Cancer Cell
Article

Colitis-Associated Colorectal Cancer Driven by T-bet Deficiency in Dendritic Cells

Wendy S. Garrett,[1,2,4,*] Shivesh Punit,[1,5] Carey A. Gallini,[1] Monia Michaud,[1] Dorothy Zhang,[1] Kirsten S. Sigrist,[1] Graham M. Lord,[1,6] Jonathan N. Glickman,[3,7] and Laurie H. Glimcher[1,2,*]
[1]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA 02115, USA
[2]Department of Medicine
[3]Department of Pathology, Brigham and Women's Hospital
Harvard Medical School, Boston, MA 02115, USA
[4]Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA 02115, USA
[5]Present address: Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37240, USA
[6]Present address: Department of Nephrology and Transplantation, King's College London and Guy's and St. Thomas' Hospital, London SE1 9RT, UK
[7]Present address: GI Pathology, Boston Caris Diagnostics, 320 Needham Street, Suite 200, Newton, MA 02464, USA
*Correspondence: wendy_garrett@dfci.harvard.edu (W.S.G.), lglimche@hsph.harvard.edu (L.H.G.)
DOI 10.1016/j.ccr.2009.07.015

SUMMARY

We previously described a mouse model of ulcerative colitis linked to T-bet deficiency in the innate immune system. Here, we report that the majority of *T-bet*$^{-/-}$ *RAG2*$^{-/-}$ ulcerative colitis (TRUC) mice spontaneously progress to colonic dysplasia and rectal adenocarcinoma solely as a consequence of *MyD88*-independent intestinal inflammation. Dendritic cells (DCs) are necessary cellular effectors for a proinflammatory program that is carcinogenic. Whereas these malignancies arise in the setting of a complex inflammatory environment, restoration of *T-bet* selectively in DCs was sufficient to reduce colonic inflammation and prevent the development of neoplasia. TRUC colitis-associated colorectal cancer resembles the human disease and provides ample opportunity to probe how inflammation drives colorectal cancer development and to test preventative and therapeutic strategies preclinically.

INTRODUCTION

The three highest risk groups for developing colorectal cancer (CRC) are individuals with ulcerative colitis (UC), familial adenomatous polyposis, and hereditary nonpolyposis colon cancer syndrome. Among UC patients, the relative risk of developing CRC correlates with the extent and duration of disease—18% will have developed CRC after 30 years of disease (Eaden et al., 2001; Xie and Itzkowitz, 2008). The sequence of genetic events in colitis-associated CRC (caCRC) differs from that observed in sporadic CRC. In caCRC, chromosomal instability (CIN) and DNA damage can precede and predict the development of dysplasia, and alterations in *p53* expression occur early in the oncogenic pathway, not as is depicted in the classic adenoma-carcinoma sequence (Cho and Vogelstein, 1992; Clausen et al., 2001; Itzkowitz, 2003; Yoshida et al., 2003). In addition, in contrast to sporadic CRC, alterations in β-catenin localization reflecting *APC* mutations occur very late in the caCRC transformation process. Sporadic CRC carcinogenesis is typified by the transformation of the adenoma to an adenocarcinoma (ACA); however, in caCRC, invasive carcinomas frequently arise in flat areas of dysplasia. This feature of caCRC makes clinical surveillance of this at risk population particularly challenging and speaks to the distinct biology of these neoplasias.

Mouse models of intestinal cancer have been instrumental in understanding oncogenesis and have shed light on the role of

SIGNIFICANCE

Inflammatory bowel disease (IBD) is one of the three highest risk factors for colorectal cancer (CRC). The molecular pathogenesis of IBD-associated colorectal cancer (caCRC) differs from that of sporadic CRC. The findings reported here demonstrate that the *T-bet*$^{-/-}$ *RAG2*$^{-/-}$ ulcerative colitis model of caCRC is a robust system for studying the human disease. This model establishes the importance of the innate immune system and specifically dendritic cells as key cellular effectors in inflammation that drives neoplasia. Our data also demonstrate that there are *MyD88*-independent pathways to caCRC. Finally, the observation that overexpression of *T-bet* in innate immune cells prevents caCRC provides an additional explanation for why colorectal cancer patients with intratumoral *T-bet* expression may have improved survival and generates interest in immune-based cancer therapeutics.

innate immunity and the commensal microbiota in colon cancer. We recently described a model of commensal-dependent UC termed *T-bet*$^{-/-}$ *RAG2*$^{-/-}$ UC (TRUC) that results from T-bet deficiency in the innate immune system (Garrett et al., 2007). *T-bet* is a T-box family transcription factor that controls chemokine, chemokine receptor, and cytokine expression; regulates host-commensal homeostasis in the colon; and is expressed only in immune cells (Glimcher, 2007; Ma, 2007). TRUC mice develop a severe and highly penetrant colitis, driven in part by loss of *TNF-α* regulation in the colon, that can be reversed by antibiotics, TNF-α blockers, or transfer of T regulatory cells. CRC has been documented in other inflammatory bowel disease (IBD) mouse models including *IL-10*$^{-/-}$, *TCRα*$^{-/-}$, *Gαi2*$^{-/-}$, *IL-2*$^{-/-}$ × *β2m*$^{-/-}$, and *αv*$^{-/-}$ mice (Berg et al., 1996; Dianda et al., 1997; Lacy-Hulbert et al., 2007; Rudolph et al., 1995; Shah et al., 1998). This observation coupled with the finding that increased levels of T-bet in human colorectal tumors correlate with increased patient survival spurred us to determine if TRUC mice, whose colitis is mechanistically distinct from other IBD models, would develop caCRC (Pages et al., 2005).

RESULTS

TRUC Mice Develop Dysplasia and Carcinoma Resembling Human IBD-Associated CRC

In our colony, TRUC manifests a juvenile colitis that starts rectally, has 100% penetrance, and can result in bacteremia and death as early as 10 weeks of age. We monitored large cohorts (>400 TRUC mice during a 3 year period) to robustly survey whether dysplasia and carcinoma would develop over time. At 3 months of age, 50% of TRUC mice had dysplastic lesions and ACA was present in 3/40 cases. By 6 months of age, over 96% of mice had dysplastic lesions and 42% had ACA (Figure 1A). Low-grade dysplasia (LGD) predominated in younger mice (75% of cases), while 6 month olds manifested high-grade dysplasia (HGD) (89% of cases) (Figure 1B). In 12/30 of the HGD cases there were adjacent regions of LGD (1–3 mm) (data not shown), suggesting progression to the higher grade. Similarly, ACA (size range 2–7 mm) were flat and usually arose in the rectum within regions of dysplastic mucosa (25/31 cases), similar to what is observed in UC patients. The number of mice with more advanced submucosal invasive cancers increased over time (Figure 1C). Hence, malignant transformation of intestinal epithelial cells occurs in virtually all TRUC mice by 6 months of age and progresses to frank ACA in a significant proportion of animals.

On gross examination, areas of colitis with dysplasia and ACA appeared more vascular and engorged as compared with colons with similar inflammatory scores but no dysplasia or cancer (Figure 1D). We noted the occurrence of fixed anorectal prolapse as it can complicate the diagnosis of cancer and dysplasia (10/21 intramucosal and 2/10 submucosal ACA cases). Photomicrographs are shown from representative cases with carcinoma (Figure 1E, upper panel; dysplasia [middle panel: left, HGD; right, LGD], and no dysplasia [bottom panel: left, colitis; right, no colitis]).

Immunohistochemistry for PCNA revealed a high degree of proliferation in mice with ACA and dysplasia, consistent with neoplastic transformation (Figure 1F, upper left panel). To determine whether TRUC caCRC phenotypically resembled human caCRC, we assessed the expression of additional markers by immunohistochemistry. Similar to human caCRC, and in contrast to what is observed in sporadic CRC, β-catenin localization was membranous in nondysplastic and dysplastic lesions (Figure 1F, upper right panel) and nuclear (indicative of APC loss of function) in carcinoma (Figure 1F, upper right panel). As is observed in human caCRC, we saw high levels of epithelial cell and immune cell COX-2 expression in inflamed mucosa that was nonneoplastic and neoplastic (Figure 1F, middle panel) (Agoff et al., 2000). Approximately 30% of nonneoplastic crypts were positive for COX-2 and >90% of neoplastic crypts were positive (Figure 1F, middle panel); thus *COX-2* epithelial expression is a premalignant feature of TRUC transformation. Intense nuclear staining for p53 (CM5 clone: detects both mutant and wild-type forms) was detected in the neoplastic epithelium and in nondysplastic crypts; an observation that is highly suggestive of *p53* mutations (Rodrigues et al., 1990) (Figure 1F, bottom panel). To assess if this increased *p53* expression was a consequence of mutations resulting in loss of p53 function, we performed two complementary experiments examining both the DNA binding capability of p53 and induction of p53 target genes. Specifically, we tested p53 DNA binding activity and expression of the p53 target genes, *p21* and *APAF1*, in colonic epithelial cells (CECs) from control, nonneoplastic TRUC, and neoplastic TRUC in response to treatment with doxorubicin, an anthracycline chemotherapeutic that inhibits topoisomerase II, induces double-stranded DNA breaks, and strongly activates *p53* (Ravizza et al., 2004). Doxorubicin treatment induced p53 binding to a consensus site oligonucleotide in *RAG2*$^{-/-}$ (control) CECs in a specific fashion but was reduced in TRUC nonneoplastic CECs and markedly diminished in neoplastic TRUC CECs (Figure 1G, upper panel). *p21* expression was induced approximately 250-fold in *RAG2*$^{-/-}$ CECs treated with doxorubicin; however, this induction was more modest in TRUC nonneoplastic CECs (70.2-fold) and substantially reduced in neoplastic TRUC CECs (2.23-fold) (Figure 1G, lower left panel). APAF1 levels in response to doxorubicin were upregulated most in *RAG2*$^{-/-}$ (1321-fold), approximately an order of magnitude less in TRUC nonneoplastic (100.3-fold) and substantially reduced in TRUC neoplastic (1.42-fold) samples (Figure 1G, lower right panel). Thus the TRUC neoplastic process does resemble human caCRC in several aspects of its molecular pathogenesis, specifically in both early loss of function of *p53* and increased epithelial *COX-2* expression and later *APC* mutations.

TRUC Mice Develop Colonic Epithelial Aneuploidy prior to Dysplasia, and the TRUC Mucosa Is Rich in ROS, Colonic Epithelial DNA Adducts, and Cytokines, Similar to IBD Patients Who Develop Dysplasia and Cancer

CIN and its resulting aneuploid DNA content are features of human caCRC. In fact, CIN has been detected in UC patient colonic biopsies that are nondysplastic, dysplastic, and malignant; and aneuploidy predicts dysplasia (Clausen et al., 2001; Meling et al., 1991a, 1991b; Rubin et al., 1992). To search for CIN and aneuploidy in TRUC mice with and without dysplasia and cancer, we measured DNA content in CECs from TRUC colons by flow cytometry. While no aneuploid cell populations were detected in 6 month old *RAG2*$^{-/-}$ mice (data not shown), aneuploidy was a feature of nondysplastic and dysplastic