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Living with It:

A Review of the Host / Microbiome Interactions

By J.F. Treml

Our understanding of gut / microbiome interactions has developed rapidly in the new century. This in no small part due to the increased availability of next generation sequencing and gene chip techniques that allow for a greater understanding of the makeup of our microbiota and the molecular mechanisms that mediate the impact these organisms have on their hosts. One unexpected finding is the magnitude of influence our diet and environment have on the diversity and richness of the gut microbiome. The benefits and harms of these diets extend well beyond the intestinal environment via the production of microbial products which enter the host's circulation or other direct interactions influencing allergy, obesity, type 2 diabetes, hypertension, and pulmonary inflammation. The following reviews highlight recent advances in our understanding of the microbiome and its effects in health and disease. They are further informed by a required undergraduate journal club course at the University of Kansas' Edwards Campus for which, the Spring 2024 reading list can be found following the reviews.

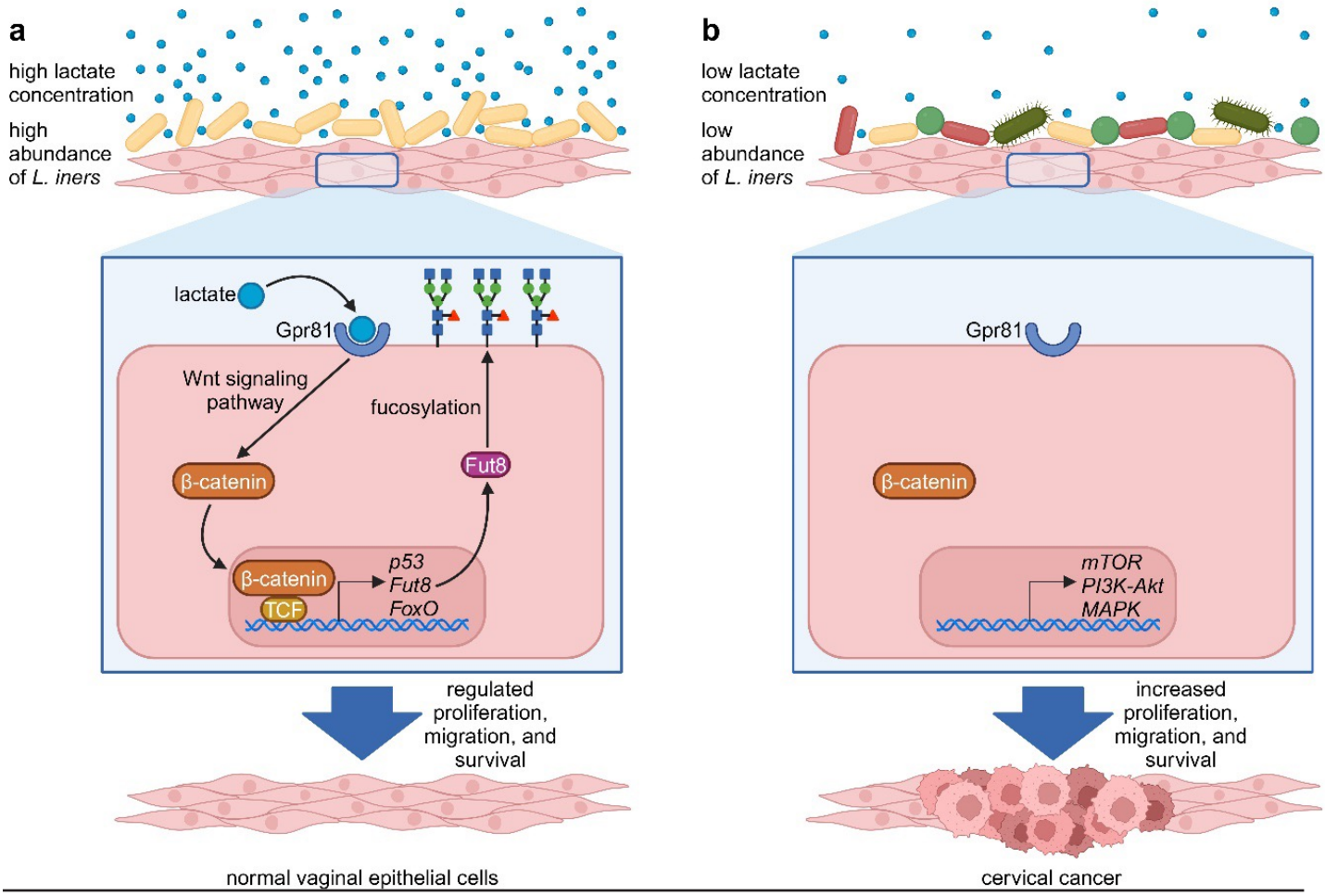
Giving Cancer Cells a Taste of Their Own Medicine: Lactic Acid from Vaginal Microbiota Protects Against Cancer

By Kaitlyn Sy

Cancer is a leading cause of morbidity and mortality worldwide, with cervical cancer ranking among the top four most common malignant tumors in females. Tumor microenvironments surrounding solid tumors, including cervical cancer, have often been found to contain high concentrations of lactic acid due to a preference for aerobic glycolysis, termed the Warburg effect. Several studies have implicated lactic acid in solid tumors' ability to evade immune defenses, but lactic acid's role in the human body is far from being one-sided. Lactobacillus species, the most common bacteria in the vaginal tract, have long been known to play a protective role in the vaginal microbiota by secreting lactic acid that inhibits the growth of pathogens. Recent research by Fan et al. uncovered that the beneficial effects of lactic acid produced by Lactobacillus species not only prevent vaginal infections but also protect against cervical cancer by regulating the fucosylation of vaginal epithelial cells. Hence, vaginal Lactobacillus gives cervical cancer cells a taste of their own medicine: using high doses of the very molecule that cancer cells may use to evade the immune system to suppress the cancer cells' growth.

Among females, cervical cancer ranks among the top four malignant tumors in both morbidity and mortality.[1] Consequently, understanding the processes that lead to cervical cancer and developing preventive measures and treatments is vital.

Cancer is characterized by uncontrolled proliferation of abnormal cells. It is a complex disease, affecting not only the malignant cells but also modulating the function of the surrounding cells. Solid tumors, including cervical cancer, have long been known to secrete lactic acid into the tumor microenvironment (TME) as a byproduct of their shift from aerobic respiration to fermentation, a phenomenon known as the Warburg effect.[2,3] Lactic acid quickly dissociates into lactate and H+. Lactate concentrations as high as 40 mM and pH levels as low as [5,6] have been reported in the TME, compared to the <3 mM lactate and pH 7.4 generally found under normal physiological conditions.[2] Lactic acid in the TME has been implicated in solid tumors' ability to evade immune defenses. For example, recent studies have reported that acidic conditions and high concentrations of lactate could independently impact the immune responsiveness of T cells,[4,5] and others have reported that tumor-derived lactate affects the phenotypes of natural killer cells6 and dendritic cells.[7] Despite the harmful effects of lactic acid on immune responsiveness in the TME, the role of lactic acid in the body is far from being one-sided, as lactic acid plays beneficial roles as well. Lactobacillus species, the predominant bacteria in the vaginal tract, have long been known to play a protective role in the vaginal microbiota by secreting lactic acid that inhibits the growth of pathogens.[8] In individuals where lactobacilli dominate the vaginal microbiota, pH levels around 3.5 and lactate concentrations above 110 mM are common.[9] Interestingly, the beneficial effects of lactobacilli seem to extend beyond protection against vaginal infections but also encompass anti-cancer defenses. Cervical cancer patients have been reported to be colonized by significantly fewer lactobacilli compared to healthy females, resulting in vaginal dysbiosis,[10] and changes in the vaginal mi-



crobiota have been proposed as a diagnostic marker for cervical cancer,[11] although neither a causal connection nor mechanism of action for the protective effects of lactobacilli against cervical cancer have been determined. However, groundbreaking research published by Fan et al. recently uncovered that lactic acid produced by Lactobacillus iners, a predominant member of the vaginal microbiota, protects against cervical cancer by regulating the fucosylation of epithelial cells in the vaginal mucosa.[12] Consistent with prior reports, Fan et al. observed that, compared to healthy fe-

Figure 1 | L. iners regulates fucosylation of vaginal epithelial cells and protects against cervical cancer.
(a) Lactobacillus species, especially L. iners, dominate the vaginal microbiota and secrete lactic acid that inhibits the growth of other bacteria. The lactate activates the Wnt signaling pathway via the lactate-Gpr81 complex, leading to the activation of TCF/β-catenin, which enhances the transcription of Fut8, p53, and genes involved in the FoxO signaling pathway. Fut8 mediates the fucosylation of vaginal epithelial cells, suppressing their transformation to a malignant phenotype and thereby inhibiting the growth of cervical cancer. **(b)** When the abundance of Lactobacillus species is decreased in a dysbiotic vagina, the deficiency of lactate prevents the normal fucosylation of vaginal epithelial cells. This leads to the upregulation of genes involved in the mTOR, PI3K-Akt, and MAPK signaling pathways, resulting in increased proliferation, migration, and survival, which could ultimately lead to cervical cancer if left unchecked. in HCMV-infected cells.

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males, cervical cancer patients had lower levels of protein core fucosylation in both cervical tissue and serum. Fucosylation is a post-translational modification comprising the attachment of a fucose residue to N-glycans and O-glycans most commonly mediated by α-1,6 fucosyltransferase (Fut8)[13] and has been reported to be decreased in cervical cancer patients, compared to healthy females.[14] To assess the role of fucosylation in cervical cancer, Fan et al. knocked out Fut8 in SiHa cells, a cell line derived from uterine tissue collected from a 55-year-old female patient with squamous cell carcinoma. Fut8-/- SiHa cells displayed increased migration as determined by scratch assay compared to Fut8+/+ SiHa cells, a phenotype reversible via the reintroduction of Fut8. Transcriptomic analyses revealed that knockout of Fut8 led to changes in gene expression in SiHa cells. Genes involved in the TNF signaling pathway were downregulated, whereas genes involved in the mTOR, PI3K-Akt, and MAPK signaling pathways and migration were upregulated. These changes in gene expression were predicted to lead to a more malignant phenotype, characterized by decreased apoptosis and increased growth, proliferation, migration, and survival. Fan et al. verified these effects in vivo by injecting SiHa cells into athymic female mice. Fut8-/- SiHa cells produced larger and heavier tumors than Fut8+/+ SiHa cells. Together, these results indicate that Fut8 plays a regulatory role in inhibiting the growth and progression of cervical cancer.

Considering that fucosylation of mucosal epithelial cells had previously been linked to the microbiota,[15] Fan et al. sought to elucidate the connections between the vaginal microbiota, fucosylation of epithelial cells in the vaginal mucosa, and cervical cancer. 16S rRNA sequencing showed that Lactobacillus was the only dominant bacterial genus in the vaginal microbiota of healthy females, whereas Clostridium, Staphylococcus, and Bacteroides dominated the vaginal microbiota of cervical cancer patients. Consistent with prior reports, the abundance of Lactobacillus—and especially L. iners—was significantly decreased in cervical cancer patients, compared to healthy females. This vaginal dysbiosis resulted in significantly higher vaginal pH and lower lactate levels in cervical cancer patients, due to decreased abundance of Lactobacillus metabolites. To assess the effects of L. iners metabolites on the growth of cervical cancer, Fan et al. treated SiHa cells with the supernatant and lysate of L. iners, resulting in decreased proliferation, central carbon metabolism, and survival. Transcriptomic analyses of SiHa cells indicated that L. iners metabolites upregulated IL-17, p53, Fut8, and genes involved in the FoxO signaling pathway. In vivo, abundance of L. iners in the vagina was positively correlated with serum levels of core fucosylation, suggesting that L. iners metabolites regulate the activity of Fut8.

In a previous study, this same research group reported that microbiota can upregulate core fucosylation of epithelial cells by activating the Wnt pathway,[16] so they sought to trace the mechanism by which L. iners metabolites regulate Fut8. While treatment of SiHa cells with L. iners metabolites increased the activity of TCF/β-catenin (transcription factors that significantly enhance the activity of Fut8), treatment with L. iners metabolites and DKK-1 (an inhibitor of the Wnt pathway) negated these effects, leading Fan et al. to suspect that lactic acid produced by L. iners activates the Wnt pathway via the lactate-Gpr81 complex. To test this hypothesis, Fan et. al compared levels of core fucosylation, wnt3, and β-catenin in SiHa cells treated with L. iners metabolites with and without 3-OBA, an antagonist of Gpr81. While SiHa cells treated with L. iners metabolites alone displayed increased levels of wnt3, β-catenin, and core fucosylation, these effects were negated in SiHa cells treated with L. iners and 3-OBA, supporting the authors' hypothesis.

The results reported by Fan et al. piece together a clear picture of the connections between the vaginal microbiota, fucosylation of epithelial cells in the vaginal mucosa, and cervical cancer (Figure 1). Lactobacillus species, especially L. iners, normally dominate the vaginal microbiota and secrete lactic acid that inhibits the growth of other bacteria. The lactate activates the Wnt signaling pathway via the lactate-Gpr81 complex, leading to the activation of TCF/β-catenin, which enhances the transcription of Fut8, p53, and genes involved in the FoxO signaling pathway. Fut8 mediates the fucosylation of vaginal epithelial cells, suppressing their transformation to a malignant phenotype and thereby inhibiting the growth of cervical cancer. When the abundance of Lactobacillus species is decreased in a dysbiotic vagina, the deficiency of lactate prevents the normal fucosylation of vaginal epithelial cells. This leads to the upregulation of genes involved in the mTOR, PI3K-Akt, and MAPK signaling pathways, resulting in increased proliferation, migration, and survival, which could ultimately lead to cervical cancer if left unchecked.

With these results in mind, several questions arise. For example, via what mechanisms does fucosylation of epithelial cells in the vaginal mucosa protect against cervical cancer? Or, considering the immunosuppressive effects of lactate, how do the high concentrations of lactate in a normobiotic vagina affect immune responsiveness in this area of the body? Although these questions are not addressed by Fan et al. in this paper, their discovery of the pivotal role of Lactobacillus species in protecting against cervical cancer has several implications. Considering the potentially harmful consequences of a dysbiotic vagina, clinical tests to assess the composition of the vaginal microbiota may be a useful measure for preventive screening and diagnosis of cervical cancer. Additionally, returning a dysbiotic vagina to normobiosis may have potential as a treatment for cervical cancer, alone or alongside other therapeutics. Conversely, providers must exercise caution when prescribing antibiotics for patients with cervical cancer or risk for cervical cancer, as microbial shifts in the vagina could not only lead to opportunistic infections but could also increase the chance of cancerous growth.

In conclusion, while questions remain to be answered, this study by Fan et al. significantly expanded the current understanding of the connections between the vaginal microbiota and cervical cancer, paving the way for future research.

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