Supplemental Information

Hypoxic tumor-derived exosomal circular RNA SETDB1 promotes invasive growth and EMT via the miR-7/Sp1 axis in lung adenocarcinoma

Li Xu, Wei-Lin Liao, Qi-Jue Lu, Peng Zhang, Ji Zhu, and Ge-Ning Jiang
Figure S1.

(A) The expression of circSETDB1 was up-regulated in LUAD cell lines after normalizing to BEAS-2B and the expression in A549 and PC-9 were higher than others. (B-E) The effect of hypoxic exosomes on the EMT of LUAD cells was analyzed by Western blot analysis. The data were adjusted to β-actin expression (integral optical density of objective protein versus that of β-actin protein). Experiments were
performed in triplicate, respectively.
Figure S2.

(A) Real-time PCR analyzing of cellular and exosomal circSETDB1 levels in control and sh-circSETDB1 treated cells. Experiments were performed in triplicate. (B) Real-time PCR analyzing of circSETDB1 and linear-SETDB1 expression in sh-circSETDB1 treated cells. Experiments were performed in triplicate. (C) Effect of hypoxia-induced exosomes on cell invasion were abrogated by sh-circSETDB1 in Matrigel Transwell invasion assays. (D) Effect of hypoxia-induced exosomes on cell proliferation were abrogated by sh-circSETDB1 in EdU incorporation assay. (E) The expression of exosomal circ_0032674 and circ_0004921 in LUAD patients circulating blood versus their expression in healthy samples circulating blood. (F) ROC curve of serum exosomal circSETDB1 as a potential biomarker for prediction of LUAD in comparison groups. (G-J) The effect of hypoxic exosomes co-transfection with sh-circSETDB1 on the EMT of LUAD cells was analyzed by western blot analysis. The data were adjusted to β-actin expression (integral optical density of objective protein versus that of β-actin protein). Experiments were performed in triplicate, respectively.
Figure S3

(A) The effect HIF-1α of PBS and YC-1 treatment of hypoxic LUAD cells was analyzed by Western blot analysis. (B) Quantitative real-time PCR validated the decrease of exosomal circSETDB1 when treated with YC-1 in hypoxic LUAD cells. Experiments were performed in triplicate. (C) Quantitative real-time PCR validated the decrease of cellular circSETDB1 when treated with YC-1 in hypoxic LUAD cells. Experiments were performed in triplicate.
Figure S4.

(A) Ago2 RIP assay for the amount circSETDB1 in PC-9 cells. The expression levels of circSETDB1 were measured by RT-PCR. Experiments were performed in triplicate. (B) Over-expresive miR-7 inhibits the capacity of LUAD cell invasion in Matrigel Transwell invasion assays. And the effects of lenti-circSETDB1 improvement on cell invasion were eliminated by miR-7 minic. Experiments were performed in triplicate. (C) Over-expresive miR-7 inhibits the capacity of LUAD cell proliferation in EdU incorporation assay. And the effects of lenti-circSETDB1 improvement on cell proliferation were eliminated by miR-7 minic. Experiments were performed in triplicate. (D-G) The effect of miR-7 and co-transfection with lenti-circSETDB1 on the EMT of LUAD cells was analyzed by Western blot analysis. The data were adjusted to β-actin expression (integral optical density of objective protein versus that of β-actin protein). Experiments were performed in triplicate, respectively.
Figure S5.

(A) The expression of Sp1 in A549 cells was detected by western blot analysis. (B) The expression of Sp1 in LUAD cells was detected by western-blot analysis. The data were adjusted to β-actin expression (IOD of objective protein versus IOD of β-actin protein). Experiments were performed in triplicate,
respectively. (C) Cells were transfected with control vector, miR-7 minic, lenti-circSETDB1 with or without miR-7 minic, normoxic exosomes and hypoxic exosomes. Experiments were performed in triplicate, respectively. (D, E) The migration ability of A549 and PC-9 cells was enhanced by lenti-circSETDB1 and was partially eliminated siSp1 transfection. Experiments were performed in triplicate. (F, G) The proliferation ability of A549 and PC-9 cells was enhanced by lenti-circSETDB1 and was partially eliminated siSp1 transfection. Experiments were performed in triplicate. (H, I) The invasion ability of A549 and PC-9 cells was enhanced by lenti-circSETDB1 and was partially eliminated siSp1 transfection. Experiments were performed in triplicate. (J-N) The effect of si-SP1 and co-transfection with lenti-circSETDB1 on the EMT of LUAD cells was analyzed by Western blot analysis. The data were adjusted to β-actin expression (integral optical density of objective protein versus that of β-actin protein). Experiments were performed in triplicate, respectively.
Figure S6.

(A-C) CircSETDB1 and Sp1 relative expression was upregulated, whereas miR-7 relative expression was downregulated in LUAD tumor tissues compared with in adjacent normal tissues. A paired two tailed t test was used for statistical analysis. (D) Sp1 expression was upregulated in LUAD tumor tissues compared with in adjacent normal tissues detected by western-blot. (E) CircSETDB1 relative expression was negatively correlated with miR-7 relative expression in lung adenocarcinoma tissues. (F) CircSETDB1 relative expression was positive correlated with Sp1 relative expression in lung adenocarcinoma tissues.
| **hsa_circ_0003439**          | Forward: 5'- AAAGACCAGAAGCTCCGTGAA -3’ | Reverse: 5'- TGAAATGCGAAGTTCTCCA -3’ |
|-------------------------------|----------------------------------------|---------------------------------------|
| **hsa_circ_0032674**          | Forward: 5’- GTCACTCTGTTAATGCACCTA-3’  | Reverse: 5’- ACTCTGCAACCCTTG-3’       |
| **hsa_circ_0004921**          | Forward: 5’- AGCCAACCTTTTACTCTCTA-3’   | Reverse: 5’- TAGTGTGAAGTCATTTC-3’     |
| **SETDB1**                    | Forward: 5’- AGTCTACTCTGGGGAGCCA-3’    | Reverse: 5’- ACAATGCCAGCAGGAGCCA-3’   |
| **Sp1**                       | Forward: 5’- TGGCAGCAGTACCAATGGC-3’    | Reverse: 5’- CCAGGTAGTCCTGTCAGAATT-3’ |
| **HIF-1 α**                   | Forward: 5’- CATAAGTCTGCAACATGGAAGGT-3’| Reverse: 5’- ATTTGATGGGTGAGGAATTGT-3’ |
| **miR-7**                     | Forward: 5’- CTAGCTAGCTAGCAACCCAATAGGAGG-3’ | Reverse: 5’- GAAGATCTGATCCTCTGGAGGTGT-3’ |
| **GAPDH**                     | Forward: 5’- GCTCTCTGCTCCTCCTGTT-3’    | Reverse: 5’- ACGACCATACTCGTTGACTC-3’  |