ESCHERICHIA COLI DIFFERS IN VIRULENCE-ASSOCIATED GENES BETWEEN PATIENTS WITH COLORECTAL NEOPLASIA AND HEALTHY CONTROLS

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Background

Escherichia coli is a commensal bacterium of the human gastrointestinal tract but, at the same time, one of the most important human pathogens. Pathogenic *E. coli* emerged from nonpathogenic strains by acquisition of virulence factors. While pathogenic *E. coli* have been identified as the causative agents of urogenital and diarrheal infections, the role of *E. coli* in other conditions, such as inflammatory bowel diseases (IBDs) and colorectal cancer, remains unclear. Besides the host genetic and environmental factors, several bacteria have been involved in the pathogenesis of different neoplastic conditions, including *E. coli*, which has been abundant among these patients. Reflecting this situation, *E. coli* strains with specific sets of virulence factors (e.g., adherent invasive *E. coli*, colibactin-producing *E. coli*) are considered pathobionts rather than bacteria causing acute infection.

The aim of this study was to characterize mucosal *E. coli* isolates for the presence of genetic determinants encoding known virulence factors.

Study design

81 participants:

- healthy volunteers (HC; n = 20)
- non advanced colorectal adenoma (nCRA; n = 20)
- advanced colorectal adenoma (nCRA; n = 20)
- colorectal carcinoma (CRC; n=21)
  - bioptic samples from 3 different intestinal regions – caecum, traversum, and colon

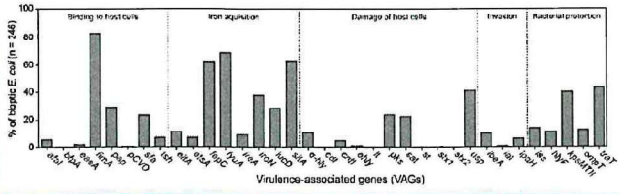
246 *E. coli* isolates from:

- healthy volunteers (HC; n = 46)
- non advanced colorectal adenoma (nCRA; n = 71)
- advanced colorectal adenoma (nCRA; n = 65)
- colorectal carcinoma (CRC; n = 64)
  - unique *E. coli* isolates (i.e., duplicate isolates per participant have been excluded)

PCR detection:

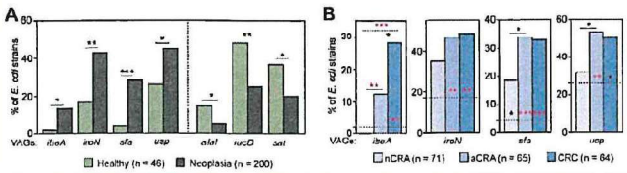
- thirty-five virulence-associated genes (VAGs; i.e., *α-hly, afaI, bfpA, cdt, cnf1, eaeA, ehly, eitA, etsA, fepC, fimA, fyuA, hlyF, iaI, ibeA, ipaH, ireA, iroN, iss, iucD, kpsMTII, it, ompT, pap, pCVD432, pks, sfa, sat, sitA, st, stx$_1$, stx$_2$, traT, tsh,* and *usp*)

1. Prevalence of VAGs among mucosal *E. coli* isolates



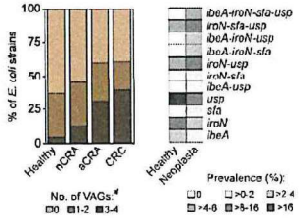
We analyzed 246 *E. coli* isolates from biopsies of patients (n = 200) and healthy controls (n = 46) for prevalence of 35 VAGs. VAGs associated with diarrheal *E. coli* pathotypes (i.e., *bfpA, ial, pCVD432, it, st, stx1,* and *stx2*) were rarely found in the set of mucosal *E. coli*. At the same time, genes encoding fimbriae type 1 and three iron acquisition systems have been found in more than half of mucosal isolates (*fimA* 82.5%, *fyuA* 68.7%, *sitA* 63.0%, and *fepC* 62.2%). The neoplasia group consisted of isolates from non-advanced colorectal adenoma (nCRA; n = 71), advanced colorectal adenoma (aCRA; n = 65), and colorectal carcinoma (CRC; n = 64).

2. *E. coli* from neoplasia patients differ in prevalence of VAGs



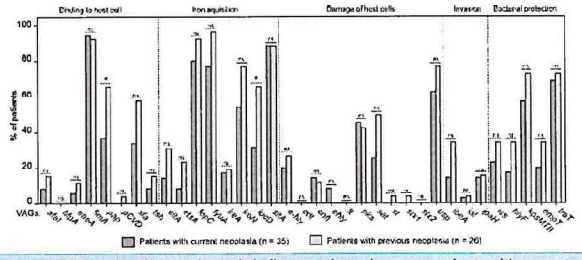
Among *E. coli* from patients, the prevalence of four VAGs was higher (left) and the prevalence of three others was lower (right) compared to isolates from healthy controls (**A**). Four VAGs with increased prevalence were mostly associated with *E. coli* from patients with advanced adenoma or carcinoma (**B**). The two-tailed *Fisher's exact* test was used for calculation of statistical significance between healthy controls and groups of patients (*p < 0.05, **p < 0.01, and ***p < 0.001). In panel B, the dotted lines represent the prevalence in *E. coli* of healthy controls, and the related statistical significance to neoplasia stages is shown by asterisks nearby. Statistical significance after correction for false discovery rate (p_{adj} < 0.0125) is shown in pink. *E. coli* from patients with non-advanced colorectal adenoma (nCRA), advanced colorectal adenoma (aCRA), and colorectal carcinoma (CRC) are subsets of neoplasia isolates.

3. Certain combinations of VAGs are associated with neoplasia



Certain combinations of neoplasia-associated genes (i.e., *ibeA*, *iroN*, *sfa*, and *usp*) occurred frequently in *E. coli* from patients. Combination *iroN-sfa-usp* has been the most frequent combination in *E. coli* from patients with advanced neoplasia (21.5% in aCRA and 12.5% in CRC). Moreover, combination of all four identified VAGs has been found exclusively in *E. coli* from patients with advanced neoplasia (9.2 and 10.9% in aCRA and CRC).

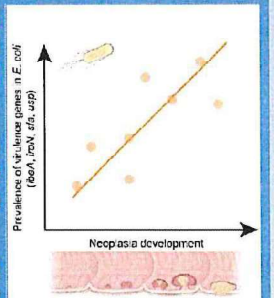
4. Prevalence of VAGs is similar between patients with current neoplasia and patients with neoplasia history



The most of the analyzed VAGs showed similar prevalence between patients with current and with previous neoplasia. Since treatment of colorectal neoplasia did not result in a significant decrease in their occurrence, the presence of specific *E. coli* strains in the intestinal microflora appears to be a cause rather than a consequence of colorectal neoplasia. The two-tailed *Fisher's exact* test was used for calculation of statistical significance between healthy controls and patients (*p < 0.05, ns – not significant). Prevalence of VAGs was analyzed on level of neoplasia patients (nCRA (n = 20), aCRA (n = 20), and CRC (n = 21)); 35 have neoplasia and 26 overcame neoplasia in the past.

Summary

- This study found a positive association between four virulence associated genes (VAGs) of *E. coli* and colorectal neoplasia.
- These VAGs were predominantly found among *E. coli* from patients with advanced adenoma or carcinoma.
- Presence of certain *E. coli* in the gut appears to be a cause rather than a consequence of colorectal neoplasia.



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