

Supplemental Table 1. Correlation of Clinicopathological Features and ESRP1 Expression in Pancreatic Cancer

ESRP1-high group showed more frequent histological grade G1/G2, whereas ESRP1-low group showed histological grade of G3 ($P=0.04$). Between ESRP1-high and -low groups there were no statistically significant differences in age, sex, T, N, M factor and stage based on UICC classification.

Supplemental Figure 1. Immunohistochemical Analysis of ESRP1 in Normal Human Pancreas

Immunohistochemical staining was carried out in serial sections focused on ductal (A), acinar/islet (B), and vascular (C) components from normal human pancreatic tissues using anti-ESRP1 antibody. ESRP1 immunoreactivity was weak or absent in pancreatic ductal cells (arrows), but was readily evident in the nuclei and cytoplasm of acinar cells and in the nuclei of islet cells, and absent in vascular endothelial cells. Staining for CK19, insulin and CD31 confirmed the ductal, islet, and endothelial nature of the cells, respectively. bar: 100 μ m.

Supplemental Figure 2. Overall Survival and Disease-free Survival of High-ESRP1 and Low-ESRP1 groups In Surgery Only patients

In 57 patients who underwent resection and did not received adjuvant chemotherapy,

both overall (A) and disease-free survival (B) tended to be longer in ESRP1-high group, but there were no statistical significance ($P=0.55$, $P=0.30$ respectively).

Supplemental Figure 3. qRT-PCR for ESRP1, FGFR-2 IIIb, and FGFR-2 IIIc mRNA in Multiple Human Pancreatic Cancer Cell Lines

ESRP1 (A), FGFR-2 IIIb (B) and FGFR-2 IIIc (C) mRNA levels were assayed in the indicated cell lines. ESRP1 (A) and FGFR-2 IIIb (B) expression was highest in Capan-1, KLM-1, PK-1 and PK-8 cells and relatively low in PANC-1, PK-45H and MIAPaCa-2 cells. By contrast, FGFR-2 IIIc (C) expression was variable and was highest in PANC-1 cells. Measurements were performed in triplicate, and results were expressed as the ratio of target to 18S rRNA.

Supplemental Figure 4. Establishment of ESRP1-transfected PANC-1 Clones

A: qRT-PCR. ESRP1 mRNA levels in ESRP1-2 and 5 cells were significantly higher than in control cells (PANC-EV2 and 9, $**P<0.01$). The ratio of FGFR-2 IIIb mRNA to FGFR-2 IIIc mRNA and of FGFR-2 IIIb mRNA to FGFR2 mRNA in ESRP1-2 and 5 tended to be higher than in control cells (PANC-EV2 and 9). B: Imaging. Phase-contrast imaging revealed a similar morphology in ESRP1-2 and 5 cells, and PANC-EV2 and 9 cells (upper panel). ESRP1 nuclear immunofluorescence (green) was increased in ESRP1-2 and 5 cells as compared with PANC-EV2 and 9 cells (lower panel). bar:

100µm, inset of lower panel: bar: 50 µm.

Supplemental Figure 5. Effects of Stably-transfected ESRP1 on Migration of PANC-1 Clones

A: Single cell movement. ESRP1-transfected clones exhibited attenuated single cell movement as compared with PANC-EV cells (* P <0.05, ** P <0.01); bar: 100 µm. B: Boyden chamber migration. ESRP1-transfected clones also showed decreased migration ability in a Boyden chamber assay, as compared with PANC-EV cells (** P <0.01); bar: 100 µm

Supplemental Figure 6. Effects of ESRP1 Silencing in KLM-1 Cells

A: qRT-PCR. ESRP1 mRNA levels in siESRP1-A and siESRP1-B cells were significantly lower than in siNeg cells and parental KLM-1 cell (* P <0.05), FGFR-2 IIIb mRNA levels were similar in all the cells, FGFR-2 IIIc mRNA levels were higher in siESRP1-A and siESRP1-B cells than in control cells. B: Immunoblotting. ESRP1 protein levels were decreased in the cytoplasm (cyt) and nuclei (nu) of siESRP1-A and siESRP1-B cells as compared to KLM-1 and siNeg cells (upper panel). rb ESRP1 and rb ESRP2 represent recombinant ESRP1 and ESRP2, respectively. C: Imaging. Phase-contrast imaging revealed a similar morphology in all the cells (upper panel). ESRP1 nuclear immunofluorescence (green) was decreased in siESRP1-A and

siESRP1-B cells as compared with KLM-1 and siNeg cells (lower panel). Upper panel; bar: 100 μ m; inset in lower panel; bar: 50 μ m.

Supplemental Figure 7. Effects of Concomitant ESRP1 and FGFR-2 IIIc Silencing in KLM-1 Cells

A: qRT-PCR. FGFR-2 IIIc mRNA levels in siESRP1-A + siNeg and siESRP1-B + siNeg cells were significantly higher than in KLM-1, siNeg, siESRP1-A + siFGFR-2 IIIc, and siESRP1-B + siFGFR-2 IIIc cell (** P <0.01). B: Cell proliferation. Proliferation was monitored using the WST-8 cell counting assay. There was a significant increase in cell proliferation in both siESRP1-A + siNeg and siESRP1-B + siNeg cells as compared with KLM-1, siNeg, siESRP1-A + siFGFR-2 IIIc, and siESRP1-B + siFGFR-2 IIIc cells (* P <0.05 ** P <0.01). C and D: Migration and invasion. Both migration and invasion were markedly increased in siESRP1-A + siNeg and siESRP1-B + siNeg cells as compared with KLM-1, siNeg, siESRP1-A + siFGFR-2 IIIc, and siESRP1-B + siFGFR-2 IIIc cells (** P <0.01). bar: 200 μ m.

Supplemental Figure 8. Expression of genes detected by proteomic analysis in PANC-1 cells

Vimentin, 14-3-3 ϵ , heat shock protein 70, and IQGAP1 mRNA levels were measured by quantitative PCR in parental PANC-1 cells, empty vector-transfected cells (PANC-EV)

and ESRP1 overexpressing cells (PANC-ESRP1). $**P<0.01$ by comparison with PANC-1 and PANC-EV.

Supplemental Figure 9. Expression of genes detected by proteomic analysis in KLM-1 cells

Expression of indicated mRNA moieties was measured by quantitative PCR in parental KLM-1 cells (KLM-1), negative controls expressing non-relevant siRNA (siNeg) and siRNAs targeting ESRP1 (siESRP1-A and siESRP1-B). $*P<0.05$ and $**P<0.01$ by comparison with siNeg and KLM-1 cells.

Supplemental Figure 10. Expression of protein detected by proteomic analysis in PANC-1 cells

IQGAP1, FINA, and PLIN3 protein levels were examined by western blot assay in parental PANC-1 cells, empty vector-transfected cells (PANC-EV) and ESRP1 overexpressing cells (PANC-ESRP1). 14-3-3 ϵ protein levels were also examined in PANC-1, PANC-EV, PANC-ESRP1, KLM-1, siNeg, siESRP-A, and siESRP-B.

Supplemental Figure 11. Effects of ESRP1 on TGF- β 1-induced EMT

A and B: Effects of TGF- β 1 on E-cadherin and vimentin expression. Parental PANC-1 cells were cultured for 24 and 48 hours with 20 ng/ml TGF- β 1. C: Effects of TGF- β 1 on cell morphology. Cells were incubated for 48h in the absence or presence of

TGF- β 1 (20 ng/ml). Bar: 100 μ m. D-E: Effects of ESRP1 on E-cadherin and vimentin levels. PANC-ESRP1 expressed higher E-cadherin levels than PANC-EV cells (* P <0.05) but lower levels of vimentin (** P <0.01 vs PANC-EV).

Supplemental Figure 12. Effects of ESRP1 on FGFR-1, FGFR-3, and CD44

Isoform Expression

A. FGFR-1. The ratio of FGFR-1 IIIb/IIIc was significantly higher in ESRP1 overexpressing PANC-1 cells than control cells (** P <0.01), and significantly lower following ESRP1 silencing in KLM-1 cells (** P <0.01). B. FGFR-3. The ratio of FGFR-3 IIIb/IIIc was similar in all PANC-1 cells and clones, whereas, the FGFR-3 IIIb/IIIc ratio was markedly decreased in ESRP1-silenced KLM-1 cells (** P <0.01). C. The ratio of CD44s/CD44 was similar in all PANC-1 cells and clones, but was markedly increased in ESRP1-silenced KLM-1 cells (** P <0.01).

Supplemental Figure 13. Subcutaneous tumor growth in nude mice

PANC-EV2 and PANC-EV9 cells and ESRP1-2 and ESRP1-5 cells (1×10^6 cells/injection) were injected subcutaneously in the flank region of nude mice, three mice per cell line. Tumor growth was monitored for three weeks. There were no statistically significant differences of tumor volumes between PANC-EV2 and PANC-EV9 cells and ESRP1-2 and ESRP1-5 tumors.