Title Page

Title: Overexpression of miR-499-5p inhibits non-small cell lung cancer proliferation and metastasis by targeting VAV3

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Figure S1
miR-499-5p expression levels in NSCLC cell lines.
(A) qRT-PCR data show lower expression of miR-499-5p in NSCLC cell lines (A549, H23, H522 and HOP62) compared with human bronchial epithelial cells (BEAS-2B). Data are presented as mean ± s.d. (B) Correlation scatter plot of miR-499-5p and VAV3 expression in A549 and H23 cells.

Figure S2
(A) Quantitation of BrdU staining results. Error bars represent the mean ± s.d. of three independent experiments. ***, p<0.001. (B) Immunofluorescence analysis of BrdU incorporation in A549 cells transfected with pre-miR-499-5p vs control vs ASO-miR-499 for 72 h.

Figure S3
miR-499-5p induces cell cycle arrest.
In miR-499-5p or NC stably transfected-A549 and H23 cells, cells in different cycle phases are detected. Cell cycle was examined by FACS. A significant increase in S-phase was observed. **P < 0.01.

Figure S4
A549 and H23 cells were transfected with pre-miR-499, miR-499-5p mimic and miR-499-3p, respectively. pre-miR-499 and miR-499-5p mimic inhibited NSCLC cells proliferation. Error bars represent the mean ± s.d. of three independent experiments. **, p<0.01; ***, p<0.001.

Figure S5
Expression of ECM and EMT-related markers in NSCLC cells.
(A) Protein expression level of MMP-2, MMP-9 and β-actin in A549 H23 cells transfected with vector, miR-499-5p and miR-499-5p inhibitor was detected by western blotting analysis. (B) Western blot analysis shows VAV3 and miR-499-5p regulates E-cadherin and vimentin expression in A549 cells.
Figure S6

The efficiency of overexpression and knockdown of miR-499-5p.
qRT-PCR was performed to confirmed the miR-499-5p expression in A549 cells after transfection with pre-miR-499 and ASO-miR-499. Error bars represent the mean ± s.d. of three independent experiments. ***, p<0.001.

Figure S7

Sequence alignment of miR-499-5p with the 3’ UTR of the VAV3 gene.
Figure S2

(A) Bar graph showing BrdU incorporation (%) at different time points (24h, 48h, and 72h) for pre-miR-499-5p, control, and ASO-miR-499 groups. The error bars indicate standard deviation.

(B) Immunofluorescence images showing DAPI and BrdU staining for pre-miR-499-5p, control, and ASO-miR-499 groups.
Figure s3
Figure s4

The graphs illustrate the growth of cells over time (24h, 48h, 72h, 96h) under different conditions:

- Control
- miR-499-3p mimic
- miR-499-5p mimic
- Pre-miR-499

The graphs are labeled with A549 and H23, indicating the cell lines used in the experiment.

Statistical significance is marked with symbols: * (p < 0.05), ** (p < 0.01), *** (p < 0.001).
Figure s6

![Graph showing relative level of miR-499-5p for different treatments: Vector, pre-miR-499, ASO-NC, ASO-miR-499. The graph indicates significant differences (***) between pre-miR-499 and the other treatments.](image-url)
Figure s7

VAV3 Wild-Type 5’ UAG---CCCUCCUAAGUCUUAA 3’

hsa-miR-499-5p 3’ UUUGUAGUGACGUUCAGAAUU 5’

VAV3 Mutan-Type 5’ UAG---CCCUCCUACGGCGUAA 3’