Supplementary Data

Hypoxia-induced MIR31HG expression promotes partial EMT and basal-like phenotype in pancreatic ductal adenocarcinoma based on data mining and experimental analyses

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Supplementary Data include:

Supplementary Materials and Methods

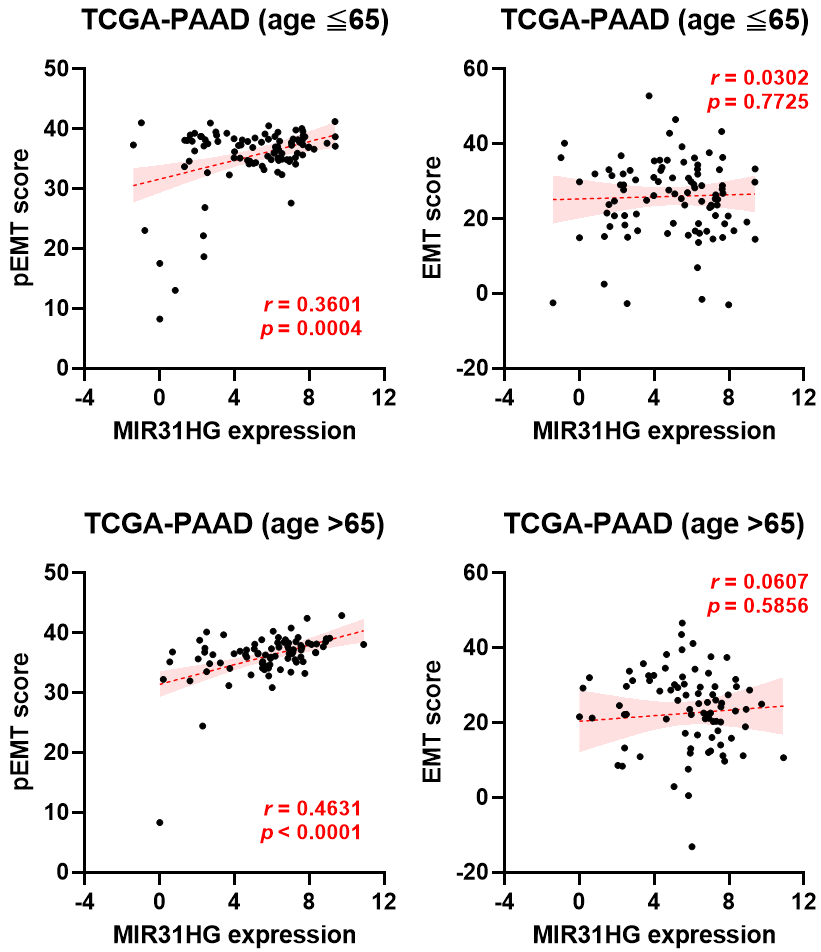
Supplementary Figures S1-S3

Supplementary Materials and Methods

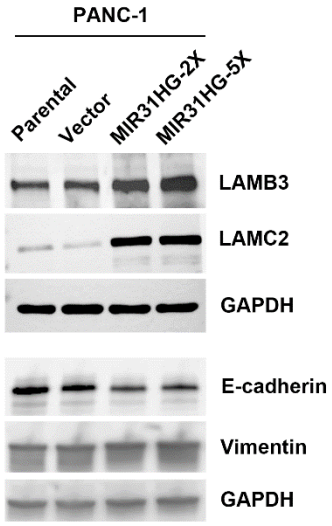
Western blot analysis

Total protein lysates were extracted using the RIPA lysis and extraction buffer (#89901; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with a 1× concentration of protease (#11873580001) and phosphatase (#04906837001) inhibitor cocktails (Roche, Indianapolis, IN, USA). Proteins were then separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto nitrocellulose membranes. The membranes were blocked with 5% skimmed milk in TBST buffer (20 mM Tris-base, 150 mM NaCl, and 0.05% Tween-20). They were then incubated with a specific primary antibody followed by a horseradish peroxidase (HRP)-conjugated secondary antibody. Protein bands were visualized using the Western Lightning Plus ECL detection reagent (#NEL105001EA; PerkinElmer, Waltham, MA, USA) and detected with the GE Amersham Imager 600 (GE Healthcare Life Sciences, Marlborough, MA, USA). E-cadherin antibody (#3195) was purchased from Cell Signaling Technology (Berkeley, CA, USA). Vimentin (#GTX100619), LAMB3 (#GTX103736), and LAMC2 (#GTX113765) antibodies were purchased from GeneTex (Hsinchu City, Taiwan). HRP-conjugated anti-rabbit secondary antibody (#111-035-003) was from Jackson ImmunoResearch (West Grove, PA, USA).

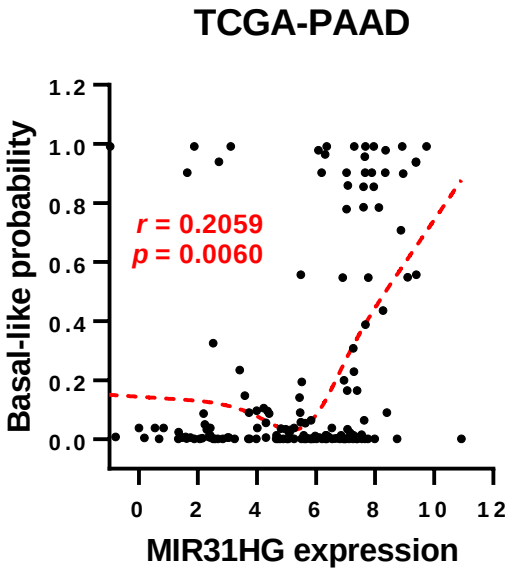
Supplementary Figures



Supplementary Figure S1. The correlation between MIR31HG and partial EMT in PDAC patients of different age groups. Data from PDAC patients (TCGA-PAAD dataset; n = 177) were obtained from the cBioPortal website (https://www.cbioportal.org/). Patients were divided into two age groups: age ≦65 (n = 94) and >65 (n = 83). EMT and partial EMT (pEMT) scores were calculated as described in the Materials and Methods section, and their correlation with MIR31HG gene expression was analyzed.



Supplementary Figure S2. Effect of MIR31HG overexpression on EMT and partial EMT marker expression. The protein expression levels of EMT markers (E-cadherin and vimentin) and partial EMT markers (LAMB3 and LAMC2) in parental PANC-1, PANC-1-Vector, PANC-1-MIR31HG-2X, and PANC-1-MIR31HG-5X cells were analyzed by western blotting.



Supplementary Figure S3. The correlation between MIR31HG and basal-like probability in PDAC patients. (A) Data from PDAC patients (TCGA-PAAD dataset; n = 177) were obtained from the cBioPortal website (https://www.cbioportal.org/). The basal-like probability was calculated using the PurIST classifier and plotted against MIR31HG expression. Their relationship was analyzed using Spearman's rank correlation.