IN THE SPOTLIGHT

Immunotherapy for Pancreatic Cancer: More Than Just a Gut Feeling

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Summary: Development of pancreatic cancer in spontaneous murine models is associated with enrichment of specific strains of gut and intratumoral bacteria that induce a tolerogenic immunosuppressive microenvironment favoring cancer progression and resistance to immunotherapies. Ablation of the microbiome with antibioticsreshapes the tumor microenvironment, inducing T-cell activation, improving immune surveillance, and increasing sensitivity to immunotherapy in established tumors. Cancer Discov; 8(4): 386-8. ©2018 AACR.

See related article by Pushalkar et al., p. 403 (11).

The emergence of next-generation sequencing methodologies has allowed extensive characterization of the microbiota in various pathologic conditions, including cancer. For example, bacteria-induced inflammation in the gastrointestinal tract has been linked to colorectal cancer initiation and progression (1-4). Similarly, Urbaniak and colleagues detected the presence of diverse bacterial communities in mammary tissue, which could modulate the risk of developing breast cancer (5). Recently, several studies have also shed light on the key role of the gut and tumor microbiome in modulating responses to chemotherapy, as well as immunotherapies like immune checkpoint inhibition using anti-PD-1 antibodies (6-9). The first study to show that the gut microbiota could influence the response to chemotherapy by activating the immune system was reported by Viazul and colleagues (10). In addition, the composition of the gut microbiota has enabled stratification of patients into responders and nonresponders, thereby allowing the use of the microbiota composition as a predictive biomarker of sensitivity to immunotherapy in established tumors. The authors highlighted the significance of the microbiome beyond the gut, specifically intratumoral bacteria, in altering the natural history of this cancer.

In this issue, Pushalkar and colleagues (11) investigate the role of the gut and intratumoral microbiome in pancreatic cancer progression and in modulating responses to immunotherapies. Furthermore, they elucidate the immunologic mechanisms that underlie the variable responses to systemic therapy upon changes in the microbiome. The authors detected the presence of abundant intratumoral bacteria in mice and human PDAC samples compared with the normal pancreas, suggesting the existence of a bacterial translocation from the intestinal tract into the tumors. By deriving a preinvasive spontaneous murine model p48Cre;LSL-KrasG12D (KC) in germ-free conditions and performing bacterial ablation with antibiotics on invasive orthotopic PDX1Cre;LSL-KrasG12D;Tyrp3R172H (KPC)–derived tumor cell models, they showed a delay in tumor initiation and progression compared with non-germ-free KC mice and mice implanted with KPC with intact bacterial content, respectively. These data strongly suggest that bacteria promote pancreatic oncogenesis in both preinvasive and invasive models. A longitudinal analysis between age-matched KC and wild-type mice showed that certain bacterial populations were enriched in KC mice, with the most abundant species being Bifidobacterium.

With respect to immunologic mechanisms, the study reported that bacterial ablation with antibiotics in the orthotopic KPC model is associated with an increase in tumor...
infiltration with CD4+ and CD8+ T cells, a reduction of the myeloid-derived suppressor cells fraction, and changes in macrophage polarization from the immune-suppressive M2-like tumor-associated macrophages (TAM) toward predominately M1-like TAMs. Antibiotic ablation enhanced Th1 polarization of CD4+ T cell and increased the acquisition of cytotoxic CD8+ T cells. Antibiotics induce upregulation of PD-1 expression in both CD4+ and CD8+ T cells, and a synergistic antitumoral effect is observed when PD-1 blockade is combined with microbial ablation, resulting in a significant tumor size reduction.

To explain the microbiome-induced tolerogenic microenvironment, the authors demonstrated the transfer of feces from PDAC-bearing KPC mice. T-cell activation after antibiotic ablation was also functionally assessed by adoptive transfer into mice challenged with subcutaneous KPC tumors, which resulted in reduced tumor burden. Subsequently, the authors demonstrated that this immunogenic phenotype can be reversed by the transfer of feces from PDAC-bearing KPC mice. T-cell activation after antibiotic ablation was also functionally assessed by adoptive transfer into mice challenged with subcutaneous KPC tumors, which resulted in reduced tumor burden. Similarly, extracts from KC mice–derived gut bacteria induced higher activation of diverse pattern recognition receptors (PRR) in tumor macrophages. On the contrary, pancreatic cancers growing in antibiotic-ablated hosts exhibited markedly lower expression of PRRs. In vivo inhibition of Toll-like receptor (TLR) signaling by blocking TRAF6 abrogated the cancer-promoting effects of repopulating antibiotic-ablated mice with KPC feces, suggesting that an important mechanism to explain the microbiome-induced tolerogenic microenvironment might be the activation and expression of TLRs in macrophages (Fig. 1). Together, these experiments strongly implicate PDAC-associated bacteria as modulator of the immune-suppressive microenvironment that characterizes this malignancy.

Because bacteria ablation upregulated PD-1 in T cells, the authors tested the combination of antibiotics and PD-1 blockade and were able to show synergistic antitumoral effect that was associated with T-cell activation. This is in contrast to a recent study reported by Routy and colleagues, where the authors reported that changes in the gut composition following antibiotic usage decreased responses to immunotherapy in patients with lung, bladder, and kidney cancers (9). These confounding results suggest that each cancer type may induce a distinct compendium of alterations in the gut and tumor microbiome composition that may either attenuate or facilitate the function of immune checkpoint inhibitors.

Most immunotherapeutic agents that have proven efficacy in other malignancies have not been successful thus far against PDAC, a rarely curable disease. Immune tolerance mechanisms have been implicated as the main barrier to efficacious antitumor immunotherapy. Further understanding of how the microbiota contributes to the establishment and maintenance of immune tolerance and the search for novel strategies targeting the cancer-associated microbiome to circumvent these
mechanisms would be relevant avenues to induce immunotherapy efficacy. The use of antibiotics to overcome these barriers could be one “generic” avenue. However, a detailed analysis of the microbiota in large cohorts of human pancreatic cancer is likely necessary to determine the specific communities that contribute positively or negatively in the development and progression of pancreatic cancer, allowing a more selective approach to modulating therapy response. The first trials of fecal (“global”) or selective bacterial supplementation are just beginning in diseases like melanoma, and with further knowledge, similar strategies might be attempted for pancreatic cancer.

In conclusion, the experimental data from Pushalkar and colleagues provide compelling preclinical evidence for the role of the gut and tumor microbiota in the local and systemic activation of the immune system, which not only affects the natural history of the tumor, but also converts an immunotherapy-refractory tumor into a significantly more responsive one.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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