Editorial Commentary

Peroxisome proliferator-activated receptor gamma (PPAR-γ) activation through gut microbiota modulation as a novel therapeutic approach against anastomotic leak after colorectal cancer surgery

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Comment on: Hajjar R, Oliero M, Fragoso G, *et al.* Modulating Gut Microbiota Prevents Anastomotic Leak to Reduce Local Implantation and Dissemination of Colorectal Cancer Cells after Surgery. Clin Cancer Res 2024;30:616-28.

Keywords: Anastomotic leak (AL); colorectal cancer (CRC); peroxisome proliferator-activated receptor gamma (PPAR-γ); microbiota

Submitted Apr 28, 2024. Accepted for publication Sep 13, 2024. Published online Oct 14, 2024.
doi: 10.21037/jgo-24-314
View this article at: https://dx.doi.org/10.21037/jgo-24-314

Colorectal cancer (CRC) is one of the most common cancers worldwide with a high economic burden associated. It represents about 10% of all cancer causes and, despite the advances in diagnosis and treatment, it is associated with high rates of morbidity and mortality remaining as the second leading cause of cancer-related deaths (1). Although several efforts have been made to understand the mechanisms involved in CRC development and progression, the exact etiology of CRC remains uncertain (2). The majority of CRC cases occur sporadically, with lifestyle factors such as obesity, consumption of diets rich in fat and/or red and processed meat, toxic habits (smoking and alcohol abuse), and environmental pollution being linked to the onset and progression of CRC (3). In addition, alterations in gut microbiota profiles characterized by an increase in opportunistic pathogens alongside reduced levels of beneficial butyrate-producing bacteria such as Bifidobacteria or *Faecalibacterium prausnitzii*, have been associated with the development and progression of CRC (3,4).

Surgical resection remains the gold-standard treatment for patients with CRC, which involves the removal of the affected segment of the colon or rectum along with an adequate margin of healthy issue. This typically requires the reconnection (anastomosis) of the healthy ends of the gastrointestinal tract to restore continuity (5). However, postoperative complications are not uncommon in these patients, including wound infections or bleeding, which contribute to increased morbidity and mortality rates. Among these complications, anastomotic leak (AL) remains one of the most challenging following CRC surgery. AL refers to the unpredicted breakdown or failure of the surgical connection between two segments of the intestine. It is associated with severe events such as permanent stoma formation, worse prognosis, reduced quality of life and increased cancer recurrence together with lower overall survival (OS) rates (6). The estimated rates of AL vary widely, ranging from 2% to 30% depending on factors such as the site of anastomosis, patient comorbidities, and the selected surgical procedure. This variability underscores the multifactorial nature of the problem (6,7). AL results from a complex, dynamic interplay of several factors, including individual genetics, the immune system, and the

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gut microbiota (8,9). All this underscores the need for a thorough understanding of the underlying mechanisms in anastomotic healing, as well as the risk factors that favor the occurrence of AL. Such insights are crucial for developing individualized preventive strategies and optimizing management.

Despite advances in treatment and improvements in surgery techniques, which have led to a reduction in cancer recurrence after CRC surgery, high rates of both local (at the anastomotic site and surrounding tissues) and distant recurrence persist (10). In this context, the occurrence of local recurrences is particularly significant, as it emphasizes the potential role of surgical technique and wound healing in preventing disease relapse. However, the exact role of AL in increased recurrence, as well as the mechanisms involved, remains unclear.

In a recent study published by Hajjar *et al.*, insights into the pathophysiology of AL in CRC were provided, along with the identification of novel strategies for its management and prevention (11). The authors revealed that patients who experienced AL after undergoing CRC surgery had lower OS and recurrence-/progression-free survival (RFS/PFS) compared to those without AL, independently of the site of anastomosis. Furthermore, these results remained consistent even after adjusting for confounding factors such as age, sex, cancer stage, or body mass index. Additionally, patients with AL exhibited higher systemic inflammation rates, as evidenced by significantly higher levels of C-reactive protein compared to those without AL. These patients with AL also had a lower probability of stoma reversal and a decreased rate of stoma closure at 5 years post-surgery, which might indicate that the aforementioned inflammatory response may play a key role in suboptimal recovery. Furthermore, patients with AL showed recurrent or progressive tumors in both proximal and distal CRC, affecting various sites including the colon, peritoneum, liver or lungs, lymph nodes, bone, and skin. These results were in line with previous studies suggesting that the occurrence of AL is associated with an increased risk of local recurrence, reduced OS, and disease-free survival (12,13). Overall, these findings demonstrated the detrimental effects of AL on the postoperative prognosis and quality of life of CRC patients, highlighting the urgent need for strategies to prevent and manage AL.

To further explore the potential mechanisms linking AL to cancer recurrence, the authors performed a series of animal studies using an experimental model of AL. First, they analyzed the impact of AL on local CRC recurrence. For that purpose, the authors used mice underwent colonic surgery, where the colotomy was closed with either six interrupted sutures or 10 sutures, creating two different levels of anastomotic healing. Following surgery, mice were administered murine CT26 carcinoma cells into the colon via enema. The control group received CT26 cells without undergoing surgery. The results revealed that while the control group showed no tumor incidence, mice with poor anastomotic healing (6 sutures) exhibited a higher number and larger abdominal tumors compared to those with improved healing. Furthermore, mice with weaker anastomoses showed a higher prevalence of hemorrhagic ascites, leading to increased cancer burden and a higher peritoneal cancer index. Interestingly, no spontaneous leaks were observed in any of the groups. These results suggest that poor anastomotic healing may delay the healing process, thereby increasing the risk of AL and local recurrence. Overall, these findings highlight the importance of optimizing surgical techniques to ensure adequate anastomotic healing and therefore, reduce AL risk and local recurrence.

The peroxisome proliferator-activated receptor gamma (PPAR-γ) is involved in the regulation of different physiological processes such as adipocyte differentiation, glucose homeostasis and inflammation (14-16). Dysregulation of PPAR-γ signaling has been described to play a critical role in inflammatory bowel disease (IBD) and, more recently, in CRC, where has been associated with increased intestinal inflammation and tumor progression (17). In a previous study, the same authors suggested that the reduction of PPAR-γ activation driven by alterations in gut microbiota profiles could underlie the observed causal relationship gut microbiota and anastomotic healing (18,19). This led the authors to hypothesize that modulation of the gut microbiota targeting PPAR-γ modulation prior to CRC surgery might represent a potential promising therapeutic strategy for reducing AL occurrence. In order to explore this proof-of-concept therapeutic strategy, the authors carried out a series of elegant *in vitro* and animal studies. To confirm whether the microbiota of patients who developed AL was able to reduce the activation of the PPAR-γ, a mouse model of fecal microbiota transplantation (FMT) from patients who did or did not develop AL was used. Notably, mice colonized using samples from AL patients showed lower activation of PPAR-γ confirming the author's previous results. These findings have opened a new window of opportunity to develop a preoperative therapeutic strategy based on the use of a low-risk dietary supplement

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of inulin targeting this pathway. Inulin is a commonly used prebiotic that serves as substrate for commensal bacteria of the gut microbiota that are known to be beneficial for host health such as *Bifidobacterium* and *Lactobacillus* (20). These bacteria are responsible for metabolizing inulin in the gut into different short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate. Since inulin is not metabolized by the host but by gut bacteria, any potential effects resulting from its supplementation are likely due to some of the metabolites produced during fermentation. Butyrate, for example, is a microbial metabolite derived from the fermentation of dietary fibers, including inulin, and is well-known for its beneficial effects on colonic health, such as anti-inflammatory activity. For instance, in this study butyrate was able to reduce the proliferation of different CRC cell lines (HT-29 human CRC cells and CT26 murine CRC cells), and to inhibit the activation of the inflammatory nuclear factor kappa B (NF-κB) pathway while promoting the activation of the PPAR-γ pathway. These finding support the hypothesis that butyrate exerts both antineoplastic and anti-inflammatory effects in CRC cells.

To assess the potential impact of preoperative supplementation on anastomotic healing, a murine model of poor healing (six sutures after colonic surgery) was used. Mice were divided into three groups: one receiving a control diet, another receiving a control diet supplemented with 10% inulin, and a third receiving a control diet supplemented with 0.6% 5-aminosalicylic acid (5-ASA), a PPAR-γ activator commonly used for IBD treatment. The mice were fed their respective diets for 14 days prior to undergoing colonic surgery. Following surgery, CT26 cells were administered via enema, and the mice were monitored for 2 weeks. Supplementation with either inulin or 5-ASA resulted in a lower number of smaller and less disseminated abdominal tumors, along with a decreased prevalence of hemorrhagic ascites compared to the control group. Additionally, mice receiving inulin or 5-ASA exhibited an improved gut barrier function, as evidenced by a reduction in bacteria translocation, as measured by the number of bacteria detected in the spleen. This effect on gut barrier function was further confirmed in a model of healthy mice supplemented with the same diets followed by administration of fluorescein isothiocyanate-dextran (FITC-dextran) to assess gut permeability. Both 5-ASA and inulin supplementation led to a decrease in the fluorescent signal in plasma samples along with an increase in fecal samples compared to the control group, which indicates a reduction in intestinal permeability.

The disruption of the gut microbiota has been recognized as a promoter of CRC progression and has emerged as a significant contributor to CRC metastasis (21). Indeed, the translocation of gut bacteria has been suggested to play a role in the occurrence and progression of metastasis, although the underlying mechanisms remain to be fully explored (21). The effect of inulin and 5-ASA supplementation were further explored in a mouse model of surgically-induced liver metastasis. Prior to undergoing open abdominal surgery with intrasplenic injection of CT26 cells, the animals received the dietary supplementation for 2 weeks. The results showed that supplementation with inulin and 5-ASA reduced the burden of hepatic colorectal tumors and the peritoneal carcinomatosis index, indicating decreased tumor dissemination. Furthermore, these mice showed lower translocation of gut bacteria to mesenteric lymph nodes indicating, increased gut barrier integrity.

Finally, inulin and 5-ASA supplementation demonstrated to have an impact on systemic inflammation, as evidenced by reduced serum levels of pro-inflammatory cytokines such as interleukin 1β and tumor necrosis factor α. In addition, exposure to serum from these mice led to lower levels of NF-kB activation in monocytic THP-1 cells harboring the luciferase reporter gene. These results reinforce the potential of inulin and 5-ASA to alleviate systemic inflammation and colorectal metastatic disease to the liver.

In summary, this study highlights the association between AL and worsened CRC postoperative outcomes, including reduced OS and disease-free survival. In addition, the finding shed light on the critical role of the gut microbiota and PPAR-γ pathway in inflammation, anastomotic healing, and CRC progression and recurrence. The study underscores a possible link between gut microbiota composition and the risk of AL development after CRC surgery, highlighting the therapeutic potential of low-risk dietary interventions aimed at modulating gut microbiota. Dietary supplementation with inulin or 5-ASA was able to promote gut barrier integrity, improved anastomotic healing, reduced local tumor spread and mitigated systemic inflammation. These effects may potentially contribute to a reduction in disease recurrence and metastasis. Overall, this study highlights the intricate interplay among AL, gut microbiota and PPAR-γ in CRC progression and recurrence, paving the way toward the development of dietary therapeutic strategies aimed to modulate gut microbiota and activate PPAR-γ pathway as a novel approach to improve postoperative CRC outcomes.

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It is important to note that despite these promising results found in animal models of AL the findings of the study may not be generalizable to all CRC patients due to variations in diet, genetics, and other environmental factors that influence gut microbiota. To address this limitation, the same authors have registered an ongoing human clinical trial (NCT05860322) to validate these results in a clinical setting. The outcome of this trial will help confirm the effects of preoperative dietary supplementation with inulin on anastomotic leakage and systemic inflammation in CRC patients.

Acknowledgments

Funding: This study was supported by the Institute of Health Carlos III (ISCIII) (award No. PI23/00171); a PFIS predoctoral fellowship from the ISCIII (FI21/00003 to M.A.M.S.; Fondo Europeo de Desarrollo Regional-FEDER); and the "Miguel Servet" program of the ISCIII (CP23/00051 to M.A.N.S. and CP19/00098 to B.R.M., respectively; Fondo Europeo de Desarrollo Regional-FEDER).

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Journal of Gastrointestinal Oncology*. The article has undergone external peer review.

Peer Review File: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-24-314/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-24-314/coif). B.R.M. reports that this study was supported by the Institute of Health Carlos III (award No. PI23/00171). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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References

1. Morgan E, Arnold M, Gini A, et al. Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. Gut 2023;72:338-44.
2. Dougherty MW, Jobin C. Intestinal bacteria and colorectal cancer: etiology and treatment. Gut Microbes 2023;15:2185028.
3. Martínez-Sánchez MA, Núñez-Sánchez MÁ, Balaguer-Román A, et al. Gut Microbiome Modification through Dietary Intervention in Patients with Colorectal Cancer: Protocol for a Prospective, Interventional, Controlled, Randomized Clinical Trial in Patients with Scheduled Surgical Intervention for CRC. J Clin Med 2022;11:3613.
4. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Microbiol 2014;12:661-72.
5. Tsalikidis C, Mitsala A, Mentonis VI, et al. Predictive Factors for Anastomotic Leakage Following Colorectal Cancer Surgery: Where Are We and Where Are We Going? Curr Oncol 2023;30:3111-37.
6. Zarnescu EC, Zarnescu NO, Costea R. Updates of Risk Factors for Anastomotic Leakage after Colorectal Surgery. Diagnostics (Basel) 2021;11:2382.
7. Foppa C, Ng SC, Montorsi M, et al. Anastomotic leak in colorectal cancer patients: New insights and perspectives. Eur J Surg Oncol 2020;46:943-54.
8. Liu Y, Li B, Wei Y. New understanding of gut microbiota and colorectal anastomosis leak: A collaborative review of the current concepts. Front Cell Infect Microbiol 2022;12:1022603.
9. Martínez-Montoro JI, Martínez-Sánchez MA, Balaguer-Román A, et al. Dietary modulation of gut microbiota in patients with colorectal cancer undergoing surgery: A review. Int J Surg 2022;104:106751.
10. Qaderi SM, Galjart B, Verhoef C, et al. Disease recurrence after colorectal cancer surgery in the modern era: a population-based study. Int J Colorectal Dis 2021;36:2399-410.
11. Hajjar R, Oliero M, Fragoso G, et al. Modulating Gut Microbiota Prevents Anastomotic Leak to Reduce Local

Implantation and Dissemination of Colorectal Cancer Cells after Surgery. Clin Cancer Res 2024;30:616-28.
12. Brito da Silva F, Lopes P, Cavadas D, et al. The impact of anastomotic leakage after curative colon cancer resection on long-term survival: A retrospective cohort study. Cir Esp (Engl Ed) 2024;102:3-10.
13. Karim A, Cubas V, Zaman S, et al. Anastomotic leak and cancer-specific outcomes after curative rectal cancer surgery: a systematic review and meta-analysis. Tech Coloproctol 2020;24:513-25.
14. Sun L, Ji S, Xie X, et al. Deciphering the interaction between Twist1 and PPARγ during adipocyte differentiation. Cell Death Dis 2023;14:764.
15. Gutting T, Weber CA, Weidner P, et al. PPARγ-activation increases intestinal M1 macrophages and mitigates formation of serrated adenomas in mutant KRAS mice. Oncoimmunology 2018;7:e1423168.
16. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature 2007;447:1116-20.
17. Schöckel L, Woischke C, Surendran SA, et al. PPARG activation promotes the proliferation of colorectal cancer cell lines and enhances the antiproliferative effect of 5-fluorouracil. BMC Cancer 2024;24:234.
18. Hajjar R, Fragoso G, Oliero M, et al. Basal levels of microbiota-driven subclinical inflammation are associated with anastomotic leak in patients with colorectal cancer. Gut 2024;73:1031-3.
19. Hajjar R, Gonzalez E, Fragoso G, et al. Gut microbiota influence anastomotic healing in colorectal cancer surgery through modulation of mucosal proinflammatory cytokines. Gut 2023;72:1143-54.
20. Ahmed W, Rashid S. Functional and therapeutic potential of inulin: A comprehensive review. Crit Rev Food Sci Nutr 2019;59:1-13.
21. Wu J, Dong W, Pan Y, et al. Crosstalk between gut microbiota and metastasis in colorectal cancer: implication of neutrophil extracellular traps. Front Immunol 2023;14:1296783.

Cite this article as: Núñez-Sánchez MÁ, Martínez-Sánchez MA, Ramos-Molina B. Peroxisome proliferator-activated receptor gamma (PPAR-γ) activation through gut microbiota modulation as a novel therapeutic approach against anastomotic leak after colorectal cancer surgery. J Gastrointest Oncol 2024;15(5):2338-2342. doi: 10.21037/jgo-24-314

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