Global metabolic reprogramming of colorectal cancer occurs at adenoma stage and is induced by MYC

Kiyotoshi Satoh1, Shinichi Yachida2, Masahiro Sugimoto2, Minoru Oshima2, Toshitaka Nakagawa3, Shintaro Akamoto3, Sho Tabata3, Kaori Saitoh3, Keiko Kato3, Saya Sato4, Kaori Igarashi3, Yumi Aizawa4, Rie Kajino-Sakamoto5, Yasushi Kojima6, Teruki Fujishita4, Ayame Enomoto3, Akiyoshi Hirayama7, Takamasa Ishikawa8, Makoto Mark Taketo4, Yoshio Kushida4, Reiji Haba9, Keiichi Okano4, Masaru Tomita9, Yasuyuki Suzuki9, Shinni Fukuda9, Masahiro Aoki8, and Tomoyoshi Soga1,10

1Institute for Advanced Biosciences, Keio University, Kakuganjii, Tsuruoka 997-0052, Japan; 2National Cancer Center Research Institute, Chuo-ku, Tokyo 104-0045, Japan; 3Gastroenterological Surgery, Faculty of Medicine, Kagawa University, Kagawa 761-0793, Japan; 4Life Science Center, Kagawa University, Kagawa 761-0793, Japan; 5Division of Molecular Pathology, Aichi Cancer Center Research Institute, Chikusa-Ku, Nagoya, Aichi 464-8861, Japan; and 6Department of Pharmacology, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan

Edited by Tak W. Mak, The Campbell Family Institute for Breast Cancer Research at Princess Margaret Cancer Centre, University Health Network, Toronto, Canada, and approved August 9, 2017 (received for review June 9, 2017)

Cancer cells alter their metabolism for the production of precursors of macromolecules. However, the control mechanisms underlying this reprogramming are poorly understood. Here we show that metabolic reprogramming of colorectal cancer is caused chiefly by aberrant MYC expression. Metabolomics-based analyses of paired normal and tumor tissues from 275 patients with colorectal cancer revealed that metabolic alterations occur at the adenoma stage of carcinogenesis, in a manner not associated with specific gene mutations involved in colorectal carcinogenesis. MYC expression induced at least 215 metabolic reactions by changing the expression levels of 121 metabolic genes and 39 transporter genes. Further, MYC negatively regulated the expression of genes involved in mitochondrial biogenesis and maintenance but positively regulated genes involved in DNA and histone methylation. Knockdown of MYC in colorectal cancer cells reset the altered metabolism and suppressed cell growth. Moreover, inhibition of MYC target pyrimidine synthesis genes such as CAD, UMPS, and CTPS blocked cell growth, and thus are potential targets for colorectal cancer therapy.

Significance

Metabolic reprogramming is one of the hallmarks of cancer. However, the underlying mechanisms that regulate cancer metabolism are poorly understood. Here we performed metabolomics-based analysis of paired normal–tumor tissues from patients with colorectal cancer, which revealed that the protooncogene protein MYC regulated global metabolic reprogramming of colorectal cancer by modulating 215 metabolic reactions. Importantly, this metabolic reprogramming occurred in a manner not associated with specific gene mutations in colorectal carcinogenesis. For many years, small-molecule or biologic inhibitors of MYC have been required. Here we demonstrate that knockdown of MYC downstream pyrimidine synthesis genes contributes to the suppression of colorectal cancer cell proliferation similar to MYC, and thus pyrimidine synthesis pathways could be potential targets for colorectal cancer therapy.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission. Freely available online through the PNAS open access option.

Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, https://www.ncbi.nlm.nih.gov/geo (accession nos. GSE89076, GSE89077, and GSE87693).

1To whom correspondence should be addressed. Email: soga@fc.keio.ac.jp.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1710366114/-/DCSupplemental.
Fig. 1. Metabolite levels are altered at the adenoma stage of colorectal tumors. (A) Heat map of metabolite levels in paired normal and tumor tissues obtained from 275 patients with CRC. Each metabolite was normalized by dividing by the median of the normal tissue. Data colored in the red–white–blue scheme indicate a relatively higher, average, and lower concentration, respectively. Data are horizontally arranged by Union for International Cancer Control (UICC) cancer staging (seventh edition) and vertically arranged by the fold change in median values of paired tumor and normal tissue. N and T indicate normal and paired tumor tissues, respectively. Ad and Stg indicate adenoma and stage, respectively. (B) Score plots of principal component analysis of normal (green) and tumor colorectal tissue (red) based on metabolome data (n = 274 each). Samples were grouped by UICC cancer staging. (C and D) Comparison of metabolite levels in normal and tumor tissues (n = 274) at each stage (adenoma, n = 5; stage 0, n = 2; stage I, n = 36; stage II, n = 101; stage III, n = 85; and stage IV, n = 45). PCA of the PC1 and PC2 values for normal (blue) and tumor tissue (red) at each stage. Data outside the 5 and 95 percentiles were plotted as dots. Error bars represent standard deviation. (D) Levels of representative metabolites in normal and tumor tissues at each stage.
tissue (Fig. 1 and SI Appendix, Fig. S1). Glucose was the second most decreased metabolite in tumor tissue, whereas lactate was increased (Fig. 1 and SI Appendix, Fig. S1), implying activation of glycolysis, termed the Warburg effect (1). Interestingly, every amino acid except glutamine, the main substrate in glutaminolysis, was significantly accumulated in tumor tissue (SI Appendix, Fig. S1).

CRC progression is associated with mutations in oncogenes and tumor suppressor genes such as APC, KRAS, and TP53 (12). We applied next-generation and Sanger sequencing technologies to detect somatic mutations in cancer-related genes in the tumor tissue. The mutation frequencies in APC, TP53, and KRAS were 76, 68, and 46%, respectively (Fig. 2A), which are comparable to those in previous reports (13–15). Other mutations were found throughout the cancer stages, but these major mutations were not associated with metabolite levels in the CRC tissue (Fig. 2B and C).

Next, we applied transcriptomic analysis to paired normal and tumor tissues and observed overexpression of LAT1, the proto-oncogene MYC, and inflammatory cytokine genes in CRC tissue (Fig. 3A). Similar to the metabolome data (Fig. 1A), the expression levels of metabolic genes were constant throughout the stages (Fig. 3B). Taken together with the results of our metabolomic and transcriptomic analysis, we propose that the metabolic shift occurs at the adenoma stage of CRC, although further validation on an independent and larger cohort of normal/CRC samples particularly at this earliest stage is necessary.

**Analyses of Tissue from Apc Mutant Mice.** Because human clinical samples are heterogeneous entities, we performed metabolome and DNA microarray analyses on normal and adenomatous tissue obtained from the large intestines of Apc−/−/Apc−/−/Apc−/− mice, a genetically engineered mouse model of familial adenomatous polyposis that develops benign adenomas in the intestines (16), and on normal tissue from wild-type C57BL6/6N mice. Considerable differences were observed in metabolite levels (SI Appendix, Fig. S2A and B) that correlated with those found in the CRC tissue (SI Appendix, Fig. S2C). The transcriptome data were also similar to the clinical samples, demonstrating abnormal expression of Myc and inflammatory cytokine genes in adenoma tissue (SI Appendix, Fig. S3).

**Aberrant MYC Expression Correlates with Metabolic Reprogramming.** MYC is one of the most frequently deregulated oncogenes and is estimated to regulate the expression of 15% of all genes (17, 18), including various metabolic genes (19, 20). In cancerous cells, deregulation of MYC expression occurs via many mechanisms (19, 21). We found that MYC expression was up-regulated in all cancer stages, including adenomas, irrespective of the presence or absence of Apc mutations (Fig. 3C and D). We then explored metabolic genes that were co-regulated with MYC (Spearman rank-order correlation coefficient: > 0.4) (Fig. 3E) and identified 231 unique metabolic genes (SI Appendix, Table S3). Consistently, partial correlation analysis showed no significant direct relationship between MYC and a specific metabolic gene (SI Appendix, Table S3). The results indicate that MYC expression is a highly correlated expression of a variety of metabolic genes.

The 231 genes were involved in a total of 346 metabolic reactions and included transporters in major metabolic pathways, including purine/pyrimidine synthesis, the pentose phosphate pathway, MAPK signaling pathway, and fatty acid oxidation pathway (Fig. 3F and SI Appendix, Table S3). Among them, almost all metabolic genes of the de novo purine/pyrimidine synthesis pathway were up-regulated, correlating with MYC expression (Fig. 3G and SI Appendix, Fig. S4A and B). Several genes in the glycolysis and pentose phosphate pathways were up-regulated, whereas those in the TCA cycle were down-regulated with aberrant MYC expression (SI Appendix, Fig. S4C). Many genes involved in fatty acid synthesis were also up-regulated, while those participating in fatty acid oxidation were down-regulated in CRC tissue (SI Appendix, Fig. S4C).

One-carbon metabolism involving the folate and methionine cycles has attracted attention as a driver of oncogenesis (22). Nine one-carbon metabolism genes and three genes related to one-carbon transport, including SLC25A42 (mitochondrial folate transporter: MFT), SLC7A5 (LAT1), and SLC7A8 (LAT2), were highly expressed in conjunction with MYC expression (SI Appendix, Fig. S4C). In addition, MYC expression is likely to be associated with DNA and histone methylation activity through increases in one carbon-related metabolites and genes [i.e., SAM (Fig. 1L), DNMT1, DNMT3B (correlation coefficient with MYC: > 0.309)] and histone-lysine N-methyltransferase enzyme (EZH2) and a decrease in TET2 DNA demethylase (Fig. 3H). Various genes involved in amino acid metabolism were also up- or down-regulated together with aberrant MYC expression (SI Appendix, Fig. S4C).

Consistent with the transcriptome data, we observed increased levels of lactate, the final product of glycolysis, and many metabolic intermediates in de novo purine and pyrimidine synthesis in the tumor tissues. In addition, most of the metabolites in one-carbon metabolism-related pathways, including serine, one-carbon and transsulfuration metabolism (SI Appendix, Fig. S1), and the products of fatty acid synthesis (i.e., palmitate and oleate) were significantly increased (Fig. 3I).

**Aberrant MYC Expression Reduces Mitochondrial Homeostasis.** PINK1, a central regulator gene for mitochondrial maintenance, and a master autophagy regulator gene, TFEB (correlation coefficient with MYC: > 0.367), demonstrated the highest inverse correlation with MYC (Fig. 3E and 4A), implying inhibition of mitophagy in tumor tissue. The expression of PGC-1α, a master regulator of mitochondrial biogenesis (23, 24), was also inversely correlated with MYC (Fig. 3E), and the expression levels of these genes were markedly reduced in tumor tissues (Fig. 4A).

Although mitochondrial content was little altered (Fig. 4B and C), transmission electron microscopy (TEM) revealed an accumulation of abnormal mitochondria in cancerous (Fig. 4D–F) and adenomatous tissues (Fig. 4G), with severe mitochondrial swelling, disappearance of cristae, and matrix clearing. We found that the expression of acetyltransferase gene, a regulator of mitochondrial biogenesis, was increased in CRC tissue (Fig. 4H). Interestingly, every amino acid except glutamine, the main substrate in glutaminolysis, was hypermethylated (Fig. 4I) and thus had a decreased expression level (Fig. 4J), resulting in suppression of PGC-1α expression (Fig. 4A).

**MYC Knockdown Resets Metabolism and Suppresses Cell Growth.** To further establish the role of MYC in metabolic reprogramming, we suppressed MYC expression by siRNA in the human colorectal cancer cell line (Fig. 5A). MYC knockdown dramatically changed the expression signatures of genes involved in major metabolic pathways, transporters, and mitochondrial biogenesis/maintenance, as well as those related to DNA and histone methylation (Fig. 5 and SI Appendix, Figs. S5 and S6). The levels of LAT1, LAT2, DNMT3B, and EZH2 decreased, while the level of TET2 was elevated in MYC-knockdown cells (Fig. 5A and B and SI Appendix, Fig. S5B). The expression levels of most metabolic genes highly correlated with MYC in CRC tissue were reversed when MYC was inhibited by siRNAs in HCT116 human colorectal carcinoma cells (Fig. 5C and SI Appendix, Figs. S5A and S6), which indicates that almost all of these gene expressions are regulated by MYC. Overall, we found that MYC regulated at least 215 metabolic reactions in major metabolic pathways, including de novo purine/pyrimidine synthesis and one-carbon metabolism, controlling 121 metabolic genes and 39 transporters (Fig. 6 and SI Appendix, Table S3). Regarding glucose metabolism, aberrant
MYC expression activated glycolysis through up-regulation of GPI, PFKM, ENO1, and LDHB (Figs. 5C and 6C and SI Appendix, Fig. S5A) and down-regulation of PEPCK expression (Figs. 5C and 6C and SI Appendix, Fig. S5A), the rate-limiting enzyme in gluconeogenesis, suggesting that MYC expression induces the Warburg effect.

Fig. 2. Colorectal cancer metabolic reprogramming occurs in a manner not associated with specific gene mutations involved in colorectal carcinogenesis. (A) Mutations in oncogenes and tumor suppressor genes (adenoma, n = 5; stage 0, n = 1; stage I, n = 8; stage II, n = 8; stage III, n = 11; and stage IV, n = 8). Mutated tissues are indicated as colored boxes. Hatched boxes indicate genes not determined. (B) The effect of major mutations on the levels of representative metabolites, namely glucose, lactate, and SAM. Although the levels of glucose, lactate, and SAM in normal and tumor tissues (n = 41) were significantly different, there were no significant differences between wild-type tumor tissue (red) and mutated tumor tissue (brick). (C) PCA of wild-type tumor tissue (open circles) and mutated tumor tissue (filled circles) based on metabolome data. Kruskal–Wallis and Dunn’s posttest (8). ***P < 0.001, **P < 0.01, and *P < 0.05.
Fig. 3. MYC regulates global metabolic reprogramming of colorectal cancer. (A) Ranking of genes expressed in colorectal tumor tissue compared with paired normal tissue. (B) Heat map of gene expression levels in metabolic pathways obtained from 41 paired normal and tumor colorectal tissues (Top) and mutations in APC, β-catenin, KRAS, NRAS, BRAF, and TP53 (Bottom Right). Mutated tissue samples are indicated as colored boxes. These samples were collected from 39 patients; of these, 2 patients provided one normal and two tumor samples at different disease stages. (C and D) Gene expression levels of MYC in normal and tumor colorectal tissues obtained by DNA microarray and the mutation status of APC and β-catenin at each cancer stage. Samples with mutations are depicted as filled boxes. (E) Ranking of metabolism-related genes that were positively (black) or inversely (blue) correlated with MYC expression (Spearman rank-order correlation coefficient: $r^2 > 0.4$). Ranking (green) and median and 95% confidence intervals of bootstrap analyses of each rank (black and orange) are shown. (F, Left) A total of 172 metabolic reactions are regulated by 116 unique metabolic genes showing a positive correlation with MYC expression. (F, Right) A total of 174 metabolic reactions are regulated by 119 unique metabolic genes showing an inverse correlation with MYC. (G) Heat map of expression levels of metabolic genes involved in purine and pyrimidine biosynthesis pathways in normal and tumor colorectal tissue. Genes highlighted in orange have correlation coefficients ($r^2$) greater than 0.4 for MYC. (H) DNA microarray analysis of the expression levels of genes involved in DNA methylation in normal and tumor colorectal tissue. (I) LC-MS/MS analysis of palmitate and oleate levels in normal (blue) and tumor (red) colorectal tissue ($n = 44$ each). The Wilcoxon signed-rank test was used to determine statistical significance (D, H, and I). ***$P < 0.001$. Satoh et al. PNAS Early Edition | 5 of 10
Fig. 4. *MYC* is involved in the transcriptional regulation of mitochondrial biosynthesis and maintenance. (A) DNA microarray analysis of the expression levels of *PINK1*, *ATG4A*, and *PGC-1α* and *PGC-1β* in normal (blue) and tumor (red) colorectal tissue. (B) The ratio of mitochondrial DNA (mtDNA) to nuclear DNA determined by qRT-PCR analysis of paired normal and tumor tissues obtained from 11 patients with CRC (adenoma, n = 2; stage I, n = 3; stage II, n = 2; stage III, n = 2; and stage IV, n = 2). (C) Immunohistochemistry for TOMM20 (a mitochondrial outer-membrane marker) and COX IV (a mitochondrial inner-membrane marker) of normal and tumor tissue samples obtained from a CRC patient in stage II. Sections were counterstained with hematoxylin. (Scale bars, 100 μm.) (D–G) TEM images of paired normal and tumor tissues obtained from a colorectal cancer patient at stage IIIb (D and E), stage II (F), and adenoma stage (G). (D, Lower) Higher magnification of boxed areas (Upper). Arrows indicate abnormal mitochondria. (H and J) DNA microarray analysis of the expression levels of *GCN5* (H) and *IRF4* (J) in normal and tumor colorectal tissues. (I) MeDIP-seq of the promoter region of the *IRF4* gene, a transcription factor for *PGC-1α* in normal (n = 9) and tumor (n = 11) colorectal tissues. The promoter region of *IRF4* was hypermethylated through the adenoma stage. The arrow, red boxes, and black lines indicate the transcription start site, exons, and introns, respectively. The Wilcoxon signed-rank test was used to assess statistical significance (A, H, and J). ***P < 0.001.
Subsequently, we analyzed changes in intra- and extracellular metabolite levels between control and MYC-knockdown HCT116 cells. When MYC was suppressed, intra- and extracellular glucose levels increased, while lactate levels decreased (Fig. 7 A and B); this pattern is known as the “reverse Warburg effect.” Additionally, the levels of many metabolic intermediates were consistently decreased in MYC-knockdown HCT116 cells, including those involved in serine synthesis, one-carbon metabolism (Fig. 7C), the start of de novo purine/pyrimidine metabolism, and amino acid metabolism (SI Appendix, Fig. S7). However, several metabolites, such as 3PG, citrate, Asp, Glu, and Pro, showed inconsistent patterns (SI Appendix, Figs. S1 and S7). A recent study demonstrated that bacterial communities were different between normal and CRC tissues (27). This different microbe composition might be associated with the inconsistent results of the metabolites. Principal component analysis (PCA) showed that the metabolic profiles of CRC tumor tissue, ApoE+/-Δ716 adenomatous tissue, and HCT116 cells were similarly shifted toward the positive PC1 direction (Fig. 7 D and E). Taken together, these results suggest an essential role for MYC in metabolic reprogramming through the regulation of important metabolic genes.

As described above, PGC-1α (28) and PGC-1β (29), MYC target genes, have been proposed to be master regulators of cancer metabolism (24, 30). We therefore investigated the possible involvement of PGC-1α and PGC-1β in global regulation of colorectal cancer metabolism. However, knockdown of PGC-1α...
and PGC-1β caused little alteration in metabolic gene expression in a colorectal normal cell line (Fig. 7 F and G), leading us to conclude that MYC is the master regulator of colorectal metabolism. Knockdown of MYC considerably reduced growth of HCT116 and RKO human colon carcinoma cells (Fig. 7 H–K). Moreover, we investigated several MYC downstream metabolic genes (SI Appendix, Table S3) and observed that in addition to TYMS, a target enzyme of 5-fluorouracil (Fig. 6C), knockdown of

![Diagram](image-url)
pyrimidine synthesis genes such as **CAD**, the rate-limiting enzyme in de novo pyrimidine synthesis, **UMPS**, and **CTPS** (Fig. 6B) blocked HCT116 and RKO cell proliferation (Fig. 7H–K). However, knockdown of purine synthesis genes such as **PPAT**, the rate-limiting enzyme in de novo purine synthesis, **GART**, and **ATIC** (Fig. 6B) had no significant effect on their proliferation (Fig. 7H–K).

**Discussion**

Our multiomics analyses of paired normal and tumor tissues from patients with CRC and tissues from **Apc** mutant mice highlight the critical role of MYC in reprogramming CRC tissue metabolism. We obtained clear evidence that MYC regulates global metabolic reprogramming of colorectal tumor metabolism through the modulation of 215 major metabolic reactions, controlling 121 metabolic genes and 39 transporters (**SI Appendix**, Table S3), and facilitates production of cellular building blocks. MYC also reprograms several cellular processes, including those modulating mitophagy and DNA/histone methylation. Knockdown of **MYC** in colorectal cancer cells can reset the altered metabolism and suppress cell growth. MYC can drive cell proliferation, and our current data alone may not formally exclude the possibility that MYC indirectly regulates the metabolic genes through its effect on proliferation. However, MYC has been

---

Satoh et al. PNAS Early Edition | 9 of 10
demonstrated to directly control transcription of various key metabolic genes, including LDH-A and CAD (31, 32). We thus propose that MYC is the master regulator of colorectal tumor metabolism and an attractive therapeutic target.

MYC is a target gene of the Wnt signaling pathway, and thus MYC overexpression was caused by APC or β-catenin mutations in most of the colorectal tumor tissues (Fig. 3C). However, interestingly, elevated MYC expression occurred even without these mutations (Fig. 3C). In addition to Wnt signaling, MYC deregulation in cancer has been as a consequence of several abnormalities, including gross genetic abnormalities and aberrant activity of transcriptional factors, PI3K/AKT/mTOR signaling pathways, receptor tyrosine kinases, hormones, and growth factors (19, 21). Therefore, we speculate that these factors may induce MYC expression, resulting in metabolic reprogramming of colorectal cancer metabolism.

Here we propose that a sufficient nutrient supply in the pre-cancerous stage may be indispensable for cancer development and growth. These findings may have implications for future cancer prevention and therapeutic approaches targeting MYC-regulated metabolism. Unfortunately, however, finding small-molecule or biologic inhibitors of MYC has proved difficult because MYC is localized within the nucleus and does not have a deep surface-binding pocket (33). Therefore, MYC is not amenable to blockade by small molecules or accessible to neutralization by antibodies.

Here we have demonstrated that knockdown of MYC or MYC target pyrimidine synthesis genes such as CAD, UMPS, and CTPS, but not purine synthesis genes, can suppress colorectal cancer cell proliferation (Fig. 7 H–K). This provides the foundation for a potential anticancer strategy in which pyrimidine synthesis pathways downstream of MYC could be an alternative target for colorectal cancer therapy.

**Materials and Methods**

**Clinical Samples.** We conducted all experiments according to a study protocol approved by the Institutional Ethics Committee of Kagawa University (Heisei 24-040), upon obtaining informed consent from all subjects. The tumor and surrounding grossly normal-appearing tissue were obtained from 275 colorectal cancer patients at the time of surgery. The normal tissues were obtained from colorectal mucosa. Regarding the tumor tissues, to minimize the effect of other cells, we excluded CRC tissues with excessive stroma or infiltrating lymphocytes using hematoxylin-eosin staining. Clinicopathologic information is listed in SI Appendix, Table S1.

**Mouse Strains.** Construction of an Apc<del>1438-776</del> strain has been described previously (34). The strain was backcrossed to the C57BL/6N background for >20 generations. C57BL/6N mice were purchased from CLEA Japan. Mice were kept under a 12-hour light–dark cycle at ~22 °C and fed ad libitum with a CLEA CE-2 chow diet. All animal experiments were conducted according to protocols approved by the Animal Care and Use Committee of the Aichi Cancer Center Research Institute.

**ACKNOWLEDGMENTS.** We thank Kumi Suzuki for technical assistance, and Dr. Josephine Galpin for critical reading and English editing of the manuscript. This work was partially supported by AMED-CREST from the Japan Agency for Medical Research and Development (AMED) (S.Y., M.A., and T.S.), a research program of the Project for Development of Innovative Research on Cancer Therapeutics (P-Direct) and AMED (K.O. and T.S.), as well as research funds from the Yamagata prefectural government and the City of Tsuruoka.