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

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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 Comm-
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 and colorectal cancer (8, 10, 11). *E. coli* are divided into four phylogenic 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 cycломodulins such as cytotoxic distending toxins (CDT), cytotoxic necrotizing factor (CNF), cycle inhibiting factor, and colibactin (6). Cycломodulins 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 pls genomic island, has genotoxic properties causing DNA double-strand breaks and chromosomal instability in human eukaryotic cells (18, 19). With regard to the cycломodulin-producing *E. coli*, a recent study revealed an increased prevalence of cycломodulin-producing B2 *E. coli* in colon tumor biopsies, suggesting a possible role of such pathogenic *E. coli* in colon carcinogenesis (20).

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 cyclomodulin-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 (noncancer controls; *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 mucosa adjacent to the tumor and the mucosa in a patient with colon cancer, using the multiple intestinal neoplasia (Min) mice animal model.

**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...
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-Apc<sup>Min+/−</sup> (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 peroral bacteria (∼1 × 10<sup>8</sup> bacteria in PBS) or PBS alone (controls).

Two *E. coli* strains were tested: the ampicillin- and kanamycin-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.

### 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>Patients with colon cancer</th>
<th>Patients with diverticulosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>70.5 ± 11.4</td>
<td>58.0 ± 11.9&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td></td>
</tr>
<tr>
<td>28/22</td>
<td>13/20</td>
</tr>
<tr>
<td>Presence of colon-associated <em>E. coli</em>, n (%)</td>
<td></td>
</tr>
<tr>
<td>Mucosa</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>45 (90)</td>
</tr>
<tr>
<td>No</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Tumor&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>41 (90)</td>
</tr>
<tr>
<td>No</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Presence of colon-associated <em>E. coli</em>, n (%)</td>
<td></td>
</tr>
<tr>
<td>Mucosa</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>27 (54)</td>
</tr>
<tr>
<td>No</td>
<td>23 (46)</td>
</tr>
<tr>
<td>Tumor&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>38 (86)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>No</td>
<td>6 (14)</td>
</tr>
<tr>
<td>Presence of B2 phylotype <em>E. coli</em> strains</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>31 (65)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>No</td>
<td>17 (35)</td>
</tr>
<tr>
<td>Presence of cyclomodulin-positive <em>E. coli</em> strains</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26 (60)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>No</td>
<td>17 (40)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Six are undetermined.

<sup>b</sup>*P* = 0.03.

<sup>c</sup>*P* < 0.0002; tumor versus mucosa of patients with colon cancer or diverticulosis.

<sup>d</sup>*P* = 0.04; in both the mucosa and tumors of patients with colon cancer versus diverticulosis.

<sup>e</sup>*P* = 0.01; in both the mucosa and tumors of patients with colon cancer versus diverticulosis.

AICC/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.
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 embols (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>3 (21%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Yes</td>
<td>11 (79%)</td>
<td>14 (93%)</td>
<td>18 (95%)</td>
</tr>
<tr>
<td>Phylgroup, n (% of patients)</td>
<td>A</td>
<td>6 (43%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>B1</td>
<td>5 (36%)</td>
<td>4 (27%)</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>B2</td>
<td>6 (43%)</td>
<td>12 (60%)</td>
<td>13 (68%)</td>
</tr>
<tr>
<td>D</td>
<td>5 (36%)</td>
<td>4 (27%)</td>
<td>5 (26%)</td>
</tr>
<tr>
<td>Cyclomodulin-positive strain(b), n (% of patients)</td>
<td>+</td>
<td>5 (45%)</td>
<td>9 (64%)</td>
</tr>
<tr>
<td>–</td>
<td>6 (55%)</td>
<td>5 (36%)</td>
<td>6 (33%)</td>
</tr>
<tr>
<td>pks-positive strain, n (% of patients)</td>
<td>+</td>
<td>4 (36%)</td>
<td>8 (57%)</td>
</tr>
<tr>
<td>–</td>
<td>7 (64%)</td>
<td>6 (43%)</td>
<td>9 (50%)</td>
</tr>
</tbody>
</table>

*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.
$^cP < 0.05$.
$^dP < 0.05$.
$^eP < 0.05$.
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.
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(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 bacteria colonization in either tumor or normal mucosa. In addition, we measured the percentage of the proliferative index. The proliferative index of the mucosa did not correlate with the TNM characteristics of the corresponding tumors (Supplementary Fig. SA). However, the proliferative index significantly correlated with the levels of both mucosa-associated and internalized *E. coli* (*P* < 0.02 and *P* < 0.04, respectively; Fig. 3).

We then extended the study by searching for a correlation between the presence and characteristics of the *E. coli* strains with regards to TNM classification. Of note, the frozen subcultures of mucosa-associated bacteria from two patients, one with TNM stage I and the other with stage III, were not viable. No *E. coli* was isolated in 21%, 7%, and 5% of patients with stage I (3 of 14), II (1 of 15), and III/IV (1 of 18) tumors, respectively (Table 2). In most cases, similar clones of *E. coli* strains were found in the normal mucosa and tumor tissue of patients with colon cancer and most strains were from the B2 phylogroup. Interestingly, the proportion of mucosa and tumor colonization by B2 phylogroup *E. coli* was significantly increased in patients with stage II (80%) and III/IV disease (68%) compared with stage I (43%; *P* < 0.05; cf. Table 2). TNM stage was not associated with other phylogroups. As previously reported for tumor tissue (20), *E. coli* strains harboring cyclomodulin-encoding genes (cdt, cif, and/or cnf) and/or the *pks* island were significantly more frequently observed in normal mucosa of patients with colon cancer (26/43) than diverticulosis (6 of 23; 60% vs. 26%, respectively; *P* = 0.01). Of great interest, the proportion of mucosa and tumor colonization by cyclomodulin-positive *E. coli* strains was significantly higher in TNM stage II (64%) and III/IV (67%) patients than stage I patients (45%; *P* < 0.05; cf. Table 2). The presence of B2 phylogroup or cyclomodulin-positive *E. coli* did not influence the overall level of *E. coli* colonization in the mucosa or tumors (Supplementary Figs. SB and SC).

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 *E. coli* 11G5 stimulates 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 × 10^7 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 × 10^7 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

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.
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 CEACAM6, 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* occurs in increased in mice, a strong association between the levels of *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 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, B. Pereira, 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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References
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Colonization of the Human Gut by *E. coli* and Colorectal Cancer Risk

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