Gut and Intratumoral Microbiomes in Tumor Metastasis

Subjects: Oncology

Contributor: Aneta Sevcikova, Sona Ciernikova*, Beata Mladosievicova, Michal Mego

Cancer cell dissemination involves invasion, migration, resistance to stressors in the circulation, extravasation, colonization, and other functions responsible for macroscopic metastases. By enhancing invasiveness, motility, and intravasation, the epithelial-to-mesenchymal transition (EMT) process promotes the generation of circulating tumor cells and their collective migration. Preclinical and clinical studies have documented intensive crosstalk between the gut microbiome, host organism, and immune system. According to the findings, polymorphic microbes might play diverse roles in tumorigenesis, cancer progression, and therapy response. Microbial imbalances and changes in the levels of bacterial metabolites and toxins promote cancer progression via EMT and angiogenesis. In contrast, a favorable microbial composition, together with microbiota-derived metabolites, such as short-chain fatty acids (SCFAs), can attenuate the processes of tumor initiation, disease progression, and the formation of distant metastases.

Keywords: gut microbiome ; intratumoral microbiota ; cancer progression ; metastasis ; epithelial-to-mesenchymal transition ; angiogenesis ; microbiota modulation

1. Introduction

The emerging trend of microbiome research in oncology results from studies uncovering the role of microorganisms in the etiology of several malignancies. Preclinical and clinical studies have also revealed a significant impact of the gut and tumor microbiomes on the efficacy of antitumor therapy and treatment-induced toxicity [1]. Moreover, mounting research focuses on the analysis of the microbiome composition in metastatic disease [2]. The significant role of the microbiome in oncogenesis and treatment underlines the fact that polymorphic microbiomes, including intestinal, oral, skin, tumor, lung, and vaginal microbiomes, were added to the extended comprehensive concept termed “The Hallmarks of Cancer”, which summarizes the key characteristics of tumors. The microbiome directly interacts positively or negatively with other hallmarks of malignancies, such as inflammation, immune impairment, genomic instability, and resistance to antitumor therapy [3].

The study of metastasis biology at the cellular, molecular, biochemical, and physical levels has undergone dramatic growth over the last 20 years. While the precise pathways are still under investigation, recent research has indicated new roles of cancer cells, which involve promoting genes with metastasis-driving mutations, cancer stem cells, circulating tumor cells (CTCs), epithelial-to-mesenchymal transition (EMT), and the metastatic dormancy and dynamic plasticity of cancer cells [4][5]. Various studies also demonstrated that the following drive metastatic spread: systemic inflammation; immune system modulation; specific interactions between cancer cells, immune cells, and cells in the tumor microenvironment; the avoidance of anoikis; immune checkpoint regulation; self-seeding, and other mechanisms. Mounting research highlights the role of the intratumoral and mucosal microbiomes in the progression of metastatic processes.

2. The Mechanisms of Tumor Progression and Metastasis

Tumor progression and metastasis represent multi-step processes, resulting in cancer cell changes that enable them to grow, spread, and establish secondary tumors at distant body sites (**Figure 1**).

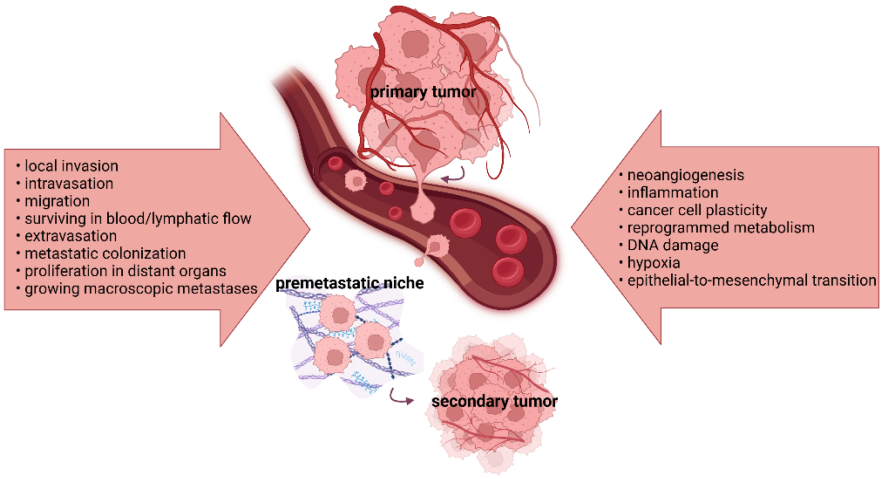

Figure 1. The key processes involved in tumor progression and metastasis. A deep understanding of the crucial events and corresponding mechanisms leading to the formation of distant metastases is essential for developing treatment modalities to target different stages of cancer development and improve patient outcomes.

The activation of invasion and metastasis is initiated by epigenetic changes, cell–cell interactions, growth factors, cytokines, signals from extracellular matrix components, extracellular matrix mechanical pressures, and the intratumoral microbiota [6].

The metastatic cascade includes the detachment of cancer cells from the primary tumor and the gaining of an invasive phenotype, local invasion into surrounding tissue, intravasation into the circulation, systemic transportation, extravasation, and the formation of colonies at distant sites, with adaptation and proliferation in secondary organs.

CTCs typically arise from epithelial tumor cells that undergo EMT, resulting in the loss of cell–cell adhesion and apical–basal polarity, the reorganization of the cytoskeleton, acquiring properties of tumor stem cells, and resistance to therapy. This process is regulated by transcription factors in tumor cells (Snail 1, Slug, ZEB1, Twist, FOXC2, etc.) and signaling pathways from the tumor microenvironment (WNT, Notch, Hedgehog, TGFβ, FGF, EGF, HGF signaling, etc.). Additionally, the hypoxia and activation of specific signaling pathways, including PI3K, WNT/β-catenin, and MAPK, affect EMT regulation [7][8]. Many studies focus not only on CTC detection and enumeration but also on CTC biomarkers, among which EMT markers are of great interest [9][10][11]. The most aggressive CTCs are related to the infiltration of the primary tumor or established metastasis in a process of “self-seeding”. Self-seeding in metastasis is the recruitment of cancer cells and the re-seeding of primary tumors and existing metastases by aggressive cancer cell clones [12][13].

Cancer cells can induce neutrophils to release neutrophil extracellular traps (NETs), which sequester CTCs and promote the metastatic process [14][15][16][17][18]. A certain number of CTCs can be eliminated by anoikis, the programmed apoptosis of cells [19]. However, cells can develop an anoikis-resistant state via oncogene activation (e.g., *ERBB2* and *RAS*), an integrin switch (e.g., the downregulation of αvβ3 integrin expression), the constitutive activation of antiapoptotic pathways (e.g., the PI3K/Akt signaling pathway), the triggering of EMT, microRNAs (e.g., the downregulation of the miR200 family), high oxidative stress (e.g., activated growth factor receptors increase intracellular reactive oxygen species production by activating enzymes such as NADPH oxidase and lipoxygenase), hypoxia, the modulation of extracellular matrix stiffness, and the metabolic reprogramming of cancer cells [20]. Tumor cells can attach to specific distant organs/tissues and form colonies through distinct adhesion molecules, including proteoglycans (e.g., CD44), mucins (e.g., MUC16), integrins (e.g., α2β1), and the members of the immunoglobulin superfamily (e.g., ICAM1, VCAM1, and L1CAM) [21].

Before the arrival of tumor cells from primary tumors to the premetastatic niche [22], hematopoietic progenitor cells (VEGFR1-positive) travel from the bone marrow into the circulation and establish themselves in secondary organs, where they adhere to fibronectin, produced by fibroblasts and fibroblast-like cells [23]. The adherence is mediated by the integrin VLA-4, expressed by hematopoietic progenitor cells [24]. The nidation of tumor cells is primarily influenced by stromal-derived factor 1 (SDF-1), binding to the chemokine receptor CXCR4 [25]. CXCR4 receptor expression on breast cancer tumor cells is a typical determinant of bone metastasis [26][27]. Its activation results in pseudopodia formation and integrin modulation, followed by the recruitment of endothelial cells (VEGFR2-positive) to the distant site [28].

Cancer cells and the tumor microenvironment produce factors that influence angiogenic processes, with the key drivers being VEGF-A [29][30] binding to VEGFR2 receptor [31]. Alterations of protooncogenes (*RAS* and *SRC*) and tumor suppressor genes (*TP53* and *VHL*) correlate with VEGF overproduction by tumor cells. Hypoxia is the principal stimulator of VEGF production, and hypoxia-inducible transcription factors (HIF-1α and HIF-2α) play a central role in VEGF

regulation. Other angiogenesis inductors, such as FGF1, EGF2, PDGF-B, PDGF-C, and EGF, bind to their respective receptors on blood vessel endothelial cells and induce proliferation and migration [32]. Besides the conventional angiogenic mediators, BMP9 signaling and Shh signaling also participate in the process [33]. In addition, exosomes released by cancer and immune cells may transport various proangiogenic molecules like VEGF, MMPs, and microRNAs [34].

3. The Relationship between Microbiome and Cancer Progression-Related Processes

In recent years, the correlation between the microbiome, cancer, and metastatic disease has gained more attention (**Figure 2**). Many studies confirmed that certain microbes and their metabolites are associated with a better/worse therapy response and patient outcomes.

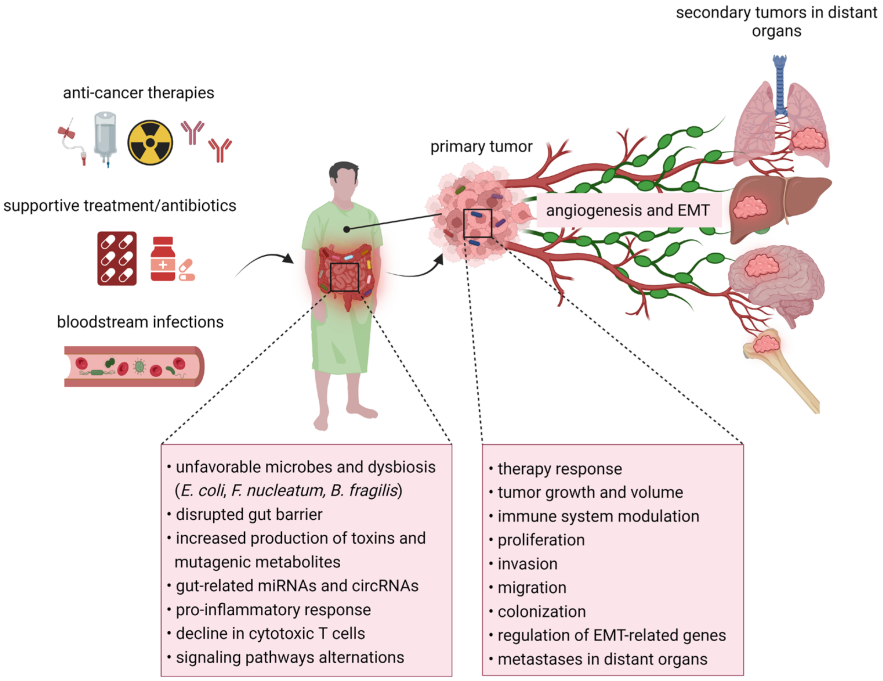

Figure 2. The involvement of the gut and intratumoral microbiome in metastatic processes. Not only cancer development but also the type of anti-cancer therapy affects the diversity of microbial composition in the gastrointestinal tract and alters microbial-associated metabolites. The deregulated thickness of the gut mucosal layer might be responsible for bacterial translocation and development of bloodstream infections. Gastrointestinal dysbiosis results in the inflammation that promotes cancer cell spread due to changed immune responses. Microbes within the tumor microenvironment affect the progression of cancer via modulated immunity and changed inflammatory signaling pathways. Moreover, studies observed the relationship between intratumoral bacteria and metastasis via increased resistance to mechanical stress. Abbreviations: EMT, epithelial-to-mesenchymal transition.

Understanding the mechanisms by which unfavorable microbes have an impact on tumor progression is an active area of recent research. Therefore, intensive research in numerous ongoing clinical trials might shed more light on prognostic microbial markers for treatment outcomes in metastatic disease (**Table 1**). The identification of microbial biomarkers will help to understand how the microbiome is implicated in cancer progression.

Table 1. Exploring the microbial markers associated with treatment outcomes in advanced or metastatic cancer patients (according to https://ClinicalTrials.gov/, accessed on 18 October 2023).

Study	Study Design	Disease	Purpose	Patients (n)	Intervention	Study Status
NCT03941080	An observational prospective study	Metastatic CRC	To confirm the microbial taxa associated with treatment response and side effects in metastatic or irresectable disease	300 adults/ older adults	Enrolled patients will be newly diagnosed with an indication for standard palliative systemic treatment.	Recruiting
NCT04579484	An observational prospective study	Metastatic breast cancer	To determine the gut microbiome in fecal samples of patients with ER^+ $HER2^-$ breast cancer and assess the relationship between dietary factors and microbiome	20 adults/ older adults	Patients will receive endocrine therapy with an aromatase inhibitor combined with an inhibitor of cyclin-dependent kinases 4 and 6.	Recruiting
NCT04804956	An observational prospective study	Metastatic rectal cancer	To identify the profile of the mesorectal microbiome and correlation with poor prognosis prediction	100 adults	Participants will receive neoadjuvant treatment.	Recruiting
NCT04579978	An observational prospective study	Metastatic solid cancer	To study changes in the gut microbial community after ICI and evaluate bacterial species associated with treatment efficacy	60 adults/ older adults	Patients will be enrolled in the study for planned standard-of-care ICI.	Recruiting
NCT05878977	An interventional open-label study	Metastatic melanoma	To define novel markers for the prediction of therapy response	150 adults/ older adults	Immunotherapy will consist of PD-1 and CTLA-4 inhibitors.	Recruiting
NCT05635149	An observational prospective study	Metastatic CRC	To assess the composition of the gut microbiome and its association with treatment efficacy	100 adults/ older adults	Patients will be treated with Fruquintinib, ICI plus RT, or Fruquintinib and ICI alone.	Recruiting

Study	Study Design	Disease	Purpose	Patients (n)	Intervention	Study Status
NCT05753839	An interventional randomized open-label study with parallel assignment	Metastatic clear cell renal cell carcinoma/kidney cancer	To correlate the gut and urine microbiome compositions with OS, PFS, and ORR	40 adults/ older adults	Patients will receive ICI followed by maintenance therapy with ICI or cytoreductive nephrectomy ± metastasectomy after ICI.	Recruiting
NCT04090710	An interventional randomized study with parallel assignment	Metastatic renal cell carcinoma	To investigate the changes in the gut microbiome via analysis of stool samples	78 children/ adults/ older adults	Patients will undergo cytoreductive stereotactic body RT with a combination of ICIs vs. one ICI alone.	Recruiting
NCT04243720	An observational prospective study	Metastatic solid cancer	To determine changes in the gut microbiome associated with resistance to immunotherapy	100 adults/ older adults	Only participants who progressed on immunotherapy will be enrolled in this study.	Recruiting
NCT04148378	An observational case-only prospective study	CRC neoplasms/ metastatic CRC/ colorectal sarcoma/ adenocarcinoma	To correlate microbiome composition with type of disease	100 children/ adults/ older adults	There is no intervention for the study.	Unknown
NCT04516135	An interventional randomized open-label study with parallel assignment	Metastatic gynecologic cancers	To describe overall diversity, richness, and specific microbial dynamics in the gut and vaginal microbiomes	108 adults/ older adults	Females will be treated with 3D conformal RT/intensity-modulated RT/volume-modulated arc therapy at the physician’s discretion for 1 fraction in the absence of RT-induced toxicities or progression.	Recruiting

Study	Study Design	Disease	Purpose	Patients (n)	Intervention	Study Status
NCT04214015	An observational case-only prospective study	Metastatic mesothelioma	To analyze the relative abundance of bacterial members in the gut microbiome	100 children/ adults/ older adults	There is no intervention for the study.	Unknown
NCT03818061	An interventional non-randomized study with parallel assignment	Metastatic HNSCC	To characterize the gut microbiome in immunotherapy using whole-metagenome sequencing	33 adults/ older adults	Patients with/without human papillomavirus will receive atezolizumab combined with bevacizumab.	Active, but not recruiting
NCT03698461	An interventional open-label study with single-group assignment	Metastatic neoplasms/ colorectal neoplasms/ colonic neoplasms/ rectal neoplasms	To determine fecal microbial profile in different time frames	20 adults/ older adults	Anti-cancer treatment will consist of atezolizumab with bevacizumab, levoleucovorin, oxaliplatin, and 5-fluorouracil.	Active, but not recruiting
NCT03977571	An interventional randomized open-label study with parallel assignment	Metastatic renal cell carcinoma/ kidney cancer/ synchronous neoplasm	To correlate the gut microbiome with OS, PFS, and ORR	400 adults/ older adults	Patients will receive deferred cytoreductive nephrectomy/no surgery following nivolumab with ipilimumab or tyrosine kinase inhibitors.	Recruiting
NCT04636775	An observational prospective study	Metastatic non-small-cell lung cancer	To assess the correlations between gut microbiome composition and adverse effects and differences between responders and non-responders	46 adults/ older adults	Patients will be treated with immunotherapy using ICI.	Recruiting

Study	Study Design	Disease	Purpose	Patients (n)	Intervention	Study Status
NCT04219137	An observational prospective study	Metastatic EGA	To study the microbiome in feces and rectal swab samples	120 adults/ older adults	Participants will undergo platinum-based chemotherapy.	Unknown
NCT03161756	An interventional non-randomized study parallel assignment	Metastatic melanoma	To explore associations between the gut microbiome and therapy response	72 adults/ older adults	Nivolumab alone or in combination with ipilimumab will be administered intravenously plus denosumab subcutaneously.	Active, but not recruiting
NCT04720768	An interventional open-label study with sequential assignment	Metastatic melanoma	To identify fecal biomarkers associated with therapy response/resistance	78 adults/ older adults	Patients will receive combined treatment with encorafenib, binimetinib, and palbociclib.	Recruiting
NCT03340129	An interventional randomized open-label study with parallel assignment	Metastatic melanoma	To observe the diversity and composition of the gut microbiome and to determine the correlation between mucosal integrity and microbes	218 adults/ older adults	Treatment will include ipilimumab and nivolumab with concurrent intracranial stereotactic RT or ipilimumab and nivolumab alone.	Recruiting

Abbreviations: CRC, colorectal cancer; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; EGA, esophagogastric adenocarcinoma; HNSCC, head and neck squamous cell carcinoma; ICI, immune checkpoint inhibitors; ORR, overall response rate; OS, overall survival; PD-1, programed cell death protein 1; PFS, progression-free survival; RT, radiotherapy.

4. The Studies of Microbiome Composition in Metastatic Disease

Recently, Hilmi et al. studied samples obtained from the lymph nodes, lungs, and livers of patients suffering from different cancer types, such as breast, lung, and colorectal malignancies. A higher presence of *F. nucleatum* was specific to lung metastases. The microbial load in lymph node metastases was lower than in liver and lung metastases. However, the authors did not observe a relationship between the type of primary tumor and the microbial composition in metastases [35]. The level of *Eubacterium halli* in stool samples is negatively associated with fatigue in patients with advanced, metastatic, unresectable colon, ovarian, cervical, and non-small-cell lung cancers [36]. In vivo experiments confirmed that gut microbial depletion via a broad-spectrum antibiotic cocktail reduced the incidence of metastases in melanoma, pancreatic, or colon cancer murine models [37]. Spakowicz et al. performed a retrospective analysis of 690 patients treated with immunotherapy for metastatic melanoma or non-small-cell lung cancer. The results showed that antibiotics and corticosteroids reduced overall survival (OS), but no direct microbiome measurements were performed [38].

The fundamental studies focusing on the microbiome composition in metastatic disease are summarized in **Table 2**.

Table 2. Detection of specific microorganisms in advanced/metastatic cancer. The table summarizes fundamental preclinical/clinical studies and their major findings.

Malignancy	Study Type Preclinical/Clinical	Intervention	Changes in Microbial Composition	Major Findings	Ref.
CRC with liver/lung metastases	Patients	Regorafenib plus toripalimab	*Fusobacterium*, *Alistipes*, *Bilophila*, and *Acidaminococcus*	A higher level of specific bacteria was observed in non-responders. Shorter PFS correlated with a higher amount of *Fusobacterium*.	[39]
FAP	Patients/ mice	No intervention provided	*E. coli* and *ETBF*	Both bacterial taxa were biofilm members in FAP tissues from patients. Colonization with *E. coli* and *ETBF* increased DNA damage and IL-17 production in carcinogen-treated mice.	[40]
CRC with liver/lung metastases	Patients	Quxie capsules	Actinobacteria, *Oscillibacter*, *Eubacterium*, and *Lachnospiraceae*	Capsules increased butyrate-producing, immunity-stimulating, and anti-cancer bacterial taxa and enhanced Th cells, both CD4 and CD8 cells.	[41]
PDAC with lymph node metastases	Patients	No intervention	*Leuconostoc*, *Sutterella*, *Comamonas*, and *Turicibacter*	Lower levels of *Leuconostoc* and *Sutterella* were documented in tumors with a size ≥3 cm. An increase in lymph node metastases correlated with a higher abundance of *Comamonas* and *Turicibacter*. On the contrary, *Streptococcus* dominated recurrence-free tumors.	[42]
Hepatocellular carcinoma	Mice	NpRg3	Bacteroidetes, Verrucomicrobia, and Firmicutes	Developed NpRg3 remodeled gut microbiome via reduced Firmicutes and increased Bacteroidetes and Verrucomicrobia in stool samples. Moreover, NpRg3 attenuated tumor development and lung metastatic formation in dimethylnitrosamine-induced spontaneous murine carcinoma.	[43]

Malignancy	Study Type Preclinical/Clinical	Intervention	Changes in Microbial Composition	Major Findings	Ref.
Lung cancer	Patients	Systemic therapy/surgical resection	*Legionella* and *Thermus*	*Thermus* was abundant in the lung microbiome in patients with advanced cancer stages, while *Legionella* was enriched in patients with developed metastases. Alpha diversity in tumor tissues was lower than in non-malignant lung tissue samples.	[44]
Hormone receptor-positive breast cancer	Mice	Antibiotic cocktail (vancomycin, ampicillin, metronidazole, neomycin, and gentamicin)	*Blautia*, *Alistipes*, *Blautia*, *Escherichia/Shigella*, and *Bilophila*	Orally gavaged antibiotics caused commensal dysbiosis with a higher abundance of specific genera in poorly metastatic mice. Antibiotics promoted tumor cell dissemination to the lungs/peripheral blood/and lymph nodes.	[45]
Breast cancer	Patients	No intervention	*Streptococcus*, *Campylobacter*, *Moraxellaceae*, *Lactobacillales*, *Bacilli*, *Epsilonproteobacteria*, *Veillonella*, *Acinetobacter*, *Pseudomonadales*, *Megamonas*, and *Akkermansia*	Listed bacteria, except for *Megamonas* and *Akkermansia*, were increased in stool samples of patients with bone metastases. However, the results showed lowered levels of *Megamonas* and *Akkermansia.* Bacterial diversity was reduced in the order of normal controls, patients without metastases, and patients with bone metastases.	[46]
Breast cancer	Patients	Neoadjuvant chemotherapy	*Streptococcus*, *Pseudomonas*, *Brevundimonas*, and *Staphylococcus*	Chemotherapy decreased intratumoral *Streptococcus* and increased *Pseudomonas.* The development of distant metastases correlated with a higher presence of *Brevundimonas* and *Staphylococcus* in primary breast tumors.	[47]

Malignancy	Study Type Preclinical/Clinical	Intervention	Changes in Microbial Composition	Major Findings	Ref.
Oral squamous cell carcinoma	Patients	Therapeutic neck dissection due to positive lymph node metastases	*Tannerella*, *Fusobacterium*, *Prevotella*, *Stomatobaculum*, *Bifidobacterium*, *Finegoldia Peptostreptococcaceae*, and *Shuttleworthia*	Two taxa—*Tannerella* and *Fusobacterium*—were enriched in the oral microbiome of patients without metastases. Other genera from the listed panel increased in patients with developed lymph node metastases. Differences in alpha diversity between the oral microbiome of 2 analyzed groups were not significant.	[48]
Castrate-resistant prostate cancer	Patients	Immune checkpoint inhibitor (pembrolizumab)	*A. muciniphila*, *B. thetaiotaomicron*, *B. fragilis*, *and R. unassigned*	*A. muciniphila* was depleted in pembrolizumab responders, while other listed microbes were higher in responding patients.	[49]
Renal cell carcinoma	Patients	Immune checkpoint inhibitor (nivolumab or nivolumab plus ipilimumab	*A. muciniphila*, *B. adolescentis*, *B. intestinihominis*, *Odoribacter splanchnicus*, *Bacteroides ovatus*, and *Eggerthella lenta*	*A. muciniphila*, *B. adolescentis*, *B. intestinihominis*, and *O. splanchnicus* correlated with clinical benefit in metastatic patients, while *B. ovatus* and *E. lenta* were associated with no clinical benefit from immunotherapy.	[50]
Renal cell carcinoma	Patients	Immune checkpoint inhibitor	*Akkermansia*	The presence of *Akkermansia* was documented in both responding and non-responding patients to immunotherapy. Therefore, host-specific or tumor factors might affect therapy response.	[51]
Melanoma	Patients	Immune checkpoint inhibitor	*Lactobacillales*, *Clostridiales/Ruminococcaceae*, *Faecalibacterium*, *Bacteroidales*, *B. thetaiotaomicron*, *E. coli*, and *Anaerotruncus colihominis*	*Lactobacillales* dominated the oral microbiome of all metastatic patients. *Clostridiales/Ruminococcaceae*, *Faecalibacterium*, and alpha diversity were greater in responders, while *Bacteroidales*, *B. thetaiotaomicron*, *E. coli*, and *A. colihominis* were abundant in non-responders.	[52]

References

1. Sevcikova, A.; Izoldova, N.; Stevurkova, V.; Kasperova, B.; Chovanec, M.; Ciernikova, S.; Mego, M. The Impact of the Microbiome on Resistance to Cancer Treatment with Chemotherapeutic Agents and Immunotherapy. Int. J. Mol. Sci. 2022, 23, 488.
2. Cullin, N.; Azevedo Antunes, C.; Straussman, R.; Stein-Thoeringer, C.K.; Elinav, E. Microbiome and cancer. Cancer Cell 2021, 39, 1317–1341.
3. Hanahan, D. Hallmarks of Cancer: New Dimensions. Cancer Discov. 2022, 12, 31–46.
4. Massague, J.; Ganesh, K. Metastasis-Initiating Cells and Ecosystems. Cancer Discov. 2021, 11, 971–994.
5. Gerstberger, S.; Jiang, Q.; Ganesh, K. Metastasis. Cell 2023, 186, 1564–1579.
6. Fares, J.; Fares, M.Y.; Khachfe, H.H.; Salhab, H.A.; Fares, Y. Molecular principles of metastasis: A hallmark of cancer revisited. Signal Transduct. Target. Ther. 2020, 5, 28.
7. Thiery, J.P. Epithelial-mesenchymal transitions in tumour progression. Nat. Rev. Cancer 2002, 2, 442–454.

Melanoma	Study Type	Intervention	Changes in Microbial Composition	Major Findings	Ref.
	Preclinical/Clinical				
Melanoma	Patients	Immune checkpoint inhibitor	*B. longum*, *C. aerofaciens*, *F. faecium*, *R. obeum*, and *R. intestinalis*	The authors observed a higher abundance of *R. obeum* and *R. intestinalis* in non-responders, while the other 3 species were enriched significantly in the responder gut microbiome.	[53]
Melanoma	Patients	Immune checkpoint inhibitor	*Clostridiales* and *Bacteroidales*	Higher bacterial diversity with the prevalence of *Clostridiales* was observed in the gut microbiome of responding patients. However, the dominance of *Bacteroidales* within the gut microbiome characterized non-responders.	[54]
Melanoma	Patients	No intervention	*Corynebacterium*	In swab samples, *Corynebacterium* was the most detected taxa in advanced-stage patients. However, the authors did not detect associations between cutaneous microbiome and cancer stage.	[55]

8. Yang, J.; Antin, P.; Berx, G.; Blanpain, C.; Brabletz, T.; Bronner, M.; Campbell, K.; Cano, A.; Casanova, J.; Christofori, G.; et al. Guidelines and definitions for research on epithelial-mesenchymal transition. Nat. Rev. Mol. Cell Biol. 2020, 21, 341–352.
9. Giuliano, M.; Giordano, A.; Jackson, S.; Hess, K.R.; De Giorgi, U.; Mego, M.; Handy, B.C.; Ueno, N.T.; Alvarez, R.H.; De Laurentiis, M.; et al. Circulating tumor cells as prognostic and predictive markers in metastatic breast cancer patients receiving first-line systemic treatment. Breast Cancer Res. 2011, 13, R67.
10. Mego, M.; Karaba, M.; Minarik, G.; Benca, J.; Sillova, M.; Gronesova, P.; Sedlackova, T.; Manasova, D.; Kalavska, K.; Pindak, D.; Cristofanilli, M.; et al. Circulating Tumor Cells with Epithelial-to-mesenchymal Transition Phenotypes Associated with Inferior Outcomes in Primary Breast Cancer. Anticancer Res. 2019, 39, 1829–1837.
11. Fridrichova, I.; Kalinkova, L.; Ciernikova, S. Clinical Relevancy of Circulating Tumor Cells in Breast Cancer: Epithelial or Mesenchymal Characteristics, Single Cells or Clusters? Int. J. Mol. Sci. 2022, 23, 12141.
12. Kim, M.Y.; Oskarsson, T.; Acharyya, S.; Nguyen, D.X.; Zhang, X.H.; Norton, L.; Massague, J. Tumor self-seeding by circulating cancer cells. Cell 2009, 139, 1315–1326.
13. Comen, E.; Norton, L. Self-seeding in cancer. Recent Results Cancer Res. 2012, 195, 13–23.
14. Park, J.; Wysocki, R.W.; Amoozgar, Z.; Maiorino, L.; Fein, M.R.; Jorns, J.; Schott, A.F.; Kinugasa-Katayama, Y.; Lee, Y.; Won, N.H.; et al. Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. Sci. Transl. Med. 2016, 8, 361ra138.
15. Cools-Lartigue, J.; Spicer, J.; McDonald, B.; Gowing, S.; Chow, S.; Giannias, B.; Bourdeau, F.; Kubes, P.; Ferri, L. Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis. J. Clin. Investig. 2013, 123, 3446–3458.
16. Zhong, W.; Wang, Q.; Shen, X.; Du, J. The emerging role of neutrophil extracellular traps in cancer: From lab to ward. Front. Oncol. 2023, 13, 1163802.
17. Adrover, J.M.; McDowell, S.A.C.; He, X.Y.; Quail, D.F.; Egeblad, M. NETworking with cancer: The bidirectional interplay between cancer and neutrophil extracellular traps. Cancer Cell 2023, 41, 505–526.
18. Hu, W.; Lee, S.M.L.; Bazhin, A.V.; Guba, M.; Werner, J.; Niess, H. Neutrophil extracellular traps facilitate cancer metastasis: Cellular mechanisms and therapeutic strategies. J. Cancer Res. Clin. Oncol. 2023, 149, 2191–2210.
19. Nepali, P.R.; Kyprianou, N. Anoikis in phenotypic reprogramming of the prostate tumor microenvironment. Front. Endocrinol. 2023, 14, 1160267.

Abbreviations: ETBF, enterotoxigenic *B. fragilis*; FAP, familial adenomatous polyposis; NpRg3, nanoparticle conjugation of ginsenoside Rg3; PDAC, pancreatic ductal adenocarcinoma; PFS, progression free survival; Th cells, T helper cells.

20. Paoli, P.; Giannoni, E.; Chiarugi, P. Anoikis molecular pathways and its role in cancer progression. Biochim. Biophys. Acta (BBA) Mol. Cell Res. 2013, 1833, 3481–3498.
21. Pretzsch, E.; Bösch, F.; Neumann, J.; Ganschow, P.; Bazhin, A.; Guba, M.; Werner, J.; Angele, M. Mechanisms of Metastasis in Colorectal Cancer and Metastatic Organotropism: Hematogenous versus Peritoneal Spread. J. Oncol. 2019, 2019, 7407190.
22. Peinado, H.; Zhang, H.; Matei, I.R.; Costa-Silva, B.; Hoshino, A.; Rodrigues, G.; Psaila, B.; Kaplan, R.N.; Bromberg, J.F.; Kang, Y.; et al. Pre-metastatic niches: Organ-specific homes for metastases. Nat. Rev. Cancer 2017, 17, 302–317.
23. Kaplan, R.N.; Riba, R.D.; Zacharoulis, S.; Bramley, A.H.; Vincent, L.; Costa, C.; MacDonald, D.D.; Jin, D.K.; Shido, K.; Kerns, S.A.; et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 2005, 438, 820–827.
24. Huang, H. Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances. Sensors 2018, 18, 3249.
25. Kryczek, I.; Wei, S.; Keller, E.; Liu, R.; Zou, W. Stroma-derived factor (SDF-1/CXCL12) and human tumor pathogenesis. Am. J. Physiol. Cell Physiol. 2007, 292, C987–C995.
26. Xu, C.; Zhao, H.; Chen, H.; Yao, Q. CXCR4 in breast cancer: Oncogenic role and therapeutic targeting. Drug Des. Dev. Ther. 2015, 9, 4953–4964.
27. Muller, A.; Homey, B.; Soto, H.; Ge, N.; Catron, D.; Buchanan, M.E.; McClanahan, T.; Murphy, E.; Yuan, W.; Wagner, S.N.; et al. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001, 410, 50–56.
28. Kaplan, R.N.; Rafii, S.; Lyden, D. Preparing the "soil": The premetastatic niche. Cancer Res. 2006, 66, 11089–11093.
29. De Palma, M.; Biziato, D.; Petrova, T.V. Microenvironmental regulation of tumour angiogenesis. Nat. Rev. Cancer 2017, 17, 457–474.
30. Kim, H.J.; Ji, Y.B.; Lee, Y.M. Crosstalk between angiogenesis and immune regulation in the tumor microenvironment. Arch. Pharm. Res. 2022, 45, 401–416.

5. Microbiota Modulation and Cancer Progression

Gut microbiome modulation leading to increased intestinal barrier and anti-inflammatory responses might inhibit pro-tumorigenic processes, including cancer progression, migration, invasion, and angiogenesis [56][57]. The intragastric administration of *Lactobacillus reuteri* FLRE5K1 inhibited the incidence of tumors in BALB/C mice injected with melanoma cells. The results indicated that *L. reuteri* FLRE5K1 might restrain the development of tumors due to the blockade of the migration and colonization of cancer cells [58]. A preclinical study noted that fecal transplants from obese mice to lean mice with B16F10 tumors stimulated melanoma development and supported cancer progression [59]. The aim of recent studies is to assess the effect of probiotic supplementation on tumor progression.

Chen et al. showed that probiotics composed of *B. longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* attenuated the development of lung metastases and prolonged survival in melanoma-bearing mice. Probiotic supplementation led to increased levels of *Lachnospiraceae*, *Streptococcus*, and *Lachnoclostridium* [60]. Aerosolized probiotic *Lactobacillus rhamnosus*, which reached lung murine tissue, reduced the number of lung metastases. Moreover, aerosolized *L. rhamnosus* and *Bifidobacterium bifidum* increased the effect of conventional chemotherapeutic dacarbazine in melanoma-bearing mice [61]. A probiotic product composed of *L. rhamnosus* GG, *E. coli* Nissle 1917, and VSL#3 (1:1:1) orally administered to mice bearing hepatocellular carcinoma retarded tumor growth and inhibited angiogenesis. Probiotic administration altered the gut microbiome toward valerate producer *Oscillibacter* and propionate producer *Prevotella*, both known for their ability to reduce Th17 polarization and support Treg/Tr1 cells in the gut. The results showed that probiotics reduced the recruitment of Th17 cells, which secrete pro-inflammatory cytokines to the tumor microenvironment. Additionally, several angiogenic factors, including ANG2, FLT-1, KDR, TEK, and VEGF-A, were downregulated in the probiotic-supplemented group [62]. Daily oral administration of CBM588 containing *Clostridium butyricum* prolonged PFS in patients with metastatic renal cell carcinoma treated with nivolumab and ipilimumab. The

In conclusion, the gut and intratumoral microbiomes can influence cancer progression and metastatic processes in various ways, including inducing inflammation and immune system modulation, affecting metabolism and providing energy for cancer cell spread, promoting the angiogenesis caused by microbial metabolites, and impacting cancer treatment efficacy to control metastatic disease. Particular attention should be paid to addressing the ability of specific microorganisms and microbiota-derived metabolites to shape the immune system and tumor microenvironment, potentially promoting the growth and spread of cancer cells.

38. Spakowicz, D.; Hoyd, R.; Muniak, M.; Husain, M.; Bassett, J.S.; Wang, L.; Tinoco, G.; Patel, S.H.; Burkart, J.; Miah, A.; et al. Inferring the role of the microbiome on survival in patients treated with immune checkpoint inhibitors: Causal modeling, timing, and classes of concomitant medications. BMC Cancer 2020, 20, 383.

39. Wang, F.; He, M.M.; Yao, Y.C.; Zhao, X.; Wang, Z.Q.; Jin, Y.; Luo, H.Y.; Li, J.B.; Wang, F.H.; Qiu, M.Z.; et al. Regorafenib plus toripalimab in patients with metastatic colorectal cancer: A phase Ib/II clinical trial and gut microbiome analysis. Cell Rep. Med. 2021, 2, 100383.

40. Dejea, C.M.; Fathi, P.; Craig, J.M.; Boleij, A.; Taddese, R.; Geis, A.L.; Wu, X.; DeStefano Shields, C.E.; Hechenbleikner, E.M.; Huso, D.L.; et al. Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. Science 2018, 359, 592–597.

41. Sun, L.; Yan, Y.; Chen, D.; Yang, Y. Quxie Capsule Modulating Gut Microbiome and Its Association with T cell Regulation in Patients with Metastatic Colorectal Cancer: Result From a Randomized Controlled Clinical Trial. Integr. Cancer Ther. 2020, 19, 1534735420969820.

42. Jeong, J.Y.; Kim, T.B.; Kim, J.; Choi, H.W.; Kim, E.J.; Yoo, H.J.; Lee, S.; Jun, H.R.; Yoo, W.; Kim, S.; et al. Diversity in the Extracellular Vesicle-Derived Microbiome of Tissues according to Tumor Progression in Pancreatic Cancer. Cancers 2020, 12, 2346.

43. Ren, Z.; Chen, X.; Hong, L.; Zhao, X.; Cui, G.; Li, A.; Liu, Y.; Zhou, L.; Sun, R.; Shen, S.; et al. Nanoparticle Conjugation of Ginsenoside Rg3 Inhibits Hepatocellular Carcinoma Development and Metastasis. Small 2020, 16, e1905233.

44. Yu, G.; Gail, M.H.; Consonni, D.; Carugno, M.; Humphrys, M.; Pesatori, A.C.; Caporaso, N.E.; Goedert, J.J.; Ravel, J.; Landi, M.T. Characterizing human lung tissue microbiota and its relationship to epidemiological and clinical features. Genome Biol. 2016, 17, 163.

45. Buchta Rosean, C.; Bostic, R.R.; Ferey, J.C.M.; Feng, T.Y.; Azar, F.N.; Tung, K.S.; Dozmorov, M.G.; Smirnova, E.; Bos, P.D.; Rutkowski, M.R. Preexisting Commensal Dysbiosis Is a Host-Intrinsic Regulator of Tissue Inflammation and Tumor Cell Dissemination in Hormone Receptor-Positive Breast Cancer. Cancer Res. 2019, 79, 3662–3675.

46. Wenhui, Y.; Zhongyu, X.; Kai, C.; Zhaopeng, C.; Jinteng, L.; Mengjun, M.; Zepeng, S.; Yunshu, C.; Peng, W.; Yanfeng, W.; et al. Variations in the Gut Microbiota in Breast Cancer Occurrence and Bone Metastasis. Front. Microbiol. 2022, 13, 894283.

47. Chiba, A.; Bawaneh, A.; Velazquez, C.; Clear, K.Y.J.; Wilson, A.S.; Howard-McNatt, M.; Levine, E.A.; Levi-Polyachenko, N.; Yates-Alston, S.A.; Diggle, S.P.; et al. Neoadjuvant Chemotherapy Shifts Breast Tumor Microbiota Populations to Regulate Drug Responsiveness and the Development of Metastasis. Mol. Cancer Res. 2020, 18, 130–139.

48. Eun, Y.G.; Lee, J.W.; Kim, S.W.; Hyun, D.W.; Bae, J.W.; Lee, Y.C. Oral microbiome associated with lymph node metastasis in oral squamous cell carcinoma. Sci. Rep. 2021, 11, 23176.

49. Peiffer, L.B.; White, J.R.; Jones, C.B.; Slottke, R.E.; Ernst, S.E.; Moran, A.E.; Graff, J.N.; Sfanos, K.S. Composition of gastrointestinal microbiota in association with treatment response in individuals with metastatic castrate resistant prostate cancer progressing on enzalutamide and initiating treatment with anti-PD-1 (pembrolizumab). Neoplasia 2022, 32, 100822.

50. Salgia, N.J.; Bergerot, P.G.; Maia, M.C.; Dizman, N.; Hsu, J.; Gillece, J.D.; Folkerts, M.; Reining, L.; Trent, J.; Highlander, S.K.; et al. Stool Microbiome Profiling of Patients with Metastatic Renal Cell Carcinoma Receiving Anti-PD-1 Immune Checkpoint Inhibitors. Eur. Urol. 2020, 78, 498–502.
51. Agarwal, A.; Modliszewski, J.; Davey, L.; Reyes-Martinez, M.; Runyambo, D.; Corcoran, D.; Dressman, H.; George, D.J.; Valdivia, R.H.; Armstrong, A.J.; et al. Investigating the role of the gastrointestinal microbiome in response to immune checkpoint inhibitors (ICIs) among patients (pts) with metastatic renal cell carcinoma (mRCC). J. Clin. Oncol. 2020, 38, 730.
52. Gopalakrishnan, V.; Spencer, C.N.; Nezi, L.; Reuben, A.; Andrews, M.C.; Karpinets, T.V.; Prieto, P.A.; Vicente, D.; Hoffman, K.; Wei, S.C.; et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018, 359, 97–103.
53. Matson, V.; Fessler, J.; Bao, R.; Chongsuwat, T.; Zha, Y.; Alegre, M.L.; Luke, J.J.; Gajewski, T.F. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 2018, 359, 104–108.
54. Wargo, J.A.; Gopalakrishnan, V.; Spencer, C.; Karpinets, T.; Reuben, A.; Andrews, M.C.; Tetzlaff, M.T.; Lazar, A.; Hwu, P.; Hwu, W.J.; et al. Association of the diversity and composition of the gut microbiome with responses and survival (PFS) in metastatic melanoma (MM) patients (pts) on anti-PD-1 therapy. J. Clin. Oncol. 2017, 15, 3008.
55. Mizuhashi, S.; Kajihara, I.; Sawamura, S.; Kanemaru, H.; Makino, K.; Aoi, J.; Makino, T.; Masuguchi, S.; Fukushima, S.; Ihn, H. Skin microbiome in acral melanoma: Corynebacterium is associated with advanced melanoma. J. Dermatol. 2021, 48, e15–e16.
56. Ciernikova, S.; Mego, M.; Hainova, K.; Adamcikova, Z.; Stevurkova, V.; Zajac, V. Modification of microflora imbalance: Future directions for prevention and treatment of colorectal cancer? Neoplasma 2015, 62, 345–352.
57. Wang, Z.; Li, L.; Wang, S.; Wei, J.; Qu, L.; Pan, L.; Xu, K. The role of the gut microbiota and probiotics associated with microbial metabolisms in cancer prevention and therapy. Front. Pharmacol. 2022, 13, 1025860.
58. Luo, M.; Hu, M.; Xu, F.; Wu, X.; Dong, D.; Wang, W. Preventive effect of Lactobacillus reuteri on melanoma. Biomed. Pharmacother. 2020, 126, 109929.
59. Pereira, F.V.; Melo, A.C.L.; Silva, M.B.; de Melo, F.M.; Terra, F.F.; Castro, I.A.; Perandini, L.A.; Miyagi, M.T.; Sato, F.T.; Origassa, C.S.T.; et al. Interleukin-6 and the Gut Microbiota Influence Melanoma Progression in Obese Mice. Nutr. Cancer 2021, 73, 642–651.
60. Chen, L.; Zhou, X.; Wang, Y.; Wang, D.; Ke, Y.; Zeng, X. Propionate and Butyrate Produced by Gut Microbiota after Probiotic Supplementation Attenuate Lung Metastasis of Melanoma Cells in Mice. Mol. Nutr. Food Res. 2021, 65, e2100096.
61. Le Noci, V.; Guglielmetti, S.; Arioli, S.; Camisaschi, C.; Bianchi, F.; Sommariva, M.; Storti, C.; Triulzi, T.; Castelli, C.; Balsari, A.; et al. Modulation of Pulmonary Microbiota by Antibiotic or Probiotic Aerosol Therapy: A Strategy to Promote Immunosurveillance against Lung Metastases. Cell Rep. 2018, 24, 3528–3538.
62. Li, J.; Sung, C.Y.; Lee, N.; Ni, Y.; Pihlajamaki, J.; Panagiotou, G.; El-Nezami, H. Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. Proc. Natl. Acad. Sci. USA 2016, 113, E1306–E1315.
63. Dizman, N.; Meza, L.; Bergerot, P.; Alcantara, M.; Dorff, T.; Lyou, Y.; Frankel, P.; Cui, Y.; Mira, V.; Llamas, M.; et al. Nivolumab plus ipilimumab with or without live bacterial supplementation in metastatic renal cell carcinoma: A randomized phase 1 trial. Nat. Med. 2022, 28, 704–712.
64. Jakubauskas, M.; Jakubauskiene, L.; Leber, B.; Horvath, A.; Strupas, K.; Stiegler, P.; Schemmer, P. Probiotic Supplementation Attenuates Chemotherapy-Induced Intestinal Mucositis in an Experimental Colorectal Cancer Liver Metastasis Rat Model. Nutrients 2023, 15, 1117.
65. Shang, F.; Jiang, X.; Wang, H.; Chen, S.; Wang, X.; Liu, Y.; Guo, S.; Li, D.; Yu, W.; Zhao, Z.; et al. The inhibitory effects of probiotics on colon cancer cells: In vitro and in vivo studies. J. Gastrointest. Oncol. 2020, 11, 1224–1232.
66. Baruch, E.N.; Youngster, I.; Ben-Betzalel, G.; Ortenberg, R.; Lahat, A.; Katz, L.; Adler, K.; Dick-Necula, D.; Raskin, S.; Bloch, N.; et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science 2021, 371, 602–609.