Mevalonate Pathway Provides Ubiquinone to Maintain Pyrimidine Synthesis and Survival in p53-Deficient Cancer Cells Exposed to Metabolic Stress

Irem Kaymak1, Carina R. Maier1, Werner Schmitz1, Andrew D. Campbell2, Beatrice Dankworth1, Carsten P. Ade1, Susanne Walz2, Madelon Paauwe2, Charis Kalogirou4, Hecham Marouf1, Mathias T. Rosenfeldt5,6, David M. Gay2,7, Grace H. McGregor2,7, Owen J. Sansom2, and Almut Schulze1,6

ABSTRACT

Oncogene activation and loss of tumor suppressor function changes the metabolic activity of cancer cells to drive unrestricted proliferation. Moreover, cancer cells adapt their metabolism to sustain growth and survival when access to oxygen and nutrients is restricted, such as in poorly vascularized tumor areas. We show here that p53-deficient colon cancer cells exposed to tumor-like metabolic stress in spheroid culture activated the mevalonate pathway to promote the synthesis of ubiquinone. This was essential to maintain mitochondrial electron transport for respiration and pyrimidine synthesis in metabolically compromised environments. Induction of mevalonate pathway enzyme expression in the absence of p53 was mediated by accumulation and stabilization of mature SREBP2. Mevalonate pathway inhibition by statins blocked pyrimidine nucleotide biosynthesis and induced oxidative stress and apoptosis in p53-deficient cancer cells in spheroid culture.

Moreover, ubiquinone produced by the mevalonate pathway was essential for the growth of p53-deficient tumor organoids. In contrast, inhibition of intestinal hyperproliferation by statins in an Apc/KrasG12D-mutant mouse model was independent of de novo pyrimidine synthesis. Our results highlight the importance of the mevalonate pathway for maintaining mitochondrial electron transfer and biosynthetic activity in cancer cells exposed to metabolic stress. They also demonstrate that the metabolic output of this pathway depends on both genetic and environmental context.

Significance: These findings suggest that p53-deficient cancer cells activate the mevalonate pathway via SREBP2 and promote the synthesis of ubiquinone that plays an essential role in reducing oxidative stress and supports the synthesis of pyrimidine nucleotide.

Introduction

The metabolic activity of cancer cells is controlled by genetic alterations and by the tumor microenvironment. Under metabolic stress, defined by reduced access to nutrients and oxygen present in poorly vascularized solid tumors, cancer cells need to adapt their metabolic activity to maintain cell proliferation and survival. One important factor in the adaptation to metabolic stress is the hypoxia inducible factor (HIF), which is stabilized and activated in the absence of oxygen, and promotes the uptake of glucose and its fermentation to lactate while reducing oxidative metabolism (1). However, poor access to the vascular network not only rewrites their metabolic activity to be able to adapt to these conditions.

The p53 tumor suppressor is a master regulator of cellular metabolism (2, 3). It reduces glucose uptake (4) and alters glycolysis and the flux of metabolites into the pentose phosphate pathway (5–8). Conversely, p53 enhances mitochondrial metabolism by promoting the assembly of cytochrome c oxidase (complex IV) and increasing respiration (9). It has been shown that p53 allows cancer cells to adapt to nutrient deprivation, in particular, the absence of the amino acid serine and glutamine (10, 11). Thus, loss of p53 function can increase the sensitivity of cancer cells toward metabolic stress, resulting in a selective vulnerability that could be exploited therapeutically.

In this study, we have investigated the role of p53 in the regulation of metabolic processes in colon cancer cells exposed to metabolic stress. To recreate the simultaneous reduction in oxygen and nutrient availability found in tumors, we cultured cancer cells as multicellular tumor spheroids. Under these conditions, we find that p53-deficient cancer cells activate the expression of enzymes of the mevalonate pathway via the sterol regulatory element binding protein 2 (SREBP2). Moreover, inhibition of mevalonate pathway activity with statins selectively induced apoptosis in p53-deficient cancer cells exposed to metabolic stress. This effect was mediated by reduced generation of ubiquinone (CoQ10), which p53-deficient cells require to maintain tricarboxylic acid (TCA) cycle activity, respiration, and the synthesis of pyrimidine nucleotides. Our study thus reveals a novel link between the regulation of isoprenoid synthesis and the modulation of electron transfer mediated by ubiquinone in cancer cells. Mevalonate pathway

1Theodor-Boveri-Institute, Biocenter, Würzburg, Germany. 2Cancer Research UK Beatson Institute, Glasgow, United Kingdom. 3Comprehensive Cancer Center Mainfranken, Core Unit Bioinformatics, Biocenter, University of Würzburg, Würzburg, Germany. 4Department of Urology, University Hospital Würzburg, Würzburg, Germany. 5Department of Pathology, University Hospital Würzburg, Würzburg, Germany. 6Comprehensive Cancer Center Mainfranken, Würzburg, Germany. 7Institute of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom.

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Corresponding Author: Almut Schulze, Division of Tumor Metabolism and Microenvironment, German Cancer Research Center, Heidelberg, Germany. Phone: 4962-2142-5423; Fax: 4962-2142-5467; E-mail: almut.schulze@dkfz-heidelberg.de

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activity is essential for p53-deficient cancer cells to proliferate and survive under the metabolic constraints of the tumor microenvironment.

**Materials and Methods**

**Tissue culture and reagents**

HCT116 p53-isogenic cells were obtained from B. Vogelstein (Johns Hopkins University, Baltimore, MD) and HCT116 p21-isogenic cells from M. Dobbelstein (Georg-August University, Göttingen, Germany). RKO p53-isogenic lines were a gift from K. Vousden (Beatson Institute, Glasgow, United Kingdom). All other cell lines were from CRUK LRI Research Services, authenticated by short tandem repeat profiling and used at low passage. Unless stated otherwise, cells were cultured in DMEM with 10% FBS (Gibco), 4 mMol/L l-glutamine, and 1% penicillin-streptomycin, at 37°C in a humidified incubator at 5% CO2 and regularly tested for absence of Mycoplasma. Etoposide, (R)-Mevalonic acid lithium salt, SB216732, CHIR99021, simvastatin, zoleodrlic acid monohydrate, coenzyme Q10, NAC, water-soluble cholesterol, uridine, lefumidine, and 5-FU were all from Sigma. MG132 and MK2206 were from Bertin Pharma, rapamycin from Cayman Chemicals, mevastatine, and 5-FU were all from Sigma. MG132 and MK2206 were from Bertin Pharma, rapamycin from Cayman Chemicals, mevastatin and YM-3601 from Biomol, and nucleosides (EmbryoxMax 100×) from Merck-Milipore.

**Spheroid formation, flow cytometry, and histology**

For spheroid formation, 10,000 cells/well were placed in 96-well ultralow attachment plates (Corning CORN7007) followed by centri-12100 from Bertin Pharma, rapamycin from Cayman Chemicals, mevastatine, and 5-FU were all from Sigma. MG132 and MK2206 were from Bertin Pharma, rapamycin from Cayman Chemicals, mevastatin and YM-3601 from Biomol, and nucleosides (EmbryoxMax 100×) from Merck-Milipore.

**Spheroid formation, flow cytometry, and histology**

For spheroid formation, 10,000 cells/well were placed in 96-well ultralow attachment plates (Corning CORN7007) followed by centri-fugation at 850 × g for 10 minutes. Spheroids were cultured for 12-14 days, during which, medium was replaced every three days. Monolayer and spheroid cells were incubated with 20 μM/L BrdU (Sigma) for 24 hours, trypsinized, and fixed in 80% EtOH. Cells incubated in 2 mol/L HCl with 0.5% Triton X-100 for 30 minutes at room temperature, neutralized with Na2B4O7, and incubated with anti-BrdU-FITC antibodies (Biozol). Cells were washed and treated with RNase A (24 μg/mL) and propidium iodide (54 μmol/L) for 30 minutes. Analysis was performed on a BD FACSCanto II using FACSDIVA software.

Spheroids were fixed with 3.7% paraformaldehyde, incubated in 70% ethanol for 16 hours, mixed with low-melting agarose, and paraffin embedded. Four-micron-thick sections were deparaffinized and rehydrated. Antigen retrieval was performed with citrate buffer (pH 6.0) in a microwave oven for 6 minutes. Sections were stained with anti-Ki67 (SP6, Thermo Fisher) and anti-cleaved caspase 3 (Cell Signaling Technology) in PBS/1% BSA at 4°C and biotinylated secondary antibody (Vector Laboratories). Slides were developed with 3,3’-diaminobenzidine (Cell Signaling Technology) and counterstained with Gilmore 3 hematoxylin. For TUNEL staining, sections were heated in citrate buffer (pH 6.0) for 2 minutes. TUNEL reactions were developed for 1 hour (In Situ Cell Death Detection Kit, Sigma) and counterstained with Hoechst (Sigma). Archival tumor tissue (8) was stained with anti-Ki67 as above.

**RNA sequencing**

RNA was extracted using RNeasy columns (Qiagen) including DNAse I digestion. mRNA was isolated using NEBNext Poly(A) mRNA Magnetic Isolation Module and library preparation was performed with NEBNext Ultra RNA Library Prep Kit for Illumina following the manufacturer’s instructions. Libraries were size-selected using Agencourt AMPure XP Beads (Beckman Coulter), followed by amplification with 12 PCR cycles. Library quantification and size determination was performed with an Experion system (BioRad) and libraries were sequenced with NextSeq500 (Illumina).

**RNA extraction and qRT-PCR**

Total RNA was isolated using PeqGOLD Trifast, followed by reverse transcription into cDNA using M-MLV Reverse Transcriptase (Promega) and random hexamer primers. Real-time PCR was performed using Power-up SYBR Green Master Mix (Thermo Fisher Scientific) using Quantitert primers (Qiagen) or custom primers as follows: human ACTB forward 5’-GACTCGGTTTGGCCGAT-3’ and reverse 5’-CGGCGGATATCATATCC-3’; and human CDKN1A forward 5’T-CACTGTCCTGTACCCATTGC-3’ and reverse 5’-CTTTGGAGTGTTAGAAA-3’ (Sigma). All qPCR reactions were performed in technical duplicate on three biologically independent replicate samples. Relative mRNA amounts were calculated using the comparative C threshold method after normalization to β-actin (ACTB).

**Western blotting**

Cells were lysed in lysis buffer (1% Triton X100, 50 mmol/L Tris pH 7.5, 300 mmol/L NaCl, 1 mmol/L EGTA, 1 mmol/L DTT, 1 mmol/L NaVO4 with protease inhibitors for 30 minutes) for 30 minutes, cleared by centrifugation, and quantified using BCA assay (Thermo Fisher). Nuclear extraction of SREBP2 was performed as described previously (12). Proteins were separated on SDS-PAGE and blotted onto polyvinylidine difluoride membrane (Immobilon), blocked with blocking solution (LI-COR), and incubated with primary and secondary antibodies. Signals were detected on an Odyssey scanner and quantified using ImageJ. The following antibodies were used: p53 (DO-1), p21 (C-19), CCND1 (DSC-6; from Santa Cruz Biotechnology), HMGC-S1 (#ab155787), histone-3 (#ab1791, from Abcam), SREBP-2 (1D2), ABCA1 (from Novus Biologicals), SREBP-2 (R&D Systems), GSK3 (4G-1E; from Milipore), PKD1, ACS52, p-GSK3β (Ser21/9), β-actin (AC-15), FDFTI, and vinculin (from Sigma). Secondary antibodies were from LI-COR Biosciences.

**Stable isotope labeling and mass spectrometry**

Monolayer cells or spheroids were washed with PBS and medium was replaced with either complete medium or glucose-free medium with 25 mmol/L [U-13C]-glucose (Cambridge Isotope Laboratories). Cells were incubated for the indicated times, washed with cold 154 mmol/L ammonium acetate, and snap frozen. For tissue extraction, 150 mg of frozen tissue was homogenized in 3 mL of H2O using an UltraTurrax.

For water-soluble metabolites, samples were extracted with ice-cold MeOH/H2O (80/20, v/v) containing 0.1 μmol/L l-amnvidine (Sigma) and separated by centrifugation. Supernatants were transferred to a Strata C18-E column (Phenomenex) that has been conditioned with 1 mL of CH3CN and 1 mL of MeOH/H2O (80/20, v/v). The eluate was dried and dissolved in 50 μL of a mixture of CH3CN and 5 mmol/L NH4OAc (25/75, v/v).

For cholesterol and ubiquinone, samples were extracted with ice-cold MeOH/H2O (80/20, v/v) containing 1 mmol/L CoQ9 (Sigma) and separated by centrifugation. Supernatants were extracted twice with 0.4 mL of hexane, collected and taken to dryness under nitrogen at 35°C. Samples were dissolved in 150 μL of hexane and transferred to Strata SI-1 columns (Phenomenex), washed with 750 μL hexane, and...
500 μL hexane/acetic acid ethyl ether (18/1 v/v). Ubiquinone was eluted with 0.5 mL hexane/acetic acid ethyl ether (9/1, v/v). Cholesterol was fully eluted with 0.5 mL hexane/acetic acid ethyl ether (9/1, v/v). Eluates were dried under nitrogen at 35°C and dissolved in 50 μL ipOH.

Metabolites were analyzed by LC-MS using setting provided in Supplementary Methods.

**Seahorse assays**

Mouse small intestines were isolated from wild-type, VillinCreER^ER^ Ap^+/0^ or VillinCreER^ER^ Ap^+/0^ Kras^G12D/+^ mice sacrificed 3 days post-infection with tamoxifen, opened longitudinally, and washed with PBS. Crypts were isolated as described previously (13), mixed with 20 μL Matrigel (BD Biosciences), and plated in 24-well plates in Advanced DMEM/F12 (Thermo Fisher Scientific) supplemented with 1% penicillin/streptomycin, 10 mmol/L HEPES, 2 mmol/L glutamine, N2 (Thermo Fisher Scientific), B27 (Thermo Fisher Scientific), 100 ng/mL Noggin, and 50 ng/mL EGF (both from PeproTech). Growth factors were added every two days. Experiments were performed on two biologically independent samples. Genotyping was performed using primers: p53^B^ 5'-GAGA- GAAAGGGGAGG-3' and 5'-AAGGGGTATGAGGGACAAGG-3'; Kras^G12D^ 5'-GCTTTTCGCCACGACAGTC-3', 5'-CTCTTGTCC-TACGCAACACGT-3' and 5'-ACGCTAGCCACACTGGCTTGAGTAAGTCTGCA-3'.

**Mice**

All animal experiments were performed under UK Home Office guidelines using project licenses 70-8645 or 70-8646. Experimental protocols were subject to the University of Glasgow animal welfare and ethical review board approval. VillinCreER^ER^ Ap^+/0^ and VillinCreER^ER^ Ap^+/0^ Kras^G12D/+^ mice have been described previously (14). For induction of intestinal hyperproliferation, mice were given a single intraperitoneal injection of 80 mg/kg tamoxifen on one occasion (VillinCreER^ER^ Ap^+/0^ Kras^G12D/+^), or on two consecutive days (VillinCreER^ER^ Ap^+/0^). Mice were treated with a daily dose of 50 mg/kg simvastatin in 0.5% methylcellulose/5% DMSO or vehicle or a daily dose of 35 mg/kg leflunomide in 100 μL 0.15% carboxymethylcellulose via oral gavage from one day post initial tamoxifen injection. For 2H2O tracing, mice were exposed to 8% 2H2O in their drinking water for 4 days. Mice were given an intraperitoneal injection of 250 μL of cell proliferation reagent (RP201, GF Healthcare/Amersham) 2 hours prior to sacrifice and tissue sections were stained for BrdU as described in Supplementary Methods.

**Statistical analysis**

Statistical details for each experiment are stated in the figure legends. Graphs were generated using GraphPad Prism 6.0 (GraphPad software). Unless otherwise indicated, statistical significance was calculated using the unpaired two-tailed Student t test.

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**Results**

**Spheroid culture induces tumor-like transcriptional signatures and leads to complex metabolic reprogramming**

To determine the influence of conditions of the tumor microenvironment on colon cancer cells, we used isogenic HCT116 lines that are either wild-type (wt) for p53 or an isogenic derivative in which the TP53 gene had been deleted by homologous recombination (15). These cells do not express detectable levels of p53 and fail to induce p21 upon treatment with the DNA-damaging agent etoposide (Supplementary Fig. S1A). Both cell lines were cultured either as subconfluent monolayers (MLC) for 48 hours or as large three-dimensional tumor spheroids (diameter >600 μm), thereby exposing cancer cells to gradients of oxygen and nutrient depletion (16). Spheroid cultures (SPC) showed an overall reduction in proliferation compared with MLC, which was similar in both genotypes (Fig. 1A). However, staining for the proliferation marker Ki67 revealed that p53 wt SPC show proliferation only in the outer regions (Fig. 1B), while SPC of p53-deficient cells present Ki67 positivity throughout their cross-sections (Fig. 1B). Similarly, subcutaneous xenograft colon tumors formed by p53 wt HCT116 cells displayed more heterogeneous proliferation patterns compared with their p53-deficient counterparts (Fig. 1C). This suggests that p53 is required for cell-cycle arrest induced by the nutrient and oxygen-depleted conditions found in SPC and tumors.

We next performed transcriptome analysis of p53 wt and deficient cells cultured as SPC, MLC, or xenograft tumors. Principal component analysis showed that global gene expression in SPC is more similar to those in tumors rather than MLC (mainly in PC1 accounting for 80% of variance; Fig. 1D). Gene set enrichment analysis (GSEA) revealed reduced proliferation (Hallmark_E2F_targets) and induction of interferon response and hypoxia signatures as major transcriptional phenotypes in both SPC and tumors compared with MLC (Fig. 1E and F). Moreover, analysis of the cell-cycle regulator cyclin D1 (CCND1) and the HIF target pyruvate dehydrogenase kinase (PDHK) confirmed reduced proliferation and induction of hypoxia in SPC compared with MLC (Fig. 1G).

We next performed metabolomic analysis to determine differences in metabolism between genotypes and culture conditions (Supplementary Fig. S1B). Stable isotope tracing using [U-13C]-glucose showed that SPC increases glucose-dependent lactate synthesis in both p53 wt and deficient cells (Fig. 1H). Time-course experiments revealed that the labeling of TCA cycle metabolites as well as alanine, glutamate, and aspartate reached steady state more rapidly in SPC compared with MLC, as maximal labeling was reached much earlier (Supplementary Fig. S1E). Moreover, fractions of labeled metabolites were reduced, indicating that the contribution of precursors other than glucose, most likely glutamine, to the TCA cycle is higher in SPC compared with MLC. We also found evidence for pyruvate-dependent anaplerosis, as M+3 isotopologues for succinate, fumarate, and malate were formed more rapidly in SPC compared with MLC (Supplementary Fig. S1D). This pyruvate-dependent anaplerosis supported the production of aspartate, indicated by the high M+1 to M+2 ratio for aspartate in SPC (Fig. 1I).

While most metabolic differences between MLC and SPC were found in both genotypes, the total levels of aspartate were higher in SPC from p53 wt cells compared with p53-deficient SPC and also compared with MLC (Fig. 1J). Aspartate is a precursor for pyrimidine nucleotide synthesis and thus essential for proliferation (17). Consistently, glucose-derived labeling of uridine monophosphate (UMP), a central metabolite in pyrimidine biosynthesis, while overall reduced...
Spheroid cultures replicate tumor-like transcriptional profiles and show pyruvate-dependent anaplerosis. A, HCT116 p53<sup>+/+</sup> and p53<sup>−/−</sup> cells were cultured as subconfluent monolayer cultures (MLC) for 48 hours or as multilayered tumor spheroid cultures (SPC) for 14 days. Cells were incubated with BrdU for 24 hours and analyzed by FACS. B, HCT116 p53<sup>+/+</sup> and p53<sup>−/−</sup> cells were cultured as SPC for 14 days, fixed, and embedded in paraffin. Histologic sections were analyzed for expression of the proliferation marker Ki67. Representative images of three spheroids analyzed per condition are shown. C, Analysis of proliferation in HCT116 p53<sup>+/+</sup> and p53<sup>−/−</sup> xenograft tumor tissue from ref. 8 using Ki67. Representative images of tumors from 6 animals per group are shown. D, HCT116 p53<sup>+/+</sup> and p53<sup>−/−</sup> cells were cultured as MLC for 48 hours or as SPC for 14 days. RNA was analyzed by RNA-seq and compared with RNA-seq data from HCT116 p53<sup>+/+</sup> and p53<sup>−/−</sup> cells grown as xenograft tumors (8). Principal component analysis showed overall higher similarity in gene expression signatures between tumors (T) and SPC compared with MLC. E, GSEA comparing MLC and SPC cultures of HCT116 p53<sup>+/+</sup> and p53<sup>−/−</sup> cells. Enrichment plots for HALLMARK_E2F_TARGETS, BROWNE_INTERFERON_RESP_GENES, and MANALO_HYPOXIA_UP are shown. F, Enrichment plots for the same gene sets as in E comparing MLC and tumors (T) of HCT116 p53<sup>+/+</sup> and p53<sup>−/−</sup> cells. G, Western blots showing levels of cyclin D1 (CCND1) and pyruvate dehydrogenase kinase 1 (PDK1) in HCT116 p53<sup>+/+</sup> or p53<sup>−/−</sup> cells grown as MLC or SPC. Vinculin is shown as loading control. M–J, HCT116 p53<sup>+/+</sup> and p53<sup>−/−</sup> cells were cultured as MLC or SPC and labeled for 16 hours with [U13C]-glucose. Cells were extracted and metabolites were analyzed by LC-MS. Data show mean ± SEM of three independent biological replicates. Results from time-resolved experiments are provided in Supplementary Fig. S1. H, Relative peak intensities for lactate. I, Ratios of M+3 and M+2 isotopologues for aspartate. J, Relative peak intensities of aspartate.
compared with MLC, was higher in p53-deficient SPC compared with their wt counterparts (Supplementary Fig. S1F), potentially reflecting higher demand of nucleotides for proliferation.

Together, transcriptomic and metabolic analyses demonstrated that SPC induces hypoxic reprogramming of cellular metabolism in cancer cells. However, oxidative reactions required to generate substrates for anabolic reactions (i.e., aspartate) are still supported through anaplerosis.

**Loss of p53 activates the mevalonate pathway via SREBP2**

We next compared gene expression signatures between p53 wt and deficient cells under the different culture conditions. The major signatures associated with wt p53 status in all conditions were inflammation and IFNγ response (Supplementary Fig. S2A). Signatures associated with p53 deficiency in MLC mapped to TGFβ signaling, spermatogenesis, and cell cycle (Supplementary Fig. S2A, left). In contrast, loss of p53 in SPC and xenograft tumors resulted in the induction of genes associated with cholesterol homeostasis (Supplementary Fig. S2A, middle and right part), many of which are regulated by the SREBP transcription factors (18). Moreover, SREBP target genes (Horton_SREBP_targets; ref. 19) showed strong enrichment in p53-deficient cells in SPC and xenograft tumors but not in MLC (Fig. 2A), suggesting that the combined effect of environment and loss of p53 leads to the upregulation of these genes. As cholesterol homeostasis is preferentially regulated by SREBP2, rather than the closely related SREBP1a or SREBP1c isoforms (19), we next investigated expression of canonical SREBP2 target genes. This showed increased expression of HMGCS1, MVD, HMGR, DHCR7, and FDFT1 mRNA in p53-deficient SPC compared with MLC, which was further increased upon loss of p53, while expression of SREBP2 mRNA was increased in SPC compared with MLC of p53-deficient cells (Fig. 2B). Moreover, HMGCS1, FDTF1, and ACS5S showed increased protein levels in p53-deficient SPC from a second isogenic colon cancer cell line, RKO (Fig. 2C).

We also investigated whether expression of SREBP2 target genes is associated with TP53 mutation in human colorectal adenocarcinoma. Analysis of a The Cancer Genome Atlas (TCGA) dataset (20) revealed higher expression of canonical SREBP2 targets in TP53-mutant tumors (Fig. 2D) and increased expression of HMGCS1 in high-grade colorectal adenocarcinoma (Fig. 2E). Moreover, two colon cancer cell lines expressing mutant TP53 (HT29 and DLD1) displayed stronger induction of HMGCS1 expression upon SPC compared with p53 wt cell lines (LS174T and LOVO; Fig. 2F), corroborating that loss of normal p53 function either through mutation or deletion increases the expression of mevalonate pathway genes.

Wild-type p53 was shown to inhibit mevalonate pathway genes through induction of the cholesterol transporter ABCA1 (21). In agreement with this study, we found that ABCA1 mRNA expression was strongly reduced in p53-deficient cells both in MLC and SPC (Supplementary Fig. S2B). However, levels of ABCA1 protein were higher in p53-deficient MLC and completely absent in SPC (Supplementary Fig. S2C). Interestingly, ABCA1 is a target for miRNA-33, which is encoded by an intron within the SREBP2 gene (22, 23). It is therefore possible that ABCA1 is repressed in SPC via a miRNA-dependent mechanism.

To address the mechanism of mevalonate pathway regulation in our system, we first confirmed that increased expression of HMGCS1 protein in p53-deficient SPC is abolished upon shRNA-mediated silencing of SREBP2 (Supplementary Fig. S2D and S2E). We also established that p53-deficient SPC contain high levels of the 55 kDa mature form of SREBP2 (Fig. 2G), which represents the active transcription factor. Accumulation of mature SREBP2 and enhanced target expression was also observed in MLC of p53-deficient HCT116 cells cultured in lipid-reduced medium (Supplementary Fig. S2F and S2G), a condition that induces SREBP processing (18). Nuclear accumulation of mature SREBP2 is mediated by increased processing of the precursor or by stabilization of the mature protein. As SREBP processing is induced by mTORC1 (24, 25), we first investigated the activity of this pathway. We found that phosphorylation of the mTORC1 substrate p70S6K (indicated by the higher relative abundance of the upper band) and its downstream target S6 ribosomal protein (S6RB), is strongly increased in SPC compared with MLC (Fig. 2H, Supplementary Fig. S2H). This was surprising as hypoxia, a major feature of SPC, inhibits the mTORC1 pathway (26, 27). Indeed, exposure of HCT116 MLC to hypoxia decreased S6RB phosphorylation and slightly reduced HMGC51 expression (Supplementary Fig. S2I).

As increased mTORC1 activity was observed in SPC from both genotypes, we also addressed whether loss of p53 alters protein stability of mature SREBP2. Treatment with the proteasome inhibitor MG132 only increased mature SREBP2 in p53 wt SPC, confirming that mature SREBP2 is more stable when p53 is absent (Fig. 2I). Mature SREBP2 is phosphorylated by glycogen synthase kinase 3 (GSK3), leading to its ubiquitination and degradation (28). We found an overall increase in GSK3 phosphorylation on serine 21 (GSK3α) and serine 9 (GSK3β) in SPC compared with MLC, with a further increase in p53-deficient cells (Fig. 2J), indicating reduced activity of the kinase upon p53 loss. Consistently, treatment of p53 wt SPC with GSK3 inhibitors increased levels of mature SREBP2 and restored expression of HMGCS1 mRNA to the same level found in p53-deficient cells (Fig. 2K and L). Treatment with the mTORC1 inhibitor rapamycin reduced mature SREBP2 and HMGC51 mRNA in p53-deficient SPC (Fig. 2K and M). However, this was independent of AKT, as treatment with MK2206 did not affect GSK3 or S6RB phosphorylation (Supplementary Fig. S2J).

Together, this suggests that loss of p53 in SPC induces nuclear accumulation of mature SREBP2 through activation of mTORC1 and inhibition of GSK3.

**Inhibition of mevalonate synthesis induces apoptosis in p53-deficient spheroids**

We next tested whether the mevalonate pathway contributes to cancer cell survival in the metabolically compromised environment of SPC. We used statins, a class of lipid-lowering drugs that inhibit the activity of HMGGR, the rate-limiting enzyme of the pathway (Fig. 3A). Statin treatment increased the expression of SREBP2 target genes, due to inactivation of the negative feedback loop (29), and resulted in global downregulation of cell cycle and epithelial-to-mesenchymal transition (EMT) expression signatures regardless of genotype (Supplementary Fig. S3A). Protein levels of the S-phase proteins cyclin A (CCNA1) and aurora kinase A (AURKA) were also reduced (Supplementary Fig. S3B), confirming that the mevalonate pathway contributes to proliferation (30) and disruption of tissue architecture (31).

When investigating the effect of mevalonate pathway inhibitors on cell viability, we found strong inhibition of proliferation in MLC, irrespective of genotype (Supplementary Fig. S3C). In contrast, in SPC only, p53-deficient cells were sensitive to mevastatin treatment, indicated by TUNEL staining, while p53 wt cells were largely resistant to this treatment (Fig. 3B and C). Mevastatin-induced apoptosis in p53-deficient cells was blocked by addition of mevalonate, the product of the HMGCR reaction (Fig. 3B and C), confirming the specificity of the inhibitor. Apoptotic cells positive for TUNEL and cleaved caspase-
Loss of p53 induces enzymes of the mevalonate pathway via activation of SREBP2. A, Enrichment plots for HORTON_SREBF_TARGETS (19) for HCT116 p53+/+ and p53−/− cells cultured as MLC or SPC. Data show mean ± SEM of three independent biological replicates. **, P < 0.01; ****, P < 0.0001, compared by unpaired two-tailed Student t test. B, Expression of canonical mevalonate pathway genes and SREBF2 in HCT116 p53−/− or p53+/+ cells grown as MLC or SPC. Data show mean ± SEM of three independent biological replicates.**, P < 0.01; ****, P < 0.0001, compared by unpaired two-tailed Student t test. C, Western blots showing levels of HMGCS1, Fdft1, Acss2, and p53 in RKO p53+/+ and p53−/− cells grown as SPC. Vinculin is shown as loading control. D, Combined z-score for the expression of canonical mevalonate pathway genes (HMGCS1, HMGCR, MVD, DHCR7, ACSS2, FDFT1, and SREBF2) was calculated from tumors of the TCGA colorectal adenocarcinoma dataset. Mevalonate pathway signature values were compared between all p53 wt (n = 94) and p53-mutant (n = 88) tumors. P = 0.0051 was determined using an unpaired two-tailed Student t test. E, Expression of HMGCS1 in colorectal adenocarcinoma tumors from the TCGA dataset according to stage (PT1–4). F, Expression of HMGCS1 in TPS3 wt and mutant colon cancer cell lines grown as MLC or SPC. G, Western blot analysis showing expression of HMGCS1 and mature SREBF2 in HCT116 p53−/− or p53+/+ cells grown as MLC or SPC. Actin is shown as loading control. H, Western blots showing levels of phosphorylated ribosomal protein S6 (P-S6RB) and total ribosomal protein S6 expression (S6RB) in HCT116 p53−/− or p53+/+ cells grown as MLC or SPC. (Continued on the following page.)
Mevalonate Pathway Supports Ubiquinone Synthesis in Cancer

![Diagram showing selected metabolites of the mevalonate pathway and HMGCR, the molecular target of statins. B, HCT116 p53+/+ or p53−/− cells were grown as SPC and treated with 10 μmol/L mevavastatin (MST) or solvent (DMSO) either alone or in combination with 0.5 mmol/L mevalonate (MVL) for 72 hours. Spheroids were fixed and histologic sections were analyzed for the presence of apoptotic cells by TUNEL staining. Images show representative results of three spheroids analyzed per condition. C, Quantitation of data shown in B. Data are presented as mean ± SEM of at least three spheroids analyzed per condition. *; P < 0.05, **; P < 0.001, unpaired two-tailed Student t test. D, HCT116 p53+/+ and p53−/− cells grown as SPC were treated with 10 μmol/L simvastatin (SIM) or solvent (DMSO) for 24 or 72 hours. Expression of p21 (CDKN1A) mRNA was determined by qPCR. Data show mean ± SEM of three independent biological replicates. *, P < 0.05; **, P < 0.001, unpaired two-tailed Student t test. E, HCT116 p53+/+ and p53−/− cells were grown as SPC and treated with 0.5 mmol/L simvastatin (SIM) or solvent (DMSO) either alone or in combination with 0.5 mmol/L mevalonate for 72 hours. Expression of p21 protein was determined by Western blotting. Vinculin is shown as loading control. F, HCT116 p21−/− cells were grown as SPC and treated with 10 μmol/L simvastatin (SIM) or solvent (DMSO) either alone or in combination with 0.5 mmol/L mevalonate (MVL) for 72 hours. Expression of p21 protein was determined by Western blotting. Vinculin is shown as loading control.](cancerres.aacrjournals.org)
pathway activity, we treated SPC of p53 wt and depleted prenoid units and functions as electron transfer molecule between the tyrosine linked to a tail comprising 10 (human) or 9 (mouse) isoprene units. Ubiquinone consists of a benzoquinone ring derived from the synthesis of dolichol, heme A, and ubiquinone (Ref. 32). Ubiquinone is an essential component of the mitochondrial electron transport chain (ETC) where it shuttles electrons between NADH-CoQ reductase (complex I) and succinate dehydrogenase (complex II) and CoQH₂-cytochrome c reductase (complex III; Fig. 5A). Using stable isotope tracing with [U-13C]glucose and determined label incorporation into different metabolites, we found that simvastatin reduced labeled and unlabeled fractions of aspartate and maximal OCRs in SPC of p53-deficient cells, which was rescued by mevalonate addition. In contrast, SPC from p53 wt cells only displayed a small reduction in maximal respiration upon statin treatment (Fig. 5C).

Reduced availability of oxygen as final electron acceptor of the ETC can lead to electron leakage and the formation of reactive oxygen species (35). We therefore reasoned that inhibition of ubiquinone synthesis could cause oxidative stress under the hypoxic conditions in SPC, which could lead to the induction of apoptosis. Indeed, replenishing spheroid cultures either with ubiquinone or the antioxidant N-acetyl-cysteine (NAC) was as effective as mevalonate in preventing statin-induced apoptosis in SPC (Fig. 5D and E), while cell-permeable cholesterol had no effect (Supplementary Fig. S5B). In addition, the viability of statin-treated MLC was not restored by the addition of ubiquinone (Supplementary Fig. S5C), indicating that multiple products of this pathway are needed to support the rapid proliferation of cancer cells observed in MLC.

**Production of ubiquinone by the mevalonate pathway supports pyrimidine nucleotide biosynthesis**

Ubiquinone also functions as an electron acceptor for dihydroorotate dehydrogenase (DHODH), an essential enzyme for the generation of pyrimidine nucleotides for DNA and RNA synthesis (Fig. 6A). Stable isotope tracing showed higher incorporation of glucose-derived carbons into UMP in SPC of p53-deficient cells (Fig. 6B). This was detected in the M+5 fraction, representing labeling via ribose, but also in the M+7/M+8 fractions, representing ribose plus either two or three labeled carbons derived from aspartate (Fig. 6B). Treatment with statins significantly lowered labeling and overall levels of UMP in SPC from both genotypes (Fig. 6B and C). This was restored by supplementing statin-treated SPC with either mevalonate or ubiquinone (Fig. 6C), confirming that ubiquinone is rate-limiting for pyrimidine synthesis under these conditions. Moreover, addition of nucleosides or uridine, which can readily be taken up by cells and used to replenish the nucleotide pool via the salvage pathway, was sufficient to block the induction of apoptosis by statins in p53-deficient SPC (Supplementary Fig. S6A).

The antimitabolite drug 5-fluorouracil (5-FU), which is standard-of-care for advanced colorectal adenocarcinoma, exerts its effect mostly through inhibition of thymidylate synthase (TYMS; Ref. 36). TYMS converts dUMP to dTMP for DNA synthesis, and 5-FU treatment leads to DNA damage and cell death. We therefore investigated whether statins alter 5-FU sensitivity of cancer cells under the metabolic constraints of SPC. Interestingly, while p53 wt HCT116 cells showed remarkable resistance toward 5-FU, most likely due to the low proliferation of these cells in this condition, the drug sensitized the cells to simvastatin treatment (Fig. 6E). In contrast, p53-deficient cells already showed induction of apoptosis in response to statin alone, which was not further increased by 5-FU (Fig. 6E).

Collectively, these results demonstrate that ubiquinone production by the mevalonate pathway is essential for pyrimidine biosynthesis in cancer cells. Inhibition of ubiquinone synthesis blocks the viability of p53-deficient cells under the metabolic constraints of SPC. In contrast, p53 wt cells are initially resistant to statin treatment but can be sensitized by the antimitabolite 5-FU, which blocks dTMP synthesis and causes DNA and RNA damage.

**The metabolic output of the mevalonate pathway depends on environmental context**

To investigate the role of the mevalonate pathway under different conditions resembling the tumor microenvironment, we used...
Figure 4.
Inhibition of mevalonate synthesis blocks the production of ubiquinone in colon cancer cells. A, Schematic showing the branching of the mevalonate pathway into cholesterol biosynthesis, the generation of isoprenoids for protein prenylation, and the synthesis of dolichol, heme A, and ubiquinone (CoQ10). B and C, HCT116 p53+/+ or p53−/− cells were grown as SPC and labeled with [U-13C] glucose for 16 hours before extraction and analysis of mevalonate isotopologues. Data show mean ± SEM of three independent biological replicates. B, Relative peak intensities of labeled and unlabeled fractions for mevalonate. C, Relative peak intensities of individual labeled fractions for mevalonate. D–G, HCT116 p53+/+ or p53−/− cells were grown as SPC and treated with 10 μmol/L simvastatin (SIM) or solvent (DMSO) for 72 hours. For the last 16 hours, cells were labeled with [U-13C]-glucose before cells were extracted and metabolites were analyzed by LC-MS. Data show mean ± SEM of three independent biological replicates. D, Relative peak intensities of labeled and unlabeled fractions for cholesterol. E, Relative peak intensities of individual isotopologues for cholesterol. F, Relative peak intensities of labeled and unlabeled fractions for ubiquinone (CoQ10). G, Relative peak intensities of individual isotopologues for ubiquinone. H, Xenograft tumors from HCT116 p53+/+ or p53−/− cells were extracted and levels of cholesterol, 7-dihydroxycholesterol (7-DHC), and ubiquinone (CoQ10) were determined by LC-MS. Data are shown as mean ± SEM of six p53+/+ and five p53−/− colon tumors. *, P < 0.05, unpaired two-tailed Student t test.
organoid cultures of intestinal epithelial cells from mice carrying conditional alleles of Apc, Trp53, or KrasG12D, together with Villin-CreERT2. Efficient recombination of the Trp53 and Kras locus were confirmed by PCR (Supplementary Fig. S7A). Placed in organoid culture medium, these cells grow as large cysts without any signs of differentiation (13). Interestingly, while simvastatin only had a minor effect on the growth of Apc-deficient organoids, Apc/p53 double-deficient cells showed a severe reduction in organoid growth, which was fully restored by mevalonate supplementation (Fig. 7A and B). Reduced organoid growth was accompanied by induction of PARP cleavage, a marker of apoptotic cell death (Supplementary Fig. S7B). Similar results were also obtained for Apc691/KrasG12D and Apc691/p53fl/fl/KrasG12D organoids (Fig. 7A and B), demonstrating that the deletion of Trp53 of Apc-p53−/− cells sensitizes the organoids toward mevalonate pathway inhibition. Moreover, inhibition of organoid growth in Apc/p53−/− and Apc691/p53fl/fl/KrasG12D+ cells was robustly restored by addition of either ubiquinone or nucleosides (Fig. 7C and D), confirming that the provision of ubiquinone for nucleotide biosynthesis is an essential function of the mevalonate pathway in colorectal adenocarcinoma tumor organoids.

We next assessed the ability of simvastatin to suppress intestinal hyperproliferation induced by acute deletion of Apc and activation of Kras in vivo. This was achieved by crossing mice carrying conditional alleles of Apc or an activated allele of Kras (Apc691 or Apc691, KrasG12D+/+) to mice bearing the VillinCreERT2 transgene (37). After induction of CRE-dependent recombination, mice were treated for 4 days with simvastatin or vehicle and with [13C]-glucose before cells were extracted and metabolite levels were analyzed by LC-MS. Relative peak intensities of isotope log levels for aspartate are shown. Data show mean ± SEM of three independent biological replicates. C, HCT116 p53−/− or p53−/− cells were grown as SPC and treated with 10 μmol/L simvastatin (SIM) or solvent (DMSO) either alone or in the presence of 0.5 mmol/L mevalonate for 72 hours. Oxygen consumption rates (OCR) were determined using the Seahorse Bioanalyzer. Oligomycin (oligo), FCCP, and rotenone/antimycin A (R/A) were added to determine ATP-dependent, maximal, and basal respiration. Data are presented as mean ± SEM of at least 14 spheroids analyzed per condition. D, HCT116 p53−/− or p53−/− cells were grown as SPC and treated with 10 μmol/L simvastatin (SIM) or solvent (DMSO) either alone or in combination with 0.5 mmol/L mevalonate (MVL), 10 μmol/L ubiquinone (CoQ10), or 5 mmol/L N-acetylcysteine (NAC) for 72 hours. Spheroids were fixed and histologic sections were analyzed for the presence of apoptotic cells by TUNEL staining. Images show representative results of three spheroids analyzed per condition. E, Quantitation of data shown in D. Data are presented as mean ± SEM of at least 3 spheroids analyzed per condition. **, P < 0.001; ****, P < 0.0001, unpaired two-tailed Student’s t test.

**Discussion**

Metabolic gradients in tumors are likely to simultaneously limit access to oxygen and nutrients, making adaptation by metabolic compensation challenging (40). One potential response of cancer cells...
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Figure 6.
Mevalonate pathway activity is essential for pyrimidine nucleotide biosynthesis and survival in colon cancer cells. A, Diagram showing the role of ubiquinone (CoQ10) in the conversion of dihydroorotate to orotate during pyrimidine biosynthesis. B, HCT116 p53⁻/⁻ or p53⁺/⁺ cells were grown as SPC and treated with 10 μmol/L simvastatin (SIM) or solvent (DMSO) for 72 hours. For the last 16 hours, cells were labeled with [U⁻¹³C]-glucose before metabolites were extracted and analyzed by LC-MS. Relative peak intensities (left) and total labeled fractions (right) for UMP are shown. Data show mean ± SEM of three independent biological replicates. *, P < 0.05; **, P < 0.01, unpaired two-tailed Student t test. C, HCT116 p53⁻/⁻ or p53⁺/⁺ cells were grown as SPC and treated with 10 μmol/L simvastatin (SIM) or solvent (DMSO) for 72 hours either alone or in combination with 0.5 mmol/L mevalonate (MV) or 10 μmol/L ubiquinone (CoQ10). For the last 16 hours, cells were labeled with [U⁻¹³C]-glucose before metabolites were extracted and analyzed by LC-MS. Relative peak intensities (left) and total labeled fractions (right) for UMP are shown. Data show mean ± SEM of three independent biological replicates. *, P < 0.05; **, P < 0.01, unpaired two-tailed Student t test; n.s., nonsignificant. D, HCT116 p53⁻/⁻ or p53⁺/⁺ cells were grown as SPC and treated with solvent (DMSO) or 10 μmol/L simvastatin (SIM) either alone or in combination with nucleosides (150 μmol/L each of cytidine, guanosine, adenosine, uridine, and 50 μmol/L of thymidine = NCL) for 72 hours. Spheroids were fixed and histologic sections were analyzed for the presence of apoptotic cells by TUNEL staining. Data are presented as mean ± SEM of at least three spheroids analyzed per condition. *, P < 0.05; **, P < 0.01, unpaired two-tailed Student t test.
Simvastatin reduces growth of p53-deficient tumor organoids and blocks proliferation in Apc/p53-deficient Kras-transformed intestinal crypts. A, Primary mouse intestinal cells derived from VillinCREERT2Apcfl/fl;KrasG12D/+; or VillinCREERT2Apcfl/fl;KrasG12D/+; animals were treated with 10 μmol/L simvastatin (SIM) either alone or in combination with 0.5 mmol/L mevalonate (MEV) for 48 hours. Images show representative microscopic fields from three independent replicate cultures. B, Quantitation of data shown in A. Data are presented as mean ± SEM of microscopic fields from three independent cultures. *P < 0.05; **P < 0.01; ***P < 0.0001, unpaired two-tailed Student t test. C, Apcfl/fl; p53fl/fl or Apcfl/fl; p53fl/fl;KrasG12D/+ organoids were treated with 10 μmol/L simvastatin either alone or in combination with 10 μmol/L ubiquinone (CoQ10) or nucleosides (150 μmol/L each of cytidine, guanosine, adenosine, uridine, and 50 μmol/L of thymidine) for 48 hours. Images show representative microscopic fields from three independent replicate cultures. D, Quantitation of data shown in C. Data are presented as mean ± SEM of microscopic fields from three independent cultures. ****P < 0.0001, unpaired two-tailed Student t test. E, VillinCREERT2Apcfl/fl and VillinCREERT2Apcfl/fl;KrasG12D/+ mice were treated with a single intraperitoneal injection of 80 mg/kg of tamoxifen on one occasion (VillinCREERT2Apcfl/fl;KrasG12D/+ or on two consecutive days (VillinCREERT2Apcfl/fl). From one day post-induction, mice were treated with a daily dose of 50 mg/kg simvastatin (in 0.5% methylcellulose/5% DMSO). After four days, mice were sacrificed and intestinal mucosa was fixed, paraffin embedded, and histologic sections were stained for BrdU incorporation. Representative images are shown. F, Three intestinal crypts for each genotype and treatment were scored for BrdU-positive cells. G, Fraction of cholesterol and ubiquinone (CoQ9) containing deuterated water in intestinal mucosa from the different genotypes. *P < 0.05 unpaired two-tailed Student t test.
to nutrient deprivation is cell-cycle arrest, which alleviates the metabolic demand of nucleotide biosynthesis for DNA replication, allowing cancer cells to survive until nutrients become available, for example, after formation of new blood vessels or engagement of metabolic symbiosis (41, 42). Using spheroid cultures (SPC) as model, we show here that p53 wt colon cancer cells respond to metabolic deprivation by reducing proliferation. In contrast, p53-deficient colorectal adenocarcinoma cells are able to maintain proliferation in the spheroid center, where nutrient and oxygen supply is restricted. Contrary to monolayer cultures, gene expression signatures in SPC are characteristic of cell-cycle arrest and induction of hypoxia, similar to those found in tumor tissue. Moreover, stable isotope tracing showed that SPC engage in hypoxic remodeling of their metabolism, with reduced glucose oxidation, enhanced lactate production, and increased TCA cycle anaplerosis from pyruvate. Pyruvate anaplerosis promotes glutamine-independent growth of cancer cells (43) and supports aspartate synthesis in succinate dehydrogenase (SDH)-deficient cancer cells (44). We found that SPC of p53-deficient colon cancer cells showed reduced aspartate levels, indicating its enhanced usage for pyrimidine biosynthesis.

Importantly, p53-deficient colorectal adenocarcinoma cells in SPC or grown as xenograft tumors increase expression of mevalonate pathway enzymes and upregulation of SREBP2 targets was observed in p53-mutant colorectal adenocarcinoma patient samples and cell lines. Previous studies have shown that mutant p53 can bind to SREBP2 and promoting its transcriptional activity during disruption of mammary tissue architecture (31), and that wt p53 represses the mevalonate pathway through ABCA1-dependent inhibition of SREBP2 processing (21). We demonstrate here that loss of p53 in SPC promotes expression of SREBP2 target genes by activating mTORC1 signaling, which drives the processing of SREBP2 (24), and by limiting the activity of GSK3, which controls the phosphorylation-dependent degradation of mature SREBP2 (28). The combination of mTORC1 activation and inhibition of GSK3 results in the accumulation of mature SREBP2 and increases the expression of its target genes.

Our study also shows that tumor-like metabolic stress alters the sensitivity of cancer cells toward mevalonate pathway inhibition. In monolayer, both genotypes were highly sensitive to mevalonate pathway inhibitors, most likely because cells require cholesterol for rapid proliferation (32). However, when exposed to metabolic stress, p53-proficient cells were largely resistant to statin treatment, while p53-deficient cancer cells showed induction of apoptosis. Cell death was restricted to the nutrient- and oxygen-deprived center of the spheroids, indicating that the mevalonate pathway provides essential metabolic functions under these conditions. Surprisingly, sensitivity toward mevalonate pathway inhibition was independent of p21, suggesting that the protective effect of wt p53 is independent of its role as transcriptional inducer of this target. Indeed, it has been shown that an acetylation-deficient form of p53 that is unable to induce p21 expression retains important tumor-suppressive functions (45).

We also demonstrate that p53-dependent metabolic rewiring of the mevalonate pathway supports the synthesis of ubiquinone, an important electron transport molecule of the ETC (46). Previous studies indicate that nutrient deprivation increases dependency of cancer cells on ETC activity (47, 48), particularly for the generation of aspartate as precursor for pyrimidine synthesis (49). DHODH, the enzyme converting dihydroorotate to orotate during UMP synthesis, requires electron transfer via ubiquinone and has been shown to support the growth of respiration-deficient tumors (39). This suggests that ubiquinone synthesis by the mevalonate pathway supports pyrimidine synthesis, particularly when efficient electron transport is hampered by low oxygen availability. Ubiquinone deprivation also induces oxidative stress, especially when demand for biosynthetic reactions that deliver electrons to the ETC is high. We found that statin-induced cell death was prevented by antioxidants or by the addition of nucleosides or uridine, which allow cells to switch to the salvage pathway, suggesting that reducing de novo pyrimidine synthesis prevents ROS formation and cell death. Furthermore, the antimetabolite 5-FU, which blocks dTMP synthesis and induces DNA and RNA damage, sensitized p53 wt SPC to statin treatment. 5-FU may impose additional strain on pyrimidine biosynthesis and/or increase oxidative stress, both of which would enhance the dependency of cancer cells on ubiquinone. While clinical trials combining statins with 5-FU in colorectal adenocarcinoma have produced some promising results (50), our study suggests that p53 status could determine the outcome of mevalonate pathway inhibition in colorectal adenocarcinoma.

The dependence of p53-deficient cancer cells on mevalonate pathway activity was also confirmed in apc/−/− intestinal tumor organoids, where deletion of p53, either alone or in combination with Kras activation, induced sensitivity toward statin treatment. Addition of ubiquinone or nucleosides restored growth of p53-deficient tumor organoids in the presence of statins, suggesting that cells require mevalonate pathway-derived ubiquinone to counteract oxidative stress and support biosynthetic reactions. Indeed, LGR5+ intestinal stem cells are enriched for gene expression signatures linked to purine and pyrimidine metabolism (51) and are highly dependent on mitochondrial metabolism (52).

We also found that statins block Kras-dependent hyperproliferation in Apc-deficient intestinal crypts in vitro, in contrast to our findings in SPC and organoids, this was associated with reduced cholesterol rather than ubiquinone synthesis. Cholesterol is required for membrane synthesis (32) and could be the major metabolic output of the mevalonate pathway in rapidly proliferating tissues. Moreover, cells within the intestinal mucosa may not be exposed to metabolic deprivation, as they have access to the nutrient-rich contents of the intestinal lumen, including nucleosides generated by the degradation of diet-derived nucleic acids.

Together, our findings reveal a novel function of the mevalonate pathway in supporting the synthesis of ubiquinone for electron transfer and pyrimidine biosynthesis in p53-deficient cancer cells exposed to environmental stress. However, our results also show that the dependence on mevalonate pathway-derived metabolites is determined by environmental context. Mevalonate pathway inhibition may therefore be most effective under conditions of nutrient and oxygen deprivation. Beneficial effects of mevalonate pathway inhibitors have already been demonstrated in several cancer entities, including colorectal adenocarcinoma (53, 54). The results of this study indicate that mevalonate pathway inhibitors may need to be combined with treatments that induce metabolic stress, such as antiangiogenic therapy.

Disclosure of Potential Conflicts of Interest

O.J. Sansom reports receiving a commercial research grant from AstraZeneca, Novartis, and Cancer Research Technology. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: I. Kaymak, O.J. Sansom, A. Schulte
Development of methodology: I. Kaymak, W. Schmitt
Acquisition of data (provided animals, collected data, provided facilities, etc.): I. Kaymak, C.R. Maier, W. Schmitt, A.D. Campbell, B. Dankworth, C.P. Ade, M. Pauwe, H. Marouf, D.M. Gay, G.H. McGregor

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Analysis and interpretation of data (e.g., statistical analysis, biosististics, computational analysis): I. Kaymak, C.R. Maier, W. Schmitz, A.D. Campbell, B. Dankworth, C.P. Ade, S. Walz, M. Pauwae, C. Kalogiourou, M.T. Rosenfeldt, D.M. Gay, A. Schulze

Writing, review, and/or revision of the manuscript: I. Kaymak, W. Schmitz, A.D. Campbell, C.R. Maier, D.M. Gay, O.J. Sansom, A. Schulze

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): I. Kaymak

Study supervision: M.T. Rosenfeldt, O.J. Sansom, A. Schulze

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