Supporting Information (SI)

Materials and Methods

Mouse strains

To create a *Palb2* conditional knockout mouse model, we constructed a gene targeting vector in which exons 2 and 3 of the mouse *PALB2* gene are flanked by loxP sites. The *Palb2* gene locus was targeted in V6.5 ES cells. Germline-transmitted animals were crossed to a FLP-deleter strain (FLPeR, Jackson Laboratory) to delete the drug (neomycin) selection cassette. Details of the above procedures are published elsewhere (1). The *Palb2flox/flox* mice were crossed to strains carrying *Trp53flox2-10* (2), *Becn1*-KO (3) and *Wap-Cre* (4) alleles to generate all the genotypes in this study. Females of desired genotypes were mated to go through two rounds of pregnancy and lactation, and the mice were monitored biweekly for tumor development over a period of 750 days. Tumors were recorded when they reach a diameter of 0.5 cm and latency was counted starting from the date of the first delivery.

Mouse embryo fibroblasts (MEFs) and mouse mammary tumor cell cultures

Primary mouse embryo fibroblast (MEF) cells were generated from E13.5 embryos following standard protocols and cultured in DMEM medium containing 10% fetal bovine serum, 100 U/ml of penicillin, 100 µg/ml of streptomycin, and 1% of glutamine. To delete *Palb2* and *Trp53* genes in MEFs, freshly generated cells with floxed alleles were infected with a Cre-encoding retrovirus (pLXSP-Myc-Cre (5)) for 3 times during a 2 day period. Cells were then selected with puromycin (2.5 µg/ml) for 2 days prior to various analyses shown in Fig. 2. Mammary tumor cells were generated from tumor specimens dissociated with collagenase and grown in complete DMEM/F12 (1:1) medium supplemented with 5µg/ml insulin, 5 ng/ml EGF, 5 ng/ml Cholera toxin and antibiotics. All cells were cultured at 37°C in a humidified chamber with 5% CO_2.

Antibodies and chemicals

Antibodies used to detect mouse PALB2 were raised in rabbits against residues 1-200 and affinity purified. Other antibodies were purchased from the following vendors- ERα (Santa Cruz, sc-7207), PR (Santa Cruz, sc-538), p53 (Santa Cruz, sc-6243), 8-oxo-dG (Trevigen, 4354-MC-050), NRF2 (EP1808Y, Epitomics), 53BP1 (A300-272A, Bethyl Labs), α-Tubulin (T9026,

Sigma), p62/SQSTM1 (PW9860, Enzo Lifesciences), LC3B (NB600-1384, Novus Biologicals), cleaved Caspase3 (ASP175, Cell Signaling Technology), and phospho-H2A.X (Ser139, 07-164, Millipore). Olaprib (KU-0059436) was a generous gift from Dr. Graeme Smith (Kudos Pharmaceuticals, Cambridge, UK). Mitomycin C was purchased from Sigma (M4287).

Western blotting

Whole-cell extract was prepared by lysing cells in a NETNG-250 buffer (20 mM Tris-HCl [pH7.5], 250 mM NaCl, 0.5% NP-40, 10% Glycerol) containing the Complete® proteases inhibitor cocktail (Roche). 15-20 μg of each cell lysate was resolved by SDS-PAGE on 4-12% Tris-glycine gels. Resolved proteins were transferred to nitrocellulose membranes and detected following standard protocols.

Olaparib and mitomycin C (MMC) sensitivity assays

Cells were seeded into 96-well plates at a density of 1,000 cells per well in 100 μl medium. Twenty-four hours after seeding, 50 μl of Olaparib- or MMC-containing media were added to achieve desired final concentrations. Cells were then incubated for 96 hr, and survival was determined by the CellTiterGlo® cell proliferation assay (Promega).

Immunofluorescence staining

Cells were grown on glass coverslips in 12-well plates to ~80% confluence. Cells were washed with PBS and fixed with 3% paraformaldehyde in PBS at RT for 10 min. Fixed cells were washed with PBS and permeablized with PBS containing 0.5% Triton X-100 at RT for 5 min. Cells were then washed and incubated at 37°C for 20 min with primary antibodies in PBS containing 5% goat serum. Coverslips were washed and then incubated with Rhodamine (TRITC)- or Fluorescein (FITC)-conjugated secondary antibodies (Jackson Immunoresearch) for 20 min at 37°C. Finally, coverslips were washed 3 times with PBS and mounted on glass slides with VECTASHIELD® Mounting Media with DAPI (Vector Labs).

Reactive oxygen species (ROS) measurement

Exponentially growing cells in 6-well plates were washed twice with PBS and then incubated with fresh phenol red-free DMEM medium containing 10% fetal bovine serum and 100 μM DCF

(2′,7′-Dichlorofluorescin diacetate, Sigma, #D6883) at 37°C for 20 min. Cells were washed twice with PBS and trypsinized. Harvested cells were washed and resuspended in PBS at ~10^6 cells/ml. ROS levels were measured by flow cytometry with excitation at 488 nm and emission at 515-545 nm. All steps were performed in dark whenever possible.

Senescence-associated β-galactosidase assay

Senescence β-Galactosidase Staining Kit was purchased from Millipore. Briefly, cells were washed once with PBS (pH7.2), fixed with 0.5% glutaraldehyde/PBS and washed in PBS (pH7.2) supplemented with 1 mM $MgCl_2$. Cells were stained in X-gal solution (1 mg/ml X-gal, 0.12 mM $K_3Fe[CN]_6$, 0.12 mM $K_4Fe[CN]_6$, 1 mM $MgCl_2$ in PBS at pH 6.0) overnight at 37℃. Three to five hundred cells were counted from each plate.

Detection of apoptotic cells

Apoptotic cells were detected using the Guava Nexin® Annexin V Assay and quantitated on a Guava EasyCyte® microcapillary flow cytometer. Approximately 20,000 cells in complete medium were added to 96-well plates. Assay reagents were added to the wells and incubated for 20 min at room temperature in the dark. Plates were loaded onto the flow cytometry system and 2,000 apoptosis events per well were acquired.

Immunohistochemistry (IHC)

Dissected mammary tumors were fixed in 10% formalin, embedded in paraffin, cut into 5 μm sections and adhered to glass slides. IHC was carried out using the DAKO LSAB2 System-HRP (DAKO) following manufacturer's instructions.

References

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