Supplementary methods and references

Drug repositioning and bioinformatics approach

In brief, a SCLC disease signature was obtained using a previously described method to identify human disease-associated experiments with normal controls in Gene Expression Omnibus (GEO) repository (1). This process identified experiments GSE11969 and GSE1037, which were analyzed using the RankProd (2) method to identify a set of genes differentially expressed across the two experiments. All genes differentially expressed between SCLC affected and control classes having an estimated false discovery rate (FDR) ≤ 5%. The set of differentially expressed genes defined the SCLC signature, and therapeutic activity scores were estimated between the SCLC signatures and 1,300 drug signatures from the Connectivity Map (3, 4). Top scoring drug hits were mapped to DrugBank (5) records by a basic string-matching approach using the drug chemical name to identify drug targets associated with the compounds. Pathway enrichment analysis of targets was performed using the DAVID tool (6) using the set of known drug targets in DrugBank as the background. P-values for the enrichment analysis were adjusted for multiple hypothesis-testing using the Benjamini-Hochberg (BH) method as implemented in DAVID.

Annexin V staining, ROS measurement, and receptor binding assays

Quantification of cell death was performed using AnnexinV-FITC and Propidium Iodide (PI) according to the manufacturer’s instructions (BD Biosciences). FACS data were analyzed using FlowJo software and the percentage of cell death was determined as the sum of AnnexinV positive cells, PI positive cells, and double positive cells. For ROS measurement, we performed FACS analysis of fluorescent DCF (2’,7’-dichlorofluorescein) labeling of SCLC cells stained for 30 minutes in Opti-MEM medium per the manufacturer’s instructions (Invitrogen). Surface
expression of muscarinic receptors was measured by single-point saturation binding assay with [3H]-N-methyl scopolamine (NMS; Perkin Elmer). mSCLC (Kp3) and hSCLC (H69) cells were resuspended in phosphate buffered saline and diluted five-fold into a reaction mix of phosphate buffered saline supplemented with 10nM [3H]-NMS and 0.1% bovine serum albumin. Non-specific binding was measured in the presence of 20μM atropine. To calculate receptor expression non-specific binding was subtracted from total counts, and the resulting counts were compared to radioactivity of a [3H]-NMS standard.

**Liquid chromatography - Mass spectrometry analysis**

SCLC cells were trypsinized and washed twice in PBS before incubation for four hours in PBS at 37°C (4x10⁶ cells in 1ml). The supernatants were filtered through 0.22 µm filters and frozen at -20°C before analysis. Purified epinephrine, norepinephrine, serotonin, and histamine (all purchased from Sigma-Aldrich) were used to prepare calibration curve standards (ten points covering ranges from 0.2 fmol to 5 pmol). Each standard (and each sample for analysis) was spiked with an equal volume of a stable isotope-labeled histamine (d4-histamine) internal standard (200nM in 0.1% formic acid). The samples were analyzed on LC-MS/MS system at the Vincent Coates Foundation Mass Spectrometry Laboratory at Stanford University. Chromatographic separation was achieved on HP1100 HPLC with Eclipse Plus Phenyl-Hexyl column (2.1mm x 150mm, 5µm, Agilent) under a gradient elution. Mobile phase A consisted of water/0.1% formic acid and mobile phase B consisted of acetonitrile/0.1% formic acid. The total run time for each sample analysis was 8 minutes. Analyte detection was obtained using a Quattro Premier triple quadrupole mass spectrometer (Waters) operated under positive electrospray ionization (ESI) using selected reaction monitoring (SRM) mode. Further details of the analysis are available upon request. The low limit of detection (LLOD) was defined as a signal-to-noise
ratio of 3:1, which corresponds to 5 fmol for histamine, 5 fmol for Norepinephrine, 0.5 fmol for epinephrine, and 0.2 fmol for serotonin.

**Microarray analyses**

The normalized human SCLC datasets used in this study were Bhattacharjee (7) and GSE6044 (8) for primary tissue samples, and Wooster (9) for SCLC cell lines. We downloaded the gene expression data sets from the above GEO sets. Each data set was manually curated to select only tissue biopsy samples from patients with SCLC and other types of neuroendocrine tumors. For each study, we used the sample phenotypes as defined by the corresponding original published study. Each oligonucleotide data set was normalized using gcRMA (10). Microarray probes in each data set were mapped to Entrez Gene identifiers (IDs). If a probe matched more than one gene, the expression data for the probe were expanded to add one record for each mapped gene (11). For data sets that did not provide raw data, we used gene expression data as available from the GEO.

The normalized primary mouse SCLC microarrays were described elsewhere (12). The normalized neuroendocrine tumors datasets used were as follows: GSE22396 for Merkel Cell Carcinoma (13), GSE16476 for neuroblastoma (14), GSE2841 for pheochromocytoma (15), and GSE271162 for midgut carcinoid tumors (16). The normalized expression data was extracted for the genes of interest from the above data sets and the data was imported into TMEV (http://www.tm4.org/) to generate the expression heat maps.
References


