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Microbiota and cancer therapy

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Abstract: The current article is a review of the most important, accessible, and relevant literature published between April 2018 and March 2019 on the gut microbiota and cancer therapy. The first part of the review focuses on literature describing changes in the gut microbiota in response to therapeutic interventions, including checkpoint inhibitors, radiotherapy, and microbial therapeutics whilst the subsequent section focuses on the mechanistic aspects of the process.

Keywords: Gut microbiota, Cancer therapy, Immune checkpoint inhibitors, Microbial manipulation, Radiation therapy.

INTRODUCTION

Cancer is a global health burden and despite significant advances in treatment, resistance to medical therapies remains a major cause of therapeutic failure. The gut microbiota is known to impact on both chemotherapy and immunotherapy strategies either directly or indirectly. In addition, anticancer treatments are also known to affect the gut microbiota. In the last few years, a number of studies have demonstrated a role for gut microbiota in shaping immune checkpoint blockade treatments in various cancers, highlighting that treatment responders have different microbiota signatures compared to non-responders. These outcome responses have also been shown to be transferable *via* faecal microbial transplantation (FMT) or directed gut microbiota manipulation. These findings have opened the door to exploring the gut microbiota in the context of other cancer therapies, such as chemotherapy, including antibiotics, surgery, and radiation therapy, as well as delineating mechanistic explanations for their efficacy.

EFFECTS OF ANTIBIOTICS AND PROBIOTICS ON CANCER THERAPY

Antibiotics are frequently prescribed alongside chemotherapy and cancer surgery. Antibiotics are known to affect the gut microbiota, depending on the antibiotic class/specific drug they can cause loss of community structure, including general loss of taxonomic richness, as well as reduction in distinct community members favouring expansion of other components which will also affect microbiota functioning and host-microbial inter-dependence. With this in mind, Derosa et al[1], assessed the impact of antibiotic use prior to immune checkpoint inhibitor (ICI) treatment in independent cohorts of renal cell carcinoma (RCC) and non small-cell lung cancer (NSCLC) followed up in two US cancer centres and demonstrated that antibiotic usage was associated with worse outcomes in both cancer types. Whilst microbiota analysis was not undertaken as this was a retrospective study, sub-group analysis, to look at whether timing of the antibiotic course impacted the outcome, indicated that antibiotic usage within 30 days of ICI had more impact than antibiotic courses given 30 to 60 days before ICI. A possible explanation for this could be the partial restoration of the gut microbiota which is known to be mainly restored within 1 to 3 months

of antibiotic treatment. A similar response was seen in a retrospective study of stage IV melanoma patients, from the same group, as well as an additional retrospective Japanese study on 90 NSCLC patients treated with nivolumab with/without prior antibiotic treatment[2,3]. In the Elkrief et al study[2], patients who received antibiotics in the 30 days prior to ICI had worse clinical outcomes, including significantly shorter progression free survival compared to ICI only patients (2.4 *vs.* 7.3 months, HR 0.28, 95% confidence interval (CI), 0.10-0.76, p=0.01). In the Hakozaki et al study[3], median progression-free survival time in patients treated with antibiotics was 1.2 months [95% CI, 0.5-5.8], while the time for patients who were not treated with antibiotics was 4.4 months (95% CI, 2.5-7.4). Whilst further studies are necessary, including specific detail on gut microbiota signatures affected by antibiotics, which are directly impacting ICI efficacy, the studies add to the growing body of evidence which demonstrates that a dysbiotic gut microbiota is sub-optimal for cancer patients and provides additional evidence that microbial profiling/manipulation are required as part of decision-making in order to improve cancer treatment success rates.

Probiotics have been investigated in the context of numerous gastrointestinal diseases, in attempts to supplement a dysbiotic gut microbiota. Several studies have suggested that regular consumption of probiotics may improve the functioning of the intestinal microbiota, reducing ensuing gut inflammation and the production of carcinogenic compounds which contribute to the carcinogenic process. Various studies have demonstrated that individuals who consume probiotic products have a lower risk of developing cancer or experiencing disease relapse. This has been reported in colorectal cancer development, breast cancer relapse, and bladder cancer relapse, although caution is required when administering probiotics to cancer patients and immunosuppressed individuals, due to the increased risk of probiotic bacteraemia in these patients. It has been speculated that probiotic supplementation may reduce chemotherapy-induced diarrhoea (CID). To assess this, Tian et al[4] conducted a prospective clinical trial to assess the impact of giving *Clostridium butyricum* to patients with lung cancer undergoing chemotherapy, compared to placebo. *C. butyricum* has previously been shown to regulate gut homeostasis, promote proliferation of beneficial species, including Bifidobacteria and Lactobacilli, reduce inflammation, and reduce stool frequency in disorders, including inflammatory bowel disease and antibiotic-associated diarrhoea. Patients were given probiotic/placebo tablets 3 times a day for 3 weeks, commencing prior to their chemotherapy regimen. *C. butyricum* reduced chemotherapy-induced diarrhoea, maintaining gut microbiota diversity. Whilst changes in immune function were assessed, findings were not statistically significant, suggesting that 3 weeks of probiotic administration may be insufficient to have an impact on the immune response.

IMMUNE CHECKPOINT INHIBITOR-INDUCED COLITIS AND FAECAL MICROBIOTA TRANSPLANTATION

Immune checkpoint inhibitors can induce a number of adverse events[5]. One of the most frequent adverse events is ICI-associated diarrhoea and colitis[6]. The exact pathogenesis of ICI-associated colitis is not completely understood, but antibiotic use and microbiota composition are thought to play a role. A study by Liu et al[7] aimed to investigate the association between intestinal microbiota and immune-related diarrhoea in patients receiving ICI. The study included faecal samples from 26 lung cancer patients before the first dose of ICI therapy with anti-PD-1 antibodies. Out of 26 patients, eight experienced diarrhoea after the treatment of anti-PD-1 antibodies. The study demonstrated that at the phylum level, Bacteroidetes were more abundant in diarrhoea-free patients, while Firmicutes were found at reduced levels in these patients. *Bacteroides* and *Parabacteroides* species belonging to the Bacteroidetes phylum and *Phascolarctobacterium* species of the Firmicutes phylum were more abundant in patients without diarrhoea. Meanwhile, *Veillonella* species (Proteobacteria phylum) were less frequently observed in lung cancer patients without diarrhoea[7]. This small observational study suggests that microbiome composition might be linked with the subsequent development of immune-related diarrhoea.

Modulation of gut microbiota through faecal microbiota transplantation (FMT) is thought to mediate different immune responses[8]. FMT is now indicated for treatment of recurrent *C. difficile* infections; however, this treatment modality is also being explored in a number of different disorders. Microbiota disturbances in ICI induced diarrhoea and colitis have been described in multiple previous studies[7,9,10]. A research team from the United States took this observation further to the clinical level. Wang et al[11] reported the first case series of FMT in ICI-associated colitis. Although this

study included only two patients, the success of the reported cases encourages further research in the field. In the first patient, complete resolution of ICI colitis symptoms occurred gradually within two weeks, while the second patient experienced complete resolution of symptoms after a second FMT. Endoscopic evaluation demonstrated significant mucosal inflammation and ulceration in both patients near the time of the diagnosis of ICI-associated colitis, without substantial improvement after systemic corticosteroids, anti-TNF, and anti-integrin agents. Following FMT, marked improvement was evident on endoscopic evaluation, with reduced inflammation and resolution of ulcerations. The study also showed that FMT was able to restore gut microbiota diversity and to induce an increase in the proportion of regulatory T-cells within the colonic mucosa. In the first patient, bacteria had effectively colonized the intestinal tract with nearly 75% of the sequences uniquely attributable to the donor microbiota and a notably higher abundance of *Akkermansia* species. In the second patient there was a significant increase in the abundance of *Blautia* and *Bifidobacterium* species after FMT, which have been associated with reduced intestinal inflammation. This preliminary report provides evidence[11] that modulation of the gut microbiota may limit ICI-associated colitis; however, further randomized controlled trials are needed to investigate whether this clinical condition could become yet another indication for the use of FMT in clinical practice.

RADIATION THERAPY AND DYSBIOSIS

Radiation therapy is known to cause intestinal damage through induction of epithelial cell apoptosis with ensuing mucosal inflammation, ulceration, fibrosis, and disorganised vascularisation resulting in diarrhoea, bleeding, and pain. Changes in gut microbiota composition, including reduced Firmicutes and Bacteroidetes diversity and increased Proteobacteria levels, have previously been documented following radiotherapy. However, the mechanistic impact of radiotherapy-induced dysbiosis and its association with tissue damage has been lacking. Using a mouse model of localised directed rectal radiation, Gerassy-Vainberg et al[12] evaluated both luminal and mucosal-associated microbial signatures, interplay with the host immune system, and tissue damage in radiation-induced tissue damage. Radiotherapy caused a dramatic shift in faecal microbiota composition with 6-week post-radiation samples more greatly impacted compared to 2-week post-radiation samples, suggesting that ensuing intestinal damage contributed to the dysbiosis. Genera, including *Akkermansia*, *Bacteroides*, *Parabacteroides, Sutterella, Turicibacter* genera, and an unclassified genus belonging to the RF32 order, all increased in abundance in mice that developed radiation proctitis compared to control mice. Comparison of mucosally associated bacterial communities indicated that changes correlated with radiation-induced tissue damage and transmission of susceptibility to radiation proctitis, as well as demonstrated colitis. The results also show, for the first time, that a radiotherapy-induced dysbiotic microbiota increases mucosal interleukin-1 (IL-1) levels which contribute at least in part to the ensuing colonic damage. Manipulation of the dysbiotic microbiota or direct inhibition of increased IL-1 levels offer potential novel therapeutic options.

INTERACTIONS BETWEEN IMMUNE CHECKPOINT INHIBITOR MEDIATED MOLECULAR PATHWAYS AND THE GUT MICROBIOTA

Previous studies[13,14] have clearly shown that the effectiveness of cancer immunotherapy in melanoma and epithelial cancer patients is affected by the host microbiota. The exact mechanism of this subtle interaction between microbes and ICI, however, is not yet completely understood. Several recent studies looking at PD-1 and CTLA-4 pathways have provided important insights within the molecular pathways that are involved in this phenomenon[15-18].

Diosgenin is a natural steroidal saponin derived from the genus *Dioscorea* that can modify bacterial composition[19]. A study[15] from China using a melanoma mouse model showed that anti-melanoma effects of diosgenin were related to $CD4^+/CD8^+$ T-cell infiltration and may influence the composition of the intestinal microbiota. In this *in vivo* study the researchers showed that antibiotics may impair the therapeutic efficacy and immunity responses of diosgenin through microbiota community disruption. They further showed that combined administration of PD-1 antibody with diosgenin reinforced tumour necrosis responses and apoptosis by eliciting augmented T-cell responses in the mice[15]. A further study[16] from the United States examined the significance of the microbiota

in pancreatic adenocarcinoma. The study found that pancreatic cancer tissues had a more abundant microbiota when compared with normal pancreas tissue in both mice and humans. In an elegant cell line and mice experiment the group showed that treatment exposure to antibiotics prevented invasive features of pancreatic adenocarcinoma. Furthermore, they found that bacterial suppression increased the potential efficacy for ICI targeted immunotherapy by upregulating PD-1 expression. This study[16] suggests that the microbiota may disable certain important immune pathways and needs to be considered in the development of novel therapeutic approaches.

The study reported by Su et al[17] looked at the potential therapeutic effect of the polysaccharide derived from spores of Ganoderma lucidum (SGP) given alongside paclitaxel (PTX) in a murine 4T1-breast cancer model. Flow cytometry analysis showed that the combination therapy in murine breast cancer models recovered the exhausted tumour infiltration lymphocytes (TILs) *via* inhibiting the expression of immune checkpoints (PD-1 and Tim-3), while PTX alone increased CTLA-4 expression. Interestingly, 16S rRNA sequencing analysis showed that the combination therapy had positive effects on gut microbiota composition. After treatment, Bacteroides and Ruminococcus species levels were significantly enriched while Desulfovibrio and Odoribacter species (considered cancer-risk genera) were decreased[17]. The same study group also explored additional anticancer effects of an extract derived from the sporoderm-breaking spores of G. lucidum[18]. In mice experiments researchers showed that treatment with this extract regulated PD-1 in the spleen, and CTLA-4 in tumours. Furthermore, mice faecal microbiota analysis revealed that the extract remodeled the overall structure of the gut microbiota from tumour-bearing mice towards that of the healthy mice together with impacting on host gene expression, including several genes that are responsible for signaling pathways involved in metabolism, cellular processes, and environmental information processing[18]. A short report from Derosa et al[20] assembled the major publications in the field of the gut microbiota and response to cancer immunotherapy and defined a list of 14 bacterial genera or species associated with favourable responses to PD-1 blockade.

CONCLUDING REMARKS

Accumulating evidence points towards undisputable effects of the microbiota on cancer immunotherapy. An increasing number of studies show that antibiotic use prior to ICI therapy has profound effects and is associated with a decreased survival of cancer patients. 16S rRNA analysis data suggest that individual microbiota composition may predict ICI-associated diarrhoea, while FMT could be a treatment option for patients with refractory ICI-associated colitis. Emerging fundamental studies in animal models and cell lines highlight the importance of the microbiota for immune check-point inhibitor related pathways, but further research is needed to translate all these observations into clinically relevant solutions for cancer patients.

Conflict of Interest

The authors declare no conflict of interest.

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