ABCB1 transcription[185]. Conversely, CircFAM114A2 promotes oxaliplatin sensitivity in gastric cancer cells by sponging miR-421 to upregulate ATM expression[186].

Doxorubicin is an anthracycline topoisomerase inhibitor. Resistant breast cancer tissues and cells demonstrated higher circPRELID2 expression via miR-7-5p/RAF1 to upregulate downstream MEK/ERK signaling[187]. CircLARP4 is associated with enhanced doxorubicin sensitivity in breast cancer[105] and osteosarcoma but not methotrexate sensitivity[109]. CircKDM4C is another potential biomarker of doxorubicin sensitivity via the miR-548p/PBLD axis[188].

CircRNAs have predicted taxane sensitivity and resistance. In analysis of paclitaxel resistant lung cancer cells, 11,281 circRNAs were differentially expressed with 2909 circRNAs upregulated[189]. In lung adenocarcinoma, circARFGEF2 was implicated in miR-326 mediated docetaxel resistance[190]. CircPVT1 expression in gastric cancer tissues predicted paclitaxel resistance. In vitro, circPVT1 inhibited miR-124-3p to increase ZEB1 expression to promote EMT[191]. In breast cancer, circABCB10 expression was comparatively higher in paclitaxel-resistant tissues. In cells, circABCB10 inhibited miRNA let-7a-5p to increase expression of DUSP7, a MAP kinase inhibitor[192]. CircAMOTL1 was also implicated in paclitaxel resistance in breast cancer cells by binding and inhibiting AKT phosphorylation[193]. In ovarian cancer, circCERS1 expression was increased in paclitaxel-resistant tissue via miR-1252/FOXR2[194]. In nasopharyngeal cancer, circCRIM1 predicts docetaxel resistance to docetaxel via the miR-422a/FOXQ1 axis[195]. Conversely, circPTK2 expression correlates with taxane chemosensitivity in NSCLC. This circRNA sponges multiple miRNAs and enhances paclitaxel sensitivity by inhibiting miR-182-5p to regulate GRB2, FOXO1, and FOXO3 downstream[196]. CircFOXO3 expression reduced docetaxel resistance in vitro and in vivo and enhanced linear FOXO3 expression[197].

CircRNA also predicts gemcitabine response in pancreatic and bladder cancer. Two studies evaluated differential circRNA expression in gemcitabine resistant pancreatic cancer cell lines. 26 circRNAs were upregulated and 55 downregulated circRNAs in one study and 68 upregulated and 58 downregulated circRNAs in the other[198, 199]. CircHIPK3 expression was higher in gemcitabine-resistant pancreatic cancer tissues. In cells, circHIPK3 sponged miR-330-5p to upregulate RASSF1 expression[200]. Conversely, circHIPK3 overexpression resensitized previously gemcitabine resistant bladder cancer cells[201]. Likewise, circSMARCA5 overexpression enhanced chemosensitivity in gemcitabine and cisplatin-treated lung cancer cells[202].

**Targeted Therapies**

CircRNAs have also been implicated in resistance to molecularly targeted agents. Microarray-based expression analysis on two lung cancer cell lines resistant to osimertinib, a third generation EGFR tyrosine kinase inhibitor (TKI), revealed 7966 upregulated and 7538 downregulated circRNAs. The most highly differentially expressed circRNA mediated effects on p53 and mTOR, both previously implicated in
CircCCDC66 was highly expressed in EGFR-mutated resistant cell lines[204]. CircCDK14 overexpression in lung cancer cells conferred resistance to gefitinib, another EGFR-targeting TKI, via the miR-1183/PDK1 axis[205]. Alternatively, expression analysis on serum from gefitinib-sensitive NSCLC patients revealed circZNF117 and circZNF91 overexpression, correlating with improved progression free survival[206]. In oral squamous cell carcinoma, circGDI2 overexpression increased sensitivity to cetuximab, an anti-EGFR antibody, by promoting apoptosis and regulating EGFR expression[207].

Immune checkpoint inhibition treats multiple cancers, but patients often develop resistance. In NSCLC, patients who progressed on anti-PD-1 therapy exhibited comparatively higher circFGFR1 expression. CircFGFR1 increases CXCR4 expression, thereby reducing CD8+ T-cell recruitment[208]. In pancreatic cancer, four circRNAs (circUBAP2, circCLEC17A, circHIBADH, and circTADA2A) regulate CXCR4 and ZEB1, two proteins that correlated with CTLA4 and PD-1 expression[209].

**Endocrine Therapies**

CircRNAs have been proposed that predict endocrine therapy response in breast, ovarian, and prostate cancer. Higher circCNOT2 expression in breast cancer indicated earlier progression on aromatase inhibitors, whereas linear CNOT2 was not predictive[95]. CircGAPDH also induces tamoxifen sensitization in vitro and in vivo through miR-182-5p/FOXO3[210].

Enzalutamide is an antiandrogen that treats prostate cancer. Screening circRNAs in enzalutamide resistant cells revealed 230 upregulated and 465 downregulated circRNAs in a highly resistant clone and 60 upregulated and 175 downregulated circRNAs in a moderately resistant clone. One downregulated circRNA, CircRBM39, is derived from a parental gene in the U2AF65 family that regulates ARv7, previously described in enzalutamide resistance[211]. CircRNA17 expression is decreased in high grade prostate cancer and also decreases enzalutamide resistance by enhancing miR-181c-5p stability to modulate ARv7 expression[212].

**Treatment Side Effects**

CircRNAs may detect adverse treatment toxicities, including doxorubicin-mediated cardiotoxicity. CircTTN, CircFHOD, and CircSTRN3 promote protective effects of QKI against doxorubicin induced cardiotoxicity[213]. CircPan3 was also downregulated along with QKI in a model of doxorubicin cardiotoxicity, whereas miR-31-5p was upregulated[214]. In a model of cisplatin-induced acute kidney injury, circZNF644 expression was upregulated via miR-494/ATF3 with downstream effects on IL-6, a pro-inflammatory factor[215]. High circRSF1 expression may signify radiation-induced hepatic injury by sponging miR-146a-5p to increase proinflammatory cytokines in irradiated liver cells[216]. Microarray analysis for circRNA in irradiated hepatic stellate cells found 179 upregulated and 630 downregulated circRNAs, of which circPALLD inhibits proliferation after radiation[217].

**CircRNA Non-Invasive Detection**
Perhaps the greatest cancer biomarker potential for circRNA is non-invasive detection with clinical implications for early detection and serial monitoring. Advantages of using circRNA for early cancer detection include stability, lineage specificity, conservation, and abundance. Due to exoribonuclease degradation resistance, circRNA is more stable than corresponding linear mRNA. CircRNA has been detected in exosomes, plasma, saliva, and urine[53, 218, 219]. CircRNA could also potentially facilitate a more accurate diagnostic platform than circulating tumor DNA. One circulating tumor DNA early detection platform, CancerSEEK, demonstrated strong sensitivity for some cancers but inaccuracy for others, especially earlier stage cancers[220]. Utilization of cancer-specific circRNA may overcome these inaccuracies. Challenges in developing plasma circRNA-based detection assays include selecting abundantly expressed backspliced isoforms and determining clinically relevant targets, given variable expression between circRNA and parental linear transcripts[95]. Nonetheless, non-invasively detected cancer-specific circRNA have been reported.

**CircRNA and Early Detection**

Several circRNA biomarkers for early cancer detection have been proposed. Sensitivity and specificity at a determined cutoff point and area under the receiver operative curve (AUC) for non-invasively detected circRNA biomarkers across several cancers is represented in Table 3. In NSCLC, F-circEA is generated from backsplicing EML4-ALK fusion gene exons. This circRNA was detected in EML4-ALK positive lung cancer plasma, whereas the corresponding linear mRNA was not[221]. In plasma from 153 lung cancer patients, 83 of whom had stage I disease, circYWHAZ and circBNC2 exhibited differential expression in cancer patients compared to healthy controls. Sensitivity and specificity AUC for lung cancer detection using both circRNA was 0.81 for the entire cohort and 0.83 for stage I patients[222]. CircFARSA also has been detected in NSCLC patient plasma. Expression was upregulated in cancer and moderate correlation was observed between plasma and lung tissue circFARSA expression (\(\rho = 0.64\)). Sensitivity and specificity AUC for lung cancer detection was 0.71[70]. Another circRNA biomarker proposed for early lung adenocarcinoma detection is circACP6. This circRNA exhibits upregulated expression in lung adenocarcinoma, with reported sensitivity and specificity AUC 0.794 for plasma-based lung adenocarcinoma identification[69]. As lung cancer does not currently have clinically useful non-invasive biomarkers for early detection, plasma circRNA biomarkers are promising.

Although serum breast cancer biomarkers exist, they are not commonly used for early detection. CircELP3 is a proposed plasma breast cancer biomarker, as expression was significantly increased compared to normal controls. In a cohort of 57 breast cancer patients and 17 age-matched healthy controls, sensitivity and specificity AUC of circELP3 expression was 0.784, compared to CEA (AUC 0.562) and CA15-3 (AUC 0.629). Sensitivity and specificity improved to AUC 0.839 with combined circRNA and protein biomarkers[223].

One study performed RNA-seq on serum from 11 colorectal cancer patients and healthy controls. Compared to healthy controls, colorectal cancer serum contained 257 likely cancer-specific circRNAs, 53 of which correlated to known genes upregulated in colorectal cancer tissues. Among these, circKLHDC10 was further validated as significantly cancer-specific[218]. A microarray study analyzed circRNA in plasma from 156 colorectal cancer patients, including 66 stage I patients. 204 differentially expressed circRNA between cancer and normal plasma were identified, of which 178 were upregulated. Further qPCR identification identified two upregulated circRNAs, circFAM71F2 and circFLI1, and one
downregulated circRNA, circALDH1A2, in serum from stage I colorectal cancer patients compared to normal controls. All three circRNA biomarkers combined had the highest sensitivity and specificity for non-invasive colorectal cancer detection[224]. CircRNA expression analysis in colorectal cancer patient plasma found decreased circCCDC66, circABCC1, and circSTIL expression compared to healthy controls. In a separate validation cohort, these circRNA markers yielded better sensitivity and specificity for detection than CEA. Sensitivity and specificity of the assay improved to AUC 0.855 by combining these circRNA biomarkers with CEA. These circRNAs retained diagnostic value in subanalysis of early stage, CEA-negative, and CA19-9 negative colorectal colon cancer[225].

Differential expression analysis on blood from gastric cancer patients found 172 upregulated and 171 downregulated circRNAs, of which only seventeen were differentially expressed in gastric cancer tissue. Furthermore, plasma expression of two downregulated circRNAs, circXPO1 and circNRP1, was validated by droplet-digital RT-PCR measurement. When combined, their specificity and sensitivity AUC for diagnosing gastric cancer in serum was 0.912, which was superior to the same assay in tissue (AUC 0.779)[75]. A diagnostic assay with circLMO1 and circUBXN7 also distinguished gastric cancer patients from controls. When combined with CEA, sensitivity and specificity AUC was 0.7988[226]. Individual circRNAs investigated for non-invasive gastric cancer diagnosis include circRPL6, which had downregulated expression with sensitivity and specificity AUC 0.733. When combined with protein biomarkers CEA, CA19-9, and CA72-4, AUC improved to 0.825. In patients exhibiting normal protein biomarker levels, sensitivity and specificity was inferior (AUC 0.692), indicating potential for circRNA to detect cancer underdiagnosed by conventional means[227]. CircCNIH4 was also downregulated in serum and tissue from gastric cancer patients compared to normal controls, although sensitivity and specificity for cancer detection was higher for tissue than plasma[228]. Plasma circSPECC1 expression was also significantly different between cancer patients and controls with AUC 0.775 in combination with CEA[229]. Another reported early gastric cancer detection biomarker is circTATDN3. Expression was significantly decreased in gastric cancer patients with cutoff point sensitivity 99.0% but poor specificity at 20.6% (AUC 0.582)[230].

In pancreatic cancer, circLDLRAD3 has been evaluated for non-invasive detection. In plasma from 31 pancreatic cancer patients, circLDLRAD3 expression was upregulated compared to healthy controls. Plasma expression level correlated with CA19-9, metastasis, lymphatic and venous invasion, and stage. Independently, circLDLRAD3 had sensitivity and specificity AUC 0.67 for non-invasive detection. Combining circLDLRAD3 with CA19-9 improved sensitivity and specificity of this established pancreatic cancer protein biomarker from AUC 0.83 to 0.87[231].

In hepatocellular carcinoma (HCC), a validated diagnostic cohort with three circRNA biomarkers, circHPCAL1, circRABGGTA, and circMTM1, was developed to distinguish cancer patients from healthy and HCC precursor (hepatitis B and cirrhosis) control patients. This panel outperformed AFP in distinguishing HCC from non-HCC in two validation sets, and accuracy further improved with circRNAs and AFP combined. The circRNA panel also had high diagnostic accuracy in detecting small HCC tumors and AFP-negative HCC, often missed by conventional diagnostic techniques[232]. In another study, CircSMARCA5 expression was significantly decreased in HCC patients compared to healthy and HCC precursor control patients. Sensitivity and specificity AUC for HCC detection compared to healthy controls was 0.938, although the assay was less specific and sensitive when differentiating HCC from precursor diseases. Sensitivity and specificity AUC of circSMARCA5 expression in patients with AFP < 200 ng/mL compared to hepatitis and cirrhosis patients was 0.847 and 0.706, respectively. Therefore, this
noninvasive marker could detect patients who may not be diagnosed otherwise[233]. Another circSMARCA5 expression assay also found decreased plasma expression in HCC patients. Sensitivity and specificity AUC for HCC diagnosis compared to healthy controls, hepatitis B, and cirrhosis patients was 0.970, 0.877, and 0.743, respectively[234].

CircRNA specific to genitourinary tumors can be detected in urine. In a study supported by the early detection research network (EDRN), exome capture RNA-seq on urine obtained from prostate cancer patients detected 6788 circRNAs, 1092 of which were detected in prostate cancer tissue[53]. In bladder cancer, circPRMT5 was detected in urinary exosomes, and higher expression positively correlated with lymph node metastasis and tumor progression[235].

Noninvasive circRNA detection can occur in saliva for head and neck cancers. In a study of 93 oral cancer patients and healthy controls, differential expression was detected in 32 circRNA, with 12 upregulated and 20 downregulated. Two circRNAs, circBICD2 and circFAM126A, effectively differentiated between oral cancer and oral leukoplakia. These two circRNA combined demonstrated excellent sensitivity and specificity for cancer diagnosis (AUC 0.895)[236].

CircRNA and Disease Monitoring

Another potential use for circRNA biomarkers is non-invasive monitoring for recurrent and progressive disease. When CircRNA expression normalizes post-operatively, subsequent significant changes in expression may indicate recurrent disease[223, 224, 226, 227, 236]. A validated circRNA model was developed to predict postoperative recurrence in stage II and III colon cancer. Candidate circRNAs were determined by RNA-seq on resection tissue from known recurrences and nonrecurrences. Four circRNAs, circPLOD2, circAGTPBP1, circISPD, and circPRKAR1B, comprised a recurrence risk score that predicted post-surgical disease-free and overall survival, which could potentially guide decisions about adjuvant therapy[237]. A model of stage III gastric cancer recurrence utilized four circRNAs, circTMCO3, circCDK14, circNEK6, and circLPHN2, determined by differential expression on tissue. This model’s prediction of disease recurrence within one year had sensitivity and specificity AUC 0.711 in the validation set, which improved to 0.818 when combining circRNAs and tumor stage[238].

CircRNA may also predict progression on chemotherapy in non-invasive assays. Differential circRNA expression on plasma from gemcitabine sensitive and resistant patients revealed two circRNAs, circSNORD114-1 and circDCUN1D4, that predicted resistance[199]. In serum exosomes derived from ovarian cancer patients, Cdr1as expression level was significantly higher in cisplatin-sensitive patients than resistant patients[172]. Therefore, circRNA biomarkers could help determine development of chemotherapy resistance in cases where progression is otherwise clinically uncertain.
Discussion

CircRNA has emerged as an intriguing multifaceted cancer biomarker. Inherent biologic properties, including stability due to exoribonuclease resistance and potential for tissue specificity underlie the potential for utilizing circRNA in non-invasive detection, likely its most useful application. Hundreds of potential prognostic, predictive, and diagnostic circRNA biomarkers have been described. Several of these, including Cdr1as, circITCH, circPVT1, and circHIPK3 are expressed in multiple cancers. Some circRNAs also have divergent implications among cancer subtypes, for example circPVT1, which signifies good gastric cancer prognosis and poor colorectal cancer prognosis. Further studies are necessary to independently verify these trends and better understand the biological mechanisms behind each circRNA. Moreover, it remains unclear which reported circRNAs are truly tissue and cancer specific. While specific circRNA/miRNA/mRNA interactions have been described, many circRNAs have multiple purported miRNA binding sites and can target multiple genes. The functional relationship between circRNAs and parental mRNA needs to be further clarified, given their variable correlation. While circRNAs offer great promise as cancer biomarkers, especially for non-invasive detection, prospective validation is necessary before clinical application is feasible.
Acknowledgments

This work was supported by the Prostate Cancer Foundation (PCF), Early Detection Research Network (U01 CA214170) and NCI Prostate SPORE (P50 CA186786). A.M.C. is an NCI Outstanding Investigator, Howard Hughes Medical Institute Investigator, A. Alfred Taubman Scholar, and American Cancer Society Professor.
References

1. Sanger, H.L., et al., *Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures.* Proc Natl Acad Sci U S A, 1976. 73(11): p. 3852-6.

2. Hsu, M.T. and M. Coca-Prados, *Electron microscopic evidence for the circular form of RNA in the cytoplasm of eukaryotic cells.* Nature, 1979. 280(5720): p. 339-40.

3. Cocquerelle, C., et al., *Mis-splicing yields circular RNA molecules.* FASEB J, 1993. 7(1): p. 155-60.

4. Nigro, J.M., et al., *Scrambled exons.* Cell, 1991. 64(3): p. 607-13.

5. Capel, B., et al., *Circular transcripts of the testis-determining gene Sry in adult mouse testis.* Cell, 1993. 73(5): p. 1019-30.

6. Pasman, Z., M.D. Been, and M.A. Garcia-Blanco, *Exon circularization in mammalian nuclear extracts.* RNA, 1996. 2(6): p. 603-10.

7. Salzman, J., et al., *Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types.* PLoS One, 2012. 7(2): p. e30733.

8. Jeck, W.R., et al., *Circular RNAs are abundant, conserved, and associated with ALU repeats.* RNA, 2013. 19(2): p. 141-57.

9. Memczak, S., et al., *Circular RNAs are a large class of animal RNAs with regulatory potency.* Nature, 2013. 495(7441): p. 333-8.

10. Zhang, Y., et al., *Circular intronic long noncoding RNAs.* Mol Cell, 2013. 51(6): p. 792-806.

11. Zhou, B. and J.W. Yu, *A novel identified circular RNA, circRNA_010567, promotes myocardial fibrosis via suppressing miR-141 by targeting TGF-beta1.* Biochem Biophys Res Commun, 2017. 487(4): p. 769-775.

12. Burd, C.E., et al., *Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk.* PLoS Genet, 2010. 6(12): p. e1001233.

13. Zhao, Y., et al., *Deficiency in the Ubiquitin Conjugating Enzyme UBE2A in Alzheimer's Disease (AD) is Linked to Deficits in a Natural Circular miRNA-7 Sponge (circRNA; ciRS-7).* Genes (Basel), 2016. 7(12).

14. Chen, B.J., et al., *Characterization of circular RNAs landscape in multiple system atrophy brain.* J Neurochem, 2016. 139(3): p. 485-496.

15. Stoll, L., et al., *Circular RNAs as novel regulators of beta-cell functions in normal and disease conditions.* Mol Metab, 2018. 9: p. 69-83.

16. Hansen, T.B., J. Kjems, and C.K. Damgaard, *Circular RNA and miR-7 in cancer.* Cancer Res, 2013. 73(18): p. 5609-12.

17. Kristensen, L.S., et al., *Circular RNAs in cancer: opportunities and challenges in the field.* Oncogene, 2018. 37(5): p. 555-565.

18. Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation.* Cell, 2011. 144(5): p. 646-74.

19. Su, M., et al., *Circular RNAs in Cancer: emerging functions in hallmarks, stemness, resistance and roles as potential biomarkers.* Mol Cancer, 2019. 18(1): p. 90.

20. Li, F., et al., *Circular RNA ITCH has inhibitory effect on ESCC by suppressing the Wnt/beta-catenin pathway.* Oncotarget, 2015. 6(8): p. 6001-13.

21. Pan, H., et al., *Overexpression of Circular RNA ciRS-7 Abrogates the Tumor Suppressive Effect of miR-7 on Gastric Cancer via PTEN/PI3K/AKT Signaling Pathway.* J Cell Biochem, 2018. 119(1): p. 440-446.

22. Xu, H., et al., *NFIx Circular RNA Promotes Glioma Progression by Regulating miR-34a-5p via Notch Signaling Pathway.* Front Mol Neurosci, 2018. 11: p. 225.
23. Chen, L.L. and L. Yang, *Regulation of circRNA biogenesis*. RNA Biol, 2015. 12(4): p. 381-8.
24. Wang, Y. and Z. Wang, *Efficient backsplicing produces translatable circular mRNAs*. RNA, 2015. 21(2): p. 172-9.
25. Salzman, J., et al., *Cell-type specific features of circular RNA expression*. PLoS Genet, 2013. 9(9): p. e1003777.
26. Liang, D. and J.E. Wilusz, *Short intronic repeat sequences facilitate circular RNA production*. Genes Dev, 2014. 28(20): p. 2233-47.
27. Ivanov, A., et al., *Analysis of intron sequences reveals hallmarks of circular RNA biogenesis in animals*. Cell Rep, 2015. 10(2): p. 170-7.
28. Conn, S.J., et al., *The RNA binding protein quaking regulates formation of circRNAs*. Cell, 2015. 160(6): p. 1125-34.
29. Li, X., et al., *Coordinated circRNA Biogenesis and Function with NF90/NF110 in Viral Infection*. Mol Cell, 2017. 67(2): p. 214-227 e7.
30. Barrett, S.P., P.L. Wang, and J. Salzman, *Circular RNA biogenesis can proceed through an exon-containing lariat precursor*. Elife, 2015. 4: p. e07540.
31. Xia, S., et al., *Comprehensive characterization of tissue-specific circular RNAs in the human and mouse genomes*. Brief Bioinform, 2017. 18(6): p. 984-992.
32. Westholm, J.O., et al., *Genome-wide analysis of drosophila circular RNAs reveals their structural and sequence properties and age-dependent neural accumulation*. Cell Rep, 2014. 9(5): p. 1966-1980.
33. Jeck, W.R. and N.E. Sharpless, *Detecting and characterizing circular RNAs*. Nat Biotechnol, 2014. 32(5): p. 453-61.
34. Suzuki, H. and T. Tsukahara, *A view of pre-mRNA splicing from RNase R resistant RNAs*. Int J Mol Sci, 2014. 15(6): p. 9331-42.
35. Yu, L., et al., *The Circular RNA Cdr1as Act as an Oncogene in Hepatocellular Carcinoma through Targeting miR-7 Expression*. PLoS One, 2016. 11(7): p. e0158347.
36. Du, W.W., et al., *Induction of tumor apoptosis through a circular RNA enhancing Foxo3 activity*. Cell Death Differ, 2017. 24(2): p. 357-370.
37. Chen, C.Y. and P. Sarnow, *Initiation of protein synthesis by the eukaryotic translational apparatus on circular RNAs*. Science, 1995. 268(5209): p. 415-7.
47. Yang, Y., et al., Novel Role of FBXW7 Circular RNA in Repressing Glioma Tumorigenesis. J Natl Cancer Inst, 2018. 110(3).

48. Zheng, X., et al., A novel protein encoded by a circular RNA circPPP1R12A promotes tumor pathogenesis and metastasis of colon cancer via Hippo-YAP signaling. Mol Cancer, 2019. 18(1): p. 47.

49. Wang, Z., M. Gerstein, and M. Snyder, RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet, 2009. 10(1): p. 57-63.

50. Xiao, M.S. and J.E. Wilusz, An improved method for circular RNA purification using RNase R that efficiently removes linear RNAs containing G-quadruplexes or structured 3’ ends. Nucleic Acids Res, 2019. 47(16): p. 8755-8769.

51. Giannoukos, G., et al., Efficient and robust RNA-seq process for cultured bacteria and complex community transcriptomes. Genome Biol, 2012. 13(3): p. R23.

52. Cieslik, M., et al., The use of exome capture RNA-seq for highly degraded RNA with application to clinical cancer sequencing. Genome Res, 2015. 25(9): p. 1372-81.

53. Vo, J.N., et al., The Landscape of Circular RNA in Cancer. Cell, 2019. 176(4): p. 869-881 e13.

54. Qu, S., et al., Microarray expression profile of circular RNAs in human pancreatic ductal adenocarcinoma. Genom Data, 2015. 5: p. 385-7.

55. Cieslik, M., et al., Circular RNAs: Methodological challenges and perspectives in cardiovascular diseases. J Cell Mol Med, 2018. 22(11): p. 5176-5187.

56. Shao, Y., et al., Global circular RNA expression profile of human gastric cancer and its clinical significance. Cancer Med, 2017. 6(6): p. 1173-1180.

57. Sand, M., et al., Circular RNA expression in cutaneous squamous cell carcinoma. J Dermatol Sci, 2016. 83(3): p. 210-8.

58. Peng, N., et al., Microarray profiling of circular RNAs in human papillary thyroid carcinoma. PLoS One, 2017. 12(3): p. e0170287.

59. Li, Y., et al., CircHIPK3 sponges miR-558 to suppress heparanase expression in bladder cancer cells. EMBO Rep, 2017. 18(9): p. 1646-1659.

60. Zhong, Z., M. Lv, and J. Chen, Screening differential circular RNA expression profiles reveals the regulatory role of circTF25-miR-103a-3p/miR-107-CDK6 pathway in bladder carcinoma. Sci Rep, 2016. 6: p. 30919.

61. Lu, L., et al., Identification of circular RNAs as a promising new class of diagnostic biomarkers for human breast cancer. Oncotarget, 2017. 8(27): p. 44096-44107.

62. Zhu, M., et al., Circular BANP, an upregulated circular RNA that modulates cell proliferation in colorectal cancer. Biomed Pharmacother, 2017. 88: p. 138-144.

63. Shi, P., et al., Profiles of differentially expressed circRNAs in esophageal and breast cancer. Cancer Manag Res, 2018. 10: p. 2207-2221.

64. Wang, S., et al., Circular RNA FOXP1 promotes tumor progression and Warburg effect in gallbladder cancer by regulating PKLR expression. Mol Cancer, 2019. 18(1): p. 145.

65. Sui, W., et al., Circular RNA and gene expression profiles in gastric cancer based on microarray chip technology. Oncol Rep, 2017. 37(3): p. 1804-1814.

66. Zhu, J., et al., Differential Expression of Circular RNAs in Glioblastoma Multiforme and Its Correlation with Prognosis. Transl Oncol, 2017. 10(2): p. 271-279.

67. Fan, Y., et al., Circular RNA Expression Profile in Laryngeal Squamous Cell Carcinoma Revealed by Microarray. Cell Physiol Biochem, 2018. 50(1): p. 342-352.

68. Huang, X.Y., et al., Comprehensive circular RNA profiling reveals the regulatory role of the circRNA-100338/miR-141-3p pathway in hepatitis B-related hepatocellular carcinoma. Sci Rep, 2017. 7(1): p. 5428.
69. Zhu, X., et al., *hsa_circ_0013958: a circular RNA and potential novel biomarker for lung adenocarcinoma.* FEBS J, 2017. 284(14): p. 2170-2182.

70. Hang, D., et al., *A novel plasma circular RNA circFARSA is a potential biomarker for non-small cell lung cancer.* Cancer Med, 2018. 7(6): p. 2783-2791.

71. Jin, M., et al., *Upregulated circRNA ARHGAP10 Predicts an Unfavorable Prognosis in NSCLC through Regulation of the miR-150-5p/GLUT-1 Axis.* Mol Ther Nucleic Acids, 2019. 18: p. 219-231.

72. Li, H., et al., *Circular RNA Expression Profile of Pancreatic Ductal Adenocarcinoma Revealed by Microarray.* Cell Physiol Biochem, 2016. 40(6): p. 1334-1344.

73. Xia, Q., et al., *Circular RNA Expression Profiling Identifies Prostate Cancer-Specific circRNAs in Prostate Cancer.* Cell Physiol Biochem, 2018. 50(5): p. 1903-1915.

74. Chen, D.F.Z.L.J., Tan K., Jing Q., *Application of droplet digital PCR in quantitative detection of the cell-free circulating circRNAs.* Biotechnology & Biotechnological Equipment, 2018. 32(1): p. 116-123.

75. Li, T., et al., *Plasma circular RNA profiling of patients with gastric cancer and their droplet digital RT-PCR detection.* J Mol Med (Berl), 2018. 96(1): p. 85-96.

76. Geiss, G.K., et al., *Direct multiplexed measurement of gene expression with color-coded probe pairs.* Nat Biotechnol, 2008. 26(3): p. 317-25.

77. Dahl, M., et al., *Enzyme-free digital counting of endogenous circular RNA molecules in B-cell malignancies.* Lab Invest, 2018. 98(12): p. 1657-1669.

78. Vromman, M., J. Vandesompele, and P.J. Volders, *Closing the circle: current state and perspectives of circular RNA databases.* Brief Bioinform, 2020.

79. Glazar, P., P. Papavasileiou, and N. Rajewsky, *circBase: a database for circular RNAs.* RNA, 2014. 20(11): p. 1666-70.

80. Ji, P., et al., *Expanded Expression Landscape and Prioritization of Circular RNAs in Mammals.* Cell Rep, 2019. 26(12): p. 3444-3460 e5.

81. Dong, R., et al., *CIRCpedia v2: An Updated Database for Comprehensive Circular RNA Annotation and Expression Comparison.* Genomics Proteomics Bioinformatics, 2018. 16(4): p. 226-233.

82. Liu, M., et al., *Circbank: a comprehensive database for circRNA with standard nomenclature.* RNA Biol, 2019. 16(7): p. 899-905.

83. 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.

84. Liu, Y.C., et al., *CircNet: a database of circular RNAs derived from transcriptome sequencing data.* Nucleic Acids Res, 2016. 44(D1): p. D209-15.

85. Chen, X., et al., *circRNADb: A comprehensive database for human circular RNAs with protein-coding annotations.* Sci Rep, 2016. 6: p. 34985.

86. Meng, X., et al., *CircPro: an integrated tool for the identification of circRNAs with protein-coding potential.* Bioinformatics, 2017. 33(20): p. 3314-3316.

87. Meng, X., et al., *CircFunBase: a database for functional circular RNAs.* Database (Oxford), 2019. 2019.

88. Yao, D., et al., *Circ2Disease: a manually curated database of experimentally validated circRNAs in human disease.* Sci Rep, 2018. 8(1): p. 11018.

89. Ghosal, S., et al., *Circ2Traits: a comprehensive database for circular RNA potentially associated with disease and traits.* Front Genet, 2013. 4: p. 283.

90. Fan, C., et al., *CircR2Disease: a manually curated database for experimentally supported circular RNAs associated with various diseases.* Database (Oxford), 2018. 2018.

91. Xia, S., et al., *CSCD: a database for cancer-specific circular RNAs.* Nucleic Acids Res, 2018. 46(D1): p. D925-D929.
92. Ruan, H., et al., Comprehensive characterization of circular RNAs in ~1000 human cancer cell lines. Genome Med, 2019. 11(1): p. 55.
93. Zuo, Z., et al., BBCancer: an expression atlas of blood-based biomarkers in the early diagnosis of cancers. Nucleic Acids Res, 2020. 48(D1): p. D789-D796.
94. Li, S., et al., exoRBase: a database of circRNA, lncRNA and mRNA in human blood exosomes. Nucleic Acids Res, 2018. 46(D1): p. D106-D112.
95. Smid, M., et al., The circular RNome of primary breast cancer. Genome Res, 2019. 29(3): p. 356-366.
96. Nair, A.A., et al., Circular RNAs and their associations with breast cancer subtypes. Oncotarget, 2016. 7(49): p. 80967-80979.
97. Wang, C., et al., RNA-Seq profiling of circular RNA in human lung adenocarcinoma and squamous cell carcinoma. Mol Cancer, 2019. 18(1): p. 133.
98. 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.
99. 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.
100. Wang, S., et al., Upregulation of circ-UBAP2 predicts poor prognosis and promotes triple-negative breast cancer progression through the miR-661/MTA1 pathway. Biochem Biophys Res Commun, 2018. 505(4): p. 996-1002.
101. Tang, H., et al., circKIF4A acts as a prognostic factor and mediator to regulate the progression of triple-negative breast cancer. Mol Cancer, 2019. 18(1): p. 23.
102. Yang, R., et al., The circRNA circAGFG1 acts as a sponge of miR-195-5p to promote triple-negative breast cancer progression through regulating CCNE1 expression. Mol Cancer, 2019. 18(1): p. 4.
103. Wang, Y., et al., Upregulated Circular RNA circ-UBE2D2 Predicts Poor Prognosis and Promotes Breast Cancer Progression by Sponging miR-1236 and miR-1287. Transl Oncol, 2019. 12(10): p. 1305-1313.
104. Li, Y. and H. Li, Circular RNA VRK1 correlates with favourable prognosis, inhibits cell proliferation but promotes apoptosis in breast cancer. J Clin Lab Anal, 2020. 34(1): p. e22980.
105. Zhang, X., et al., Circular RNA La-related RNA-binding protein 4 correlates with reduced tumor stage, as well as better prognosis, and promotes chemo sensitivity to doxorubicin in breast cancer. J Clin Lab Anal, 2020: p. e23272.
106. Zhang, J., et al., Circular RNA LARP4 inhibits cell proliferation and invasion of gastric cancer by sponging miR-424-5p and regulating LATS1 expression. Mol Cancer, 2017. 16(1): p. 151.
107. Chen, Z., et al., circLARP4 induces cellular senescence through regulating miR-761/RUNX3/p53/p21 signaling in hepatocellular carcinoma. Cancer Sci, 2019. 110(2): p. 568-581.
108. Zou, T., et al., Circular RNA LARP4 is lower expressed and serves as a potential biomarker of ovarian cancer prognosis. Eur Rev Med Pharmacol Sci, 2018. 22(21): p. 7178-7182.
109. Hu, Y., et al., Circular RNA LARP4 correlates with decreased Enneking stage, better histological response, and prolonged survival profiles, and it elevates chemosensitivity to cisplatin and doxorubicin via sponging microRNA-424 in osteosarcoma. J Clin Lab Anal, 2020. 34(2): p. e23045.
110. Li, Y., et al., Upregulated circular RNA circ_0016760 indicates unfavorable prognosis in NSCLC and promotes cell progression through miR-1287/GAGE1 axis. Biochem Biophys Res Commun, 2018. 503(3): p. 2089-2094.
111. Qi, Y., et al., Upregulation of circular RNA hsa_circ_0007534 predicts unfavorable prognosis for NSCLC and exerts oncogenic properties in vitro and in vivo. Gene, 2018. 676: p. 79-85.
112. Liu, T., Z. Song, and Y. Gai, *Circular RNA circ_0001649 acts as a prognostic biomarker and inhibits NSCLC progression via sponging miR-331-3p and miR-338-5p*. Biochem Biophys Res Commun, 2018. **503**(3): p. 1503-1509.

113. Yao, Y., Q. Hua, and Y. Zhou, *CircRNA has_circ_0006427 suppresses the progression of lung adenocarcinoma by regulating miR-6783-3p/DKK1 axis and inactivating Wnt/beta-catenin signaling pathway*. Biochem Biophys Res Commun, 2019. **508**(1): p. 37-45.

114. Li, L., et al., *Circular RNA circAGO2 drives cancer progression through facilitating HuR-repressed functions of AGO2-miRNA complexes*. Cell Death Differ, 2019. **26**(7): p. 1346-1364.

115. Du, W., et al., *Circ-PRMT5 promotes gastric cancer progression by sponging miR-145 and miR-1304 to upregulate MYC*. Artif Cells Nanomed Biotechnol, 2019. **47**(1): p. 4120-4130.

116. Wang, S., et al., *circLMTK2 acts as a sponge of miR-150-5p and promotes proliferation and metastasis in gastric cancer*. Mol Cancer, 2019. **18**(1): p. 162.

117. Tseng, Y.Y., et al., *PVT1 dependence in cancer with MYC copy-number increase*. Nature, 2014. **512**(7512): p. 82-6.

118. Hsiao, K.Y., et al., *Noncoding Effects of Circular RNA CCDC66 Promote Colon Cancer Growth and Metastasis*. Cancer Res, 2017. **77**(9): p. 2339-2350.

119. Fang, G., et al., *CircRNA_100290 promotes colorectal cancer progression through miR-516b-induced downregulation of FZD4 expression and Wnt/beta-catenin signaling*. Biochem Biophys Res Commun, 2018. **504**(1): p. 184-189.

120. Yuan, Y., et al., *CircRNA AGO2-1 interacts with AGO2 and modulates its activity*. Biochem Biophys Res Commun, 2018. **503**(2): p. 870-875.

121. Ge, Z., et al., *CircRNA_10114717 is downregulated in colorectal cancer and inhibits tumor growth by promoting p16 expression*. Biomed Pharmacother, 2018. **98**: p. 775-782.

122. Huang, X.Y., et al., *CircRNA-100338 Is Associated With mTOR Signaling Pathway and Poor Prognosis in Hepatocellular Carcinoma*. Front Oncol, 2019. **9**: p. 392.

123. Tan, A., Q. Li, and L. Chen, *CircHIPK3 promotes colorectal cancer progression through regulating miR-3619-5p/CTNNB1 axis and activating Wnt/beta-catenin pathway*. Arch Biochem Biophys, 2019. **661**: p. 196-202.
133. Dong, W., et al., *The RNA-binding protein RBM3 promotes cell proliferation in hepatocellular carcinoma by regulating circular RNA SCD-circRNA 2 production*. EBioMedicine, 2019. **45**: p. 155-167.

134. Wang, G., et al., *Three isoforms of exosomal circPTGR1 promote hepatocellular carcinoma metastasis via the miR449a-MET pathway*. EBioMedicine, 2019. **40**: p. 432-445.

135. Meng, J., et al., *Twist1 Regulates Vimentin through Cul2 Circular RNA to Promote EMT in Hepatocellular Carcinoma*. Cancer Res, 2018. **78**(15): p. 4150-4162.

136. Yu, J., et al., *Circular RNA cSMARCA5 inhibits growth and metastasis in hepatocellular carcinoma*. J Hepatol, 2018. **68**(6): p. 1214-1227.

137. Qiu, L., et al., *Circular RNA profiling identifies circADAMTS13 as a miR-484 sponge which suppresses cell proliferation in hepatocellular carcinoma*. Mol Oncol, 2019. **13**(2): p. 441-455.

138. Han, D., et al., *Circular RNA circMTO1 acts as the sponge of microRNA-9 to suppress hepatocellular carcinoma progression*. Hepatology, 2017. **66**(4): p. 1151-1164.

139. Guo, W., et al., *Polymorphisms and expression pattern of circular RNA circ-ITCH contributes to the carcinogenesis of hepatocellular carcinoma*. Oncotarget, 2017. **8**(29): p. 48169-48177.

140. Zhang, P.F., et al., *CircRNA circTRIM33-12 acts as the sponge of MicroRNA-191 to suppress hepatocellular carcinoma progression*. Mol Cancer, 2019. **18**(1): p. 105.

141. Zhong, Z., et al., *Circular RNA MYLK as a competing endogenous RNA promotes bladder cancer progression through modulating VEGFA/VEGFR2 signaling pathway*. Cancer Lett, 2017. **403**: p. 305-317.

142. Bi, J., et al., *Circ-BPTF promotes bladder cancer progression and recurrence through the miR-31-5p/RAB27A axis*. Aging (Albany NY), 2018. **10**(8): p. 1964-1976.

143. Su, H., et al., *Circular RNA cTFRC acts as the sponge of MicroRNA-107 to promote bladder carcinoma progression*. Mol Cancer, 2019. **18**(1): p. 27.

144. Okholm, T.L.H., et al., *Circular RNA expression is abundant and correlated to aggressiveness in early-stage bladder cancer*. NPJ Genom Med, 2017. **2**: p. 36.

145. Lu, Q., et al., *Circular RNA circSLC8A1 acts as a sponge of miR-130b/miR-494 in suppressing bladder cancer progression via regulating PTEN*. Mol Cancer, 2019. **18**(1): p. 111.

146. Li, Y., et al., *Circular RNA circMTO1 suppresses bladder cancer metastasis by sponging miR-221 and inhibiting epithelial-to-mesenchymal transition*. Biochem Biophys Res Commun, 2019. **508**(4): p. 991-996.

147. Liu, H., et al., *Circular RNA circUBXN7 represses cell growth and invasion by sponging miR-1247-3p to enhance B4GALT3 expression in bladder cancer*. Aging (Albany NY), 2018. **10**(10): p. 2606-2623.

148. Lin, G., et al., *cicrlPAR1 is a novel biomarker of prognosis for muscle-invasive bladder cancer with invasion and metastasis by miR-762*. Onclet, 2019. **17**(3): p. 3537-3547.

149. Chen, D., et al., *Circular RNA circHIPK3 promotes cell proliferation and invasion of prostate cancer by sponging miR-193a-3p and regulating MCL1 expression*. Cancer Manag Res, 2019. **11**: p. 1415-1423.

150. Huang, E., X. Chen, and Y. Yuan, *Downregulated circular RNA itchy E3 ubiquitin protein ligase correlates with advanced pathologic T stage, high lymph node metastasis risk and poor survivals in prostate cancer patients*. Cancer Biomark, 2019. **26**(1): p. 41-50.

151. Hu, Y. and B. Guo, *Circ-MTO1 correlates with favorable prognosis and inhibits cell proliferation, invasion as well as miR-17-5p expression in prostate cancer*. J Clin Lab Anal, 2020. **34**(3): p. e23086.

152. Liu, N., et al., *CircHIPK3 is upregulated and predicts a poor prognosis in epithelial ovarian cancer*. Eur Rev Med Pharmacol Sci, 2018. **22**(12): p. 3713-3718.
153. Sun, Y., et al., 
*circPIP5K1A* serves as a competitive endogenous RNA contributing to ovarian cancer progression via regulation of miR-661/IGFBP5 signaling. J Cell Biochem, 2019. **120**(12): p. 19406-19414.

154. Chen, Y., et al., 
*Circular RNA ABCB10* correlates with advanced clinicopathological features and unfavorable survival, and promotes cell proliferation while reduces cell apoptosis in epithelial ovarian cancer. Cancer Biomark, 2019. **26**(2): p. 151-161.

155. Sun, D., J. Liu, and L. Zhou, 
Upregulation of circular RNA *circFAM53B* predicts adverse prognosis and accelerates the progression of ovarian cancer via the miR646/VAMP2 and miR647/MDM2 signaling pathways. Oncol Rep, 2019. **42**(6): p. 2728-2737.

156. Zhang, L., et al., 
*CircPLEKHM3* acts as a tumor suppressor through regulation of the miR-9/BRCA1/DNAJB6/KL4/4AKT1 axis in ovarian cancer. Mol Cancer, 2019. **18**(1): p. 144.

157. Hu, J., et al., 
The *circular RNA circ-ITCH* suppresses ovarian carcinoma progression through targeting miR-145/RASA1 signaling. Biochem Biophys Res Commun, 2018. **505**(1): p. 222-228.

158. Su, H., et al., 
Profiling and bioinformatics analyses reveal differential circular RNA expression in radioresistant esophageal cancer cells. J Transl Med, 2016. **14**(1): p. 225.

159. Liu, J., et al., 
*CircRNA_100367* regulated the radiation sensitivity of esophageal squamous cell carcinomas through miR-217/Wnt3 pathway. Aging (Albany NY), 2019. **11**(24): p. 12412-12427.

160. Yu, D., et al., 
Comprehensive circular RNA expression profile in radiation-treated HeLa cells and analysis of radioresistance-related circRNAs. PeerJ, 2018. **6**: p. e5011.

161. Fan, L., et al., 
Aberrant miR-1246 expression promotes radioresistance in non-small cell lung cancer: a potential prognostic biomarker and radiotherapy sensitization target. Am J Cancer Res, 2020. **10**(1): p. 314-335.

162. Li, Y.H., et al., 
*circMTDH.4/miR-630/AEG-1* axis participates in the regulation of proliferation, migration, invasion, chemoresistance, and radioresistance of NSCLC. Mol Carcinog, 2020. **59**(2): p. 141-153.

163. Wang, L., et al., 
Inhibition of hsa_circ_0001313 (circCCDC66) induction enhances the radiosensitivity of colon cancer cells via tumor suppressor miR-338-3p: Effects of circ_0001313 on colon cancer radio-sensitivity. Pathol Res Pract, 2019. **215**(4): p. 689-696.

164. Shuai, M., et al., 
Upregulation of *circRNA_0000285* serves as a prognostic biomarker for nasopharyngeal carcinoma and is involved in radiosensitivity. Oncol Lett, 2018. **16**(5): p. 6495-6501.

165. Chen, L., H. Zhou, and Z. Guan, 
*CircRNA_000543* knockdown sensitizes nasopharyngeal carcinoma to irradiation by targeting miR-9/platelet-derived growth factor receptor B axis. Biochem Biophys Res Commun, 2019. **512**(4): p. 786-792.

166. Yang, J., et al., 
*Curcumin* enhances radiosensitization of nasopharyngeal carcinoma by regulating circRNA network. Mol Carcinog, 2020. **59**(2): p. 202-214.

167. Xia, X., et al., 
A novel tumor suppressor protein encoded by circular AKT3 RNA inhibits glioblastoma tumorigenicity by competing with active phosphoinositide-dependent Kinase-1. Mol Cancer, 2019. **18**(1): p. 131.

168. 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.

169. Mao, Y. and R. Xu, 
*Circular RNA CDR1-AS* contributes to pemetrexed and cisplatin chemoresistance through EGFR/P13K signaling pathway in lung adenocarcinoma. Biomed Pharmacother, 2020. **123**: p. 109771.
171. Yuan, W., et al., *Circular RNA Cdr1as sensitizes bladder cancer to cisplatin by upregulating APAF1 expression through miR-1270 inhibition*. Mol Oncol, 2019. **13**(7): p. 1559-1576.

172. Zhao, Z., et al., *Circular RNA Cdr1as Upregulates SCAI to Suppress Cisplatin Resistance in Ovarian Cancer via miR-1270 Suppression*. Mol Ther Nucleic Acids, 2019. **18**: p. 24-33.

173. Xu, Y., et al., *CircAKT3 inhibits glycolysis balance in lung cancer cells by regulating miR-516b-5p/STAT3 to inhibit cisplatin sensitivity*. Biotechnol Lett, 2020.

174. Huang, X., et al., *Circular RNA AKT3 upregulates PIK3R1 to enhance cisplatin resistance in gastric cancer via miR-198 suppression*. Mol Cancer, 2019. **18**(1): p. 71.

175. Dong, Y., et al., *Circ_0076305 regulates cisplatin resistance of non-small cell lung cancer via positively modulating STAT3 by sponging miR-296-5p*. Life Sci, 2019. **239**: p. 116984.

176. Chen, J., et al., *Androgen receptor-regulated circFNTA activates KRAS signaling to promote bladder cancer invasion*. EMBO Rep, 2020. **21**(4): p. e48467.

177. Chi, B.J., et al., *Downregulation of hsa_circ_0000285 serves as a prognostic biomarker for bladder cancer and is involved in cisplatin resistance*. Neoplasma, 2019. **66**(2): p. 197-202.

178. Su, Y., et al., *Hypoxia-elevated circELP3 contributes to bladder cancer progression and cisplatin resistance*. Int J Biol Sci, 2019. **15**(2): p. 441-452.

179. Li, H., F. Liu, and W. Qin, *Circ_0072083 interference enhances growth-inhibiting effects of cisplatin in non-small-cell lung cancer cells via miR-545-3p/CBLL1 axis*. Cancer Cell Int, 2020. **20**: p. 78.

180. Liu, F., et al., *Circular RNA EIF6 (Hsa_circ_0060060) sponges miR-144-3p to promote the cisplatin-resistance of human thyroid carcinoma cells by autophagy regulation*. Aging (Albany NY), 2018. **10**(12): p. 3806-3820.

181. Huang, X.X., et al., *A novel circular RNA circFN1 enhances cisplatin resistance in gastric cancer via sponging miR-182-5p/CBL1 axis*. J Cell Biochem, 2020.

182. Abu, N., et al., *Identification of differentially expressed circular RNAs in chemoresistant colorectal cancer*. Epigenomics, 2019. **11**(8): p. 875-884.

183. Lin, Y.C., et al., *Oxaliplatin-Induced DHX9 Phosphorylation Promotes Oncogenic Circular RNA CCDC66 Expression and Development of Chemoresistance*. Cancers (Basel), 2020. **12**(3).

184. Wang, X., et al., *Exosome-delivered circRNA promotes glycolysis to induce chemoresistance through the miR-122-PKM2 axis in colorectal cancer*. Mol Oncol, 2020. **14**(3): p. 539-555.

185. Li, J., et al., *Circular RNA circFBXO11 modulates hepatocellular carcinoma progression and oxaliplatin resistance through miR-605/FOXO3/ABCB1 axis*. J Cell Mol Med, 2020.

186. Wu, Q., et al., *Hsa_circ_0001546 acts as a miRNA-421 sponge to inhibit the chemoresistance of gastric cancer cells via ATM/Chk2/p53-dependent pathway*. Biochem Biophys Res Commun, 2020. **521**(2): p. 303-309.

187. Gao, D., et al., *Screening circular RNA related to chemotherapeutic resistance in breast cancer*. Epigenomics, 2017. **9**(9): p. 1175-1188.

188. Liang, Y., et al., *circKDM4C suppresses tumor progression and attenuates doxorubicin resistance by regulating miR-548p/PBLD axis in breast cancer*. Oncogene, 2019. **38**(42): p. 6850-6866.

189. Xu, N., et al., *Profiles and Bioinformatics Analysis of Differentially Expressed Circrnas in Taxol-Resistant Non-Small Cell Lung Cancer Cells*. Cell Physiol Biochem, 2018. **48**(5): p. 2046-2060.

190. Yu, W., et al., *Hsa_circ_0003998 Promotes Chemoresistance via Modulation of miR-326 in Lung Adenocarcinoma Cells*. Oncol Res, 2019. **27**(5): p. 623-628.

191. 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).

192. Yang, W., et al., *Circ-ABCB10 Contributes to Paclitaxel Resistance in Breast Cancer Through Let-7a-5p/DUSP7 Axis*. Cancer Manag Res, 2020. **12**: p. 2327-2337.
193. Ma, J., et al., *Posttranscriptional regulation of AKT by circular RNA angiomotin-like 1 mediates chemoresistance against paclitaxel in breast cancer cells*. Aging (Albany NY), 2019. **11**(23): p. 11369-11381.

194. Zhang, S., et al., *circCELSR1 (hsa_circ_0063809) Contributes to Paclitaxel Resistance of Ovarian Cancer Cells by Regulating FOXR2 Expression via miR-1252*. Mol Ther Nucleic Acids, 2020. **19**: p. 718-730.

195. Hong, X., et al., *Circular RNA CRIM1 functions as a ceRNA to promote nasopharyngeal carcinoma metastasis and docetaxel chemoresistance through upregulating FOXQ1*. Mol Cancer, 2020. **19**(1): p. 33.

196. Li, X., et al., *Hsa_circ_0002483 inhibited the progression and enhanced the Taxol sensitivity of non-small cell lung cancer by targeting miR-182-5p*. Cell Death Dis, 2019. **10**(12): p. 953.

197. Shen, Z., et al., *Reduction of circular RNA Foxo3 promotes prostate cancer progression and chemoresistance to docetaxel*. Cancer Lett, 2020. **468**: p. 88-101.

198. Xu, C., Y. Yu, and F. Ding, *Microarray analysis of circular RNA expression profiles associated with gemcitabine resistance in pancreatic cancer cells*. Oncol Rep, 2018. **40**(1): p. 395-404.

199. Shao, F., et al., *Circular RNA Signature Predicts Gemcitabine Resistance of Pancreatic Ductal Adenocarcinoma*. Front Pharmacol, 2018. **9**: p. 584.

200. Liu, Y., et al., *CircHIPK3 Promotes Gemcitabine (GEM) Resistance in Pancreatic Cancer Cells by Sponging miR-330-5p and Targets RASSF1*. Cancer Manag Res, 2020. **12**: p. 921-929.

201. Xie, F., et al., *Circular RNA CircHIPK3 Promotes Gemcitabine Sensitivity in Bladder Cancer*. J Cancer, 2020. **11**(7): p. 1907-1912.

202. Tong, S., *Circular RNA SMARCA5 may serve as a tumor suppressor in non-small cell lung cancer*. J Clin Lab Anal, 2020: p. e23195.

203. Chen, T., et al., *Comprehensive analysis of circular RNA profiling in AZD9291-resistant non-small cell lung cancer cell lines*. Thorac Cancer, 2019. **10**(4): p. 930-941.

204. Joseph, N.A., et al., *The role of HGF-MET pathway and CCDC66 cirRNA expression in EGFR resistance and epithelial-to-mesenchymal transition of lung adenocarcinoma cells*. J Hematol Oncol, 2018. **11**(1): p. 74.

205. Zhou, Y., et al., *Circular RNA hsa_circ_0004015 regulates the proliferation, invasion, and TKI drug resistance of non-small cell lung cancer by miR-1183/PDPK1 signaling pathway*. Biochem Biophys Res Commun, 2019. **508**(2): p. 527-535.

206. Liu, Y.T., et al., *Circular RNA profiling identified as a biomarker for predicting the efficacy of Gefitinib therapy for non-small cell lung cancer*. J Thorac Dis, 2019. **11**(5): p. 1779-1787.

207. Su, W., et al., *Hsa_circ_0005379 regulates malignant behavior of oral squamous cell carcinoma through the EGFR pathway*. BMC Cancer, 2019. **19**(1): p. 400.

208. Zhang, P.F., et al., *Circular RNA circFGFR1 promotes progression and anti-PD-1 resistance by sponging miR-381-3p in non-small cell lung cancer cells*. Mol Cancer, 2019. **18**(1): p. 179.

209. Zhao, R., et al., *CircUBAP2-mediated competing endogenous RNA network modulates tumorigenesis in pancreatic adenocarcinoma*. Aging (Albany NY), 2019. **11**(19): p. 8484-8501.

210. Gupta, S.K., et al., *Quaking Inhibits Doxorubicin-Mediated Cardiotoxicity Through Regulation of Cardiac Circular RNA Expression*. Circ Res, 2018. **122**(2): p. 246-254.
214. Ji, X., et al., MicroRNA-31-5p attenuates doxorubicin-induced cardiotoxicity via quaking and circular RNA Pan3. J Mol Cell Cardiol, 2020. 140: p. 56-67.

215. Cao, Y., et al., Transcriptome sequencing of circular RNA reveals a novel circular RNA- has_circ_0114427 in the regulation of inflammation in acute kidney injury. Clin Sci (Lond), 2020. 134(2): p. 139-154.

216. Chen, Y., et al., Circular RNA RSF1 promotes inflammatory and fibrotic phenotypes of irradiated hepatic stellate cell by modulating miR-146a-5p. J Cell Physiol, 2020.

217. Chen, Y., et al., Microarray profiling of circular RNAs and the potential regulatory role of hsa_circ_0071410 in the activated human hepatic stellate cell induced by irradiation. Gene, 2017. 629: p. 35-42.

218. Li, Y., et al., Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. Cell Res, 2015. 25(8): p. 981-4.

219. Bahn, J.H., et al., The landscape of microRNA, Piwi-interacting RNA, and circular RNA in human saliva. Clin Chem, 2015. 61(1): p. 221-30.

220. Cohen, J.D., et al., Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science, 2018. 359(6378): p. 926-930.

221. Tan, S., et al., Circular RNA F-circEA produced from EML4-ALK fusion gene as a novel liquid biopsy biomarker for non-small cell lung cancer. Cell Res, 2018. 28(6): p. 693-695.

222. Liu, X.X., et al., A two-circular RNA signature as a noninvasive diagnostic biomarker for lung adenocarcinoma. J Transl Med, 2019. 17(1): p. 50.

223. Yin, W.B., et al., Circulating circular RNA hsa_circ_0001785 acts as a diagnostic biomarker for breast cancer detection. Clin Chim Acta, 2018. 487: p. 363-368.

224. Ye, D.X., et al., A 3-circular RNA signature as a noninvasive biomarker for diagnosis of colorectal cancer. Cancer Cell Int, 2019. 19: p. 276.

225. Lin, J., et al., Plasma circular RNA panel acts as a novel diagnostic biomarker for colorectal cancer. Clin Biochem, 2019. 74: p. 60-68.

226. Han, L., et al., A Dual-Circular RNA Signature as a Non-invasive Diagnostic Biomarker for Gastric Cancer. Front Oncol, 2020. 10: p. 184.

227. Lu, J., et al., Circular RNA hsa_circ_0006848 Related to Ribosomal Protein L6 Acts as a Novel Biomarker for Early Gastric Cancer. Dis Markers, 2019. 2019: p. 3863458.

228. Chen, S., et al., Using circular RNA hsa_circ_0000190 as a new biomarker in the diagnosis of gastric cancer. Clin Chim Acta, 2017. 466: p. 167-171.

229. Huang, M., et al., Circular RNA hsa_circ_0000745 may serve as a diagnostic marker for gastric cancer. World J Gastroenterol, 2017. 23(34): p. 6330-6338.

230. Zhao, Q., et al., Clinical values of circular RNA 0000181 in the screening of gastric cancer. J Clin Lab Anal, 2018. 32(4): p. e22333.

231. Yang, F., et al., Circular RNA circ-LDLRAD3 as a biomarker in diagnosis of pancreatic cancer. World J Gastroenterol, 2017. 23(47): p. 8345-8354.

232. Yu, J., et al., Plasma circular RNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma: A large-scale, multicenter study. Int J Cancer, 2020. 146(6): p. 1754-1763.

233. Li, Z., et al., Using circular RNA SMARCA5 as a potential novel biomarker for hepatocellular carcinoma. Clin Chim Acta, 2019. 492: p. 37-44.

234. Zhang, X., et al., The Circular RNA hsa_circ_0001445 Regulates the Proliferation and Migration of Hepatocellular Carcinoma and May Serve as a Diagnostic Biomarker. Dis Markers, 2018. 2018: p. 3073467.

235. Chen, X., et al., PRMT5 Circular RNA Promotes Metastasis of Urothelial Carcinoma of the Bladder through Sponging miR-30c to Induce Epithelial-Mesenchymal Transition. Clin Cancer Res, 2018. 24(24): p. 6319-6330.
236. Zhao, S.Y., et al., Salivary Circular RNAs Hsa_Circ_0001874 and Hsa_Circ_0001971 as Novel Biomarkers for the Diagnosis of Oral Squamous Cell Carcinoma. Cell Physiol Biochem, 2018. 47(6): p. 2511-2521.

237. Ju, H.Q., et al., A circRNA signature predicts postoperative recurrence in stage II/III colon cancer. EMBO Mol Med, 2019. 11(10): p. e10168.

238. Zhang, Y., et al., Circular RNAs signature predicts the early recurrence of stage III gastric cancer after radical surgery. Oncotarget, 2017. 8(14): p. 22936-22943.
### Tables

**Table 1:** Summary of publicly available circRNA databases

| Database           | Number of Samples | Total Human CircRNA | Human/Cancer Specific? | Detection Tool                  | Brief Description                                                                 | Ref. |
|--------------------|-------------------|---------------------|------------------------|---------------------------------|-----------------------------------------------------------------------------------|------|
| Circ2Disease       | N/A               | 237                 | Yes/No                 | N/A                             | CircRNA associated with disease, reports interaction with miRNA                   | [88] |
| Circ2Traits        | 4                 | 1,953               | Yes/No                 | find_circ                       | Associate circRNA with disease and traits                                         | [89] |
| circAtlas 2.0      | 240               | 421,501             | No/No                  | CIRI2, CIRCexplorer, DCC, MapSplice | Diversity and similarity among circRNA and expression from multiple species      | [80] |
| circBank           | 34                | 140,790             | Yes/No                 | Multiple sources                | Comprehensive database with predicted miRNA binding sites                          | [82] |
| circBase           | 20                | 92,375              | No/No                  | Multiple sources                | Database of circRNA identified by RNaseq on cell lines from multiple studies      | [79] |
| CircFunBase        | N/A               | 3,799               | No/No                  | N/A                             | Compendium of circRNA from various species with focus on reported functions       | [87] |
| CircInteractome    | 34                | 140,790             | Yes/No                 | N/A                             | Interaction between circRNA, miRNA, and potential transcripts                     | [83] |
| circNet            | 464               | 212,950             | Yes/No                 | find_circ                       | CircRNA-miRNA-gene networks and reports circRNA expression                         | [84] |
| CIRCpedia v2       | 70                | 183,493             | No/No                  | CIRCexplorer2, MapSplice         | CircRNA across 6 different species                                               | [81] |
| CircR2Disease      | N/A               | 661                 | Yes/No                 | N/A                             | CircRNA with known association with disease                                       | [90] |
| circRNADb          | N/A               | 32,914              | Yes/No                 | Multiple sources                | Reports circRNA and possible transcripts as well as protein-coding potential      | [85] |
| exoRBase           | 92                | 58,330              | Yes/No                 | ACFS, find_circ                 | Characterize RNA (circRNA, IncRNA, mRNA) found in exosomes                       | [94] |
| TCSD               | 60                | 1,184,752           | No/No                  | CIRI, circRNA_finder, find_circ | Tissue-specific circular RNA in adult and fetal human and mouse tissue           | [32] |
Table 2: Summary of publicly available cancer specific circRNA databases

| Database   | Number of Samples | Total Human CircRNA | Cancer Specific? | Detection Tool | Brief Description                                                                 | Ref.   |
|------------|--------------------|---------------------|------------------|----------------|----------------------------------------------------------------------------------|-------|
| BBCancer   | 7184               | 175,508             | Yes              | CIRI, find_circ | Blood-based biomarkers including circRNA in liver, colorectal, and pancreatic cancer | [93]  |
| circPro    | 1                  | 2,036               | Yes              | CIRI2          | Ribosome-associated CircRNA with potential for translation into protein           | [86]  |
| CircRic    | 935                | 92,589              | Yes              | CIRI2, CIRCexplorer2, circRNA_finder, find_circ | CircRNA among cancer cell lines with integrative analysis and potential drug response | [92]  |
| CSCD       | 228                | 1,394,023           | Yes              | CIRI2, find_circ, circRNA_finder, CIRCexplorer | Cancer-specific circRNA in cell lines and potential function                     | [91]  |
| MiOncoCirc | 2000+              | 160,120             | Yes              | CIRCexplorer  | CircRNA in human cancer tissues by exome capture sequencing                      | [53]  |
Table 3: Sensitivity and specificity of reported differential circRNA expression in cancer plasma compared to normal patients for early cancer detection

| Primary Cancer | CircRNA                                                                 | Sensitivity | Specificity | AUC      | Ref.  |
|----------------|------------------------------------------------------------------------|-------------|-------------|----------|-------|
| Lung           | circYWHAZ (hsa_circ_0005962) circBNC2 (hsa_circ_0086414)               | 77.8%       | 72.2%       | 0.81     | [222] |
| Lung           | circFARSA                                                              | Not reported| Not reported| 0.71     | [70]  |
| Lung           | circACP6 (hsa_circ_0013958)                                            | 75.5%       | 79.6%       | 0.815    | [69]  |
| Breast         | circELP3 (hsa_circ_0001785)                                            | 76.4%       | 69.9%       | 0.784    | [223] |
| Colorectal     | circFAM7IF2 (hsa_circ_0082182) circFLI1 (hsa_circ_0000370) circALDH1A2 (hsa_circ_0035445) | Not reported| Not reported| 0.835    | [224] |
| Colorectal     | circCCDC66 circABCC1 circSTIL                                          | 64.4%       | 85.2%       | 0.780    | [225] |
| Gastric        | circXPO1 (hsa_circ_0001017) circNRP1 (hsa_circ_0061276)                 | 84.7%       | 96.6%       | 0.912    | [75]  |
| Gastric        | circLMO1 (hsa_circ_0021087) circUBXN7 (hsa_circ_0005051)                | Not reported| Not reported| 0.773    | [226] |
| Gastric        | circRPL6                                                               | Not reported| Not reported| 0.733    | [227] |
| Gastric        | circCNIH4 (hsa_circ_0000190)                                           | 41.4%       | 87.5%       | 0.60     | [228] |
| Gastric        | circSPECC1 (hsa_circ_0000745)                                          | 85.5%       | 45%         | 0.683    | [229] |
| Gastric        | circTATDN3 (hsa_circ_0000181)                                          | 99.0%       | 20.6%       | 0.582    | [230] |
| Pancreatic     | circLDLRAD3                                                            | 57.4%       | 70.5%       | 0.67     | [231] |
| Liver          | circHPICAL1 (hsa_circ_0000976) circRABGGTA (hsa_circ_0007750) circMTM1 (hsa_circ_0139897) | 87.5%       | 85.5%       | 84.0%    | 89.5%  | 0.858 (Set 1) 0.875 (Set 2) | [232] |
| Liver          | circSMARCA5                                                            | 86.7%       | 89.3%       | 0.938    | [233] |
| Liver          | circSMARCA5 (hsa_circ_0001445)                                         | 71.2%       | 94.2%       | 0.862    | [234] |
| Oral (saliva)  | circBICD2 (hsa_circ_0001874) circFAM126A (hsa_circ_0001971)             | 92.7%       | 77.8%       | 0.922    | [236] |
**Figure Legends**

**Figure 1.** Biogenesis and Stability of CircRNA. CircRNAs are formed from backsplicing the 5’ end of one exon to the 3’ end of another, in this figure exon 5 onto 4 and exon 3 onto itself. Backspliced exons typically have longer flanking introns, as is the case between exon 3 and 4. The backspliced exons form covalently closed structures that may consist of one exon or multiple exons. Intronic DNA may be present as well. CircRNAs are resistant to degradation by exoribonucleases due to a lack of 3’ poly(A) tail. This enables them to be stable in plasma, urine, and saliva.

**Figure 2.** Proposed CircRNA Biologic Functions. A. Function as competing endogenous RNA. CircRNA can bind miRNAs that negatively regulate mRNA to increase mRNA expression. Many circRNA/miRNA/mRNA axes have been reported with potential biologic significance. B. Function as protein scaffold. CircRNA can help form scaffolds to stabilize protein complexes, including those involving RNA binding proteins. C. Function as template for translation. Some circRNA have internal ribosomal entry sites (IRES) and open reading frames (ORF) enabling translation. CircRNAs can also undergo multiple consecutive rounds of translation.
Figure 2:

A

Competing endogenous RNA (circRNA/miRNA/mRNA axis)

B

Protein scaffold

C

Translation

Expression

Expression

RBP

RBP

Ribosome

tRNA

IRES

AUG
