A research article titled "The Oral Tumor Cell Exosome miR-10b Stimulates Cell Invasion and Relocation via AKT Signaling" by Xiang Li, Ting Yang, and Chuanji Shu. The article discusses the role of oral cancer-derived exosomes in tumor metastasis, with a focus on miR-10b. The authors investigate the effect of exosomal miR-10b on oral cancer cell behaviors and find that AKT signaling is involved in regulating exosome-mediated invasion and migration of oral cancer cells. They also observe that miR-10b knockdown reduces the inhibitory effect of exosomal miR-10b.
2. Materials and Methods

2.1. Cell Culture. Oral cancer cell lines HSC-6 and THP-1 were purchased from Chinese Academy of Sciences (Shanghai, China). HSC-6 and THP-1 were cultured in a medium containing 100 μg/mL streptomycin, 100 U/mL penicillin (Hyclone Laboratories, Inc, Logan, UT, USA) and 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) in DMEM (Hyclone Laboratories, Inc, Logan, UT, USA) and cultured in a constant temperature cell incubator (37°C, CO2 volume fraction 5%).

2.2. Cell Transfection. HSC-6 cells were transfected with miR-10b inhibitor and NC (GenePharma) using Lipofectamine 2000 reagent (Invitrogen, USA). The transfection was measured by RT-qPCR.

2.3. Exosome Isolation. Exosomes were obtained from HSC-6 cells by Ribo™ Exosome Isolation Reagent (RiboBio, C10130-1) according to the instruction and identified by scanning electron microscopy.

2.4. Exosome Uptake. Exosomes isolated from oral cancer cells were labeled with PKH67 dye, and DAPI was used for nuclear staining. After incubation, exosome uptake was examined by confocal microscopy.

2.5. Invasion and Migration Assay. Evaluate cell invasion and migration. HSC-6 cells (2×10⁴ cells per well) were seeded in the upper chamber of the Transwell, serum-free culture medium was placed in the lower chamber, and SDF-1 was added to the culture medium. HSC-6 cells were removed after 12 hours of incubation, cleaned, fixed, crystallized, and quantified using Image Pro Plus.

2.6. Western Blot. Cellular proteins were isolated using RIPA buffer, and proteins were then denatured by boiling. The total concentration of protein was determined by a bicinechonic acid assay (Beyotime Institute of Biotechnology). The protein was separated using electrophoresis at 90 V for 100 min and then transferred to a nitrocellulose membrane at 110 V for 75 min. The membrane was blocked with skimmed milk for 2 h and then incubated with the following antibodies: anti-AKT (1:1000; AA326; Beyotime), anti-p-AKT (1:1000; AA329; Beyotime), and anti-GAPDH (1:5000; 10494-1-AP; Proteintech). Shake on a decolorization shaker at 4°C. Subsequently, we washed the membranes (three times for 15 min, each wash) with TBST and incubated them with goat anti-rabbit (1:3,000, Abcam, Cambridge, MA, USA) for 2 h at 26 ± 2°C. Afterward, the membranes were rinsed with TBST (three times/15 min each). We then visualized Western blot bands using the ECL detection system (Bio-Rad).

2.7. qRT-PCR. Total RNA was isolated and reversely transcribed to cDNA followed by real-time (RT)-PCR using SYBR Green Master Mix (Biosharp; BL705A). Gene level was analyzed using the ΔΔCt quantification method. The primers were U6 sense 5’-CTCCTTGGCAGCAC-3’, U6 antisense 5’-AACCTTCCGAATTTGCGT-3, miR-10b sense 5’-ACACTCACGTTGACCTTGAAGATC-3’, and miR-10b antisense 5’-CTCACTGGTGTCATGA-3.

2.8. Statistical Method. All data analyses were conducted using IBM SPSS Statistics for Windows, version 27.0 (IBM Corporation, Armonk, NY, USA). The Western blots were placed into ImageJ for analysis. Plots were generated using Prism 8.0 software (Graphpad Software, Inc., San Diego, CA, USA). The data are presented as mean ± standard deviation, as determined by one-way variance analysis with Tukey’s test. P < 0.05 was considered statistically significant.

3. Results

3.1. Characterization of Exosomes from Oral Cancer Cells. The exosomes were obtained by Ribo™ Exosome Isolation Reagent. To identify exosomes, we performed electron microscopy assays. Figure 1 demonstrates the characterization of exosomes secreted by oral cancer cells. As shown in Figure 1(a), the specific exosome morphology and size were about 100 nm in diameter. To further determine the maturity of oral cancer cell-derived exosomes, we next measured the exosome-specific makers including CD63, Arg-1, and TGS101 by Western blotting. The expression of CD63, Arg-1, and TGS101 was higher in exosomes than in cell lysate, as shown in Figure 1(b).

3.2. High miR-10b in Exosomes from Oral Cancer Cells. We then determine whether exosomes can be internalized by oral cancer cells, and exosomes were labeled with PKH67 dye (Green). Figure 2 shows a high miR-10b level in exosomes from oral cancer cells. The uptake of exosomes was visualized under a confocal microscope, as shown in Figure 2(a). A number of studies reported the role of cancer cell-derived miR-10b in cancer development [14], and we next measured its role in oral cancer and found significantly upregulated miR-10b in exosomes from oral cancer cells, as shown in Figure 2(b).

3.3. miR-10b Is Transferred to Recipient Cells by Exosomes. Figure 3 shows that miR-10b was transferred to recipient cells by exosomes and promoted oral cancer cell invasion and migration. Exosome administration increased miR-10b expression, as shown in Figure 3(a), and promoted oral cancer cell invasion and migration, as shown in Figure 3(b).

3.4. Inhibition of miR-10b Attenuates Oral Cancer Cell Behaviors. We next reduced miR-10b expression in an oral cancer cell by transfection of miR-10b inhibitor and found decreased miR-10b expression after inhibitor treatment, as shown in Figures 4(a) and 4(b). mir-10b that inhibits exosome secretion and nonsense inhibitors were used to coculture with oral cancer cells, and it was found that mir-
10b significantly decreased in oral cancer cells when mir-10b secreted by exosomes was inhibited, as shown in Figure 4(c). More importantly, transwell assays showed that incubation with miR-10b inhibitor exosomes attenuated oral cancer cell invasion and migration, as shown in Figure 4(d). The data suggested that oral cancer cell-derived exosomal miR-10b could enhance oral cancer cell invasion and migration.

3.5. Exosomal miR-10b Promotes Oral Cancer Cell Behaviors via AKT Signaling. miR-10b is involved in AKT signaling activation [15]. In addition, AKT contributed to the malignant potential of oral cancer [16, 17]. Thus, we sought to explore whether exosomal miR-10b regulates oral cancer cells via activating AKT signaling. Figure 5 shows that exosomal miR-10b promotes oral cancer cell behaviors via AKT signaling. The expression of phosphorylated AKT was significantly increased after oral cancer cell-derived exosome treatment; however, inhibition of miR-10b reduced phosphorylated AKT level, as shown in Figure 5(a). We next treated exosomes incubated oral cancer cells with AKT activator, SC79, as shown in Figure 5(b). The protein expressions of AKT and p-Akt in exosome-cultured oral cancer cells treated with AKT activator (SC79) were significantly higher than those in exosome cultured oral cancer cells, as shown in Figure 5(c). The data indicated that exosomal miR-10b promoted oral cancer cell behaviors via activating AKT.
4. Discussion

Several studies indicated that exosomes derived from cancer cells participate in cancer progression, such as malignant melanoma [18], lung cancer [19], and pancreatic cancer [20]. Of note, exosomes carrying miRNAs are important to tumorigenesis [21].

It is well known that exosomes contribute to oral cancer angiogenesis [22] and regulate natural killer cells and tumor immunity [23]. In addition, exosomes containing miRNAs are also involved in oral cancer progression. Exosomal miR-21 from hypoxic oral squamous cell carcinoma cells promotes prometastatic behaviors of tumor cells [24]. miR-10b has exosome-mediated cancer cell malignant properties. Qian et al. demonstrated that miR-10b-5p was delivered by hypoxic glioma exosomes to normoxic glioma cells to affect cell behaviors [25]. Singh et al. showed that exosomal miR-10b promotes breast cancer cell invasion [12]. Of note, miR-

**Figure 3:** miR-10b is transferred to recipient cells and promotes oral cancer cell invasion and migration. (a) miR-10b level in exosome-treated HSC-6 cell was measured by RT-qPCR assay. PBS-treated HSC-6 cell was used as control (**P < 0.01). (b) Cell invasion and migration (**P < 0.01 (mean ± SD, n = 3)).

**Figure 4:** Inhibition of miR-10b attenuates oral cancer cell invasion and migration. (a) miR-10b expression in HSC-6 cell with NC inhibitor exosome or miR-10b inhibitor exosome administration was measured by RT-qPCR assay (**P < 0.0001). (b) miR-10b level in NC inhibitor or miR-10b inhibitor-transfected HSC-6 cells was detected by RT-qPCR assay (**P < 0.001). (c) miR-10b expression (*) < 0.05). (d) The invasion and migration of HSC-6 cell with NC inhibitor exosome or miR-10b inhibitor exosome administration (**P < 0.01; ***P < 0.001).
miR-10b is upregulated in oral cancer [11]. Our study found an increased miR-10b level in oral cancer cell-derived exosomes and it could be transferred to the oral cancer cell. The administration of exosomal miR-10b promotes cancer cell behaviors, which were impaired by miR-10b inhibition, indicating that exosomal miR-10b contributes to the progression of oral cancer.

miRNAs exert effects through modulating downstream targets or different signaling pathways. Numerous targets of miR-10b and their role in cancer development have been reported, such as hyaluronan synthase 3 (HAS3) in prostate cancer [26] and phosphatase and tensin homolog (PTEN) in colorectal cancer [27]. In addition, accumulating evidence indicated that miR-10b modulates cancer cell malignancy
via AKT signaling. For example, miR-10b regulates breast cancer stem cells via activating AKT [28]. miR-10b attenuates radiosensitivity of glioblastoma cells via AKT [29]. miR-10b facilitates gastric cancer cell invasion. Thus, we sought to explore whether exosomal miR-10b affects oral cancer cells. The results reveal that oral cancer cell-derived exosomal miR-10b can enhance AKT signaling. Moreover, inhibition of miR-10b decreases reduced phosphorylated AKT level and activation of AKT signaling could recover the inhibitory effect of miR-10b knockdown. mir-10b secreted by exosomes can increase the invasion and migration of oral cancer cells by regulating the AKT signaling pathway.

5. Conclusions

Exosomes derived from oral cancer cells promote cancer cell invasion and migration. The forced effect on cell invasion and migration is mainly attributed to miR-10b, which is transferred to recipient cells by oral cancer-derived exosomes. The experimental results show that miR-10b transfer mediated by exosome facilitates oral cancer cell invasion and migration by activating AKT signaling. In our future work, the new function of oral cancer-derived exosomes in regulating oral cancer invasion and migration will be explored in depth.

Data Availability

The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] G. S. Sarode, C. S. Sarode, N. Maniyar, R. Anand, and S. Patil, "Oral cancer databases: a comprehensive review," Journal of Oral Pathology & Medicine, vol. 47, no. 6, pp. 547–556, 2018.
[2] Z. Huang, M. Yang, Y. Li, F. Yang, and Y. Feng, "Exosomes derived from hypoxic colorectal cancer cells transfer Wnt4 to normoxic cells to elicit a prometastatic phenotype," International Journal of Biological Sciences, vol. 14, pp. 2094–2102, 2018.
[3] K. Zhao, Z. Wang, X. Li, J. L. Liu, L. Tian, and Jq Chen, "Exosome-mediated transfer of CLIC1 contributes to the vincristine-resistance in gastric cancer," Molecular and Cellular Biochemistry, vol. 462, no. 1-2, pp. 97–105, 2019.
[4] U. Rai, R. Kosuru, S. Prakash et al., "Tetramethylpyrazine prevents diabetes by activating PI3K/Akt/GLUT-4 signalling in animal model of type-2 diabetes," Life Sciences, vol. 236, p. 116836, 2019.
[5] W. T. Huang, I. W. Chong, H. L. Chen et al., "Pigment epithelium-derived factor inhibits lung cancer migration and invasion by upregulating exosomal thrombospondin 1," Cancer Letters, vol. 442, pp. 287–298, 2019.
[6] M. Xiao, J. Zhang, W. Chen, and W. Chen, "M1-like tumor-associated macrophages activated by exosome-transferred THBS1 promote malignant migration in oral squamous cell carcinoma," Journal of Experimental & Clinical Cancer Research, vol. 37, no. 1, p. 143, 2018.
[7] Z. Sun, K. Shi, S. Yang et al., "Effect of exosomal miRNA on cancer biology and clinical applications," Molecular Cancer, vol. 17, no. 1, p. 147, 2018.
[8] J. Huang, M. Shen, M. Yan, Y. Cui, Z. Gao, and X. Meng, "Exosome-mediated transfer of miR-1290 promotes cell proliferation and invasion in gastric cancer via NKD1," Acta Biochimica et Biophysica Sinica, vol. 51, no. 9, pp. 900–907, 2019.
[9] C. Song, L. He, J. Zhang et al., "Fluorofenidone attenuates pulmonary inflammation and fibrosis via inhibiting the activation of NALP3 inflammasome and IL-1β/IL-1R1/MyD88/NF-κB pathway," Journal of Cellular and Molecular Medicine, vol. 20, no. 11, pp. 2064–2077, 2016.
[10] Z. Wen, L. Lian, H. Ding et al., "LncRNA ANCR promotes hepatocellular carcinoma metastasis through upregulating HNRNPA1 expression," RNA Biology, vol. 17, no. 3, pp. 381–394, 2020.
[11] Y. C. Lu, Y. J. Chen, H. M. Wang et al., "Oncogenic function and early detection potential of miRNA-10b in oral cancer as identified by microRNA profiling," Cancer Prevention Research, vol. 5, pp. 665–674, 2012.
[12] R. Singh, R. Pochampally, K. Watabe, Z. Lu, and Y. Y. Mo, "Exosome-mediated transfer of miR-10b promotes cell invasion in breast cancer," Molecular Cancer, vol. 13, no. 1, p. 256, 2014.
[13] X. P. Tian, C. Y. Wang, X. H. Jin et al., "Acidic microenvironment up-regulates exosomal miR-21 and miR-10b in early-stage hepatocellular carcinoma to promote cancer cell proliferation and metastasis," Theranostics, vol. 9, no. 7, pp. 1965–1979, 2019.
[14] L. Zhen, J. Li, M. Zhang, and K. Yang, "MiR-10b decreases sensitivity of glioblastoma cells to radiation by targeting AKT," Journal of Biological Research-Thessaloniki, vol. 23, no. 1, p. 14, 2016.
[15] I. Bahena-Ocampo, M. Espinosa, G. Ceballos-Cancino et al., "miR-10b expression in breast cancer stem cells supports self-renewal through negative PTEN regulation and sustained AKT activation," EMBO Reports, vol. 17, no. 5, pp. 648–658, 2016.
[16] K. Wu, Y. Hu, K. Yan et al., "microRNA-10b confers cisplatin resistance by activating AKT/mTOR/P70S6K signaling via targeting PPARγ in esophageal cancer," Journal of Cellular Physiology, vol. 235, no. 2, pp. 1247–1258, 2020.
[17] S. Zhang, H. Bian, X. Li et al., "Hydrogen sulfide promotes cell proliferation of oral cancer through activation of the COX2/AKT/ERK1/2 axis," Oncology Reports, vol. 35, no. 5, pp. 2825–2832, 2016.
[18] J. L. Palacios-Ferrer, M. B. García-Ortega, M. Gallardo-Gómez et al., "Metabolomic profile of cancer stem cell-derived exosomes from patients with malignant melanoma," Molecular Oncology, vol. 15, no. 2, pp. 407–428, 2021.
[19] A. Jouida, C. McCarthy, A. Fabre, and M. P. Keane, "Exosomes: a new perspective in EGFR-mutated lung cancer," Cancer and Metastasis Reviews, vol. 40, no. 2, pp. 589–601, 2021.
[20] Z. Ye, Z. Zhu, J. Xie et al., "Hsa_circ_0000069 knockdown inhibits tumorigenesis and exosomes with downregulated hsa_circ_0000069 suppress malignant transformation via inhibition of STIL in pancreatic cancer," International Journal of Nanomedicine, vol. 15, pp. 9859–9873, 2020.
[21] B. Li, Y. Cao, M. Sun, and H. Feng, "Expression, regulation, and function of exosome-derived miRNAs in cancer
progression and therapy,” *Federation of American Societies for Experimental Biology Journal: Official Publication of the Federation of American Societies for Experimental Biology*, vol. 35, p. 21916, 2021.

[22] H. Wang, L. Wang, X. Zhou et al., “OSCC exosomes regulate miR-210-3p targeting EFNA3 to promote oral cancer angiogenesis through the PI3K/AKT pathway,” *BioMed Research International*, vol. 2020, pp. 1–13, 2020.

[23] X. Zhu, X. Qin, X. Wang et al., “Oral cancer cell-derived exosomes modulate natural killer cell activity by regulating the receptors on these cells,” *International Journal of Molecular Medicine*, vol. 46, no. 6, pp. 2115–2125, 2020.

[24] L. Li, C. Li, S. Wang et al., “Exosomes derived from hypoxic oral squamous cell carcinoma cells deliver miR-21 to normoxic cells to elicit a prometastatic phenotype,” *Cancer Research*, vol. 76, no. 7, pp. 1770–1780, 2016.

[25] M. Qian, Z. Chen, X. Guo et al., “Exosomes derived from hypoxic glioma deliver miR-1246 and miR-10b-5p to normoxic glioma cells to promote migration and invasion,” *Laboratory Investigation*, vol. 101, no. 5, pp. 612–624, 2021.

[26] E. D. Czyrnik, M. Wiesehöfer, J. T. Dankert, and G. Wennemuth, “The regulation of HAS3 by miR-10b and miR-29a in neuroendocrine transdifferentiated LNCaP prostate cancer cells,” *Biochemical and Biophysical Research Communications*, vol. 523, no. 3, pp. 713–718, 2020.

[27] Y. Cheng, C. Yu, W. Li, Y. He, and Y. Bao, “Matrine inhibits proliferation, invasion, and migration and induces apoptosis of colorectal cancer cells via miR-10b/PTEN pathway,” *Cancer Biotherapy & Radiopharmaceuticals*, vol. 34, no. 11, pp. 299–304, 2020.

[28] C. Théry, S. Amigorena, G. Raposo, and A. Clayton, “Isolation and characterization of exosomes from cell culture supernatants and biological fluids,” *Current Protocols in Cell Biology*, vol. Chapter 3, no. 1, pp. 322–333, 2006.

[29] H. Chen, Y. Fan, W. Xu et al., “miR-10b inhibits apoptosis and promotes proliferation and invasion of endometrial cancer cells via targeting HOXB3,” *Cancer Biotherapy & Radiopharmaceuticals*, vol. 31, pp. 225–231, 2016.