The metabolic dialogue between intratumoural microbes and cancer: implications for immunotherapy

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eBioMedicine
2025;115: 105708
Published Online xxx
https://doi.org/10.1016/j.ebiom.2025.105708

Summary

The tumour microenvironment (TME) exerts a profound influence on cancer progression and treatment outcomes. Recent investigations have elucidated the crucial role of intratumoural microbiota and their metabolites in shaping the TME and modulating anti-tumour immunity. This review critically assesses the influence of intratumoural microbial metabolites on the TME and cancer immunotherapy. We systematically analyse how microbial-derived glucose, amino acid, and lipid metabolites modulate immune cell function, cytokine secretion, and tumour growth. The roles of specific metabolites, including lactate, short-chain fatty acids, bile acids, and tryptophan derivatives, are comprehensively examined in regulating immune responses and tumour progression. Furthermore, we investigate the potential of these metabolites to augment the efficacy of cancer immunotherapies, with particular emphasis on immune checkpoint inhibitors. By delineating the mechanisms through which microbial metabolites influence the TME, this review provides insights into novel microbiome-based therapeutic strategies, thereby highlighting a promising frontier in personalised cancer medicine.

Keywords: Tumour microenvironment; Intratumoural microbiota; Metabolites; Immunotherapy; Immune checkpoint inhibitors

Introduction

The tumour microenvironment (TME) represents a highly complex system that encompasses cellular components (such as cancer cells, stromal cells, and immune cells) and non-cellular components (such as blood vessels and signalling molecules).[1–3] These diverse components engage in complex interactions with tumour cells and undergo dynamic changes during tumour progression. These components function not only as markers of tumour progression but also actively regulate tumour growth and metastasis through microenvironmental modification.[1] Notably, intratumoural microbes remain distinct from gut microbiota, although both populations are closely associated with tumour development and progression. Specifically, gut microbiota encompasses microorganisms colonizing the intestinal tract, whereas

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intratumoural microbes constitute those present within tumour cells and immune cells in tumour tissues.[4] However, it is currently widely accepted that there are three primary mechanisms by which microbes transform into intratumoural microbes: 1) Translocation through mucosal barriers into tumour tissues. In colorectal cancer, pancreatic cancer, and other tumours growing in organs with luminal connections to the external environment, mucosal damage—either intrinsic or caused by tumour growth—provides a portal for microbes to enter tumour tissues. A bacterial driver-passenger model applicable to gastric and colorectal cancers has been proposed, suggesting that certain gut microbes can disrupt the mucosal barrier, thereby creating favourable conditions for microbial colonization of tumour tissues.[4,5] Additionally, studies have found that gut microbes may translocate via the pancreatic duct into pancreatic ductal adenocarcinoma[6]; 2) Origination from normal tissues surrounding the tumour, a perspective substantiated by studies revealing high similarity between the microbial composition of tumour tissues and that of surrounding tissues[7]; 3) Translocation via blood and lymphatic vessels, with multiple studies identifying gut microbes translocating through blood or lymph to lung cancer, melanoma, and other tumour tissues.[8,9] Further research has demonstrated that *Escherichia coli* colonizing tumour tissues can disrupt vascular barriers and translocate via blood to the liver.[10,11] These translocated gut microbes subsequently become an integral part of the intratumoural microbiota. Notably, numerous questions regarding translocation mechanisms of intratumoural microbes remain unresolved; for instance, the aforementioned translocation modes may not apply to all microbes colonizing tumours, and even within the same translocation pattern, different microbes may exhibit distinct molecular action mechanisms.[12] Emerging research has increasingly demonstrated that intratumoural microbes represent a crucial component of the tumour microenvironment. These translocated gut microbes subsequently become an integral part of the intratumoural microbiota. Emerging research has increasingly demonstrated that intratumoural microbes represent a crucial component of the tumour microenvironment.[13] Recent investigations have systematically elucidated the metabolic activities of intratumoural microbes within tumours. The diverse metabolites synthesized by these microbes exert multiple effects within the tumour microenvironment, encompassing direct modulation of tumour cell growth and immune cell function, thus establishing themselves as crucial regulatory factors in the tumour microenvironment[14] (Table 1, Figs. 1 and 2).

Immune checkpoint inhibitors have emerged as a crucial approach in tumour immunotherapy.[23–25] The efficacy and prognosis of immunotherapy are intricately linked to the TME. As a result, an increasing number of studies have focused on targeted regulation of the TME in tumour immunotherapy. The TME comprises a complex and diverse cellular composition, including cancer cells, stromal cells, and various immune cells (such as macrophages, dendritic cells, T cells, and B cells).[26–29] These cells participate in continuous and complex interactions with one another.[30] Stromal cells regulate the function of immune cells through the secretion of cytokines. Concurrently, cytokines secreted by immune cells can elicit pro-inflammatory or anti-inflammatory responses, thus influencing tumour cell growth.[31–33]

Recent studies have demonstrated that intratumoural microorganisms establish intricate connections with the TME via their metabolic activities. Studies have revealed distinct microbial profiles in various cancer types: colorectal cancer (CRC) shows high abundance of *Fusobacterium*; pancreatic ductal adenocarcinoma (PDAC) is dominated by *Proteobacteria* and *Bacteroidetes*; breast cancer (BC) is enriched with *Proteobacteria* and *Firmicutes*; lung cancer (LC) harbours *Campylobacter*, *Enterobacter*, *Debaryomyces*, and *Fusobacterium*; while lung squamous cell carcinoma (LUSC) exhibits a higher abundance of Acidovorax.[34–38] The metabolic products of these microorganisms encompass a range of bioactive molecules, including lactate, amino acids, short-chain fatty acids (SCFAs), bile acids, trimethylamine N-oxide, and inosine. These metabolites, when released into the TME, can influence immune cell function through the modulation of cytokine secretion.

Considering the significant role of intratumoural microbial metabolites in the tumour microenvironment, these bioactive compounds may substantially impact cancer immunotherapy outcomes. Although extensive research has focused on gut microbiota-derived metabolites in tumour progression and cancer treatment, it is crucial to distinguish between metabolites from intratumoural microbes and those produced by gut microbiota. In this context, this review provides a systematic analysis of the role of intratumoural microbes in the tumour microenvironment and cancer immunotherapy, specifically examining how intratumoural microbes modulate the tumour microenvironment through: 1) glucose metabolites, 2) amino acids and their metabolites, 3) lipid metabolites, and 4) other metabolites, ultimately affecting the clinical benefits of immunotherapy. Furthermore, this review explores potential therapeutic strategies targeting microbial metabolites and their potential synergistic interactions with immunotherapy. This review presents a systematic and comprehensive analysis of the mechanisms by which intratumoural microbial metabolites function in the tumour microenvironment and investigates the potential connections between these metabolites and immunotherapy efficacy, with the objective of establishing theoretical foundations and research directions for novel cancer treatment

Evidence for microbial origin	Metabolites	Associated microbiota	Tumour type	Implications	Reference
Metabolites definitively proven to be of microbial origin through rigorous experimental validation	L-Arginine	Engineered bacterium *Escherichia coli* Nissle 1917	CRC	Increasing the number of tumour-infiltrating T cells and enhancing the efficacy of PD-L1 blocking antibodies	15
	Butyrate	*Clostridium butyricum*	PDAC	Enhanced susceptibility to iron death in PDAC	16
	TMAO	*Clostridiales*	TNBC	Induction of tumour cell pyroptosis and enhancement of CD8+ T cell-mediated anti-tumour immunity	17
Metabolites strongly associated with microbial presence but requiring further validation	N-Acetyl-L-aspartic acid	*Paraburkholderia fungorum*	ICC	Inhibition of tumour cell migration	18
	Indole-3-propionic acid	Collaboration of *Lactobacillus johnsonii* and *Clostridium sporogenes*	Melanoma, Breast Cancer, CRC	Enhancing the efficacy of CD8+ T cell-mediated αPD-1 immunotherapy	19
	butyric acid	*Eubacterium* *Butyricicoccus* *Roseburia* *Ruminococcus* *Faecalibacterium* *Clostriduim*	GC	Activation of cytotoxic CD8+ T cells enhances the effect of anti-PD1 therapy	20
Metabolites detected in the presence of microbes but potentially derived from host cells	TUDCA	*Akkermansia muciniphila*	HCC	Promoting tumour cell apoptosis, increasing the number of CD8+ T cells within TME, and enhancing the efficacy of PD-1mAb in HCC	21
	Bile acid metabolites	*Hymenobacter* *Anaerococcus* *Collimonas*	lymphoma	Increased infiltration of CD8+ T cells, CD4+ T cells, Th1 cells and M1-type macrophages	22

Abbreviations: ICC, intrahepatic cholangiocarcinoma; PDAC, pancreatic ductal adenocarcinoma; CRC, colorectal cancer; GC, Gastric Cancer; HCC, Hepatocellular carcinoma; TMAO, trimethylamine-N-oxide; TME, tumour microenvironment; TNBC, triple-negative breast cancer; TUDCA, tauroursodeoxycholic acid.

Table 1: **Intratumoural microbial metabolites alter the tumour immune microenvironment.**

strategies, while fostering interdisciplinary integration among microbiology, oncology, and immunology.

Metabolites from intratumoural microbes affecting TME or cancer immunotherapy

Glucose metabolism-related products

In traditional metabolic paradigms, lactate serves as the primary glucose metabolic product in normal tissue cells under hypoxic conditions. However, owing to the elevated energy consumption profile of tumour tissues, lactate persists as the primary glucose metabolic product in tumour cells even under normoxic conditions.[39] This enhanced glycolytic process in tumour cells, yielding lactate as the end product, produces significantly more Adenosine triphosphate (ATP) than conventional glycolysis in normal cells, a metabolic adaptation termed the Warburg effect.[40–43] Emerging evidence indicates that lactate exhibits a crucial role in mediating the immunosuppressive effects of glucose metabolism within the tumour microenvironment.[44] In addition to lactate produced through cancer cell glucose metabolism, tumour-colonizing anaerobic bacteria (such as *lactobacilli*) contribute to lactate production through their metabolic activities.[45] Upon entering the tumour microenvironment (TME), these lactate molecules act as critical immunomodulatory mediators, orchestrating the formation of anti-tumour immune barriers through multiple mechanisms, ultimately influencing tumour progression.[46]

Primarily, anaerobic microbial communities within tumour tissues facilitate the conversion of pyruvate to lactate, wherein the subsequent lactate accumulation enhances tumour tissue progression.[45] This tumour-promoting mechanism predominantly manifests through its effects on immune cells and vasculature in the tumour microenvironment. Within the TME, lactate exerts dual immunomodulatory effects: it interacts with G protein-coupled receptors on macrophage surfaces to induce M2 phenotype polarisation and suppresses NFAT expression on CD8+ T cells and NK (natural killer) cells, resulting in reduced IFN-γ (interferon-gamma) production and subsequent tumour growth promotion.[47–49] In addition to its immunomodulatory effects, lactate accumulation stimulates tumour angiogenesis.[47] Enhanced tumour vasculature facilitates the expansion of oxygen-accessible tumour cells near blood vessels (normoxic tumour cells), while these normoxic tumour cells utilise MCT1 to import lactate from the TME for ATP generation, thereby augmenting tumour tissue growth.[42] Conversely, depletion of lactate in the TME results in regulatory T cell dysfunction, leading to tumour tissue growth suppression.[50] Significantly, the acidic tumour microenvironment facilitates anaerobic microbiota colonization, while these microorganisms reciprocally maintain environmental acidity through lactate production, establishing a self-reinforcing cycle.[45] Therefore, the bidirectional relationship between intratumoural anaerobic microbiota and the acidic

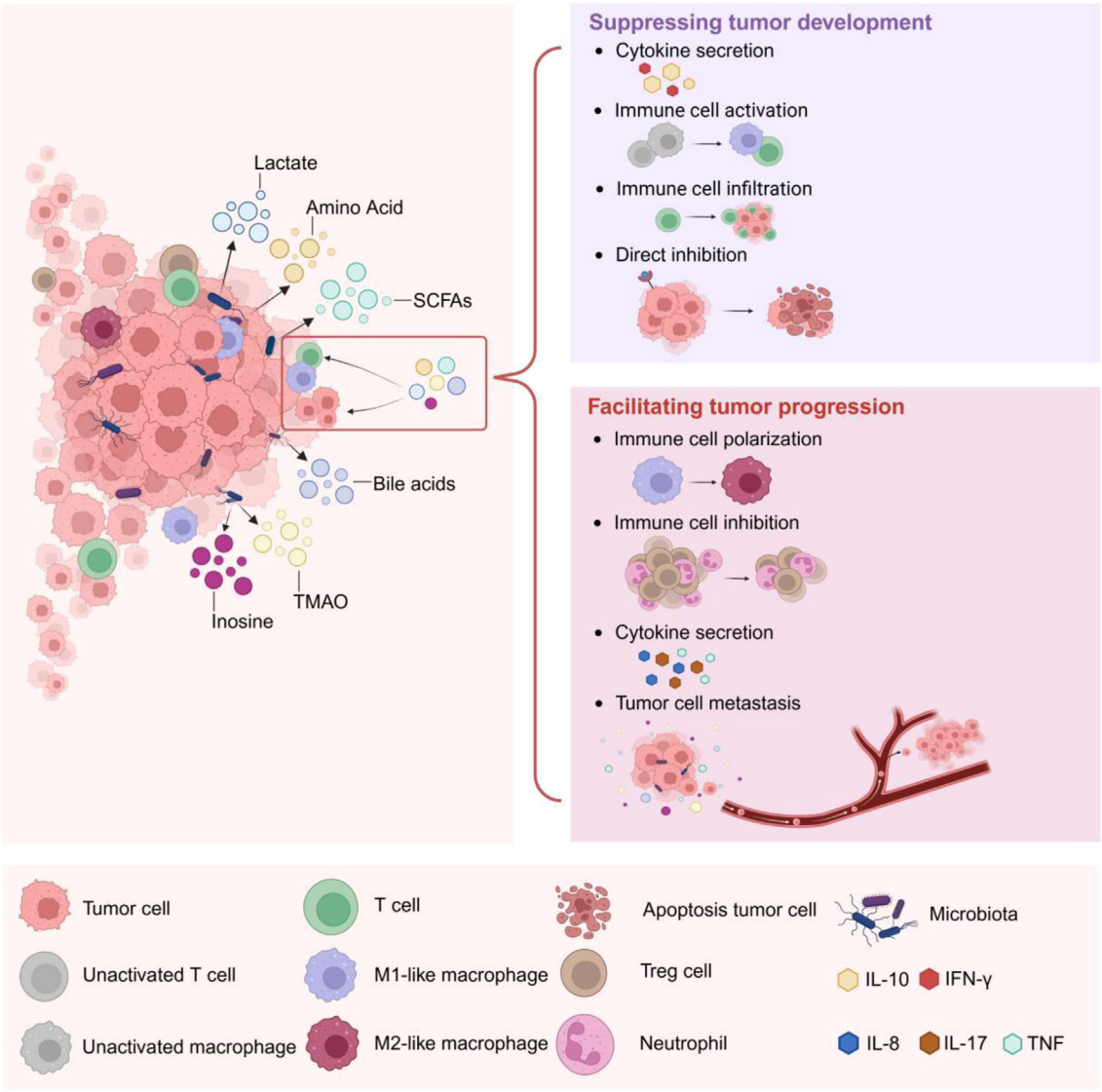

Fig. 1: Metabolites from intratumoural microbes exert significant regulatory effects on the tumour microenvironment (TME) and cancer immunotherapy. Various intratumoural microbes can influence the TME through a diverse array of metabolites including lactate, amino acids, short-chain fatty acids (SCFAs), bile acids, trimethylamine N-oxide (TMAO), and inosine. These metabolites affect the TME through diverse mechanisms: 1) Lactate primarily plays a tumour-promoting role in the TME by inhibiting IFN-γ production and/or influencing the emergence of M2 macrophages and regulatory T cells (Tregs), thereby facilitating cancer progression. Notably, lactate can also significantly promote intratumoural angiogenesis. The increased intratumoural vasculature not only facilitates energy acquisition by cancer cells but also provides critical opportunities for haematogenous metastasis. 2) Amino acids, encompassing various derivatives produced through intratumoural microbial metabolism, primarily inhibit tumour development by enhancing T cell infiltration in tumour tissues and regulating cytokine secretion. It is noteworthy that certain amino acid derivatives can mediate the reduction of neutrophils in the TME, thereby exerting potent tumour growth inhibitory effects. 3) SCFAs, intratumoural microbial metabolites that predominantly inhibit tumour progression, not only regulate the production and secretion of various cytokines (IL-10, IL-17, IFN-γ) but also directly interact with cancer cell membrane surface receptors to promote cancer cell apoptosis. 4) Bile acids, as intratumoural microbial metabolites that play significant roles in the TME, exhibit complex functions. Different bile acids exhibit distinct mechanisms of action, exerting differential effects on tumour progression through the enhancement of enzyme activity, modulation of cytokine secretion, and regulation of tumour cell proliferation. 5) TMAO and inosine, two key metabolites of intratumoural microbes, influence cancer progression by modulating immune cell activity.

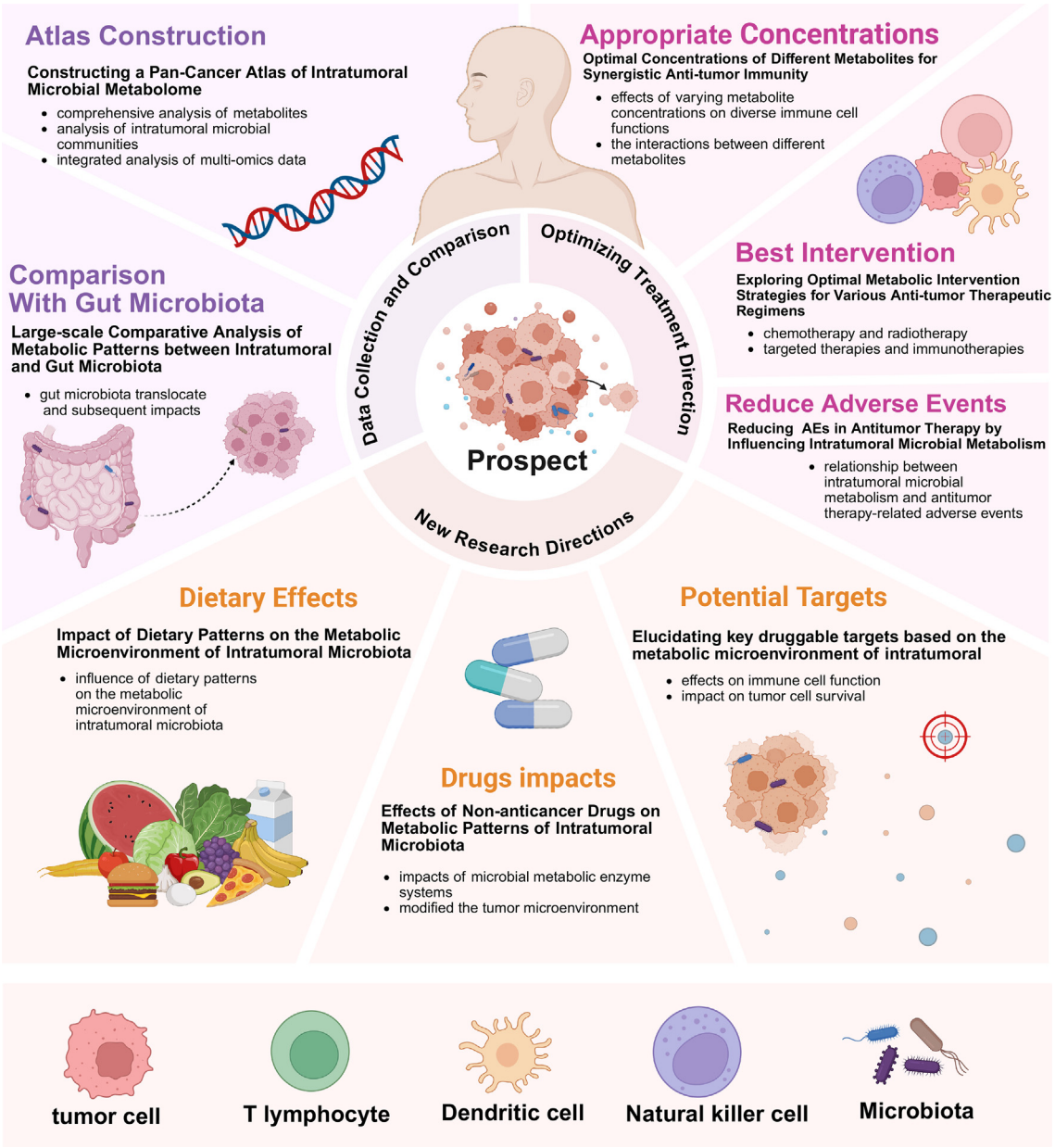

Fig. 2: Future perspectives on intratumoural microbial metabolites in the tumour microenvironment: 1) Construction of a pan-cancer atlas of the intratumoural microbial metabolome, which involves optimizing metabolite extraction and analysis protocols, conducting comprehensive analysis of metabolites in different types of tumour tissues, and integrating multi-omics data, 2) Comparison between intratumoural and gut microbiota, systematically identifying distinctive characteristics of intratumoural microbes compared to gut microbiota through diverse sequencing technologies, 3) Determination of optimal concentrations for synergistic anti-tumour immunity, necessitating the establishment of precise metabolite quantification methods to accurately measure the concentrations of various metabolites in the tumour microenvironment, 4) Development of optimal metabolic intervention strategies for anti-tumour therapeutic regimens, through comprehensive investigation of how metabolites influence drug target expression, cellular signalling pathways, and gene expression, 5) Reduction of adverse events through modulation of intratumoural microbial metabolism, which entails exploring strategies to prevent or mitigate adverse reactions by regulating harmful or harmless metabolites in the tumour microenvironment through the modulation of intratumoural microbial metabolism, 6) Investigation of the impact of dietary patterns, encompassing further studies on how specific dietary components directly influence the growth and metabolism of intratumoural microbes, how diet-induced host metabolic changes indirectly affect intratumoural microbes, and how diet impacts the tumour microenvironment, which will enhance our understanding of the complex diet-microbe-tumour relationship, 7) Evaluation of the effects of non-anticancer drugs, systematically examining how drugs directly act on microbial metabolic enzyme systems, how drugs affect microbial metabolism by altering the tumour microenvironment, and how drug-induced host metabolic changes reciprocally influence

microenvironment represents a promising therapeutic target for inhibiting tumour progression.

Amino acid metabolism-related products

Amino acids and their derivatives constitute another class of nutrients implicated in anti-tumour immune barriers, playing crucial roles in the TME by modulating cytokine secretion and influencing immune cells, especially T cell function. Among these amino acids, tryptophan and arginine are of particular significance in anti-tumour immune barriers.[51–53] Moreover, being essential nutrients, amino acids can be directly absorbed by tumour cells, thus promoting tumour cell growth.[54] Recent advancements in research have preliminarily elucidated the mechanisms by which amino acids influence tumour development.

Tryptophan

Research demonstrates that elevated tryptophan consumption by tumour cells results in tryptophan depletion within the tumour microenvironment, inducing T cell apoptosis and consequently suppressing tumour development.[55–57] Despite the production of diverse tryptophan derivatives by different intratumoural microorganisms, these metabolites often converge on similar biological outcomes. Specifically, *Lactobacillus johnsonii* (*L. johnsonii*) converts tryptophan into indole-3-propionic acid (IPA), which potentiates CD8+ T cell function during anti-PD-1 immunotherapy, resulting in tumour growth inhibition.[19] In the context of melanoma, *Lactobacillus reuteri* (*L.reuteri*) synthesizes indole-3-aldehyde from tryptophan, which selectively activates AhR on CD8+ T cells, stimulating the generation of IFNγ-producing CD8+ T cells and subsequently mediating anti-tumour effects.[9] These divergent outcomes likely stem from the distinct indole derivatives synthesized through intratumoural microbiota metabolism and their differential activation of AhR receptors on various immune cell populations. In addition to the indole metabolism pathway, the kynurenine metabolism pathway emerges as another critical metabolic circuit. Within immune cells, indoleamine-2,3-dioxygenase (IDO) catalyses the conversion of tryptophan to kynurenine, whose accumulation enhances regulatory T cell generation, thus promoting tumour development.[58,59] Collectively, distinct tryptophan metabolism pathways in the tumour microenvironment yield diverse biological outcomes, and elucidating mechanisms to modulate intratumoural microbiota's tryptophan utilization represents a promising avenue for cancer therapeutic development.

Arginine

Over the past decade, arginine has established itself as a critical target in cancer research, with particular emphasis on the impact of arginine methylation on tumour biology.[60,61] With advancing insights into intratumoural microbiota, the significance of microbially-derived arginine in tumour tissues has gained substantial recognition. Analogous to tryptophan's biological functions, arginine synthesized through intratumoural microbial metabolism demonstrates bidirectional effects. Experimental evidence indicates that increased arginine concentrations within the tumour microenvironment facilitate T cell infiltration. Clinical investigations reveal that a genetically engineered *E. coli* Nissle 1917, following tumour colonization, catalyses the conversion of ammonia to arginine, subsequently promoting T cell infiltration in tumour tissues and enhancing immunotherapy efficacy.[15] Conversely, research has demonstrated that elevated arginine levels can modulate glucose, lipid, and amino acid metabolism, thereby fostering tumour formation.[62] Consequently, arginine exhibits dual capacity to promote tumour growth and potentiate anti-tumour therapy, with these divergent effects contingent upon intratumoural arginine concentrations, highlighting the need for comprehensive research to establish optimal therapeutic strategies.[63]

Other amino acids

Beyond the tumour-suppressing effects of tryptophan and arginine under defined conditions, emerging research has revealed that alanine, aspartate, glutamate, and valine, when processed through intratumoural *Paraburkholderia fungorum* metabolism, demonstrate tumour growth inhibitory properties. Nevertheless, the underlying molecular mechanisms by which these amino acids exert their anti-tumour effects remain incompletely characterized, warranting further investigation.[18]

Lipid metabolism-related products

Short-chain fatty acids (SCFAs)

Similar to lactic acid, SCFAs are metabolites predominantly produced by various anaerobic bacteria, with the primary SCFAs being acetic acid, propionic acid, and butyric acid.[16,45,64–67] These SCFA-producing anaerobic bacteria encompass various genera, including but not limited to *Lactobacillus*, *Clostridium*, *Escherichia*, *Coprococcus*, members of the family *Lachnospiraceae*, and *Akkermansia*. SCFAs generated by intratumoural anaerobic bacteria exert their influence on tumour progression primarily via two mechanisms: direct action on cancer cells and indirect action through modulation of

the metabolic activities of intratumoural microbes, which will contribute to a more comprehensive understanding of drug-microbe-host interactions, 8) Elucidation of key druggable targets. Rigorous investigation of how these metabolites directly affect tumour cell survival and proliferation, and how they regulate immune cell function, will facilitate the evaluation of the potential therapeutic efficacy of targeting relevant metabolites or their production pathways. Abbreviation: AEs, Adverse Events.

immune responses. The majority of studies indicate that both mechanisms result in inhibitory effects of SCFAs on tumour development and progression.[45]

SCFAs predominantly exert their anti-tumour effects by orchestrating multiple intracellular signalling pathways that govern tumour cell apoptosis and proliferation. Within the tumour microenvironment, SCFAs interact with G protein-coupled receptors, particularly the low-affinity butyrate receptor GPR109A, subsequently inhibiting AKT and mTOR signalling pathways in tumour cells. This cascade initiates reactive oxygen species (ROS) generation, promotes oxidative stress, and ultimately results in mitochondrial membrane dysfunction and cellular apoptosis.[16,68] Furthermore, SCFAs synthesized by intratumoural anaerobic bacteria suppress cancer cell proliferation through inducing G1 phase cell cycle arrest.[45]

Compared to their direct effects on cancer cells, the modulation of immune cells by SCFAs produced by intratumoural anaerobic bacteria appears to play a more critical role in inhibiting tumour development and progression. Accumulating evidence suggests that SCFAs can modulate cytokine secretion by regulating various immune cells, thereby influencing the function of other cells in the TME. For instance, emerging evidence indicates that *Fusobacterium nucleatum* (*F. nucleatum*) colonizing CRC can increase the infiltration of Th17 cells within tumours in an free fatty acid receptors 2 (FFAR2)-dependent manner, subsequently enhancing the secretion of interleukin-17A (IL-17A) and interleukin-17F (IL-17F).[69] Considering that IL-17 can induce the production of angiogenic factors and is closely associated with tumour invasion, the reduction of IL-17 induced by propionate may play a crucial role in inhibiting tumour invasion.[70] In the context of intestinal lymphoma, *Eubacterium rectale* exerts an antitumour effect by reducing tumour necrosis factor (TNF) levels.[71] Additionally, recent investigations have revealed that SCFAs can induce the upregulation of interferon-γ receptor 1 (IFN-γ-R1) on immune cells while concurrently suppressing the function of NK cells and CD8+ T cells, ultimately leading to the inhibition of tumour cell growth.[72]

Furthermore, research has demonstrated that butyrate present within gliomas can regulate microglia through epigenetic modifications, consequently exerting an inhibitory effect on tumour growth.[73,74] Moreover, SCFAs produced by intratumoural anaerobic bacteria not only modulate the secretion of diverse cytokines but also function as a carbon source, supplying energy for immune cell activities.[75] In summary, SCFAs in the TME can exert anti-tumour effects in multiple cancers, including CRC and glioma, by potentiating the functions of immune cells such as T cells, antigen-presenting cells, and brain-resident microglia.[73,76]

While SCFAs produced by intratumoural anaerobic bacteria demonstrate significant antitumour effects, some pro-tumour effects may also occur when the concentration of SCFA is altered. Conversely, studies have demonstrated that excessively low concentrations of butyrate may suppress the proliferation of immune cells.[77] Consequently, the antitumour efficacy of SCFAs appears to be intricately linked to their concentration within the TME. Nevertheless, the optimal SCFA concentration for maximizing antitumour therapeutic efficacy remains to be elucidated through further comprehensive research.

Bile acids

Bile acids constitute a class of water-soluble compounds, predominantly classified into primary and secondary bile acids.[77] In recent years, there has been a surge in research focussing on the role of bile acids in various tumour tissues, including hepatocellular carcinoma, gallbladder carcinoma, and CRC. The underlying mechanisms, encompassing immunomodulation and direct effects on tumour cells, are being progressively elucidated.[78–82] Emerging evidence suggests that bile acids function as signalling molecules within the TME, exerting both direct and indirect effects on immune cell surface receptors. This modulates the activation status of various immune cells, including NK cells and macrophages, consequently influencing tumour progression.[83–86] In clinical settings, intratumoural bile acid concentrations have emerged as a potential prognostic biomarker for the survival of patients with cancer. Generally, patients exhibiting lower intratumoural bile acid concentrations demonstrate a more favourable prognosis compared to those with elevated levels.[22,81]

Previous studies have demonstrated that bile acids and their metabolites, which are derived from gut microbiota, exhibit diverse roles in various tumour tissues.[78] The predominant active secondary bile acids are synthesized by bacteria such as *Clostridium difficile* and *Bacteroides*, which possess 7-α-hydroxylase or dehydroxylase.[81,84,87] However, in tumour tissues, the microorganisms that regulate bile acid metabolism differ from gut microbiota, and bile acid metabolism demonstrates distinct roles in different tumour microenvironments. Through a comprehensive cohort analysis of breast cancer, researchers identified that three bacteria–*Hymenobacter*, *Anaerococcus*, and *Collimonas*–were enriched in the low bile acid metabolism group of breast cancer, which demonstrated more significant immune cell infiltration, particularly M1 macrophage infiltration.[22] In the tumour microenvironment of hepatocellular carcinoma, colonization by *Akkermansia muciniphila* may convert ursodeoxycholic acid (UDCA) to tauroursodeoxycholic acid (TUDCA), thereby enhancing immunosuppressive effects against tumours, potentially benefiting patients with liver cancer.[21] This anti-tumour effect may be attributed to intratumoural TUDCA's ability to suppress the expression of interleukin-8 (IL-8) and interleukin-1α (IL-1α) while inhibiting NF-κB-mediated inflammatory responses.[88]

Other metabolism-related products of intratumoural microbiota

Trimethylamine-N-oxide (TMAO)

TMAO is a metabolite derived from the metabolism of carnitine and choline, primarily synthesized by gut microbiota, notably *F. nucleatum*.[17,89] Recent studies have extensively demonstrated TMAO's role in promoting atherosclerosis, establishing it as a crucial research target for cardiovascular diseases, especially hypertension.[90–92] Furthermore, TMAO has been implicated in the development of various chronic conditions, encompassing diabetes, heart failure, chronic kidney disease, Alzheimer's disease, and diverse types of cancer.[93,94] Emerging research has elucidated a close relationship between TMAO and tumour tissues. Substantial evidence demonstrates that TMAO levels in plasma and tumour tissues are significantly correlated with the progression of various cancers, such as cervical, colorectal, and BC. Consequently, elucidating TMAO's mechanisms of action in the TME holds great potential for advancing innovative tumour treatment strategies.[17,95,96]

Current research primarily focuses on TMAO generated through gut microbial metabolism, while gut microbiota modulates intratumoural microbiota through direct translocation or indirect regulation of the microbial environment.[97] TMAO generated via intratumoural microbial metabolism demonstrates significant anti-tumour effects. In tumour tissues such as breast cancer and pancreatic ductal adenocarcinoma, TMAO has been demonstrated to activate effector T cells and anti-tumour M1 macrophages in the tumour microenvironment via the activation of type I interferon signalling pathway.[17,97,98] Additionally, TMAO derived from intratumoural microbial metabolism simultaneously activates endoplasmic reticulum stress kinases to promote cancer cell apoptosis and interacts with α-casein in breast cancer to inhibit tumour progression.[17,99] However, in specific tumour tissues such as hepatocellular carcinoma and colorectal cancer, TMAO may promote tumour progression by activating the MAPK signalling pathway–a critical pathway in cancer treatment response–to enhance inflammatory responses and facilitate cancer cell proliferation and migration.[51,100]

Inosine

Inosine, a crucial metabolite in purine metabolism, functions not only as an essential messenger molecule in signal transduction processes of various cellular activities but also contributes to RNA modification, thereby modulating the transcription and translation processes in tumour cells.[101,102] Moreover, studies have demonstrated that inosine serves as a crucial signalling molecule in the mitochondrial respiration process within tumour cells.[103] In humans, inosine is synthesized through metabolic processes by microorganisms, notably *Bifidobacterium pseudolongum*.[104] Numerous studies have demonstrated that the presence of inosine in the TME of various cancers, including CRC, bladder cancer, and melanoma, is strongly associated with tumour progression.[102]

A growing body of research evidence suggests that inosine, produced by intratumoural microbial metabolism, may plays a crucial role in the TME. Specifically, inosine functions not only as a carbon source for effector T cells but also as a modulator of responses to immune checkpoint inhibition therapy in tumours. By interacting with the A2A receptor, inosine enhances the activity of anti-tumour T cells, thereby improving the efficacy of immunotherapy.[105,106] In summary, studies suggest that inosine demonstrates significant anti-tumour potential in cancer treatment.

Nucleosides

The metabolic modulator 5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR), a well-characterized AMPK activator, demonstrates significant effects on tumour progression across multiple cancer types, including haematological malignancies and prostate cancer.[107,108] Recent investigations in cervical cancer have revealed that intratumoural *Lactobacillus iners* (*L. iners*) substantially elevates AICAR concentrations, leading to the downregulation of glycolytic metabolism, consequently inhibiting tumour cell proliferation and inducing apoptosis.[109]

Microbial metabolites and cancer therapy

As early as 1891, researchers discovered that injecting microorganisms into tumours could potentially aid in cancer treatment. Subsequently, the mechanisms underlying direct or indirect tumour therapy mediated by microorganisms have been gradually elucidated.[110,111] Recent advancements in research have provided increasing evidence indicating that microbial metabolites are closely associated with tumour progression within the TME. Consequently, targeting these metabolites as a strategy for cancer therapy holds significant potential clinical value.

Microbial metabolites and chemoradiotherapy

The metabolism of intratumoural microbiota significantly impacts the therapeutic efficacy of various chemoradiotherapy regimens. In CRC, for example, intratumoural *F. nucleatum* metabolizes the chemotherapeutic agent 5-fluorouracil (5-FU) into non-toxic dihydrofluorouracil (DHFU), thereby diminishing local drug efficacy.[112] SCFAs, metabolic products of *Coprococcus*, a genus of Firmicutes present in rectal cancer, potentially attenuate the response to neoadjuvant chemoradiotherapy.[67] L-lactate, a metabolite of *L. iners* in cervical cancer, enhances the metabolism of cervical cancer cells, consequently diminishing the efficacy of chemoradiotherapy against these cells.[113] Conversely, indole-3-acetic acid (I3A), a metabolic product of intratumoural microbiota in PDAC, enhances the

chemosensitivity of PDAC cells, thus augmenting chemotherapy efficacy.[114]

Synergistic effects in immunotherapy

Recent research has demonstrated the synergistic effects of various intratumoural microbial metabolites in cancer immunotherapy. These synergistic effects are primarily achieved through the induction of anti-tumour immune cell infiltration by intratumoural microbial metabolites, thereby potentiating the efficacy of anti-PD-L1, PD-1, and CTLA-4 immunotherapies.[9,35,65,111] Studies have demonstrated that various microbial metabolites, including butyrate produced by *Eubacterium*, *Butyricicoccs*, *Roseburia*, *Ruminococcs* and *Faecalibacterium*, indole-3-propionic acid (IPA) produced by *L. johnsonii*, TMAO produced by *F. nucleatum*, and inosine produced by *Bifidobacterium*, can augment the activity and tumour infiltration capacity of CD8+ T cells, thereby eliciting significant synergistic effects in anti-PD-L1 immunotherapy.[9,17,19,20,104,106,115] Additionally, studies have revealed that genetically engineered *E. coli* can metabolically produce arginine, thereby elevating the number of tumour-infiltrating T cells and further potentiating the efficacy of anti-PD-L1 therapy.[15] Another study indicates that *A. muciniphila* may augment the efficacy of anti-PD-1 monoclonal antibody (PD-1 mAb) immunotherapy by modulating the content of TUDCA in the TME, thereby elevating the level of intratumoural CD8+ T cells.[21] Furthermore, multiple in vivo and in vitro experiments have demonstrated that microorganisms can potentiate checkpoint blockade immunotherapy.[104,116–119]

Moreover, in addition to its synergistic effects in checkpoint blockade immunotherapy, inosine has been demonstrated to enhance the function of chimeric antigen receptor T (CAR-T) cells, thereby potentially augmenting the efficacy of immunotherapy.[120] Notably, recent studies have reported that the ablation of intratumoural microbiota may enhance the efficacy of immunotherapy in certain tumour types, such as PDAC. However, the specific anti-tumour mechanisms underlying this effect remain to be fully elucidated and require further investigation.[6,116] With the continuous advancement of research techniques, the molecular mechanisms underlying the influence of intratumoural microbial metabolites on cancer immunotherapy are being progressively elucidated. These emerging insights are expected to provide a crucial theoretical foundation for the development of more effective and personalised cancer immunotherapy strategies.

Methods for profiling microbe-derived metabolites in cancer research

Currently, numerous studies on intratumoural microbes employ methodologies combining 16S rRNA sequencing with culturomics.[17,18] The 16S rRNA represents a highly conserved and specific microbial sequence that enables identification of intratumoural microbes predominantly at the genus level. Genetic sequencing combined with culturomics, which allows high-throughput bacterial cultivation, can effectively reveal microbes colonizing tumours.[86,121] Furthermore, another genetic sequencing approach, metagenomic sequencing, also plays a pivotal role in intratumoural microbiome research. Unlike 16S rRNA, DNA sequencing-based metagenomics can identify microbes at the strain level, providing substantially more refined results for research.[122] Simultaneously, metabolomics plays a vital role in elucidating the metabolites of intratumoural microbes. Through metabolomics, various metabolites in tumour tissues at specific time points can be identified, thereby enabling inference of changes in the metabolic activities of intratumoural microbes.[123,124]

Despite the rapid development of various detection and analysis techniques, numerous significant challenges remain to be overcome. Environmental microbial contamination during intratumoural microbe sample collection, low biomass of collected intratumoural microbes, and variations in ex vivo culture conditions of microbe-containing tumour tissues may all substantially compromise the accuracy of sample analysis results. Additionally, existing metabolomics methodology lacks mature techniques to track the dynamic processes of intratumoural metabolite production and cannot be directly applied to determine the precise roles of metabolites produced by intratumoural microbes in tumour tissues.[125,126] Resolving the aforementioned issues necessitates the persistent efforts of numerous researchers across multiple disciplines.

Future perspectives

As research into the role of intratumoural microbiota and their metabolites in the TME progresses, our understanding of tumour development and treatment continues to evolve. Nevertheless, numerous critical issues remain to be addressed. This section aims to provide a multi-faceted outlook on future research directions, offering insights into further exploration of the mechanisms of action of intratumoural microbial metabolites in the TME and their potential clinical applications (Fig. 2).

Constructing a pan-cancer atlas of intratumoural microbial metabolome

The construction of a comprehensive pan-cancer atlas of the intratumoural microbial metabolome represents a critical direction for future research in oncology. At present, our understanding of intratumoural microbes and their associated metabolites is predominantly confined to a limited number of cancer types. Limited by

small sample sizes and technical challenges in metabolite tracing detection, most studies have only investigated the specific microbial composition in different tumour tissues.[6,127–129] To gain a comprehensive understanding of the role of intratumoural microbial metabolites across diverse tumour types, it is imperative to establish standardized sampling and analysis methodologies. This endeavour encompasses the optimization of tumour tissue collection, storage, and processing techniques, as well as the establishment of unified protocols for metabolite extraction and analysis. The crux of this research will entail comprehensive analysis of metabolites in diverse tumour tissue types utilizing state-of-the-art high-throughput metabolomics technologies, including but not limited to liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy. Concurrently, the analysis of intratumoural microbial communities employing advanced techniques such as 16S rRNA sequencing and metagenomic sequencing will facilitate the elucidation of associations between microbial metabolites and specific bacterial populations. The integrated analysis of multi-omics data, encompassing metabolomics, genomics, transcriptomics, and proteomics, will facilitate the deciphering of complex interaction networks among intratumoural microbial metabolites, tumour cells, and their microenvironment. Through these comprehensive efforts, we aim to identify signature metabolites and pivotal metabolic pathways across diverse tumour types, thereby establishing a robust foundation for subsequent targeted therapy research and potential clinical applications.

Large-scale comparative analysis of metabolic patterns between intratumoural and gut microbiota

Large-scale comparative analysis of metabolic patterns between intratumoural and gut microbiota constitutes another crucial research direction. Although no studies have conducted large-scale comparisons of compositional and metabolic similarities and differences between gut microbiota and intratumoural microbiota. Significant differences are likely to exist in the metabolic patterns of intratumoural and gut microbiota, and an in-depth investigation of these differences has the potential to provide valuable biological insights. The foundation for this work lies in establishing large-scale clinical cohort studies, involving the simultaneous collection of tumour tissue and gut samples from patients. Analysis of community composition and functional genes of intratumoural and gut microbiota can be performed utilizing techniques such as 16S rRNA sequencing and metagenomic sequencing. Simultaneously, comprehensive analysis of metabolites in both sample types can be conducted employing metabolomics techniques. This paired analysis has the potential to identify unique metabolic characteristics of intratumoural microbiota. An in-depth investigation of the interrelationship between intratumoural and gut microbiota, including exploring whether gut microbiota can translocate to tumour tissues and the subsequent impact on the TME, constitutes another crucial aspect of this research direction. Through this comparative study, researchers may identify unique metabolic pathways and key enzymes of intratumoural microbiota, thereby providing a foundation for developing novel anti-tumour therapeutic targets.

Optimal concentrations of different metabolites for synergistic anti-tumour immunity

Numerous studies have documented that potential metabolites from intratumoural microbes and those definitively produced by intratumoural microbes can significantly influence tumour immunotherapy.[9,105,130] Elucidating the optimal concentrations of diverse metabolites that promote synergistic anti-tumour immunity is crucial for enhancing therapeutic strategies. Various microbial metabolites play significant roles in modulating the TME and anti-tumour immune responses; however, these effects frequently exhibit concentration dependence. The establishment of precise metabolite quantification methods for accurately measuring the concentrations of various metabolites in the TME is fundamental to this line of research. The development of cutting-edge technologies, including single-cell metabolomics and spatial metabolomics, has the potential to provide researchers with the ability to capture the spatial heterogeneity of metabolite distribution in the TME. Systematic in vitro and in vivo experiments investigating the effects of varying metabolite concentrations on diverse immune cell functions (e.g., CD8+ T cells, NK cells, and dendritic cells), including their impact on immune cell activation, proliferation, cytokine secretion, and cytotoxic function, constitute critical steps in determining optimal concentrations. Concurrently, the interactions between different metabolites necessitate in-depth investigation, exemplified by the synergistic effects of SCFAs and bile acids. These comprehensive studies aim to identify the optimal concentration ranges of metabolites that enhance anti-tumour immunity, thereby providing a foundation for precise modulation of the TME.

Exploring optimal metabolic intervention strategies for various anti-tumour therapeutic regimens

The exploration of optimal metabolic intervention strategies for diverse anti-tumour therapeutic regimens will be a critical focus of future research. As our understanding of the role of intratumoural microbial metabolites in the TME deepens, the development of optimal metabolic intervention strategies for various anti-tumour therapeutic regimens becomes increasingly feasible. The foundation of this research lies in the systematic investigation of how intratumoural microbial metabolites influence various anti-tumour therapeutic regimens, encompassing conventional chemotherapy

and radiotherapy, as well as emerging targeted therapies and immunotherapies. Recent studies have demonstrated that intratumoural microbial metabolites can substantially influence the efficacy of radiotherapy and chemotherapy.[109,114,131] An in-depth examination of the synergistic mechanisms between microbial metabolites and diverse treatment modalities—including their influence on drug target expression, cellular signalling pathways, and gene regulation—will contribute significantly to the optimization of therapeutic strategies. A crucial objective in this field is the development of personalised metabolic intervention strategies, which involve predicting optimal intervention plans based on individual patients' tumour types, microbial compositions, and metabolic profiles. The key to achieving clinical applications lies in effectively integrating metabolic intervention strategies with existing treatment modalities. This integration may involve developing nanocarriers capable of co-delivering anti-tumour drugs and specific metabolites, or engineering bacterial strains to produce desired metabolites in situ within the TME.

Reducing adverse events (AEs) in antitumour therapy by influencing intratumoural microbial metabolism

Modulating intratumoural microbial metabolism to mitigate a AEs in antitumour therapy represents a crucial approach to enhance treatment tolerability. Previous research has established that certain metabolites in the tumour microenvironment are closely associated with poor prognosis, and these metabolites can be produced not only by tumour cells but also by the metabolic activities of intratumoural microbes.[80,81,132] The foundation of this research encompasses a systematic investigation into the relationship between intratumoural microbial metabolism and various antitumour therapy-related AEs, coupled with a thorough examination of the molecular mechanisms through which these metabolites contribute to adverse reactions. The core focus of this research direction centres on exploring strategies to prevent or mitigate adverse reactions by modulating intratumoural microbial metabolism. These strategies include: (1) utilizing specific probiotics or metabolic precursors to promote the production of beneficial metabolites, (2) employing specific antibiotics or metabolic inhibitors to reduce the generation of harmful metabolites, (3) developing methods to selectively eliminate specific harmful metabolites. An additional critical objective of this research involves developing predictive models based on patients' microbiome and metabolome profiles to identify high-risk individuals and subsequently formulate personalised prevention and intervention strategies.

Impact of dietary patterns on the metabolic microenvironment of intratumoural microbiota

Elucidating the influence of dietary patterns on the metabolic microenvironment of intratumoural microbiota will establish a foundation for developing targeted dietary intervention strategies to augment antitumour therapies. For example, modulating dietary tryptophan can influence anti-tumour immune responses in the tumour microenvironment through the metabolism of intratumoural microbes.[9] The cornerstone of this research rests on establishing comprehensive, large-scale prospective cohort studies that meticulously document patients' long-term dietary habits while simultaneously collecting tumour tissue samples for in-depth microbiome and metabolome analyses. Comprehensive investigations into the mechanisms by which dietary components modulate the metabolic activity of intratumoural microbiota are essential. These studies should encompass: (1) the direct effects of specific dietary components on the proliferation and metabolism of intratumoural microbes, (2) the indirect impacts of diet-induced alterations in host metabolism on intratumoural microbiota, (3) the influence of diet on the TME. Such multifaceted research will significantly enhance our understanding of the intricate interplay between diet, microbiota, and tumour biology. A pivotal objective in this field is to elucidate how targeted dietary interventions can optimise intratumoural microbial metabolism to potentiate the efficacy of anti-tumour treatments or attenuate adverse effects. This may involve the development of tailored functional foods or nutraceuticals specifically designed to modulate the intratumoural microbiome.

Effects of non-anticancer drugs on metabolic patterns of intratumoural microbiota

The impact of non-anticancer drugs on the metabolic patterns of intratumoural microbiota represents an important area worthy of in-depth investigation. To initiate this research, a systematic screening of various commonly used non-anticancer drugs, including those affecting glucose and lipid metabolism as well as antibiotics, should be conducted to assess their effects on intratumoural microbial metabolism. Apart from the direct antimicrobial effect of antibiotics on intratumoural microbes, no studies have yet elucidated mechanisms by which other non-anticancer drugs directly influence the metabolites of intratumoural microbes.[133] Furthermore, an in-depth study of the molecular mechanisms underlying the influence of these non-anticancer drugs on intratumoural microbial metabolism is necessary. This should encompass how drugs directly affect microbial metabolic enzyme systems, how they alter microbial metabolism by modifying the TME, and how drug-induced changes in host metabolism reciprocally impact the metabolic activities of intratumoural microbiota. Such investigations will contribute to a more comprehensive understanding of drug-microbe-host interactions. A key objective in this research direction is to explore how to leverage the microbial metabolic regulatory effects of these non-

anticancer drugs to optimise antitumour therapies. This includes investigating the potential of certain hypoglycemic agents to enhance the efficacy of chemotherapeutic drugs by altering glucose metabolism patterns in intratumoural microbiota, and the possibility of certain antibiotics enhancing immunotherapy effectiveness through selective elimination of specific intratumoural microbes.

Elucidating key druggable targets based on the metabolic microenvironment of intratumoural microbiota

Elucidating key druggable targets based on the metabolic microenvironment of intratumoural microbiota represents a critical avenue for future research.[134,135] Current research demonstrates cross-reactivity between intratumoural immune cells and microbial antigens, thereby revealing potential targets for tumour therapy.[136,137] However, these studies predominantly focus on gut microbes rather than intratumoural microbes and their metabolites. The foundation of this work lies in identifying potential targets through large-scale multi-omics studies metabolomic analysis of intratumoural microbial metabolites, integrated with transcriptomic and proteomic data, to elucidate metabolites and metabolic pathways closely associated with tumour progression or treatment response. Rigorous investigation of the biological functions and mechanisms of these potential targets, including their direct impact on tumour cell survival and proliferation, as well effects on immune cell function, will facilitate the evaluation of the potential therapeutic effects of targeting these metabolites or their production pathways. The development of intervention strategies targeting these entities, such as small molecule inhibitors or neutralizing antibodies specifically targeting metabolites, inhibitors directed at key microbial enzymes responsible for metabolite production, or the utilization of engineered bacterial strains to modulate specific metabolite levels, constitutes a critical component of this research direction. Concurrently, it is imperative to investigate how to enhance immune cell function and infiltration via these targets to optimise anti-tumour effects.

Limitations of existing studies

Despite significant advancements in understanding the role of intratumoural microbes and their metabolites in cancer biology, the current research landscape is characterized by several critical limitations. First, methodological inconsistencies in microbiome sampling, processing, and analysis pose substantial challenges for reproducibility and cross-study comparisons.[35] Contamination during sample collection and processing remains a significant concern, as it can introduce artifacts that confound the interpretation of results, particularly given the relatively low microbial biomass in tumour tissues compared to gut samples. Second, most studies employ correlation-based approaches that merely demonstrate associations between specific microbial metabolites and cancer outcomes without establishing causal relationships. While emerging research utilizing gnotobiotic animal models has begun to address this limitation, establishing definitive causal relationships between specific intratumoural microbial metabolites and immunotherapy responses necessitates further investigation using mechanistic approaches. Third, current research frequently focuses on individual metabolites in isolation, rather than considering the complex metabolic networks and interactions that collectively determine biological outcomes. The synergistic and antagonistic effects of multiple metabolites that act concurrently within the tumour microenvironment remain inadequately characterized. Additionally, the spatial heterogeneity of metabolite distribution within tumours is seldom accounted for in current studies, despite its considerable implications for therapeutic responses. Fourth, the translation of preclinical findings to clinical applications faces substantial challenges owing to species-specific differences in microbiome composition and immune system functionality. The metabolic activities of intratumoural microbes observed in laboratory models may not faithfully recapitulate those occurring in patients with cancers. Finally, although technological advances have enabled the identification of numerous microbial metabolites within the tumour microenvironment, our comprehension of their receptors, signalling pathways, and downstream effects on immune and cancer cells remains incomplete. Furthermore, the dynamic nature of the tumour microbiome in response to treatment interventions, particularly the temporal alterations in metabolite production, represents an understudied aspect that may profoundly influence therapeutic outcomes.[138] Overcoming these limitations necessitates interdisciplinary approaches that integrate advanced metabolomics, single-cell technologies, spatial profiling methods, and computational modelling to comprehensively map the complex interactions between intratumoural microbes, their metabolites, and host cells. Such efforts are imperative for translating the promising findings in this field into efficacious clinical strategies for cancer immunotherapy.

In conclusion, future investigations will elucidate the role of intratumoural microbial metabolites in the TME and their potential clinical applications from multiple perspectives. By constructing a pan-cancer metabolomic atlas of intratumoural microbiota, comparing metabolic profiles between intratumoural and gut microbiota, determining optimal synergistic concentrations of metabolites, evaluating optimal metabolic intervention strategies, investigating the effects of diet and non-antitumour drugs, and identifying novel druggable targets, we aim to develop more effective personalised antitumour treatment strategies. These studies will not

Search strategy and selection criteria

A comprehensive literature search was conducted in PubMed to identify relevant content for this review. Search terms included combinations of keywords related to intratumoural microbiota, microbial metabolism and cancer. Articles and reviews published in English from 1980 to 2025 were included, and references from selected articles were further examined to identify additional relevant studies. The manually screened articles were all related to intratumoural microbial metabolism and its impacts on tumour microenvironment, development and response to cancer immunotherapy.

only advance our understanding of tumour biology but also offer novel therapeutic prospects for patients with cancers. However, these studies also face numerous challenges, including limitations in technical methodologies, the complexity of inter-individual variability, and the translation of basic research findings into clinical applications. Addressing these challenges requires multidisciplinary collaboration and continuous innovation. As research progresses, we anticipate that intratumoural microbial metabolites will emerge as a significant breakthrough in the field of cancer therapy, potentially leading to improved treatment outcomes and quality of life for patients with cancers.

Outstanding questions

Although intratumoural microbiota and cancer immunotherapy have attracted considerable attention from the scientific community, many challenges remain to be addressed. For instance, the collection of intratumoural microbial samples faces significant challenges such as susceptibility to contamination, low biomass yield and stringent cultivation requirements. Furthermore, current static metabolomic analyses are unable to track the dynamic processes of intratumoural metabolite production in real time. These challenges necessitate concerted efforts from the broader scientific community.

Contributors

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Declaration of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

Funding: This work was supported by grants from the Natural Science Foundation of Guangdong Province (2021A1515012593), the National Natural Science Foundation of China (82373129, 82172750, 82172811, and 82260546), the Guangdong Basic and Applied Basic Research Foundation (Guangdong-Guangzhou Joint Funds) (2022A1515111212), the Science and Technology Program of Guangzhou (2023A04J1257), and Hunan Youth Science and Technology Talent Project (No. 2023RC3074).

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