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Research paper

DNA damage response genes mark the early transition from colitis to neoplasia in colitis-associated colon cancer

Stephen P. Sharp[a,1], Robert A. Malizia[a,1], Travis Walrath[b], Shanti S. D'Souza[b], Carmen J. Booth[c], Brittany J. Kartchner[b], Edward C. Lee[a], Steven C. Stain[a], William O'Connor Jr.[b,*]

[a] Department of Surgery, Albany Medical College, Albany, NY, USA
[b] Department of Immunology and Microbial Disease, Albany Medical College, Albany, NY, USA
[c] Department of Comparative Medicine, Yale University, New Haven, CT, USA

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ABSTRACT

Chronic intestinal inflammation predisposes patients with Inflammatory Bowel Disease (IBD) to Colitis-Associated Cancer (CAC). In the setting of chronic inflammation, microsatellite instability (MSI) results from early loss of DNA damage response (DDR) genes, ultimately leading to tumor formation. Despite continued efforts to improve early detection of high risk, pre-dysplastic regions in IBD patients, current macroscopic and genetic surveillance modalities remain limited. Therefore, understanding the regulation of key DDR genes in the progression from colitis to cancer may improve molecular surveillance of CAC. To evaluate DDR gene regulation in the transition from colitis to tumorigenesis, we utilized the well-established Azoxymethane/Dextran Sodium Sulfate (AOM/DSS) pre-clinical murine model of CAC in C57BL/6 mice. In order to assess colonic tumor burden in the setting of mutagen and intestinal irritation, tumors were visualized and graded in real time through high-resolution murine colonoscopy. Upon sacrifice, colons were opened and assessed for macroscopic tumor via high magnification surgical lenses (HMSL). Tissues were then sectioned and separated into groups based on the presence or absence of macroscopically visible tumor. Critical DDR genes were evaluated by semi-quantitative RT-PCR. Interestingly, colon tissue with macroscopically visible tumor (MVT) and colon tissue prior to observable tumor (the non-macroscopically visible tumor-developing group, NMVT) were identical in reduced mRNA expression of *mlh1*, *anapc1*, and *ercc4* relative to colitic mice without mutagen, or those receiving mutagen alone. Colitis alone was sufficient to reduce colonic *ercc4* expression when compared to NMVT mice. Therefore, reduced *ercc4* expression may mark the early transition to CAC in a pre-clinical model, with expression reduced prior to the onset of observable tumor. Moreover, the expression of select DDR genes inversely correlated with chronicity of inflammatory disease. These data suggest *ercc4* expression may define early stages in the progression to CAC.

1. Introduction

Colorectal cancer (CRC) is the third most common malignancy worldwide and the fourth leading cause of cancer-related deaths in the United States (Jemal et al., 2009, 2011; Tenesa and Dunlop, 2009). Moreover, CRC accounts for roughly 15% of IBD-associated deaths (Lakatos and Lakatos, 2008; Mattar et al., 2011). Chronic colitic episodes associated with these disease processes predispose individuals to the development of colitis-associated cancer (CAC) (Grivennikov, 2013; Munkholm, 2003). Ulcerative colitis (UC) and Crohn's disease (CD)

Abbreviations: DDR, DNA Damage Response; IBD, Inflammatory Bowel Disease; CAC, Colitis-Associated Cancer; MSI, microsatellite instability; AOM, Azoxymethane; DSS, Dextran sodium sulfate; HMSL, high magnification surgical lenses; MVT, macroscopically visible tumor; NMVT, non-macroscopically visible tumor; *mlh1*, MutL homolog 1; *anapc1*, Anaphase Promoting Complex Subunit 1; *ercc1*, Excision Repair Cross-Complementation Group 1; *ercc4*, Excision Repair Cross-Complementing Rodent Repair Deficiency, Complementation Group 4; *msh2*, MutS Homolog 2; *msh6*, MutS Homolog 6; *pms2*, Postmeiotic Segregation Increased 2; WNT, Wingless-related integration site; *apc*, Adenomatous polyposis coli; β-catenin, Beta Catenin; *hprt*, Hypoxanthine Phosphoribosyltransferase1; XPF, Xeroderma Pigmentosum, Complementation Group F; CRC, Colorectal Cancer

* Corresponding author at: Department of Immunology and Microbial Disease, Albany Medical College, 47 New Scotland Avenue, MC-151, Albany, NY 12208, USA.
E-mail addresses: sharps@amc.edu (S.P. Sharp), malizir@amc.edu (R.A. Malizia), walratt@amc.edu (T. Walrath), dsouzas2@amc.edu (S.S. D'Souza), carmen.booth@yale.edu (C.J. Booth), kartchb@amc.edu (B.J. Kartchner), leee@amc.edu (E.C. Lee), stains@amc.edu (S.C. Stain), oconnow@amc.edu (W. O'Connor).
[1] Contributed equally to this work.

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both confer an increased risk of CAC development, directly correlating with duration of disease (Buchner and Lichtenstein, 2016). Within 30 years of IBD onset, > 20% of IBD patients will develop carcinoma (Farraye et al., 2010). CAC has proven very difficult to treat and is associated with a higher mortality than sporadic colorectal cancer (Munkholm, 2003). Given the propensity for chronic inflammation to progress ultimately to CAC, stringent surveillance is warranted to attempt early detection and improve prognosis. Current guideline recommendations illustrate that surveillance colonoscopy should be initiated after 8–10 years of disease onset and occur yearly for duration of the patient's life (Mattar et al., 2011; Zisman and Rubin, 2008). During colonoscopy, multiple random biopsy specimens should be obtained at 10 cm intervals throughout the entirety of the colon (Mattar et al., 2011; Zisman and Rubin, 2008). Histopathological confirmation of dysplasia within any tissue specimen(s) typically warrants total colectomy (Mattar et al., 2011; Farraye et al., 2010; Zisman and Rubin, 2008).

Retrospective data suggest that frequent colonoscopic surveillance reduces CAC-associated mortality, yet this approach has associated risks and utilizes abundant healthcare resources (Ananthakrishnan et al., 2015). Concomitantly, the current methodology is limited in providing predictive localization of high-risk regions susceptible to dysplastic transformation following colitis despite advances in chromo-endoscopy techniques (Mattar et al., 2011; Buchner and Lichtenstein, 2016; Zisman and Rubin, 2008; Trivedi and Braden, 2013). Ideally, the combination of surveillance colonoscopy and genetic analysis could synergistically improve diagnostic potential. Personalized biomarker panels could allow enhanced detection of pre-dysplastic regions in high-risk patient populations; currently, there are no such genetic panels available in the screening for CAC (Farraye et al., 2010).

Although the exact mechanistic proceedings in the development of CAC remain to be fully characterized, several distinct differences exist when compared to the pathogenesis of sporadic CRC. Exploration of key differences may provide insight and development of novel screening modalities. The well-established adenoma to carcinoma sequence in human sporadic CRC involves early loss of the tumor suppressor gene adenomatous polyposis coli (APC) and associated increase in activity of the canonical Wnt/β-catenin pathway (Dhir et al., 2008; Fearon and Vogelstein, 1990). These changes are known to be followed by mutation of the proto-oncogene K-ras and loss of heterozygosity of chromosome region 18q. Ultimately, loss of tumor suppressor gene p53 culminates in progression to invasive carcinoma (Zisman and Rubin, 2008; Fearon and Vogelstein, 1990; Armaghany et al., 2012; Waldner and Neurath, 2015).

Interestingly, in the inflammation-dysplasia-carcinoma pathway of CAC, variations occur in the timing of mutagenesis (Waldner and Neurath, 2015). Prolonged exposure to inflammation results in extensive cytokine stimulation, mucosal barrier injury, and stimulation of multiple signaling pathways (Lasry et al., 2016). Concomitantly, production of radical oxygen species promotes oxidative stress, leading to early loss of p53 and alterations in DNA repair mechanisms (Hussain et al., 2003). These pathogenic changes promote initiation of dysplasia. Subsequently, later occurrences of K-ras and APC inactivation in human CAC lead to increasing dysplastic grade and ultimately adenocarcinoma (Mattar et al., 2011; Lasry et al., 2016; Colotta et al., 2009; Mariani et al., 2014).

Several DNA damage response (DDR) genes including *ercc1*, *ercc4* (XPF), and *anapc1* have been shown to be important in the pathogenesis of sporadic CRC, but remain poorly characterized in the development of CAC (Facista et al., 2012). *Ercc1* and *ercc4* form a heterodimer endonuclease complex that is critical for nucleotide excision and interstrand DNA crosslink repair (Wang et al., 2011; Houtsmuller et al., 1999; Rubin et al., 1985; Liu et al., 1993; Sijbers et al., 1996; Manandhar et al., 2015). Increased expression of ERCC1 is associated with increased chemotherapeutic resistance (McNeil and Melton, 2012; Chen et al., 2010; Kirschner and Melton, 2010). Additionally, loss of critical DNA mismatch repair genes (*mlh1*, *pms2*, *msh2*, *msh6*) results in MSI and tumor formation, as evidenced in Lynch Syndrome (Ma et al., 2018; Papadopoulos et al., 1994; Kosinski et al., 2010; Truninger et al., 2005; Koinuma et al., 2005). MSI is proposed to occur early in the transition from colitis to CAC (Mattar et al., 2011; Mariani et al., 2014; Romano et al., 2016). Recent evidence suggests acute and chronic colitis may negatively impact critical genes governing MSI, specifically *mlh1* (Casorelli et al., 2010). In addition to factors governing MSI, the regulation of cellular mitotic processes is critical to ensuring genomic integrity. In brief, the anaphase-promoting complex, additionally known as the cyclosome (APC/C), functions as an E3 ubiquitin ligase to degrade mitotic cyclins and allow for proper chromosome segregation (Casorelli et al., 2010; Pines, 2011; Primorac and Musacchio, 2013; Wang et al., 2003). The *anapc1* gene (also known as *apc1*) encodes the largest subunit of the APC/C complex (Jorgensen et al., 2001). Aberrancies in numerous subunits associated with this complex have been reported in human colorectal cancer (Wang et al., 2003).

Given the critical roles of *ercc1*, *errc4*, *anapc1* and *mlh1*, highlighted above, it is imperative to evaluate DDR gene regulation in the transition from colitis to cancer. One such method to delineate the role of DDR genes in the transition of colitis to neoplasia may be through murine pre-clinical models. The AOM/DSS murine model for CAC provides a cost effective, reproducible, and well-validated system to evaluate pathogenesis of CAC, resulting in short interval tumorigenesis with distribution and histopathologic similarities to human disease (Parang et al., 2016; Tanaka et al., 2003). This system allows for meaningful correlation of genetic alterations associated with the linear histologic progression from colitis to tumor (adenoma) (De Robertis et al., 2001). Interestingly, a paucity of data exists regarding evaluation of DDR genes in the AOM/DSS model for CAC. Therefore, the mouse pre-clinical model may provide extensive insight into the understanding of how altered DNA damage response elements contribute to the progression of neoplasia in the human IBD patient population. Our data suggest select DNA damage response genes associated with human CAC may predict neoplastic transformation in colitic colon. Additionally, the AOM/DSS pre-clinical model for CAC is an effective tool capable of characterizing changes in a subset of DDR genes that may mark early differentiation from chronic colitis to cancer.

2. Materials and methods

2.1. Mice and experimental design

All animal studies were performed in accordance with institutional guidelines for laboratory animal care and ethical standards. The protocol for this study was approved by the Albany Medical College Institutional Animal Care and Use Committee. All mice were housed in our institutional vivarium. Given previous data indicating an increased risk for development of CAC and mortality in male IBD patients (Lee et al., 2016), we elected to utilize male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME). The AOM/DSS murine model for CAC was utilized to evaluate tumorigenesis in adult age, male mice. The mice were divided into four groups (Fig. 1A): 1) control (untreated, n = 5), 2) AOM treated (n = 5), 3) DSS treated (n = 5), and 4) AOM/DSS treated (n = 14). The AOM only and AOM/DSS groups received a single intraperitoneal injection of the mutagen Azoxymethane (AOM) (7.5 mg/kg, Sigma-Aldrich, St. Louis, MO) on day 0. The AOM/DSS and DSS groups each received two five-day cycles of 2.5% DSS (TdP Consultancy, Uppsala, Sweden; MW 36–50 kDa) administered in their drinking water. Upon conclusion of each 5 day DSS course, the mice were placed back on regular drinking water as shown. Mice underwent high resolution murine colonoscopy on Days 21 and 35. The protocol was terminated on Day 35 after achieving the desired tumor burden. Additionally, acute colitis was evaluated utilizing thirteen, adult age male C57BL/6 mice. The mice were divided into two groups (Fig. 4A): 1) control (untreated, n = 5), and 2) DSS treated for 7 days (n = 8). The