Research Article
Circular RNA circHIPK3 Promotes Cell Metastasis through miR-637/STAT3 Axis in Osteosarcoma

Zhongyue Huang,1 Chunyan Yuan,2 Huijie Gu,1 Xiangyang Cheng,1 Kaifeng Zhou,1 Jun Xu,1 Xiaofan Yin (✉),1 and Jiangni Xia1

1Department of Orthopaedics, Minhang Hospital, Fudan University, 170 Xin-Song Road, Shanghai 201199, China
2Department of Pathology, Minhang Hospital, Fudan University, 170 Xin-Song Road, Shanghai 201199, China

Correspondence should be addressed to Xiaofan Yin; yin_xiaofan@fudan.edu.cn and Jiangni Xia; jiangnixiaxs1@126.com

Received 24 April 2020; Accepted 29 June 2020; Published 25 July 2020

Guest Editor: Tao Huang

Copyright © 2020 Zhongyue Huang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Recent studies have suggested that circular RNAs play an important role in the progression of various cancers. However, few studies have revealed the great value of circRNAs in the diagnosis and prognosis prediction of osteosarcoma (OS). In this study, we performed experiments with the human OS cell lines and the results showed that the expression of circHIPK3 in OS cell lines was significantly upregulated compared to that in the normal cell line. In addition, the results showed that circHIPK3 could promote the migration, invasion, and growth of OS cells. Furthermore, miR-637 was identified as a target of circHIPK3, while STAT3 was targeted by miR-637. circHIPK3 could promote STAT3 expression via interacting with miR-637 in OS cells. In conclusion, our research uncovered an important role of the circHIPK3/miR-637/STAT3 pathway in the migration and invasion of OS cells and suggested that circHIPK3 may be a prognostic marker and a promising therapeutic target for OS.

1. Background

Osteosarcoma (OS) is a primary malignancy that develops in the long bones of the extremities [1]. The incidence of OS worldwide is approximately one to three cases per million people per year [2]. OS is characterized by a difficult etiology, high-frequency local invasion, and rapid metastatic potential [3]. Despite advances in treatment technology and medical development, the treatment of osteosarcoma remains one of the challenges facing doctors [4]. Current treatment strategies have failed to make more progress in improving patient survival and quality of life. Therefore, more advanced protocols and drugs are still needed [5].

With the development of technology and further research, various small molecules in cells have been found to be able to regulate cell activities [6, 7]. Among them, circRNA is a kind of RNA without coding potential that has been widely reported to regulate cell growth, metabolism, and metastasis [8, 9] and could be implicated in RNA or protein sponging to modulate gene expression [10]. For example, circRNA HRCR serves as a miR-223 sponge to suppress its activity, thereby repressing miR-223’s target expression and ultimately inhibiting cardiac hypertrophy and heart failure [11]. Circ-abcb10 was found to have a spongy effect on miR-1271 in breast cancer cells, promoting cell proliferation and inhibiting cell apoptosis [8]. Circ_101222 in blood cells can be used to predict early pre-eclampsia [9]. circRNA_102171 overexpression promotes PTC progression through activating the Wnt/β-catenin pathway in a CTNNBIP1-dependent way [12].

circHIPK3 consists of two targeting sites of miR-558, and the binding between circHIPK3 and miR-558 could significantly inhibit the expression of heparinase, thereby reducing the invasion, migration, and angiogenesis of cancer cells [13]. Meanwhile, circHIPK3 can decrease miR-7 in colorectal cancer cells, thus promoting the growth, migration, and invasion
of colorectal cancer cells [14]. It has also been shown that silencing circHIPK3 suppresses the growth of liver cancer cells while ectopic overexpression of circHIPK3 can promote the growth of gallbladder cancer cells [15, 16]. However, the roles of circHIPK3 in OS remained unclear.

In vitro functional assay showed that circHIPK3 can promote the proliferation of osteosarcoma cells and enhance cell migration and invasion. We found that circHIPK3 could sponge on miR-637, thus significantly reducing the effect of miR-637 on its downstream gene STAT3, thus promoting the growth and spread of cancer cells.

2. Materials and Methods

2.1. Patient Samples. Ten patients with OS were included in our study with written informed consent. Both the matched noncancerous tissues and OS tissues were obtained from each patient. The project was approved by the research ethics committee of Minhang Hospital, Fudan University.

2.2. Cell Culture and Transfection. U2OS and SW1353 cells were from ATCC and maintained in RPMI-1640 medium with 10% FBS. Cells were transfected with circHIPK3 siRNA (si-circHIPK3) (GenePharma, Suzhou, China) and control siRNA (si-NC) using Lipofectamine 2000 (Thermo Fisher Scientific) [17].

2.3. qRT-PCR Analysis. Total RNA was isolated from OS cells using TRIzol reagent. Total RNA was reverse transcribed to cDNA, and then, qPCR was conducted by using a SYBR Green PCR Kit (Takara, Otsu, Japan). All primer sequences were designed and synthesized by GenePharma (Shanghai, China). GAPDH was chosen as the reference gene for circRNA and mRNA. Gene expression was calculated with the 2−ΔΔCt method.

2.4. Cell Invasion and Cell Migration. A Transwell plate coated with Matrigel or without Matrigel was used for cell invasion assays or migration assays. The cells were preincubated in a serum-free medium for 6 hours and then resuspended seeded to the upper chamber of the Transwell in a medium containing 1% FBS, and the medium containing 20% FBS in the lower chamber was used as the chemoattractant. After incubation for 48 hours, Matrigel and the cells in the upper chamber were removed and the cells on the lower surface were fixed and stained with DAPI (Solarbio, Beijing, China). The cell number was counted in 5 random microscopic fields (×200).

2.5. CCK-8 Assay. Cells were seeded in 96-well plates and CCK-8 (10 μl/well) was added; the absorbance OD 450 of each well was measured with a microplate reader after incubation for 2 hours [18].

2.6. Dual-Luciferase Reporter Assay. The miR-637 target sequence in circHIPK3 or STAT3 was amplified and inserted into the luciferase vector. 293T cells were transfected with WT/Mut-circHIPK3 or WT/Mut-STAT3 and miR-637 mimics or control miRNA (miR-NC) using Lipofectamine 2000 (Invitrogen) [19]. The luciferase activities were measured through the Dual-Luciferase Assay System (Promega).

2.7. Statistical Analysis. The analysis of experimental data was calculated with SPSS 19.0 statistical software. The comparison between the two groups was calculated using Student’s t-test or the one-way analysis of variance (ANOVA) method. We considered p < 0.05 was statistically significant [20].

3. Results

3.1. circHIPK3 Was Upregulated in OS Tissues and Cells. Through qPCR assay, we found that circHIPK3 was significantly upregulated in OS tissues and cell lines compared to that in control tissues and cells (Figures 1(a) and 1(b)) (p < 0.001).

3.2. Knockdown of circHIPK3 Inhibited OS Cell Proliferation. To investigate circHIPK3’s role in osteosarcoma, we examine U2OS and SW1353 cell proliferation rate after circHIPK3 knockdown using CCK-8 assay. The result showed that the cell growth rate of U2OS and SW1353 cells was significantly suppressed after circHIPK3 knockdown compared to the negative control in series time points (Figures 2(a) and 2(b)), demonstrating that circHIPK3 could promote OS cell proliferation. The knockdown efficiency is shown in Figure 1(c).

3.3. Knockdown of circHIPK3 Inhibited OS Cell Migration and Invasion. We further performed a Transwell assay to investigate the functional roles of the circHIPK3 role in OS progression. The result showed that the silencing of circHIPK3 greatly inhibited U2OS and SW1353 cell migration and invasion compared to control groups (Figures 2(c)–2(f)).

3.4. circHIPK3 Promoted STAT3 Expression by Reducing miR-637 in OS through Sponging. Using localization analysis, circHIPK3 is reported to be mainly located in the cytoplasm (Figures 3(a) and 3(b)). To explore the mechanisms, we next analyzed the downstream targets of circHIPK3 through bioinformatics analysis and found circHIPK3 may bind to miR-637. Luciferase reporter assay demonstrated the luciferase activity was significantly reduced in 293T cells after the cotransfection of miR-637 mimic with WT-circHIPK3. We also showed no significant difference after cells cotransfected with miR-637 mimic and Mut-circHIPK3 (Figure 3(c)). Moreover, knockdown of circHIPK3 induced a significant increase of miR-637 in both SW1353 and U2OS cells (Figure 3(d)). Interestingly, we found miR-637 could suppress circHIPK3 levels in OS cells (Figure 3(e)).

Previous studies suggested that STAT3 is one potential target site of miR-637. To verify the interaction between miR-637 and STAT3, we cloned the 3′UTR of STAT3 or mutant sequences into the pmirGLO vector and then cotransfected into 293T with miR-637 mimic or negative controls. Luciferase activity was significantly lower in cells cotransfected with miR-637 mimic and WT-STAT3 than that in cells with negative controls (Figure 4(a)). Besides, miR-637 significantly decreased STAT3 expression in OS cells.
epithelial cells increased the expression of miR-193a, significantly accelerated the apoptosis of human lens epithelial cells under oxidative stress, and inhibited cell proliferation [25]. circHIPK3 is also highly expressed in human lung tissues, under oxidative stress, and inhibited cell proliferation [25]. For example, in oral squamous cell carcinoma [13], prostate cancer [23], colorectal cancer [14], and six types of normal tissues, circHIPK3 was highly expressed. It was found that circHIPK3 contained more than one binding site of multiple miRNAs, which could inhibit the activity of miRNA [24]. Previous reports have shown circHIPK3 is abundant in multiple cancers and can enhance the growth of cancer cells [25–27]. For example, in oral squamous cell carcinoma, downregulation of the circHIPK3 gene can reduce the adsorption of miR-124, which can normally regulate the downstream target gene and suppress cancer cell growth [28]. circHIPK3 is also expressed in human lens epithelial cells increased the expression of miR-193a, significantly accelerated the apoptosis of human lens epithelial cells under oxidative stress, and inhibited cell proliferation [25]. circHIPK3 is also highly expressed in human lung tissues, inhibiting miR-338-3p activity through serving as an endogenous miR-338-3p sponge, improving the fibroblast-to-myofibroblast transition, and inhibiting the proliferation of fibroblasts [27]. However, circHIPK3 shows different effects in some cells. According to studies in bladder cancer, circHIPK3 is significantly downregulated in bladder cancer, which leads to the adsorption of miR-558 that is significantly weakened, leading to a decreased targeting effect of miR-558 on the heparinase gene, thereby promoting bladder cancer progression. In contrast, the forced overexpression of circHIPK3 reverses these regulatory processes and inhibits the growth and development of bladder cancer cells [13]. In the present study, we found that circHIPK3 was overexpressed in OS tissues and cell lines and acted as an oncogene in OS by promoting cancer cell proliferation, migration, and invasion.

4. Discussion

circHIPK3 is a circRNA produced by direct reverse splicing of the coding sequence of the HIPK3 gene [21]. circHIPK3 has an intracellular half-life of more than 24 hours, indicating its resistance to nucleic acid exonuclease [22]. Studies have reported that in seven types of cancers including bladder epithelial carcinoma [13], prostate cancer [23], colorectal cancer [14], and six types of normal tissues, circHIPK3 was highly expressed. It was found that circHIPK3 contained more than one binding site of multiple miRNAs, which could inhibit the activity of miRNA [24]. Previous reports have shown circHIPK3 is abundant in multiple cancers and can enhance the growth of cancer cells [25–27]. For example, in oral squamous cell carcinoma, downregulation of the circHIPK3 gene can reduce the adsorption of miR-124, which can normally regulate the downstream target gene and suppress cancer cell growth [28]. circHIPK3 is also expressed in human lung tissues, under oxidative stress, and inhibited cell proliferation [25]. circHIPK3 is also highly expressed in human lung tissues, inhibiting miR-338-3p activity through serving as an endogenous miR-338-3p sponge, improving the fibroblast-to-myofibroblast transition, and inhibiting the proliferation of fibroblasts [27]. However, circHIPK3 shows different effects in some cells. According to studies in bladder cancer, circHIPK3 is significantly downregulated in bladder cancer, which leads to the adsorption of miR-558 that is significantly weakened, leading to a decreased targeting effect of miR-558 on the heparinase gene, thereby promoting bladder cancer progression. In contrast, the forced overexpression of circHIPK3 reverses these regulatory processes and inhibits the growth and development of bladder cancer cells [13]. In the present study, we found that circHIPK3 was overexpressed in OS tissues and cell lines and acted as an oncogene in OS by promoting cancer cell proliferation, migration, and invasion.

miRNAs are characterized by their small molecular weight, consisting of approximately 22 ribonucleotides [29]. miRNAs mainly play a regulatory role in cells, regulating the translation process by binding to specific sites of miRNA and affecting the expression of related genes [30]. Most circRNAs were found to have sites homologous with miRNAs and can act as sponges to adsorb target miRNAs, thus inhibiting the miRNAs' regulation [31]. Extensive evidence suggests that some circRNAs may act as competitive endogenous RNA (ceRNA) competing with matched miRNAs for binding sites on the latter, thereby affecting the normal functioning of miRNAs. For example, human circRNA can significantly reduce the efficiency of miR-7 binding to its target mRNA by binding to miR-7 as a sponge [32]. More surprisingly, a single circRNA has more than one binding site. For example, studies in a variety of cancer tissues and normal tissues have found that circHIPK3 has up to 18 potential binding sites, which can regulate nine miRNAs from different sources and play the role of sponge [24]. Therefore, circRNAs are complex and powerful, which is also related to their special ring structure. Its stable structure allows it to avoid being rapidly broken down by intracellular degradation mechanisms [33]. Our study found that circHIPK3 can also play a spongy role of miR-637, and the knockdown of circHIPK3 significantly increased the expression of miR-637.
miR-637 can play various roles in cellular processes [34–36]. Exogenous miR-637 can regulate the expression of RING1 in order to intervene to reverse the proliferation of cervical cancer cells mediated by C5orf66-AS1 and inhibit cancer cell growth [36]. miR-637 could regulate c-reactive protein (CRP) in the acute phase. miR-637 competitively binds HuR to CRP mRNA by interacting with CRP 3′UTR to reduce the protein abundance of CRP expression level, which is an important inflammatory marker [35]. In addition, studies have shown that miR-637 inhibits the growth of human mesenchymal stem cells (hMSCs) and induces s-phase arrest. At the same time, miR-637 significantly enhanced adipocyte differentiation in hMSC and inhibited osteoblasts by directly inhibiting Osterix expression, thus playing an important role in maintaining the balance between adipocytes and osteoblasts [34]. Our study found that miR-637 binds to the mRNA of oncogene STAT3 and regulates its expression, respectively, confirmed by double luciferase

![Graphical representation of experimental results](image-url)
reporter assay and measured by qRT-PCR. STAT3 has been extensively demonstrated to be activated by structural phosphorylation in v-src transforming cells, which plays a central role in the process of malignant transformation [37]. Therefore, our study suggests that miR-637 can inhibit the growth and development activity of osteosarcoma cells by inhibiting the mRNA activity of STAT3.

In this study, we measured the abnormal increase of circHIPK3 expression in OS samples and cells by qRT-PCR and confirmed that circHIPK3 can promote the growth and metastasis of OS cells (SW1353 and U2OS) in vitro. The above research results. We also proved the mechanism of action of circFAT1 (e2) in osteosarcoma. However, our research still has certain limitations. In follow-up studies, we will conduct animal model experiments and increase the number of clinical samples to further verify that circHIPK3 affects the tumorigenesis and development of osteosarcoma in vivo. Also, we realized there was a lack of comprehensive identification of circRNAs in OS. We plan to collect the clinical samples and apply RNA-sequencing in the future study.

In summary, compared with normal people, we found that circHIPK3 was upregulated in OS. Knocking out circHIPK3 could inhibit the proliferation and metastasis of OS. We further found that circHIPK3 could sponge miR-637, thereby inducing STAT3 expression and promoting OS progression. Also, circHIPK3 played a vital role in the

![Figure 3: circHIPK3 served as a sponge for miR-637. (a, b) Comparison of the abundance of circHIPK3 in nuclear and cytoplasmic. (c) Luciferase reporter assay showed that miR-637 mimics dramatically reduced luciferase activity of the Wt-circHIPK3 group. (d) miR-637 was enriched by the downregulation of circHIPK3 compared with the negative control. (e) Compared with the control group, miR-637 overexpression downregulated circHIPK3. *p < 0.05 and **p < 0.01.](image-url)
development of osteosarcoma. Our research revealed a novel regulatory pathway in OS, which may provide a new strategy for the diagnosis and treatment of OS.

Data Availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval
The experimental protocol was established according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of Minhang Hospital, Fudan University.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
Zhongyue Huang and Chunyan Yuan contributed equally to this work.

Acknowledgments
This work is supported by the National Natural Science Foundation of China (Grant Number 81772433), applicant: Xiaofan Yin.
References

[1] G. Ottaviani and N. Jaffe, “The epidemiology of osteosarcoma,” in Pediatric and Adolescent Osteosarcoma, vol. 152 of Cancer Treatment and Research, pp. 3–13, Springer, Boston, MA.

[2] M. Kansara, M. W. Teng, M. J. Smyth, and D. M. Thomas, “Translational biology of osteosarcoma,” Nature Reviews Cancer, vol. 14, no. 11, pp. 722–735, 2014.

[3] J. Kirpensteijn, M. KIK, E. Teske, and G. R. Ruttenman, “TP53 gene mutations in canine osteosarcoma,” Veterinary Surgery, vol. 37, no. 5, pp. 454–460, 2008.

[4] R. A. V. Griend, “Osteosarcoma and its variants,” The Orthopedic Clinics of North America, vol. 27, no. 3, pp. 575–581, 1996.

[5] D. J. Harrison, D. S. Geller, J. D. Gill, V. O. Lewis, and R. Gorlick, “Current and future therapeutic approaches for osteosarcoma,” Expert Review of Anticancer Therapy, vol. 18, no. 1, p. 39, 2017.

[6] K. J. Boyce, M. J. Hynes, and A. Andrianopoulos, “The Ras and Rho GTPases genetically inter-.co-co-ordinate regulatory cell polarity during development in Penicillium marneffei,” Molecular Microbiology, vol. 55, no. 5, pp. 1487–1501, 2005.

[7] H. Luo, Y. Li, B. Liu, Y. Yang, and Z.-Q. D. Xu, “MicroRNA-15b-5p targets ERK1 to regulate proliferation and apoptosis in rat PC12 cells,” Biomedicine & Pharmacotherapy, vol. 92, pp. 1023–1029, 2017.

[8] H. F. Liang, X. Z. Zhang, B. G. Liu, G. T. Jia, and W. L. Li, “Circular RNA circ-ABC101 promotes breast cancer proliferation and progression through sponging miR-1271,” American Journal of Cancer Research, vol. 7, no. 7, pp. 1566–1576, 2017.

[9] Y. G. Zhang, H.-L. Yang, Y. Long, and W.-L. Li, “Circular RNA in blood corpuscles combined with plasma protein factor for early prediction of pre-eclampsia,” BJOG, vol. 123, no. 13, pp. 2113–2118, 2016.

[10] Z. Shabaninejad, A. Vafadar, A. Movahedpour et al., “Circular RNAs in cancer: new insights into functions and implications in ovarian cancer,” Journal of Ovarian Research, vol. 12, no. 1, p. 84, 2019.

[11] K. Wang, B. Long, F. Liu et al., “A circular RNA protects the heart from pathological hypertrophy and heart failure by targeting miR-223,” European Heart Journal, vol. 37, no. 33, pp. 2602–2611, 2016.

[12] W. Bi, J. Huang, C. Nie et al., “CircRNA circRNA_102171 promotes papillary thyroid cancer progression through modulating CTNNBIP1-dependent activation of β-catenin pathway,” Journal of Experimental & Clinical Cancer Research, vol. 37, no. 1, article 275, 2018.

[13] Y. Li, F. Zheng, X. Xiao et al., “CircHIPK3 sponges miR-558 to suppress heparanase expression in bladder cancer cells,” Embryo Reports, vol. 18, no. 9, pp. 1646–1659, 2017.

[14] K. Zeng, X. Chen, M. Xu, X. Liu, and S. Wang, “CircHIPK3 promotes colorectal cancer growth and metastasis by sponging miR-7,” Cell Death & Disease, vol. 9, no. 4, article 417, 2018.

[15] G. Chen, Y. Shi, M. Liu, and J. Sun, “circHIPK3 regulates cell proliferation and migration by sponging miR-124 and regulating AQP3 expression in hepatocellular carcinoma,” Cell Death & Disease, vol. 9, no. 2, article 175, 2018.

[16] D. Kai, L. Yannian, C. Yitian, G. Dinghao, Z. Xin, and J. Wu, “Circular RNA HIPK3 promotes gallbladder cancer cell growth by sponging microRNA-124,” Biochemical and Biological Research Communications, vol. 503, no. 2, pp. 863–869, 2018.

[17] J. Jin, A. Chen, W. Qiu et al., “Dysregulated circRNA_100876 suppresses proliferation of osteosarcoma cancer cells by targeting microRNA-136,” Journal of Cellular Biochemistry, vol. 120, no. 9, pp. 15678–15687, 2019.

[18] C. Jiang, X. Fang, H. Zhang et al., “AMD3100 combined with triptolide inhibit proliferation, invasion and metastasis and induce apoptosis of human U2OS osteosarcoma cells,” Bio- medicine & Pharmacotherapy, vol. 86, pp. 677–685, 2017.

[19] L. Liang, J. Zhu, N. G. Zaoisky et al., “MicroRNA-223 enhances radiation sensitivity of U87MG cells in vitro and in vivo by targeting ataxia telangiectasia mutated,” International Journal of Radiation Oncology • Biology • Physics, vol. 88, no. 4, pp. 955–960, 2014.

[20] Y. Z. Song and J. F. Li, “Circular RNA hsa_circ_0001564 regulates osteosarcoma proliferation and apoptosis by acting miRNA sponge,” Biochemical and Biophysical Research Communications, vol. 495, no. 3, pp. 2369–2375, 2018.

[21] M. Xiao-Long, Z. Kun-Peng, and Z. Chun-Lin, “Circular RNA circ_HIPK3 is down-regulated and suppresses cell proliferation, migration and invasion in osteosarcoma,” Journal of Cancer, vol. 9, no. 10, pp. 1856–1862, 2018.

[22] H. Ni, W. Li, Y. Zhuge et al., “Inhibition of circHIPK3 prevents angiogenesis II-induced cardiac fibrosis by sponging miR-29b-3p,” International Journal of Cardiology, vol. 292, pp. 188–196, 2019.

[23] C. Cai, Y. Zhi, K. Wang et al., “CircHIPK3 overexpression accelerates the proliferation and invasion of prostate cancer cells through regulating miRNA-338-3p,” OncoTargets and Therapy, vol. 12, pp. 3363–3372, 2019.

[24] Q. Zheng, C. Bao, W. Guo et al., “Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs,” Nature Communications, vol. 7, no. 1, p. 11215, 2016.

[25] X. Liu, B. Liu, M. Zhou et al., “Circular RNA HIPK3 regulates human lens epithelial cells proliferation and apoptosis by targeting the miR-193a/CRYAA axis,” Biochemical and Biophysical Research Communications, vol. 503, no. 4, pp. 2277–2285, 2018.

[26] Y. Xie, X. Yuan, W. Zhou et al., “The circular RNA HIPK3 (circHIPK3) and its regulation in cancer progression: review,” Life Sciences, vol. 254, article 117252, 2020.

[27] Z. J-x, J. Lu, H. Xie et al., “circHIPK3 regulates lung fibroblast-to-myofibroblast transition by functioning as a competing endogenous RNA,” Cell Death & Disease, vol. 10, no. 3, article 182, 2019.

[28] J. Wang, S. Y. Zhao, S. S. Ouyang, Z. K. Huang, Q. Luo, and L. Liao, “Circular RNA circHIPK3 acts as the sponge of microRNA-124 to promote human oral squamous cell carcinoma cells proliferation,” Zhonghua Kou Qian Yi Xue Za Zhi, vol. 53, no. 8, pp. 546–551, 2018.

[29] W. Lukiw, T. Andreeva, A. Grigorenko, and E. Rogaveg, “Studying micro RNA function and dysfunction in Alzheimer’s disease,” Frontiers in Genetics, vol. 3, no. 327, 2013.

[30] G. C. Shukla, J. Singh, and S. Barik, “MicroRNAs: processing, maturation, target recognition and regulatory functions,” Molecular and Cellular Pharmacology, vol. 3, no. 3, pp. 83–92, 2011.

[31] F. R. Kulcheski, A. P. Christoff, and R. Margis, “Circular RNAs are miRNA sponges and can be used as a new class of biomarker,” Journal of Biotechnology, vol. 238, pp. 42–51, 2016.
[32] T. B. Hansen, J. Kjems, and C. K. Damgaard, “Circular RNA and miR-7 in cancer,” *Cancer Research*, vol. 73, no. 18, pp. 5609–5612, 2013.

[33] X. B. Zheng, M. Zhang, and M. Q. Xu, “Detection and characterization of ciRS-7: a potential promoter of the development of cancer,” *Neoplasma*, vol. 64, no. 3, pp. 321–328, 2017.

[34] F. Zhang J-f, “MiR-637 maintains the balance between adipocytes and osteoblasts by directly targeting Osterix,” *Molecular Biology of the Cell*, vol. 22, no. 21, pp. 3955–3961, 2011.

[35] Y. Kim, N. Noren Hooten, D. F. Dluzen, J. L. Martindale, M. Gorospe, and M. K. Evans, “Posttranscriptional regulation of the inflammatory marker C-reactive protein by the RNA-binding protein HuR and microRNA 637,” *Molecular and Cellular Biology*, vol. 35, no. 24, pp. 4212–4221, 2015.

[36] X. Rui, Y. Xu, X. Jiang, W. Ye, Y. Huang, and J. Jiang, “Long non-coding RNA C5orf66-AS1 promotes cell proliferation in cervical cancer by targeting miR-637/RING1 axis,” *Cell Death & Disease*, vol. 9, no. 12, p. 1175, 2018.

[37] D. E. Levy and C.-k. Lee, “What does Stat3 do?,” *The Journal of Clinical Investigation*, vol. 109, no. 9, pp. 1143–1148, 2002.