Gut microbes modulate host response to immune checkpoint inhibitor cancer immunotherapy

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Ever since their discovery in the1670s, and proof of their link with disease in the 1870s, bacteria have had a bad name and are often called pathogens. They are regarded as the cause of diseases and things to be avoided. However, there are an estimated 100 trillion of bacteria, ten times more than the number of cells in a human body. These commensal microbes (the microbiota) live on all surface barriers of a human body and are particularly abundant and diverse in the gut. Therefore, bacteria are something that we cannot avoid.

The good news is that not all microbes are bad. In fact, we now know that commensal microbes co-exist with human cells in a mutually beneficial way. These microbes digest food such as fiber to generate nutrients, synthesize certain vitamins among many other beneficial functions (1,2). Further extending these essential functions of gut microbiota in human health, an article in the November 2 issue of Science reported an intriguing and exciting discovery that the gut microbiota influences the efficacy of immune checkpoint inhibitor (ICI) immunotherapy against human non-small-cell-lung cancer (NSCLC) and renal cell carcinoma (RCC) (3). Cancer immunotherapy represents a breakthrough in human cancer research and treatment. For the first time in human cancer treatment history, durable and complete responses have been achieved in many types of human cancers including metastatic melanoma, RCC and NSCLC (4). However, despite this amazing advance, not all cancer patients respond to ICI immunotherapy. Various mechanisms have been explored to explain the non-response to ICI, including low tumor antigen load, low mutational burdens, poor antigen presentation, immune checkpoint-independent immune suppression and exhaustion of tumor-specific T cells (4,5). The study by Routy *et al.* indicates that a non-host factor, particularly a specific host gut microbe, shapes patient response to ICI immunotherapy and use of antibiotics during ICI immunotherapy may dampen patient response to the therapy. This finding not only identified a novel mechanism underlying resistance to ICI immunotherapy but also had tremendous implications in extending ICI immunotherapy benefits to those non-responding patients.

This new finding is a translation of previous findings by the same research group and others in mouse tumor models that gut microbiota modulates tumor-bearing mouse response to ICI immunotherapy (6,7). Prior to human studies, Routy *et al.* determined that treatment of antibiotics significantly increased tumor sizes and decreased survival of sarcoma and melanoma-bearing mice that were treated with PD-1 blockade monotherapy or combined PD-1 and CTLA-4 blockade therapies, thereby validating the critical role of host microbiota in ICI immunotherapy efficacy in mouse tumor models.

Mice are not human and observations made in mice are not always translated to human. In this case, when Routy *et al.* examined clinical data of patients with non-small-cell-lung cancer (n=140), renal cell carcinoma (n=67) or urothelial carcinoma (UC, n=42) who received PD-1 or PD-L1 blockade ICI immunotherapy, the result is crystal clear. Out of the 249 patients, 69 patients who were prescribed antibiotics for routine other reasons before and

soon after the 1st ICI immunotherapy exhibited significantly shorter progression-free survival (PFS) and overall survival (OS) than the rest patients who did not receive antibiotics treatment. In contrast, proton pump inhibitors, a medication that can also alter the microbiota composition (8), did not affect the PFS and OS in these patients. These observations thus indicate that the microbiome modulates host response to ICI immunotherapy and that particular microbes, not the composition of microbiota, dictate the response.

It is therefore logically to identify the microbes linked to the clinical response to ICI immunotherapy. Using quantitative metagenomics by shotgun sequencing of DNA samples from stools of 100 NSCLC and RCC patients, the researchers identified *Akkermansia muciniphila* (*A. muciniphila*), a commensal species associated with the gut mucus lining, as the microbe that is most significantly associated with favorable clinical outcome in both NSCLC and RCC patients. This finding is consistent in principle with another recent report that specific commensal microbes modulate cancer patient response to ICI immunotherapy (9). However, gut microbe modulation of patient response to ICI immunotherapy might be cancer type-dependent. In melanoma patients, responders had a more diverse microbiome and more specific microbes associated with the favorable response to ICI immunotherapy than NSCLC and RCC patients (9).

These studies clearly demonstrated that gut microbiota modulate host response to ICI immunotherapy in NSCLC, RCC and melanoma patients. To establish a cause-effect relationship, mice were treated with antibiotics and then re-colonized with fecal microbiota transplantation (FMT) by patient stool to create "avatar" mice. Tumor-bearing avatar mice with FMT from clinical responder patients are sensitive whereas avatar mice with FMT from clinical non-responder patients are resistant to ICI immunotherapy. This study clearly indicates that it is the gut microbes that confer cancer patient response to ICI-unleashed T cell immunity and thus has tremendous translational implication. For example, fecal transplants or specific bacterial colonization may overcome resistance to ICI immunotherapy and extend the benefit to non-responders. In addition, simply avoiding antibiotics while undergoing ICI immunotherapy will likely increase the efficacy in responders and the response rate in non-responders. One issue remained to be solved is whether the host immune-modulating microbes are tumor-type-specific (3,9).

ICI immunotherapy works through unleashing the immune suppressed tumor-specific T cells to repress tumor growth (4,10-13). Therefore, commensals such as *A. muciniphila* must in some way modulate the tumor-reactive T cells either directly or indirectly (14-17). To link the gut microbial content to the systemic immune response, T cell recall memory response was tested. Circulating CD4+ and CD8+ T cells were collected from NSCLC and RCC patients under PD-1 blockade immunotherapy and co-cultured with autologous monocytes pre-incubated with distinct commensals. IFNg release was identified as the factor associated with PFS in these patients. Additionally, colonizing intestine with *A. muciniphila* alone or combined *A. muciniphila* and another commensal *Enterococcus hirae* reinstated the anti-tumor effect of PD-1 ICI in the melanoma and Lewes lung carcinoma mouse models. The increased anti-tumor effects are associated with accumulated central memory CD4+ T cells expressing CCR9 and/or CXCR3 in mesenteric LN, tumor-draining LN, and tumor beds, increased CD4/FoxP3 ratios in tumor and secretion of IL-12 from dendritic cells. This is indication that the specific commensal microbes shape patient response to ICI immunotherapy at least partially through modulating the host anti-tumor immune response. On the other hand, it seems that specific gut commensal bacteria may modulate the host immune response in different types of cancer through different mechanisms (3,9). An outstanding question is what are the cellular and molecular links between the commensal bacteria-elicited immune response and the tumor antigen-specific T cells in the context of ICI immunotherapy.

Tumor cells are the final targets of the ICI-unleashed cytotoxic T lymphocytes (CTLs). CTLs kill tumor cells through induction of apoptosis by the perforin/granzyme and Fas/FasL effector mechanisms (18). Therefore, for CTLs to kill tumor cells, tumor cells must be sensitive to apoptosis induction. Unfortunately, resistance to apoptosis is one of the hallmarks of human cancer cells (19,20). It is known that commensal bacteria may generate metabolites or secrete signal molecules to directly modulate tumor cell growth and apoptosis (17). Therefore, it is possible that, in addition to their immune modulatory effect, commensal microbes may also secrete modulators or generate metabolites to potentiate tumor cells sensitivity to apoptosis induction and thereby rendering cancer patient response to ICI immunotherapy, which remains to be determined.

Footnote

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