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ADDENDUM

Targeting colorectal cancer-associated bacteria: A new area of research for personalized treatments

T. Faïs^{a,b,c}, J. Delmas^{a,b,c}, A. Cougnoux^{a,c}, G. Dalmaso^{a,c}, and R. Bonnet^{a,b,c}

^aMicrobes, Inflammation, Intestin et Susceptibilité de l'Hôte (M2iSH), Clermont Université, Université d'Auvergne, Inserm U1071, INRA USC2018, Clermont-Ferrand, France; ^bLaboratoire de Bactériologie, Center Hospitalier Universitaire, Clermont-Ferrand, France; ^cInstitut Universitaire de Technologie, Université d'Auvergne, Aubière, France

ABSTRACT

Most cases of colorectal cancer (CRC) are sporadic, and numerous studies have suggested that gut microbiota may play a crucial role in CRC development. *Escherichia coli* is a member of the gut microbiota frequently associated with colorectal tumors. CRC-associated *E. coli* strains frequently harbor the *pks* genomic island. This genomic island is responsible for the synthesis of colibactin genotoxin, which increases tumor numbers in CRC mouse models. We recently showed that targeting ClbP, a key enzyme involved in colibactin synthesis, blocks the deleterious effect of this toxin *in vitro* and leads to a significant decrease in tumor numbers *in vivo*. Altogether, our results suggest that the personalized treatment of CRC should also take into consideration the bacteria associated with the tumor in order to limit their deleterious effects.

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ClbP; colorectal cancer; colibactin; *E. coli*; microbiota; *pks*

Colorectal cancer (CRC) is the third most frequent cancer in the world, with an incidence of 1.2 million new cases and more than 600,000 deaths a year.¹ Inherited forms of the disease represent only 5 to 10% of cases while 1 to 2% occur in a context of inflammatory bowel diseases. Almost all cases (about 90%) of CRC are therefore sporadic and influenced by environmental factors such as diet or intestinal microbiota.^{2,3} Overall CRC survival rate at 5 years at any stage of the disease is 50%, but only 10% in metastatic stages. It is therefore essential to prevent, detect and treat this cancer efficiently before the occurrence of metastasis. Resecting surgery is usually the primary or first treatment. Chemotherapy and radiotherapy can also be proposed to patients, as an alternative to surgery if the latter is impossible, or in addition to surgery (which is frequently the case for stage III patients and stage II patients with high recurrence risk). In stage IV patients (when the cancer has spread to distant organs/tissues), surgery is unable to cure the disease and most patients receive chemotherapy.⁴

Chemotherapy is thus an important part of CRC treatment and numerous chemotherapy regimens are

currently available for patients.^{5,6} Recently, the therapeutic arsenal available for CRC has doubled with the emergence of targeted therapies.² Indeed, in addition to the 4 chemotherapy molecules classically used (5-fluorouracil, capecitabine, irinotecan and oxaliplatin), 5 targeted therapies have recently been FDA approved (bevacizumab, aflibercept, cetuximab, panitumumab, and regorafenib). These new treatments target specific biological functions or alterations found in cancer cells or pathways that are crucial for cancer development such as vascular endothelial growth factor (VEGF), a pro-angiogenic pathway, and epidermal growth factor receptor (EGFR). The combined use of these molecules with cytotoxic molecules has improved patient survival.^{7–10} However, it has been observed that the efficacy of the treatment depends on the type of mutations found in the tumor. For example, chemotherapy adjuvant treatment was not beneficial in patients with microsatellite instability/mismatch repair (MSI/MMR) and is therefore not recommended in the MSI/MMR CRCs.^{5,11} Anti-EGFR molecules should only be used in patients with RAS wild-type tumors. Thus,

CONTACT R. Bonnet  rbonnet@chu-clermontferrand.fr  M2iSH Inserm U1071/University of AUvergne, CBRV, 28 Place Henri Dunant, 63000 Clermont-Ferrand, France

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investigating *ras* mutation status has become an important aspect of diagnosis and pre-therapeutic evaluation and is an indispensable prerequisite in choosing the most suitable treatment.⁶⁻⁸ Many studies now take into account tumor pathophysiology in order to propose a “personalized medicine” approach, in which treatment is adapted to each patient. However, markers investigated in CRC are still mainly exclusion criteria. The goal today is to identify new biomarkers so that a therapeutic course can be tailored for each patient.

Although genetics plays an important role in cancers, increasing evidence supports the part played by infections in the occurrence of cancers.¹² They can induce tumorigenesis and/or sustain tumor growth and progression. Gut microbiota is highly suspected to play an important role in CRC and therefore has become in recent years a wide area of investigation. Studies comparing bacteria associated with tumor tissue and with tumor-adjacent mucosa of CRC patients, and bacteria associated with the mucosa of healthy patients have demonstrated the existence of a dysbiotic microbiota associated with CRC.¹³⁻¹⁷ Although it is still difficult to identify a “typical” dysbiotic microbiota associated with CRC, the abnormal abundance of certain species in colonic tissues of CRC patients has been found in numerous studies.^{13,15} Thus, studies of CRC have observed a specific enrichment in *Fusobacterium* and *Bacteroides*,^{13,15,17} bacteria that can potentially play a role in colorectal carcinogenesis.^{18,19} In contrast, microbiota from healthy patients seems to be enriched in *Bifidobacterium*,¹³ which are generally anti-inflammatory bacteria. Interestingly, early signs of dysbiosis have been reported at the adenoma stage,¹⁵ and there is a significant increase in some species (such as *Bacteroides*) from healthy to advanced adenoma and from advanced adenoma to carcinoma.¹³ However, these studies were not able to identify adenoma-associated bacterial communities predictive of cancer progression. This interesting point still remains a challenge for the future. In addition to the analysis of microbiota in terms of species, studies have been performed to investigate microbiota composition in terms of metabolic capacities. They revealed enrichments in some bacterial functions (xenobiotic metabolism, utilization of polyamines and degradation of polycyclic aromatic compounds) in meta-communities associated with CRC.¹⁵ These functions may lead to the production of pro-

tumorigenic metabolites, which are potentially important for cancer development. Thus, gut microbiota could be a new element to consider in the personalized treatment of CRC. The first step is to identify “harmful” bacterial genes so that they or their products can be targeted to limit their deleterious effects.

In this search for relevant members of the microbiota affecting the fate of CRC, it has been observed that human CRC biopsies are highly colonized by *E. coli*.²⁰ Interestingly, molecular analyses of these strains have revealed that they frequently harbor in their genome one or several pathogenic islands responsible for the production of toxins.^{21,22} These toxins can induce DNA damage and/or affect the cellular cycle. *E. coli* harboring cytotoxic necrotizing factor (Cnf) and cytolethal distending toxin (Cdt) are significantly associated with CRC biopsies.²¹ However, the toxin most frequently associated with *E. coli* colonizing CRC is colibactin.^{21,22} Colibactin is a genotoxic polyketide non-ribosomal peptide (PK-NRP) not yet purified and synthesized by the *pks* genomic island.²³ Colibactin-producing *E. coli* (*E. coli* clb+) increased the number of tumors in different CRC mouse models.^{22,24} *E. coli* clb+ induced DNA damage such as interstrand cross-links, which leads to double-strand breaks,²⁵ cell cycle arrest²³ and cellular senescence.^{24,26} Senescent cells produce a senescence-associated secretory phenotype (SASP) comprising cytokines, chemokines and growth factors in particular hepatocyte growth factor (HGF), a marker of poor prognosis in CRC that was involved in the growth of human tumor xenografts in nude mice transiently infected with colibactin-producing *E. coli*.²⁴ For all these reasons, targeting colibactin synthesis could be of interest in reducing the impact of *E. coli* clb+ on CRC development.

To date, the colibactin synthesis pathway has been only partially characterized. However, our laboratory has clearly shown the role of ClbP peptidase in colibactin maturation and activation.^{27,28} Structural studies of this periplasmic protein revealed an active serine site, which is accessible to inhibitors and makes ClbP a potential target.^{27,28} We therefore aimed at identifying small molecules able to bind and block the catalytic pocket of ClbP.²⁹

First, *in silico* docking experiments identified 2 boron-based compounds with computed ligand efficiency values consistent with results expected for medicinal chemistry leads. The crystalline structure of ClbP in complex with the compounds confirmed that

they bound the active site of ClbP, in the immediate vicinity of the active serine. The efficiency of these compounds was then assessed *in vitro* with an *E. coli* clb+ clinical strain isolated from a human CRC biopsy.²⁴ The compounds did not alter bacterial growth and had no cytotoxic activity against eukaryotic cells. Interestingly, they were able to block the genotoxic activity of the *E. coli* clb+ in a dose-dependent manner. This resulted in the abolition of *E. coli* clb+-induced cellular senescence and consequently of SASP-induced proliferation of uninfected cells. The efficiency of the compounds was then confirmed *ex vivo* and *in vivo*. In a murine colon loop model, compounds were able to block the genotoxic activity of colibactin. They were also able to inhibit tumor growth and HGF expression in a xenograft model. Finally, administration of the compounds in a CRC mouse model did not modify bacterial colonization of the tumors but did significantly reduce the number of macroscopic tumors. Remaining tumors harbored less DNA damage, less cellular senescence, and lower expression levels of cell proliferation markers and of HGF than tumors from mice which did not receive the compounds. These findings open up new avenues of research in targeted therapies against CRC. To date very few studies have proved that specifically targeting microbial protein might be useful in CCR. To our knowledge, only our study²⁹ and a study published by Wallace and colleagues have shown the feasibility of this approach.³⁰ Wallace and colleagues demonstrated *in vivo* that targeting bacterial β -glucuronidase abrogated the diarrhea induced by irinotecan (a secondary effect of chemotherapeutic drugs used in CRC).

In the light of these different levels of the involvement of bacteria in colorectal carcinogenesis, some authors have hypothesized that modulating the microbiota could be a lead for new targeted therapies and refer to this practice as “microbiome-targeted therapy” or “cancer bacteriotherapy.” Ideas on how to do so include antibiotics, modulation of the gut microbiota with a change of diet, probiotics, and fecal transplantation.³¹⁻³³ The ultimate goal is to decrease the amount of deleterious bacteria to the profit of beneficial bacteria. Indeed, it should be kept in mind that gut microbiota does not play a solely deleterious role in CCR but can be highly beneficial. For example, we can cite 2 elegant studies performed in mice showing that gut microbiota, and especially Gram positive bacteria (*Lactococcus*, *Enterococcus*, *Clostridium*), plays a

crucial role in the success of chemotherapeutic treatment by modulating the immune system.^{34,35} Thus, our work is evidence that another possible area of research is to specifically target the bacterial functions of particular species involved in CRC development.

In conclusion, CRC treatment is taking the turn of personalized medicine. The diversity of cancers and their varying responses to treatments are such that the aim is now to determine the most effective and suitable treatment for the patient. The stage of the cancer is the first element to consider. Molecular characterization of the tumor is also part of the pre-treatment medical evaluation. In the coming years, new markers currently undergoing trial will be validated. Although the roles of the microbiota are complex, still poorly characterized and require further investigation recent insights into the involvement of microbiota in CRC indicate that, in addition to investigating tumor cell genetics, it would be interesting to identify patients harboring a deleterious gut microbiota. Our preclinical data show that it is feasible to specifically target bacteria to prevent their pro-carcinogenic effects, and suggest that a bacteria-targeting treatment in CRC may be a useful adjuvant therapy in CRC treatment or its prevention.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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