Potential Dual Role of Activating Transcription Factor 3 in Colorectal Cancer

XIAOJING JIANG*, KUI-JIN KIM*, TAEKYU HA and SEONG-HO LEE

Department of Nutrition and Food Science, College of Agriculture and Natural Resources, University of Maryland, College Park, MD, U.S.A.

Abstract. *Background/Aim: Activating transcription factor 3 (ATF3) is a member of the ATF/CREB transcription factor family and has been proposed as a molecular target for cancer therapy. The present study was undertaken in order to investigate whether ATF3 influences cancer-related phenotypes in colorectal cancer. Materials and Methods: ATF3 was overexpressed in human colorectal cancer cells and the effects of ATF3 on apoptosis, cell cycle, cell migration and epithelial mesenchymal transition (EMT) were investigated. B-cell lymphoma-2 (Bcl-2) promoter was cloned and used for luciferase assay in cells transfected with control or ATF3 expression vector. Results: ATF3 down-regulated the expression of Bcl-2 and promoter activity of the Bcl-2 gene. ATF3 increased collective cell migration and expression of cluster of differentiation 44 (CD44) and decreased retinoblastoma (Rb) expression. In addition, ATF3 down-regulated EMT-inducing transcription factors and β-catenin. Conclusion: ATF3 may play a dichotomous role in apoptosis and metastasis in human colorectal cancer cells.*

Colorectal cancer (CRC) occupies the third place in cancer incidence and mortality in the United States (1). Understanding of molecular targets is very important for the development of effective prevention and therapeutic strategies. During carcinogenesis, cells undergo and respond to numerous cellular and physical stresses, and the failure to restrain and eliminate stress signals can increase the risk of cancer (2). Activating transcription factor 3 (ATF3) is a member of the ATF/CREB family and contains basic region-leucine zipper (bZip) DNA binding domain (3). Basal expression of ATF3 is low in normal cells and can be induced by various stress stimuli and signals that damage cells or tissues (4). These stress signals include hypoxia, anoxia, carcinogens, DNA damage, UV exposure and radiation (5). In addition, ATF3 is responsible for adaptation to different extra- and intra-cellular stimuli (6, 7).

In previous studies, we found that ATF3 could be a molecular target of many anticancer compounds such as PI3K inhibitor (8), epicatechin gallate (9), indole-3-carbinol (10), conjugated linoleic acid (11), and tolfenamic acid (12), that mediate compounds-stimulated apoptosis (9-11). However, the role of ATF3 in cancer seems to be complex and dichotomous. Recently Dr. Kitajima's group reported that ATF3 mediated ER stress-induced sensitization of colon cancer cells to TRAIL-mediated apoptosis (13, 14) and regulated synergistic anticancer activity of a HDAC inhibitor and anti-DR5 antibody in human colon cancer cells (15). Stable or transient overexpression of ATF3 increased caspase activity and enhanced the etoposide- or camptothecin-induced apoptosis in HeLa cells (16). However, ATF3 inhibited apoptosis through activating the AKT pathway in PC12 cells (17). Recently, Yin *et al*. reported that ATF3 enhanced apoptosis in the untransformed MCF10A mammary epithelial cells, whereas protected the aggressive MCF10CA1a cells and enhanced cell motility (18). In addition, an *in vivo* study using a xenograft mouse model showed that ATF3 possessed either tumor suppressive or oncogenic activity (19-22). The role of ATF3 in apoptosis depends on the cell type, tissue context and stage of tumorigenesis.

The current study was undertaken in order to investigate the effect of ATF3 expression on tumorigenicity to better understand the role of ATF3 in human colon cancer progression.

Materials and Methods

Cell culture and antibodies. Human colorectal adenocarcinoma cells (HCT116, HCT15, Caco-2, LoVo, HT29 and SW480) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and grown in Dulbecco's modified Eagle's medium (DMEM/F12) supplemented with 10% fetal bovine serum (FBS).

This article is freely accessible online.

*These Authors contributed equally to this study.

Correspondence to: S-H. Lee, Department of Nutrition and Food Science, University of Maryland, College Park, MD, 20742, U.S.A. Tel: +1 301 405 4532, Fax: +1 301 314 3313, e-mail: slee2000@umd.edu

Key Words: Activating transcription factor 3, Bcl-2, apoptosis, migration, colorectal cancer.

Table I. *Sequences of PCR primers used for RT-PCR.*

Genes	Forward primers	Reverse primers
ATF3	5'-GTTTGAGGATTTTGCTAACCTGAC-3'	5'-AGCTGCAATCTTATTTCTTTCTCGT-3'
GAPDH	5'-GGGCTGCTTTTAACTCTGGT-3'	5'-TGGCAGGTTTTTCTAGACGG-3'
Bcl-2	5'-GAGGATTGTGGCCTTCTTTG-3'	5'-CAGCCAGGAGAAATCAAACAG-3'
Bak	5'-TCTGGCCCTACACGTCTACC-3'	5'-ACAAACTGGCCCAACAGAAC-3'
Slug	5'-GGGGAGAAGCCTTTTTCTTG-3'	5'-TCCTCATGTTTGTGCAGGAG-3'
Snail	5'-CCTCCCTGTCAGATGAGGAC-3'	5'-CCAGGCTGAGGTATTCCTTG-3'

Recombinatnt human epithermal growth factor (EGF), basic fibroblast growth factor (bFGF) and propidium iodide (PI) were purchased from Sigma (St. Louis, MO, USA). The antibodies for Bcl-2 and ATF3 were purchased from BD Biosciences (San Jose, CA, USA) and Santa Cruz (Santa Cruz, CA, USA), respectively. Antibodies for β-actin, Bak, Bax, cyclin D1, p21, p27, Rb, CD44, ZO-1, GSK3 β, and β-catenin were purchased from Cell Signaling (Beverly, MA, USA).

Cloning of Bcl-2 promoter and measurement of luciferase activity. Human Bcl-2 promoter region spanning from -1000 to +715 base pairs was amplified from human genomic DNA by PCR. PCR primers used are as follows: (F: 5'-CGATCTAAGTAAGC TTGCCCTCCCCGGCCGCGGC-3', R: 5'-CCGGAATGCCAAGC TTTGTCAATCCGCAGGAATCCCAA-3'). PCR products were cloned into pGL3-basic vector (Promega, Madison, WI, USA) by using In-Fusion cloning method (Clontech, Mountain View, CA, USA). The luciferase activity was measured using a dual luciferase assay kit (Promega) as we described previously (12). The promoter activity was expressed as ratio of Bcl-2 promoter activity/pGL3-basic activity.

Cloning of ATF3 expression vector and establishing ATF3-expressing stable cell line. Human ATF3 plasmid DNA was amplified with following primers (F: 5'-TCTCGAGCTCAAGCTTCGATGAT GCTTCAACACCCAGGCC-3', R: 5'-GCAGAATTCGAAGCTTT TAGCTCTGCAATGTTCCTTCT-3') and PCR products were inserted to pAcGFP-C1 by using In-Fusion cloning method from Clontech. To create a cell clone overexpressing human ATF3 gene, the pAcGFP-C1-ATF3 vectors were transfected into HCT15 cells using Lipofectamine 2000, and the cells were maintained with G418 for two weeks to eliminate the untransfected cells.

Transient transfection and overexpression of ATF3. Transient transfections were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) or PolyJet (SignaGen Laboratories, Rockville, MD, USA), as we previously described (12). The pCG-ATF3 expression construct was described previously (12). pcDNA3.1 V5/His empty vector were used as control. The cells were plated in 6-well plates at the concentration of 4×10^5 cells/well and grown overnight. The next day, plasmid mixtures containing 2.5 μg of control or ATF3 expression vector were transfected for 48 h.

Western blot and RT-PCR analysis. Western bot and RT-PCR were performed as described previously (12). For western blot, Chemiluminescence was visualized by ChemiDoc MP Imaging system (Bio-Rad, Hercules, CA, USA). For RT-PCR, total RNA was extracted and cDNA was synthesized using Verso cDNA kit (Thermo Scientific). PCR was carried out using ReadyMix Taq polymerase (Sigma). The sequences of PCR primers are indicated in Table I.

Apoptosis and cell-cycle analysis. Apoptosis and cell-cycle analysis were measured using flow cytometer, as we described previously (23). For apoptosis, Annexin V positive/PI positive and Annexin V positive/PI negative cell populations were determined as apoptotic cells from the total gated cells.

Tumorsphere forming assay. Stable cells overexpression control or ATF3 expression vector were plated onto 1% methylcellulose on poly–HEMA coated 6-well plates at the concentration of $2x10^4$/well and grown in DMEM supplemented with 10% FBS, 20 ng/mL of human recombinant bFGF and 20 ng/mL of human recombinant EGF. Suspension cultures were performed for 14 days and then microscope images were taken by phase-contrast microscope.

Statistical analysis. Statistical analysis was performed with the unpaired Student's *t*-test. Data were expressed as means±SD and differences were considered significant at $p\leq0.05$.

Results

ATF3 modulates the expression of Bcl-2 family members in human colorectal cancer cells. It is known that basal expression of ATF3 is low in normal cells and its expression is stimulated by stress and signals that damage cells (3). To observe differences in basal expression of ATF3 among different human colorectal cancer cell lines, we performed western blot analysis to compare protein levels of endogenous ATF3 in multiple human colorectal cancer cell lines with different genetic backgrounds. As shown in Figure 1, a low level of ATF3 was detected in HCT116, HCT15, HT29 and SW480, whereas higher expression of ATF3 was observed in Caco-2 and LoVo cells. RTPCR results also showed a similar pattern; low *ATF3* mRNA in HCT116 and SW480 cells and increased mRNA in LoVo and Caco-2 cells (data not shown). In a previous study, we observed that overexpression of ATF3 induced apoptosis over 2-fold in HCT116 cells (8). To elucidate a potential anti-apoptotic mechanism of *ATF3* gene, we tested if ATF3 overexpression affects the expression of Bcl-2 family proteins. Overexpression of ATF3 decreased the protein levels of Bcl-

2 in HCT116 and HCT15 cells transfected with pCG-ATF3 expression vector while Bcl-2 band was not detected in SW480 cells (Figure 2A). RT-PCR data showed that *Bcl-2* mRNA was decreased in HCT116, HCT15 and SW480 cells overexpressed ATF3 (Figure 2B). We also tested if knockdown of ATF3 reverses expression of Bcl-2. Knockdown of ATF3 using small interfering RNA (siRNA) elevated expression of Bcl-2 (data not shown). On the other hand, ATF3 overexpression increased the expression of proapoptotic protein, Bak in both protein and mRNA levels (Figure 2C and D). No changes were found in the expression of other Bcl-2 family members including Bax and Bcl-xL. Next, in order to investigate if decreased expression of Bcl-2 is associated with transcriptional down-regulation of the *Bcl-2* gene, we cloned Bcl-2 promoter spanning from –1000 to +715 (Figure 3A). The promoter was co-transfected into HCT116 cells with control or *ATF3* expression vector and then luciferase activity was measured. Results indicated a significant decrease of Bcl-2 promoter activity. Taken together, these data demonstrate that a decrease of Bcl-2 and an increase of Bak expression could be potential mechanisms of increased apoptosis in ATF3-overexpressing human colorectal cancer cells.

ATF3 does not affect cell cycle of human colorectal cancer cells. Since anticancer activity of ATF3 is mediated *via* increased G_1/S arrest in HeLa cells (24), we tested if ATF3 overexpression changes sub-population of each cycle in human colorectal cancer cells. The fraction of each phase in cell cycle was analyzed using PI staining and FACS analysis in HCT116 cells transfected with control or ATF3 expression vector for 48 h. As shown in Figure 4A, ATF3 overexpression (HCT116 OA) resulted in a slight decrease in G_1 phase and an increase in S and G_2/M phase compared to control (HCT116 OC). We also tested the effect of ATF3 knockdown on cell cycle. Inversely, suppression of ATF3 in HCT116 cells resulted in minimal G_1 arrest, and decreased S and G_2/M phase. However, we did not observe any changes in cell cycle-regulatory genes such as *cyclin D1* and cyclin-dependent kinase (CDK) inhibitors, *p21* and *p27* (Figure 4B). These data suggest that ATF3 may show no or minimal effect on the cell-cycle regulation.

ATF3 overexpression leads to an increase of collective cell invasion phenotype. During metastasis, invasive cancer cells divide rapidly, increase the size of primary tumor mass, invade the surrounding microenvironment and migrate. Cell migration occurs in two major modes. Single-cell migration with no cell-cell adhesion and collective cell migration with retained cell-cell junctions (25). To test the effect of ATF3 on the initial stage of metastasis, we established a stable cell line overexpressing ATF3 and analyzed the single cell migration using a Boyden chamber. The result indicates that

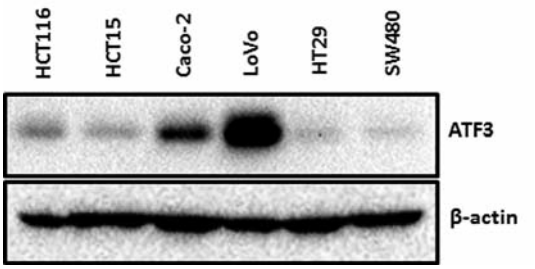

Figure 1. *Basal expression of ATF3 in different human colorectal cancer cell lines. Different human colorectal cancer cell lines (HCT116, HCT15, Caco-2, LoVo, HT-29 and SW480) were harvested and western blot analyses were performed to analyze the expression of ATF3 and β-actin.*

ectopic expression of ATF3 in HCT116 and HCT15 cells does not affect single-cell motility (data not shown). Next, to test the effect of ATF3 on tumorigenic and metastasis potential of HCT116, we performed a tumor sphere-forming assay under non-adherent conditions. As shown in Figure 5A, ATF3-overexpressing HCT116 cells were typically approximately 200-300 μm in diameter after 14 days, whereas control HCT116 cells were 100-150 μm in diameter after 14 days. In addition, ATF3-overexpressing HCT116 cells showed more condensed cell density and collective invasion budding phenotype sites. Our result suggests that overexpression of ATF3 can promote tumor growth and collective invasion of human colorectal cancer cells. Tumorigenic cells are enriched in the fraction of cells that express high levels of CD44 (26). CD44 variant isoforms switching is associated to primary tumor growth and metastatic phenotype in colorectal cancer (27, 28). Thus, we performed western blotting to analyze the protein expression of CD44 and gain a better understating over the role of ATF3 in colorectal cancer. As shown in Figure 5B, overexpression of ATF3 increased the expression of both standard and variant splice forms of CD44 and suppressed the expression of Rb and ZO-1 compared to control, respectively. These changes are crucial for initiating collective cell invasion and metastatic progression (29). *CD44* splice variant switching is required to induce EMT in human epithelium (30).

Down-regulation of EMT-related genes by ATF3 in human colorectal cancer cells. EMT is an important cellular and molecular event during cancer progression and metastasis. We next examined whether ectopic expression of ATF3 in human colorectal cancer cells (HCT116, SW480 and HT29) affect the Snail family members Snail and Slug which has been known to trigger EMT during tumorigenesis (31). Unexpectedly, we found the Snail and Slug transcription factors were dramatically suppressed in HCT116, SW480 and HT29 cells transfected with ATF3 expression vector. The

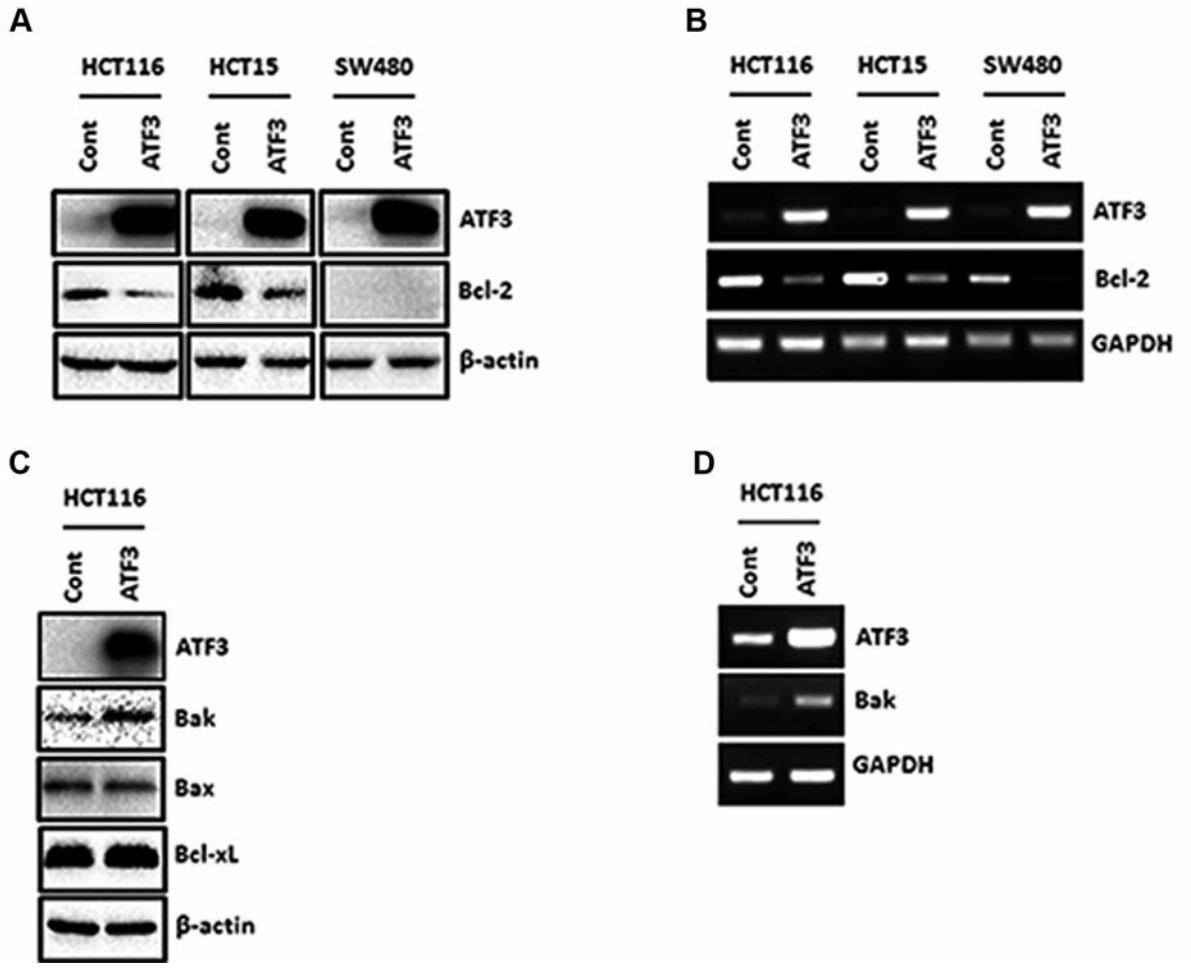

Figure 2. *ATF3 inversely regulates the expression of Bcl-2 in human colorectal cancer cells. (A, C) Control or pCG-ATF3 expression vector was transfected into human colon cancer cell lines using lipofectamine 2000 for 48 h and then the cells were harvested. Western blot analysis was performed against the indicated antibodies. (B, D) Control or pCG-ATF3 expression vector was transfected into human colorectal cancer cell lines using lipofectamine2000 for 48 h and then RT-PCR was performed using primers (Table I) for indicated genes.*

activation of GSK3β can negatively regulate Snail and Slug (32, 33) and inhibit β-catenin expression (34). To determine the suppression of Snail and Slug transcription factors through regulation of GSK3β in ATF3-overexpressing HCT116, we examined the expression of GSK3β and β-catenin, another downstream target of GSK3β by western blot. As a result, ATF3 overexpression increased GSK3β and decreased β-catenin expression in HCT116 cells (Figure 6B). Our data suggested that ectopic expression of ATF3 promotes tumorsphere formation with collective invasion phenotype, but not EMT.

Discussion

ATF3 is a stress-inducible gene and responds to a variety of signals including DNA damage, hypoxia, anoxia, chemicals and microenvironment (3). Therefore, ATF3 is considered an adaptive-response gene that participates in cellular processes to adapt to extra- and/or intra-cellular changes and responds to signals disrupting homeostasis.

In this study, we cloned *Bcl-2* promoter and tested if ATF3 directly influences *Bcl-2* promoter activity. ATF3 suppressed the promoter activity and decreased the expression of Bcl-2 protein. Therefore, ATF3 may regulate Bcl-2 transcription by direct binding to the promoter region of Bcl-2. We identified three potential ATF3 binding sites in the promoter region we cloned (-841 to -830, -797 to -791, and -614 to -608). Further study using internal deletion clones of each CREB binding site is required to elucidate which cis-acting element is pivotal for ATF3-mediated Bcl-2 down-regulation. On the other hand, Bak, the pro-apoptosis protein from Bcl-2 family, is activated by ATF3.

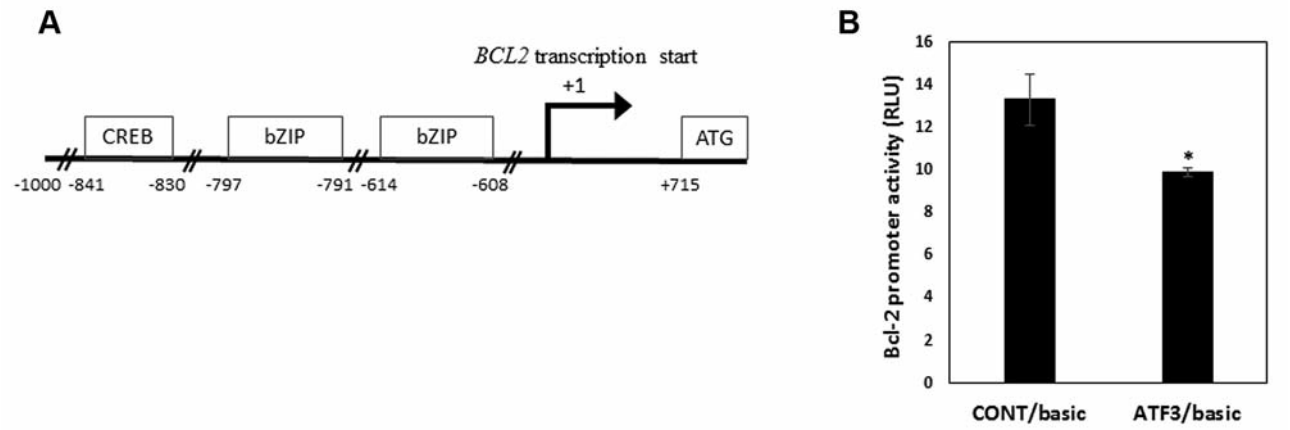

Figure 3. *ATF3 suppresses transcriptional activity of Bcl-2 promoter. (A) Schematic diagram of human Bcl-2 promoter. Human Bcl-2 promoter spanning from -1000 to +715 base pairs was cloned into pGL3-basic vector (Promega) using In-Fusion cloning method (Clontech). Three potential binding sites for ATF3 are indicated. (B) HCT116 cells were co-transfected with human Bcl-2 promoter or pGL-basic (empty) promoter with control or ATF3 expression vector. The cell lysates were isolated and luciferase activity was measured. Values are means±SD, n=3. *Significant at p≤0.05.*

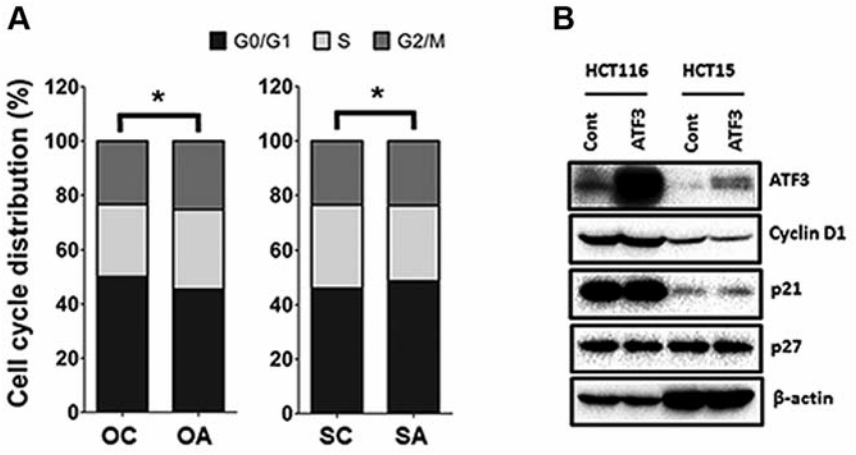

Figure 4. *ATF3 shows a minimal effect on cell-cycle regulation. (A) HCT116 cells were transfected with control (OC) or ATF3 expression vector (OA) using PolyJet for 48 h. HCT116 cells were also transfected with control (SC), or ATF3 siRNA (SA) using TransIT-TKO 48 h. The cell cycle was analyzed by FACS. Values are means±SD, n=3. (B) Control or ATF3 expression vectors were transfected into the cells using PolyJet for 48 h and then the cells were harvested. Western blot analysis was performed against indicated antibodies. *Significant at p≤0.05.*

Our data indicate that increase of ATF3 expression has a minimal effect on cell-cycle regulation and no changes in cell cycle-regulating proteins such as cyclins and cyclin-dependent kinases (CDKs) and CDK inhibitors, p21 and p27. However, Fan *et al*., reported that overexpression of ATF3 using the tetracycline-inducible system moderately reduced progression of cells from G_1 to S phase in HeLa cells, indicating that the ATF3 protein might be a candidate in the control of cell-cycle arrest (24). This might be due to the different cell type and different induction system.

One of interesting findings of the current study is that ectopic expression of ATF3 in colon cancer cells increases invasion phenotype, a hub of biological network to promote cancer progression and metastasis. According to our result, increased tumorsphere formation and CD44-positive cells were also found in ATF3-overexpressing breast cancer cells (35). Recently, Wu *et al*. reported that knockdown of ATF3 in LoVo and Caco-2 cells suppressed *in vitro* cancer cell migration and invasion as well as *in vivo* tumor growth and liver metastasis (36). We also found that ATF3 overexpression decreased Rb and stimulated CD44, markers of colon cancer stem cell. A sub-population of cancer stem cells and interaction with stromal signals are pivotal for metastatic colonization and tumor expansion. To elucidate

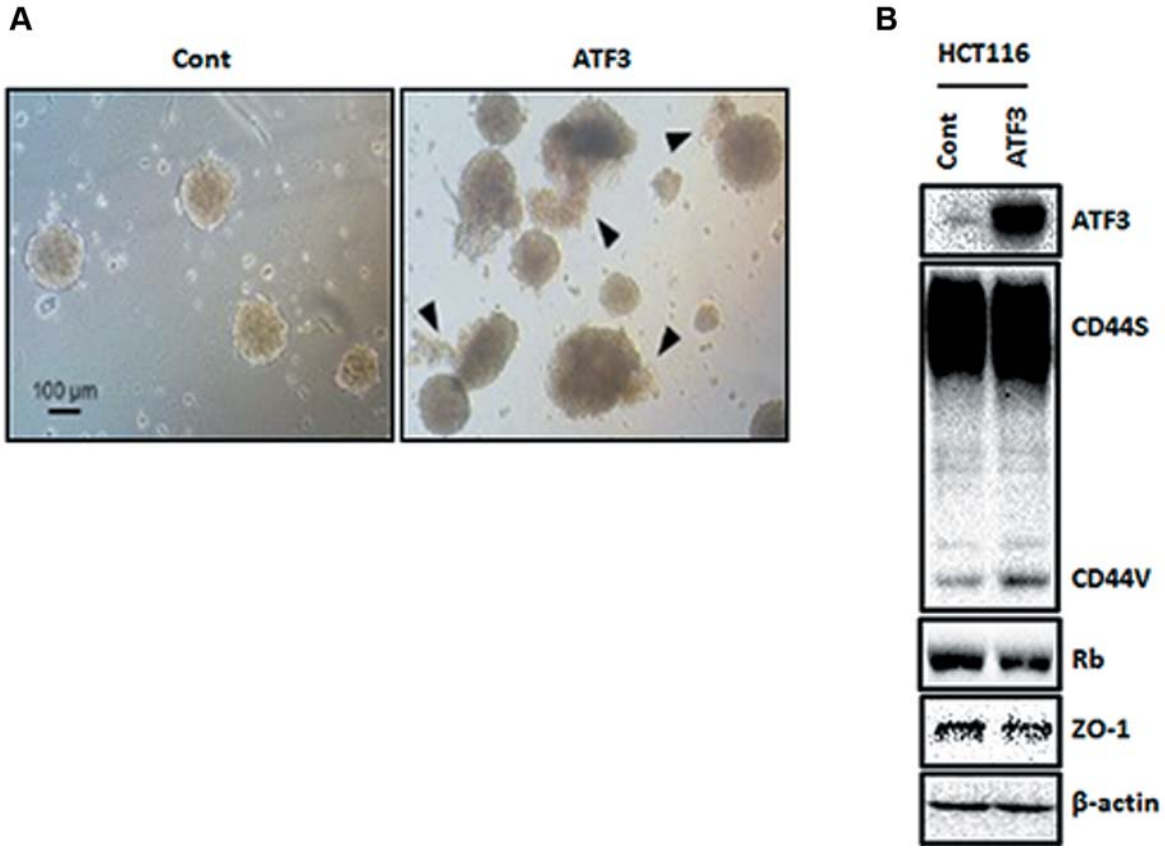

Figure 5. *ATF3 increases collective cell invasion. (A) Phase contrast image of tumorsphere-forming assay. HCT116 cells overexpressing control or ATF3 expression vector were used to test the collective invasion potency. (B) Western blot was performed with the indicated antibodies.*

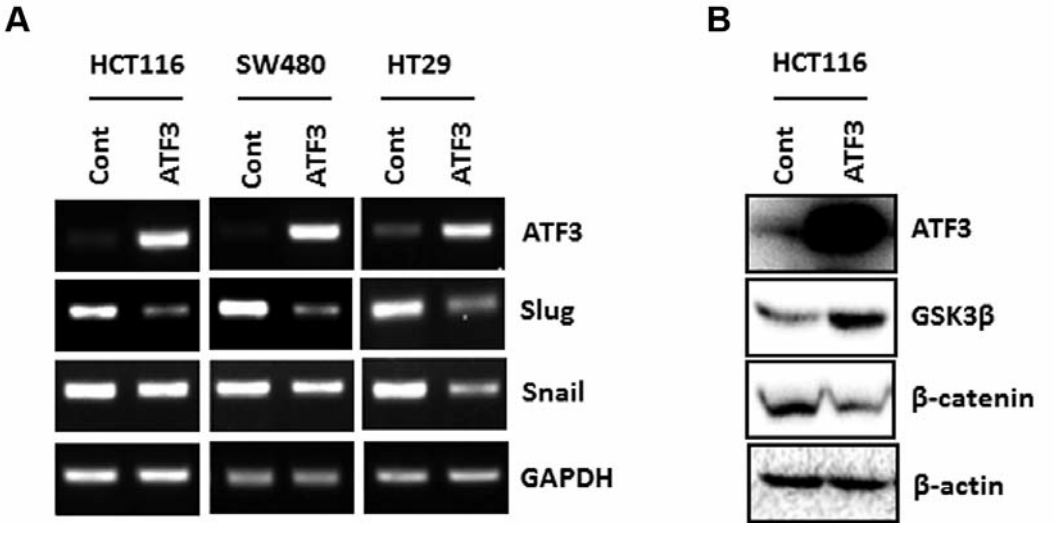

Figure 6. *ATF3 down-regulates EMT inducers and β-catenin. (A) Human colorectal cancer cells (HCT116, SW480, HT29) were transfected with control or ATF3 expression vector and RT-PCR was performed for indicated genes. (B) Western blot was performed to analyze the expression level of indicated proteins in HCT116 cells transfected with control or ATF3 expression vector.*

the mechanism of cell migration, further study to examine cell surface proteases including MMP2 is required, because this is an early event of collective cell movement and associated with ECM remodeling.

Another interesting finding is that ATF3 suppresses the expression of EMT-associated genes in human colorectal cancer cells. We speculate that enhanced tumorsphere-forming and invasion phenotype by ATF3 overexpression might be of an EMT-independent manner. Recently, EMT-independent migration pathways have been documented (37, 38). Herein, we propose two mechanisms. Firstly, ATF3 overexpression leads to the suppression of Wnt pathway with increase of GSK-3β and subsequently decrease of β-catenin expression (Figure 6B). In fact, GSK3β promotes proteasomal degradation of β-catenin and inhibits translocation of β-catenin to the nucleus and transcription of their target genes including EMT-suppressing genes (39). Secondly, ATF3 may suppress TGFβ-induced EMT. To test this hypothesis, we studied the effect of ATF3 overexpression on TGFβ-induced expression of EMT-related gene. However, we did not see morphological changes for EMT in human colorectal cancer cells treated with TGFβ (data not shown). It is probably due to mutation of TGFβ type II receptor in colorectal cancer (40).

Regarding the clinicopathological features of patients, Wu *et al*., observed that expression of ATF3 was much higher in colon tissues of cancer patients compared to matched non-cancerous colon tissues, supporting a potential cancer-promoting activity of ATF3 (36).

Interestingly, we observed that another colorectal cancer cell line, HCT15 showed different expression profile in EMT-related genes. Unlike HCT116, SW480 and HT29 cells, HCT15 cells had elevated *Slug*, *Snail* and *vimentin* mRNA expression level after ATF3 overexpression (data not shown). We do not know why HCT15 responds to ATF3 in a different way in terms of EMT. Further investigation is required to address this issue. Given the importance of ATF3 in *in vitro* cancer phenotypes, it is crucial to investigate how ATF3 functions in *in vivo* situation because mechanisms for biological activities of ATF3 are complex and different from *in vitro* conditions.

Conclusion

ATF3 inversely modulated the expression of Bcl-2 in different colon cancer cells. In addition, ectopic expression of ATF3 enhanced the tumorsphere-forming ability with collective invasion phenotype through activation of a variant from of CD44 and inactivation of Rb and ZO-1 protein and silencing of the EMT-associated transcription factors including Snail and Slug. Hence, these findings provide evidence to support a dual role of ATF3 in colorectal cancer progression.

Acknowledgements

The Authors thank Elizabeth Leahy for her critical reading of manuscript. This work was supported by start-up (S-H Lee) funds from University of Maryland.

References

1 Siegel RL, Miller KD and Jemal A: Cancer statistics, 2015. CA Cancer J Clin *65*: 5-29, 2015

2 Yadav RK, Chae SW, Kim HR and Chae HJ: Endoplasmic reticulum stress and cancer. J Cancer Prev *19*: 75-88, 2014.

3 Hai T, Wolford CC and Chang YS: ATF3, a hub of the cellular adaptive-response network, in the pathogenesis of diseases: is modulation of inflammation a unifying component? Gene Exp *15*: 1-11, 2010.

4 Liang G, Wolfgang CD, Chen BP, Chen TH and Hai T: ATF3 gene. Genomic organization, promoter, and regulation. J Biol Chem *271*: 1695-1701, 1996.

5 Ameri K, Hammond EM, Culmsee C, Raida M, Katschinski DM, Wenger RH, Wagner E, Davis RJ, Hai T, Denko N and Harris AL: Induction of activating transcription factor 3 by anoxia is independent of p53 and the hypoxic HIF signalling pathway. Oncogene *26*: 284-289, 2007.

6 Tanaka Y, Nakamura A, Morioka MS, Inoue S, Tamamori-Adachi M, Yamada K, Taketani K, Kawauchi J, Tanaka-Okamoto M, Miyoshi J, Tanaka H and Kitajima S: Systems analysis of ATF3 in stress response and cancer reveals opposing effects on pro-apoptotic genes in p53 pathway. PLoS One *6*: e26848, 2011.

7 Hai T, Jalgaonkar S, Wolford CC and Yin X: Immunohistochemical detection of activating transcription factor 3, a hub of the cellular adaptive-response network. Methods Enzymol *490*: 175-194, 2011.

8 Yamaguchi K, Lee S-H, Kim JS, Wimalasena J, Kitajima S and Baek SJ: Activating transcription factor 3 and early growth response 1 are the novel targets of LY294002 in a phosphatidylinositol 3-kinase-independent pathway. Cancer Res *66*: 2376-2384, 2006.

9 Baek SJ, Kim JS, Jackson FR, Eling TE, McEntee MF and Lee S-H: Epicatechin gallate-induced expression of NAG-1 is associated with growth inhibition and apoptosis in colon cancer cells. Carcinogenesis *25*: 2425-2432, 2004.

10 Lee S-H, Kim J-S, Yamaguchi K, Eling TE and Baek SJ: Indole-3-carbinol and 3, 3'-diindolylmethane induce expression of NAG-1 in a p53-independent manner. Biochem Biophys Res Commun *328*: 63-69, 2005.

11 Kim KJ, Lee J, Park Y, Lee S-H: ATF3 Mediates Anti-Cancer Activity of Trans-10, cis-12-Conjugated Linoleic Acid in Human Colon Cancer Cells. Biomol Ther *23*: 134-140, 2015.

12 Lee S-H, Bahn JH, Whitlock NC and Baek SJ: Activating transcription factor 2 (ATF2) controls tolfenamic acid-induced ATF3 expression *via* MAP kinase pathways. Oncogene *29*: 5182-5192, 2010.

13 Edagawa M, Kawauchi J, Hirata M, Goshima H, Inoue M, Okamoto T, Murakami A, Maehara Y and Kitajima S: Role of activating transcription factor 3 (ATF3) in endoplasmic reticulum (ER) stress-induced sensitization of p53-deficient human colon cancer cells to tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis through up-regulation of death receptor 5 (DR5) by zerumbone and celecoxib. J Biol Chem *289*: 21544-21561, 2014.

14 Taketani K, Kawauchi J, Tanaka-Okamoto M, Ishizaki H, Tanaka Y, Sakai T, Miyoshi J, Maehara Y and Kitajima S: Key role of ATF3 in p53-dependent DR5 induction upon DNA damage of human colon cancer cells. Oncogene *31*: 2210-2221, 2012.

15 Liu J, Edagawa M, Goshima H, Inoue M, Yagita H, Liu Z and Kitajima S:Role of ATF3 in synergistic cancer cell killing by a combination of HDAC inhibitors and agonistic anti-DR5 antibody through ER stress in human colon cancer cells. Biochem Biophys Res Commun *445*: 320-326, 2014.

16 Mashima T, Udagawa S and Tsuruo T: Involvement of transcriptional repressor ATF3 in acceleration of caspase protease activation during DNA damaging agent-induced apoptosis. J Cell Physiol *188*: 352-358, 2001.

17 Nakagomi S, Suzuki Y, Namikawa K, Kiryu-Seo S and Kiyama H: Expression of the activating transcription factor 3 prevents c-Jun N-terminal kinase-induced neuronal death by promoting heat shock protein 27 expression and Akt activation. J Neurosci *23*: 5187-5196, 2003.

18 Yin X, Dewille J and Hai T: A potential dichotomous role of ATF3, an adaptive-response gene, in cancer development. Oncogene *27*: 2118-2127, 2008.

19 Lu D, Wolfgang CD and Hai T: Activating transcription factor 3, a stress-inducible gene, suppresses Ras-stimulated tumorigenesis. J Biol Chem *281*: 10473-10481, 2006.

20 Bottone FG, Moon Y, Kim JS, Alston-Mills B, Ishibashi M and Eling TE: The anti-invasive activity of cyclooxygenase inhibitors is regulated by the transcription factor ATF3 (activating transcription factor 3). Mol Cancer Ther *4*: 693-703, 2005.

21 Ishiguro T, Nagawa H, Naito M and Tsuruo T: Inhibitory effect of ATF3 antisense oligonucleotide on ectopic growth of HT29 human colon cancer cells. Cancer Sci *91*: 833-836, 2000.

22 Bandyopadhyay S, Wang Y, Zhan R, Pai SK, Watabe M, Iiizumi M, Furuta E, Mohinta S, Liu W and Hirota S: The tumor metastasis suppressor gene Drg-1 down-regulates the expression of activating transcription factor 3 in prostate cancer. Cancer Res *66*: 11983-11990, 2006.

23 Choi J, Jiang X, Jeong JB and Lee SH: Anticancer activity of protocatechualdehyde in human breast cancer cells. J Med Food *17*: 842-848, 2014.

24 Fan F, Jin S, Amundson SA, Tong T, Fan W, Zhao H, Zhu X, Mazzacurati L, Li X and Petrik KL: ATF3 induction following DNA damage is regulated by distinct signaling pathways and over-expression of ATF3 protein suppresses cells growth. Oncogene *21*: 7488-7496, 2002.

25 Ewald AJ, Brenot A, Duong M, Chan BS and Werb Z: Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. Dev Cell *14*: 570-581, 2008.

26 Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, Shelton AA, Parmiani G, Castelli C and Clarke MF: Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci USA *104*: 10158-10163, 2007.

27 Banky B, Raso-Barnett L, Barbai T, Timar J, Becsagh P and Raso E: Characteristics of CD44 alternative splice pattern in the course of human colorectal adenocarcinoma progression. Mol Cancer *11*: 83, 2012.

28 Ropponen KM, Eskelinen MJ, Lipponen PK, Alhava E and Kosma VM: Expression of CD44 and variant proteins in human colorectal cancer and its relevance for prognosis. Scand J Gastroenterol *33*: 301-309, 1998.

29 Kim KJ, Godarova A, Seedle K, Kim MH, Ince TA, Wells SI, Driscoll JJ and Godar S: Rb suppresses collective invasion, circulation and metastasis of breast cancer cells in CD44-dependent manner. PLoS One *8*: e80590, 2013.

30 Brown RL, Reinke LM, Damerow MS, Perez D, Chodosh LA, Yang J and Cheng C: CD44 splice isoform switching in human and mouse epithelium is essential for epithelial-mesenchymal transition and breast cancer progression. J Clin Invest *121*: 1064-1074, 2011.

31 Nieto MA: The snail superfamily of zinc-finger transcription factors. Nat Rev Mol Cell Biol *3*: 155-166, 2002.

32 Kao SH, Wang WL, Chen CY, Chang YL, Wu YY, Wang YT, Wang SP, Nesvizhskii AI, Chen YJ, Hong TM and Yang PC: GSK3beta controls epithelial-mesenchymal transition and tumor metastasis by CHIP-mediated degradation of Slug. Oncogene *33*: 3172-3182, 2014.

33 Liu CW, Li CH, Peng YJ, Cheng YW, Chen HW, Liao PL, Kang JJ and Yeng MH: Snail regulates Nanog status during the epithelial-mesenchymal transition *via* the Smad1/Akt/GSK3beta signaling pathway in non-small-cell lung cancer. Oncotarget *5*: 3880-3894, 2014.

34 Hu D, Bi X, Fang W, Han A and Yang W: GSK3beta is involved in JNK2-mediated beta-catenin inhibition. PLoS One *4*: e6640, 2009.

35 Yin X, Wolford CC, Chang YS, McConoughey SJ, Ramsey SA, Aderem A and Hai T: ATF3, an adaptive-response gene, enhances TGFβ signaling and cancer-initiating cell features in breast cancer cells. J Cell Sci *123*: 3558-3565, 2010.

36 Wu ZY, Wei ZM, Sun SJ, Yuan J and Jiao SC: Activating transcription factor 3 promotes colon cancer metastasis. Tumour Biol *35*: 8329-8334, 2014.

37 Gao J, Zhu Y, Nilsson M and Sundfeldt K: TGF-beta isoforms induce EMT independent migration of ovarian cancer cells. Cancer Cell Int *14*: 72, 2014.

38 Fang JH, Zhou HC, Zhang C, Shang LR, Zhang L, Xu J, Zheng L, Yuan Y, Guo RP, Jia WH, Yun JP, Chen MS, Zhang Y and Zhuang SM: A novel vascular pattern promotes metastasis of hepatocellular carcinoma in an epithelial-mesenchymal transition-independent manner. Hepatology *62*: 452-465, 2015.

39 Lamouille S, Xu J and Derynck R: Molecular mechanisms of epithelial–mesenchymal transition. Nat Rev Mol Cell Biol *15*: 178-196, 2014.

40 Xu Y and Pasche B: TGF-beta signaling alterations and susceptibility to colorectal cancer. Hum Mol Genet *16*: R14-20, 2007.

Received December 14, 2015
Revised January 16, 2016
Accepted January 18, 2016