IN THE SPOTLIGHT

Immunotherapy for Pancreatic Cancer: More Than Just a Gut Feeling

Erick Riquelme[1], Anirban Maitra[2], and Florencia McAllister[1,3]

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 antibiotics reshapes 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 Viaud and colleagues (10). In addition, the composition of the gut microbiome has enabled stratification of patients into responders and nonresponders, thereby allowing the use of the microbiota composition as a predictive biomarker of response to immunotherapy (7–9). Although the emerging preclinical data strongly suggest that the gut microbiota can modulate tumor progression and responses to therapies systemically, the molecular basis for this regulation is still being elucidated.

The functional relationship between the gut or intratumoral bacteria and treatment responses in pancreatic cancer is still in its relative infancy. Recently, Geller and colleagues described that bacterial species belonging to the *Gammaproteobacteria* class were able to metabolize the chemotherapeutic agent gemcitabine (2′,2′-difluorodeoxycytidine) into its inactive form, 2′,2′-difluorodeoxyuridine. Using a murine model of pancreatic cancer, they showed that the presence of intratumoral *Gammaproteobacteria* was responsible for inducing resistance to gemcitabine and that this effect was abolished by the use of antibiotics (6). Because gemcitabine has been used for advanced pancreatic cancer, they postulated that the presence of this type of bacteria could confer resistance to this drug. Indeed, they detected *Gammaproteobacteria* on 76% (86/113) of the tissue specimens analyzed from patients with human pancreatic ductal adenocarcinoma (PDAC), suggesting the tumor samples contain bacteria that can potentially modulate tumor sensitivity to gemcitabine (6). This study 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 $p48^{Cre}$;*LSL-Kras*^{G12D} (KC) in germ-free conditions and performing bacterial ablation with antibiotics on invasive orthotopic $PDX1^{Cre}$;*LSL-Kras*^{G12D};*Trp53*^{R172H} (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

[1]Department of Clinical Cancer Prevention, The University of Texas MD Anderson Cancer Center, Houston, Texas. [2]Sheikh Ahmed Pancreatic Cancer Research Center, The University of Texas MD Anderson Cancer Center, Houston, Texas. [3]Department of GI Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas.

Corresponding Author: Florencia McAllister, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 1360, Houston, TX 77030. E-mail: fmcallister@mdanderson.org

doi: 10.1158/2159-8290.CD-18-0123

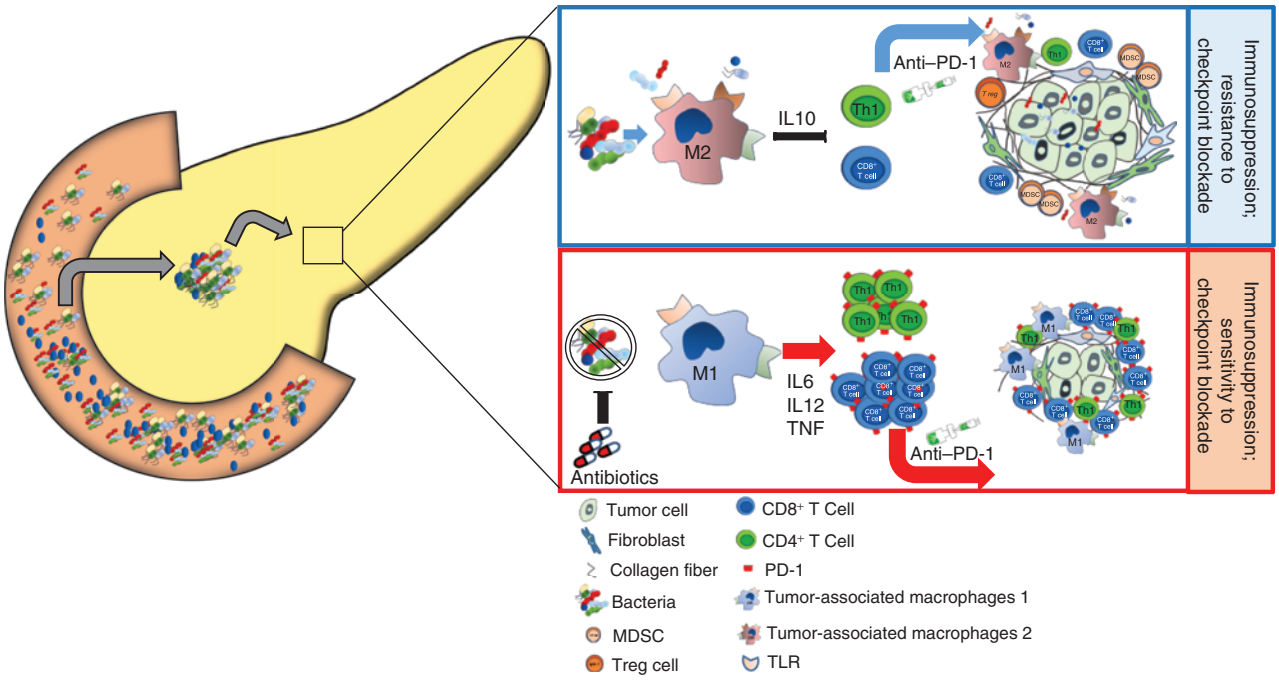

Figure 1. Gut and pancreatic microbiome induce tumor progression, and bacterial ablation is protective and sensitizes PDAC to checkpoint blockade. Gut microorganisms can translocate to the pancreas and induce a suppressive microenvironment that contributes to the progression of the PDAC. Microbiome ablation with antibiotics improves tumor immune surveillance as well as antitumor immunity against established PDAC and improves responses to PD-1 blockade. The microbial ablation with antibiotics reduces the myeloid-derived suppressor cells (MDSC), induces macrophage polarization from the immune-suppressive M2-like tumor-associated macrophages (TAM) toward predominantly M1-like TAMs, and increases tumor infiltration of Th1$^-$ CD4$^+$ T cell and 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.

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 predominantly M1-like TAMs. Antibiotic ablation enhanced Th1 polarization of CD4$^+$ T cell and increased the acquisition of cytotoxic CD8$^+$ T-cell phenotype, as demonstrated by increased expression of T-BET, TNFα, IFNγ, CD38, PD-1, and CD44. In a very compelling experiment, 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. Subsequently, the authors demonstrated that macrophages entrained by gut bacterial extracts from pancreatic cancer–bearing hosts prevented the activation of CD4$^+$ and CD8$^+$ T cells, and deterioration of the antigen-presenting capacity of the macrophages was the mechanism implicated. 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.

Acknowledgments

F. McAllister is a Paul Calabresi K12 clinical scholar (NCI grant awarded to MDACC K12CA088084-16A1) and a V Scholar. A. Maitra and F. McAllister have received funding through the MD Anderson Moonshot program. F. Maitra is also funded by the Khalifa Bin Zayed Al Nahyan Foundation, U01CA196403, and U01CA200468.

Published online April 2, 2018.

REFERENCES

1. Housseau F, Sears CL. Enterotoxigenic bacteroides fragilis (ETBF)-mediated colitis in Min (Apc+/–) mice: a human commensal-based murine model of colon carcinogenesis. Cell Cycle 2010;9:3–5.
2. Sears CL, Garrett WS. Microbes, microbiota, and colon cancer. Cell Host Microbe 2014;15:317–28.
3. Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat Med 2009;15:1016–22.
4. Chung L, Thiele Orberg E, Geis AL, Chan JL, Fu K, Shields CED, et al. Bacteroides fragilis toxin coordinates a pro-carcinogenic inflammatory cascade via targeting of colonic epithelial cells. Cell Host Microbe 2018;23:203–14e5.
5. Urbaniak C, Cummins J, Brackstone M, Macklaim JM, Gloor GB, Baban CK, et al. Microbiota of human breast tissue. Appl Environ Microbiol 2014;80:3007–14.
6. Geller LT, Barzily-Rokni M, Danino T, Jonas OH, Shental N, Nejman D, et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science 2017;357:1156–60.
7. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018;359:97–103.
8. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 2018;359:104–8.
9. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 2018;359:91–7.
10. Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillere R, Hannani D, et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 2013;342:971–6.
11. Pushalkar S, Hundeyin M, Daley D, Zambirinis CP, Kurz E, Mishra A., et al. The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. Cancer Discov 2018;8:403–16.

AACR American Association for Cancer Research

CANCER DISCOVERY

Immunotherapy for Pancreatic Cancer: More Than Just a Gut Feeling

Erick Riquelme, Anirban Maitra and Florencia McAllister

Cancer Discov 2018;8:386-388.