illumina®

The Microbiome Emerges as a Key Player in Cancer

New discoveries in microbiome and cancer research are possible with next-generation sequencing applications.

The Microbiome and Human Health

Humans have a symbiotic relationship with trillions of microbes residing within the gastrointestinal tract.[1] Thousands of species forming this community are collectively known as the microbiome. Interactions between host and microbiome can have dramatic effects on health by aiding digestion, regulating metabolism, conferring resistance against pathogens, and modulating host immunity.[1,2] Balanced microbial communities can positively contribute to training the immune system and maintaining immune homeostasis.[3,4] Dysbiosis is a change in the normal microbiome composition that can initiate chronic inflammation, epithelial barrier breaches, and overgrowth of harmful bacteria. All these factors have been associated with carcinogenesis.[2]

Historically, members of microbial communities were identified by culture and subsequent staining, which precluded the investigation of nonculturable species and strains. However, next-generation sequencing (NGS) methods have revolutionized the study of the microbiome.[5] Without the need to culture or clone individual organisms, NGS enables simultaneous analysis of thousands of species within a microbial community. With the development of bioinformatic tools to manage large volumes of new information, it became possible to assess species diversity and measure dynamic fluctuations in microbial communities. As institutions began to recognize the important role microbes play in health and disease, new initiatives were launched to aid the research community.[6]The NIH-funded Human Microbiome Project (HMP)[7] and the European Metagenomics of the Human Intestinal Tract (MetaHIT)[8] both use NGS-based data to establish valuable reference genome databases for the human microbiome.

Numerous microbial species have been implicated in promoting tumor growth associated with local inflammation.[9-12] More recently, microbes have been shown to have systemic effects on the host that influence the efficacy of anticancer drugs.[13-16] Though the mechanisms of these effects are not fully characterized, modulation of the immune system is frequently a factor. The ability of NGS to analyze complex microbial communities may help to uncover new mechanisms of host–microbe interactions that promote cancer or promote drug efficacy. This application note highlights several key discoveries regarding the influence of the microbiome on cancer development, and reviews the technologies that can be used to help further investigate host–microbe interactions.

Microbial Sequencing Methods

Unlike capillary sequencing or PCR-based approaches, NGS allows researchers to sequence thousands of organisms in parallel. With the ability to obtain high sequence coverage per sample, NGS-based metagenomic sequencing can detect low-abundance members of the microbial community that may be missed or are too expensive to identify using other methods.

Illumina provides streamlined workflows for several NGS methods (Figure 1) that can provide critical genetic insight into cataloging microbiome species and monitoring their dynamics. Three basic NGS applications are available for microbiome studies: 1) 16S rRNA sequencing, 2) shotgun metagenomic sequencing, and 3) metatranscriptome analysis. High-quality reagents and user-friendly software enable efficient progress through each sequencing workflow. The choice of application depends on the research question being asked.

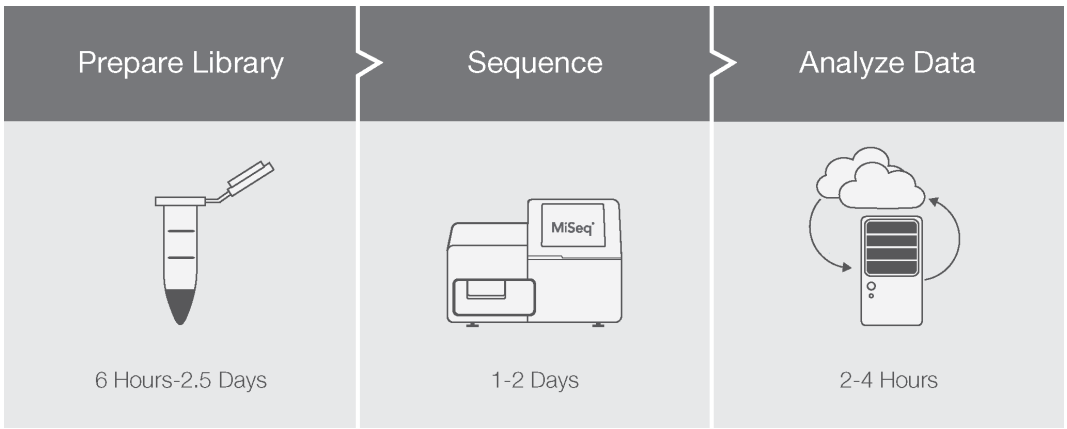

Figure 1: NGS Workflow—The Illumina NGS workflow includes three general steps: library preparation, sequencing, and analysis.

16S rRNA Sequencing

16S ribosomal RNA (rRNA) sequencing is an amplicon-based sequencing method that targets a genetic marker found in all bacteria. It is commonly used to identify bacteria present within a given sample down to the genus and/or species level. 16S rRNA gene sequencing is a well-established method for studying phylogeny and taxonomy of samples from complex microbiomes or environments that can be difficult to study.

Shotgun Metagenomic Sequencing

Shotgun metagenomic sequencing is used to sequence all genomic content in a microbial sample for species identification and functional analysis. With high sequence coverage, shotgun metagenomic sequencing can detect rare and low-abundance members of the microbial community. The method enables biologists to evaluate microbial diversity and detect the abundance of microbes in various environments.

Metatranscriptome Analysis

Metatranscriptomes encompass all RNAs encoded by a group of organisms in a complex sample. Metatranscriptome analysis applies RNA sequencing (RNA-Seq) to microbial samples to determine which species are there, what they are expressing, and how they respond to changes in the environment. Unlike hybridization-based methods, microbial RNA-Seq enables unbiased strand-specific identification of common and novel transcripts. Metatranscriptome information can be used to quantify gene expression changes, predict antibiotic resistance, understand host-pathogen interactions, and track disease progression.

Microbiome Applications in Cancer Research

Cancer can be caused by changes in the microbiome. The roles of individual microbial species in cancer progression have been identified long ago for various tissue types. Localized inflammation has frequently been found in tissues directly exposed to the microbes (Figure 2).[9-12] Alternatively, some tissues have been identified where the microbial population had a localized protective effect.[12,17] Furthermore, some immune-modulatory therapies and conventional therapies rely upon the inflammatory response, which is suppressed in the absence of microbial components.[14]

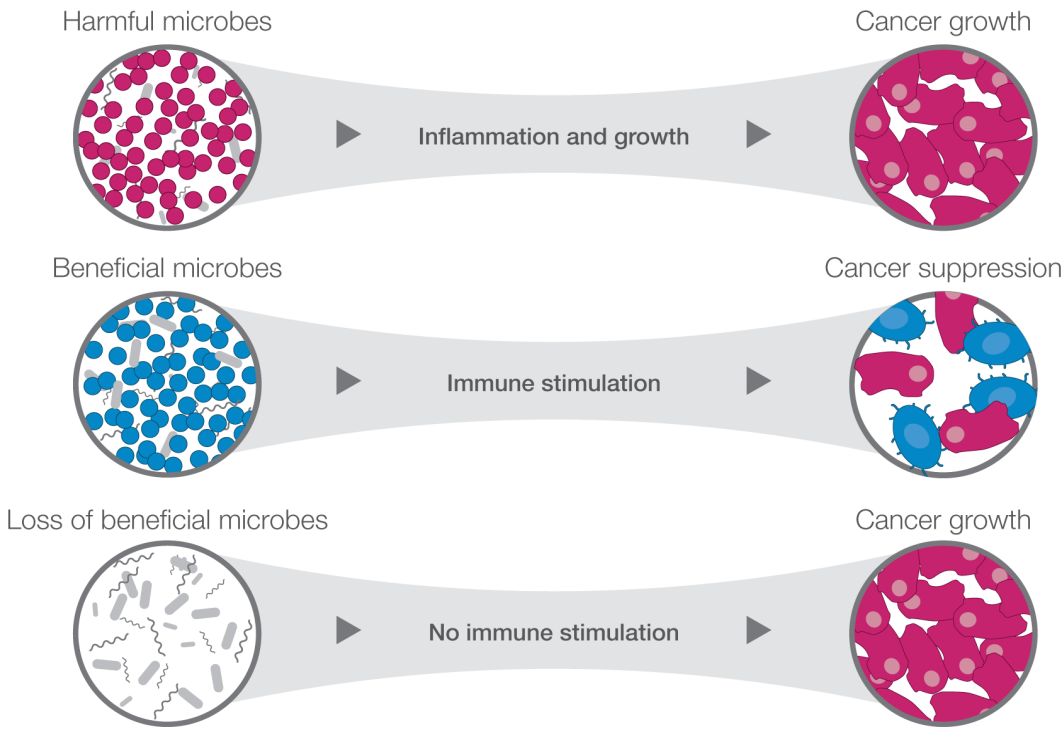

Figure 2: Microbes Can Exert Varied Effects on Cancer Progression—Microbes that influence cytokine release and inflammation can exert either a positive or negative effect on tumor growth, or the ability of the immune system to suppress tumor growth. Profiling of microbial communities in different contexts is critical for the identification of species or conditions that may be targeted for developing new therapeutic approaches.

More recently, studies have emerged describing microbiomes acting at a distance to influence sterile tumor environments,[13-16] and can affect both natural autoimmunity and immune-modulating anticancer therapies. Response to CpG-oligonucleotide immunotherapy and platinum chemotherapy was impaired in sterile or antibiotic-treated mice that exhibited poor tumor infiltration by myeloid-derived cells and low cytokine production.[13] Systemic effects influencing the T-cell repertoire were possibly mediated by cross-reactivity between microbial antigens and tumor antigens. Other studies in mice have demonstrated that hematopoietic differentiation in bone can be impacted by gut microbiome perturbations caused by high-fat diet.[18] This study underscores the possibility of incorporating specific dietary instructions into future models of disease treatment. An improved understanding of microbial influences on the immune system as it relates to cancer could impact both conventional cancer therapies and immune-modulating therapies in the future.

Microbiome Effects on Immunotherapy

Recent studies in mouse models have demonstrated the benefit of assessing microbiomes together with immunotherapy approaches, discovering that key species in the gut microbiome exert systemic influences on the efficacy of immune-modulatory drugs. Two studies have implicated specific bacteria that impact the ability of checkpoint inhibitor drugs to strengthen the immune response. In one study, a negative outcome using CTLA-4 blockade therapy was associated with the absence of a specific gut bacterium.[16] However, the outcome improved with several combinatorial approaches, such as gavage with the bacteria, using bacterial antigens for immunization, or adoptive transfer of antigen-specific T-cells. An independent study used 16S rRNA sequencing to identify another microbe that mediated the effects of anti-PD-L1 treatment.[15] Similarly, a combinatorial approach significantly reduced tumor growth associated with accumulation of T-cells in the tumor microenvironment. Together these studies highlight the potential of identifying beneficial species and using combinatorial approaches involving microbiota manipulation.

Conclusion

Collectively these studies have revealed that both progression of cancer and the efficacy of cancer treatments can be significantly influenced by microbes living in the host. The diversity of the microbiome can be perturbed by diet and drugs, while key species in the microbiome can cause local or systemic influences on host immunity. There is hope that new methods of treatment will be developed that combine existing cancer therapies with methods to encourage growth of beneficial microbes or eliminate harmful ones. However, the variation between individual hosts will likely require profiling of many more microbial communities and their properties to decipher new mechanisms and methods of treatment.

As NGS-based research continues to explore host–microbiome interactions, Illumina strives to align trends in microbiology with the evolution of genomic technologies that compliment and enable the promise of this field. The scale of data delivered by Illumina sequencing systems supports a broad range of cancer research goals. With various NGS applications available, Illumina provides flexible, accurate, and reliable options for analyzing the microbiome. BaseSpace® Informatics Suite offers user-friendly informatics tools for data storage, analysis, and sharing. Through partnerships with leading oncology experts and collaboration with national and international cancer organizations, Illumina continues to expand the portfolio of cancer-focused research solutions.

References

1. Zama D, Biagi E, Masetti R, et al. Gut microbiota and hematopoietic stem cell transplantation: where do we stand? *Bone Marrow Transplant.* 2017;52 (1):7-14.
2. Schwabe R, Jobin C. The microbiome and cancer. *Nat Rev Cancer.* 2013;13 (11):800-812.
3. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science.* 2010; 330:1768–1773.
4. Candela M, Turroni S, Biagi E, Carbonero F, Rampelli S, Fiorentini C et al. Inflammation and colorectal cancer, when microbiota-host mutualism breaks. *World J Gastroenterol.* 2014; 20:908–922.
5. Franzosa EA, Hsu T, Sirota-Madi A, et al. Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. *Nat Rev Microbiol.* 2015;13(6):360-372.
6. Gevers D, Knight R, Petrosino J, et al. The Human Microbiome Project: a community resource for the healthy human microbiome. *PLoS Biol.* 2012;10 (8):e1001377. doi: 10.1371/journal.pbio.1001377.
7. NIH Human Microbiome Project (HMP). hmpdacc.org/ Accessed February 7, 2017.
8. Metagenomics of the Human Intestinal Tract. www.metahit.eu/. Accessed February 7, 2017.
9. NIH National Cancer Institute. https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/h-pylori-fact-sheet. Accessed February 15, 2017.
10. Hieken TJ, Chen J, Hoskin TL, et al. The Microbiome of Aseptically Collected Human Breast Tissue in Benign and Malignant Disease. *Sci Rep.* 2016;6:30751. doi: 10.1038/srep30751.
11. 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.* 2013;14(2):195-206.
12. Pfirschke C, Garris C, Pittet MJ. Common TLR5 mutations control cancer progression. *Cancer Cell.* 2015;27(1):1-3.
13. Zitvogel L, Ayyoub M, Routy B, Kroemer G. Microbiome and Anticancer Immunosurveillance. *Cell.* 2016;165(2):276-287.
14. Iida N, Dzutsev A, Stewart CA, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science.* 2013;342(6161):967-970.
15. Sivan A, Corrales L, Hubert N, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science.* 2015;350 (6264):1084-1089.
16. Vétizou M, Pitt J, Daillère R, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science.* 2015;350(6264):1079-1084.
17. Pevsner-Fischer M, Tuganbaev T, Meijer M, et al. Role of the microbiome in non-gastrointestinal cancers. *World J Clin Oncol.* 2016;7(2):200-213
18. Luo Y, Chen GL, Hannemann N, et al. Microbiota from Obese Mice Regulate Hematopoietic Stem Cell Differentiation by Altering the Bone Niche. *Cell Metab.* 2015;22(5):886-894.

Illumina, Inc. • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

For Research Use Only. Not for use in diagnostic procedures.

illumina®