Colonization of the Human Gut by E. coli and Colorectal Cancer Risk

Mathilde Bonnet, Emmanuel Buc, Pierre Sauvanet, Claude Darcha, Damien Dubois, Bruno Pereira, Pierre Déchelotte, Richard Bonnet, Denis Pezet, and Arlette Darfeuille-Michaud

Abstract

**Purpose:** The intestinal microbiota is potentially involved in the development of colorectal carcinoma via various mechanisms. *Escherichia coli* are commensal bacteria of the human gut microbiota, but some pathogenic strains have acquired the ability to induce chronic inflammation and/or produce toxins, such as cyclomodulin, which could participate in the carcinogenesis process. Here, we analyzed the *E. coli* population associated with mucosa of patients with colon cancer in relation to clinicopathologic characteristics. We assessed carcinogenic properties of a colon cancer–associated *E. coli* strain in multiple intestinal neoplasia (Min) mice.

**Experimental design:** Mucosa-associated or internalized *E. coli* were quantified and characterized from tumors and mucosa of patients with colon cancer and the healthy mucosa of diverticulosis controls. Min mice were inoculated with a colon cancer–associated *E. coli* strain (11G5). The number of colonic polyps was evaluated at 7 weeks after infection.

**Results:** An increased level of mucosa-associated and internalized *E. coli* was observed in the tumors compared with normal tissue. A relationship between poor prognostic factors for colon cancer (tumor–node–metastasis stage) and colonization of mucosa by *E. coli* was observed. Pathogenic cyclomodulin-positive *E. coli* strains were more prevalent on mucosa of patients with stages III/IV than those with stage I colon cancer. Proliferative index and *E. coli* colonization level of the mucosa distant from the tumor significantly correlated. Min mice infected with the *E. coli* strain 11G5 displayed a marked increase in the number of visible colonic polyps compared with controls.

**Conclusion:** These findings support that pathogenic *E. coli* could be a cofactor in pathogenesis of colorectal cancer. *Clin Cancer Res;* 20(4); 859–67. ©2013 AACR.

Introduction

Colorectal cancer is the third most commonly diagnosed cancer worldwide and is responsible for more than 600,000 deaths every year. Because of its high incidence and mortality rate, colorectal cancer is a major public health problem. Survival and risk of recurrence are conditioned by the extent of the tumor, stratified according to the prognostic classification of the American Joint Committee on Cancer (AJCC; ref. 1). When the tumor is confined to the colon (stages I and II), surgery is curative with a 5-year survival rate up to 80%; however, in the case of dissemination (nodal involvement, metastatic disease) the prognosis is dramatically reduced (2). Thus, the development of new diagnostic tools for the early detection of lesions is attractive.

Colorectal carcinogenesis was first defined as a classic adenoma–carcinoma sequence by Fearon and Vogelstein in 1990 and several risk factors were identified to favor this tumoral development (3). Among them, pathogens seem to play a major role. Indeed, approximately 20% of the global cancer burden can be linked to infectious agents (4, 5). Bacteria and their related products could participate in initiation or progression of sporadic colon cancer by driving a variety of mechanisms, including the induction of proinflammatory and procarcinogenic pathways in epithelial cells, the production of genotoxins and reactive oxygen species, and the conversion of procarcinogenic dietary factors into carcinogens (6–8). In colorectal cancer, various bacteria have been associated with carcinogenesis, including *Streptococcus bovis*, *Enterococcus spp.*, *Helicobacter pylori*, the enterotoxigenic *Bacteroides fragilis*, and various
pathogenic *Escherichia coli* (7–11). Despite the fact that *E. coli* is a commensal bacteria of the human microbiota and represents the most common cultivable, gram-negative, aero–anaerobic bacteria, various studies have demonstrated a clear link between mucosa-adherent *E. coli* and colorectal cancer (8, 10, 11). *E. coli* are divided into four phylogenetic groups (A, B1, B2, and D) according to the acquisition of factors of virulence. *E. coli* from phylogroups A and B1 are generally not pathogenic, whereas phylogroups B2 and D are involved in intestinal and extra-intestinal diseases. Interestingly, some strains of *E. coli* from phylogroup B2 are associated with Crohn disease, a chronic inflammatory bowel disease known to be a risk factor for colorectal cancer (12, 13). Moreover, two studies showed that mucosa-associated and mucosa-internalized *E. coli* were observed more often in patients with colorectal cancer than in controls, supporting the central role of these bacteria in the development of colorectal cancer (10, 11).

Pathogenic *E. coli* strains synthesize various virulence factors, including several toxins called cyclopeptides such as cytolethal distending toxins (CDT), cytotoxic necrotizing factor (CNF), cycle inhibiting factor, and colibactin (6). Cyclomodulins are genotoxic and/or modulate cell-cycle progression, proliferation, cell differentiation, and apoptosis (14–19). In particular, colibactin, a hybrid polyketide-nonribosomal peptide encoded by the *pks* genomic island, has genotoxic properties causing DNA double-strand breaks and chromosomal instability in human eukaryotic cells (18, 19). With regard to the cyclopeptin-producing *E. coli*, a recent study revealed an increased prevalence of cyclopeptin-producing B2 *E. coli* in colon tumor biopsies, suggesting a possible role of such pathogenic *E. coli* in colon carcinogenesis (20).

Translational Relevance

The gut microbiota and dysbiosis have recently been associated with colorectal cancer. We observed here a significant relationship between the colonization of mucosa by pathogenic *Escherichia coli* and poor prognostic factors for colorectal cancer, such as tumor–node–metastasis stage, suggesting that this colonization by *E. coli* could be an additional prognostic factor of poor outcome in patients. In addition, a strong association was observed between the proliferative index of epithelial cells and the colonization of the mucosa by *E. coli*, suggesting a role for these bacteria in regenerative epithelial cell growth and tumor progression. We demonstrated that infection with a pathogenic colon cancer–associated *E. coli* strain accelerates tumor development in multiple intestinal neoplasia mice, thereby providing evidence of the strain’s procarcinogenic properties. Our findings show that mucosa colonization by pathogenic *E. coli* could be a crucial factor in colorectal carcinogenesis and serve as a new prognostic marker.

The aim of the present study is to compare the bacterial colonization of the colonic mucosa in patients screened for colorectal cancer with noncolon cancer controls. Given that *E. coli* represent the majority of the cultivable bacteria in the colon and are positively selected for during chronic inflammation (21), we restricted our analysis to these bacteria. We evaluated two parameters of mucosa-associated and internalized *E. coli* in the normal mucosa and tumors of patients with colon cancer compared with the normal mucosa of patients receiving an operation due to diverticulosis, a nontumoral intestinal pathology. We analyzed the relationship between the levels of mucosa-associated *E. coli*, including genotypic characterization of the isolated strains (phylogroup and search for cyclopeptin-encoding genes), and clinicopathologic characteristics. Finally, we assessed the carcinogenic properties of an *E. coli* strain colonizing the tumor and the mucosa in a patient with colon cancer, using the multiple intestinal neoplasia (Min) mice animal model.

Materials and Methods

Patients and resection specimen

Patients with resectable colon cancer (*n* = 50) and uncomplicated diverticulosis (*n* = 33) were prospectively included in the Surgical Digestive Unit of Clermont-Ferrand (France) between March 2007 and July 2010. All patients were adult volunteers and signed informed consent. Patients with diverticulosis were operated on to prevent any inflammatory recurrence at least 10 weeks after an episode of diverticulitis. Exclusion criteria included neo-adjuvant chemotherapy, history of previous colonic resection, emergency surgery, and use of antibiotics within 4 weeks before surgery. The clinical data were collected from medical files and are summarized in Tables 1 and 2. The sex ratio (M/F) was 1.22 and 0.74 for patients with colon cancer and diverticulosis, respectively. The age range was 35 to 95 years for cancer patients (median age, 70 years) and 34 to 81 years for controls (median age, 58 years). Of note, the diverticulosis group comprised younger subjects than the colorectal cancer group (Table 1).

Surgery was performed either by laparotomy or laparoscopy. None of the patients received mechanical bowel preparation before the surgery. All patients received cefoxitin (2 g i.v.) at the time of incision. A specimen of macroscopically normal mucosa (absence of diverticules) was collected from the patients with diverticulosis and a non-necrotic fragment from the peripheral areas of the tumor was collected from the patients with colon cancer. In parallel, fresh normal mucosa samples adjacent to the tumor (10 cm from the tumor) were collected from the patients with colon cancer.

Pathologic analysis

After colonic resection, fresh specimens were transported to the pathology laboratory, fixed in buffered 4% formalin, embedded in paraffin, cut into 5-µm slices, and stained with hematoxylin/eosin/safranin. For the patients with colon cancer, tumors were staged according to the seventh
Table 1. Clinical data from patients with colon cancer and diverticulosis in the search for mucosa- or tumor-associated and internalized E. coli

<table>
<thead>
<tr>
<th>Presence of E. coli, n (%)</th>
<th>Patients with colon cancer</th>
<th>Patients with diverticulosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosa</td>
<td>Yes 45 (90)</td>
<td>29 (88)</td>
</tr>
<tr>
<td>No</td>
<td>5 (10)</td>
<td>4 (12)</td>
</tr>
<tr>
<td>Tumor</td>
<td>Yes 41 (93)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3 (7)</td>
<td></td>
</tr>
<tr>
<td>Presence of B2 phylotype E. coli strains</td>
<td>Yes 31 (65)</td>
<td>13 (39)</td>
</tr>
<tr>
<td>No</td>
<td>17 (35)</td>
<td>20 (61)</td>
</tr>
<tr>
<td>Presence of cyclomodulin-positive E. coli strains</td>
<td>Yes 26 (60)</td>
<td>6 (28)</td>
</tr>
<tr>
<td>No</td>
<td>17 (40)</td>
<td>17 (74)</td>
</tr>
</tbody>
</table>

Six are undetermined.

bP = 0.03.
cP = 0.04; in both the mucosa and tumors of patients with colon cancer versus diverticulosis.
dP = 0.01; in both the mucosa and tumors of patients with colon cancer versus diverticulosis.

eAICC/Union for International Cancer Control (UICC) tumor-node–metastasis classification (TNM stage; ref. 1). The acronym is indicative of the following criteria: (T) tumor size and nearby tissue invasion, (N) regional lymph node involvement, and (M) distant metastasis. The degree of tumor differentiation (poor, moderate, or high), perineural invasion, vascular emboli, the mucinous colloid component, and the presence of necrosis were also assessed. In patients with diverticulosis, the extent of mucosal inflammation (congestion of the mucosa or the submucosa, ulcerations, polymuclear infiltration, or abscesses) and presence of polyps or tumors were assessed.

Immunohistochemical staining of the human Ki-67 antigen was performed on the colonic mucosa sections from patients with colon cancer. Five-micrometer sections of paraffin-embedded, healthy mucosa were labeled with the anti-Ki-67 MB-1 antibody (Dako) and Ultra View Detection Kit (Ventana) on Benchmark XT stainer (Ventana). Sections were counterstained with Harris’s hematoxylin.

For each patient, the proliferation index was calculated as the percentage of Ki-67–positive epithelial cells in proportion to the total number of cells in a minimum of 10 entire crypt columns as previously reported (22). Ki-67 expression was then classified as a low (Ki-67–positive cells ≤10%) or high (Ki-67–positive cells >10%) proliferative index.

Microbiologic analyses

Specimens (tumor, normal mucosa) were immediately processed for the analysis of mucosa-associated and internalized E. coli colonization as previously described (20). The genotypic characteristics (phylogroup typing and presence of cyclomodulin-encoded genes) of these E. coli strains were assessed using PCR, and cyclomodulin expression was determined by assessing a specific cytopathic effect on cultured epithelial HeLa cells (20).

Electron microscopy

Human epithelial cell (T84) monolayers were infected with the colon cancer–associated E. coli strain 11G5 in vitro for 3 hours at a multiplicity of infection (MOI) of 30 bacteria per cell. The cells were then fixed in 1.6% glutaraldehyde for 1 hour, washed in cacodylate buffer (0.2 mol/L, pH 7.2), and postfixed in 1% OsO4 for 1 hour. After washing, the samples were dehydrated in graded alcohol and embedded in epoxy resin. Thin sections (60 nm) cut with a diamond knife in an ultramicrotome were stained with uranyl acetate and lead citrate and observed using a Hitachi H7650 TEM (Hitachi High Technologies Corporation). Sample preparations and observations were performed in the Centre Imagerie Cellulaire Santé (CICS) platform.

Animal model

Studies were performed using 5- to 6-week-old C57BL/6J-ApcMin/+ (Min mice; The Jackson Laboratory) or wild-type (WT) C57BL/6J female mice in accordance with the French and European Economic Community guidelines (86-60, EEC) for care of laboratory animals. Studies were approved by the French Regional Ethical Animal Use Committee (No. CE-2912). All mice were housed in specific pathogen-free conditions at the animal care facility of the Université d’Auvergne (Clermont-Ferrand, France). To enhance E. coli strain colonization, we administered streptomycin (2.5 g/L) for 3 days before inoculations of poreral bacteria (~1 × 108 bacteria in PBS) or PBS alone (controls).

Two E. coli strains were tested: the ampicillin- and kana-mycin-resistant E. coli strain 11G5 isolated from a patient with colon cancer as the colon cancer–associated strain and the commensal rifampicin-resistant E. coli strain K-12 MG1655 (laboratory stock) as the nonpathogenic control. We periodically quantified fecal bacterial colonization as colony-forming units (CFU) per stool as previously described (23). Fifty days after bacterial inoculation, the mice were sacrificed. The colons were then removed from the caecum to the rectum, flushed in PBS, and splayed longitudinally. Then, the tumors were counted. The colons were swiss-rolled from the distal to proximal end and fixed.
overnight in 10% formalin. Paraffin-embedded sections were cut into 5 μm sections, and the tissue sections were prepared for hematoxylin–phloxin staining and routine pathologic analysis.

Statistical analysis

The bacterial colony counts among multiple disease populations were compared using the Kruskal–Wallis non-parametric analysis followed by the Dunn posttest. The Fisher exact test or $\chi^2$ analysis was used for comparisons of the proportion of patient samples positive for E. coli across multiple disease populations. Unpaired Student $t$ tests and Mann–Whitney tests were used for comparisons of 2 groups where appropriate (normality verified by the Shapiro–Wilk test and homoscedasticity by the Fisher–Snedecor test). All tests were performed using Graph Pad Prism 5 STATA (StataCorp) or R software (http://cran.r-project.org/). We considered $P$ values of $<0.05$ as statistically significant.

Results

Patients and pathologic data

Fifty patients with adenocarcinoma (22 proximal and 28 distal samples) and 33 with diverticulosis were prospectively included. Most patients with colon cancer had tumors invading the peritoneal serosa (T3 stage, 60%) and negative lymph nodes (N0, 62%; Supplementary Table SA). Tumors were most often classified as moderate or well-differentiated (42% and 54%, respectively) adenocarcinoma as well as lacking perinervous encasement (91%), vascular embol (82%), mucinous component (84%), or intratumoral necrosis (64%; Supplementary Table SA). Acute ($n = 5$) or chronic ($n = 6$) inflammation was observed in 11 patients with diverticulosis. None of the patients with diverticulosis displayed signs of neoplastic transformation or adenomatous polyps.

Mucosa-associated and internalized E. coli in colon cancer and noncancerous tissues

Mucosa-associated E. coli were detected in 88%, 90%, and 93% of diverticulosis mucosa, colon cancer mucosa, and colon cancer tumor specimens, respectively (Table 1). A significant increase in mucosa-associated E. coli was observed in colon cancer tumors compared with colon cancer and diverticulosis mucosa ($P < 0.01$ and $P < 0.001$, respectively; Fig. 1A). Mucosa-internalized E. coli were detected in 48%, 54%, and 86% of diverticulosis, colon cancer mucosa, and colon cancer tumor specimens, respectively ($P < 0.0002$). In addition, the number of internalized E. coli was significantly increased in colon cancer tumors compared with colon cancer and diverticulosis mucosa ($P < 0.001$; Fig. 1B).

Colonization and clinical data

Colonization by associated and internalized E. coli was assessed according to the patients' clinical data (Supplementary Table SA). The sex and the age of the patients with diverticulosis and colon cancer did not influence the colonization by associated and internalized E. coli (data not shown).

Table 2. Characteristics of E. coli strains isolated from the colon cancer samples according to TNM status

<table>
<thead>
<tr>
<th>TNM status</th>
<th>I</th>
<th>II</th>
<th>III/IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients*, n</td>
<td>14</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Presence of E. coli, n (% of patients)</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (21%)$^a$</td>
<td>1 (7%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td></td>
<td>11 (79%)</td>
<td>14 (93%)</td>
<td>18 (95%)</td>
</tr>
<tr>
<td>Phylogroup, n (% of patients)</td>
<td>A</td>
<td>B1</td>
<td>B2</td>
</tr>
<tr>
<td></td>
<td>6 (43%)</td>
<td>5 (36%)</td>
<td>6 (43%)</td>
</tr>
<tr>
<td></td>
<td>2 (13%)</td>
<td>4 (27%)</td>
<td>12 (80%)$^b$</td>
</tr>
<tr>
<td></td>
<td>6 (32%)</td>
<td>4 (21%)</td>
<td>18 (95%)</td>
</tr>
<tr>
<td>Cyclomodulin-positive strain$^b$, n (% of patients)</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5 (45%)</td>
<td>6 (55%)</td>
<td>4 (36%)</td>
</tr>
<tr>
<td></td>
<td>9 (64%)$^c$</td>
<td>5 (36%)</td>
<td>8 (57%)$^d$</td>
</tr>
<tr>
<td></td>
<td>12 (67%)$^c$</td>
<td>6 (33%)</td>
<td>9 (50%)$^d$</td>
</tr>
<tr>
<td>pks-positive strain, n (% of patients)</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4 (36%)</td>
<td>7 (64%)</td>
<td>4 (36%)</td>
</tr>
<tr>
<td></td>
<td>8 (57%)$^d$</td>
<td>6 (43%)</td>
<td>9 (50%)</td>
</tr>
</tbody>
</table>

$^a$Undetermined for 2 patients (one patient with stage I tumor and other with stage III tumor).

$^b$The presence of genes coding for the cyclomodulins CNF, CDT, CIF, and colibactin (pks island) was analyzed.

$^c$P < 0.05.

$^d$P < 0.05.
Colonization was associated with the TNM stage and each parameter separately (T, N). Given the low number of patients with metastasis (n = 3), statistical analysis was not performed on this category. Colonization by associated *E. coli* was significantly increased in the normal mucosa distant from the tumor at advanced stages (stages III/IV) compared with stage I (P < 0.01; Fig. 2A). No significant variation in internalized bacteria was observed in relation to TNM stages.

Figure 1. Mucosa-associated and internalized *E. coli* are significantly increased in colon cancer tumors. Quantification of colon-associated (A) and internalized (B) *E. coli* in human colonic mucosa of patients with diverticulosis and colon cancer as well as the tumors of patients with colon cancer. *; P < 0.05.

Figure 2. Significant relationship between mucosal colonization by *E. coli* and TNM stage classification or its parameters T and N. A, mucosa-associated *E. coli* according to the TNM stage (from the TNM classification of AJCC/UICC). B and C, mucosa-associated *E. coli* according to T (B) or N status (C). D, mucosa-internalized *E. coli* according to T status. *; P < 0.05.
(data not shown). When each parameter of the TNM classification was considered, colonization by associated *E. coli* was significantly increased in nontumor mucosa of patients with locally advanced tumors (T3 and T4; *P* = 0.004) and lymphatic spreading (N+; *P* = 0.03; Fig. 2B and C). With regard to associated *E. coli*, the level of internalized bacteria was increased in nontumor mucosa of patients with advanced tumors (T3 and T4) compared with noninvasive tumor (T1 and T2; *P* = 0.045; Fig. 2D). In the tumor, no significant variation in the colonization by either associated or internalized *E. coli* was observed in relation to TNM classification or parameters. For other clinical data (differentiation status, perinervous encasement, vascular invasion, mucinous component, and intratumoral necrosis), no significant association was observed with associated or internalized *E. coli* colonization in either tumor or normal mucosa. In addition, we measured the percentage of the *E. coli* colonization in either tumor or nontumor mucosa of patients with colon cancer with a low (left) or high (right) proliferative index. B and C, mucosa-associated (B) and mucosa-internalized (C) *E. coli* according to the proliferative index.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Significant relationship between colonization of the mucosa by *E. coli* and the mucosal proliferative index. A, representative Ki-67 immunostainings from the normal colonic mucosa of patients with colon cancer with a low (left) or high (right) proliferative index. B and C, mucosa-associated (B) and mucosa-internalized (C) *E. coli* according to the proliferative index.

Colon cancer–associated *E. coli* 11G5 stimulates colon tumor development in Min mice

The *E. coli* strain 11G5, which exhibited high level colonization in the healthy mucosa and tumor tissue of a patient with an advanced stage tumor (T4) and liver metastasis, was selected as a representative colon cancer–associated *E. coli* strain. The strain belongs to phylogroup B2 and possesses the *pks* island encoding colibactin. Electron microscopy analysis of infected T84 intestinal cells indicated that 11G5 induces the elongation of eukaryotic cell membranes at bacterial adhesion sites. In addition, bacteria were internalized within endocytic vacuoles (Fig. 4A). Infection of Min mice with *E. coli* 11G5 resulted in high-level colonization of the gut as indicated by the number of bacteria in the feces (4.95 × 10⁷ CFUs per gram feces) 2 days after infection (Fig. 4B). Similar levels of colonization were measured in the stools of WT and Min mice (*P* = 0.79). In contrast, significantly reduced bacterial levels (1.69 × 10⁷ CFUs per gram feces) were detected in stools of mice infected with *E. coli* K-12 (*P* < 0.001; Fig. 4C). At the time of sacrifice (50 days after infection), colonic polyps were detected in 92% of Min mice infected with *E. coli* 11G5, 57% of Min mice with K-12 *E. coli*, and 50% of uninfected Min mice (*P* < 0.05). The histologic analysis indicated that all polyps were adenocarcinomas (Fig. 4D) and no sign of obvious inflammation in the 11G5-colonized mice was
detected. Infection experiments with strain 11G5 in WT mice did not lead to any neoplastic changes (data not shown). Markedly significant increases in the number and in the size of tumors were observed in the 11G5-colonized Min mice (Fig. 4E and F).

Discussion

Bacterial and viral infections have long been established as important factors in the etiology of several human cancers (4, 5). More recently, the gut microbiota and dysbiosis have been associated with colon cancers. This article presents evidence for the presence of pathogenic *E. coli* strains in the mucosa and tumors of patients with colon cancer that could play a role in colorectal carcinogenesis. We observed that mucosa-adherent *E. coli* were not restricted to the tumor site of carcinoma as previously reported (8, 10, 24). We demonstrated that colon cancer tumors have increased numbers of associated *E. coli* than colon cancer and diverticulosis mucosa. It could be argued that local modifications of the mucosal properties induced by the tumor, such as loss of the mucosal barrier and epithelial cell polarity as well as expression of abnormal surface antigens in patients with cancer, promote increased adhesion and internalization of bacteria in the tumor tissue. Although colon cancer–specific surface antigens remain uncharacterized, we can hypothesize that the carcino-embryonic antigen cell adhesion-related molecule 6 (CEACAM6), which is upregulated in patients with colorectal cancer (25), could be involved. Indeed, we have...
previously reported, in Crohn disease that type I pili are among the bacterial virulence factors potentially implicated in increased E. coli adhesion based on binding to CEA-CAM6, which is abnormally expressed in mucosa of these patients (26). Despite noting no significant variation in the total number of E. coli between the normal mucosa of patients with colon cancer and diverticulosis, we observed changes in the E. coli populations. We previously showed that pathogenic cyclomodulin-positive E. coli strains belonging to B2 phylogroup were significantly more prevalent in patients with colon cancer tumors than in the mucosal samples of diverticulosis (20). We demonstrated here that pathogenic E. coli strains were also detected on normal mucosa of patients with colon cancer. Our findings are consistent with results by Arthur and colleagues in a smaller cohort of patients with colon cancer (8). This finding prompted us to explore the effect of colon cancer–associated E. coli infection in the Min mice model for which the microbiota has been reported to play a role in carcinogenesis (27). The representative pathogenic pks-positive E. coli strain (11G5) significantly induced tumorigenicity with an increase in the size and number of adenocarcinoma tumor. The genotoxic effects of colibactin requires bacteria–host cell contact (18, 19), and it has been reported that mRNA expression of the pks island occurs in the bacteria in closest contact with the epithelial cells (28). We observed similar levels of colonization with E. coli 11G5 in Min and WT mice, indicating that tumor development induced by infection with pks-positive bacteria requires a first hit, such as an apc mutation which is consistent with results previously observed in the interleukin (IL)-10(−/−) murine model (8, 29, 30). In parallel to these results observed in mice, a strong association between the levels of E. coli colonization in human normal mucosa and the proliferative index (Ki-67 immunostaining) was noted, suggesting that these bacteria play a role in regenerative epithelial cell growth and tumor progression which is previously demonstrated in murine models (24, 27). We also observed a relationship between the levels of mucosa-associated E. coli colonization and the tumor cell dissemination process (axillary lymph node invasion and invasive tumor status) and a higher prevalence of the pathogenic strains in aggressive form of cancer. Given that abnormal bacterial colonization was previously reported on the mucosa of patients with adenoma (10, 24), all these data on human mucosa suggest that pathogenic E. coli could promote the development of an aggressive form of carcinoma at the early steps of enterocyte transformation by cyclomodulin genotoxic agent effect, such as colibactin, and/or the induction of chronic inflammatory reactions.

We report, for the first time, a statistically significant relationship between high levels of mucosa-associated E. coli and poor colorectal carcinoma prognostic factors, such as TNM stages. The TNM staging system is still the most widely used in patient selection for adjuvant chemotherapy after curative resection. At present, it is not possible to accurately differentiate between patients with a favorable or poor prognosis in stages II and III colon cancer (31). Various clinical factors have been associated with a higher risk of recurrence in stage II and III colon cancer, including tumor obstruction and/or perforation, the number of lymph nodes identified in the surgical specimen, T4 stage, lymphovascular invasion, undifferentiated histology, and lymph node micrometastasis (32–36). None of these markers are able to clearly separate patients into distinct prognostic groups. Although other microbes likely participate in the progression of colon cancer, our findings demonstrate that mucosal colonization by pathogenic E. coli could serve as a new and crucial prognostic factor of poor outcome. Large multicenter prospective clinical studies are needed to evaluate whether pathogenic E. coli colonization in the mucosa is a prognostic marker.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: M. Bonnet, E. Buc, R. Bonnet, A. Darfeuille-Michaud
Development of methodology: M. Bonnet, P. Sauvanet, P. Déchelotte, R. Bonnet
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Bonnet, E. Buc, P. Sauvanet, D. Dubois, P. Déchelotte, D. Pezet
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Bonnet, E. Buc, P. Sauvanet, A. Darfeuille-Michaud
Writing, review, and/or revision of the manuscript: M. Bonnet, E. Buc, P. Sauvanet, B. Pereira, R. Bonnet, A. Darfeuille-Michaud
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Bonnet, E. Buc, P. Sauvanet, D. Pezet
Study supervision: D. Pezet, A. Darfeuille-Michaud
Histologic analysis: C. Darcha

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