Supplementary information, Data S1

Materials and Methods

GENOMIC DNA EXTRACTION

Fresh tumor tissues and peripheral blood samples were collected from each patient during surgery from the department of neurosurgery at Huashan Hospital affiliated to Shanghai Medical College, Fudan University. None of the patients received radiotherapy prior to surgery. DNA was extracted from fresh tumor tissues or peripheral blood lymphocytes using the AllPrep DNA/RNA Mini Kit (Qiagen) according to the manufacturer's instructions. For formalin-fixed, paraffin-embedded samples, DNA was obtained using the QIAamp DNA FFPE Tissue Kit (Qiagen). DNA concentration and purity were quantified using the Qubit dsDNA HS Assay kit with the Qubit 2.0 Fluorometer (Life Technologies) before being constructed into sequencing libraries. Mass ARRAY (Sequenom) spectrometric genotyping with well-established 48 SNPs panels was used to double-confirm the identity of tumor and normal pairs, which were derived from the same patient.

LIBRARY PREPARATION

The qualified genomic DNA was randomly fragmented by an ultrasonicator Covaris E-220 (Covaris). DNA fragments 200-300bp in size were purified using Agencourt AMPure XP beads (Beckman), and then the adaptors with sample-specific barcodes were ligated to the resulting fragments at both ends. The adapter-ligated products 200-300bp in size were further purified using Agencourt AMPure XP beads and amplified by ligation-mediated polymerase chain reaction (LM-PCR). The resulting products were combined into pools of six for solution phase hybridization using the Illumina TruSeq™ (Illumina) Exome Enrichment Kit to obtain exome-enriched DNA

fragments. Captured LM-PCR products were subjected to an Agilent 2100 Bioanalyzer to estimate the magnitude of enrichment. Each library was then loaded on the HiSeq 2500 platform for sequencing.

VARIANT DETECTION

Raw sequencing data were first checked by FastQC v0.11.2 (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc) for quality control and then trimmed by Trimmomatic v0.30 to filter low quality reads [1]. Clean reads that passed quality control were aligned to reference human genome sequences (UCSC, hg19 build) using the Burrows-Wheeler Aligner with default parameters (BWA v0.7.7). Duplication was removed from aligned outputs by the Picard v1.110 MarkDuplicate subroutine (http://picard.sourceforge.net). Local realignment and base quality score recalibration steps were performed with the Genome Analysis Toolkit (GATK) v2.8-1 subroutine Indel Realignment and BaseRecalibrator[2]. Somatic single-nucleotide variants (SNVs) were detected by MuTect v1.1.5 with a contaminant fraction of 10% and a minimum Base Quality score of 20[3]. Somatic indels were identified by VarScan v2.3.6[4] with a strand filter, a minimum Base Quality score of 20 and tumor purity of 90%, and copy number variants (CNVs) were also identified by this software with default parameters. The standards to reduce false positives were as follows: (1) a minimum depth of 10x in both tumors and normal pairs; (2) read depths of variant alleles in tumors should be more than 4x; (3) allelic fractions in tumors should be more than 20%. All of the above standards were written in Perl scripts. Filtered variants were manually checked using a visualization tool Integrative Genomics Viewer (IGV, Broad Institute, Cambridge, MA, USA). Finally, all high quality somatic variants were annotated with RefSeq genes, dbSNP v138, 1000 Genomes Project, as well as the COSMIC database v68 by the Annovar software[5] to predict potential functional changes in encoded proteins (synonymous missense, nonsense, splicing, and so on)

and to search for known mutations in annotated databases.

SANGER SEQUENCING VALIDATION

Point mutations and indels were validated by PCR amplification and Sanger sequencing. Oligonucleotide PCR primers were designed with the Primer3 program (http://frodo.wi.mit.edu/primer3/) using GRCh37/hg19 as the reference sequence. For PCR amplification of the *USP8* mutagenesis, the following Oligonucleotide primers pairs were used: Forward 5'-ATTCACCCACCAACACTGTTCATA-3', Reverse 5'-TTGTTTTCCCGATTAACTGTTGGA-3'.

PCR was performed on a Dual 384-well GeneAmp PCR System9700 (Applied Biosystems), 20 ng template DNA from each sample was used per reaction. The DNA fragments were amplified with the 2X Taq PCR Master Mix (Lifefeng Biotech). The sequencing reactions were performed utilizing the Big Dye Terminator v.3.1 kit (Applied Biosystems). The products were sequenced by the 3730xl DNA Analyzer (Applied Biosystems).All sequences were analyzed by the Chromas Software (Technelysium). If the mutations were successfully confirmed in the tumors but not identified in the matched normal blood DNA samples in this step, then the somatic mutation was considered to be successfully validated.

IMMUNOCHEMISTRY

5 μm tissue sections were prepared, cleaned in xylene and hydrated through a descending alcohol series to distilled water. After antigen retrieval, endogenous peroxidase (HRP) activity was blocked by treating the sections with 3% hydrogen peroxide in methanol for 20 min. Tissue sections were then incubated with anti-USP8 (1:500, Abcam, ab170025), anti-EGFR (1:100, Abcam, ab52894), anti-C-Met (1:300, Cell signaling technology, cat#8198) or anti-ErbB3 antibodies (1:200, Cell signaling

technology, cat#12708). After washing, the sections were stained with the ABC staining kit (Vector Laboratories) according to the manufacturer's protocol, and HRP activity was detected using the DAB kit (Vector Laboratories). Finally, the sections were counterstained with hematoxylin.

IMMUNOBLOT ANALYSIS

Cells and tumor tissues were lysed in buffer (25 mM Tris•HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, protease inhibitor cocktail, 50 mM NaF and 2 mM Na_3VO_4) and subjected to SDS-PAGE, followed by electrotransfer to nitrocellulose or PVDF membranes. The membranes were incubated with primary antibody overnight and secondary antibody for 1 h at room temperature. Proteins were detected using an enhanced chemiluminescence (ECL) kit from PerkinElmer Life Sciences, Inc (Woodbridge, Canada).

IMMUNOPRECIPITATION

HeLa cells transfected with the indicated expression vectors were lysed in buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, protease inhibitor cocktail, 50 mM NaF, 2 mM Na_3VO_4 and 1 μM MG132 (Sigma)). After centrifugation at 12,000×g for 15 min, the supernatant was incubated with anti-EGFR antibody at 4 °C overnight. After incubation, Protein A beads (Roche) were added to capture the EGFR-associated protein complex. After washing, the immunoprecipitation complex was subjected to immunoblot analysis for ubiquitin and

EGFR expression.

CELL CULTURE, LENTIVIRUS EXPRESSION VECTOR, TRANSFECTION, VIRUS PRODUCTION AND INFECTION

Cell lines HeLa and 293T were maintained in medium containing 90% DMEM and 10% fetal calf serum (FCS) and supplemented with antibodies. shRNA against human USP8 was cloned into PLKO.1 lentivivrus vector (Sigma). The sequence of indicated shRNA is listed in **Supplementary information, Table S4**. Transfection was performed using Lipofectamine® 2000 Reagent (Invitrogen) according to manufacture's manual. Viral production and infection were followed according to the standard protocol. Three days after infection, puromycin-resistant cells were selected by adding 2 mg/ml puromycin (Invitrogen) for two days.

CULTURE OF PRIMARY TUMOR CELLS DERIVED FROM HUMAN ACTH-SECRETING PAS AND RIA

Human ACTH-secreting PAs (from the Department of Neurosurgery at HuaShan hospital, Shanghai Pituitary Tumor Center) were obtained at the time of surgery and transferred in ice 0.5% FBS-containing DMEM. After wash, tumor tissue were minced into 1-2 mm pieces, and digested with DMEM containing 0.3% collagenase (Sigma) and 0.15%hyaluronidase for 30 minutes at 37 °C. The mixture was filtered with cell strainer to remove undigested tissues and centrifuged at 1300 rpm for 5 minutes. The cell pellet was suspended in 10% FBS-containing DMEM and plated in 24-well plates.

After 24-hour culture, tumor cells were incubated with Gefitinib (1μM) for 48 hours before analysis for ACTH secretion. Otherwise, tumor cells were infected with Lentivirus for three days and further selected with 2 mg/ml puromycin (Invitrogen) for two days. Tumor cells were centrifuged for 5 minutes and suspended in medium. Live cells were calculated and replated in 24-well plates. The supernatant was collected for analysis for ACTH secretion after 24-hour culture. ACTH levels were measured using IMMULITE 1000 Immunoassay system (SIEMENS) according to the manufacture's protocol.

REVERSE TRANSCRIPTION QUANTITATIVE POLYMERASE CHAIN REACTION (RT-QPCR) ANALYSIS

Total RNA was extracted with TRIzol reagent (Invitrogen) from freshly frozen tumor tissues and cDNA was synthesized using Superscript reverse transcriptase and random primers (Invitrogen). QPCR was performed using Power SYBR Green PCR master mix (Applied Biosystems) to examine the mRNA abundance. The primers are listed in **Supplementary information, Table S4.**

GST PULL-DOWN ASSAY

GST-14-3-3ε fusion protein was expressed in Escherichia coli BL21 (DE3) and affinity-purified using Glutathione-Sepharose 4B beads (GE Healthcare). Purified GST-14-3-3ε fusion protein was immobilized on Glutathione-Sepharose beads and incubated with 293T cell lysates for 16 h at 4°C in the following buffer: 25 mM Tris-HCl,

pH 7.4, 100 mM NaCl, 0.5% Triton-X-100, 10% glycerol, protease inhibitor cocktail, 50 mM NaF and 2 mM Na_3VO_4). The beads were washed, and bound proteins were eluted with boiling SDS-PAGE sample buffer and detected by immunoblotting.

REFERENCE RANGES FOR ENDOCRINE PARAMETERS

Our study used the normal range for plasma ACTH (0-46 pg/ml), urinary free cortisol (30.15-129.13 μg/24 hours) and awake midnight serum cortisol (<7.5 μg/dl). Serum cortisol less than 5 μg/dl after the administration of 1 mg of dexamethasone is considered suppressive, while more than a 50% decrease of urinary free cortisol in the classic high-dose dexamethasone suppression test is considered suppressive.

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