ROSA LILIANA SOLIS CASTRO

DETERMINAÇÃO DOS PRINCIPAIS PATÓTIPOS DE *Escherichia coli* ISOLADAS DE PACIENTES COM CÂNCER DE RETO

Dissertação apresentada ao Programa de Pós-Graduação em Microbiologia do Instituto de Ciências Biomédicas da Universidade de São Paulo, para obtenção do Título de Mestre em Ciências

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RESUMO

CASTRO, R. L. S. **Determinação dos principais patótipos de *Escherichia coli* isoladas de pacientes com câncer de reto.** 2017. 106 f. Dissertação (Mestrado em Microbiologia) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2017.

No Brasil, os cânceres de cólon e de reto são considerados as neoplasias gastrintestinais mais comumente observadas na população. Nos últimos anos vêm se relatando na literatura nacional e internacional a possível relação da presença de microrganismos com o desenvolvimento de câncer; entretanto, ainda não se observam evidências científicas convincentes dessa interação. Este estudo teve como objetivo determinar a presença e participação dos diferentes patótipos de *Escherichia coli* em pacientes com e sem câncer de reto. Foram coletadas amostras fecais de pacientes com neoplasia de reto, e de indivíduos sadios sem sinais de câncer (pólipos e/ou tumor), usadas como controle. Uma porção fecal foi semeada em ágar MacConkey isolando-se aletaoriamente quatro colônias de cada amostra. A identificação em nível de espécie, e dos patótipos, assim como dos fatores de virulência das cepas extra-intestinais de *E. coli* foi realizada por PCR convencional. Para a caracterização molecular das *E. coli* foi usada a técnica de ERIC-PCR. Os pacientes com neoplasia de reto apresentaram idade média de 63 anos de idade ($P < 0,001$). Nos pacientes com câncer de reto, a ocorrência de *E. coli* extra-intestinal (ExPEC; 53 cepas, 44,1%) foi menor do que as cepas de *E. coli* diarreiogênicas (DEC; 59 cepas, 49,2%). A presença de *E. coli* enteroagregativa típica (tEAEC) foi observada em 44,1% das cepas DEC (59 cepas) isoladas de amostras fecais de pacientes com câncer de reto e em 12,9% das cepas DEC isoladas dos indivíduos sadios (31 cepas) ($P = 0,003$); entretanto, as *E. coli* enteropatogênicas atípicas (aEPEC) foram isoladas em ambos os grupos de pacientes (câncer: 22 cepas, 37,3%; sadios: 15 cepas, 48,4%). O gene *afa/dra* da adesina Afa/Dr foi observado em maior prevalência nas ExPEC (35 cepas, 66,0%) isoladas de pacientes com câncer de reto ($P < 0,001$). As cepas de *E. coli* mostraram combinações gênicas que variaram de 2 a 8 genes, observando-se 39 e 24 combinações gênicas nas cepas de pacientes com câncer de reto e indivíduos sadios, respectivamente. Pelo ERIC-PCR observou-se elevada diversidade genética em todas as cepas, e clusters de cepas clone foram observadas em cepas isoladas do mesmo paciente. Foi observada a presença dos oito filogrupos de *E. coli,* sendo o filogrupo B2 (55,2%) o mais predominante. Os filogrupos D e E não agruparam cepas de indivíduos sadios. Os resultados sugerem maiores estudos para determinar o papel das DEC, particularmente das aEPEC, tEAEC e ExPEC de forma individual ou em associação, avaliando-se o provável sinergismo e/ou a co-infecção de diferentes patótipos nesses processos, assim como sua presença no trato intestinal em pacientes assintomáticos com câncer de reto.

Palavras-chave: Câncer de reto. *Escherichia coli*. Patótipos. Fatores de virulência Filogrupos. ERIC-PCR.

ABSTRACT

CASTRO, R. L. S. **Determination of the main pathotypes of *Escherichia coli* in patients with rectal cancer.** 2017. 106 p. Master thesis (Microbiology) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2017.

In Brazil, colon and rectal cancer are increasing and they are considered the gastrointestinal neoplasia most commonly observed in the population. In recent years, national and international literatures have shown a possible correlationship among the presence of microorganisms with the development of cancer; however, no convincing scientific evidence of this interaction has been observed. This study aimed to determine the presence and participation of different pathotypes of *Escherichia coli* isolated from patients with or without rectal cancer. Fecal samples were collected from patients with rectal cancer and healthy individuals with no signs of cancer (polyps and/or tumor) used as a control. Feces were plated onto agar MacConkey and four strains were randomly selected from each sample. Conventional PCR was used for identification of *E. coli* and pathotypes, as well as to detect virulence genes in extra-intestinal strains. The molecular characterization of *E. coli* was performed by ERIC-PCR. Patients with rectal cancer were mean age of 63 years old ($P < 0.001$). Diarrheogenic *E. coli* (DEC, 59 strains, 49.17%) were more prevalent than extra-intestinal *E. coli* (ExPEC, 53 strains, 44.17%). The presence of tEAEC was observed in 44.1% of the DEC strains (59 strains) isolated from patients with rectal cancer and in 12.9% of the DEC strains isolated from the healthy patients (31 strains) ($P = 0.003$). Atypical enteropathogenic *E. coli* (aEPEC) strains were isolated in both patient groups (cancer: 22 strains, 37.3%; healthy: 15 strains, 48.4%). The gene *afa/dra* for adhesin Afa/Dr was observed in higher prevalence in the ExPEC strains (35 strains, 66.0%) isolated from patients with rectal cancer ($P < 0.001$). *E. coli* strains showed genetic combinations from 2 to 8 genes, showed 39 and 24 genetic combinations in strains from cancer and healthy patients, respectively. All strains showed high genetic diversity by ERIC-PCR, and some clusters grouped clone strains isolated from the same patient. It was observed presence of eight filogroups and B2 filogroup (55.2%) was the most prevalent. Filogroups D and E were absent in strains from healthy. The results suggest further studies to determine the role of DEC, particularly aEPEC, tEAEC, and ExPEC, individually or in combination, and the synergism and co-infection of different pathotypes in these processes, as well as its presence in the intestinal microbiota in asymptomatic patients with rectal cancer.

Keywords: Rectal cancer. *Escherichia coli*. Pathotypes. Virulence factors. Phylogroups. ERIC-PCR.

INTRODUÇÃO

Bactérias pertencentes à microbiota residente intestinal humana participam de diversos processos infecciosos, cujo papel tem despertado o interesse de microbiologistas e clínicos visando-se obter melhor conhecimento da etiologia e desenvolvimento dos diferentes processos em que participam, incluindo os vários tipos de câncer que acometem o ser humano.

O câncer intestinal mais conhecido é o colorretal (CCR). Esta patologia é observada em maior frequência em populações de países ocidentais. O CCR afeta a população de países desenvolvidos e em desenvolvimento, aparecendo aproximadamente um milhão de casos por ano, distribuídos em 550.000 homens e 470.000 mulheres. A incidência é variada em diferentes partes do mundo, devido às variações genéticas, populacionais, hábitos dietéticos e ambientais (ARVELO; SOJO; COTTE, 2015).

A principal característica no CCR é o aparecimento dos adenomas ou pólipos, que podem ser malignos. Aproximadamente 70% das mutações precoces se originam no gene *adenomatous polyposis coli*(*apc*) (BRENNER; KLOOR; POX, 2014). Entretanto, para a sua progressão é necessário que ocorra uma série de mutações adicionais no gene *Tumor protein 53* supressor de tumor (*tp53*) e do oncogene *Kirstein rat sarcoma*(*k-ras)* (KHEIRELSEID; MILLER; JERIN, 2013).

No Brasil, o CCR é o quarto tumor maligno mais frequente em homens (12.490 novos casos por ano) e o terceiro em mulheres (14.500 novos casos por ano), sendo observado entre 50 a 70 anos de idade; entretanto, esse risco aumenta a partir dos 40 anos (BEDANI; ROSSI, 2009). Em 2015, o número de casos de câncer de cólon e/ou reto no Brasil alcançou a marca aproximada de 33 mil, com um risco estimado de 17 novos casos a cada 100 mil homens e a cada 100 mil mulheres (FACINA, 2014). Para o ano 2016, foi de 16.660 homens e de 17.620 mulheres (INSTITUTO NACIONAL DO CÂNCER, INCA, 2016).

Dentre os cânceres intestinais, o câncer de reto (CR) constitui-se o segundo (28%) tipo mais frequentemente observado no intestino grosso, depois do câncer proximal (42%) (LI; LAI, 2009; SIEGEL; DESANTIS; JEMAL, 2014). Estudos epidemiológicos consideram esses tipos de câncer como uma única entidade clínica, sendo denominado de câncer colorretal (FAZELI; KERAMATI, 2015; Li; LAI, 2009).

Alguns fatores abióticos estão relacionados ao desenvolvimento do câncer de cólon e/o reto. Entre eles podem ser mencionados as características genéticas, idade, hábitos alimentares, obesidade, consumo de álcool e fumo, sedentarismo e inflamação crônica do trato intestinal (ARVELO; SOJO; COTTE, 2015; COMPARE; NARDONE, 2014; LABIANCA et al., 2010).

Dos fatores que estariam relacionados ao desenvolvimento do carcinoma intestinal podemos mencionar também a microbiota. A microbiota residente intestinal desempenha função importante na homeostase e equilíbrio desse ecossistema. Entretanto, em casos de desequilíbrio ou disbiose da microbiota, podem ser observadas mudanças metabólicas microbianas. (AHN et al., 2013; ARTHUR et al., 2012; KEKU et al., 2015; NEISCH, 2009). Estudos mais recentes têm mostrado a participação de alguns microrganismos denominados de patobiontes, onde sua proliferação no ecossistema intestinal produziria a diminuição da função protetora da microbiota residente (WU et al., 2013, ZACKULAR et al., 2015). Dentre esses microrganismos patobionticos são destacadas as espécies toxigênicas de *Bacteroides fragilis* e *Clostridium perfringens* (LEUNG; TSOI; YU, 2014; TOPRAK et al., 2006).

Outro grupo bacteriano intestinal com enorme participação na homeostase intestinal, éconstituído pela *Escherichia coli* e seus diferentes patótipos, que podem se apresentar como agentes etiológicos em vários processos infecciosos, extra e intra-intestinais; essas bactérias têm sido também relacionadas ao câncer colorretal (ARTHUR et al., 2014; BUC et al., 2013; SWIDSINSKI et al., 1998). Estudos fenotípicos, genotípicos e epidemiológicos têm mostrado que cepas de *E. coli* são classificadas em diferentes patótipos, devido à presença de genes que codificam a produção de fatores de virulência, sendo agrupadas em *E. coli* diarreogênicas (DEC) e *E. coli* extra-intestinais (ExPEC). Cepas com a ausência desses genes são consideradas não patogênicas ou comensais (KAPER; NATARO; MOBLEY, 2004).

Também, mediante o uso da técnica de Multilocus Sequence Typing (MLST), as *E. coli* são divididas em quatro grupos filogenéticos (A, B1, B2 e D). Membros pertencentes aos filogrupos A e B1 são, geralmente, não patogênicos; enquanto que, os filogrupos B2 e D estão envolvidos em infecções extra e intra-intestinais, incluindo a doença de Crohn e a doença intestinal inflamatória crônica, considerada como principal fator de risco para o câncer colorretal (BOUDEAU et al., 1999; CLERMONT; BONACORSI; BINGEN, 2000).

Em nível mundial, as cepas DEC são consideradas importantes na diarreia endêmica e epidêmica, sendo classificadas em seis patótipos: *E. coli* enteropatogênica (EPEC), *E. coli* enterotoxigênica (ETEC), *E. coli* enteroagregativa (EAEC), *E. coli* enteroinvasiva (EIEC), *E. coli* com aderência difusa (ADEC) e *E. coli* enterohemorrágica (EHEC) (NATARO; KAPER, 1998). Darfeuille-Michaud et al (1998) relataram que a cepa *E. coli* aderente invasiva (AIEC) está fortemente associada às doenças inflamatórias intestinais, como a doença de Crohn; e mais recentemente foi informado que essa cepa esta também relacionada com o CCR (ARTHUR et al., 2012; RAISCH et al., 2014). Estudos relacionando os diferentes patótipos de *E. coli* com o câncer de cólon e/o reto ainda são escassos na literatura.

Nas últimas décadas, com o desenvolvimento de técnicas moleculares para a detecção, identificação e caracterização de bactérias patogênicas, o diagnóstico de doenças infecciosas tem se tornado mais rápido e eficiente. O aprimoramento da reação em cadeia da polimerase (PCR) tem permitido classificar de forma mais eficiente os grupos microbianos com elevada similaridade genética. O método *Enterobacterial Repetitive Intergenic Consensus-PCR* (ERIC-PCR) tem sido muito utilizado na determinação de sequências nucleotídicas pequenas, repetidas e dispersas no genoma bacteriano, e na análise filogenética de enterobactérias (ATEBA; MBEWE, 2014; VERSALOVIC; KOEUTH; LUPSKI, 1991).

Tendo em vista a diversidade bacteriana encontrada no trato intestinal, assim como nos diversos processos infecciosos que ocorrem nesse ecossistema, e nos diferentes tipos de câncer, especialmente de cólon e reto, este estudo certamente fornecerá dados microbiológicos de interesse clínico para estabelecer estratégias para o melhor diagnóstico e tratamento desse tipo de carcinoma intestinal que tanto acomete à população mundial.

CONCLUSÕES

Os dados deste estudo permitem concluir que:

1. Em pacientes com câncer de reto as cepas *E. coli* diarreiogênicas (DEC) predominam na microbiota fecal, sendo as aEPEC e tEAEC as mais significativas, e

2. O filogrupo B2 foi observado em maior proporção nas ExPEC, independente da diversidade genética observada entre as cepas analisadas.

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