Circular RNAs Are Promising Biomarkers in Liquid Biopsy for the Diagnosis of Non-small Cell Lung Cancer

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The high incidence and mortality of lung cancer make early detection of lung cancer particularly important. At present, the diagnosis of lung cancer mainly depends on diagnostic imaging and tissue biopsy. However, current diagnostics are not satisfactory owing to the low specificity and inability of multiple sampling. Accumulating evidence indicates that circular RNAs (circRNAs) play a critical role in cancer progression and are promising cancer biomarkers. In particular, circRNAs are considered novel specific diagnostic markers for non-small cell lung cancer (NSCLC). Liquid biopsy is an important method in the early diagnosis of cancer due to its high sensitivity and specificity, as well as the possibility of performing multiple sampling. circRNAs are stably present in exosomes and sometimes become part of circulating nucleic acids, making them ideal for liquid biopsy. In this review, we summarize the advances in the research on circRNAs in NSCLC, and also highlight their potential applications for NSCLC detection.

Keywords: circular RNAs, exosome, liquid biopsy, non-small cell lung cancer, biomarker

INTRODUCTION

Cancer is a major public health problem worldwide. In China, lung cancer is one of the most common cancers and has the highest incidence and mortality rates among all cancers (Chen, 2015). The 5-year survival rate of patients with lung cancer is 54% when diagnosed at an early stage (Bach et al., 2012). However, this rate decreases to only 18% in patients with advanced cancer (Siegel et al., 2018). Non-small cell lung cancer (NSCLC), which is historically divided into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, accounts for approximately 80% of lung cancers. Using current diagnostics, NSCLC patients are usually diagnosed at the middle or late stages. To increase the survival rate of NSCLC patients, it is necessary to discover new biomarkers for its early diagnosis.

Abbreviations: circRNAs, circular RNAs; NSCLC, non-small cell lung cancer; CTCs, circulating tumor cells; EVs, extracellular vesicles; TEPs, tumor-educated platelets; miRNAs, microRNAs; IncRNAs, long non-coding RNAs; mRNAs, messenger RNAs; RBP, RNA-binding proteins; Cdk2, cyclin-dependent kinase 2; snRNA, small nuclear RNA; RNA-seq, RNA sequencing; RT-qPCR, real-time fluorescent polymerase chain reaction; rRNA, ribosomal RNA; RNase R, ribonuclease R; cDNAs, complementary DNAs; RT, reverse transcription; RCA, rolling circle amplification; RNA-ISH, RNA in situ hybridization; CHA, catalyzed hairpin assembly; LDN, linear DNA nanostructure; CEA, embryonic antigen; SCCA, squamous cell carcinoma antigen; CYFRA21-1, cytokeratin 19 fragment; ROC, receiver operating characteristic; AUC, area under the ROC curve; CI, confidence interval; PD-1, programmed cell death protein-1; exo-circRNAs, exosomal circRNAs; siRNA, small interfering RNA.
Precision oncology aims to improve the diagnosis and treatment of cancer (Ashley, 2016; Kumar-Sinha and Chinnaiyan, 2018). Studies on the comprehensive molecular signatures of cancers at the DNA, RNA, protein, and epigenetic levels deepen our understanding of the molecular events in different cancer types. Accurate identification of tumor subtypes and drug targets based on molecular signature profiling is a prerequisite for precision oncology (Heitzer et al., 2019). In the past few decades, molecular signatures have usually been identified by tissue biopsies. Histological examination is the gold standard for tumor diagnosis and grading (Chen, 2015). However, it is difficult to sample tumor tissues multiple times, and it is also difficult to conduct real-time tumor monitoring. In addition, cancer heterogeneity impedes the complete revelation of tumor information. Therefore, researchers have become increasingly interested in liquid biopsy, because liquid biopsy not only provides information equivalent to that provided by tissue biopsy, but also performs better than tissue biopsy in the minimally invasive, time-sensitive, and dynamic monitoring of cancers. With the advancement of research, liquid biopsy has played a significant role in the clinical testing of cancers.

In oncology, liquid biopsy refers to the sampling and measurement of analytes in various biological fluids (e.g., blood, urine, ascites, pleural effusions) (Siravegna et al., 2017). Peripheral blood analytes include circulating tumor cells (CTCs), circulating nucleic acids, circulating extracellular vesicles (EVs), tumor-educated platelets (TEPs), proteins, and metabolites. They can reveal several types of tumor information that are usually obtained by traditional tissue biopsy. Recent studies show that liquid biopsy could be a powerful tool for the early diagnosis of cancer (Cohen et al., 2018; Pantel and Alix-Panabière, 2019).

Circular RNAs (circRNAs) are a type of endogenous non-coding RNAs (Shabaninejad et al., 2019; Borran et al., 2020; Naei et al., 2020). Other types of non-coding RNAs include microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) (Vafadar et al., 2019; Sadri Nahand et al., 2020; Razavi et al., 2021). circRNAs are stably present in exosomes or circulating RNAs, making them an important target for liquid biopsy. Recent studies show that liquid biopsy could be a powerful tool for the early diagnosis of cancer (Cohen et al., 2018; Pantel and Alix-Panabière, 2019).

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CHARACTERISTICS AND FUNCTIONS OF CIRCULAR RNAs

Biogenesis and Characteristics of circRNAs
Circular RNAs were discovered by Nigro et al. (1991) in their analysis of human DCC gene sequencing. The 3′ and 5′ ends of circRNAs are not free, but covalently linked to each other. This linking occurs at a site flanked by canonical splice signals. To yield a circRNA, a splice donor must be linked to an upstream splice acceptor (Jeck et al., 2013; Memczak et al., 2013). This splicing product can be divided into two categories according to the differences in their structure and function: (1) circRNAs consisting exclusively of coding sequences and (2) circRNAs in which an intron is retained (exon-intron ciRNAs, EicirRNAs) (Xia et al., 2017).

circRNAs are widespread in eukaryotic cells. Evidence suggests that circRNAs have the following characteristics: (1) high stability. circRNAs are more stable than lncRNAs because of their circular structure. They are not easily degraded by exonucleases and thus have a longer half-life (Bachmayr-Heyda et al., 2015; Wu et al., 2019). (2) High abundance. Memczak et al. (2015) observed a higher expression of circRNAs relative to corresponding linear RNAs, as evidenced by the high circular-to-linear RNA ratio in the blood. In some cases, the ratio was more than 10-fold. (3) Evolutionary conservation. The sequences of circRNAs are evolutionarily conserved among different species (Jeck et al., 2013). (4) Specific expression. circRNAs are often expressed in a tissue- or developmental-stage-specific manner (Memczak et al., 2013).

Functions of circRNAs
Previous studies have indicated that circRNAs have four major functions: act as miRNA sponges, interact with RNA-binding proteins, regulate gene transcription, and encode polypeptides (Figure 1).

Acting as miRNA Sponges
MicroRNA sponges are RNA transcripts containing multiple high-affinity binding sites that associate with and sequester specific miRNAs to prevent them from interacting with their target messenger RNAs (mRNAs). Some circRNAs contain a large number of miRNA binding sites and function as miRNA sponges to indirectly regulate gene expression. One of the representative circRNAs of this type is the ciRS-7 (Hansen et al., 2013). CircS7 has been shown to contain 470 conserved binding sites of miR7, thus it is considered a “sponge” of miR7 (Hansen et al., 2013). However, only a small fraction of circRNAs function as potential miRNA sponges in mammals (Chen, 2016).

Interacting With RNA-Binding Proteins
Several circRNAs can bind to RNA-binding proteins (RBPs) and function as RBP sponges. For example, circFoxo3 has been reported to interact with cyclin-dependent kinase 2 (Cdk2) and p21 to inhibit the G1-S transition in the cell cycle, resulting in cell cycle arrest (Du et al., 2016).

Regulating Gene Transcription
Circular RNAs can also regulate gene transcription. For instance, EicirRNAs (Li Z. et al., 2015) interact with U1 small nuclear RNA (snRNA) to enhance the expression of their parental genes in cis (Li Z. et al., 2015). circRNAs that regulate gene transcription are mainly located in the nucleus.
**FIGURE 1** | The functions of circRNAs. In the nucleus, circRNAs can interact with transcription complexes in the promoter region of their host gene to induce gene transcription by interacting with U1 snRNP. circRNAs can directly interact with transcription complexes on host genes to induce their transcription. In the cytoplasm, circRNAs can interact with RBPs and affect their functions and translocations. circRNAs can act as miRNA sponges to inhibit miRNA activity by interacting with miRNA-Ago2 complexes. Some circRNAs have protein-coding capacity and can encode proteins. circRNAs can be loaded into exosomes, released by donor cells, and enter recipient cells through endocytosis, thus modulating gene expression in recipient cells.

**Encoding Polypeptides**

Although circRNAs are non-coding RNAs, a few circRNAs encode polypeptides that exert regulatory functions. This feature of circRNAs increases the complexity of the transcriptome and proteome. circ-ZNF609 can be translated into proteins in a splicing-dependent and cap-independent manner (Legnini et al., 2017). Further analysis revealed that consensus m\(^6\)A motifs were enriched in circRNAs, and a single m\(^6\)A site was sufficient to drive translation initiation. The study also stated that the m\(^6\)A-driven translation of circRNAs is widespread, and hundreds of endogenous circRNAs have translational potential (Yang et al., 2017).

**IDENTIFICATION AND VALIDATION OF CIRCULAR RNAs**

**Identification of circRNAs**

RNA sequencing (RNA-seq) is an important method for genome-wide circRNA research. High-throughput sequencing allows for deeper sequencing and longer read lengths, making circRNA detection possible (Jeck and Sharpless, 2014). RNA-seq library preparation and bioinformatics analysis of RNA-seq data are the main challenges of high-throughput circRNA sequencing (Szabo and Salzman, 2016). RNA purification is crucial for library preparation and subsequent circRNA detection. Currently, circRNAs are typically purified by either poly(A)-selected or depleted ribosomal RNA (Szabo and Salzman, 2016; López-Jiménez et al., 2018). Various software packages, such as CIRI and circRNA_finder, can be used to analyze circRNAs from RNA-seq data, including 11 algorithms (Zeng et al., 2017). However, complicated data analysis methods and high cost severely limit the application of RNA-seq in circRNA detection. Compared with RNA-seq, a circRNA microarray is a more sensitive and efficient platform for circRNA identification. Combined with enzymatic linear RNA removal, a circRNA microarray uses unique circular junction-specific probes to enrich, capture, and quantify circRNAs with high sensitivity and specificity (Xu et al., 2011).

**Validation of circRNAs**

Real-time fluorescent quantitative polymerase chain reaction (RT-qPCR) is essential for the verification of differentially expressed circRNAs after genome-wide sequencing or microarray analysis (Starke et al., 2015; Panda et al., 2017). In addition, RT-qPCR can conveniently quantify the expression of circRNAs used as biomarkers in clinical research (Guria et al., 2020). In a typical RT-qPCR analysis, total RNAs including linear RNAs (such as mRNAs and miRNAs) are extracted from cells, and linear RNAs are removed by ribosomal RNA (rRNA)
consumption or ribonuclease R (RNase R) (an exoribonuclease) digestion. Next, complementary DNAs (cDNAs) of circRNAs are obtained by reverse transcription (RT). Finally, the fluorescent signal is accumulated and acquired by PCR amplification (Jeck and Sharpless, 2014). However, current methods for rRNA consumption and RNase R digestion cannot completely remove linear RNAs, especially linear RNAs with extensive secondary structures. Amplification of residual linear RNAs hinders quantitative and qualitative analysis of circRNAs (Panda et al., 2017). Furthermore, during the RT process, rolling circle amplification (RCA) using circRNAs as templates produces technical artifacts, leading to irreversible false-positive results (Szabo and Salzman, 2016).

Northern blotting enables the specific detection and characterization of circRNAs (Hansen et al., 2013; Zhang et al., 2014). Detection of specific circRNAs by northern blotting can be accomplished by short probes spanning the circular splice junction, or by longer probes covering as much as an entire circularized exon (Yang et al., 2018). Compared with RT-qPCR, northern blotting is laborious and time-consuming, but it is still an important tool for circRNA characterization (Schneider et al., 2018). circRNA in situ hybridization (RNA-ISH) can locate and quantify circRNAs by using specific probes that bind to back-spliced junction sites (Yang et al., 2020). However, it is also time-consuming and labor-intensive. In addition, RNA-ISH requires complex experimental steps and yields low detection signals. Therefore, advanced microscopic technology is required to enhance the signals to detectable levels.

Considering the growing interest in the comprehensive characterization of circRNAs, it is essential to develop better circRNA detection methods. The detection and imaging method based on catalyzed hairpin assembly (CHA) is a promising strategy. In CHA, two complementary nucleic acid hairpins are designed to kinetically hinder their spontaneous hybridization by embedding complementary regions in hairpin stems. In the presence of a target input chain, one hairpin is opened as a result of the toehold-mediated strand displacement, which further induces the assembly of the two hair clips. Finally, similar to a catalyst, the target chain is spontaneously replaced and recycled to trigger more hairpin assembly events (Jung and Ellington, 2014). The CHA-based method has high sensitivity and programmability. It has been proven to be a versatile tool for the detection of various molecules and events, including nucleic acids, small molecules, proteins, enzyme activity, metal ions, and...
cancer cells (Liu J. et al., 2019). Using this method, Jin et al. constructed a linear DNA nanostructure (LDN) to directly detect circRNAs in complex samples and even in cells (Jiao et al., 2020). They can accurately identify circRNAs without linear RNA-derived interference and image intracellular circRNAs in situ. Hence, CHA provides a simple, effective, and stable method for the detection and quantification of circRNAs. This may facilitate the clinical application of circRNAs as biomarkers.

CIRCULAR RNAs IN NSCLC

Circular RNAs are closely related to the initiation and development of cancer (Figure 2). Genome-wide analysis has revealed that circRNAs are differentially expressed in various cancer tissues and cell lines (Dou et al., 2016; Shang et al., 2016; Wan et al., 2016; Chen et al., 2017; Zhao et al., 2017). Cancer cell lines express a more diverse pattern of circRNAs than non-cancer cell lines (Hsiao et al., 2017; Liu X-X. et al., 2019).

Circular RNAs may be involved in the onset of lung cancer. By comparing the expression of circRNAs between tumor samples and paired adjacent normal tissues, researchers found that 357 circRNAs were dysregulated in patients with early-stage lung adenocarcinoma (Zhao et al., 2017). Indeed, high expression of certain circRNAs is positively correlated with the progression of lung cancer. For example, circ_0043278 promotes NSCLC cell proliferation, invasion, and migration by directly inhibiting miR-520f to increase the expression of ROCK1, CDKN1B, and AKT3 (Cui et al., 2019). Furthermore, there is a close correlation between the upregulated circRNA_100876, lymph node metastasis and tumor staging in NSCLC. The overall survival time of NSCLC patients with high circRNA_100876 expression was significantly shorter than that of patients with low circRNA_100876 expression (Yao et al., 2017).

Circular RNAs can also play a role in cancer inhibition (Wan et al., 2016). For instance, ectopic expression of circ-ITCH markedly elevated the expression of its parental cancer-suppressive gene ITCH and inhibited the proliferation of lung cancer cells. The molecular mechanism underlying this effect is that circ-ITCH acts as a sponge of oncogenic miR-7 and miR-214 to enhance ITCH expression, thus suppressing the activation of Wnt/β-catenin signaling.

In conclusion, some circRNAs are closely related to the pathogenesis of NSCLC. Therefore, they may serve as targets for NSCLC therapy.

CIRCULAR RNAs AS PROMISING BIOMARKERS FOR NSCLC

Circular RNAs can be easily and repeatedly detected in blood samples, making them promising biomarker candidates for human diseases (Menczak et al., 2015). Furthermore, the discovery of circRNAs in saliva indicates the presence of circRNAs in other body fluids (Bahn et al., 2015). Compared to traditional biomarkers found in tumor tissues, circRNAs in body fluids could be used as novel biomarkers in the more convenient and non-invasive “liquid biopsy” for the diagnosis of tumors at different stages. Until recently, efforts have been made to explore the significance of circRNAs as biomarkers for various types of cancers (Wang et al., 2016). At present, there are many studies on the application of circRNA expression in peripheral blood in the diagnosis of NSCLC, which proves the effectiveness of circRNA in the diagnosis of NSCLC (Table 1).

In the past decades, numerous studies have investigated the expression profiles of miRNAs and IncRNAs in patients with NSCLC to assess if these RNA types can serve as biomarkers for the early detection of NSCLC (Wei and Zhou, 2016; Han and Li, 2018). However, the results remain controversial. Compared with other non-coding RNAs, circRNAs have superior features, such as higher stability, abundance, and evolutionary conservation, making them more suitable diagnostic biomarkers (Figure 3).

Circular RNAs as Differential Diagnosis Biomarkers for NSCLC

At present, the most commonly used serum tumor markers for the diagnosis of NSCLC are embryonic antigen (CEA), squamous cell carcinoma antigen (SCCA), and cytokeratin 19 fragment (CYFRA21-1) (Ye et al., 2020). However, the sensitivity and specificity of detecting a single circulating biomarker in the diagnosis of lung cancer are not satisfactory, especially in the early stages (Kamel et al., 2019). circRNAs can stably exist in peripheral blood, and the detection of circRNAs expression in the blood has gradually become the focus of research in the diagnosis of NSCLC. Circulating miRNAs have shown potential advantages for early screening of NSCLC. A study found that circ_0047921, circ_0056285, and circ_0007761 in serum exosomes display significant diagnostic validity in distinguishing early-stage NSCLC cases from healthy controls, chronic obstructive pulmonary disease controls, and tuberculosis controls. In distinguishing NSCLC cases from healthy controls, the panel of the aforementioned circRNAs exhibited an area under the curve (AUC) values of 0.926 [95% confidence interval (CI), 0.895–0.956] in the training set and 0.919 (95% CI, 0.877–0.962) in the validation set (Xian et al., 2020). A study on hsa_circ_0046264 showed that the area under the ROC curve (AUC) of hsa_circ_0046264 in serum was 0.915, the specificity was 0.957, and the sensitivity was 0.927 (Liu et al., 2020). The recent discovery of circRNAs in liquid biopsy samples suggests a novel and potentially useful tool for non-invasive NSCLC diagnosis.

Circular RNAs as Prognostic Biomarkers for NSCLC

Prognostic biomarkers should provide information about the patient’s overall disease outcome, such as disease recurrence, metastasis, or progression, and may help identify high-risk cases that require active measures. circ_0067934 has been reported to function as an oncogenic circRNA in NSCLC by promoting the proliferation, migration, and invasion of NSCLC cells. High circ_0067934 expression is significantly associated with TNM stage, lymph node status, and distant metastasis, thus serving as a potential prognostic biomarker and therapeutic target for NSCLC.
### TABLE 1 | circRNAs as biomarkers in liquid biopsy for the diagnosis of NSCLC.

| circRNA | Expression level | Intersection molecules and/or pathway | Effect | Sample | AUC | References |
|---------|------------------|----------------------------------------|--------|--------|-----|------------|
| circ_0008928 | Up | miR-488/HK2 axis | Regulates cisplatin sensitivity, tumor progression, and glycolysis metabolism. | Serum exosomes |  | Shi et al., 2021 |
| circ_PIP5K1A | Up | miR-101/ABCC1 axis | Regulates the progression of NSCLC and cisplatin sensitivity. | Serum and serum exosomes |  | Shao et al., 2021 |
| circCDYL | Down | miR-185-5p/TNRC6A axis | Inhibited cell viability, proliferation, and induced apoptosis. | Plasma |  | Bian et al., 2021 |
| hsa_circ_0014235 | Up | miR-520a-5p/CDK4 axis | Promotes cisplatin chemoresistance and deteriorates the development of NSCLC. | Serum exosomes |  | Xu et al., 2020 |
| hsa_circ_0060937 | Up | – | Closely associated with bone metastasis in NSCLC. | Serum |  |  |
| hsa_circ_0046264 | Up | – | Notably associated with the patient’s age, tumor size, TNM stage, and lymph node metastasis. | Serum | 0.915 | Liu et al., 2020 |
| circ-MEMO1 | Up | miR-101-3p/KRAS axis | Promotes the progression and aerobic glycolysis of NSCLC. | Serum exosomes | 0.760 | Ding et al., 2020 |
| circARHGAP10 | Up | miR-638/FA/M83F axis | Promotes proliferation, migration, invasion, and glycolysis. | Serum exosomes | – | Fang et al., 2020 |
| circPTN1 | Up | – | Associated with chemotherapy resistance. | Serum | – | Li et al., 2020 |
| hsa_circ_0002130 | Up | miR-498/GLUT1/HK2/LDHA axis | Facilitates osimertinib resistance. | Serum exosomes | – | Ma et al., 2020 |
| the panel of circ_0047921, hsa_circ_0056285, and circ_007761 | Up, Down | – | Distinguishing early-stage NSCLC cases from healthy controls, chronic obstructive pulmonary disease controls, or tuberculosis controls. | Serum exosomes | 0.919 | Xian et al., 2020 |
| circSATB2 | Up | miR-326/FSCN1 axis | Promotes the proliferation, migration, and invasion of NSCLC cells. | Serum exosomes | – | Zhang N. et al., 2020 |
| hsa_circRNA_012515 | Up | – | May be a mechanism leading to gefitinib resistance in NSCLC patients. | Peripheral blood | – | Fu et al., 2020 |
| hsa_circ_0134501 combined with hsa_circ_0109320 | Down | – | Biomarker candidates for predicting the therapeutic effect of gefitinib in NSCLC. | Plasma | 0.79 | Liu Y.-T. et al., 2019 |
| circ FECR1 | Up | miR584-3p/ROCK1 axis | Predicts survival outcomes and predicts the response to chemotherapies. | Serum exosomes | – | Li et al., 2019 |
| F-circEA | Exist | – | Could be a novel “liquid biopsy” biomarker to monitor the EML4-ALK fusion gene in NSCLC. | Plasma | – | Tan et al., 2018 |
| hsa_circ_0102533 | Up | – | Possesses an oncogenic property in the carcinogenesis. | Whole blood | 0.744 | Zhou et al., 2018 |
| circFARSA | Up | miR-330-5p/FASN or miR-326/FASN axis | Promotes cell migration and invasion. | Plasma | 0.71 | Hang et al., 2018 |
| hsa_circ_0013958 | Up | miR-134/CCND1 axis | Promotes cell proliferation and invasion and inhibits cell apoptosis. | Plasma | 0.794 | Zhu et al., 2017 |

TNM, tumor-node-metastasis; AUC, area under the ROC curve (AUC).
Potential application of circular RNAs as liquid biopsy biomarkers. circRNA biomarkers can be isolated from blood, as well as from the exosomes in blood. Detection and analysis of circRNAs can be completed using RNA-seq, qPCR, and other methods. circRNAs may serve as potential biomarkers for diagnosis, prognosis, and therapy selection of cancer.

(Wang and Li, 2018). Another study showed that by acting as a sponge for miR-1287 and regulating GAGE1 expression, circ_0016760 promoted the growth and metastasis of NSCLC cells, while inhibiting apoptosis. Moreover, the upregulation of circ_0016760 is associated with advanced TNM stages, lymph node metastasis, and poor prognosis in patients with NSCLC (Li Y. et al., 2018). Similarly, the upregulation of circRNA_100876 was tightly correlated with lymph node metastasis and advanced tumor stages in NSCLC. In addition, NSCLC patients with high circRNA_100876 expression had significantly shorter survival times than those with low circRNA_100876 expression. These observations strongly suggest the role of circRNAs as prognostic biomarkers.

Circular RNAs as Predictive Biomarkers for Cancer Therapy

In addition, circRNAs may be useful for predicting tumor responsiveness to chemotherapy or different therapeutic approaches. Resistance to targeted therapy or immunotherapy is an ongoing problem in the successful treatment of NSCLC. It is increasingly necessary to develop biomarkers to monitor and predict the efficacy of targeted therapy or immunotherapy in patients with NSCLC. Recent studies suggested that circRNAs are likely to serve this purpose.

A study demonstrated that hsa_circ_0004015 increased the resistance of the NSCLC cell line HCC827 to gefitinib (an EGFR tyrosine kinase inhibitor) (Zhou et al., 2019). In addition, the EGFR-resistant LADC cell line H1975 expresses high levels of circRNA CCDC66 (Joseph et al., 2018). A high-throughput circRNA microarray showed that in comparison with parental cells, the paclitaxel-resistant lung adenocarcinoma cell line A549 had higher levels of 2,909 circRNAs and lower levels of 8,372 circRNAs (Xu et al., 2018). A recently identified circRNA, termed F-circEA, results from the fusion of the ALK gene with the EML4 gene in NSCLC patients. For EML4-ALK-positive NSCLC patients, F-circEA could be both
a novel diagnostic biomarker and a predictive biomarker of the response to targeted therapy (Tan et al., 2018). Another study showed that overexpression of circFGFR1 resulted in resistance of NSCLC to treatment with anti-programmed cell death protein-1 (PD-1) (Zhang et al., 2019). In general, these studies suggest that dysregulated circRNAs lead to resistance to targeted therapy and immunotherapy in NSCLC patients.

Circular RNAs also influence chemotherapy resistance in patients with NSCLC. It has been reported that circRNA cESRP1 enhances SCLC8 sensitivity to chemotherapy agents by inhibiting miR-93-5p (Huang et al., 2020). Downregulation of hsa_circ_0001946 increases NSCLC cell resistance to the chemotherapeutic drug cisplatin (Huang et al., 2019), whereas overexpression of circ-ABCB10 increases lung cancer resistance to cisplatin (Wu et al., 2020). Therefore, circRNAs may be used as biomarkers to predict the sensitivity or resistance to platinum-based chemotherapy.

**Exosomal circRNAs as NSCLC Biomarkers**

Exosomes are tiny vesicles secreted by cells. They carry a variety of biomarkers such as DNA, RNA, proteins, and metabolites (Théry et al., 2002; Kahler and Kalluri, 2013; Kumar et al., 2018). Exosomal contents are functional in recipient cells and highly variable depending on the origin of the cells. Cells can produce different exosomes under distinct physiological or pathological conditions (Fujita et al., 2016). Cancer cells produce more exosomes than their non-malignant counterparts (Rodríguez et al., 2014). Cancer cell-released exosomes induce phenotypic or functional alterations in recipient cells, thereby playing a role in cancer growth, angiogenesis, and metastasis (Kosaka et al., 2016).

Exosomes can mediate cell-to-cell communication via the direct exchange of genetic material between cells (Villarroya-Beltri et al., 2014). Exosomes can be isolated from diverse biofluids, such as blood (Caby et al., 2005), urine (Bryzgunova et al., 2016), and saliva (Ogawa et al., 2008). In recent years, circulating cancer-derived exosomes have emerged as promising biomarkers for monitoring cancer progression in non-invasive cancer diagnosis (Peinado et al., 2012; Xiao et al., 2012; Camacho et al., 2013; Zhou et al., 2014; Sundararajan et al., 2018). Studies on exosomal miRNAs and lncRNAs have contributed to the great progress in cancer screening, diagnosis, and prognostic evaluation (Liu et al., 2015; Li B. et al., 2018; Lee et al., 2019). However, our understanding of exo-circRNAs remains unclear.

Jae et al. first reported and validated circRNAs in human body fluids (Bahn et al., 2015). Later, another study confirmed abundant circRNAs in exosomes. RNA-seq analyses revealed the enrichment of circRNAs in the exosomes. It has been shown that more than 1,000 circRNAs are present in human serum exosomes (Li Y. et al., 2015), highlighting circRNAs as a novel class of stable RNA molecules in exosomes. Upon the uptake of EVs by neighboring or distant cells, exo-circRNAs can contribute to cell-to-cell communication. The existence of exo-circRNAs supports the idea that the expulsion of circRNAs from cells into the extracellular space, such as through EV release, is a probable mechanism by which circRNAs are cleared (Li Y. et al., 2015; Lasda and Parker, 2016).

Interestingly, serum exo-circRNAs might distinguish cancer patients from healthy individuals, illustrating the significant possibility of translating exo-circRNAs into cancer diagnostic biomarkers (Li Y. et al., 2015). A study found that circ-MEMO1 was higher in exosomes generated from the serum of NSCLC patients than in the healthy volunteers. The AUC reached a value of approximately 0.76, with a diagnostic sensitivity and specificity of 56.67 and 96%, respectively (95% CI 0.6259–0.8941) (Ding et al., 2020). Another study showed that circ_0008928 expression was increased in serum exosomes of cisplatin-resistant NSCLC patients compared with that in the cisplatin-sensitive NSCLC patients (Shi et al., 2021). In addition, a study showed that serum exosomal circFECR1 was associated with poor survival ($P = 0.038$) and clinical response to chemotherapy (Li et al., 2019). In summary, exo-circRNAs can be used as a promising biomarker for the diagnosis of NSCLC.

**CONCLUDING REMARKS AND PERSPECTIVES**

Liquid biopsy is a rapidly growing field, and advances in detection technology provide the possibility for the discovery and clinical translation of circulating cancer biomarkers. Although there has been extensive research on the discovery of circulating markers for cancer diagnosis, most biomarkers are still in the experimental stage. Before circulating biomarkers can be used in clinical practice, many biological, technical, and clinical challenges need to be addressed.

circRNAs have a clear functional role in the occurrence and development of NSCLC, and tumor-related differential expression has been detected in the peripheral blood of NSCLC patients. Due to the complexity of blood samples, it is necessary to develop a new methodology that can accurately extract and reproducibly measure NSCLC-related circulating circRNAs and conduct extensive verification in a large number of clinical samples to prove its clinical effectiveness. Furthermore, a study reported that it is obvious that the “multiple biomarker profile” has higher sensitivity and specificity than a single biomarker (Gulati et al., 2014). In the future, it is expected to improve the accuracy of circRNA in the diagnosis, prognosis, and prediction of NSCLC through the beneficial combination of circRNAs and different levels of other molecules (combining genome, transcriptome, and proteome). The transition from a single-biomarker view to a multi-biomarker view will help promote the development of liquid biopsy.

Circular RNAs have potential applications in both laboratory and clinical cancer research. However, there are still many questions regarding circRNAs. One major question is whether the products of endogenous circRNAs have specific functions in...
eukaryotic cells. Another interesting question is how circRNAs are degraded. To answer these questions, circRNAs should be studied in depth.

Moreover, circRNAs may be considered as novel therapeutic targets. Although there are no such reports to date, the effects of circRNAs on lung cancer proliferation, metastasis, and drug resistance indicate potential for targeting of circRNAs in anti-cancer therapy (Chi et al., 2019; Dong et al., 2019; Tian et al., 2019). For example, it is possible to deliver small interfering RNAs (siRNAs) or vectors into the body to target certain circRNAs in cancer patients. In addition, research on circRNA biogenesis might identify an approach to regulate the upstream pathways of circRNA expression (Verduci et al., 2019).

Taken together, the ultimate goal of circRNA research is to develop new effective diagnostic or therapeutic strategies for cancer treatment. With the advancement of elaborate research, our understanding of circRNAs will significantly contribute to cancer prevention, diagnosis, and treatment in the coming decades.

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LH wrote the manuscript. YR conceived the review and performed bibliographic research. XT and KY prepared the figures and table. JW and FW contributed to the critical interpretation of the manuscript. All authors read and approved the final version of the manuscript.
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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