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The Role of the Microbiome in Drug Response

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Abstract

The microbiome is known to regulate many aspects of host health and disease and is increasingly being recognized as a key mediator of drug action. However, investigating the complex multidirectional relationships between drugs, the microbiota, and the host is a challenging endeavor, and the biological mechanisms that underpin these interactions are often not well understood. In this review, we outline the current evidence that supports a role for the microbiota as a contributor to both the therapeutic benefits and side effects of drugs, with a particular focus on those used to treat mental disorders, type 2 diabetes, and cancer. We also provide a snapshot of the experimental and computational tools that are currently available for the dissection of drug–microbiota–host interactions. The advancement of knowledge in this area may ultimately pave the way for the development of novel microbiota-based strategies that can be used to improve treatment outcomes.

INTRODUCTION

Much has changed since Paul Ehrlich, the founding father of modern pharmacology, first developed Salvarsan as a treatment for syphilis in 1909 (1). However, his concepts still resonate today, as terms he introduced, such as chemotherapy and magic bullet, are widespread in contemporary scientific literature. The idea behind such notions is that pharmacological compounds can be intelligently designed and synthesized to target and kill specific types of unwanted cells (e.g., microbes, cancer cells) without causing harm to the healthy cells of the human body. Yet, a century of intense pharmacological research later, failure to deliver these gold standards, associated with high attrition rates in drug development (2), has led to the abandonment of this simplistic outlook. In addition, the recent emergence of powerful genomic, proteomic, and metabolomic techniques and preclinical models has led to a greater understanding of the genetic and/or environmental factors that affect drug efficacy and the identification of unintended targets and regulators of drug action. This has prompted scientists, medics, and industry to adopt new models that encompass a far more complex and comprehensive understanding of the inner workings of the human body in the context of drug treatments and its respective environment. As a result, new disciplines in pharmacology such as pharmacogenomics and pharmacometabolomics have emerged, which focus on understanding how host genetics and metabolism, respectively, affect drug efficacy. Furthermore, the recent developments in microbiome research, underscoring the power of host-associated microbes in regulating most if not all human bodily functions (3), highlight a novel frontline for pharmacology—pharmacomicrobiomics.

In this review, we provide an up-to-date account of the role of the microbiota in regulating the effects of drugs on host physiology and disease. In particular, we focus on the role of drugs used in the treatment of mental disorders, diabetes, and cancer whose therapeutic effects on the host rely intimately on gut microbiota functions. Finally, we provide a description of holistic experimental and computational tools that will aid in the development of predictive models of drug action by encompassing both host and microbial physiology as a single unit in drug action. Such models are the tip of the iceberg in understanding the complex and intertwined relationship between drugs, microbes, and host.

PHARMACOMICROBIOMICS AND THE HOLOBIONT

Pharmacomicrobiomics is a new discipline that aims at understanding the interplay between gut microbial ecology, pharmacology, environmental cues, and host genotype. Pharmacomicrobiomics, therefore, embodies the concept of the holobiont. Here, this supraentity of host and microbial cells functions as a single genomic and metabolic unit, where both physiological entities are under direct selective pressure and can equally or distinctively regulate the effects of their surrounding environment. A unique functional and phenotypic output arises from this complex interplay.

Importantly, such a holistic view of the human body also calls for a redefinition of what constitutes drug therapy in the first place. The Lipinski's rule of five, where a set of guidelines based on the molecular properties of pharmacological compounds (e.g., absorption, distribution, metabolism, and excretion, or ADME) were created to evaluate druglikeness and predict the likelihood of being orally active in humans (4), may no longer be fully fit for purpose. Given the widely acknowledged role of the microbiota in regulating host functions and the effects of many drugs (5), the scope of Lipinski's rule of five should be expanded to encompass not only compounds that target the host cells but also those that can alter gut microbial function. Moreover, one may want to consider the role of specific nutritional cues in regulating drug effects on the holobiont.

For example, research by Turnbaugh and colleagues (6) into the mode of action of the cardiac glycoside digoxin, a drug used to treat heart failure, has shown that the flavin- and [4Fe-4S] cluster-dependent reductase Cgr2 present in digoxin-metabolizing *Eggerthella lenta* inactivates digoxin and is inhibited by dietary arginine. Such results highlight the importance of considering pharmacology from the viewpoint of the holobiont to increase the likelihood of a drug initially aimed at targeting host cells to be fully effective.

THE FUNCTIONAL CAPACITY OF THE MICROBIOTA IN THE CONTEXT OF PHARMACOLOGY

The widespread role of the microbiota in regulating the health and well-being of the holobiont provides exciting avenues for therapy, but our current understanding of the complex interactions between host, microbiota, and environment is still in its infancy. Several studies have shown that the environment (e.g., nutrition and medication) rather than host genetics is the most important factor regulating microbial dynamics (7–9). In particular, recent clinical and population studies have shown that the microbiota composition can be changed by therapeutic drugs (10, 11), but the microbiota can equally impact drug availability (5). Alterations in pharmacokinetics caused by microbial biotransformation (activation, reactivation, inactivation) has been acknowledged for 80 years (12), with over 60 drugs known to be modified (5). Examples of prodrugs that are activated by the microbiota include protonsil, one of the earliest nonantibiotic antimicrobial drugs (12); sulfasalazine, used for treating rheumatoid arthritis; and lovastatin, used to lower cholesterol. Drugs that are reactivated by the microbiota include the anticancer drug irinotecan, which is modified by bacterial β-glucuronidases following biliary secretion into the gut (5), while the cardiac drug digoxin serves as an example of drug inactivation by the microbiota (6). The microbiota can also indirectly alter drug pharmacokinetics by producing metabolites that compete for the active site of host enzymes responsible for the modification of drugs [e.g., competition between bacterial *p*-cresol and the analgesic acetaminophen (5)] or by altering expression levels of liver cytochrome P450 enzymes responsible for drug action (13). Finally, therapeutic drugs like the antidiabetic drug metformin can also directly impact gut microbial communities and metabolism, leading to alterations in host metabolism (10, 14).

Overall, drug–microbiota interactions span a wide range of drug chemistry, therapeutic indications, and side effects, as demonstrated by a recent comprehensive study from the Typas lab (11). They tested over 1,000 host-targeted drugs and found that 24% inhibited the growth of representative gut bacterial strains in vitro. Drugs found to have a significant effect on bacterial growth included those aimed at the treatment of mental health disorders, type 2 diabetes (T2D), and cancer. Consequently, we focus on these three classes of therapeutics going forward and provide a detailed account of the current knowledge of drug–microbiota interactions that may underlie their effects.

Antipsychotic and Psychotropic Drugs

The connection between the gut and brain is well established, with the gut even being referred to as our second brain (15). However, the study of the microbiome has revealed that these two organs are even more intertwined than previously thought. The gut microbiota is known to regulate brain development, and alterations in its composition have been linked to mental disorders such as anxiety, schizophrenia, bipolar disorder, and depression (16). Moreover, drugs used to treat these conditions have been found to interact with the microbiota (**Figure 1**), although the mechanisms that govern such interactions remain to be fully elucidated. In particular, antipsychotic compounds were identified as being significantly overrepresented when host-targeted drugs were screened for

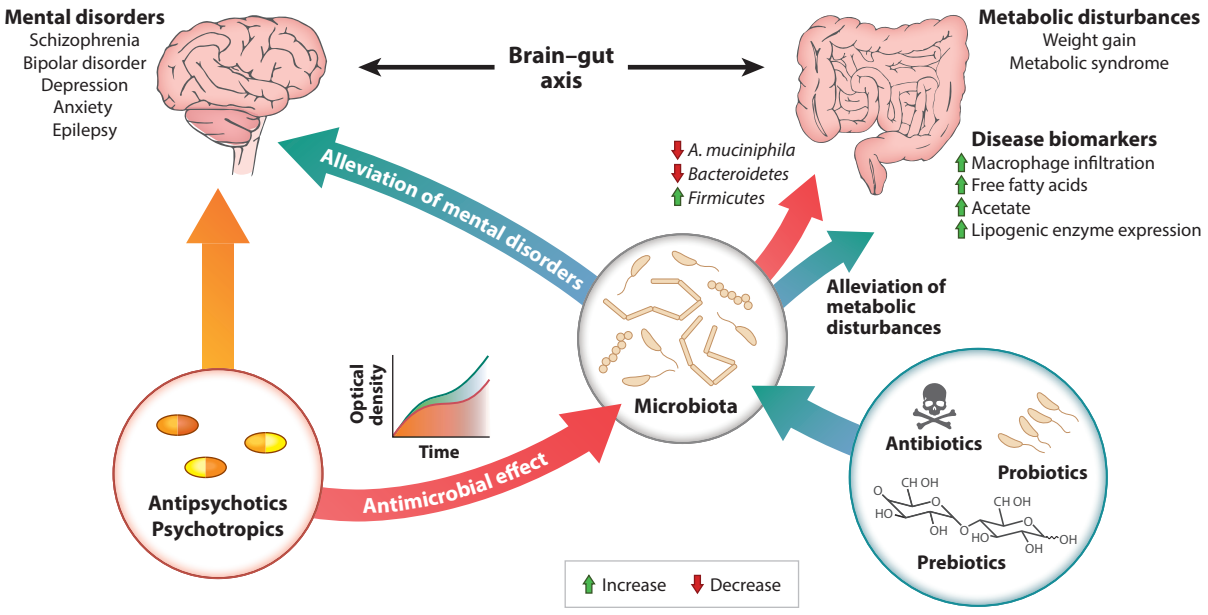

Figure 1

Illustration of the gut microbiota as a central regulator of the brain–gut axis and potential drug interactions. Antipsychotics and psychotropics have a direct effect on the gut microbiota composition, provoking dysbiosis and causing metabolic disturbances to the host. Additionally, probiotics, prebiotics, and antibiotics that target the modification of the gut microbiota community can affect host psychological status.

their ability to inhibit the growth of gut bacteria (11). This finding is supported by further studies that show that the administration of atypical antipsychotics (AAPs) induces dysbiotic alterations to the gut microbiota composition (17, 18). Interestingly, there is also evidence that the inverse relationship exists, as antibiotic-induced dysbiosis has been linked to mental disorders such as anxiety (19) and depression (20).

It has been proposed that several of the metabolic side effects associated with AAP use, including weight gain, cardiometabolic disturbances, and the development of metabolic syndrome, may result from drug action on the microbiota (21). For instance, long-term exposure to risperidone in both children (21) and mice (22) has been found to increase the Firmicutes/Bacteroidetes ratio, which is associated with obesity. Additionally, a decrease in *Akkermansia muciniphila* has been observed in AAP-treated groups (18), and this species is known to have beneficial anti-inflammatory properties and can protect against gut barrier dysfunction and fat mass development (23). Moreover, a study involving germ-free (GF) mice revealed that the gut microbiota was responsible for weight gain observed in response to olanzapine treatment (24). Similarly, olanzapine's metabolic side effects were partially revoked in female rats when coadministered with an antibiotic (25). The same study also reported that olanzapine treatment increased several markers of metabolic dysfunction, including macrophage infiltration in the adipose tissue, increased abundance of free fatty acids in the plasma, and altered expression of lipogenic enzymes. More recently, it was shown that administration of a galacto-oligosacharide prebiotic alleviated olanzapine-induced weight gain (26). Altogether, these findings demonstrate that AAPs can profoundly impact host metabolism via their effects on the gut microbiota.

Psychotropic drugs known to interact with the microbiota are not limited to those belonging to the AAP class and can trigger diverse microbial responses. This is illustrated by a study that found

that the antidepressant fluoxetine induced small alterations to the microbiota composition while other psychotropics such as lithium, aripiprazole, and valproic acid (VPA) had stronger effects (27). Interestingly, short-chain fatty acid (SCFA) levels varied depending on the drug treatment, and those changes could be mapped to bacterial taxa known to be SCFA producers (e.g., Bacteroidetes or *Clostridium* spp.). The mechanisms to account for these drug effects on the microbiota are largely unknown at present. It has been suggested that selective serotonin reuptake inhibitor antidepressants such as fluoxetine and escitalopram could be acting as antimicrobials due to their efflux pump inhibitor action (28). Conversely, certain microbial consortia are capable of degrading fluoxetine (29). A further intriguing example of drug–microbiota interaction is provided by the aforementioned VPA. This drug, which is used to treat seizures, epilepsy, and mood swings, has been shown to increase the risk of autism in children when taken by mothers during pregnancy (30). This finding has led to the development of a clinically relevant animal VPA-induced rat model of autism, whereby administration of VPA to pregnant rats generates offspring with autism-like phenotypes (30, 31). Importantly, the offspring also exhibit dysbiosis, with changes in the gut microbiota community similar to those observed in humans with autism (32). However, whether such microbial alterations cause the disorder remains to be proved.

A greater understanding of the role of the microbiota in regulating host behavior has enabled novel approaches for its modification such as the use of psychobiotics (33). For example, both prebiotics (34) and probiotics have been found to reduce depression and anxiety (35) in humans. Nevertheless, there is still much to learn about how the gut microbiota may mediate the effects of drugs used for the treatment of mental disorders. While there is compelling evidence that the microbiota can mediate the metabolic side effects associated with some psychotropic drugs, further research into the molecular basis of these drug–microbiota interactions is required. Moreover, whether the microbiota could also contribute to the therapeutic effects of these drugs remains an open question.

Antidiabetic Drugs

The first-line treatment for T2D is metformin, which belongs to the biguanide class of drugs. Until recently, research concerning metformin's mechanism of action was mostly focused on its antigluconeogenic action in the liver (36, 37). However, there is now a growing body of evidence that suggests the gut microbiota is a key mediator of metformin's therapeutic effect (**Figure 2**). Consistent with this hypothesis, intravenous administration of the drug is less effective at reducing blood glucose levels compared to oral or intraduodenal methods of delivery (38, 39). Furthermore, a delayed-release formulation of metformin that is restricted to the gut exhibits glucose-lowering properties comparable to that of standard formulations despite greatly reduced systemic exposure (40).

It is not yet fully understood how the gut microbiota contributes to metformin's ability to regulate glucose homeostasis. One possibility is that metformin induces alterations to microbial structure and function that ameliorate the dysbiosis associated with T2D. Several metagenomic studies have reported that diabetic individuals have an altered gut microbiota composition compared to nondiabetic individuals (10, 41, 42). Further stratification according to metformin treatment status revealed that the gut microbiota of untreated individuals was characterized by a depletion of butyrate-producing taxa, including *Roseburia* spp., *Subdoligranulum* spp., and *Clostridiales* spp. In contrast, metformin treatment was associated with an increase in *Escherichia* spp. and a decrease in *Intestinibacter* spp. (10). A subsequent study reported that the same changes in the microbiota of individuals with treatment-naïve T2D were observed after receiving metformin for four months, suggesting that this is a robust treatment signature (14). Remarkably, the same study also demonstrated that transplanting the microbiota of metformin-treated individuals into GF mice fed a

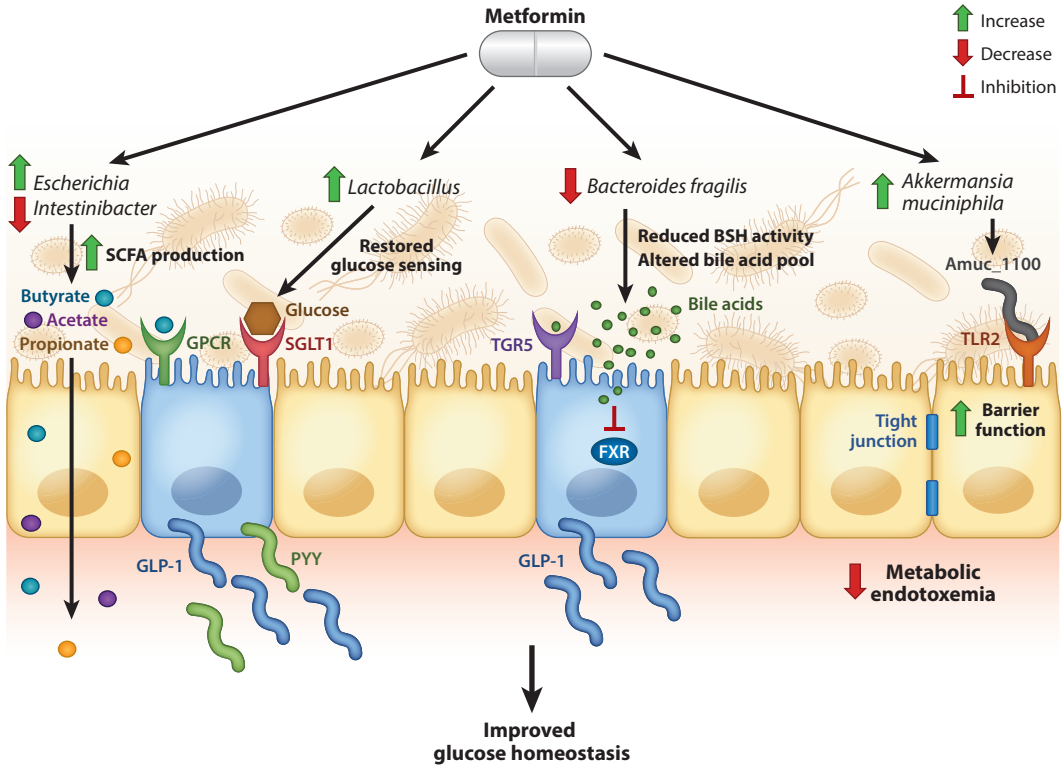

Figure 2

Metformin alters the composition of the gut microbiota to regulate glucose homeostasis. Proposed mechanisms include increased production of beneficial short-chain fatty acids and enhanced secretion of the gut hormones GLP-1 and PYY, potentially via G protein–coupled receptor activation. GLP-1 secretion may also be enhanced by restored glucose sensing via increased SGLT1 expression or by modulation of TGR5/FXR bile acid receptor activity, resulting from bile pool alterations (increased levels of glycine-ursodeoxycholic acid). Additionally, the bacterial peptide Amuc_1100 may interact with Toll-like receptor 2 to improve barrier function and reduce metabolic endotoxemia.

high-fat diet was sufficient to enhance glucose tolerance (14). It has been suggested that changes in the abundance of these bacterial taxa may mediate metformin's therapeutic action by increasing the production of SCFAs (10, 14, 43), which have been linked to improvements in glucose and energy homeostasis in rodent models (44). Conversely, modulation of microbial composition and metabolism may contribute to the drug's side effects. Gastrointestinal problems associated with metformin use have been linked to increased *Escherichia* and *Shigella* spp. (45), and metformin has been shown to disrupt bacterial folate metabolism in *Caenorhabditis elegans* (46), which may explain reduced folate status associated with its use (47).

Alternatively, metformin-induced changes to the microbiota may regulate glucose homeostasis by promoting the integrity of the intestinal barrier. Metformin has been observed to increase the relative abundance of *A. muciniphila* in both mice (48, 49) and humans (14, 43), and probiotic administration of this species enhanced glucose tolerance and improved insulin signaling (49). Interestingly, an outer membrane protein isolated from *A. muciniphila* was shown to activate Toll-like receptor 2 (TLR2) and recapitulated the positive effects of intact *A. muciniphila* on glucose and lipid metabolism in mice (23). It was suggested that TLR2 signaling may enhance intestinal barrier function and consequently correct metabolic endotoxemia associated with diabetes.

It has also been proposed that the gut microbiota mediates metformin effects by influencing the secretion of gut hormones. Individuals taking metformin have been found to exhibit increased plasma levels of the incretin hormone glucagon-like peptide 1 (GLP-1), and some studies have also reported an accompanying increase in peptide YY (PYY), which is involved in appetite control (50). A possible link between metformin's effect on gut hormone secretion and the microbiota was first highlighted when a correlation was observed between PYY levels and changes in the abundance of *Bacteroidetes* and *Firmicutes* spp. in samples from T2D individuals on metformin monotherapy (51). Furthermore, there is evidence that SCFAs can trigger the secretion of GLP-1 and PYY from enteroendocrine cells, either via interactions with G protein–coupled receptors (52), via their histone deacetylase inhibitory activity (53), or simply by acting as an energy source (54). Consequently, metformin may indirectly stimulate the release of these hormones by promoting the growth of SCFA-producing species. Additionally, increased GLP-1 secretion by metformin has been attributed to changes in the gut microbiota that affect an intestinal glucose sensor. Expression of sodium glucose cotransporter-1 (SGLT1), the major transporter responsible for glucose-stimulated GLP-1 secretion, was found to be reduced in the upper small intestine of rats fed a high-fat diet (55). However, metformin treatment restored SGLT1 expression and glucose sensing while also increasing the relative abundance of *Lactobacillus*. Crucially, transplantation of metformin-pretreated microbiota into the small intestine of rats fed a high-fat diet also restored SGLT1 expression and glucose sensing, supporting a gut microbiota-mediated mechanism. Further research is required to establish precisely how *Lactobacillus* upregulates this nutrient sensor to enhance GLP-1 secretion and lower plasma glucose levels.

The microbiota has also been linked to metformin effects on the bile acid pool that may contribute to the drug's antihyperglycemic action. Bile acids are required to solubilize lipids for intestinal uptake and can modify metabolism by binding to membrane receptor proteins such as Takeda G protein–coupled receptor 5 (TGR5) and the farnesoid X receptor (FXR). Multiple studies have demonstrated that metformin treatment induces alterations to the bile acid pool (14, 51, 56), and there is ample evidence of crosstalk between bile acids and the gut microbiota (57). In one study, increased levels of cholic acid observed in metformin-treated individuals significantly correlated with structural changes in the microbiota (51). The authors speculated that these changes may indirectly increase GLP-1 levels via increased TGR5 activity. Interestingly, a separate study showed that supplementation with cholic acid triggered the same microbial changes in mice, suggesting that metformin's effects on the microbiota are a consequence of the drug's effect on bile acid metabolism (58). However, it appears that this interaction also occurs in the reverse direction and that changes in bile acid profiles can be driven by metformin-induced alterations to the microbiota. Targeted metagenomics analysis has revealed an increase in bile salt hydrolase (*bsh*) genes in the microbiota of individuals with T2D following two months of metformin treatment (14). This could account for the observed increase in unconjugated bile acids, an effect that was significantly negatively correlated with blood glucose levels. More recent work has established a mechanism whereby metformin decreases the abundance of *Bacteroides fragilis*, resulting in increased levels of glycoursodeoxycholic acid, a bile acid reported to ameliorate insulin resistance via inhibition of intestinal FXR signaling (56).

While much research has been dedicated to understanding metformin–microbiota interactions, there is also evidence outlining a role for the microbiota in mediating the effects of other drugs used in the treatment of T2D. It has been reported that berberine, a traditional Chinese medicine with antidiabetic properties, is converted into a more absorbable form via the reducing activity of the gut bacteria (59) and that differences in the pharmacokinetic profile of berberine identified between Chinese and African populations can be attributed to variations in gut microbiota composition (60). Similar to metformin, it has been suggested that the increased production

of beneficial SCFAs (61) or the inhibition of bile acid receptor signaling (62) by the microbiota may underpin the therapeutic effects of berberine. Additionally, berberine has been linked to a decrease in the abundance of bacterial species capable of synthesizing branched-chain amino acids, metabolites implicated in the development of insulin resistance and obesity (63).

Acarbose is an α-glucosidase inhibitor that helps to regulate glucose homeostasis by delaying the digestion of complex carbohydrates in the small intestine. Since this drug impacts the substrate available for bacterial fermentation, and thus may selectively favor the growth of certain taxa, one would expect an effect on the microbiota. Indeed, several studies have demonstrated that acarbose alters the composition of bacterial communities in the gut (64–66). In a randomized controlled trial involving 52 prediabetic individuals, acarbose treatment was associated with a significant increase in *Lactobacillus* and *Dialister* spp., with the latter correlating negatively with blood glucose levels (66). This suggests that alterations to the microbiota structure may be partly responsible for the drug's therapeutic benefits. Consistent with this hypothesis, a separate study uncovered a tight association between acarbose-induced modifications to the gut microbiota and changes in the bile acid pool that were linked to improved glycemic control (65). Interestingly, the same study also found that individuals' responses to acarbose treatment could be predicted based on whether their microbiota was dominated by *Bacteroides* or *Prevotella*, a result that may have important clinical implications.

Evidence supporting a role for the gut microbiota in the mechanism of action of other T2D drugs is sparse at present. It has been shown that treatment with pioglitazone, from the thiazolidinedione family, suppressed the increase in Proteobacteria observed in rodents fed a high-fat diet (67), while rosiglitazone treatment restored the spatial distribution of bacteria along the ileal mucosa but not the composition (68). Likewise, a study investigating the effect of the sulfonylurea glipizide on the microbiota of individuals with T2D reported no significant changes in relative abundances at both the species and gene level (65). It is, however, worth noting that the bioavailability of the related drug gliclazide was found to be enhanced in diabetic rats following administration of probiotics, highlighting possible drug–microbiota interactions (69). With respect to the incretin-based class of drugs, there are several reports of an association between the GLP-1 receptor agonist liraglutide and altered gut microbiota structure (70–73). In particular, liraglutide treatment has been shown to reduce the relative abundance of obesity-related bacterial phylotypes in rodent models of diabetes and obesity (70, 72), and in one study, an observed increase in *Lactobacillus* was negatively correlated with blood glucose levels (71). An increase in *A. muciniphila* has also been observed in response to liraglutide treatment in T2D individuals, implying potential improvements to gut barrier function (73). Furthermore, some dipeptidyl peptidase-4 inhibitors that are used to increase GLP-1 levels may also exert beneficial effects via the microbiota. For example, vildagliptin treatment induced a decrease in *Oscillibacter* and an increase in both *Lactobacillus* and SCFA production in mice fed a Western diet (74), while sitagliptin was also found to partly correct the dysbiosis associated with a high-fat/high-carbohydrate diet in diabetic rats (75). Nevertheless, further research is required to evaluate whether there is a causal relationship between changes in the microbiota induced by these drugs and their therapeutic effects.

Anticancer Drugs

Microbes play a significant role in the etiology of cancer and account for approximately 20% of cancers worldwide (76). The challenges involved in understanding the complex interactions between the gut microbiota and cancer are aggravated by external factors that can influence both elements and the difficulty in discerning whether microbial alterations are a cause or consequence of the disease. For example, cancers may disrupt the microbiome directly and locally (77), indirectly

via soluble factors (e.g., CC-chemokine ligand 25) (78), and systemically via altered metabolic effects (79). Additionally, several environmental factors causing microbiota dysbiosis or influencing the production of toxins or metabolites by the microbiota can promote cancer or alter anticancer drug therapy. Furthermore, cancer and gut dysbiosis may share a common denominator that occurs upstream of both processes. For example, host lifestyle choices and immune system status can modulate both the microbiome and cancer development.

Several studies have shown that chemotherapeutics can negatively impact the gut microbiota. For example, it was found that irinotecan-based chemotherapy treatment led to dysbiosis in a colorectal cancer (CRC) rat model characterized by an increase in diarrhea-inducing bacteria such as *Clostridium* cluster XI and *Enterobacteriaceae* (80). Furthermore, non-Hodgkin lymphoma patients exposed to a five-day myeloablative chemotherapy regimen exhibited a significant increase in Proteobacteria and a concomitant decrease in Firmicutes and Actinobacteria abundance (81). Additionally, a study investigating the effect of the nucleoside analog gemcitabine on the gut microbiota of pancreatic cancer xenografted mice found a decrease in Firmicutes and Bacteroidetes populations and an increase of Proteobacteria and Verrucomicrobia spp. (82). The observed increase in bacteria associated with inflammatory processes was consistent with NF-κB upregulation in the cancer tissue, which may have a negative effect on the outcome of gemcitabine treatment (82). It is also worth noting that a study in a CRC mouse model revealed that bacteria are capable of metabolizing gemcitabine into its inactive form via cytidine deaminase enzyme activity (83). Overall, these observations highlight that various chemotherapeutic agents can have a dysbiotic influence on the gut microbiota that may contribute to detrimental side effects or reduce drug efficacy.

On the contrary, there is evidence that the gut microbiota can enhance the efficacy of certain anticancer drugs. For example, studies in GF mice have shown that the microbiota is required for optimal responses to oxaliplatin or combination immunotherapy (84, 85). Similarly, there are reports that antibiotic usage, which is known to perturb the microbial ecosystem, can negatively influence cancer treatment outcomes (85, 86). It has been demonstrated that the gut microbiota makes a major contribution to the efficacy of the antimetabolic drug 5-Fluorouracil (5-FU) (87, 88), which is used to treat multiple cancers. Research performed in *C. elegans* revealed that bacteria were capable of converting and excreting 5-FU in its most effective fluoropyrimidine forms to target the host with increased efficacy. Crucially, this mechanism was dependent on an intact bacterial ribonucleotide salvage pathway (87, 88). Moreover, knockout of the *Escherichia coli* enzyme nucleoside diphosphate kinase increased 5-FU efficacy in the host via an alternative mechanism involving bacterial deoxynucleotide pool imbalance (87). Interestingly, the gut bacteria may also play a role in the development of resistance to 5-FU and other drugs used to treat CRC. It has been reported that patients with postchemotherapy tumor recurrence have an increased abundance of *Fusobacterium nucleatum* when compared to patients that do not experience a recurrence (89). This species was found to promote chemoresistance in CRC cells via the targeting of specific microRNAs and the activation of autophagy (89). Taken together, these observations emphasize the importance of untangling the biochemical pathways involved in drug–microbiota interactions in order to improve cancer treatment outcomes.

The microbiota synthesizes a diverse array of molecules that are readily exchanged with the host, and it is known that bacterial-derived metabolites can modulate the effects of anticancer drugs. One such metabolite is the vitamin B6 precursor pyridoxine, which was found to increase the antineoplastic effect of cisplatin in a non-small-cell lung cancer mouse model (90). Bacterial-derived pyridoxal phosphate was also found to be an indirect but essential regulator of 5-FU toxicity in *C. elegans* (87). Furthermore, melanoma patients with gut bacteria that were deficient in polyamine transport and B vitamin biosynthesis were identified as being at increased risk of developing ipilimumab treatment–induced colitis (91). It is possible that SCFAs generated by the

microbiota could improve cancer treatment outcomes as they increase the abundance of *Bifidobacterium* spp., which have been linked to a reduction in tumor growth in mice (92), and may confer anti-inflammatory effects that lower the risk of CRC (93). The microbiota also secretes toxins that could promote positive treatment outcomes. In fact, bacterial toxins, such as anthracyclines (e.g., doxorubicin), are widely used chemotherapeutics due to their ability to stimulate anticancer immune responses (94). Other antibacterial peptides have been investigated for their anticancer activity based on their ability to disrupt the mitochondrial membrane (95).

Since it has been established that gut microbiota can regulate the effects of anticancer drugs (**Table 1**), interventions designed to target the microbiota may represent promising adjuvants or novel treatments for this disease. Indeed, it has been shown that the administration of probiotics can improve patient responses, particularly when used in combination with immunotherapies. For example, oral administration of *Bifidobacterium* was reported to facilitate the efficacy of PD-1 ligand 1–specific antibody therapy in mouse models of melanoma (92). Subsequently, three studies have further explored the role of the gut microbiota on the anti-PD-1 immunotherapy response (96–98). Additionally, *Bifidobacterium* supplementation was found to rescue mice from immunopathology associated with anti-CTLA-4 therapy without affecting the stimulation of antitumor immunity (99), while *Bacteroides fragilis* has also been observed to possess anticancer properties within the context of anti-CTLA-4 therapy (100). Potential benefits of probiotics are not, however, restricted to immunotherapeutics, as demonstrated by the finding that *Bifidobacterium bifidum* G9–1 ameliorated 5-FU-induced mucositis in a mouse model via the suppression of dysbiosis and attenuation of inflammatory responses (101). Moreover, *Lactobacillus acidophilus* and *Lactobacillus casei* probiotic strains were shown to increase 5-FU apoptosis capacity in vitro (102). Prebiotic compounds used to promote the growth of beneficial bacteria may also enhance the action of anticancer drugs, as it has been shown that inulin demonstrated increased antitumor effects when coadministered with the chemotherapeutic doxorubicin (103).

In addition to modifying microbial composition through the use of probiotics or prebiotics, drugs can be used to target specific processes in bacteria and provide anticancer benefits. For example, small-molecule inhibitors can be used to block the production of *E. coli*–derived colibactin, which has genotoxic properties (104). It has also been shown that bacterial β-glucuronidase inhibitors are effective at preventing the toxic reactivation of irinotecan metabolites and thus may alleviate the gastrointestinal side effects associated with this drug (105). The gut microbiota can also be targeted via the use of bacterial strains that have been genetically modified to deliver cytotoxic molecules directly to the tumor microenvironment. This strategy was successfully implemented in a study involving an attenuated *Salmonella typhimurium* strain that had been engineered to lyse in synchronous cycles upon reaching a population density threshold, resulting in the periodic release of the antitumor toxin haemolysin E (106). Oral administration of this engineered strain improved the efficacy of 5-FU in a mouse model of hepatic colorectal metastases (106). Overall, these studies provide encouraging evidence to suggest that novel microbiota-based therapies could be an effective way of improving cancer treatment outcomes going forward.

TOOLS FOR EXPLORING THE DRUG–MICROBIAL TERRA INCOGNITA

Drug–microbiota–host interactions are inherently complex and, as such, require a combination of experimental and computational approaches for their dissection. Experimental tools used for this task can be divided into two broad categories: in vitro and in vivo models. In vitro models have provided great insight into the mechanistic basis of drug–microbiota interactions (83) and can be used to screen a large number of experimental conditions simultaneously (11). However, to test the

Table 1 Microbial regulation of cancer treatment efficacy

Treatment	Bacterial species	Model	Interaction	Reference(s)
Cyclophosphamide	*Lactobacillus johnsonii* and *Enterococcus hirae*	Mouse	Translocation of these species into lymphoid organs stimulated the production of T helper 17 and T helper 1 immune responses	85
Irinotecan	*Clostridium* cluster XI and *Enterobacteriaceae* families	Rat	Therapy led to dysbiosis and increases in these families, which resulted in mucosal injury	80
	Firmicutes and Bacteroidetes phyla	NA	Bacterial β-glucuronidase activity led to toxic reactivation of irinotecan metabolites	105
Gemcitabine	Proteobacteria and Verrucomicrobia phyla	Mouse	Increased abundance of these phyla was associated with NF-κB upregulation and was detrimental to therapy outcome	82
	Gammaproteobacteria class	Mouse	Bacterial cytidine deaminase activity led to gemcitabine resistance	83
Oxaliplatin	*Alistipes shahii* and *Ruminococcus* spp.	Mouse	Bacterial species were linked to optimal treatment response via modulation of myeloid-derived cell function	84
5-FU	*Escherichia coli*	Nematode	Bacteria ribonucleotide salvage pathway was responsible for drug conversion and increased efficacy	87, 88
	Fusobacterium nucleatum	Human, cell culture	Increased abundance of this species was linked to postchemotherapy recurrence via targeting of miRNAs and autophagy processes in CRC cells	89
	Bifidobacterium bifidum G9–1 (BBG9–1)	Mouse	Administration of BBG9–1 reduced severity of 5-FU-induced mucositis	101
	Lactobacillus acidophilus and *Lactobacillus casei*	Cell culture	Administration of these species increased 5-FU-induced apoptosis of cancer cells	102
Ipilimumab	Bacteroidetes phylum	Human	Increased abundance of this phylum was linked to reduction in ipilimumab-induced colitis	91
	Bacteroides fragilis	Mouse and human	Administration of this species increased the immunostimulatory effects of CTLA-4 blockade	100
Anti-PD-1 immunotherapy	*Bifidobacterium* spp.	Mouse	Administration of these species led to improved tumor control	92
	Akkermansia muciniphila	Mouse and human	Increased abundance of these species was associated with positive treatment outcomes Fecal microbiota transplantation from these subjects into germ-free mice improved immunotherapy efficacy	96
	Bifidobacterium longum, *Collinsella aerofaciens*, and *Enterococcus faecium*			97
	Ruminococcaceae family			98

Abbreviations: 5-FU, 5-Fluorouracil; CRC, colorectal cancer; miRNA, microRNA; NA, not available.

biological relevance of these interactions on the host, one needs to go beyond in vitro approaches and utilize invertebrate model organisms that facilitate high-throughput techniques capable of shedding light on the effects of drug–microbiota interactions in vivo. *Drosophila melanogaster* has been proposed as a suitable model for both drug discovery and investigating the role of the microbiota on host physiology (107), but studies focused on the interactions between these two factors have not been performed in this model to date. In contrast, *C. elegans* is becoming an increasingly relevant player in drug–microbe–host interaction research due to its versatility, easy handling, cost, and scalability (108). For example, *C. elegans* has been successfully used to explore the microbial mechanisms responsible for the biotransformation of the anticancer drugs 5-FU (87, 88) and doxorubicin (109) and to investigate how the microbiota mediates the prolongevity effect of metformin (46). As with all animal models, *C. elegans* has both advantages and disadvantages that must be weighed when attempting to address a question related to drug–microbe–host interactions (108). While it is simple to establish microbial mono-associations in *C. elegans* that enable a high degree of experimental control and manipulation, other models are better suited to investigate how drugs interact with microbial communities to influence treatment outcomes. These include mouse models that have been extensively used in drug–microbiota research as well as the zebrafish (*Dario rerio*), which is increasingly gaining momentum within this field (110).

The study of drug–microbial community interactions requires computational methods capable of analyzing complex data sets generated from microbiota samples. Many tools exist for this purpose and have been extensively reviewed elsewhere (111, 112). Briefly, drug-induced changes at the structural level can be analyzed by 16S sequencing used in combination with platforms such as QIIME2 (113) or mothur (114) or by using metagenomics data sets with Kraken2 (115), Centrifuge (116), bioBakery (117), or CLARK-S (118). Metagenomes can be assembled using MetaVelvet (119) or metaSPAdes (120), and gene annotation can be addressed with tools like prokka (121) to identify functions differentially enriched by drug treatments. At the functional level, metatranscriptomic data can be analyzed using SAMSA2 (122), MetaTrans (123), or Anvi'o (124); metaproteomic data with MetaLab (125) or MaxQuant (126); and metabolomic data with PhenoMeNal (127) or Workflow4Metabolomics (128). Crucially, the integration of multiple data sets must occur in order to form biologically relevant and meaningful conclusions (129). For example, a multi-omic approach was effectively used to identify certain metabotypes within human microbiomes that can metabolize CRC drugs (130). Here, the authors used a combination of metabolomic and metagenomic analyses to unravel this intricate issue and extract useful biological insight.

The aforementioned tools have been developed to address a broad spectrum of questions related to microbial ecosystems. However, given the interest in drug/xenobiotic biotransformation by gut microbes, it is surprising that the number of tools available for specifically investigating this phenomenon is limited. PharmacoMicrobiomics is a web portal database released in 2011 that aims to explore the interactions between drugs and microbes (131). More recently, the Microbe-Drug Association Database was released, featuring over 5,000 entries detailing clinically or experimentally supported interactions between more than 1,000 different drugs and almost 200 different microbes (132). Furthermore, DrugBug, released in 2017, is a bioinformatic tool that encompasses the metabolic functions of 491 human gut bacteria strains and uses a random forest machine learning algorithm to predict the biotransformation of drugs by microbial enzymes present in the human gut microbiome (133). The authors used digoxin as an example to validate their tool and found that it accurately predicted both the bacterial genus and enzymes involved in the metabolism of this drug, as supported by previous in vitro and in vivo studies (6, 134).

New computational tools that aim to integrate microbial and host multi-omic data sets are an exciting computational endeavor that may provide further mechanistic insights into

drug–microbiota–host interactions. The most successful computational framework to model host metabolism is the constraint-based reconstruction and analysis (COBRA) (135), which has been continuously developed and refined for more than a decade. This framework has been used to model the metabolism of 773 different bacteria from the gut microbiome (AGORA) (136), which now forms part of a large database that encompasses both human and microbiota metabolic models (Virtual Metabolic Human) and takes into account environmental cues such as nutritional input (137). Furthermore, tools such as the Microbiome Modeling Toolbox (138), COMETS (139), or CarveMe (140), which are integrated in the COBRA toolbox (135), can be used to model the metabolic interchange between the microbes or between microbes and host. In addition, physiologically based pharmacokinetic (PBPK) models allow us to mathematically model drug kinetics across host body compartments (141). Successful examples of the integration of COBRA and PBPK models include a study of levodopa treatment on Parkinson's disease patients (142) and a computational simulation of isoniazid administration, an antibacterial used to treat *Mycobacterium tuberculosis* infections (143). However, models that integrate the role of microbes into the COBRA-PBPK models (144) are currently lacking. In the forthcoming years, computational models that integrate microbe and host metabolism in addition to external factors like drugs and nutrients will become increasingly available to us.

CONCLUSION

The work presented in this review highlights the importance of considering drug action from a microbiome perspective. Associations between drugs and microbial alterations are being increasingly reported in the scientific literature; however, direct proof of causality is often lacking, and our knowledge of the mechanisms involved is still very much in its infancy. Nevertheless, the adoption of new tools developed for the study of drug–microbiota interactions will undoubtedly lead to a more comprehensive understanding of the relationship between these complex ecosystems and will allow us to design novel, efficacious strategies for the treatment of disease.

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