

Supplementary Methods

Western Blotting. The following primary antibodies were used: phosphorylated PKR, p38MAPK, pJNK, p44/42MAPK, pEIF2, caspase-9, pFADD (Cell Signaling, Boston, MA); caspase-3, PARP, caspase-8, and FasL (BD-Pharmingen, San Diego, CA); β -actin (Sigma Chemicals, St. Louis, MO); and Fas, FAF1, FAP-1, TRADD, Bid, cytochrome C, and FLIP_{S/L} (Santa Cruz Biotechnology, Santa Cruz, CA).

Fas expression analysis. For analysis of total and cell surface Fas expression, tumor cells (1×10^5 - 10^6) treated with PBS, Ad-luc, or Ad-mda7 were analyzed by immunofluorescence and flow cytometry. For immunofluorescence, treated cells were fixed in 1% glutaraldehyde, washed with PBS two times, and stained with anti-Fas antibody (1 μ g/ml; Santa Cruz). Cells that were not treated with anti-Fas antibody or stained with an isotype-matched antibody served as negative controls. Cells were washed with PBS, and incubated with FITC-labeled anti-mouse secondary antibody (Vector Laboratories, Inc., Burlingame, CA) for 30 min. The slides were subsequently washed, covered with mounting medium, and observed under a fluorescent microscope.

For analysis of cell surface Fas expression treated cells were harvested fresh, placed on ice and were stained using FITC-labeled antihuman Fas monoclonal antibody (1 μ g; DX-2, DX-3; Biosource International, Camarillo, CA) or isotype-matched FITC-conjugated mouse IgG κ monoclonal immunoglobulin isotype control (MOPC-21; Pharmingen). At 30 min after incubation cells were washed thrice with PBS containing 0.1% sodium azide and 1% FBS, and then resuspended in 500 μ l PBS containing 0.1% sodium azide and analyzed with by flow cytometry. The percent increase in Fas expression was determined by analyzing the mean fluorescence intensity that represents antigenic density per cell basis.