Supplementary Methods

Whole exome and targeted massively parallel sequencing analysis

Sequencing reads derived from whole exome and targeted massively parallel sequencing analysis were aligned to the human reference genome GRCh37 using the Burrows-Wheeler Aligner (1). Local realignment, duplicate removal and quality score recalibration were performed using Genome Analysis Toolkit (GATK) (2). Somatic SNVs were identified using MuTect (3); small insertions and deletions (indels) were identified using Strelka (4) and VarScan 2 (5), and further curated by manual inspection. SNVs and indels with mutant allelic fraction of <1% and/or supported by <5 reads were disregarded (6,7). Variants found with >5% global minor allele frequency in dbSNP (Build 137) or that were covered by <10 reads in the tumor or <5 reads in the germline were disregarded. Variants for which the tumor variant allele fraction was <5 times than that of the normal variant allele fraction were disregarded (6,7). For SPCRP5 and SPCRP11 no matched normal tissue was available, and somatic SNVs and indels were detected using a pooled FFPE normal; mutation calling was performed as described above, and mutations found in >1% of the population in the ExAC dataset were disregarded.

2-hydroxyglutarate (2HG) assay of cell lines

Confluent cells ($MCF10A^{P}$ and $MCF10A^{H1047R}$, expressing vector control, *IDH2* wild-type or *IDH2* R172S plasmids) were collected from 6-well plates, dissolved in 220 µl cell lysis buffer (Cell signaling) and freeze-thawed three times, as previously reported (8). Cell debris samples were removed by centrifugation for 5 min at 13,000 g. Protein concentrations were determined by Qubit Assay Kit (Thermo Scientific). 100 µl lysate was deproteinized using a deproteinization kit (Biovision) following the manufacturer's instructions. For detection of 2HG in conditioned media, the cell culture supernatant was deproteinized as described above and spun down for 2 min at 13,000g. From each supernatant

(lysates and conditioned media), 96 µl were transferred to a new tube and 4 µl of neutralization solution was added. After incubation on ice for 5 min, supernatants were collected after centrifugation for 5 min at 13,000 g and stored at -20°C. This procedure was performed three times. Standard for 2HG detection in conditioned media was prepared with concentrations of 0.5, 1, 2.5, 5, 7.5, 10, 25 and 50 µM 2HG in corresponding fresh medium. Standard for 2HG detection in cell lysates was prepared with concentrations of 0.5, 1, 2.5, 5, 7.5, 10, 25 and 50 µM 2HG in distilled H_2O. All standards were prepared with the identical procedure of the corresponding samples. The total reaction volume was 100 µl. The assay solution contained 100 mM HEPES pH 8.0, 100 µM NAD^+ (Applichem), 0.1 µg D-2-Hydroxyglutaratedehydrogenase (HGDH), 5 µM resazurin (Applichem) and 0.01 U/ml diaphorase (0.01 U/ml, MP Biomedical). HGDH was a kind gift from Stefan Pusch (German Cancer Research Center). Just before use, 75 µl of assay solution was added to 25 µl sample volume and incubated at RT in the dark for 30 min in black 96-well plates (Thermo Scientific). Fluorometric detection was carried out in triplicate with 25 µl deproteinized sample analyzed in each reaction with excitation at 540 nm and emission of 610 nm (Victor X4 Multimode Plate Reader).

Methylation profiling

Methylation profiling was performed using the Infinium MethylationEPIC Kit (Illumina). DNA (300ng) from five *IDH2*-mutant SPCRPs (cases 3, 4, 5, 7 and 9), one *TET2*-mutant SPCRP (case 11) and two *IDH2*/*TET2*-wild-type invasive ductal carcinomas of no special type (IDCs; one ER-negative/HER2-negative, one ER-positive/HER2-negative) was bisulfite-converted using the EZ-96 DNA Methylation Kit (Zymo Research), and restored using the Illumina Infinium HD FFPE DNA Restore Kit. The DNA was then subjected to whole-genome amplification, and the amplified DNA enzymatically fragmented using end-point fragmentation. DNA precipitation was performed in isopropanol, followed by re-suspension in Illumina RA1 buffer. The resuspended DNA was denatured at 95^0C, and the resulting

single-stranded DNA was dispensed directly onto the Illumina BeadChip Array for hybridization (overnight at 48^0C). After hybridization, the BeadChips were washed with Illumina PB1 buffer to remove any unhybridized and non-specifically hybridized DNA. Using a Tecan Te-Flow, labeled nucleotides were dispensed over the BeadChips in Flow-Through Chambers to extend the primers hybridized to the DNA. The primers were then fluorescently stained, and the BeadChips coated in Illumina XC4 buffer to protect the fluorescence before scanning. The BeadChips were scanned, and the raw data files containing the fluorescence intensity data for each probe generated. Data quality control and analysis were performed using RnBeads (9). Hierarchical clustering of all genes was performed using complete linkage and Euclidean distance. Differential methylation analysis between SPCRPs and IDCs was performed using an FDR adjusted p-value <0.05.

Supplementary References

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