Supplementary Material

Materials and Methods

Antibodies

The following primary antibodies were used for immunoblotting: Beta-actin [(ACTB), A5441, 1:40,000, (Sigma Aldrich)], SLC2A1 [PB9435, 1:10,000, Booster, (Pleasanton, CA)], ENO1 [ab85086, 1:1,000, Abcam (Cambridge, MA)], SIRT1 [9475, 1:1000, Cell Signaling Technology, (Danvers, MA)], FLG [905801, 1:1,000, BioLegend, (San Diego, CA)], CYP1B1 [1:5000 (Walker et al., 1998)]. The following HRP-linked secondary antibodies were used for immunoblotting: goat anti-rabbit [111035144, 1:10,000, Jackson ImmunoLaboratories Research (West Grove, PA)] and goat anti-mouse (115035003, 1:10,000, Jackson ImmunoLaboratories Research). For ChIP analyses, AHR (BMLSA210, Enzo, Farmingdale, NY), was used.

Protein Quantitation

For immunoblots, protein was quantitated using the Pierce Micro BCA kit (23235, ThermoFisher Scientific). For glucose uptake, ATP, pyruvate, and lactate assays, protein was quantitated using Pierce 660 nm Protein Assay Reagent (22660, ThermoFisher Scientific) containing Ionic Detergent Compatibility Reagent (22663, ThermoFisher Scientific).

Immunoblotting, enhanced chemiluminescence, and densitometry

Following treatment, cells were collected and pellets were lysed in cold whole cell lysis buffer (0.1% SDS, 1% NP40, 5 mM EDTA, 0.5% sodium deoxycholate, 150 mM NaCl, 50 mM Tris-HCl pH 8) supplemented with phenylmethylsulfonyl fluoride and protease inhibitor cocktail (P8340, SigmaMillipore). The samples were spun for 10 minutes at 10,000 x g at 4°C and the supernatant was used for immunoblotting of SLC2A1, ENO1, SIRT1, and CYP1B1. The pellets from the whole cell lysis were boiled for 5 minutes in lysis buffer (62.5 mM Tris-HCl pH 6.8, 2% SDS, 1% 2-beta-mercaptoethanol). The samples were spun for 10 minutes at 10,000 x g at

room temperature and supernatant was used to detect FLG. Proteins were separated by PAGE (10% gel, 150 Volts) and transferred to polyvinylidene fluoride membranes (100 Volts, 90 minute, 4°C). Blocking and antibody incubations were in Tris-Buffered Saline with 0.1% Tween 20 with 5% milk. Following incubation with Clarity Western ECL Substrate (1705061, Bio-Rad) bands were visualized using the ChemiDoc Touch Imaging System (Bio-Rad). Signal density was quantitated using Bio-Rad Laboratories Image Lab (v5.2.1) software (Hercules, CA).

ChIP-Seq Library Preparation

Cross-linked (1% formaldehyde, 15 minutes) NHEKs were sonicated in lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8) using the Covaris S220 (45 min, 147 Watts, 200 cycle/burst, duty factor of 5) in tubes containing an AFA fiber (Covaris, Woburn, MA). The samples were diluted 10-fold in 18.75 mM Tris-HCL, pH 8, 1.25% Triton X-100, 187.5 mM NaCl. The AHR was immunoprecipitated from the lysate using anti-AHR antibody (10 μg). DNA libraries were prepared from 0.9 ng of antibody-enriched chromatin using NEBNext Ultra II DNA Library Preparation Kit (E7645S, NEB). Libraries were indexed using NEBNext Multiplex Oligo (E7335G, NEB). Size selected library fragments were amplified with barcoded primers and sequenced at 1 x 75 base pair read lengths using the Illumina NextSeq 500 platform and Nextseq 500/550 High Output Kit v2 (75 cycle) (Illumina, FC-404-2005). Data processing, quality control and alignment were performed with Illumina BaseSpace to generate BAM files. 20-40 million mapped reads were determined per sample. The data were deposited in NCBI Sequence Read Archive and are accessible through accession SRP126686 (https://www.ncbi.nlm.nih.gov/sra/SRP126686). Analysis was performed using Partek® software (St. Louis, MO). Peak detection used a calculated maximum fragment size of 145 and window size of 100. A Mann-Whitney test for separation of forward and reverse reads was used

to detect peaks with a maximum probability (FDR) of 0.001. Binomial *p*-values of ChIP peak significance were calculated for each peak (FDR of 0.05) (Rozowsky et al., 2009).

ChIP-PCR

Total sheared chromatin (input) and antibody-enriched chromatin was prepared as above. PCR was performed using 1 ng chromatin for input samples and 0.05 ng chromatin for antibody-enriched samples) using HotStart Taq DNA Polymerase with Q-Solution (Qiagen) and the primer pairs listed in Table S1. Signal density was quantitated using Bio-Rad Laboratories Image Lab (v5.2.1) software (Hercules, CA). Quantitation of relative binding of the AHR was determined after normalizing to the corresponding input.

RNA Isolation and Quantitative PCR

For all RNA studies, cells were treated for 24 hours. Total RNA isolation and quantitative PCR (qPCR) were performed as previously described (Sutter et al., 2009). Samples were normalized to values of tubulin, alpha 1C. Primers used in this study are listed in Table S2.

References

Rozowsky, J, Euskirchen, G, Auerbach, RK, Zhang, ZD, Gibson, T, Bjornson, R, et al. PeakSeq enables systematic scoring of ChIP-seq experiments relative to controls. Nat Biotechnol. 2009;27(1):66-75.

Sutter, CH, Yin, H, Li, Y, Mammen, JS, Bodreddigari, S, Stevens, G, et al. EGF receptor signaling blocks aryl hydrocarbon receptor-mediated transcription and cell differentiation in human epidermal keratinocytes. Proc Natl Acad Sci U S A. 2009;106(11):4266-71.

Walker, NJ, Crofts, FG, Li, Y, Lax, SF, Hayes, CL, Strickland, PT, et al. Induction and localization of cytochrome P450 1B1 (CYP1B1) protein in the livers of TCDD-treated

rats: detection using polyclonal antibodies raised to histidine-tagged fusion proteins produced and purified from bacteria. Carcinogenesis. 1998;19(3):395-402.

TABLE S1

Primer Pairs Used in ChIP-PCR

Gene	Forward Primer	Reverse Complement Primer
SLC2A1-1	CCTCACCCTGATTACCAGCA	TCCAGCATAGGCTAGGACCA
SLC2A1-2	CAGAGTCTTTTCCCAGCCCG	CCTGGAGGCTGCCTTTAGTC
SLC2A1-3	ACTTCCCCGTGTTCCATTGT	AGTTCTTCACGAAGGGTGGG
ENO1	CGCCAAAGTTGTCAGCAAGG	AACTCTGGCCCCAGATAGGA

TABLE S2

Primer Pairs Used in qPCR

Gene	Forward Primer	Reverse Complement Primer
SLC2A1	TTGCCCCTTCCCATCTCTTCCTAC	GACTGGCCCTTCCCCTCTCCT
ENO1	CGAGGCCCCCGACCAACACTT	TGGCCACCCCGGAGATGACAC
CYP1A1	GCAGCAGGATAGCCAGGAAGAGAA	CATCCCCCACAGCACAACAAGAGA
CYP1B1	AGCCCAACCTGCCCTATGTCC	CACCGCCTTTTGCCCACTG
TUBA1C	GTTGCCGTCCCTTCGCCTCCTT	CTTGCCAGCACCCGTTTCACT

TABLE S3

Primer Used in ENO1-Promoter Luciferase Assays

Gene	Forward Primer	Reverse Complement Primer
ENO1	GGGGTACCCGCCAAAGTTGTCAGCAAGG	CCCAAGCTTAACTCTGGCCCCAGATAGGAC