Circulating Circular RNAs in Lung Cancer

Subjects: Oncology

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Circular RNAs (circRNAs) are single-stranded RNAs with a covalently closed-loop structure that increases their stability; thus, they are more advantageous to use as liquid biopsy markers than linear RNAs. circRNAs are thought to be generated by back-splicing of pre-mRNA transcripts, which can be facilitated by reverse complementary sequences in the flanking introns and trans-acting factors, such as splicing regulatory factors and RNA-binding factors. circRNAs function as miRNA sponges, interact with target proteins, regulate the stability and translatability of other mRNAs, regulate gene expression, and produce microproteins. circRNAs are also found in the body fluids of cancer patients, including plasma, saliva, urine, and cerebrospinal fluid, and these “circulating circRNAs” can be used as cancer biomarkers. In lung cancer, some circulating circRNAs have been reported to regulate cancer progression and drug resistance. Circulating circRNAs have significant diagnostic value and are associated with the prognosis of lung cancer patients. Owing to their functional versatility, heightened stability, and practical applicability, circulating circRNAs represent promising biomarkers for lung cancer diagnosis, prognosis, and treatment monitoring.

Keywords: circular RNAs ; lung cancer ; liquid biopsy

1. Introduction

Despite advancements in breakthrough therapies such as targeted therapy and immunotherapy, the survival rate of lung cancer patients has failed to improve for decades. Lung cancer remains the leading cause of cancer-related death worldwide, while the 5-year survival rate of patients with distant metastatic lung cancer is only 6% [1]. Thus, these outcomes urgently require the development of an effective early diagnosis method for lung cancer. Currently, early screening methods for lung cancer in clinical practice include sputum cytology, low-dose chest computed tomography, and autofluorescence bronchoscopy [2]. For the minimal or non-invasive early diagnosis of lung cancer, blood-based biomarkers are rapidly emerging as new alternatives and include circulating tumor cells, exosomes, and circulating nucleic acids (DNAs, microRNAs, and non-coding RNAs) [3][4].

Circular RNAs (circRNAs) are single-stranded endogenous RNAs with a covalently closed-loop structure [5]. Since their discovery in the 1970s, the study of circRNAs has been limited; however, with the development of next-generation sequencing, over the past decade, there has been an increased interest and research in circRNAs. As the physiological and pathological functions of circRNAs have been discovered, many studies have attempted to use circRNAs as biomarkers in the diagnosis of cancer. Owing to their stable structure [5], circulating circRNAs are thought to be more advantageous than normal linear RNAs for application in liquid biopsies.

2. Circulating circRNAs

As previously described, circRNAs with diverse functions are involved in cancer development, progression, and metastasis. Like those of mRNAs, miRNAs, and proteins, the expression levels of circRNAs vary depending on the cell type; thus, they can be applied as diagnostic or prognostic markers in cancer patients. Numerous circRNAs have been reported to be upregulated or downregulated in various types of cancer. circRNAs have been discovered in plasma, saliva, urine, and cerebrospinal fluid, so that circulating circRNAs can be used as cancer biomarkers [6][7]. Like other linear RNAs, circRNAs can be amplified through reverse-transcription PCR (RT-PCR), which makes them more easily detectible than protein markers. Unlike linear RNAs, circRNAs lack free 5′- and 3′-ends, making them highly resistant to degradation by RNases with exonuclease activity.

It has been demonstrated that circRNAs are abundant and stable in exosomes, suggesting their significant translational potential as circulating biomarkers for cancer diagnosis [8]. In hepatocellular carcinoma, exosomal *circPTGR1* was shown to promote cancer progression through the regulation of the miR-449a/MET pathway [9]. Similarly, *circNRIP1* was also proven to be transmitted via exosomes and promoted tumorigenesis and metastasis of gastric cancer [10]. In laryngeal squamous cell carcinoma, *circRASSF2* was secreted by exosomes and promoted tumor growth through the regulation of

the miR-302b-3p/IGF-1R pathway [11]. High *circCNOT2* expression was associated with poor progression-free survival of patients with breast cancer, and *circCNOT* is detectable in cell-free RNAs from patient plasma samples [12]. In addition, circRNAs can also be detected in circulating tumor cells [13]. Furthermore, circRNAs have been shown to be highly enriched in blood platelets compared with nucleated cells, which can be used for cancer diagnosis [14]. Since protein carriers such as high-density lipoprotein and Argonaute 2 transport miRNAs [15], the circulation of circRNAs might also be mediated by certain protein carriers or RNA-binding proteins.

3. Functional Roles of Circulating circRNAs in Lung Cancer

3.1. Cancer Progression

The presence of *F-circEA* generated from the *EML–ALK* fusion gene was verified in non-small cell lung cancer (NSCLC) cells and in the plasma of NSCLC patients [16]. *F-circEA* promoted cancer cell migration and invasion, suggesting that *F-circEA* could be a novel liquid biopsy marker for NSCLC. Through circRNA profiling of serum or plasma obtained from patients, the clinical relevance of many circulating circRNAs has been explored.

Global circRNA expression can be profiled by using RNA sequencing (RNA-seq) followed by bioinformatic approaches [17]. *circFARSA* was identified as an upregulated circRNA in NSCLC tissues compared with adjacent normal tissue by analyzing back-spliced reads on RNA-seq data [18]. *circFARSA* expression was higher in the plasma from NSCLC patients than in that from healthy volunteers and showed a good diagnostic value for NSCLC (AUC = 0.71). cDNA encoding *circFARSA* was cloned into the pLCDH-ciR vector, which was specifically designed to overexpress circular transcripts [19]. *circFARSA* overexpression enhanced the migration and invasion of A549 cells. Through in silico analyses, circFARSA was predicted to sponge miR-330 and miR-326 and regulate fatty acid synthesis. This is one of the earliest studies investigating the possibility of plasma circRNAs as new biomarkers for NSCLC patients; however, it lacks functional evidence supporting the molecular mechanism of *circFARSA* in NSCLC.

Through a microarray-based screening, *circYWHAZ* (*circ_0005962*) was identified as one of the upregulated circRNAs in lung adenocarcinoma (LUAD) [20]. The knockdown of *circYWHAZ* by siRNAs significantly suppressed the proliferation of LUAD cells, implying that this circRNA can promote cell proliferation [21]. Moreover, *circYWHAZ* expression was also upregulated in plasma samples, which illustrates a good diagnostic value for LUAD patients (AUC = 0.73). After surgical resection, *circYWHAZ* expression in the plasma decreased considerably, which suggests that *circ_0005962* is potentially a good noninvasive biomarker for LUAD diagnosis [21]. miRNA-target prediction and functional enrichment analysis showed that *circYWHAZ* might function as a miRNA sponge to regulate LUAD development, which needs further validation.

circACP6 (*circ_0013958*) was also upregulated in LUAD tumors compared with nontumor tissues, which was validated by microarray and RT-PCR [22]. High expression of *circACP6* was associated with the TNM stage (p = 0.009, Cox analysis) and lymphatic metastasis (p = 0.006) in LUAD patients. Moreover, the plasma expression levels of *circACP6* distinguished LUAD from the control (AUC = 0.794, 95% CI = 0.703–0.912). Additionally, knockdown of *circACP6* inhibited the proliferation, migration, and invasion of LUAD cells. Mechanistically, *circACP6* functioned as a sponge against miR-134, which promoted the upregulation of cyclin D1, a target of miR-134.

circCXCR4 (*circ_0056616*) was identified and detected as a CXCR4-related circRNA in LUAD cells and exosomes [23]. Plasma exosome levels of *circCXCR4* were lower in LUAD patients with TNM stage III–IV or with lymphatic metastasis than in those with stage I–II or without metastasis, respectively. This suggests that *circCXCR4* might suppress the progression and metastasis of LUAD. Indeed, plasma exosomal *circCXCR4* represents a good biomarker to diagnose lymphatic metastasis of LUAD (AUC = 0.812, 95% CI = 0.720–0.903), which also needs to be validated in a larger group of patients.

Through the exoRBase database (http://www.exorbase.org; accessed on 3 June 2020), *circSATB2* (*circ_0008928*) was selected as a highly expressed circRNA in cancer exosomes [24]. The expression of *circSATB2* was higher in lung cancer cells than in normal bronchial epithelial cells. Furthermore, overexpression and knockdown experiments showed that *circSATB2* promoted the proliferation, migration, and invasion of lung cancer cells. Additionally, the packaging and transfer of *circSATB2* by exosomes influenced the proliferation and migration of the recipient cells. *circSATB2* directly bound to and inhibited miR-326, which in turn upregulated FSCN1, the presence of which has been reported as a poor prognostic marker for NSCLC patients [25]. Therefore, upregulation of FSCN1 by *circSATB2* via sponging miR-326 represents a potential mechanism through which *circSATB2* promotes NSCLC progression. In addition, serum exosomal *circSATB2*

expression was higher in NSCLC patients with metastasis than in those without, demonstrating a good diagnostic value for metastatic NSCLC (AUC = 0.797, 95% CI = 0.698–0.896).

In contrast, RNA-seq profiling demonstrated that *circ_0102537* was one of the downregulated exosomal circRNAs in LUAD, which was also retrieved from a microarray database (GSE101586). Moreover, *circ_0102537* was confirmed by quantitative RT-PCR to be downregulated in both plasma exosomes and tissues from LUAD patients. *circ_0102537* knockdown by siRNAs promoted the migration and invasion of lung cancer cells and enhanced the expression of EMT markers such as N-cadherin, Snail, and Vimentin. This suggests that *circ_0102537* might function as a tumor suppressor; however, the functional mechanism has not been presented [26]. Although many circulating circRNAs have been linked to lung cancer progression so far, further validation with more diverse groups of patients and in-depth mechanistic studies should be performed.

3.2. Anticancer Drug Response

Over a long period, numerous studies have been conducted to find predictive markers for sensitivity to EGFR inhibitors [27], and several circulating circRNAs have been proposed as candidate markers. Microarray analysis of plasma RNAs from NSCLC patients sensitive or resistant to gefitinib, an EGFR inhibitor, revealed that 1377 circRNAs were differentially expressed between the two groups [28]. Among them, *circZNF91* (*circ_0109320*) was upregulated in the gefitinib-sensitive group. The plasma levels of *circZNF91* could distinguish the gefitinib-sensitive group from the resistant group (AUC = 0.8054) and were associated with better progression-free survival in NSCLC patients treated with this EGFR inhibitor. Overall, *circZNF91* could be a predictive biomarker of the sensitivity to gefitinib treatment in NSCLC patients after comparative verification with other parameters in a wider and larger group of patients.

circC3 (*circ_0002130*) increased in NSCLC cells that acquired resistance to the EGFR tyrosine kinase inhibitor, osimertinib [29]. *circC3* knockdown inhibited proliferation, glycolysis, and tumor growth in osimertinib-resistant lung cancer cells [30]. *circC3* acted as a sponge against miR-498 to upregulate its targets, GLUT1, HK2, and LDHA, which are glycolysis-related proteins. Furthermore, an increase in *circC3* was detected in serum exosomes from osimertinib-resistant NSCLC patients with respect to those from osimertinib-sensitive patients. *circC3* provided a good diagnostic value to predict the efficacy of osimertinib treatment in NSCLC patients (AUC = 0.792, 95% CI = 0.676–0.909), suggesting circulating *circC3* as a novel biomarker. A combination of two or more circulating circRNAs with other variables such as EGFR mutations and gene copy number [27] would be a better biomarker for predicting the sensitivity to EGFR inhibitors.

circCNIH4 (*circ_0000190*) and *circSHPRH* were identified by RNA-seq to be upregulated in lung cancer cells compared with normal bronchial epithelial cells. They were also detected in conditioned media from lung cancer cells and in blood plasma samples by droplet digital PCR [31]. Furthermore, their plasma levels exhibited a poor response to immunotherapy, which might be due to the upregulation of soluble PD-L1 caused by these circRNAs [32]. Even though the detailed mechanism underlying the interplay between these circRNAs and antitumor immunity is still elusive, along with PD-L1 expression, their plasma levels could predict immunotherapy efficacy in lung cancer patients.

In-depth analysis of two GEO microarray datasets (GSE101684 and GSE101586) identified circRNAs highly expressed in LUAD samples compared with normal tissues [33]. Among them, *circ_002178* promoted PD-L1 expression via sponging miR-34a. *circ_002178* was also highly detected in plasma exosomes from LUAD patients compared with those from healthy volunteers, and exosomal *circ_002178* had a significant diagnostic value for LUAD (AUC = 0.9967). Intriguingly, *circ_002178* was transferred from cancer cells to $CD8^+$ T cells via exosomes and then promoted PD-1 expression via sponging miR-28-5p. This indicates that *circ_002178* would be a good target for immunotherapy, since it can modulate the expression of PD-1/PD-L1 in LUAD. Circulating circRNAs are highly likely to be exploited as markers for predicting the responses to anticancer drugs once their mechanisms of action are confirmed and their efficacy is validated in more diverse patients.

3.3. Cancer Diagnosis and Prognosis

As noted previously, numerous circulating circRNAs (*circFARSA*, *circYWHAZ*, *circACP6*, *circSATB2*, *circZNF91*, *circC3*, and *circ_002178*) have significant diagnostic value and are associated with prognosis in lung cancer patients. In addition, RNA-seq and subsequent RT-PCR validation confirmed that *circCD226* (*circ_0047921*) and *circRALB* (*circ_0056285*) were downregulated, while *circATXN7* (*circ_0007761*) was upregulated in serum exosomes from NSCLC patients [34]. The combination of these three circRNAs provides a noteworthy diagnostic tool, which distinguishes NSCLC from healthy control (AUC = 0.919, 95% CI = 0.877–0.962) or other lung diseases, and their expression levels were associated with NSCLC progression.

Plasma *circCNIH4* demonstrated diagnostic potentials in lung cancer patients at all TNM stages (AUC = 0.95 for stage I–IV, AUC = 0.896 for stage I–II, and AUC = 0.96 for stage III–IV) [31]. Patients with high plasma levels of *circCNIH4* exhibited poorer overall survival rates than those with low levels. Mechanistically, *circCNIH4* could modulate the EGFR/ERK pathway by sponging miR-142-5p [35]. *circPVT1* was also upregulated in tissues and sera from NSCLC patients. Both tissue and serum levels of *circPVT1* showed diagnostic potential, distinguishing NSCLC patients from controls (AUC = 0.803 and 0.794, respectively) [36]. The knockdown of *circPVT1* by siRNAs suppressed proliferation, migration, and invasion and promoted apoptosis in lung cancer cells. *circPVT1* facilitated E2F2 signaling by functioning as a sponge against miR-125b. Even though researchers did not present the effect of *circPVT1* on the survival or prognosis of NSCLC patients recruited, they proved that *circPVT1* can be used as a diagnostic marker and elucidated its working mechanism in NSCLC.

The expression levels of several circulating circRNAs are associated with major mutations found in lung cancer. For example, *F-circEA*, but not its host linear mRNA, could be detected in EML4–ALK$^+$ lung cancer plasma; thus, circulating *F-circEA* would be a novel biomarker to detect EML4–ALK fusion and to determine an effective treatment for EML4-ALK$^+$ patients [16]. LUAD patients with high plasma expression of *circBNC2* (*circ_0086414*) were revealed to harbor EGFR mutations more frequently than those with low expression (p = 0.001) [21], suggesting that plasma *circBNC2* would be a companion diagnostic marker for EGFR tyrosine kinase inhibitors. Considering the examples described above and the stable structure of circRNAs, circulating circRNAs can be novel biomarkers for diagnosis, prognosis, and treatment monitoring in lung cancer patients.

References

1. Siegel, R.L.; Miller, K.D.; Fuchs, H.E.; Jemal, A. Cancer statistics, 2021. CA Cancer J. Clin. 2021, 71, 7–33.
2. Sutedja, G. New techniques for early detection of lung cancer. Eur. Respir. J. Suppl. 2003, 39, 57s–66s.
3. Ostrin, E.J.; Sidransky, D.; Spira, A.; Hanash, S.M. Biomarkers for lung cancer screening and detection. Cancer Epidemiol. Biomarkers Prev. 2020, 29, 2411–2415.
4. Wani, J.A.; Majid, S.; Khan, A.; Arafah, A.; Ahmad, A.; Jan, B.L.; Shah, N.N.; Kazi, M.; Rehman, M.U. Clinico-pathological importance of miR-146a in lung cancer. Diagnostics 2021, 11, 274.
5. Qu, S.; Yang, X.; Li, X.; Wang, J.; Gao, Y.; Shang, R.; Sun, W.; Dou, K.; Li, H. Circular RNA: A new star of noncoding RNAs. Cancer Lett. 2015, 365, 141–148.
6. Wang, S.; Zhang, K.; Tan, S.; Xin, J.; Yuan, Q.; Xu, H.; Xu, X.; Liang, Q.; Christiani, D.C.; Wang, M.; et al. Circular RNAs in body fluids as cancer biomarkers: The new frontier of liquid biopsies. Mol. Cancer 2021, 20, 13.
7. Wang, Z.; Yu, R.; Chen, X.; Bao, H.; Cao, R.; Li, A.N.; Ou, Q.; Tu, H.Y.; Zhou, Q.; Wu, X.; et al. Clinical utility of cerebrospinal fluid-derived circular RNAs in lung adenocarcinoma patients with brain metastases. J. Transl. Med. 2022, 20, 74.
8. Li, Y.; Zheng, Q.; Bao, C.; Li, S.; Guo, W.; Zhao, J.; Chen, D.; Gu, J.; He, X.; Huang, S. Circular RNA is enriched and stable in exosomes: A promising biomarker for cancer diagnosis. Cell Res. 2015, 25, 981–984.
9. Wang, G.; Liu, W.; Zou, Y.; Wang, G.; Deng, Y.; Luo, J.; Zhang, Y.; Li, H.; Zhang, Q.; Yang, Y.; et al. Three isoforms of exosomal circPTGR1 promote hepatocellular carcinoma metastasis via the miR449a-MET pathway. EBioMedicine 2019, 40, 432–445.
10. Zhang, X.; Wang, S.; Wang, H.; Cao, J.; Huang, X.; Chen, Z.; Xu, P.; Sun, G.; Xu, J.; Lv, J.; et al. Circular RNA circNRIP1 acts as a microRNA-149-5p sponge to promote gastric cancer progression via the AKT1/mTOR pathway. Mol. Cancer 2019, 18, 20.
11. Tian, L.; Cao, J.; Jiao, H.; Zhang, J.; Ren, X.; Liu, X.; Liu, M.; Sun, Y. CircRASSF2 promotes laryngeal squamous cell carcinoma progression by regulating the miR-302b-3p/IGF-1R axis. Clin. Sci. 2019, 133, 1053–1066.
12. Smid, M.; Wilting, S.M.; Uhr, K.; Rodríguez-González, F.G.; de Weerd, V.; Prager-Van der Smissen, W.J.C.; van der Vlugt-Daane, M.; van Galen, A.; Nik-Zainal, S.; Butler, A.; et al. The circular RNome of primary breast cancer. Genome Res. 2019, 29, 356–366.
13. Zhang, Z.; Yang, T.; Xiao, J. Circular RNAs: Promising biomarkers for human diseases. EBioMedicine 2018, 34, 267–274.
14. Alhasan, A.A.; Izuogu, O.G.; Al-Balool, H.H.; Steyn, J.S.; Evans, A.; Colzani, M.; Ghevaert, C.; Mountford, J.C.; Marenah, L.; Elliott, D.J.; et al. Circular RNA enrichment in platelets is a signature of transcriptome degradation. Blood 2016, 127, e1–e11.

15. Nik Mohamed Kamal, N.; Shahidan, W.N.S. Non-Exosomal and Exosomal circulatory MicroRNAs: Which are more vali d as biomarkers? Front. Pharmacol. 2019, 10, 1500.

16. Tan, S.; Gou, Q.; Pu, W.; Guo, C.; Yang, Y.; Wu, K.; Liu, Y.; Liu, L.; Wei, Y.Q.; Peng, Y. 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, 693–695.

17. López-Jiménez, E.; Rojas, A.M.; Andrés-León, E. RNA sequencing and prediction tools for circular RNAs analysis. Adv. Exp. Med. Biol. 2018, 1087, 17–33.

18. Hang, D.; Zhou, J.; Qin, N.; Zhou, W.; Ma, H.; Jin, G.; Hu, Z.; Dai, J.; Shen, H. A novel plasma circular RNA circFARSA is a potential biomarker for non-small cell lung cancer. Cancer Med. 2018, 7, 2783–2791.

19. Zhong, Z.; Lv, M.; Chen, J. Screening differential circular RNA expression profiles reveals the regulatory role of circTCF 25-miR-103a-3p/miR-107-CDK6 pathway in bladder carcinoma. Sci. Rep. 2016, 6, 30919.

20. Chen, T.; Yang, Z.; Liu, C.; Wang, L.; Yang, J.; Chen, L.; Li, W. Circ_0078767 suppresses non-small-cell lung cancer by protecting RASSF1A expression via sponging miR-330-3p. Cell Prolif. 2019, 52, e12548.

21. Liu, X.X.; Yang, Y.E.; Liu, X.; Zhang, M.Y.; Li, R.; Yin, Y.H.; Qu, Y.Q. A two-circular RNA signature as a noninvasive diag nostic biomarker for lung adenocarcinoma. J. Transl. Med. 2019, 17, 50.

22. Zhu, X.; Wang, X.; Wei, S.; Chen, Y.; Chen, Y.; Fan, X.; Han, S.; Wu, G. hsa_circ_0013958: A circular RNA and potentia l novel biomarker for lung adenocarcinoma. FEBS J. 2017, 284, 2170–2182.

23. He, F.; Zhong, X.; Lin, Z.; Lin, J.; Qiu, M.; Li, X.; Hu, Z. Plasma exo-hsa_circRNA_0056616: A potential biomarker for ly mph node metastasis in lung adenocarcinoma. J. Cancer 2020, 11, 4037–4046.

24. Zhang, N.; Nan, A.; Chen, L.; Li, X.; Jia, Y.; Qiu, M.; Dai, X.; Zhou, H.; Zhu, J.; Zhang, H.; et al. Circular RNA circSATB2 promotes progression of non-small cell lung cancer cells. Mol. Cancer 2020, 19, 101.

25. Luo, A.; Yin, Y.; Li, X.; Xu, H.; Mei, Q.; Feng, D. The clinical significance of FSCN1 in non-small cell lung cancer. Biome d. Pharmacother. 2015, 73, 75–79.

26. Lin, S.; Xiong, W.; Liu, H.; Pei, L.; Yi, H.; Guan, Y. Profiling and integrated analysis of differentially expressed circular R NAs in plasma exosomes as novel biomarkers for advanced-stage lung adenocarcinoma. OncoTargets Ther. 2020, 13, 12965–12977.

27. Uramoto, H.; Mitsudomi, T. Which biomarker predicts benefit from EGFR-TKI treatment for patients with lung cancer? B r. J. Cancer 2007, 96, 857–863.

28. Liu, Y.T.; Han, X.H.; Xing, P.Y.; Hu, X.S.; Hao, X.Z.; Wang, Y.; Li, J.L.; Zhang, Z.S.; Yang, Z.H.; Shi, Y.K. Circular RNA pr ofiling identified as a biomarker for predicting the efficacy of Gefitinib therapy for non-small cell lung cancer. J. Thorac. Dis. 2019, 11, 1779–1787.

29. Chen, T.; Luo, J.; Gu, Y.; Huang, J.; Luo, Q.; Yang, Y. Comprehensive analysis of circular RNA profiling in AZD9291-resi stant non-small cell lung cancer cell lines. Thorac. Cancer 2019, 10, 930–941.

30. Ma, J.; Qi, G.; Li, L. A novel serum exosomes-based biomarker hsa_circ_0002130 facilitates Osimertinib-resistance in non-small cell lung cancer by sponging miR-498. OncoTargets Ther. 2020, 13, 5293–5307.

31. Luo, Y.H.; Yang, Y.P.; Chien, C.S.; Yarmishyn, A.A.; Ishola, A.A.; Chien, Y.; Chen, Y.M.; Huang, T.W.; Lee, K.Y.; Huang, W.C.; et al. Plasma level of circular RNA hsa_circ_0000190 correlates with tumor progression and poor treatment resp onse in advanced lung cancers. Cancers 2020, 12, 1740.

32. Luo, Y.H.; Yang, Y.P.; Chien, C.S.; Yarmishyn, A.A.; Adekunle Ishola, A.; Chien, Y.; Chen, Y.M.; Tsai, P.H.; Lin, T.W.; Wa ng, M.L.; et al. Circular RNA hsa_circ_0000190 facilitates the tumorigenesis and immune evasion by upregulating the e xpression of soluble PD-L1 in non-small-cell lung cancer. Int. J. Mol. Sci. 2021, 23, 64.

33. Wang, J.; Zhao, X.; Wang, Y.; Ren, F.; Sun, D.; Yan, Y.; Kong, X.; Bu, J.; Liu, M.; Xu, S. circRNA-002178 act as a ceRN A to promote PDL1/PD1 expression in lung adenocarcinoma. Cell Death Dis. 2020, 11, 32.

34. Xian, J.; Su, W.; Liu, L.; Rao, B.; Lin, M.; Feng, Y.; Qiu, F.; Chen, J.; Zhou, Q.; Zhao, Z.; et al. Identification of three circ ular RNA cargoes in serum exosomes as diagnostic biomarkers of non-small-cell lung cancer in the Chinese populatio n. J. Mol. Diagn. 2020, 22, 1096–1108.

35. Ishola, A.A.; Chien, C.S.; Yang, Y.P.; Chien, Y.; Yarmishyn, A.A.; Tsai, P.H.; Chen, J.C.; Hsu, P.K.; Luo, Y.H.; Chen, Y.M.; et al. Oncogenic circRNA C190 promotes non-small cell lung cancer via modulation of the EGFR/ERK pathway. Cancer Res. 2022, 82, 75–89.

36. Li, X.; Zhang, Z.; Jiang, H.; Li, Q.; Wang, R.; Pan, H.; Niu, Y.; Liu, F.; Gu, H.; Fan, X.; et al. Circular RNA circPVT1 prom otes proliferation and invasion through sponging miR-125b and activating E2F2 signaling in non-small cell lung cancer. Cell Physiol. Biochem. 2018, 51, 2324–2340.