Supplementary materials and methods

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Analysis of ATAC-seq

Paired-end sequencing reads were trimmed using Trimmomatic (v0.39) to remove adapters and low quality bases [1]. Trimmed reads were mapped to the human reference genome assembly GRCh37 (hg19) using Bowtie2 (v2.3.5.1) [2], allowing fragments up to 2kb to be aligned. The alignment results were further filtered by SAMtools to remove reads unmapped, not primary alignment, reads failing platform, reads mapped to mitochondria, reads mapped to ENCODE blacklisted genomic regions and multi-mapped reads [3]. PCR duplicates were removed using Picard (v2.26.8, https://github.com/broadinstitute/picard). The high quality alignment bam files were converted to tag bed files and were offset by +4bp for the "+" strand and -5bp for the "-" strand. Peaks were called by MACS2 with the parameter settings "-p 0.01 --extsize 73 --nomodel -B --SPMR". The coverage track bigwig files were generated with bin size 10 and were normalized by reads per genome coverage using deeptools [4]. Significantly differential accessible regions between C4-2R and C4-2 were identified using the R package DiffBind with edgeR statistical test (q-values < 0.05 and |fold changes| > 2) based on consensus peaks occurring at least three samples [5, 6]. GREAT (v4.0.4) was used to perform the gene set enrichment analysis [7]. All codes are available upon reasonable request.

Analysis of RNA-seq

Sequencing reads were trimmed and filtered using Trimmomatic (v0.39) to remove adapters and low quality bases. Trimmed reads were mapped to the human reference genome assembly GRCh37 (hg19) transcripts annotation using RSEM [8]. RSEM results normalization and differential expression analysis were performed using the R package DESeq2 [9]. Significantly changed genes between C4-2R and C4-2 were determined as |fold changes| >= 2 and q-values < 0.05. Transcript per million (TPM) were calculated for each gene [10]. Differentially expressed genes common in RNA-seq and ATAC-seq promoter regions were plotted in the heatmap using standardized $\log_2$ (TPM+1) values, with red depicting genes above the median level and blue depicting genes below the median level. For heatmap of Fig. 8G, the standardized values were applied k-means algorithm to split the patients into high and low subgroups, then rearranged in the descending order. Ingenuity Pathway analysis (IPA) was performed on differentially expressed genes identified by DESeq2 using QIAGEN IPA tookit. Genes with q-values less than 0.05 were used for the input of pathway analysis. A list of statistically significantly enriched canonical pathways was obtained from IPA. Gene set enrichment analyses (GSEA) were performed on default values calculated with DESeq2. MSigDB (v7.4) gene sets and GSEA software (v.4.1.0) were used in the analyses [11, 12]. The phenotype permutation was used with 1000 permutations, and no collapse was applied. Pathways used were denoted with their unique systematic numbers, and some of them were reported previously [13-19]. For correlation analysis, the TCGA dataset (https://www.cancer.gov/tcga, [20]), MSKCC dataset [21], as well as SU2C/PCF dataset [22], were downloaded from cBioportal (https://www.cbioportal.org, [23, 24]). The

Spearman's rank-order correlation coefficient and its associated 95% confidence interval were used to quantify the correlation between gene expressions of interest. All codes are available upon reasonable request.

qPCR

Total RNA was extracted with RNeasy Mini Kit (Qiagen, 74104) and quantified by NanoDrop OneC Microvolume UV-Vis Spectrophotometer (Thermo). 1ug RNA was used for reverse transcription with SuperScript IV First-Strand Synthesis System (Thermo, 18091200). qPCR detections were performed with FastStart Universal SYBR Green Master reagents (Sigma, 4913914001) and QuantStudio 5 Real-Time PCR System (Thermo). All experiments were repeated three times and one was shown.

Combination Index

Calculation of Combination Index (CI) was based on the Loewe model [25], which was described in detail here [26]. To simply the calculation and make it straightforward, we used the 50% CI ($CI_{50\%}$), under which condition 50% inhibition of cell growth was set as the target. The equation to calculate is: $CI_{50\%} = a/A + b/B$, where “A” and “B” are the individual IC_{50} of two drugs, “a” and “b” are the concentration of two drugs used in combination when 50% inhibition is reached. To further simplify our calculation, we assumed “B” was the IC_{50} of NBD or CH, “b” was set as half IC_{50} of NBD or CH, then the derived equation is: $CI_{50\%} = a/A + 0.5$. The last step was to experimentally determine “a” when half IC_{50} of NBD or CH was applied as background in the cell proliferation assay. If

$CI_{50\%}$ = 1, it means additive and the two drugs don't interfere with each other; If $CI_{50\%}$ > 1, it means subadditive or antagonism, which indicates the combination of the two drugs works less efficiently than a single drug; If $CI_{50\%}$ < 1, it means synergistic and that combination of the two drugs works better.

Luciferase assay

AhR NanoLuc luciferase reporter (pNL[NlucP/XRE/Hygro], CS186808) and control firefly luciferase vector (pGL4.54[luc2/TK], E5061) were purchased from Promega. C4-2 and C4-2R cells were co-transfected with two luciferase vectors in a ratio of 1:50 (firefly:NanoLuc). After 24 hours, the assay was performed with Nano-Glo Dual-Luciferase Reporter Assay System (Promega, N1541) according to manufacturer's instructions. The luminescence was quantified by GloMax Discover plate reader.

Flow cytometry analysis of intracellular ROS

After treatment, cells were cultured with medium containing 20μM CM-DCFDA (Thermo, C6827) for 30mins, followed by one wash with cold PBS. After trypsinization, cells were suspended in room temperature PBS and immediately analyzed by Cytoflex LX flow cytometers (Beckman Coulter) using FITC channel (488ex/525em). Results were managed with FlowJo (v.10.8.1) software. All experiments were repeated three times and one was shown.

Xenograft Experiments

The animal experiments was approved by the University of Kentucky Division of Laboratory Animal Resources. The formulations of drugs are indicated below: ENZ was dissolved in 5% benzyl benzoate and 95% peanut oil; DARO was dissolved in PEG400/propylene glycol/5% glucose (50:30:20); NBD was dissolved in 0.5% methyl cellulose (400cps). After male nude mice (Foxn1^{nu}, Jackson Lab Strain #002019) underwent castration surgery, 2 x 10^6 cells were subcutaneously injected. When the average tumor size approached 100mm^3, the mice were randomized into six groups (6 mice each group) followed with oral gavage of drugs. The treatments for the six groups are shown below: Group 1 was treated with solvent mixture of three drugs (1:1:1) every day; Group 2 was treated with 20mg/kg ENZ every day; Group 3 was treated with 20mg/kg NBD every day; Group 4 was treated with 30mg/kg DARO every day; Group 5 was treated with 20mg/kg ENZ and 20mg/kg NBD every day; Group 6 was treated with 30mg/kg DARO and 20mg/kg NBD every day. Measurement of tumor size was done by digital caliper every two days, and tumor volumes were calculated with the formula: V= L x W^2 x 0.52, where V is volume (mm^3), L is length (mm), W is width (mm). The treatment was stopped when the tumor volume reached 1000mm^3, then all mice were euthanized. Harvested tumors were fixed with 10% formalin and subject to paraffin-embedded sections.

Immunofluorescent staining

All procedures were carried out using the M.O.M. Basic Kit (Vector Laboratories, BMK-2202) according to the manufacturer's instructions. Briefly, after deparaffinization, citrate-based antigen retrival (Vector Laboratories, H-3300) and blocking steps, primary antibodies were applied to the slides for 30mins, followed with 10mins application of biotinylated secondary antibodies and 10mins application of DyLight 488 Streptavidin (Vector Laboratories, SA-5488) at 1:200 for 30min. Finally, slides were mounted with VECTASHIELD HardSet containing DAPI (Vector Laboratories, H-1500). Images were captured by Nikon confocal microscope and managed by Fiji software [27].

Hematoxylin and eosin staining.

After deparaffinization and citrate-based antigen retrival, tumor slides were sequentially stained with hematoxylin and eosin. In brief, tumor sections were immersed in the hematoxylin solution for 10 seconds, and washed with tap water until the water was clear. Next, slides were immersed in eosin solution for 30 seconds and washed with tap water until the water was clear. Finally, slides were mounted with Antifade Mounting Medium (Vector Laboratories, H-1000), followed by image acquisition by Nikon confocal microscope and management by Fiji software.

Survival analysis

All patients' survival information were downloaded from cBioportal, except for the data from PNAS 2019 (Alumkal et al., [28]), which is the courtesy of Dr. Joshi Alumkal from

University of Michigan. Kaplan-Meier survival curves and remaining time on treatment curves were generated by GraphPad Prism 8.

Table 1. List of antibodies

Name	Company	Cat. #	Source	Application	ratio
AhR	Cell Signaling	83200	Rb	IB	1:1000
β-actin	Cell Signaling	8457	Rb	IB	1:5000
CAT	Cell Signaling	12980	Rb	IB	1:1000
Cleaved Caspase 3	Cell Signaling	9664	Rb	IF	1:200
Cleaved PARP	Cell Signaling	9546	Ms	IB	1:1000
GAPDH	Cell Signaling	5174	Rb	IB	1:10000
GSTM1	Santa Cruz	sc-517262	Ms	IB	1:500
GSTM2	Santa Cruz	sc-376486	Ms	IB	1:500
GSTP1	Santa Cruz	sc-66000	Ms	IB	1:500
HA-tag	Cell Signaling	3724	Rb	IB	1:1000
p38 MAPK	Cell Signaling	9212	Rb	IB	1:1000
phospho-p38 MAPK	Cell Signaling	9211	Rb	IB	1:1000
Ki-67	Abcam	ab15580	Rb	IF	1:200
SOD1	Cell Signaling	2770	Rb	IB	1:1000
SOD2	Cell Signaling	13141	Rb	IB	1:1000
HRP-linked goat anti-Rabbit IgG	Cell Signaling	7074	Gt	IB	1:3000
HRP-linked goat anti-Mouse IgG	Cell Signaling	7076	Gt	IB	1:3000
biotinylated goat anti-rabbit IgG	Vector Laboratories	BA-1000	Gt	IF	1:200

Table 2. List of primers

Name	Forward	Reverse	Tm	Product Length	Ref
CYP1A1	GGTCAAGGAGCACTACAAAACC	TGGACATTGGCGTTCTCATCC	60	108bp	[29]
CYP1A2	TACTTGGAGGCCTTCATCCTG	TTACGAAGACACAGCATTTCTTGG	59	130bp	[30]
GSTM2	GTATGCAGCTGGCCAAACTC	GAGATGAAGTCCTTCAGGTTTGG	59	235bp	

Table 3. List of chemicals

Name	Company	Cat. #	Solvent
Enzalutamide	Selleckchem	S1250	DMSO
Apalutamide	Selleckchem	S2840	DMSO
Darolutamide	Selleckchem	S7559	DMSO

NBDHEX	MCE	HY-135318	DMSO
CH-223191	Selleckchem	S7711	DMSO
CM-DCFDA	Thermo	C6827	DMSO

Table 4. List of shRNAs

Name	Cat. #	Vector	Resistance	Targeting Region	Usage
shControl	SHC001V	pLKO.1-puro	Puromycin	/	C4-2R
shAhR #1	TRCN0000245285	pLKO.1-puro	Puromycin	CDS	C4-2R
shAhR #2	TRCN0000245287	pLKO.1-puro	Puromycin	3'-UTR	C4-2R
ShGSTM2 #1	TRCN0000151859	pLKO.1-puro	Puromycin	3'-UTR	C4-2R
ShGSTM2 #2	TRCN0000154400	pLKO.1-puro	Puromycin	CDS	C4-2R

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