

Ailanthone Induces Cell Cycle Arrest and Apoptosis in Melanoma B16 and A375 Cells

Figure Legends

Figure 2. Ailanthone inhibits cell proliferation and cell colony formation in melanoma B16 and A375 cells. (a) After treatment with different concentrations of ailanthone (0.25, 0.5, 1, 2, 4, 8, and 16 μM) for 24 h, the cell viabilities of B16 and A375 cells were determined using the cell counting kit-8 assay. (b) Statistical analysis of the number of cell colonies in ailanthone-treated B16 and A375 cells. (c) The cell colonies were stained with crystal violet and observed under an inverted microscope. The data are presented as the mean \pm SD ($n = 3$). * $p < 0.05$, ** $p < 0.01$ compared with the control B16 cells. # $p < 0.05$, ## $p < 0.01$ compared with the control A375 cells.

Figure 3. Ailanthone induces cells cycle arrest and regulates cell cycle-related proteins in melanoma B16 and A375 cells. (a) Cell cycle distributions of the B16 cells were measured by flow cytometry after treatment with various concentrations of ailanthone for 24 h. (b) Statistical analysis of the cell cycle distribution of ailanthone-treated B16 cells. * $p < 0.05$, ** $p < 0.01$ compared with the control B16 cells. (c) The levels of p21 and cyclin E in ailanthone-treated B16 cells were examined by Western blotting. (d) Quantitative analysis of the levels of p21 and cyclin E in ailanthone-treated B16 cells. * $p < 0.05$, ** $p < 0.01$ compared with the control B16 cells. (e) Cell cycle distribution of the A375 cells was measured by flow cytometry after treatment with various concentrations of ailanthone for 24 h. (f) Statistical analysis of cell cycle distributions of the ailanthone-treated A375 cells. # $p < 0.05$, ## $p < 0.01$ compared with the control A375 cells. (g) The levels of p21 and cyclin B in ailanthone-treated A375 cells were examined by Western blotting. (h) Quantitative analysis of the levels of p21 and cyclin B in ailanthone-treated B16 cells. # $p < 0.05$, ## $p < 0.01$ compared with the control A375 cells. The data are presented as the mean \pm SD ($n = 3$).

Figure 4. PI3K-Akt signaling is involved in the cell cycle arrest of ailanthone-treated melanoma B16 and A375 cells. (a) The levels of p-PI3K, PI3K, p-Akt, and Akt in ailanthone-treated B16 cells were examined by Western blotting. (b) Quantitative analysis of the levels of p-PI3K, PI3K, p-Akt, and Akt in ailanthone-treated B16 cells. * $p < 0.05$ compared with the control B16 cells. (c) The levels of p-PI3K, PI3K, p-Akt, and Akt in ailanthone-treated A375 cells were examined by Western blotting. (d) Quantitative analysis of the levels of p-PI3K, PI3K, p-Akt, and Akt in ailanthone-treated A375 cells. # $p < 0.05$, ## $p < 0.01$ compared with the control A375 cells. The data are presented as the mean \pm SD ($n = 3$).

Figure 5. Ailanthone induces cell apoptosis in melanoma B16 and A375 cells. (a) Representative morphological images of ailanthone-treated B16 cells after Hoechst 33258 staining. (b) The apoptotic rates of ailanthone-treated B16 cells were measured by flow cytometry. (c) Quantitative analysis of the apoptotic rates of ailanthone-treated B16 cells. ** $p < 0.01$ compared with the control B16 cells. (d) The representative morphological images of ailanthone-treated A375 cells after Hoechst 33258 staining. (e) The apoptotic rates of ailanthone-treated A375 cells were measured by flow cytometry. (f) Quantitative analysis of the apoptotic rates of ailanthone-treated A375 cells. ## $p < 0.01$ compared with the control A375 cells. The data are presented as the mean \pm SD ($n = 3$).

Figure 6. Ailanthone induces melanoma cell apoptosis in a caspase-dependent mechanism. (a) The expression levels of caspase-9, cleaved caspase-9, caspase-3, and cleaved caspase-3 in ailanthone-treated B16 cells were examined by Western blotting. (b) Quantitative analysis of the expression levels of caspase-9, cleaved caspase-9, caspase-3, and cleaved caspase-3 in ailanthone-treated B16 cells. * $p <$

0.05, ** $p < 0.01$ compared with the control B16 cells. (c) The expression levels of caspase-9, cleaved caspase-9, caspase-3, and cleaved caspase-3 in ailanthone-treated A375 cells were examined by Western blotting. (d) Quantitative analysis of the expression levels of caspase-9, cleaved caspase-9, caspase-3, and cleaved caspase-3 in ailanthone-treated A375 cells. # $p < 0.05$ compared with the control A375 cells. The data are presented as the mean \pm SD ($n = 3$).

Figure 7. Ailanthone induces cell apoptosis in melanoma cells via the mitochondria-mediated apoptotic signaling pathway. (a) Changes in mitochondrial membrane permeabilization in ailanthone-treated B16 cells was observed by fluorescence microscopy. (b) The expression levels of cytoplasmic cytochrome C, Apaf-1, Bcl-2, and Bax in ailanthone-treated B16 cells were examined by Western blotting. (c) Quantitative analysis of the levels of cytoplasmic cytochrome C, Apaf-1, Bcl-2, and Bax in ailanthone-treated B16 cells. * $p < 0.05$ compared with the control B16 cells group. (d) Changes in mitochondrial membrane permeabilization in ailanthone-treated A375 cells were assessed by fluorescence microscopy. (e) The expression levels of cytoplasmic cytochrome C, Apaf-1, Bcl-2, and Bax in ailanthone-treated A375 cells were examined by Western blotting. (f) Quantitative analysis of the levels of cytoplasmic cytochrome C, Apaf-1, Bcl-2, and Bax in ailanthone-treated A375 cells. # $p < 0.05$, ## $p < 0.01$ compared with the control A375 cells. The data are presented as the mean \pm SD ($n = 3$).