**Supplemental Materials and Methods**

**Pharmacokinetic study**

Nude mice were treated with a single dose of talazoparib at 0.33 mg/kg or 0.5 mg/kg. Brain tissues and blood samples were harvested at 0, 1, 2, 4, and 8 hours (n=3 mice per time point). Tissues were homogenized using Omni BEAD Ruptor 24 coupled with OmniBR CRYO, and talazoparib concentrations were determined by liquid chromatography-tandem mass spectrometry.

**In vivo Talazoparib Toxicity evaluation**

Mice without tumors were treated with or without talazoparib at 0.33mg/kg (n=3 per group), BID for 6 weeks. Body weight were measured every 2 weeks. At week 6, Blood samples were collected from facial vein and sent to Department of Veterinary Medicine & Surgery Section of Veterinary Laboratory Medicine at MD Anderson for routine hematology assay. For Blood sampling, facial vein sampling without anesthesia was performed with a 3 mm Goldenrod animal lancet (Braintree Scientific, Braintree MA, USA). Blood was collected into 100 μl EDTA microvette tubes (Sarstedt, Numbrecht Germany) and gently mixed to avoid platelet activation. Twelve microliters of whole blood were then transferred to a microcentrifuge tube containing 108 μl CellPak (Sysmex America, Lincolnshire IL, USA), as recommended by the manufacturer.

**Immunoblotting analysis**

Cells were harvested in lysis solution; the extracted proteins were subjected to immunoblotting as described previously (1), using the corresponding antibodies (see the table below for antibody information and dilution).

**Antibodies information**

|  |  |  |  |
| --- | --- | --- | --- |
| Antibodies | Source | cat# | dilution |
| EGF Receptor (D38B1) XP® Rabbit mAb | Cell Signaling | #4267 | 1:5000 for WB |
| Phospho-EGF Receptor (Tyr1068) Antibody | Cell Signaling | #2234 | 1:500 for WB |
| Phospho-Akt (Thr308) (D25E6) XP® Rabbit mAb | Cell Signaling | #13038 | 1:5000 for WB |
| PARP Antibody | Cell Signaling | #9542 | 1:5000 for WB |
| β-actin antibody | sigma | #A2066 | 1:20000 for WB |
| Stat3 antibody | Cell Signaling | #9132 | 1:1000 for WB |
| Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb | Cell Signaling | #9145 | 1:1000 for WB |
| Histone H3 Antibody | Cell Signaling | #9715 | 1:5000 for WB |
| Anti-PAR Polyclonal Antibody (rabbit) | trevigen | 4336-BPC-100 | 1:5000 for WB |
| Lamin B2 (E1S1Q) Rabbit mAb | Cell Signaling | #13823 | 1:2000 for WB |
| 53BP1 antibody | Santa Cruz | Sc-10911 | 1:200 for IF |
| Anti-gamma H2A.X (phospho S139) antibody | Abcam | ab2893 | 1:1000 for IF |
| Cleaved Caspase-3(Asp175) antibody | Cell Signaling | #9661 | 1:500 for WB |
| Cleaved PARP antibody | Cell Signaling | #5625 | 1:1000 for WB |

**Immunohistochemical staining**

Sections (5-μm thick) of formalin-fixed, paraffin-embedded whole brains from animals were stained with anti-Cleaved-Caspase 3 and anti-γH2AX (Cell Signaling Technology, Boston, MA). The sections were visualized using a diaminobenzidine substrate kit, and the slides were examined under a bright field microscope.

**TUNEL assay**

TUNEL assay was performed using *In Situ* cell death detection Kit (Roche Applied Science, Indianapolis, IN) according the manufacturer’s instruction. Briefly, tissue sections were fixed with 4% paraformaldehyde in PBS for 1 hour at room temperature and permeablized with 0.1% Triton X-100 in 0.1% sodium citrate for 2 minutes on ice. Tissue sections were labeled with TUNEL reaction mixture at 37°C for 1 hour and counterstained with Vecta shield sealant containing 4′,6-diamidino-2-phenylindole (DAPI) (Vector laboratories, California, USA).

**Flow cytometric analysis of apoptosis**

Cell apoptosis was detected using Annexin-V-fluos staining kit (Roche) according the manufacturer’s instruction. Samples were measured (10,000 events collected from each) in a BD Pharmingen FACScan (BD Pharmingen).

**Assay for 8-OHdG**

The level of 8-OHdG in extracted DNA was determined using the OxiSelect Oxidative DNA Damage ELISA Kit (Cell Biolabs, Inc.). Briefly, 5μg DNA was converted to single-stranded DNA by incubating the sample at 95°C for 5 min and rapidly chilling on ice. The samples were then digested to nucleosides by incubating the denatured DNA with 10 units of nuclease P1 for 2 h at 37°C in 20 mM sodium acetate, pH 5.2, treated with 10 units of alkaline phosphatase for 1 h at 37°C in 100 mM Tris, pH 7.5. The reaction mixture was centrifuged for 5 min at 6,000 g, and the supernatant was used for the 8-OHdG enzyme-linked immunosorbent assay (ELISA).

**Plasmids, transfection and CRISPR**

Retrovirus based EGFR wild-type (EGFR-wt) and EGFR kinase-inactive mutant (EGFR-KI) were generous gifts from Dr. Oliver Bogler (MD Anderson) (2). Retroviral particles expressing EGFR-wt or EGFR-KI mutant were produced in HEK293FT cells with the mixed set of packing plasmids, and the viruses were concentrated and tittered as previously described (3). Cells were infected with retrovirus for 2 days and selected with zeocin at 250 mg/mL.

pGIPZ-mediated expression of shRNAs (clone IDs: V3LHS\_361965 and V3LHS\_361962) targeting EGFR were purchased from GE Healthcare Dharmacon (Pittsburgh, PA). Lentiviral particles expressing targeting or control scramble shRNA (SCR) were produced in HEK293FT cells with the mixed set of packing plasmids, and the viruses were concentrated and titered as previously described (3). Cells were infected with lentivirus for 2 days and selected with 1 μg/ml puromycin.

For the CRISPR/Cas9n-mediated knock-out of EGFR in GSC262 cells, EGFR double nickase plasmid (sc-400015-NIC, Santa Cruz, Dallas, Texas) and control double nickase plasmid (sc-437281, Santa Cruz) were transfected into GSC262 cells with Lipofectamine 2000 (Invitrogen, Grand Island, NY) for 48 hours. GSC262 cells with green fluorescent protein were sorted by flow cytometry and plated into 96-well plates to form single colonies. A reverse transcription-polymerase chain reaction was performed, followed by sequencing to confirm complete allelic knock-out. The EGFR Double Nickase Plasmid consists of a pair of plasmids, each encoding a D10A mutated Cas9 nuclease and a unique, target-specific 20 nt guide RNA (gRNA). Each pair of gRNA sequences are offset by approximately 20 bp to allow for gene knockout with greater specificity. The target sequences for the two sgRNAs are “TGAGCTTGTTACTCGTGCCT” and “ATCATTTTCTCAGCCTCCAG”, respectively. Single clones were screened for EGFR expression by western blot and sequencing.

1. Wu S, Wang S, Zheng S, Verhaak R, Koul D, and Yung WK. MSK1-Mediated beta-Catenin Phosphorylation Confers Resistance to PI3K/mTOR Inhibitors in Glioblastoma. *Mol Cancer Ther.* 2016;15(7):1656-68.

2. Chumbalkar V, Latha K, Hwang Y, Maywald R, Hawley L, Sawaya R, et al. Analysis of phosphotyrosine signaling in glioblastoma identifies STAT5 as a novel downstream target of DeltaEGFR. *J Proteome Res.* 2011;10(3):1343-52.

3. Saito N, Fu J, Zheng S, Yao J, Wang S, Liu DD, et al. A high Notch pathway activation predicts response to gamma secretase inhibitors in proneural subtype of glioma tumor initiating cells. *Stem Cells.*