Supplementary information

NEDD8-conjugating enzyme E2 UBE2F confers radiation resistance by protecting lung cancer cells from apoptosis

Lisha ZHOU, Changsheng DONG, Zhuoming XU, XinranWANG, Luyi ZHANG, Siyuan CHEN, Jiahao CHEN, Yingying ZHU

Materials and methods

Cell culture, transfection, and treatment

Both HEK293T cells and human lung cancer cells A549 and H1299 were obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (Gibco, South America) and 1% penicillin-streptomycin solution (100 units/ml penicillin and 100 μg/ml streptomycin, Gibco), in 5% CO_2 atmosphere at 37 °C.

Plasmid transfection was carried out using the polyethyleneimine (PEI, Sigma-Aldrich) or lipofectamine 2000 (Invitrogen).

H_2O_2 and irradiation were used to treat lung cancer cells.

Antibodies and western blotting

Antibodies specific to cullin5 (Abcam, 18477), NOXA (Cell Signaling Technology, 14766), UBE2F (Abcam, 12932), β-actin (Huabio, EM21002) were purchased commercially. Western blot analysis was carried out according to standard methods.

Generation of stable cell lines by CRISPR/Cas9 system

Briefly, each guide RNA (gRNA) duplex specifically against UBE2F was inserted into the lenti-Guide-CRISPR-v2-puro vector. LentiCRISPR plasmid with gRNA, packaging plasmids psPAX2 and pMD2.G were co-transfected into HEK293T cells with Lipofectamine 2000 (Invitrogen). Forty-eight hours post transfection, virus supernatant was harvested, filtered (0.45 μm filter) and mixed with polybrene (Sangon, shanghai, china; 10 μg/mL) to infect A549 cell. The infected A549 cells were selected with 2 μg/mL puromycin (Invitrogen, Carlsbad, CA) for a week. Successful UBE2F KO cells were confirmed using Western Blot.

Cell survival assay

Lung cancer cells A549 were seeded in 6-well plates at a density of 500 000 cells/well. After radiation exposure (4 Gy) or H_2O_2 (100 μmol/L) treatment for 48 hours, the remaining living cells were trypsinized and counted by Bio-Rad TC20.

Animal experiments

Nude mice (nu/nu, female, 6–8 weeks old), purchased from the Nanjing Junke Bioengineering Co., Ltd. (Nanjing, China). Mouse studies were performed in specific pathogen-free (SPF) facilities with approval of the Institutional Animal Care and Use Committee

of Taizhou University. Mouse were injected subcutaneously with wild type or UBE2F knockout A549 cells (5×10^6 cells). When the size of the tumor reaches about 100 mm^3, mice were treated with radiotherapy using TRILOGY-SN5626-6X (field X: 0.8 cm; field Y: 0.8 cm; Gy: 10). Tumor size was calculated as $0.5\times length\times width^2$. At the end of the experiments, tumor xenografts were harvested for imaging and weighing.

Apoptosis assay (TUNEL staining)

Apoptosis was determined using the terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) assay.

Bioinformatics analysis

TCGA RNA-Seq was based upon data generated by TCGA Research Network (https://portal.gdc.cancer.gov). RNA-Seq analysis was performed in 535 cases of lung adenocarcinoma tissues and 59 cases of adjacent normal tissues.

Statistical analyses

The statistical significance of differences between groups was assessed using GraphPad Prism 7 (GraphPad Software, Inc., San Diego, CA, USA). For comparison of two groups of samples, the two-tailed Student's t test was used. All data are presented as mean±standard deviation (mean±SD). $P<0.05$ was considered statistically significant.

Figs. S1 and S2

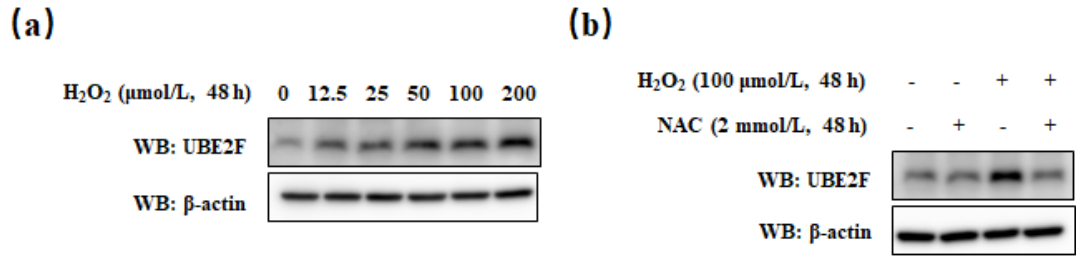

Fig. S1 H_2O_2 treatment up-regulates UBE2F expression in H1299 cells

(a) H_2O_2 treatment increases UBE2F protein levels. H1299 cells were seeded in 60-mm dish, followed by H_2O_2 treatment at different doses up to 100 μmol/L for 48 h.

(b) ROS scavenger *N*-acetyl-L-cysteine (NAC) completely blocks H_2O_2-induced upregulation of UBE2F expression. H1299 cells were treated with H_2O_2 (100 μmol/L) alone for 48 h and/or NAC (2 mmol/L) for 48 h.

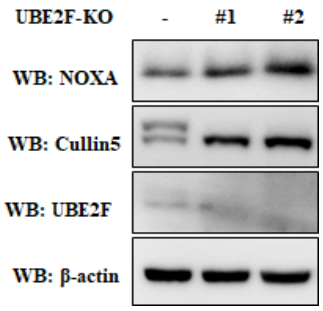

Fig. S2 UBE2F knockout cells display lower cullin5 neddylation levels and higher NOXA expression levels