Cooperative actions of p21\textsuperscript{WAF1} and p53 induce Slug protein degradation and suppress cell invasion

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Supplementary Figures and Legends (Fig. S1-S6)
Figure S1. **H1299 cells are more invasive than H460 cells.** The invasiveness of H460 and H1299 cells was compared using Matrigel-coated polycarbonate filters. Western blotting was used to confirm p53 expression in H460 cells and its absence in H1299 cells; β-actin was the loading control.
Figure S2. p53, p21, and Slug regulate the invasiveness of lung-cancer cells. H460 cells were transfected with control or 2 sets of siRNAs targeting p53, p21, or Slug. After incubation for 24 h, cellular invasiveness was compared.
Figure S3. p53 and p21 require Slug to suppress cell invasion. H460 cells were transfected with a control siRNA or siRNAs targeting p53, p21, or Slug in the indicated combinations. After incubation for 24 h, cellular invasiveness was compared.
Figure S4. p53 and p21 do not significantly influence Slug RNA levels. (A) H460 cells were transfected with the indicated siRNAs. After 40 h of recovery, p53, p21, and Slug mRNA levels were analyzed by performing RT-PCR. (B) H460 cells were treated with control or 2 sets of siRNAs targeting p53 or p21; H1299 cells were transfected with a control or p53-expressing vector. Slug mRNA levels in transfectants were compared by performing quantitative real-time PCR.
Figure S5. Co-precipitation of recombinant proteins. (A) Preparation of GST-tagged recombinant proteins. p53, p21, Slug, and Mdm2 were cloned into pGEX3-p6 vectors and expressed in the *E. coli* BL21 strain. GST-tagged proteins were purified using glutathione-Sepharose beads; SDS-PAGE and Coomassie Blue staining were used for analyzing the purified proteins. Asterisks (*) indicate GST-tagged p53, p21, Slug, and Mdm2 proteins. (B) Co-precipitation of p21, p53, Slug, and Mdm2 proteins. GST and GST-tagged Mdm2 and \(^{35}\)S-labeled Slug, p53, and p21 proteins were incubated in the indicated combinations and then subjected to pull-down analyses performed using glutathione-Sepharose beads. SDS-PAGE and autoradiography were used for analyzing the precipitated proteins. (C) Left, GST-tagged p53 proteins were incubated with \(^{35}\)S-p21 proteins. Middle, GST-p21 proteins were incubated with \(^{35}\)S-Slug proteins. Right, GST-p53 proteins were incubated with \(^{35}\)S-Slug proteins. GST served as a control in all cases. The mixtures were subjected to pull-down analyses performed using glutathione-Sepharose beads; SDS-PAGE and autoradiography were used for analyzing the precipitated proteins.
Figure S6. Deletion constructs of p21, Slug, and p53 used for *in vitro* binding assays.