SUPPLEMENTARY MATERIAL AND METHODS

Gastric cancer cell isolation

Approximately 1 cm^3 of tumour was cut into small pieces and washed with basal Advanced Dulbecco`s modified Eagle medium (DMEM)/F12 (Gibco) supplemented with Primocin 1x (Invitrogen), Glutamax 1x (Thermo Fisher) and HEPES 10 mM (Thermo Fisher) until the supernatant was clear. The tissue was digested at 37 °C using Dispase II 1 mg/ml (Roche) and Collagenase XI 0.1 mg/ml (Sigma-Aldrich). Regular gentle inversion every 5 min and frequent observation of tumour disaggregation was performed until small tumour patches became visible. Time to disaggregation varied greatly between individual cancers. On average 150 tumour patches were picked under a stereomicroscope. Tumour patches were centrifuged (5 min, 200 g), re-suspended in 20 µl Matrigel (Corning) and overlaid with human stomach medium supplemented with the ROCK Inhibitor Y-27632 10 µM (Sigma-Aldrich).

Human organoid culture and treatment

The following compounds were used for organoid cultivation: Wnt3A 50 %, Rspondin 10 %, Noggin 10 % (conditioned medium); B27 1x (Invitrogen); Nicotinamide 10 mM (Sigma-Aldrich); N2 1x (Invitrogen); N-acetyl-L-cysteine 1 mM (Sigma-Aldrich); hFGF10 200 ng/ml human, 100 ng/ml mouse (Preprotech); mEGF 50 ng/ml (Invitrogen); Gastrin 1 nM human, 10 nM mouse (Sigma-Aldrich); A83-01 2 µM (Tocris Bioscience); Cell Recovery solution (Corning).
Treatment with chemotherapeutics was performed 24 h after seeding using 5-FU (0.1, 1.0, 10.0 µM), oxaliplatin (1.0, 1.5, 2.5 µM), irinotecan (1.0, 2.0, 4.0 µM), epirubicin (1.0, 1.5, 2.0 µM) and docetaxel (0.01, 0.1, 0.3 µM).[1–5] The drugs were prediluted in human gastric cancer medium. Initial tests were performed using concentrations selected from the literature, but adaptation to higher concentrations was necessary for each drug to cover a broad response spectrum during organoid treatments. Selected cancers were treated with trastuzumab (Herceptin, Roche), palbociclib (#S1116, Selleckchem) and imatinib (#ST1571, Selleckchem). Trastuzumab treatment at concentrations of 0.01 and 0.1 µM was also performed in combination with different 5-FU concentrations (0.1, 1.0, 10.0 µM) and analysed after 72 h.

Mouse organoid culture and adenoviral infection

Mouse gastric organoids were cultured as previously described. [6] Organoids were derived from two mouse models: the first model (*Kras*$^{G12D/+}$;*Tp53*$^{R172H/+}$; CIN model) contains an inducible allele of *Kras*^{G12D} (Kras^{tm4Tyj}) and an inducible allele of *Tp53*^{R172H} (Tp53^{tm2Tyj}).[7,8] The second model (*Apc*$^{fl/fl}$;*Cdh1*$^{fl/fl}$; GS-WNT model) contains a floxed *Cdh1* allele (Cdh1^{tm2Kem}) and a floxed *Apc* allele (Apc^{tm2Rak}).[9,10] To induce the specific mutations, established gastric organoids were infected with an Adeno-CMV-Cre-GFP adenovirus (Gene Transfer Vector

Core facility, Boston, USA) with a final virus titer of $12*10^6$. Organoids were incubated with virus for 1 h at 37 °C after mechanical dissociation and spin infection for 5 min and 600 g. Successful virus infection was observed 24 h post infection via a positive GFP fluorescence signal. Organoids with an *Apc* mutation were selected by growing in Wnt and Rspondin free medium. Organoids carrying a *Kras* mutation were selected in medium without Egf. Selection started 48-72 h post infection. Successful recombination and selection was confirmed by genotyping (Suppl. Figure 4B). Wnt signalling inhibition was achieved by treating organoids with an *Apc* mutation with calphostin C (#1626, Tocris).

Organoid transplantation

Gastric cancer organoids were s.c. injected into flanks of NMRI Foxn1$^{nu/nu}$ mice. For each organoid line four mice were used, each receiving into one flank 10 wells of organoids of a 48 well plate in 50 µl Matrigel. Mouse experiments were approved by the local animal welfare commission (#DD24-9168.11/1/386); TVV 52/2012).

Classical 2D gastric cancer cell line cultivation

Gastric cancer cell lines AGS (CRL-1739™), KatoIII (HTB-103™), Snu1 (CRL-5971™) and Snu5 (CRL-5973™) were obtained from ATCC and cultured as described in the corresponding datasheet. Chemotherapy treatment was performed with 5-FU (0.001, 0.01, 0.05, 0.1, 0.15, 0.2, 2.0, 5.0, 10.0 µM), oxaliplatin (0.01, 0.025, 0.05, 0.075, 0.1, 0.5, 1.0, 1.5 µM), irinotecan (0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 4.0, 8.0 µM), epirubicin (0.05, 0.1, 0.25, 0.5, 1.0, 1.25, 1.50, 2.0 µM) and docetaxel (0.0025, 0.005, 0.01, 0.025, 0.05, 0.1, 0.5, 1.0 µM). Predilutions were made in corresponding medium. Response was analysed by plating 10^3 cells per well in a 96 well plate. 48 h after plating treatment was started and cells analysed after 24 h or 72 h (5-FU) using the Presto Blue Assay measuring fluorescence (Varioscan Lux).

Viability Assay

Cell viability was analysed using the Presto Blue Cell Viability Reagent (Invitrogen). Organoids were plated in 96 well plates with 50 µl Matrigel and covered with 100 µl human stomach medium for 24 h. For the chemotherapeutic studies using 5-FU, oxaliplatin, irinotecan, epirubicin and docetaxel, organoids were overlaid with medium for 24-72 h. For targeted treatments organoids were incubated with trastuzumab (0.1 µM) or imatinib (100, 250 and 500 µM) and viability analysed after 72 h or 24 h incubation, respectively. Presto Blue reagent (final 1x) was added, organoids incubated for 3 h at 37 °C and absorbance (Tecan Reader, Genio) or fluorescence (Varioscan Lux, Thermo Scientific) measured.

Apoptosis assay

Organoids were treated with 1 µM 5-FU for 72 h, isolated from Matrigel and incubated with Annexin V (#556419, BD) and PI (Sigma). Samples were analysed concerning necrotic and apoptotic cells using flow cytometry (LSRII, BD).

EdU proliferation assay

Organoids were treated with 5 µM palbociclib for 24 h. For EdU assay the organoids were isolated using TrypLE (Gibco) and stained using the Click-iT EdU Flow Cytometry Assay (Invitrogen). Samples were analysed with a LSRII (BD).

IHC and imaging

Organoids were fixed with 4 % PFA, after dehydration embedded in paraffin and sectioned into 2.5 µM sections. Immunohistochemistry of human organoid and tissue sections for PAS, CEA, CK7, Cadh17 and Her2 was performed according to standard procedures. Mouse slides were stained as described earlier using the following antibodies: β-catenin E247 (#ab32572, Abcam) and TP53 (CM5, Leica)[11]. Xenograft slides were stained with the human specific anti-nucleoli antibody (NM95, Abcam #190710). The Signal Stain Detection Boost IHC/HRP rabbit (#8144S, CST) or mouse (#8125S, CST) was used for detection. Imaging was performed using an EVOS FL Auto (Life Technologies).

Western Blot

For protein extraction organoids were lysed in 50-100 µl RIPA buffer and 20 µg protein was used for SDS page. Antibody incubation of blots was performed according to standard protocols. The following antibodies were used: phospho-c-Kit Tyr719 (#3391), Erk1/2 (#9102), phospho-Erk1/2 Thr202/Tyr204 (#9101), GAPDH (#2118), anti-mouse IgG HRP linked secondary antibody (#7076S) and anti-rabbit IgG HRP linked secondary antibody (#7074S; all from Cell Signalling Technology).

Genotyping via PCR

DNA was analysed as described in DNA sequencing. Standard PCR reactions were performed with Hot Start Go Taq polymerase (Promega) using the following primers:

Kras^{G12D} (Kras G12D_3: CTAGCCACCATGGCTTGAGT; Kras G12D_4: ATGTCTTTCCCCA GCACAGT; Kras G12D_5: TCCGAATTCAGTGACTACAGATG)

Tp53^{R172H} (LSL_p53_for: AGCTAGCCACCATGGCTTGAGTAAGTCTGCA; WT_p53_for: CT GTTCGTTCCATTCCGTTT; WT_p53_rev: AGCCACACTGACAATAGGAGGT)

Apc (APC_fwd: GAGAAACCCTGTCTCGAAAAAA; APC_rev: AGTGCTGTTTCTATGAGTCA AC; APC_int14R4: TTGGCAGACTGTGTATATAAGC)

Cdh1 (mCDH1_E10_f: ACTTTGGTGTGGGTCAGGAA; mCDH1_E10_r: GTGTCCCTCCAA ATCCGATAC; mCDH1_I5_f: GCCTGTGACACATGAAGCAT)

Additionally primary tumour DNA of DD109 and DD282 was analysed for selected driver mutations. Standard PCR reactions were performed with Q5® High-Fidelity DNA polymerase (NEB) using the following primers:

KIT^{E142D} (TP53Arg280*_f: CCATTTGGGCCACTAGTCAT; TP53Arg280*_r: CAGTCATGGAA CTGCCACAC)

TP53^{R280*} (TP53Arg280*_f: GAGCTTAGGCTCCAGAAAGGA; TP53Arg280*_r: CCCCAATTG CAGGTAAAACA)

ARID1A^{Q1365*} (ARID1AGln1365*_f: CCAGACTCGGGGATGTATTC; ARID1AGln1365*_r: GG CCAGTCAGGTGAGAGAAG)

MSH6^{R231H} (MSH6Arg231His_f: GGCTCTGATGTGGAATTTAAGC; MSH6Arg231His_r: TCC AATAAGAGCATCCATGTG)

PIK3CA^{R88Q} (PIK3CAArg88Gln_f: AACTATTTAAAGAAGCAAGAAAAT; PIK3CAArg88Gln_r: GGTGTTAAAAATAGTTCCATAGTTCG)

PIK3CA^{C378R} (PIK3CACys378Arg_f: GGTGAAGACTCTACATCAGTATT; PIK3CACys378Arg _r: AAAAATATACTACTTTTATGGATCA)

PIK3CA^{D805N} (PIK3CAAsp805Asn_f: TGAATTGGGAGAACCCAGAC; PIK3CAAsp805Asn_r: CTCACACAGCTAAGCAAATGA)

PCR Product was cleaned-up using PCR clean-up Gel extraction Kit (Macherey-Nagel). DNA as well as above mentioned primers were send to Eurofins Genomics for Sanger sequencing. Sequencing results were matched to the reference sequence using the Sequencing Data Analysis tool in the CLC Main Workbench v.7.7.3 (CLC). Analysis was performed using the default parameters, not including the reference sequence in the contigs with a minimum aligned read length of 5 and a low alignment stringency. Conflicts were resolved with ambiguity nucleotides.

Quantitative RT-PCR

For quantitative RT-PCR RNA was isolated using the NucleoSpin RNA Kit (Macherey-Nagel), transcribed to cDNA with the cDNA Reverse Transcription Kit (Applied Biosystems) and mRNA expression analysed via quantitative RT-PCR using SYBR Green (Applied Biosystems). The following primers were used:

Gapdh (mGAPDH_f: AGCTTGTCATCAACGGGAAG; mGAPDH_r: CGGAGATGATGACCCT TTTG)

Ccnd1 (mCCND1_f: AGACCATTCCCTTGACTGC; mCCND1_r: AAGCAGTTCCATTTGCAG C)

Axin2 (mAxin_f: GGACTGGGGAGCCTAAAGGT; mAxin_r: AAGGAGGGACTCCATCTACG C)

Whole Genome Sequencing

Sequencing data of organoid samples and normal control samples from organoids were mapped against human genome reference sequence (hg19-Ensembl) using CLC Biomedical Genomics Workbench v.3.5 (CLC BMW) with following parameters: match score 1; mismatch cost 2; affine gap cost (Insertion/deletion open cost 6; insertion/deletion extend cost 1); length fraction 0.5; similarity fraction 0.8.[12] Reads that mapped equally at multiple sites were discarded. In a parallel approach mapping was performed using BWA mem v. 0.7.15 using default settings.[13] Variants were detected via low frequency variant detection tool of CLC BMW using default parameters (minimum coverage: 10; minimum count: 2; minimum frequency: 1.0%; relative read direction filter). Variants that occurred with a count of equal 2 or less than 2 in the normal sample were removed (germline variants). Finally, only variants with a minimum frequency of at least 10% and a minimum coverage of at least 10 reads were used for subsequent analysis. Variants were annotated with ClinVar, COSMIC v.78 and ExAC v0.3 variant information. Variant effect on genes was determined via CLC BMW using human Ensembl gene information (hg19). Classification of variants into missense variants and truncation variants (including nonsense variants, frameshift variants and variants potentially causing splice site truncation) were conducted. Potential splice site variants were detected in +/- 2 nucleotides from corresponding exon. Variants were manually evaluated and pathogenic variants were identified. Copy number variations (CNVs) were investigated via CLC BMW using the CNV-detection tool with default parameters in cancer organoid sample mappings using the corresponding normal organoid sample mappings as control. Structural variations (SVs) were detected with CREST v.1.0.1.[14] Potential germline SVs were removed and only manually evaluated cancer SVs were further analysed. All manually confirmed variants were finally visualised using Circos using default parameters with manual editing the layout options.[15] Somatic signatures were determined using the R package SomaticSignatures.[16] Comparison data was obtained from GDC Data portal using the project TCGA-STAD. The MAF originating from SomaticSniper was chosen containing 441 cases. Out of the 411 cases 295 samples with known subtype annotation (obtained from cBioPortal corresponding to published data in the TCGA paper[17]) were used as comparison datasets for subsequent analyses in the SomaticSignatures pipeline. For each subtype (MSI, EBV, GS, CIN) an individual mutation spectrum was calculated. For investigated cancer organoid samples all genome-wide occurring SNVs were used for calculating individual mutations spectrum. Pie charts displaying relative frequency of particular mutation types were generated using R.

RNA-Seq read mapping and differential expression

Reads from Illumina sequencers were converted from bcl to fastq format using bcl2fastq (version v2.17.1.14) allowing for 1 barcode mismatch. Reads were trimmed for quality, sequence adapters and cropped to 75nt using trimmomatic [18] with the following parameters: TruSeq3-PE.fa:2:30:10:2:true LEADING:15 TRAILING:15 SLIDINGWINDOW:4:15 MINLEN:36 CROP:75.[18] Reads were aligned to the Gencode genome (GRCh38.p7 primary assembly) using STAR in a 2-pass mapping mode: first, an index was created using the genome sequence and gene annotation (here, Gencode GRCh38.p7 comprehensive gene annotation), against which all reads are aligned.[19] Second, all detected splice junctions of all samples are merged and used as guide for the second mapping step. The following parameters were used in both steps: --readFilesCommand zcat --alignIntronMax 500000 --alignMatesGapMax 500000 --outSAMtype BAM SortedByCoordinate --outSAMprimaryFlag OneBestScore --outFilterMultimapNmax 100 --outFilterMismatchNmax 2 --alignSJstitchMismatchNmax 5 -1 5 5. For the second step, additional parameters are: --sjdbFileChrStartEnd allSJ.out.tab --limitSjdbInsertNsj 100000000, where allSJ.out.tab denotes the collected splice junctions. Read counts of all annotated genes were extracted from the alignments using featureCounts method of the Rsubread package with the following parameters: annot.ext = gencode.gtf, isGTFAnnotationFile = T, GTF.featureType = "exon", GTF.attrType="gene_id", useMetaFeatures = T, isPairedEnd=T, requireBothEndsMapped=F, allowMultiOverlap=T, countMultiMappingReads=F, fraction=T, where gencode.gtf is the same annotation as passed to STAR.[20] Only protein-coding genes and genes with more than 1 count for all samples were considered. DESeq2 was used to find differentially expressed genes using standard parameters.[21] Only genes with multiple testing adjusted p-values (padj from DESeq2) < 0.05 were kept. A gene set enrichment analysis was done using the R package fgsea [22] and KEGG pathways and Gene Ontology terms.[23] –log10(p-value) * log2 (fold-change) was used as rank function and 100,000 permutations for p-value calculation of pathway enrichments. KEGG pathways were plotted using the R package pathview.[24]

SUPPLEMENTARY REFERENCES

1 Xu ZY, Tang JN, Xie HX, *et al.* 5-Fluorouracil Chemotherapy of Gastric Cancer Generates Residual Cells With Properties of Cancer Stem Cells. *Int J Biol Sci* 2015;**11**:284–94. doi:10.7150/ijbs.10248

2 Alizadeh-Navaei R, Rafiei A, Abedian-Kenari S, *et al.* Effect of first line gastric cancer chemotherapy regime on the AGS cell line-MTT assay results. *Asian Pacific J Cancer Prev* 2016;**17**:131–3. doi:10.7314/APJCP.2016.17.1.131

3 Florou D, Patsis C, Ardavanis A, *et al.* Effect of doxorubicin, oxaliplatin, and methotrexate administration on the transcriptional activity of BCL-2 family gene

members in stomach cancer cells. *Cancer Biol Ther* 2013;**14**:587–96. doi:10.4161/cbt.24591

4 Weinreich J, Archid R, Bajaeifer K, *et al.* Growth and Chemosensitivity of Gastric Adenocarcinoma and Non-Malignant Cell Lines in Response to Novel Anti-Cancer Drug Combinations. *Chemotherapy* 2014;**60**:346–52. doi:10.1159/000438943

5 Yuan T-M, Liang R-Y, Chueh PJ, *et al.* Role of ribophorin II in the response to anticancer drugs in gastric cancer cell lines. *Oncol Lett* 2015;**9**:1861–8. doi:10.3892/ol.2015.2900

6 Stange DE, Koo BK, Huch M, *et al.* XDifferentiated Troy+ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. *Cell* 2013;**155**:357–68. doi:10.1016/j.cell.2013.09.008

7 Olive KP. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. *Cell* 2004;**119**:847–60.http://dx.doi.org/10.1016/j.cell.2004.11.004

8 Jackson EL, Willis N, Mercer K, *et al.* Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. 2001;:3243–8. doi:10.1101/gad.943001.GENES

9 Kuraguchi M, Wang XP, Bronson RT, *et al.* Adenomatous polyposis coli (APC) is required for normal development of skin and thymus. *PLoS Genet* 2006;**2**:1362–74. doi:10.1371/journal.pgen.0020146

10 Boussadia O, Kutsch S, Hierholzer A, *et al.* E-cadherin is a survival factor for the lactating mouse mammary gland. *Mech Dev* 2002;**115**:53–62. doi:10.1016/S0925-4773(02)00090-4

11 Batlle E, Henderson JT, Beghtel H, *et al.* β-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/EphrinB. *Cell* 2002;**111**:251–63. doi:10.1016/S0092-8674(02)01015-2

12 QIAGEN. Biomedical Genomics Workbench. 2017.

13 Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;**25**:1754–60. doi:10.1093/bioinformatics/btp324

14 Wang J, Mullighan CG, Easton J, *et al.* CREST maps somatic structural variation in cancer genomes with base-pair resolution. *Nat Methods* 2011;**8**:652–4. doi:10.1038/nmeth.1628

15 Krzywinski M, Schein J, Birol I, *et al.* Circos: An information aesthetic for comparative genomics. *Genome Res* 2009;**19**:1639–45. doi:10.1101/gr.092759.109

16 Gehring JS, Fischer B, Lawrence M, *et al.* SomaticSignatures: inferring mutational signatures from single-nucleotide variants: Fig. 1. *Bioinformatics* 2015;**30**:btv408. doi:10.1093/bioinformatics/btv408

17 The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014;**513**:202–9. doi:10.1038/nature13480

18 Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;**30**:2114–20. doi:10.1093/bioinformatics/btu170

19 Dobin A, Davis CA, Schlesinger F, *et al.* STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics* 2013;**29**:15–21. doi:10.1093/bioinformatics/bts635

20 Liao Y, Smyth GK, Shi W. The Subread aligner: Fast, accurate and scalable read mapping by seed-and-vote. *Nucleic Acids Res* 2013;**41**. doi:10.1093/nar/gkt214

21 Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;**15**:550. doi:10.1186/s13059-014-0550-8

22 Sergushichev A. An algorithm for fast preranked gene set enrichment analysis using cumulative statistic calculation. *bioRxiv* Published Online First: 2016. doi:10.1101/060012

23 Luo W, Friedman MS, Shedden K, *et al.* GAGE: generally applicable gene set enrichment for pathway analysis. *BMC Bioinformatics* 2009;**10**:161. doi:10.1186/1471-2105-10-161

24 Luo W, Brouwer C. Pathview: An R/Bioconductor package for pathway-based data integration and visualization. *Bioinformatics* 2013;**29**:1830–1. doi:10.1093/bioinformatics/btt285