DNA AND CELL BIOLOGY
Volume 38, Number 4, 2019

Pp. 281–285
DOI: 10.1089/dna.2018.4579

DNACB BIT

microRNA-Mediated Tumor–Microbiota Metabolic Interactions in Colorectal Cancer

Ce Yuan[1,2] and Subbaya Subramanian[1,2]

Worldwide, colorectal cancer (CRC) is one of the leading causes of cancer-related deaths. Recent advances in high-throughput technologies have shown that the gut microbiota may have a major influence on human health, including CRC. Nonetheless, how the gut microbiota interacts with tumor cells in CRC patients is largely unknown. Studies have shown that the microbiota fills in a variety of niche metabolic pathways that the host does not possess. For example, the microbiota produces butyrate, which provides the colon's epithelial cells with about 70% of their energy needs. The typically fast proliferation of tumor cells in CRC patients drastically alters the tumor's nutrient microenvironment. Those alterations correspond to the microbiota composition and functional changes. In tumor cells, a central mediator of metabolic changes is the aberrant expression of microRNAs (miRNAs). In this study, we explored recent insights into metabolic interactions between the microbiota and tumor cells in CRC pathobiology, focusing on the role of miRNAs. These observations support our view that miRNAs may also serve as mediators of the metabolites' effects.

Keywords: host–microbiota interactions, microbiota, metabolism, metabolites, high-throughput technologies, colorectal cancer, microRNAs

Introduction

In the United States, colorectal cancer (CRC) is the third most commonly diagnosed type of cancer and the second most frequent cause of cancer-related deaths (Siegel *et al.*, 2018). In 2018, an estimated 140,250 people will be diagnosed with CRC, and 50,630 will die from it. More than a third of CRC patients will not be alive 5 years after their diagnosis. In recent years, our understanding of the microorganisms living in the intestines (collectively called the microbiota) has grown; we now know that the microbiota plays an important role in many diseases, including CRC (Burns *et al.*, 2015; Nakatsu *et al.*, 2015). An average human's intestine contains more than 10^{14} microorganisms, including commensal bacteria, pathogenic bacteria, viruses, and fungi.

The gut microbiota actively metabolizes undigested food and substances shed from the intestinal cells, thereby generating energy and sending vital nutrients back to the host (Louis *et al.*, 2014). Without the microbiota, the colon's epithelial cells will undergo autophagy and will fail to maintain their structure (Donohoe *et al.*, 2011). In the normal colon, epithelial cells primarily use butyrate as energy. Tumor cells, however, require a large amount of glucose as their energy source to sustain growth, creating a large amount of lactate as the end product in the tumor microenvironment. In addition, to support the formation of new cell membranes, the tumor has increased needs for lipid biogenesis. So the change in the energy source preferred by proliferating tumor cells profoundly alters the nutrient composition of the tumor microenvironment.

In recent years, researchers have found a consistent connection between a dysfunctional gut microbiota (dysbiosis) and CRC (Shen *et al.*, 2010; Wang *et al.*, 2012; Burns *et al.*, 2015; Nakatsu *et al.*, 2015). Yet the directionality and the mediators between CRC and dysbiosis remain unclear (Yuan *et al.*, 2018a). Given the drastic changes in the nutrient composition of the tumor microenvironment and the role of the microbiota in metabolism, there is undoubtedly a metabolic interaction between the tumor and its microbiota. In this study, we explored recent insights into metabolic interactions between the microbiota and tumor cells in CRC patients, focusing on the impact of microRNAs (miRNAs). We hypothesized that miRNAs are the mediators of the metabolites' effects (Fig. 1A).

Tumor Nutrient Microenvironment Changes

The development of CRC entails a complex interplay between the epithelial cells, the microbiota, and the immune system in the tumor microenvironment (Tjalsma *et al.*, 2012), and multiple signaling pathways play critical roles in

[1]Bioinformatics and Computational Biology Program and [2]Department of Surgery, University of Minnesota, Minneapolis, Minnesota.

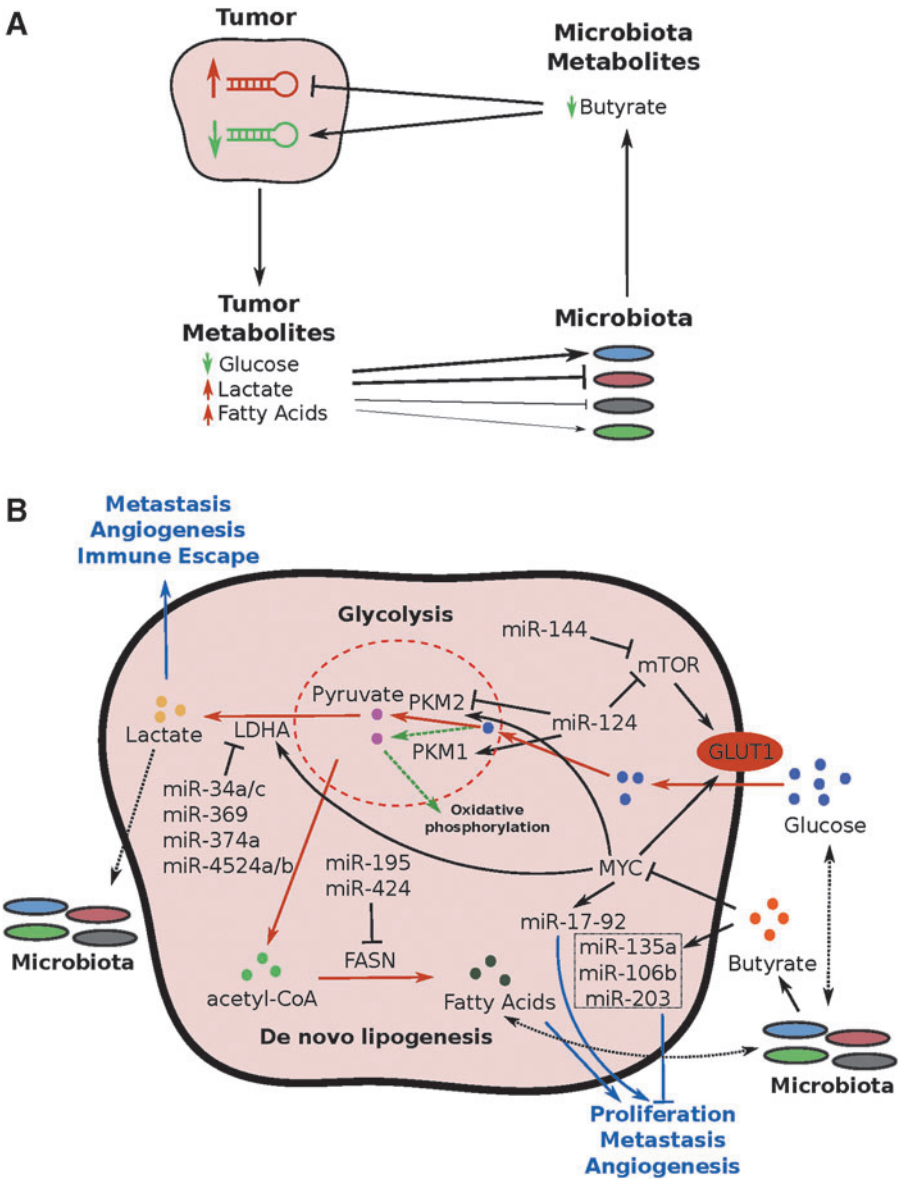

FIG. 1. Tumor–microbiota metabolic interactions. **(A)** An overview of the tumor–microbiota metabolic interactions. The thickness of the line connecting tumor metabolites and microbiota shows the relative effects of tumor metabolites on microbiota composition. **(B)** A curated map of miRNA-mediated tumor–microbiota metabolic interactions. *Red lines* indicate the pathway upregulated in colorectal cancer relative to normal tissue. *Blue lines* indicate the overall downstream effects of the miRNAs or metabolites. *Dotted green lines* indicate the oxidative phosphorylation common in the normal cells. *Dotted black lines* indicate potential effects. miRNA, microRNA.

both tumorigenesis and tumor progression. Tumor metabolism changes have been well studied and characterized. One of the hallmarks is an increase in glycolysis as the primary energy source, known as the Warburg effect (Warburg, 1956).

Several studies have found altered metabolite levels in both tissues and stools of CRC patients. In tissue samples (as compared with adjacent normal tissues), glucose levels were significantly lower, whereas levels of lactate and fatty acids were significantly higher (Hirayama *et al.*, 2009; Weir *et al.*, 2013; Brown *et al.*, 2016). In stool samples of CRC patients, amino acid levels were higher than normal; levels of fatty acids, lower (Hirayama *et al.*, 2009; Weir *et al.*, 2013; Brown *et al.*, 2016). This nutrient composition change in CRC patients correspond to the tumor's increased needs of glucose for energy and fatty acids for proliferation.

miRNAs play a critical role in regulating the metabolism of CRC patients and in sustaining the needs of tumor cells (Fig. 1B). The extracellular glucose is first transported into cells through the glucose transporter 1 (*GLUT1*) receptor, which is a downstream target of the mammalian target of rapamycin (*mTOR*) gene. In CRC patients, the *mTOR* gene is regulated by miR-144. Higher expression of miR-144 inhibits expression of the *mTOR* gene, leading to reduced glucose uptake by the tumor cells; thus, higher expression of miR-144 is associated with a good prognosis for CRC patients (Iwaya *et al.*, 2012).

After the glucose is transported into the cytosol, it undergoes glycolysis—a process regulated by the alternative splicing of pyruvate kinase (*PK*). Higher levels of two *PK* isoforms, M1 (*PKM1*) and M2 (*PKM2*), in cells will lead to increased glycolysis, instead of oxidative phosphorylation (Sun *et al.*, 2012; Taniguchi *et al.*, 2015a, 2015b). These studies have found that overexpressing miR-124 (another regulator of the *mTOR* gene) in CRC cells can lead to higher *PKM1:PKM2* ratios, thus inhibiting glycolysis and control tumor cell growth. The end product of glycolysis, pyruvate, will then be metabolized into lactate by lactate

dehydrogenase A (*LDHA*), which is commonly upregulated in CRC patients. *LDHA* is a rate-limiting enzyme of glycolysis. A loss in *LDHA* expression is thus associated with decreased adenosine triphosphate (ATP) production and cell proliferation (Wang *et al.*, 2015). In CRC cell lines, various miRNAs—including miR-34a/c, miR-369-3p, miR-374a, and miR-4524a/b—have been shown to inhibit *LDHA* expression (Wang *et al.*, 2015). The lactate produced by the tumor cells can function as signaling molecules that further affect tumor cell metastasis, angiogenesis, and immune escape (Hirschhaeuser *et al.*, 2011).

In addition to altered glucose metabolism, CRC cells also have altered macromolecule metabolism. We postulate that the reason for the higher levels of fatty acids in CRC patients' tissue samples (relative to their stool samples) is the increased need for membrane synthesis to support cell proliferation (Hirayama *et al.*, 2009; Weir *et al.*, 2013; Brown *et al.*, 2016). One of the most important genes controlling this pathway is the fatty acid synthase (*FASN*) gene. The enzyme encoded by the *FASN* gene is critical for controlling the synthesis of lipids, a process required for cell membrane formation. In breast cancer and osteosarcoma, studies have found that miR-195 and miR-424 target the *FASN* gene, thus inhibiting cell proliferation, invasion, and metastasis (Mao *et al.*, 2012; Long *et al.*, 2013; Singh *et al.*, 2015). Both of those miRNAs are significantly upregulated in CRC tissues, according to the Cancer Genome Atlas (TCGA) data set, suggesting such miRNA-mediated lipogenesis may also happen in CRC. In addition, because *FASN* is potentially important to T cell immunity (Buck *et al.*, 2015), the dynamics of the miR-424/*FASN* axis in tumor and immune cell function are currently being worked out. Other pathways with downstream effects on metabolism, such as *PTEN* and *AKT/PI3K*, are also modulated by miRNAs (Song *et al.*, 2008; Schee *et al.*, 2013; Fang *et al.*, 2014; Wang *et al.*, 2014; Wei *et al.*, 2014). Based on current evidence, it is clear that aberrant miRNA expression in CRC cells profoundly alters the nutrient composition of the tumor microenvironment.

Regulation of Host miRNAs by Microbiota Metabolites

In the healthy intestinal tract, the microbiota is dominated by the Bacteroidetes and Firmicutes phyla, which together comprise about 70% of the microbiota (Burns *et al.*, 2015). Several taxa of bacteria have been implicated in the microbiota of CRC patients. In their stool samples, at the species level, a consistently higher abundance of *Bacteroides fragilis* and *Fusobacterium nucleatum* has been found. A higher abundance of the Bacteroidetes phylum and a lower abundance of the Firmicutes phylum have been observed in CRC patients. A recent meta-analysis of various CRC microbiota data sets found, for the tissue-associated microbiota, a consistently higher abundance of *F. nucleatum, Parvimonas,* and *Streptococcus*; nine studies in that meta-analysis found a consistently lower abundance of *Faecalibacterium* and *Ruminococcaceae* (Shah *et al.*, 2018).

As mentioned previously, the microbiota is a powerhouse of metabolite production. It fills in many niche metabolic pathways that are not present in the human host. The gut microbiota produces about 70% of the energy required by the intestinal epithelial cells in the form of butyrate. Butyrate belongs to the short-chain fatty acids (SCFAs) that are produced by the gut microbiota through the fermentation of complex carbohydrates. In addition to being the major fuel source for normal intestinal epithelial cells, butyrate also functions as a histone deacetylase inhibitor (HDACi). This function is especially important in CRC patients, in part because the tumor cells switch from using butyrate to glucose as the major source of energy: the Warburg effect (Warburg, 1956). In CRC cells, high butyrate concentrations reduce *MYC* expression, which in turn reduces the levels of the miR-17-92 cluster miRNAs (Hu *et al.*, 2015). The overexpression of miR-17-92a cluster in CRC cells has been shown to lead to cell proliferation, metastasis, and angiogenesis (Dews *et al.*, 2010; Zhang *et al.*, 2014; Ke *et al.*, 2015). These suggest *MYC*/miR-17-92a cluster mediate butyrate's antitumor function in CRC cells. Butyrate also exerts antiproliferation effects on CRC *in vitro*, through directly regulating the miR-203, miR-106b, and miR-135a expression (Schlörmann *et al.*, 2015; Han *et al.*, 2016). Other members of the SCFA family, acetate and propionate, also act as HDACi. Because acetate and propionate can pass through the epithelial cells, they can exert their effect in T cells in the tumor microenvironment, by regulating the *mTOR* pathway (Park *et al.*, 2015). Those effects suggest that butyrate and other SCFA members might help slow CRC progression through modulating tumor miRNA expression and the function of tumor-infiltrating T cell. Unfortunately, in the CRC microbiota, the fecal SCFA levels and the butyrate-producing bacteria levels are all lower than in the normal microbiota (Weir *et al.*, 2013; Yuan *et al.*, 2018a).

Conclusions and Perspectives

Our current knowledge supports the belief that gut microbes can alter tumor cells in CRC patients through the metabolites being produced. These metabolites can affect miRNA expression in the tumor cells, leading to alterations in many important signaling pathways. According to recent evidence, metabolites mediate interactions between the host's microbiota and tumor cells. Our view that miRNAs are the mediators of the metabolites' effects is supported by both experimental and computational findings, as reviewed earlier (Fig. 1B).

Currently, no tissue-level data set on the microbiota's metabolites exists. So, our laboratory recently used a nonhuman primate model to analyze global interactions between the microbiota and metabolites in healthy intestines (Yuan *et al.*, 2018b). Doing so has brought us one step closer to finally understanding the metabolic interactions between the microbiota and the host. Of note, miRNAs are part of a complex highly dynamic web of interactions. Other recent studies suggest that miRNAs can directly affect the growth of bacteria and that tumor cells' miRNAs can affect the stromal and immune cells in the tumor microenvironment (Zhuang *et al.*, 2012; Kohlhapp *et al.*, 2015; Liu *et al.*, 2016; Teng *et al.*, 2018). A recent effort to develop a mouse model with humanized microbiota may further propel the field of tumor–microbiota metabolic interactions (Staley *et al.*, 2017). Future research will require incorporating humanized microbiota animal models with a dual approach

that leverages high-throughput genomics and metabolomics technologies.

Acknowledgments

We thank Dr. Mary Knatterud for assisting in article preparation. Because of space restrictions, we cannot cite many other significant contributions made by numerous researchers and laboratories in this potentially important and rapidly progressing field. S.S. is supported by research grants funded by the NIH R03CA219129 and C.Y. by the MnDrive—University of Minnesota Informatics Institute graduate fellowship.

Authors' Contributions

C.Y. and S.S. conceived the idea and wrote the article.

Disclosure Statement

The authors have no competing financial interests.

References

Brown, D.G., Rao, S., Weir, T.L., O'Malia, J., Bazan, M., Brown, R.J., *et al*. (2016). Metabolomics and metabolic pathway networks from human colorectal cancers, adjacent mucosa, and stool. Cancer Metab **4,** 11.

Buck, M.D., O'Sullivan, D., and Pearce, E.L. (2015). T cell metabolism drives immunity. J Exp Med **212,** 1345–1360.

Burns, M.B., Lynch, J., Starr, T.K., Knights, D., and Blekhman, R. (2015). Virulence genes are a signature of the microbiome in the colorectal tumor microenvironment. Genome Med **7,** 55.

Dews, M., Fox, J.L., Hultine, S., Sundaram, P., Wang, W., Liu, Y.Y., *et al.* (2010). The myc-miR-17 ~ 92 axis blunts TGF{beta} signaling and production of multiple TGF{beta}-dependent antiangiogenic factors. Cancer Res **70,** 8233–8246.

Donohoe, D.R., Garge, N., Zhang, X., Sun, W., O'Connell, T.M., Bunger, M.K., *et al*. (2011). The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metab **13,** 517–526.

Fang, L., Li, H., Wang, L., Hu, J., Jin, T., Wang, J., *et al*. (2014). MicroRNA-17-5p promotes chemotherapeutic drug resistance and tumour metastasis of colorectal cancer by repressing PTEN expression. Oncotarget **5,** 2974–2987.

Han, R., Sun, Q., Wu, J., Zheng, P., and Zhao, G. (2016). Sodium butyrate upregulates miR-203 expression to exert antiproliferation effect on colorectal cancer cells. Cell Physiol Biochem **39,** 1919–1929.

Hirayama, A., Kami, K., Sugimoto, M., Sugawara, M., Toki, N., Onozuka, H., *et al.* (2009). Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry. Cancer Res **69,** 4918–4925.

Hirschhaeuser, F., Sattler, U.G.A., and Mueller-Klieser, W. (2011). Lactate: a metabolic key player in cancer. Cancer Res **71,** 6921–6925.

Hu, S., Liu, L., Chang, E.B., Wang, J.-Y., and Raufman, J.-P. (2015). Butyrate inhibits pro-proliferative miR-92a by diminishing c-Myc-induced miR-17-92a cluster transcription in human colon cancer cells. Mol Cancer **14,** 180.

Iwaya, T., Yokobori, T., Nishida, N., Kogo, R., Sudo, T., Tanaka, F., *et al.* (2012). Downregulation of miR-144 is associated with colorectal cancer progression via activation of mTOR signaling pathway. Carcinogenesis **33,** 2391–2397.

Ke, T.-W., Wei, P.-L., Yeh, K.-T., Chen, W.T.-L., and Cheng, Y.-W. (2015). MiR-92a promotes cell metastasis of colorectal cancer through PTEN-mediated PI3K/AKT pathway. Ann Surg Oncol **22,** 2649–2655.

Kohlhapp, F.J., Mitra, A.K., Lengyel, E., and Peter, M.E. (2015). MicroRNAs as mediators and communicators between cancer cells and the tumor microenvironment. Oncogene **34,** 5857–5868.

Liu, S., da Cunha, A.P., Rezende, R.M., Cialic, R., Wei, Z., Bry, L., *et al*. (2016). The host shapes the gut microbiota via fecal microrna. Cell Host Microbe **19,** 32–43.

Long, X.H., Mao, J.H., Peng, A.F., Zhou, Y., Huang, S.H., and Liu, Z.L. (2013). Tumor suppressive microRNA-424 inhibits osteosarcoma cell migration and invasion via targeting fatty acid synthase. Exp Ther Med **5,** 1048–1052.

Louis, P., Hold, G.L., and Flint, H.J. (2014). The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Microbiol **12,** 661–672.

Mao, J.H., Zhou, R.P., Peng, A.F., Liu, Z.L., Huang, S.H., Long, X.H., *et al*. (2012). microRNA-195 suppresses osteosarcoma cell invasion and migration in vitro by targeting FASN. Oncol Lett **4,** 1125–1129.

Nakatsu, G., Li, X., Zhou, H., Sheng, J., Wong, S.H., Wu, W.K.K., *et al.* (2015). Gut mucosal microbiome across stages of colorectal carcinogenesis. Nat Commun **6,** 8727.

Park, J., Kim, M., Kang, S.G., Jannasch, A.H., Cooper, B., Patterson, J., *et al*. (2015). Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. Mucosal Immunol **8,** 80–93.

Schee, K., Lorenz, S., Worren, M.M., Günther, C.-C., Holden, M., Hovig, E., *et al*. (2013). Deep sequencing the microRNA transcriptome in colorectal cancer. PLoS One **8,** e66165.

Schlörmann, W., Naumann, S., Renner, C., and Glei, M. (2015). Influence of miRNA-106b and miRNA-135a on butyrate-regulated expression of p21 and Cyclin D2 in human colon adenoma cells. Genes Nutr **10,** 50.

Shah, M.S., DeSantis, T., Yamal, J.-M., Weir, T., Ryan, E.P., Cope, J.L., *et al*. (2018). Re-purposing 16S rRNA gene sequence data from within case paired tumor biopsy and tumor-adjacent biopsy or fecal samples to identify microbial markers for colorectal cancer. PLoS One **13,** e0207002.

Shen, X.J., Rawls, J.F., Randall, T., Burcal, L., Mpande, C.N., Jenkins, N., *et al*. (2010). Molecular characterization of mucosal adherent bacteria and associations with colorectal adenomas. Gut Microbes **1,** 138–147.

Siegel, R.L., Miller, K.D., and Jemal, A. (2018). Cancer statistics, 2018. CA Cancer J. Clin **68,** 7–30.

Singh, R., Yadav, V., Kumar, S., and Saini, N. (2015). MicroRNA-195 inhibits proliferation, invasion and metastasis in breast cancer cells by targeting FASN, HMGCR, ACACA and CYP27B1. Sci Rep **5,** 17454.

Song, B., Wang, Y., Kudo, K., Gavin, E.J., Xi, Y., and Ju, J. (2008). miR-192 regulates dihydrofolate reductase and cellular proliferation through the p53-microRNA circuit. Clin Cancer Res **14,** 8080–8086.

Staley, C., Kaiser, T., Beura, L.K., Hamilton, M.J., Weingarden, A.R., Bobr, A., *et al*. (2017). Stable engraftment of human microbiota into mice with a single oral gavage following antibiotic conditioning. Microbiome **5,** 87.

Sun, Y., Zhao, X., Zhou, Y., and Hu, Y. (2012). miR-124, miR-137 and miR-340 regulate colorectal cancer growth via inhibition of the Warburg effect. Oncol Rep **28,** 1346–1352.

Taniguchi, K., Sugito, N., Kumazaki, M., Shinohara, H., Yamada, N., Matsuhashi, N., *et al.* (2015a). Positive feedback of DDX6/c-Myc/PTB1 regulated by miR-124 contributes to maintenance of the Warburg effect in colon cancer cells. Biochim Biophys Acta **1852,** 1971–1980.

Taniguchi, K., Sugito, N., Kumazaki, M., Shinohara, H., Yamada, N., Nakagawa, Y., *et al.* (2015b). MicroRNA-124 inhibits cancer cell growth through PTB1/PKM1/PKM2 feedback cascade in colorectal cancer. Cancer Lett **363,** 17–27.

Teng, Y., Ren, Y., Sayed, M., Hu, X., Lei, C., Kumar, A., *et al.* (2018). Plant-derived exosomal MicroRNAs shape the gut microbiota. Cell Host Microbe **24,** 637–652.e8.

Tjalsma, H., Boleij, A., Marchesi, J.R., and Dutilh, B.E. (2012). A bacterial driver-passenger model for colorectal cancer: beyond the usual suspects. Nat Rev Microbiol **10,** 575–582.

Wang, J., Wang, H., Liu, A., Fang, C., Hao, J., and Wang, Z. (2015). Lactate dehydrogenase A negatively regulated by miRNAs promotes aerobic glycolysis and is increased in colorectal cancer. Oncotarget **6,** 19456–19468.

Wang, T., Cai, G., Qiu, Y., Fei, N., Zhang, M., Pang, X., *et al.* (2012). Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. ISME J **6,** 320–329.

Wang, Y., Tang, Q., Li, M., Jiang, S., and Wang, X. (2014). MicroRNA-375 inhibits colorectal cancer growth by targeting PIK3CA. Biochem Biophys Res Commun **444,** 199–204.

Warburg, O. (1956). On the origin of cancer cells. Science **123,** 309–314.

Wei, Z., Cui, L., Mei, Z., Liu, M., and Zhang, D. (2014). miR-181a mediates metabolic shift in colon cancer cells via the PTEN/AKT pathway. FEBS Lett **588,** 1773–1779.

Weir, T.L., Manter, D.K., Sheflin, A.M., Barnett, B.A., Heuberger, A.L., and Ryan, E.P. (2013). Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults. PLoS One **8,** e70803.

Yuan, C., Burns, M.B., Subramanian, S., and Blekhman, R. (2018a). Interaction between host MicroRNAs and the gut microbiota in colorectal cancer. MSystems **3,** e00205-17.

Yuan, C., Graham, M., and Subramanian, S. (2018b). Microbiota-metabolites interactions in non-human primate gastrointestinal tract. BioRxiv. [Epub ahead of print]; DOI: 10.1101/454496.

Zhang, G., Zhou, H., Xiao, H., Liu, Z., Tian, H., and Zhou, T. (2014). MicroRNA-92a functions as an oncogene in colorectal cancer by targeting PTEN. Dig Dis Sci **59,** 98–107.

Zhuang, G., Wu, X., Jiang, Z., Kasman, I., Yao, J., Guan, Y., *et al.* (2012). Tumour-secreted miR-9 promotes endothelial cell migration and angiogenesis by activating the JAK-STAT pathway. EMBO J **31,** 3513–3523.

Address correspondence to:
Subbaya Subramanian, MS, PhD
Department of Surgery
University of Minnesota
11-212 Moos Tower
Mayo Mail Code 195
420 Delaware Street SE
Minneapolis, MN 55455

E-mail: subree@umn.edu

Received for publication December 12, 2018; received in revised form December 12, 2018; accepted December 12, 2018.