Clinical Significance of Bmi1 Expression in Inflammatory Bowel Disease

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Abstract

Background: Although the stem cell marker Bmi1 is over-expressed in many malignancies, its role in inflammation-associated cancer is unclear. Colitis-associated cancer (CAC) is caused by chronic intestinal inflammation and often results from refractory inflammatory bowel disease (IBD). **Methods:** To assess the involvement of Bmi1 in the development of CAC, we analyzed the gene expression of colon tissues collected from 111 patients with IBD and CAC. **Results:** In the colonic mucosa of patients with ulcerative colitis, the expression of Bmi1 correlated significantly with the expression of inflammatory cytokines such as IL-6, IL-17, IL-23, and tumor necrosis factor α (TNF- α). In the colonic mucosa of patients with Crohn's disease, the expression of Bmi1 correlated significantly with the expression of TNF- α and IL-23. The expression of Bmi1 was enhanced in the colonic mucosae of refractory IBD, suggesting that Bmi1 expression might be related to increased cancer risk. In addition, patients with high

Bmi1 expression showed significantly lower response rates upon subsequent anti-TNF- α therapy as compared to patients with low Bmi1 expression. In human CAC specimens, the expression of Bmi1 was upregulated in nontumor tissues as well as tumors. **Conclusions:** Bmi1 expression is related to a refractory clinical course of IBD and upregulated in refractory IBD and CAC. Measurement of Bmi1 expression is a promising approach for the advanced treatment and personalized management of IBD patients.

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Introduction

Colorectal cancer is one of the most common fatal malignancies worldwide [1, 2]. Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD), is thought to result from aberrant activation of the intestinal immune system [3] and is a major risk factor for colorectal cancer, so-called colitis-associated cancer (CAC) [4]. Indeed, patients with refractory IBD are at higher risk of colorectal cancer than individuals in the general population [5]. Longstanding inflammation

induces stress response proteins such as cold-inducible RNA-binding protein (CIRP), heat shock protein A4 (HSPA4), and RNA-binding motif protein 3 (RBM3), which regulate apoptosis of inflammatory or epithelial cells and expansion of stem cells, leading to the development of CAC [6–8]. In addition, HSPA4 expression could predict poor therapeutic response to steroids in IBD patients. Recently, we reported that the oncoprotein gankyrin activates STAT3 in inflammatory cells and promotes the development of CAC. In UC patients, refractory inflammation is associated with increased gankyrin expression in the colonic mucosa, which increases the risk for CAC [9]. These data suggest that long-term inflammation would result in refractory clinical course and eventually the development of cancer in IBD.

Cancer stem cells, the microenvironment, and the immune system interact with each other through cytokines. In the context of chronic inflammation, immune responses would affect proliferation of stem cells and increase the expression of stem cell markers [10]. The expression of stress response proteins and gankyrin that functionally link between chronic inflammation and tumorigenesis in IBD is significantly correlated with that of the stem cell marker Bmi1 [6–9]. The polycomb group protein Bmi1 is frequently overexpressed in human sporadic colorectal cancer, and the degree of upregulation correlates with disease progression and is predictive of poor patient survival [11]. In mice, Bmi1 is required for intestinal tumorigenesis [12]. Here, we show a significant association between Bmi1 expression and chronic inflammation in IBD patients. This study represents the first report of the expression of Bmi1 in inflammation-associated colorectal cancer that is associated with refractory clinical course. Bmi1 might be involved in the mechanistic connection between chronic inflammation and tumorigenesis in IBD.

Materials and Methods

Human Samples

Between April 2011 and March 2013, 111 intestinal samples were obtained from IBD patients and were analyzed for the expression of several genes. CAC specimens were obtained from 10 patients who had undergone colorectal resection.

Thirty colonic mucosal samples were obtained from IBD patients before the initiation of anti-tumor necrosis factor α (anti-TNF- α) therapy, and the response to anti-TNF- α therapy was evaluated prospectively. Clinical response was assessed 8 weeks after the treatment. In UC patients, a responder was defined as a person experiencing a decrease of 3 points or more in the full Mayo score or a decrease of 2 points or more in the Mayo clinical score with a

decrease of 1 point or more in the rectal bleeding subscore. In CD, clinical response was defined as a ≥ 100 -point decrease from the baseline CDAI score, or as a CDAI score < 150 for patients with a baseline score ≤ 248 . The measurement of serum infliximab concentration was performed by KAC Co., Ltd. (Saitama, Japan).

The clinical study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the relevant institutional review boards.

Biochemical and Immunochemical Analyses

Real-time qPCR and immunohistochemistry were previously described [13–16]. The following antibodies were used: anti-Bmi1, anti-CD3, anti-CD20, and anti-CD68 (Dako, Santa Clara, CA, USA). Immunohistochemistry was performed using ImmPRESSTM reagents (Vector Laboratory, Burlingame, CA, USA) according to the manufacturer's recommendations. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI).

Statistical Analysis

Differences were analyzed using the Student *t* test. The relationship between the expression of several genes was analyzed by the Spearman rank correlation test. *p* values < 0.05 were considered significant.

Results

Association between Bmi1 and Cytokines in the Colonic Mucosa of Patients with IBD

In the colonic mucosa of IBD patients, longstanding intestinal inflammation increases the expression of stress response proteins including CIRP, RBM3, and HSPA4, whose expression correlates significantly with Bmi1 expression [6–8]. Therefore, we explored whether an association exists between Bmi1 expression and cytokine production in IBD patients. Bmi1 expression correlated weakly but significantly with TNF- α and IL-6 expression, with a linear coefficient of 0.42 and 0.48, respectively in the colonic mucosa of UC patients (Fig. 1a, b). IL-23p19 is a specific subunit of IL-23 that acts as a positive regulator of T-helper IL-17-producing cell (Th17) and other IL-17-producing cells [17]. The IL-23/IL-17 pathway has been identified to play a critical role in IBD. A significant correlation was found between Bmi1 and IL-17A or IL-23p19 mRNA expression in UC patients (Fig. 1c, d).

Currently, the biologics with regulatory approval for the management of CD include TNF antagonists and ustekinumab, a monoclonal antibody blocking the biological activity of IL-12 and IL-23 [18, 19]. Bmi1 expression correlated significantly with TNF- α and IL-23 expression, with a linear coefficient of 0.49 and 0.48, respectively in the colonic mucosa of CD patients (Fig. 2a, b).

Fig. 1. Association between Bmi1 and inflammatory cytokines in ulcerative colitis. The colonic mucosae were obtained from patients with ulcerative colitis, and gene expression was determined by quantitative real-time qPCR. Scatter plots of relative mRNA levels of Bmi1 and tumor necrosis factor α (TNF- α) (a), IL-6 (b), IL-17 (c), and IL-23 (d) are shown.

Fig. 2. Association between Bmi1 and inflammatory cytokines in Crohn's disease. The colonic mucosae were obtained from patients with Crohn's disease, and gene expression was determined by quantitative real-time qPCR. Scatter plots of relative mRNA levels of Bmi1 and tumor necrosis factor α (TNF- α) (a) and IL-23 (b) are shown.

Increased Bmi1 Expression Seen in Refractory IBD Predicts Poor Response to Anti-TNF- α Therapy

Bmi1 expression levels were increased in patients with refractory IBD associated with long-term inflammation compared with those in controls or IBD patients in remission [7]. Immunohistochemistry was performed to identify the cells expressing Bmi1 in the human intestine. Nonparenchymal cells as well as epithelial cells were found to express Bmi1 protein (Fig. 3a). In chronically inflamed mucosa, Bmi1 expression was enhanced in nonparenchymal cells (Fig. 3a).

We next explored whether an association exists between Bmi1 expression and clinical response to anti-

TNF- α therapy. Thirty colonic mucosal samples were obtained from IBD patients before the initiation of anti-TNF- α therapy, and the response to anti-TNF- α therapy was evaluated prospectively. Among the 30 IBD patients treated with anti-TNF- α therapy and evaluated prospectively, 9 turned out to be resistant to anti-TNF- α therapy. We then determined the correlation of the expression level of Bmi1 to the clinical outcome of anti-TNF- α therapy. The expression level of Bmi1 in the colonic mucosa was 2.10 ± 0.23 in patients without subsequent clinical response to anti-TNF- α therapy, whereas we detected expression levels of 1.28 ± 0.14 in patients with clinical response. Patients with high expression of

Fig. 3. Increased Bmi1 expression seen in refractory inflammatory bowel disease (IBD) predicts poor response to anti-tumor necrosis factor α (anti-TNF- α) therapy. **a** Representative images of immunohistochemical findings in human colonic mucosa of patients without IBD and those with refractory IBD using anti-Bmi1 antibody. **b** Relative levels of Bmi1 in colonic tissues of IBD patients

before the initiation of anti-TNF- α therapy, as determined by real-time qPCR (responders, $n = 21$; nonresponders, $n = 9$). The expression level in colonic mucosa of controls was set as 1. **c** The infliximab trough level was determined in patients with IBD. Each dot represents an individual point of infliximab (IFX) trough level measurements. UC, ulcerative colitis.

Bmi1 showed significantly lower response rates upon anti-TNF- α therapy as compared to those with low Bmi1 expression (Fig. 3b). There was no significant difference in age, disease duration, serum C-reactive protein, hemoglobin, or endoscopic findings evaluated using Mayo score between anti-TNF- α therapy responders and nonresponders (Table 1). During anti-TNF- α therapy, the trough levels of anti-TNF- α agents were significantly higher in responders than in nonresponders (Fig. 3c).

Bmi1 Expression Is Enhanced in Human CAC

Immunohistochemistry showed that Bmi1 was expressed in the mesenchyme of refractory IBD patients (Fig. 3a). As expected, the Bmi1-positive cell population was distinct from CD3-, CD20-, or CD68-expressing differentiated cells (Fig. 4a–c). The expression of Bmi1 was enhanced in the colonic mucosae of refractory IBD [7], suggesting that Bmi1 expression might be related to increased risk for CAC. Consistently, Bmi1 expression was increased in 9 of 10 CACs we examined. Both cancer cells and lamina propria cells expressed Bmi1 protein in human CAC (Fig. 4d).

Fig. 4. Bmi1 expression is enhanced in colitis-associated cancer. **a–c** Representative images of immunohistochemical detection of Bmi1 and CD3 (**a**), CD20 (**b**), or CD68 (**c**) in colonic tissues from refractory inflammatory bowel disease patients. **d** Representative images of immunohistochemical findings in human colonic mucosa of patients with colitis-associated cancer using anti-Bmi1 antibody. Scale bar, 100 μ m. DAPI, 4',6-diamidino-2-phenylindole.

Discussion

Inflammation is a common and important factor in the pathogenesis of cancer [20]. It has become apparent that the interaction between transformed cells and their close surrounding, including innate immune cells, fibroblasts, and endothelial cells, can be instrumental in the development of many tumors, especially in the context of chronic inflammation [21, 22]. However, the underlying molecular processes involved in this interaction remain to be elucidated. In humans, Bmi1 expression was sig-

nificantly correlated with the expression of inflammatory cytokines, including IL-17 and IL-23. IL-23/IL-17 signaling enhances the immunosuppressive activity of regulatory T cells and reduces CD8+ cells in tumor, leading to enhanced tumor initiation and promotion [23, 24]. A recent study suggested that colorectal cancer tissue-derived Foxp3+IL-17+ cells have the capacity to induce cancer-initiating cells in vitro [25]. Chronic inflammation contributes to the expansion of stem cells, leading to the development of cancer [10]. Bmi1 might act as a link between chronic inflammation and tumorigenesis in IBD.

Table 1. Clinical characteristics of patients undergoing anti-TNF-α therapy

	Responders (n = 21)	Nonresponders (n = 9)	p value
Age, years	42±18	42±19	0.98
Sex (M/F)	13/8	6/2	
Disease duration, years	4.4±4.2	7.9±8.3	0.16
Extent of disease			0.72
UC			
Pancolitis	6	4	
Left colitis	6	2	
CD			
Ileitis	6	1	
Ileocolitis	2	2	
Colitis	1	0	
CRP, mg/dL	1.7±1.5	2.7±3.8	0.27
Albumin, g/dL	3.5±0.76	3.2±0.75	0.34
CMV infection (+/-)	0/21	1/8	0.30
Mayo endoscopic score	2.7±0.43	2.8±0.44	0.96

Results are expressed as means ± SD, unless otherwise indicated. The presence of cytomegalovirus (CMV) in the colonic mucosa was assessed by PCR. UC, ulcerative colitis; CD, Crohn's disease; CRP, C-reactive protein.

IBD is associated with an increased risk of developing colorectal cancer [5]. Most cases are believed to arise from dysplasia, and surveillance colonoscopy is therefore recommended to detect dysplasia. Early detection of CAC/dysplasia is typically achieved by colonoscopic surveillance with multiple biopsies or alternatively by chromoendoscopy with targeted biopsies of all suspect areas [26]. However, such surveillance programs have a number of limitations such as low yield, high cost, invasiveness, incomplete

patient enrollment, sampling variations, and poor agreement in histopathological interpretation [27]. If a patient's specific risk for developing colorectal cancer is determined more precisely, surveillance strategies could be appropriately personalized and surveillance programs would make considerable progress. No molecular markers are routinely used to stratify IBD patients into groups at low or high risk of developing CAC/dysplasia [28]. We have shown significantly increased Bmi1 expression in mucosal specimens from patients with refractory IBD that is reported to be associated with increased cancer risk [4, 7]. High levels of Bmi1 expression represented a lower response rate upon subsequent anti-TNF-α therapy. Thus, Bmi1 expression reflects the presence of refractory inflammation and is therefore a potential marker for predicting the risk of CAC development. Indeed, Bmi1 expression was increased in human CAC tissues. Analyzing the Bmi1 level in colonoscopy specimens may be utilized to predict the risk of cancer and the prognosis of IBD patients.

Taken together, Bmi1 expression is involved in the refractory clinical course of IBD and upregulated in refractory IBD and CAC. These findings may allow us to further understand the pathogenesis of CAC and also guide personalized and stratified therapeutics.

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Disclosure Statement

The authors have no conflicts of interest to declare.

References

1 Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ: Cancer statistics, 2009. *CA Cancer J Clin* 2009;59:225–249.

2 González N, Prieto I, Del Puerto-Nevado L, Portal-Núñez S, Ardura JA, Corton M, Fernández-Fernández B, Aguilera O, Gomez-Guerrero C, Mas S, Moreno JA, Ruiz-Ortega M, Sanz AB, Sanchez-Niño MD, Rojo F, Vivanco F, Esbrit P, Ayuso C, Alvarez-Llamas G, Egido J, García-Foncillas J, Ortiz A; Diabetes-CancerConnect Consortium: 2017 update on the relationship between diabetes and colorectal cancer: epidemiology, potential molecular mechanisms and therapeutic implications. *Oncotarget* 2017;8:18456–18485.

3 Chen ML, Sundrud MS: Cytokine networks and T-cell subsets in inflammatory bowel diseases. *Inflamm Bowel Dis* 2016;22:1157–1167.

4 Itzkowitz SH, Yio X: Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am J Physiol Gastrointest Liver Physiol* 2004;287:G7–G17.

5 Ullman TA, Itzkowitz SH: Intestinal inflammation and cancer. *Gastroenterology* 2011;140:1807–1816.

6 Sakurai T, Kashida H, Watanabe T, Hagiwara S, Mizushima T, Iijima H, et al: Stress response protein Cirp links inflammation and tumorigenesis in colitis-associated cancer. *Cancer Res* 2014;74:6119–6128.

7 Adachi T, Sakurai T, Kashida H, Mine H, Hagiwara S, Matsui S, et al: Involvement of heat shock protein A4/Apg-2 in refractory inflammatory bowel disease. *Inflamm Bowel Dis* 2015;21:31–39.

8 Sakurai T, Kashida H, Komeda Y, Nagai T, Hagiwara S, Watanabe T, Kitano M, Nishida N, Fujita J, Kudo M: Stress response protein RBM3 promotes the development of colitis-associated cancer. *Inflamm Bowel Dis* 2017;23:66–74.

- 9 Sakurai T, Higashitsuji H, Kashida H, Watanabe T, Komeda Y, Nagai T, Hagiwara S, Kitano M, Nishida N, Abe T, Kiyonari H, Itoh K, Fujita J, Kudo M: The oncoprotein gankyrin promotes the development of colitis-associated cancer through activation of STAT3. *Oncotarget* 2017;8:24762–24776.
- 10 Shigdar S, Li Y, Bhattacharya S, O'Connor M, Pu C, Lin J, et al: Inflammation and cancer stem cells. *Cancer Lett* 2014;345:271–278.
- 11 Clevers H: The cancer stem cell: premises, promises and challenges. *Nat Med* 2011;17:313–319.
- 12 Sangiorgi E, Capecchi MR: Bmi1 is expressed in vivo in intestinal stem cells. *Nat Genet* 2008;40:915–920.
- 13 Sakurai T, Kashida H, Hagiwara S, Nishida N, Watanabe T, Fujita J, Kudo M: Heat shock protein A4 controls cell migration and gastric ulcer healing. *Dig Dis Sci* 2015;60:850–857.
- 14 Sakurai T, Yada N, Watanabe T, Arizumi T, Hagiwara S, Ueshima K, Nishida N, Fujita J, Kudo M: Cold-inducible RNA-binding protein promotes the development of liver cancer. *Cancer Sci* 2015;106:352–358.
- 15 Sakurai T, Kudo M, Umemura A, He G, Elsharkawy AM, Seki E, et al: p38 α inhibits liver fibrogenesis and consequent hepatocarcinogenesis by curtailing accumulation of reactive oxygen species. *Cancer Res* 2013;73:215–224.
- 16 Mine H, Sakurai T, Kashida H, Matsui S, Nishida N, Nagai T, Hagiwara S, Watanabe T, Kudo M: Association of gankyrin and stemness factor expression in human colorectal cancer. *Dig Dis Sci* 2013;58:2337–2344.
- 17 McKenzie BS, Kastelein RA, Cua DJ: Understanding the IL-23-IL-17 immune pathway. *Trends Immunol* 2006;27:17–23.
- 18 Ma C, Fedorak RN, Kaplan GG, Dieleman LA, Devlin SM, Stern N, Kroeker KI, Seow CH, Leung Y, Novak KL, Halloran BP, Huang VW, Wong K, Blustein PK, Ghosh S, Panaccione R: Long-term maintenance of clinical, endoscopic, and radiographic response to ustekinumab in moderate-to-severe Crohn's disease: real-world experience from a multicenter cohort study. *Inflamm Bowel Dis* 2017;23:833–839.
- 19 Mantzaris GJ: Anti-TNFs: originators and biosimilars. *Dig Dis* 2016;34:132–139.
- 20 Schottenfeld D, Beebe-Dimmer J: Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. *CA Cancer J Clin* 2006;56:69–83.
- 21 Balkwill F, Mantovani A: Inflammation and cancer: back to Virchow? *Lancet* 2001;357:539–545.
- 22 Coussens LM, Werb Z: Inflammation and cancer. *Nature* 2002;420:860–867.
- 23 Kortylewski M, Xin H, Kujawski M, Lee H, Liu Y, Harris T, et al: Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment. *Cancer Cell* 2009;15:114–123.
- 24 Wu D, Wu P, Huang Q, Liu Y, Ye J, Huang J: Interleukin-17: a promoter in colorectal cancer progression. *Clin Dev Immunol* 2013;2013:436307.
- 25 Yang S, Wang B, Guan C, Wu B, Cai C, Wang M, et al: Foxp3+IL-17+ T cells promote development of cancer-initiating cells in colorectal cancer. *J Leukoc Biol* 2011;89:85–91.
- 26 Rogler G: Inflammatory bowel disease cancer risk, detection and surveillance. *Dig Dis* 2012;30(suppl 2):48–54.
- 27 Gupta RB, Harpaz N, Itzkowitz S, Hossain S, Matula S, Kornbluth A, et al: Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. *Gastroenterology* 2007;133:1099–1105.
- 28 Farraye FA, Odze RD, Eaden J, Itzkowitz SH: AGA technical review on the diagnosis and management of colorectal neoplasia in inflammatory bowel disease. *Gastroenterology* 2010;138:746–774.