SR9009 induces a REV-ERB dependent anti-small-cell lung cancer effect through inhibition of autophagy

Weitao Shen1,#, Wei Zhang1,#, Weilin Ye1#, Haihong Wang2, Qingxi Zhang3, Jie Shen1, Qingsha Hong4, Xiang Li5, Ge Wen6, Ting Wei1,*; Jian Zhang1,*

Supplementary Materials

Supplementary Figures

Figure S1
SR9009 induced remarkable apoptosis in SCLC cells. (A-F) Statistics of the Cleaved-PARP expression in Figure 2A-F. Densitometric values were quantified using the ImageJ software and normalized to control. The values of control were set to 1. The data are presented as means ± SD of 3 independent experiments.
Pretreatment with SR9009 could sensitize the resistant cells to chemotherapy. (A and B) CCK-8 assays showed that pretreatment with SR9009 decreased the IC50 values to chemotherapeutic agents (CDDP or VP-16) of chemoresistant SCLC cells. The data are presented as means ± SD of three independent experiments. *P < 0.05; **P < 0.01.
REV-ERBα was involved in the antitumor effect of SR9009 in SCLC. (A and B) H69AR and H446DDP cells were dose dependently treated with SR9009 for 48 h, and the protein expression of REV-ERBα was analyzed. Densitometric values were quantified using the ImageJ software and normalized to control. The values of control were set to 1. The data are presented as means ± SD of 3 independent experiments. (C) Normal human bronchial epithelial (HBE) cells were incubated for 72 h in different concentrations of SR9009. The data are presented as means ± S.D. of three independent experiments.
Figure S4

H69AR and H446DDP cells exhibited relatively high levels of autophagy. (A and B) Western blot analysis of LC3II/I and p62 expression in two pairs of chemosensitive and chemoresistant SCLC cell lines. Densitometric values were quantified using the ImageJ software and normalized to control. The values of control were set to 1. The data are presented as means ± SD of 3 independent experiments. *P < 0.05; **P < 0.01.
Figure S5
Autophagic flux was detected by confocal microscopy. (A and C) SR9009 blocked autophagy, which resulted in reduced autophagic flux. (B and D) Quantification of autophagic vesicles (Cyto-ID). The data are presented as means ± SD of 3 independent
experiments. $^*P < 0.05$.

Figure S6

Western blot analysis of Atg5 in cells transfected with a plasmid encoding Atg5 or the corresponding negative control vector. Densitometric values were quantified using the ImageJ software and normalized to control. The values of control were set to 1. The data are presented as means ± SD of 3 independent experiments. $^*P < 0.05$; $^{**}P < 0.01$. 