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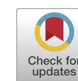
RAPID COMMUNICATION

Exosomal lncRNA LINC01268 promotes pancreatic cancer progression via the miR-217-KIF2A-PI3K/AKT axis



Pancreatic cancer (PaCa) is one of the most aggressive and lethal malignancies with rapid progression and poor prognosis with the 5-year survival rate remaining less than 5% because approximately 80% of PaCa patients are diagnosed at an advanced stage and lose the chance of curative resection.[1] Accumulated evidence has revealed that non-coding RNAs (ncRNAs) are involved in the pathogenesis of PaCa and serve as potential biomarkers for PaCa.[2,3] Exosomal lncRNAs also play a vital role in tumorigenesis and some exosomal lncRNAs have been reported to accelerate tumor progression.[4] In this study, we first employed RNA-seq of PaCa plasma samples to identify significantly differentially expressed lncRNAs in PaCa patients and revealed that LINC01268 was up-regulated in PaCa tumor tissues and plasma. LINC01268 is an emerging lncRNA associated with glioma malignancy grade.[5] Thus. we further analyzed the malignant biological behaviors of this lncRNA in PaCa and found that LINC01268 promoted cell proliferation, migration, and invasion as an oncogene, which promoted epithelial–mesenchymal transition (EMT) in PaCa via the miR-217-KIF2A-PI3K/AKT axis.

To identify potential plasma biomarkers of PaCa, five PaCa plasma samples and five control samples were analyzed by whole transcriptome sequencing. The results indicated that 466 lncRNAs were differentially expressed with a fold change $\geq$2.0 or ≤ -2.0 and a P-value <0.05 (Table S1 and Fig. S1A, B) and 634 mRNAs were significantly up-regulated, and 888 mRNAs were down-regulated (Fig. S1C, D). qRT–PCR analysis was performed to verify the significantly differential lncRNAs (LINC01268, AL132657.1, AC245595.1, and AC124854.1) in 53 pairs of PaCa tissues (adjacent normal tissues and PaCa tissues) and normal pancreatic cell lines (HPNE and HPDE) and PaCa cell lines (BxPC-3, PANC-1, AsPC-1, Capan-1, CFPAC-1, NOPR1, KP3, and SUIT2). The results revealed that four lncRNAs (AC245595.1, AC124854.1, AL132657.1, and LINC01268) were up-regulated in 53 PaCa tissues compared to normal tissues. A total of 69.8% (37 of 53), 41.5% (22 of 53), 62.3% (33 of 53), and 67.9% (36 of 53) of the PaCa tissues showed a more than 2-fold up-regulation in LINC01268, AC124854.1, AL132657.1, and LINC02802 mRNA expression levels when compared with the corresponding normal pancreatic tissues, respectively (Fig. S1E–H). We further validated that LINC01268, AL132657.1, AC245595.1, and AC124854.1 were significantly increased in some pancreatic cancer cells (Fig. S1I–L).

Next, to determine the biological function of LINC01268 in PaCa cells, we identified that knockdown of LINC01268 inhibited the growth and proliferation of AsPC-1 and PANC-1 cells (Fig. S2A, B, E, G–I), while LINC01268 overexpression promoted cell growth and proliferation in both cell lines (Fig. S2C, D, F–I) by CCK-8 and colony formation assays. The wound healing assay results showed that knockdown of LINC01268 significantly reduced migration distance in AsPC-1 and PANC-1 cells (Fig. S2J–L) and LINC01268 overexpression significantly increased the migration distance in both cell lines (Fig. S2J–L). The migration and invasion assays showed that LINC01268 overexpression increased the number of migrated AsPC-1 and PANC-1 cells, and the knockdown of LINC01268 significantly decreased the number of migrated cells (Fig. S3). As shown in Figure S4A, miR-217 was obtained by the intersection of miRcode and three GEO datasets (GSE71533, GSE163031, GSE43796). Luciferase activity was significantly decreased after co-transfection with the wild-type vector and pcDNA3.1-miR-217 (Fig. S4C, D), indicating that miR-217 could directly bind to LINC01268. We also analyzed the intersection of the differentially expressed mRNAs by RNA-seq and the downstream mRNAs of miR-217 predicted by TargetScan. Ten mRNAs (CHSY1, ABL2, C5orf15, CHST11, POLG, IKZF3, KIF2A, PCDH17, MAF, and SLC4A4) were

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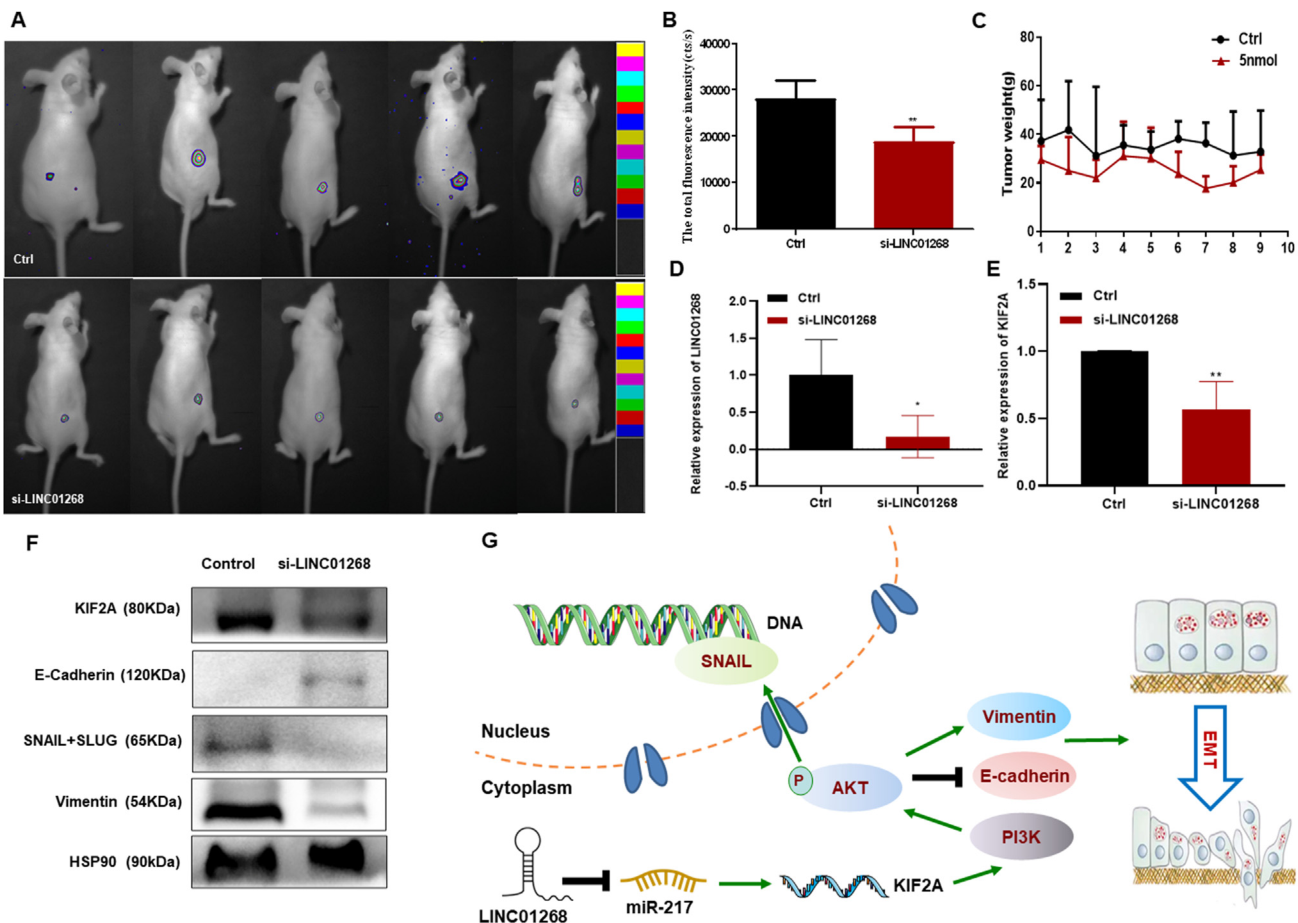

Figure 1 si-LINC01268 inhibited cell growth and proliferation *in vivo*. **(A, B)** Fluorescence intensity imaging in mice. **(C)** The weight of mice. **(D, E)** qRT—PCR analysis of LINC01268 (D) and KIF2A (E) mRNA expression levels in xenograft tumor mice with/without the intravenous injection of si-LINC01268. **(F)** Western blot analysis of KIF2A, E-Cadherin, SNAIL + SLUG, and vimentin mRNA expression in xenograft tumor mice with/without the intravenous injection of si-LINC01268. **(G)** A working model showing the interaction between LINC01268 and the KIF2A-PI3K/AKT axis in PaCa cells.

predicted as the targets of miR-217 (Fig. S4B). We also verified that KIF2A was the target of miR-217 by a luciferase reporter assay. The pmiR-KIF2A-3′UTR vector incorporating the miR-217 binding site was constructed for dual luciferase reporter gene detection. Luciferase activity was significantly decreased after co-transfection with the wild-type vector and pcDNA3.1-miR-217 (Fig. S4C, E), indicating that miR-217 could directly bind to KIF2A mRNA. We further found that miR-217 was down-regulated in PaCa cells (Fig. S4F) and PaCa tissue compared to normal tissues (Fig. S4G). However, KIF2A was notably up-regulated in CFPAC-1 and SUIT2 cells (Fig. S4H). KIF2A was up-regulated in 53 PaCa tissues compared to normal tissues (Fig. S4I). Altogether, our data demonstrated that KIF2A is a direct target of miR-217 and is up-regulated in PaCa cells. To determine whether LINC01268 could regulate KIF2A expression, we detected KIF2A expression in LINC01268 knockdown and overexpressing PaCa cells via qPCR and western blotting. KIF2A expression was significantly decreased by LINC01268 knockdown and increased by LINC01268 overexpression (Fig. S5A—D). In addition, we transfected pcDNA3.1-miR-217 or inhibitor miR-217 to overexpress or knockdown miR-217 in AsPC-1 and PANC-1 cells. As shown in Figure S5E—H, when miR-217 was overexpressed, KIF2A was increased at the mRNA and protein levels. In contrast, KIF2A was decreased at the mRNA and protein levels when the inhibitor miR-217 was transfected (Fig. S5E—H).

To further prove that LINC01268 participates in EMT progression, we identified the epithelial biomarker E-cadherin and mesenchymal biomarkers vimentin and SNAIL + SLUG at the protein level when LINC01268 was overexpressed or knocked down. Furthermore, we found that LINC01268 promoted EMT via the PI3K/AKT pathway. As shown in Figure S5C and D, vimentin, SNAIL + SLUG, phospho-PI3K, and phospho-AKT were increased, and E-cadherin was decreased when LINC01268 was overexpressed. In contrast, vimentin, SNAIL + SLUG, phospho-PI3K, and phospho-AKT were down-regulated, while E-cadherin was up-regulated when LINC01268 was knocked down. We also found that vimentin, SNAIL + SLUG, phospho-PI3K, and phospho-AKT were decreased, and E-cadherin was increased when miR-217 was overexpressed (Fig. S5G, H). The knockdown of miR-217 had the opposite effect (Fig. S5G, H). These results demonstrated that miR-217 inhibited EMT progression via the PI3K/AKT pathway.

Further to verify if LINC01268 could regulate KIF2A mRNA levels via miR-217, lenti-LINC01268 was transfected with pcDNA3.1-miR-217 in AsPC-1 and PANC-1 cells. The results showed that miR-217 overexpression decreased KIF2A mRNA levels. KIF2A mRNA expression in the lenti-LINC01268 + pcDNA3.1-miR-217 group was decreased compared with the pcDNA3.1 + lenti-LINC01268 group (Fig. S5I–L). These results suggested that KIF2A may be positively regulated by LINC01268 via miR-217. In rescue experiments, miR-217 overexpression decreased vimentin, SNAIL + SLUG, phospho-PI3K, and phospho-AKT levels, while E-cadherin was increased. Vimentin, SNAIL + SLUG, phospho-PI3K, and phospho-AKT expression in lenti-LINC01268 + pcDNA3.1-miR-217 group was down-regulated compared with that in the pcDNA3.1 + lenti-LINC01268 group (Fig. S5K, L). Conversely, E-cadherin was up-regulated in the lenti-LINC01268 + pcDNA3.1-miR-217 group compared with the pcDNA3.1 + lenti-LINC01268 group (Fig. S5K, L). Furthermore, KIF2A was related to PaCa by gene functional enrichment analysis in GSEA_4.1.0 (Fig. S6A–C) which indicated that KIF2A played key roles in mTOR, VEGF pathway, and PaCa progression.

Finally, a xenograft tumor mouse model was constructed, followed by an intravenous injection of si-LINC01268. Subcutaneous tumor formation experiments revealed that injection of si-LINC01268 had lower fluorescence intensity than the control (Fig. 1A, B). The weight of mice in the si-LINC01268 group was lower than the control (Fig. 1C). LINC01268 was knocked down 0.29-fold in the 5 nmol si-LINC01268 group (Fig. 1D). In addition, KIF2A was decreased at the mRNA and protein levels in the si-LINC01268 group (Fig. 1E), which revealed that KIF2A was regulated by LINC01268. SNAIL + SLUG and vimentin were decreased, whereas E-Cadherin was increased in the si-LINC01268 group compared with the control (Fig. 1F). In summary, si-LINC01268 represses EMT of PaCa via KIF2A *in vivo* and a working model of LINC01268 activation of EMT in PaCa via the miR-217-KIF2A-PI3K/AKT pathway is also shown in Figure 1G.

Ethics declaration

The study was conducted and performed after approval by the Clinical Research Ethics Committee of Shanghai Public Health Clinical Center. Signed informed consent was obtained from each patient.

Conflict of interests

The authors declare no potential conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.12.018.

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