

Vitamin D receptor expression is associated with colon cancer in ulcerative colitis

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Abstract. Since the 1970s, it has been well known that long-standing ulcerative colitis (UC) disposes to the development of colorectal adenocarcinoma (CRC). To date, CRC associated with UC is thought to arise along a pathway of dysplasia, however, primary factors for developing of UC-related dysplasia and cancer are unclear. Vitamin D, which works through binding the vitamin D receptor (VDR) has an important role in cancer progression and immune response. In this study, we investigated the impact of VDR expression on UC as well as colon cancer. We examined retrospectively the expression of VDR in extraction specimens of UC (n=124) patients by immunohistochemistry. We counted VDR positive cells in at least 10 fields in each case to evaluate the frequency of VDR positive cells in ductal epithelium. In addition, effect of VDR expression on inflammation was analyzed. On a normal mucosa, the expression of VDR was recognized in 58.8% of ductal cells. In UC patient, the expression of VDR was considerably decreased compared to normal mucosa, VDR positive rate was only $3.4 \pm 9.0\%$. Importantly, dysplasia and UC-CRC patients showed lower rate of VDR expression compared to non-colon cancer patients, whose expression rates were $0.6 \pm 1.3\%$ and $3.8 \pm 10\%$, respectively. Moreover, long-term UC patients (more than ten years) who were at high-risk of developing CRC showed significantly lower VDR expression than short-term patients. We did not detect direct association of VDR expression with inflammation and clinical stage of UC. These findings suggested that correlation seems to exist between the level of VDR expression and carcinogenesis in UC. VDR could be a possible marker to detect dysplasia and cancer in ulcerative colitis.

Introduction

Ulcerative colitis (UC) is one of the two major forms of inflammatory bowel diseases, which is a chronic, uncontrolled inflammatory condition of the colon and rectal mucosa. Continuous stimulation of the mucosal immune system, including luminal antigens, intestinal epithelial cells and cells of the innate and adoptive immune system which secrete mediators such as cytokines and chemokines, play a central role in the development of UC (1,2). A persistent infection with bacteria causes aggressive immune response to luminal components (3). Dendritic cells (DCs) in UC show enhanced expression of TLR4, which is associated with microbial recognition, and these activated DCs induce production of proinflammatory cytokines (4). Intestinal tissue level of TNF- α is up-regulated in UC and correlates to the grade of inflammation (5). Colorectal cancer is one of the most serious complications of UC (6). Chronic inflammation in intestinal mucosa is a risk factor for colorectal carcinoma (CRC) in UC patients. The incidence of CRC associated with UC increases dramatically among the patients with long-standing UC, reaches 5-10% after 20 years (6). At present, it is well known that the dysplasia of intestinal mucosa causes colonic cancer. Inflammation triggers abnormalities of rectal mucosa in the molecular pathways, resulting in dysplasia or cancer (7). Although possible markers for UC-related dysplasia such as Ki67, p53 and CEA have been reported, they lack precision due to certain limitations (8).

The classic function of vitamin D is to regulate calcium homeostasis. Recently other functions including effect on immune response or cellular proliferation and differentiation have been demonstrated. The biological actions of active form of vitamin D3, 1- α , 25-dihydroxyvitamin D3 [1,25-(OH) 2D3] are mediated by the nuclear vitamin D receptor (VDR) (9). VDR is a ligand-dependent transcriptional factor which can modulate the expression of various genes. VDR regulate various genes such as bone matrix formation-related genes, anti-proliferation as well as anti-inflammation-associated genes (9). Several studies have shown that VDR ligand inhibited the proliferation of prostate cancer cells, breast cancer cells and colon cancer cells (10,11). Vitamin D action has important roles in regulating differentiation of normal cells and inhibition of carcinogenesis. Several

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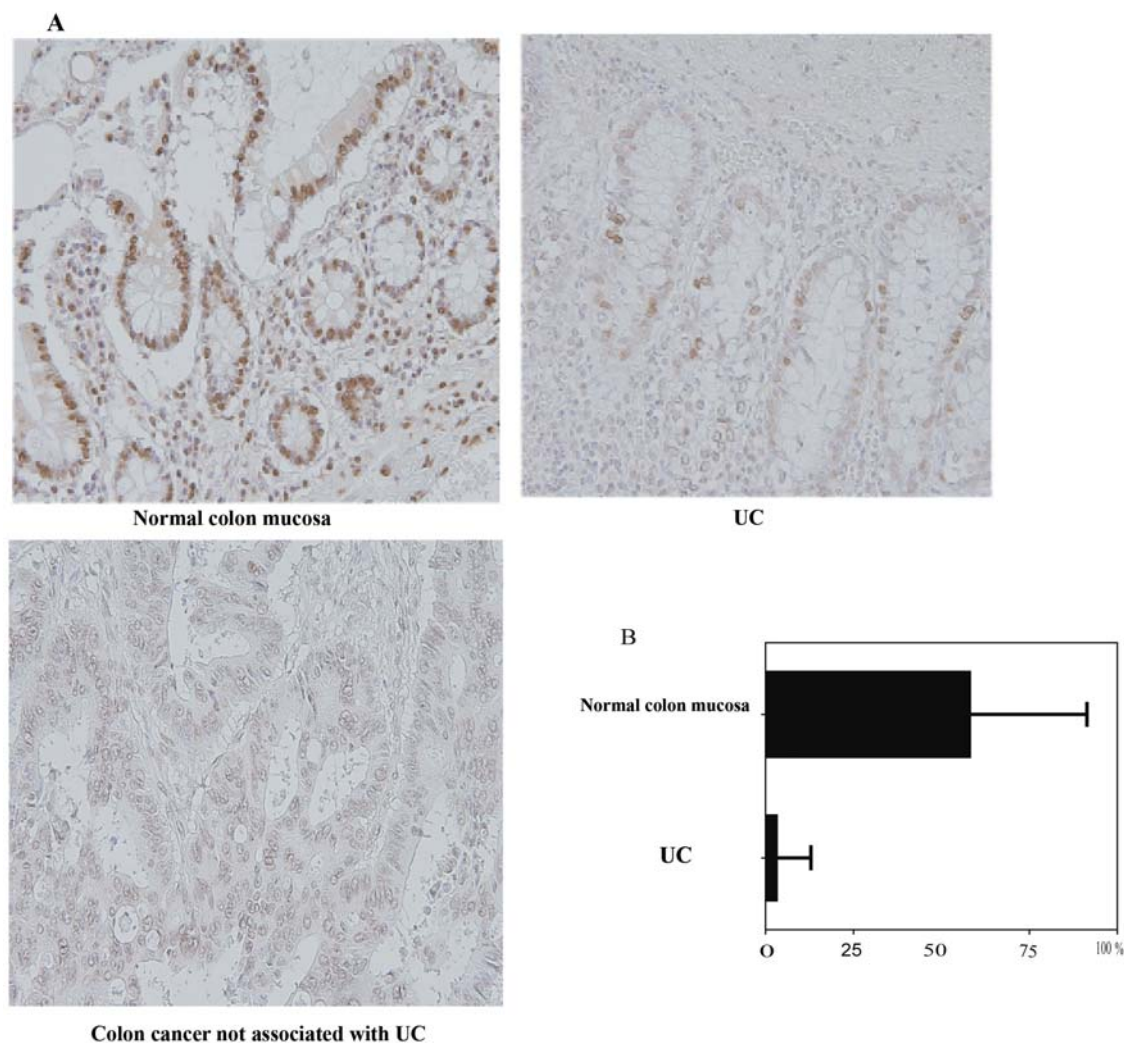


Figure 1. Expression of vitamin D receptor. (A) Immunohistochemical analysis of VDR in normal colon mucosa, ulcerative colitis (UC) and colon cancer not associated with UC. (Magnification, x400). (B) Comparison of VDR expression between normal colon mucosa and UC. Percentage of VDR expression was determined by counting positive staining cells within total ductal epithelial cells in 10 fields. VDR expression rate of UC resulted in $3.4 \pm 9.0\%$, which is significantly lower compared to that of normal mucosa. The data shown are means \pm SD. ($p < 0.05$).

epidemiological studies have revealed the relationship of CRC and vitamin D (12). The aim of this study was to examine the impact of VDR expression on UC in order to investigate the development of colon cancer.

Materials and methods

Patients and tissue samples. In total 212 specimens from 112 patients with UC and 100 with CRC who underwent operation at Osaka City Graduate School of Medicine Hospital from 1993 to 2006 were examined. According to histological diagnosis by the Riddell classification, epithelium was defined as cancer, dysplasia, or normal mucosa (13). The subjects examined in this study were: 99 cases of UC not accompanied with cancer, 7 cases of dysplasia and 6 cases of cancer associated with UC. This study was performed according to the declaration of Helsinki, and it was approved by the ethics committee of our institution. All subjects provided informed consent.

Immunohistochemistry. Paraffin-embedded specimens were taken to examine the correlation between protein in individual

ductal compartment of ulcerative colitis tissues. The VDR rabbit monoclonal antibody (Santa Cruz, CA, USA) or CD68 mouse monoclonal antibody (Abcam, MA, USA) was applied to the tissue specimens and incubated at 4°C for overnight using dilutions of 1:100. As secondary antibodies, the biotinylated donkey anti-rabbit IgG (diluted 1/100) or a biotinylated goat anti-mouse IgG was applied for 30 min. The percentage of cells showing immunoreactivity among the total number of ductal epithelial cells was determined by calculating 10 fields by two investigators.

Statistical analysis. Paired Student's t-test or Mann-Whitney U test was used and data were calculated with SPSS II software. $P < 0.05$ was considered to be statistically significant.

Results

Expression of vitamin D receptor on mucosa in ulcerative colitis. Most of VDR is present in the cytoplasm in the absence of ligand. VDR ligand induces VDR-retinoid-X-receptor heterodimerization and translocation of the complex into the

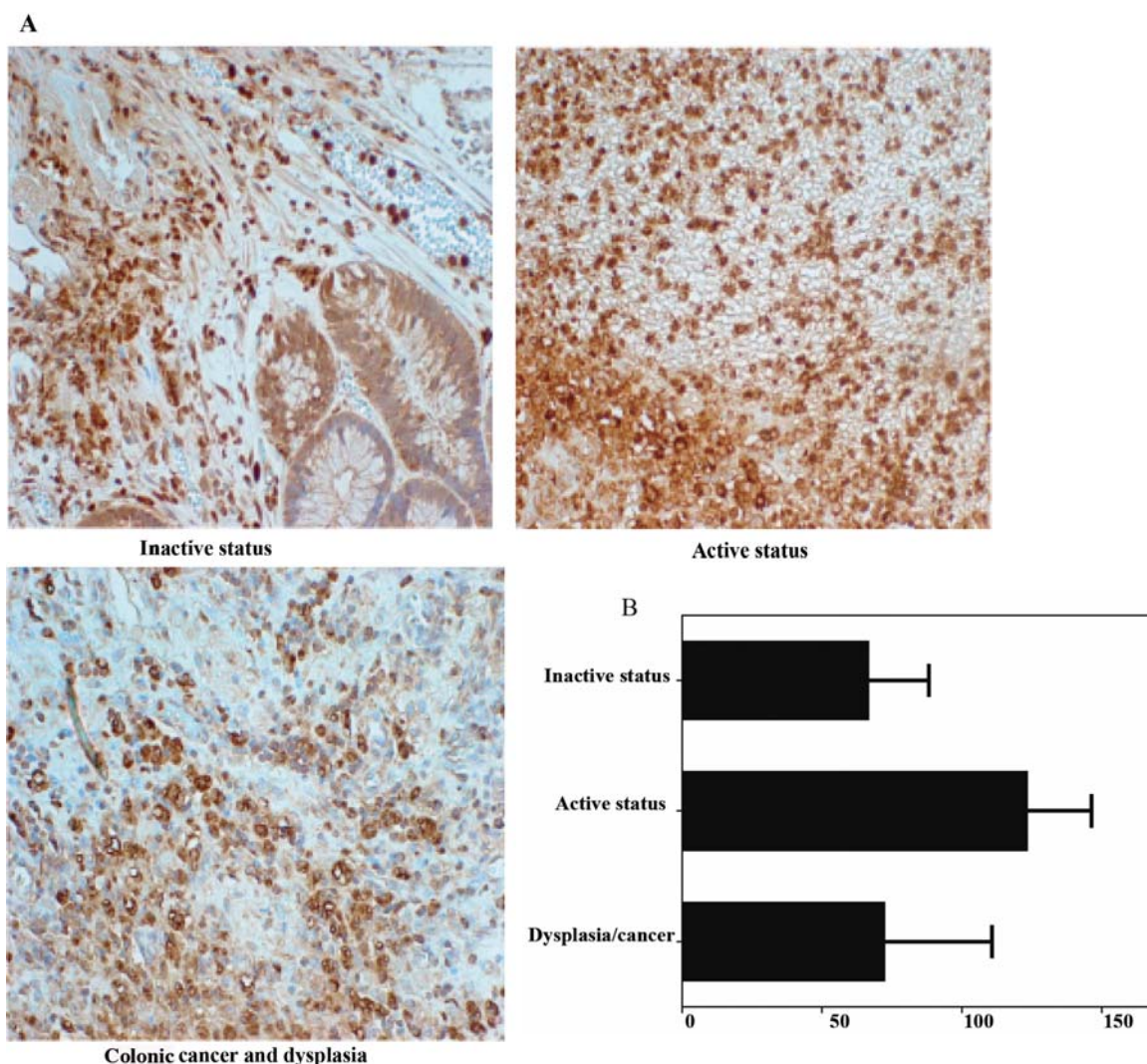


Figure 2. Macrophage infiltration into mucosal lesion. (A) Immunohistochemical analysis of CD68 in mucosa of inactive inflammatory status of UC, active inflammatory status, colonic cancer and dysplasia. (B) Comparison of macrophage infiltration among histological types. Number of CD68-positive cells was counted in 10 fields. Macrophage infiltration into mucosa was significantly increased in patients with toxic megacolon, whereas no correlation between cancer and inflammation was observed. The data shown are means \pm SD. ($p < 0.05$).

nucleus (9). Immunohistochemistry in this study revealed that VDR protein expression in nuclei of normal large intestine mucous membrane. Low expression of VDR was associated with poor progress of colon cancer (14). Our results in which VDR expression is at very low level in colon cancer did not contradict these findings. In the mucosal epithelium of UC, VDR expression clearly decreased (Fig. 1A). We counted number of VDR positive cells in epithelial ductal cells and calculated the VDR expression rate as described in Materials and methods. The VDR rate of UC resulted in $3.4 \pm 9.0\%$, which is significantly lower compared to that of normal mucosa (Fig. 1B). VDR expression rate of CRC not associated with UC resulted in 0.5% (data not shown).

Correlation of VDR expression to inflammation. To test the relevance of inflammation to VDR expression, we examined infiltration of macrophages into submucosa by immunohistochemistry using anti-CD69 antibody. Number of infiltrated macrophages into submucosa was significantly increased in patients with severe inflammation such as toxic megacolon (Fig. 2A). However, there was no difference between macro-

phage infiltration and VDR expression in submucosa. In addition, macrophage infiltration did not increase in colonic cancer and dysplasia (Fig. 2B).

Correlation of VDR expression and colonic cancer. To evaluate association with development of colon cancer, we compared VDR expression between UC accompanied with cancer or not. UC with dysplasia and colon cancer revealed lower VDR expression rate than non-cancer UC (Fig. 3A). Interestingly, VDR expression rate in patients with disease periods more than ten years was significantly decreased compared to those with less than ten years (Fig. 3B). We examined white-cell count, CRP value, LDH value, serum CA value to evaluate impact of inflammation on UC-CRC, there was no significant difference (Table I).

Discussion

We observed that expression of VDR in nuclear of colon mucosa from UC is markedly diminished compared to normal colorectal mucosa through immunohistochemistry of

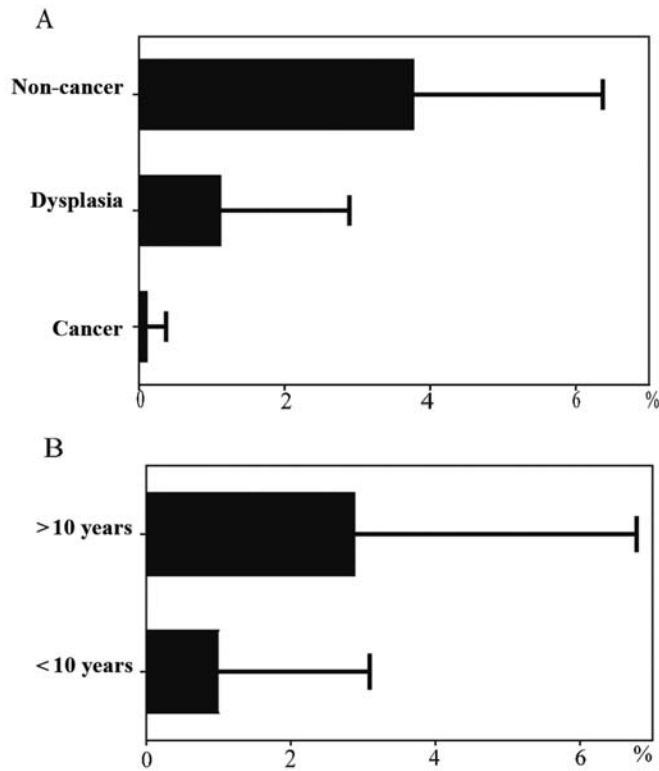


Figure 3. Impact of VDR expression on colon cancer. (A) Percentage of VDR expression was determined as described in Materials and methods. UC with dysplasia and colonic cancer revealed lower VDR expression rate than non-cancer UC. (B) Impact of disease suffering period on VDR expression. Patients with UC suffering for more than ten years exhibited lower VDR expression. The data shown are means \pm SD. ($p < 0.05$).

124 clinical samples. VDR expression was also decreased in colon cancer and dysplasia of UC. Moreover, long-term affected patients who have high-risk for development of colon cancer showed significant lower expression of VDR compared to short-time patients. Our findings were supported by several reports that VDR expression is associated with inflammatory bowel diseases. For example, Crohn disease patients had reduced expression of VDR compared with normal colonic mucosa (15) and VDR/IL-10 double knockout mice developed severe colitis like IBD (16).

Although there is convincing evidence that the patients with UC have a higher incidence of colorectal cancer than general population (17), mechanisms in development of colonic cancer are unclear. Several studies have demonstrated that vitamin D system is associated with colorectal cancer. VDR expression is repressed by SNAIL which has an important role in epithelial mesenchymal transition associated with differentiation of colon cancer cells (14,18). Clinically, mRNA expression of VDR decreases in colon cancer patient, and it is in proportion to favorable prognosis (14). Thus, vitamin D which is activated by alpha-hydroxylase has an important role in cell progression and apoptosis in epithelial cells of colorectal mucosa, and it is regulated by VDR in nuclear, indicating that VDR expression is involved in regulation of colon cancer progress. Our results suggested that correlation seems to exist between the level of VDR expression and low VDR expression might trigger carcinogenesis in ulcerative colitis.

Table I. Clinicopathological features of UC patients.

	Non-cancer UC	Dysplasia/cancer	p-value
Gender			N.S.
Male	62	7	
Female	37	6	
Age at operation	39 \pm 15	48 \pm 14	0.029
Age at diagnosis	33 \pm 15.4	36 \pm 11	N.S.
Disease period (years)	6.1 \pm 5	14.1 \pm 11	0.003
Clinical type			N.S.
Total	73	9	
Left	26	4	
Cause of operation			N.S.
Resistant to steroid	62	5	
Complication	9	0	
Severe symptom	28	2	
Cancer	0	6	
St. Marks' score	7.47 \pm 4.7	6.23 \pm 4.39	N.S.
WBC (/mm ³)	9120 \pm 6180	9067 \pm 6419	N.S.
CRP (mg/dl)	2.35 \pm 4.6	0.4 \pm 0.5	N.S.

The severity of inflammation also correlates with the risk of colorectal cancer of UC. Cyclooxygenase 2 (COX2) expression is increased in UC-related neoplasm (19) and interferon-inducible gene has been shown to be up-regulated in UC-associated cancer (20). Besides its central role in calcium, vitamin D has immunomodulatory effects on the immune system, including regulation of T cell proliferation and cytokine production. Progression of UC is correlated with Th1 cytokine production such as TNF- α , IL-12 to induce migration of macrophage into colon mucosa. We showed more infiltration of macrophages into severe inflamed mucosa such as toxic megacolon. Although there was no significance between VDR expression and infiltration of macrophages to develop UC-related cancer, we have observed that mucosa which has crypt abscess tended to have lower expression of VDR (data not shown). This result suggested that vitamin D system might have an important role in progression of ulcerative colitis with regard to mucosal inflammation.

We have not demonstrated that diminished VDR expression is the initial step of carcinogenesis in UC. In a study involving colon carcinoma cell lines, 1,25-(OH)₂D induced apoptosis by a VDR-dependent pathway as well as p38 MAPK activation (21). Holt *et al* have reported that patients who were treated with taking calcium carbonate plus vitamin D3 daily had increased apoptosis of colorectal polyps in which VDR expression significantly reduced (22). It has been reported that cancerization rate is in proportion to the length of duration of a disease period. Our results



that reduced level of VDR should improve by ration of vitamin D, which leads to protection from development of colon cancer. Our results that VDR expression was significantly reduced in long-term patients suggested that VDR in UC mucosa might be gradually decreased as time passes and result in development of dysplasia and colon cancer. In conclusion, VDR expression in mucosa might be a possible marker to estimate the development of colon cancer in ulcerative colitis.

References

1. Hanauer S: Inflammatory bowel diseases, epidemiology, pathogenesis, and therapeutic opportunities. *Inflamm Bowel Dis* 12: S3-S9, 2006.
2. Suzhen Zhang XZ and Dechun Zhang: Cellular and molecular immunopathogenesis of ulcerative colitis. *Cell Mol Immunol* 3: 35-40, 2006.
3. Campieri M and Gionchetti P: Bacteria as the cause of ulcerative colitis. *Gut* 48: 132-135, 2001.
4. Hart AL, Al-Hassi HO, Rigby RJ, Bell SJ, Emmanuel AV, Knight SC, Kamm MA and Stagg AJ: Characteristics of intestinal dendritic cells in inflammatory bowel diseases. *Gastroenterology* 129: 50-65, 2005.
5. Olsen T, Goll R, Cui G, Husebekk A, Vonen B, Birketvedt GS and Florholmen J: Tissue levels of tumor necrosis factor- α correlates with grade of inflammation in untreated ulcerative colitis. *Scand J Gastroenterol* 42: 1312-1320, 2007.
6. Wong NA and Harrison DJ: Colorectal neoplasia in ulcerative colitis-recent advances. *Histopathology* 39: 221-234, 2001.
7. Steven HI, Xianyang Y: Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am J Physiol Gastrointest Liver Physiol* 287: G7-G17, 2004.
8. Wong NA, Mayer NJ, MacKell S, Gilmour HM and Harrison DJ: Immunohistochemical assessment of Ki67 and p53 expression assists the diagnosis and grading of ulcerative colitis-related dysplasia. *Histopathology* 37: 108-114, 2000.
9. Nagpal S, Na S and Rathnachalam R: Non-calcemic actions of vitamin D receptor ligands. *Endocr Rev* 26: 662-687, 2005.
10. Skowronski RJ, Peehl DM and Feldman D: Vitamin D and prostate cancer: 1,25 dihydroxyvitamin D₃ receptors and actions in human prostate cancer cell lines. *Endocrinology* 132: 1952-1960, 1993.
11. Gilad LA, Bresler T, Gnainsky J, Smirnoff P and Schwartz B: Regulation of vitamin D receptor expression via estrogen-induced activation of the ERK 1/2 signaling pathway in colon and breast cancer cells. *J Endocrinol* 185: 577-592, 2005.
12. Garland C, Shekelle RB, Barrett-Connor E, Criqui MH, Ross AH and Paul O: Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. *Lancet* 1: 307-309, 1985.
13. Riddell RH: Dysplasia in inflammatory bowel disease. *Clin Gastroenterol* 9: 439-458, 1980.
14. Evans SR, Nolla J, Hanfelt J, Shabahang M, Nauta RJ and Shchepotin IB: Vitamin D receptor expression as a predictive marker of biological behavior in human colorectal cancer. *Clin Cancer Res* 4: 1591-1595, 1998.
15. Abreu MT, Kantorovich V, Vasilias EA, *et al*: Measurement of vitamin D levels in inflammatory bowel disease patients reveals a subset of Crohn's disease patients with elevated 1,25-dihydroxyvitamin D and low bone mineral density. *Gut* 53: 1129-1136, 2004.
16. Froicu M, Weaver V, Wynn TA, McDowell MA, Welsh JE and Cantorna MT: A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. *Mol Endocrinol* 17: 2386-2392, 2003.
17. Gyde SN, Prior P, Allan RN, Stevens A, Jewell DP, Truelove SC, Loffberg R, Brostrom O and Hellers G: Colorectal cancer in ulcerative colitis: a cohort study of primary referrals from three centres. *Gut* 29: 206-217, 1988.
18. Palmer HG, Larriba MJ, Garcia JM, *et al*: The transcription factor SNAIL represses vitamin D receptor expression and responsiveness in human colon cancer. *Nat Med* 10: 917-919, 2004.
19. Agoff SN, Brentnall TA, Crispin DA, *et al*: The role of cyclooxygenase 2 in ulcerative colitis-associated neoplasia. *Am J Pathol* 157: 737-745, 2000.
20. Hisamatsu T, Watanabe M, Ogata H, Ezaki T, Hozawa S, Ishii H, Kanai T and Hibi T: Interferon-inducible gene family 1-8U expression in colitis-associated colon cancer and severely inflamed mucosa in ulcerative colitis. *Cancer Res* 59: 5927-5931, 1999.
21. Diaz GD, Paraskeva C, Thomas MG, Binderup L and Hague A: Apoptosis is induced by the active metabolite of vitamin D₃ and its analogue EB1089 in colorectal adenoma and carcinoma cells: possible implications for prevention and therapy. *Cancer Res* 60: 2304-2312, 2000.
22. Holt PR, Bresalier RS, Ma CK, Liu KF, Lipkin M, Byrd JC and Yang K: Calcium plus vitamin D alters preneoplastic features of colorectal adenomas and rectal mucosa. *Cancer* 106: 287-296, 2006.