Pseudolaric Acid B (PAB) Induces Senescence by Activation of P53/P21 Pathway in Pancreatic Cancer Cells

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Hypothesis

Senescence has become a promising tumor suppressor therapy. Pseudolaric acid B (PAB) was shown to induce senescence by activating p53 in human lung cancer cells and upregulates p53 expression in pancreatic cancer cells.

We hypothesize that PAB could be a potential chemo-preventive agent which could activate p53 pathways to induce senescence in pancreatic cancer cells.

Materials & Methods

Major cancer cell line pance-1 cells were used to test this hypothesis. SA-β-GAL: Senescent cells were detected using SA-β-GAL staining method.

Morphological Changes: Cells were treated with PAB for indicated time periods. Then, cells were stained with DAPI to see the changes in nuclear morphology under a fluorescence microscope.

Cell Cycle: Cell Cycle distribution was analyzed by Novocye flow cytometer.

Western Blot: Expression of p53, p21, Cyclin B1, and Actin was detected using enhanced chemiluminescence kit.

siRNA Transfection: Cells were transfected with control-siRNA, p53, or p21 siRNA-Mate. Transfected cells were treated with indicated PAB for indicated time periods.

Data Analysis: All experiments were conducted at least three times independently. Statistical significance differences were determined by One-Way ANOVA and student’s t test. *P < 0.05 is considered as significant.

Results

PAB induces senescence in Panc-1 cells

24 h 48 h 72 h

Control 0.5 µM 1 µM

Figure 1. Panc-1 cells were treated with 0.5 and 1 µmol/L PAB for 24, 48, and 72 h. The senescent cells were examined by SA-β-GAL staining. Black arrows indicate SA-β-GAL positive cells.

PAB induces mitotic catastrophe in Panc-1 cells.

48 h 72 h

Control 0.5 µM 1 µM

Figure 2. Panc-1 cells were treated with 0.5 and 1 µmol/L PAB for 48 and 72 h. The cells were observed with phase contrast microscope and changes in nuclear morphology were detected by DAPI staining. The white arrows indicate multinucleated cells.

PAB induces G2/M cell cycle arrest in Panc-1 cells

48 h 72 h

Control 0.5 µM 1.0 µM

Figure 3. Panc-1 cells were treated with 0.5 and 1 µmol/L PAB for 48 and 72 h. Cell cycle distribution was analyzed using flow cytometry.

PAB induces senescence through activation of p53/p21 pathway.

48 hours 72 hours

p53 p21 Cyclin B1 Actin

Figure 4. Panc-1 cells were treated with 0.5 and 1 µmol/L PAB for 48 and 72 hours. The protein levels of p53, p21, and Cyclin B1 were evaluated by western blot.

Conclusions

PAB treatment has shown to induce senescence in pancreatic cancer cells by evidence of increased SA-β-GAL staining. PAB induced mitotic catastrophe by evidence of multinucleated cells in DAPI staining. PAB treatment also causes G2/M cell cycle arrest. Our results show PAB mediated induction of senescence by activation of p53/p21 pathway.
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PAB induces senescence through activation of p53/p21 pathway.

<table>
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<th>PAB (µM)</th>
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<th>72 hours</th>
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<td></td>
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PAB induces senescence through activation of p53/p21 pathway.

Figure 5. Transfection efficacy of P53 and P21 was examined by western blot method. Transfected cells were treated with 1 µmol/L PAB for 48 hours. Senescent cells were determined by SA-β-GAL staining. Black arrows indicate SA-β-GAL positive cells.