Supplementary Material

Flow Cytometric Analysis of cell apoptosis

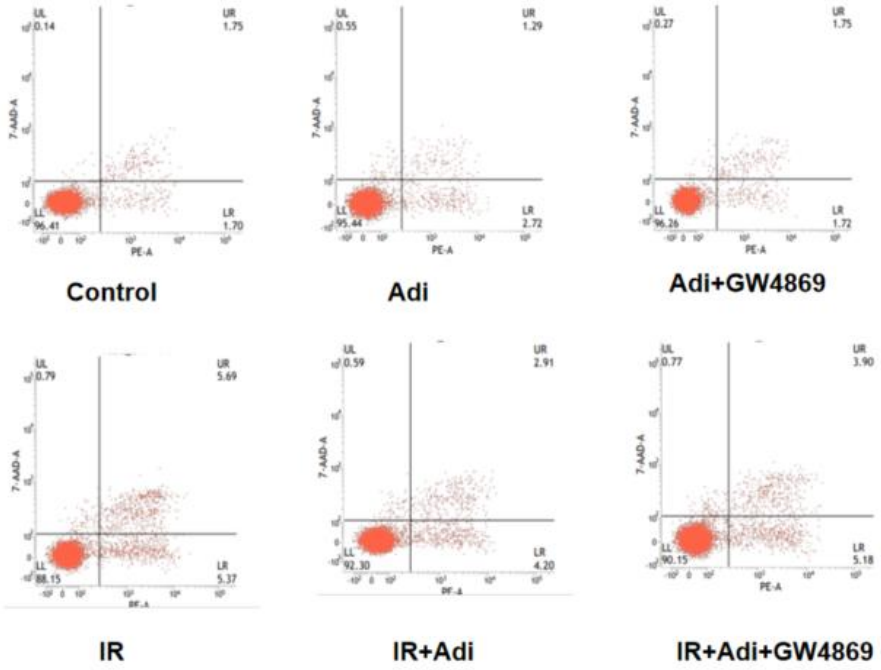
ESCC cells were seeded into six-well plates in different groups and cultured in the complete medium for 24 hours. Cells were then treated under different experimental conditions: co-cultured with adipocytes or adipocyte with GW 4869 for 24 hours followed by 6 Gy irradiation. Post-irradiation, cells were digested with trypsin for 48 hours, centrifuged to collect cell pellets, resuspended in PBS to make single-cell suspensions, centrifuged to discard the supernatant, resuspended in 1× buffer, and stained with Annexin V 7ADD/PE (BD, USA). Then, the cells were left at room temperature in dark for 15 min before apoptosis detection using flow cytometry.

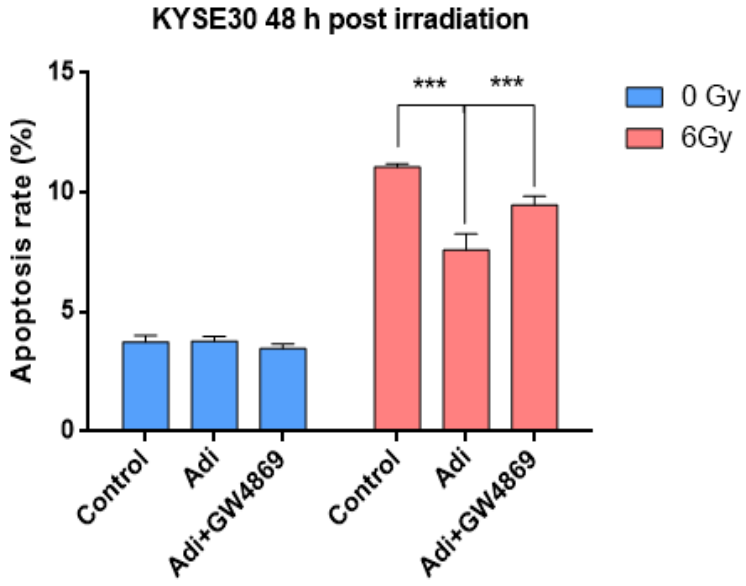
Suppl. Results

GW4869 Reverses the Radioprotective Effect of Adipocytes on Esophageal Cancer Cells.

The apoptosis rate of cells co-cultured with adipocyte-derived exosomes and GW4869 was higher than that of cells co-cultured with adipocytes 48 hours post-irradiation with co-culture (Figure 1). Adipocyte-Derived Exosomes Enhance the Radioresistance of Esophageal Cancer Cells, and the addition of the exosome inhibitor GW4869 in co-culture with adipocytes effectively increased the sensitivity to radiotherapy, counteracting the radioprotective effect induced by adipocyte-derived exosomes. This further confirms the potential role of adipocytes in mediating effects through exosomes.

Suppl. Figure 1





Supplementary Figure 1 GW4869 can reverse the radioresistance of esophageal cancer cells induced by adipocytes. Flow cytometry analysis of apoptosis in KYSE30 cells; Live cells are located in the lower left quadrant, early apoptotic cells in the lower right quadrant, and late apoptotic cells in the upper right quadrant. The total number of apoptotic cells includes both early and late apoptotic cells, and the apoptosis rates of KYSE30 cells were evaluated. Mean values from three independent experiments are presented and analyzed using the *t*-test. *$p<0.05$, **$p<0.01$, ***$p<0.001$.