Supplemental Methods

Next Generation Sequencing and Bioinformatics:

Comprehensive Genomics: The genomics study uses data from different technologies that are integrated with the purpose of understanding the molecular characteristics of that particular tumor and predicting drug sensitivity. MPseq, RNAseq and WES were performed using Mayo Clinic funds at no cost to the patient.

MPseq: Whole-genome, read-pair next generation sequencing (NGS) acquires the entire DNA sequence of an organism. DNA is fragmented, sequenced from both ends, and mapped to a reference genome. MPseq represents a variation of standard paired-end sequencing, utilizing large genomic inserts, 2-5 kb, which are circularized and fragmented to the standard paired-end protocol length, 200-500 bp. The SV detection pipeline established by Biomarker Discovery (BMD) at Mayo Clinic (http://mayoresearch.mayo.edu/center-for-individualized-medicine/biomarker-discovery-program.asp) includes guidelines for DNA preparation, library preparation, multiplex sequencing and sequencing analysis with BIMA [1] and SVAtools[2,3].

RNAseq: RNA-sequencing libraries was prepared according to the Illumina TruSeq protocol and run on the HiSeq2500platform. All transcriptome analysis was performed in the R/Bioconductor environment (https://www.bioconductor.org/). Paired end sequence fragments were aligned by the tophat aligner using the latest genome reference file (hg38) and the ensemble annotation database. Sorted "sam" files were input to the htseq program to calculate expression levels of genes in each sample. Finally, the ".count" files from the previous step were used by the edgeR program to normalize this sample against a normalized expression matrix for all samples in our database. The mRNA abundance for each interrogated gene was extracted from the normalized RNAseq data and was plotted with respect histograms constructed for all samples.

Exome sequencing (WES) was performed on the same whole genome amplified DNA as utilized for MPseq from each histological component isolated from two cases. WES libraries were sequenced as four libraries per lane on the Illumina HiSeq2500platform (2x101bp). In this study, the Mayo Clinic GenomeGPS 4.0 pipeline was utilized for exome data analysis. It includes three steps: alignment, single nucleotide variant (SNV) and small insertion/deletion (Indel) variant calling, and structural variation annotation. All tools were run under default configuration unless otherwise specified. FASTQ files were aligned to the GRCh38 reference genome using BWA 0.7.10 with the BWA-MEM algorithm for short read alignment [4,5]. Realignment was performed using GATK Version 3.4 [6] and all variants reported. Variant coding regions were functionally annotated using the BioR Version 2.5 annotation engine developed at Mayo Clinic[7].

Immunoblotting: Tissue and cells for protein analysis were prepared as previously described [8]. Protein (25ug) was separated by SDS-PAGE, transferred to Immobilon-P transfer membrane (EMD Millipore, Cat #IPVH00010) and blotted with corresponding antibodies (HER/ErbB family antibody sample kit, Cell Signaling Technology, Cat #8339; GAPDH(G-9), Santa Cruz Biotechnology, Cat #SC-365062).

Three dimensional (3D) microcancer model: Fresh surgical tissue was mechanically and enzymatically dissociated into a single cell suspension using the Miltenyi Biotec Tumor Dissociation Kit (Catalog #130-095-929) and the GentleMACS^{TM} Dissociator (Catalog #130-093-235), according to the manufacturer's instructions. Equal number of cells ($5x10^3$) were added to each well of a 96-well Insphero^{TM} hanging drop plate and incubated at 37^{o}C for 6 days in DME/F-12 media containing 10% heat inactivated horse sera, 5ng/ml insulin, 5ng/ml hydrocortisone, 10ng/ml EGF, and pen/strep. Spheroid cultures representing the cellular diversity of the original tumor (microcancers) were then transferred to the corresponding well of a Corning® 96 Well Clear Round Bottom Ultra Low Attachment 96 well plate and incubated in the presence of indicated concentrations of single agents, combination treatments, or vehicle control at 37^{o}C. Overall cell viability was measured 6 days later using the Cell Titer Glow Luminescent Cell Viability Assay (#G7571). Results were expressed as nM ATP by comparing to a standard curve, transformed into Log_{10}, and plotted in GraphPad PRISM using a four-parameter dose-response curve. IC50s were calculated using GraphPad PRISM.

References

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