SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Chemical Compounds

Chemicals were obtained from the following sources: NVP-BEZ235, LC Laboratories; rapamycin, Cell Signaling. NVP-BEZ235 and rapamycin were dissolved in dimethylformamide (DMF) and ethanol, respectively, at > 500 times the final concentration used in experiments *in vitro*.

Genotyping PCR reactions

For *PB-Cre4*: Primer 1 (5'-TCT GCA CCT TGT CAG TGA GG-3') and primer 2 (5'-GCA AAC GGA CAG AAG CAT TT-3') were used. Thermocycling conditions were 28 cycles of 94^{o}C for 45 sec, 55^{o}C for 30 sec and 72^{o}C for 1 min.

For *p53*$^{Flox/Flox}$: Primer1 (5'-CAC AAA AAC AGG TTA AAC CCA G-3') and primer 2 (5'-AGC ACA TAG GAG GCA GAG AC-3') were used. To detect the deleted allele, D*p53* primer (5'-GAA GAC AGA AAA GGG GAG GG-3') and primer 1 were used. Thermocycling conditions were 35 cycles of 94^{o}C for 1 min, 60^{o}C for 45 sec and 72^{o}C for 30 sec.

For *Pten*$^{Flox/Flox}$: Primer 1 (5'- CTT CGG AGC ATG TCT GGC AAT GC-3') and primer 2 (5'-CTG CAC GAG ACT AGT GAG ACG TGC- 3') were used. To detect the deleted allele, D*Pten* primer (5'-AAG GAAGAG GGT GGG GAT AC-3') and primer 1 were used. Thermocycling conditions were 30 cycles of 94^{o}C for 1 min, 62^{o}C for 45 sec and 72^{o}C for 1 min.

Real-time PCR for mRNA

Total RNA was extracted using RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. cDNA was synthesized from 2 micrograms of total RNA using Superscript III First-Strand Synthesis kit with oligo dT primers (Invitrogen). Quantitative real-time PCR was performed with 7500 Real-Time PCR System (Applied Biosystems) using SYBR Green Real-

Time PCR Master Mix (Applied Biosystems). Amplification cycles were 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec). The qRT-PCR results were normalized to β actin and analyzed as relative RNA levels of the Ct (cycle threshold) value, then converted to fold change. Data represent the mean ± SD of three independent experiments. The mRNA specific primer sequences are provided in Supplemental Table 1.

Real-time PCR for miRNA

Total RNA was isolated using Tri Reagent (Molecular Research Center, Inc.) according to the manufacturer's protocol. cDNA was synthesized from 400 ng of total RNA using miScript Reverse Transcription Kit (Qiagen). Real-time PCR was performed with 7500 Real-Time PCR System (Applied Biosystems) using miScript SYBR Green PCR Kit (Qiagen) according to the manufacturer's protocol. Amplification cycles were 95ºC for 15 min, followed by 45 cycles of 94ºC for 15 sec, 55ºC for 30 sec and 70ºC for 40 sec. The qRT-PCR results were normalized to miR-15a and analyzed as relative miRNA levels of the Ct (cycle threshold) value, then converted to fold change. Data represent the mean ± SD of three independent experiments. The miRNA specific primer sequences are provided in Supplemental Table 1.

Supplemental Table 1 Primers Used For Real-Time PCR

Name		Primer (5'-3')
Human HK2	Forward	5'-AAG GCT TCA AGG CAT CTG-3'
	Reverse	5'-CCA CAG GTC ATC ATA GTT CC-3'
Mouse HK2	Forward	5'-TGA TCG CCT GCT TAT TCA CGG 3'
	Reverse	5'-AAC CGC CTA GAA ATC TCC AGA-3'
Mouse HK1	Reverse	5'-GCACGATGTTCTCTGGGGTG-3'
	Forward	5'-CGTCAAGATGCTGCCAACCT-3'
Human β actin	Forward	5'-CAT GTA CGT TGC TAT CCA GGC-3'
	Reverse	5'-CTC CTT AAT GTC ACG CAC GAT-3'
Mouse β actin	Forward	5'-GGC TGT ATT CCC CTC CAT CG-3'
	Reverse	5'-CCA GTT GGT AAC AAT GCC ATG T-3'
miScript Universal Primer	Reverse	miScript SYBR Green PCR Kit (Qiagen)
miR-143	Forward	5'-TGA GAT GAA GCA CTG TAG CTC-3'

Cell Culture

MEFs of different genotypes were generated and cultured as previously described (Deng et al., 2009). RWPE-1 immortalized non-tumorigenic human prostate cells and human prostate cancer cell lines (PC3, LNCaP and DU145) were obtained from American Type Culture Collection. HCT116 $p53^{+/+}$ and $p53^{-/-}$ isogenic colorectal carcinoma cell lines were generous gifts of Dr. Bert Vogelstein (Johns Hopkins University). RWPE-1 cells were maintained in Keratinocyte Serum Free Medium (K-SFM). PC3 cells were maintained in F-12K supplemented with 10% fetal bovine serum. LNCaP and DU145 cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum. HCT116 cells were maintained in McCoy's 5A medium supplemented with 10% fetal bovine serum. Mouse tumor derived polyclonal cancer cells (UMN-4240P) were grown in DMEM containing 10% FBS at standard tissue culture condition.

Clonogenic Survival and Soft Agar Anchorage-Independent Assays

Cells were plated in triplicate at 2×10^3 cells per 6 cm culture dish. After two weeks of growth, cell colonies were fixed in 10% formalin and stained with crystal violet (0.1% w/v). An anchorage-independent growth assay was performed using soft agar as a supporting matrix. A base layer of 0.6% agarose was placed on 6 cm culture dishes. Cells were seeded at 2×10^4 cells per 6 cm culture dish in 0.3% top agarose containing medium. In cases where cells were transfected with shRNA, cells were placed in puromycin selection for 48 hr before seeding. Medium was changed every 3-4 days. After 21 days, colonies were counted in three random fields with a low power objective under the microscope. Experiments were performed in triplicate and repeated three times.

Glucose Consumption and Lactate Production

Cells were seeded at 4-6 x 10^4 cells per well in a 12-well plate. The glucose and lactate concentrations in the medium during a period of 72 hr were determined using the Glucose Assay Kit (Eton Bioscience) and Lactate Assay Kit II (Biovision), respectively. Glucose consumption was determined by the difference between the original glucose concentration and the measured glucose concentration at 72 hr. As the medium itself does not contain lactate, the measured lactate concentration reflects the lactate production by the cells. The values of glucose consumption and lactate production were converted into actual rate.

Immunoblotting

Cells were lysed with TEB150 buffer (50 mM HEPES pH 7.4, 150 mM NaCl, 2 mM $MgCl_2$, 5 mM EGTA pH 8.0, 1 mM dithiothreitol, 0.5% Triton X–100, 10% glycerol, 1mM Na_3VO_4, 1 μM microcystin–LR and protease/phosphatase inhibitor cocktail). Lysates from mouse prostates were prepared by homogenizing mouse prostates in modified RIPA buffer (20 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 5 mM EDTA and protease/phosphatase inhibitor cocktail). Insoluble material was removed by centrifugation. Proteins were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% non-fat milk in phosphate-buffered saline and then probed with the appropriate primary antibody in 5% non-fat milk overnight at 4ºC [phospho-Akt (Ser473), Akt, 4E-BP1, phospho-4E-BP1 (Thr37/46), Hexokinase II(HK2), Raptor, p53, PTEN, p70 S6 kinase and phospho-p70 S6 kinase from Cell Signaling; and β Tubulin (G-8) from Santa Cruz Biotechnology. Subsequently, membranes were washed in phosphate-buffered saline with 0.3% Tween 20, and incubated with secondary antibody conjugated to horseradish peroxidase. Proteins were visualized by enhanced chemiluminescence.

Immunohistochemistry (IHC)

All immunohistochemical analyses were conducted as previously described (Wang et al., 2009). The following antibodies were used: HK2, cleaved caspase-3, and PTEN from Cell Signaling; p53 (DO-1) from Santa Cruz, Ki-67 from Thermo Scientific; and CK8 (TROMA-I) from Developmental Studies Hybridoma Bank at University of Iowa. Cleaved caspase-3 antibody was used at 1:50 dilution and the other primary antibodies were used at 1:100 dilution.

Tissue Microarrays and Image Analysis

Cylindrical human prostate tissues measuring 1.0 mm diameter were purchased from US Biomax. (Rockville, MD) or obtained from Dr. Shahriar Koochekpour at Roswell Park Cancer Institute (Buffalo, NY). The tissue microarray contained both normal human prostate tissue and human prostate adenocarcinoma with different Gleason score. The expression profile of HK2 was characterized by IHC and histology scoring was carried out using Image-Pro plus (6.3) as previously described (Wang et al., 2009).

Polyribosome Isolation and HK2 mRNA Analysis

Fractions containing polyribosome or postpolyribosome (monosome) were separated using a method modified from previous reports (Mukhopadhyay *et al.*, 2003). Briefly, cells were treated with 0.1 mM cycloheximide for 30 min, and then lysed in a buffer containing 0.5% NP40, 0.1 M NaCl, 10 mM $MgCl_2$, 2 mM DTT, 50 mM Tris-HCl (pH 7.5), 200 U/mL SUPERase, 100 μg/mL cycloheximide, and 200 μg/mL heparin. Cellular debris and nuclei were removed from the cell lysates by centrifugation at 12 000 *g* for 10 min. A portion of the supernatant was saved for isolation of total cytoplasmic RNA (T). Polysomes in the supernatant were precipitated by ultracentrifugation at 100 000 *g* for 1 h in a 75 Ti rotor (Beckman) at 4°C, and the supernatant after ultracentrifugation was used as the source of postpolysomal (monosomal) fraction. The pellet from the above step was resuspended in 200 ul of polysomal buffer, and the resuspended

solution was used as polysomal fraction. The RNAs from polysomal and the total cytoplasmic extracts were isolated using TRI-reagent LS (Molecular Research Center Inc.). The recovered RNAs were further purified by an RNeasy Mini kit (Qiagen, Valencia, CA, USA), treated by RNase-free DNase (Qiagen), and were subjected to reverse transcription–real time polymerase chain reaction (RT–PCR) analysis for the HK2 mRNA.

Plasmids and Viral Transfections

Plasmid cDNA for human Trp53 was generated using methods previously described (Deng et al., 2002). PURO-Cre-GFP was a generous gift from Dr. Pandoffi at Harvard Medical School (Chen et al., 2005). pSIF-copGFP control vector and pSIF-copGFP-miR143 were obtained from Dr. Hongbin Ji (Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences) (Fang et al., 2012). PLZW plasmids expressing human HK2 and HK1, and tetracyclin-inducible lentivirus vector expressing mutant 4E-BP1 were obtained from Addgene. All other shRNAs were obtained from the BioMedical Genomics Center at The University of Minnesota. These are lentiviral shRNAs and are as follows: Raptor (TRCN0000039772 and TRCN0000039768 for human; TRCN0000077468 and TRCN0000077470 for mouse), AKT1 (TRCN0000039793 and TRCN0000039794 for human; TRCN0000022934 and TRCN0000022936 for mouse), human Pten (TRCN0000002747 and TRCN0000002749), HK2 (TRCN0000037670 and TRCN0000037673 for human; TRCN0000012545 and TRCN0000012546 for mouse), human p53 (TRCN0000003754 and TRCN0000003755). The negative control vector was the pLKO.1 vector backbones that has no hairpin insert. ShRNA-encoding plasmids were co-transfected with envelope and packaging plasmids (VSVG, REV and pMDL) into actively growing HEK-293T cells using the calcium phosphate transfection method. Virus-containing supernatants were collected 36 hr after transfection, centrifuged to remove cell debris and filtered to eliminate cells.

The target cells were infected in the presence of 8 μg/ml polybrene. Cells were selected with 8 μg/ml puromycin 24 hr later to generate stable cell lines and knockdown efficiency was confirmed by immunoblotting.

Senescence-Associated β-galactosidase (SA-β-Gal) Activity

MEFs at passage 2 were plated in triplicate at 3×10^5 cells per 6 cm culture dish. Four days later, cells were fixed in 4% paraformaldehyde and SA-β-Gal activity was detected. To quantify the percentage of SA-β-Gal positive cells, at least 300 cells were counted in random fields in each of the triplicate dishes.

Xenograft Models in NSG mice

MEFs xenograft tumors were formed by subcutaneously injecting 1 x 10^6 cells into the lower flank of NOD/SCID IL2RG (NSG) mice (005557 from The Jackson Laboratory; http://jaxmice.jax.org/strain/005557.html). The mice were monitored for tumor development and euthanized 6 weeks after injection. Xenograft tumors of mouse or human prostate cancer cells were developed by subcutaneously injecting 1.5 x 10^6 UMN-4240P or 2 x 10^6 PC3 cells with control or shRNA targeting HK2 (suspended in 100 μl of PBS) into the lower flank of NSG male mice. The mice were monitored for tumor progress and euthanized 4 weeks (PC3) or 7 weeks (UMN-4240P) after injection. Tumors were dissected and photographed. Tumor volume was measured once a week and calculated by the following formula: volume = (length × width^2)/2.

SUPPLEMENTAL REFERENCES

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