Intestinal microbiota: Unexpected alliance with tumor therapy

Intestinal Microbiota: Unexpected Alliance with Tumor Therapy

Naofumi Mukaida

Division of Molecular Bioregualtion, Cancer Research Institute, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan.

Tel.: +81 76 264 6735

Fax.: +81 76 234 4520

mukaida@staff.kanazawa-u.ac.jp

Evaluation of: Iida N, Dzutsev A, Stewart CA *et al.*: Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* 342, 967-970 (2013). Intestinal microbiota is essential for host physiological process including the maintenance of epithelial barrier and the immune functions. However, paradoxically, the intestinal microbiota can promote various types of experimental carcinogenesis. The current paper demonstrates that disruption of the microbiota impairs the response of tumors to CpG-oligonucleotide immunotherapy and platinum chemotherapy in a context-dependent manner. Thus, intestinal microbiota may have great impacts on the tumor response to chemotherapy and/or immunotherapy.

KEYWORDS: germ-free mouse, microbiota, platinum therapy, reactive oxygen species

Microbial community is present abundantly in mucosal organs including the intestine, the oral cavity, and the vagina, and is referred as the microbiota [1]. Microbiota and host form complex and intricate relationships, which confer the benefits to the host in many ways. However, this close relationship can incite diseases, when homeostasis between host and microbiota is disturbed [1]. Human intestinal microbiota consists of tens of trillions of

microorganisms, occupying 99 % of the microbial mass in the whole body, and can exert both local and long-distance effects on various types of pathological conditions. Germ-free (GF) mice without intestinal microbiota are resistant to various types of experimental carcinogenesis in intestine, stomach, and liver [2-4]. Thus, the intestinal microbiota can promote carcinogenesis, probably by activating pathogen-associated molecular pattern recognition receptors such as Toll-like receptors (TLRs) [4] and NOD-like receptors (NLRs) [5], generating bacteria-derived genotoxins [6] and virulence factors [7], and modulating the metabolism in host [8].

Summary of methods & results

In the current paper [9], Iida *et al.* revealed that intestinal commensal bacteria could control the responsiveness of tumors to immunotherapy using CpG-oligodeoxynucleotides (ODN) or platinum chemotherapy. Combined treatment with CpG-ODN, a ligand for TLR9, and anti-IL-10 receptor (R) antibody, retards tumor growth and prolongs survival in MC38- or B16-tumor bearing mice by inducing intra-tumoral myeloid cell-derived TNF-α-mediated hemorrhagic necrosis. An antibiotic cocktail (ABX) of vancomycin, imipenem, and neomycin reduces anti-IL-10R/CpG-ODN-mediated tumor necrosis and subsequent tumor retardation in wild-type (WT) but not *Rag1*$^{-/-}$ mice. ABX significantly reduced TNF expression, TNF-expressing cell numbers, and the expression of other pro-inflammatory cytokines but not anti-inflammatory cytokine expression in MC38 tumor sites. Likewise, when tumor-bearing GF and specific pathogen-free (SPF) mice were treated with anti-IL-10R/CpG-ODN, tumors of GF mice produced significantly lower amount of TNF and IL-12, compared with SPF mice. Gavage administration of LPS restored TNF expression in tumors of ABX-treated WT but not *Tlr4*$^{-/-}$ mice. Moreover, *Tlr4* but not *Tlr2* deficiency resulted in lower induction of TNF expression and reduced tumor regression after

immunotherapy. Thus, intestinal microbiota primes tumor-associated myeloid cells for anti-IL-10R/CpG-ODA-induced inflammatory cytokine production, mainly by using TLR4 pathway.

The examination on fecal microbiota composition under several conditions revealed that *Alispteps* and *Lactobacillus* genii correlated positively and negatively with TNF production, respectively. The administration of cultured *Alispteps shaii* by gavage to mice pre-exposed to ABX reconstituted the ability of tumor-associated myeloid cells to produce TNF while that of *Lactobacillus fermentum* to intact mice attenuated the response to anti-IL-10R/CpG-ODN. Thus, these species can influence the tumor response to CpG-ODN by affecting tumor-associated myeloid cells.

The authors examined also the effects of intestinal microbiota on the response to therapy using platinum agents such as oxaliplatin and cisplatin. Oxaliplatin eradicated most subcutaneous EL4 tumors and prolonged survival, but ABX-treated animals exhibited significantly reduced tumor regression and survival. Likewise, GF animals failed to respond to oxaliplatin treatment. Similar observations were also obtained on the effects of ABX on the response of MC38-bearing mice to oxaliplatin and that of EL4-bearing mice to cisplatin. Both oxaliplatin and cisplatin induce the formation of platinum DNA adducts and intra-strand cross-links, and eventually generate reactive oxygen species (ROS) to cause DNA damage and apoptosis [10]. Despite of few effects on the levels of DNA-bound platinum, ABX treatment reduced platinum-induced ROS generation together with reduction in gene expression of *Nox1* and *Cybb* encoding ROS-generating NADPH oxidase 2 (NOX2) and that of ROS-responsive *Nos2*, *Sod1*, and *Sod2*. Moreover, oxaliplatin increased ROS production in tumor-infiltrating neutrophils and macrophages, while this response was impaired in ABX-treated or *Cybb*$^{-/-}$ mice. Furthermore, depletion of myeloid cells impaired the ability of oxaliplatin to induce tumor regression and to increase survival. *Myd88* deficiency impaired

the early antitumor effect of oxaliplatin, while *Tlr4* deficiency had a partial effect on long-term tumor growth and survival. Thus, tumor-infiltrating myeloid cells produce most of the ROS required for oxaliplatin genotoxicity, by sensing the intestinal commensal bacteria.

Discussion & significance

Intestinal microbiota is essential for maintenance of the intestinal epithelial barrier and development of the immune system. Paradoxically, GF mice are resistant to various types of carcinogenesis, indicating the tumor-promoting effects of intestinal microbiota. The findings of Iida *et al.*, however, indicate that intestinal microbiota has crucial roles in inducing optimal responses to tumor therapy. CpG-ODN immunotherapy requires TNF production by myeloid cells primed with commensal bacteria, while platinum chemotherapy needs ROS generation by tumor-infiltrating inflammatory cells, which are stimulated with commensal bacteria. In the same issue of Science, Viaud *et al*. reported that cyclophosphamide translocates selected species of Gram-positive bacteria in intestinal microbiota into secondary lymphoid organs, where the bacteria stimulate memory Th1 and Th17 immune responses [11]. They further demonstrated that tumor-bearing GF or antibiotics-treated mice exhibited reduced responses to cyclophosphamide. Thus, intestinal microbiota can have great impacts on not only tumor immunotherapy but also various types of systemic chemotherapy. Moreover, cancer patients frequently receive wide-spectrum antibiotic treatment, which can potentially eradicate or disturb intestinal microbiota. Thus, the present observations may raise an alarm on the use of wide-spectrum antibiotics for cancer patients on chemotherapy and/or immunotherapy.

Future Perspective

The current paper by Iida *et al.* and the accompanying paper by Viaud *et al*. revealed the crucial involvement of intestinal commensal microbiota in the tumor response to some types

of chemotherapy. However, there remain several questions to be clarified. First, it is necessary to clarify whether intestinal microbiota have similar roles in tumor response to other types of chemotherapy than platinum agents and cyclophosphamide. Second, it is also required to elucidate how microbiota can induce a good response to chemotherapy at cellular and molecular levels. The most important question is whether intestinal microbiota as a whole or a specific species can confer the responsiveness to tumor therapy. If the latter is the case, it is mandatory to identify the responsible species. Despite the advent of next-generation sequencing technology, which has greatly expanded our knowledge on the composition of the intestinal microbiota, only a third of intestinal commensal bacteria can be cultured *in vitro* at present. Thus, the more detailed characterization of intestinal commensal bacteria at species levels will greatly advance our knowledge on the effects of intestinal microbiota on tumor therapy and will pave the way to improve the efficacy of chemotherapy and/or immunotherapy by modulating intestinal microbiota.

Financial & competing interests disclosure

There is no conflict of interests to disclose.

Executive summary

・Intestinal commensal microbiota is essential for many host physiological processes and contributes to promoting carcinogenesis.

・The paper under evaluation revealed that intestinal microbiota is crucially involved in optimal tumor response to CpG-ODN immunotherapy and platinum chemotherapy.

・The observations may raise the possibility that the modulation of intestinal microbiota can improve the efficacy of chemotherapy and/or immunotherapy.

Bibliography

1. Kamada N, Chen GY, Inohara N, Núñez: Control of pathogens and parabionts by the gut microbiota. *Nat. Immuol.* 14, 685-690, 2013.
2. Reddy BS, Narisawa T, Wright P, Vukusich D, Weisburger JH, Wynder EL: Colon carcinogenesis with azoxymethane and dimethylhydrazine in germ-free rats. *Cancer Res.* 35, 287-290, 1975.
3. Lofgren JL, Whary MT, Ge Z *et al.*: Lack of commensal flora in Helicobacter pylori-infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia. *Gastroenterology* 140, 210-220, 2011.
4. Dapito DH, Mencin A, Gwak GY *et al.*: Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* 21, 504-516, 2012.
5. Couturier-Maillard A, Secher T, Ateequr Rehman A *et al.*: NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J. Clin. Invest.* 123, 700-711, 2013.
6. Smith JL, Bayles DO. The contribution of cytolethal distending toxin to bacterial pathogenesis. *Crit. Rev. Microbiol.* 32, 227-248, 2006.
7. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW: Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. *Cell Host Microbe.* 14, 195-206, 2013.
8. Yoshimoto S, Loo TM, Atarashi K *et al.*: Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 499, 97-101, 2013.
9. Iida N, Dzutsev A, Stewart CA *et al.*: Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* 342, 967-970, 2013.
10. Laurent A, Nicco C, Chéreau C *et al.*: Controlling tumor growth by modulating endogenous production of reactive oxygen species. *Cancer Res.* 65, 948-956, 2005.
11. Viaud S, Saccheri F, Mifnot G *et al.*: The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* 342, 971-976, 2013.