RESEARCH COMMUNICATION

Activating Transcription Factor 1 is a Prognostic Marker of Colorectal Cancer

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Abstract

Objective: Identifying cancer-related genes or proteins is critical in preventing and controlling colorectal cancer (CRC). This study was to investigate the clinicopathological and prognostic value of activating transcription factor 1 (ATF1) in CRC. Methods: Protein expression of ATF1 was detected using immunohistochemistry in 66 CRC tissues. Clinicopathological association of ATF1 in CRC was analyzed with chi-square test or Fisher's exact test. The prognostic value of ATF1 in CRC is estimated using the Kaplan-Meier analysis and Cox regression models. Results: The ATF1 protein expression was significantly lower in tumor tissues than corresponding normal tissues (51.5% and 71.1%, respectively, P = 0.038). No correlation was found between ATF1 expression and the investigated clinicopathological parameters, including gender, age, depth of invasion, lymph node status, metastasis, pathological stage, vascular tumoral emboli, peritumoral deposits, chemotherapy and original tumor site (all with P > 0.05). Patients with higher ATF1 expression levels have a significantly higher survival rate than that with lower expression (P = 0.026 for overall survival, P = 0.008 for progress free survival). Multivariate Cox regression model revealed that ATF1 expression and depth of invasion were the predictors of the overall survival (P = 0.008 and P = 0.028) and progress free survival (P = 0.002 and P = 0.005) in CRC. Conclusions: Higher ATF1 expression is a predictor of a favorable outcome for the overall survival and progress free survival in CRC.

Keywords: ATF1 - coloretal cancer - prognosis

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females, with over 1.2 million estimated new cancer cases and 608,700 estimated deaths to have occurred in 2008, according to the global cancer statistics 2011 (Jemal et al., 2011). In the United States, CRC is the third leading cancer types for the estimated new cancer cases and deaths by both males and females (Siegel et al., 2012). Identifying cancer-related genes or proteins is critical in preventing and controlling colorectal cancer.

Activating transcription factor 1 (ATF1) belongs to the ATF/CREB family of transcription factors, which represents a large group of basic region-leucine zipper (bZip) proteins, specifically binding to the consensus ATF/CRE site 'TGACGTCA' (Hai and Hartman, 2001). A dimeric complex named the AP-1 (activator protein 1) transcription factor that contains members of the JUN, FOS, ATF and MAF protein families plays an important role in tumor development (Eferl and Wagner, 2003). The first report of ATF1 in cancer is in malignant melanoma of soft parts, where a fusion gene EWS/ATF1 was found and functioned as a tumor promoter (Zucman et al., 1993). The DNA binding activity of the EWS/ATF1 fusion protein is required for tumor cell viability in clear cell sarcoma (Bosilevac et al., 1999).

As an independent molecule, not a fusion protein, ATF1 is found to be over-expressed in lymphomas and in activated lymphocytes, and play an active role in cell growth and differentiation (Hsueh and Lai, 1995). Overexpression of ATF1 in nasopharyngeal carcinoma is associated with clinical stage (Su et al., 2011). Cyclin-dependent kinase (CDK) 3-mediated ATF1 phosphorylation enhances proliferation and cell transformation (Zheng et al., 2008). Expression of single chain antibody fragment anti-ATF-1 in melanoma cells reduces CRE-dependent promoter activation, and suppresses their tumorigenicity and metastatic potential in nude mice (Jean et al., 2000).

It is clear that ATF1 plays an important role in cancer development. In CRC, however, the role of ATF1 is not

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characterized. In this study, we examined the protein expression of ATF1 in CRC tissues. Clinicopathological association and prognostic value of ATF1 in CRC was further analyzed in order to investigate the role of ATF1 in CRC.

Materials and Methods

Patients and tissue samples

All 66 patients with CRC received surgical resection during 2004 at the Affiliated Cancer Hospital of Zhengzhou University. CRC and corresponding non-tumor colorectal tissues were collected at the time of surgery. The patients received no other therapies, such as radiation or chemotherapy, before the operation. All samples were pathologically diagnosed as CRC by two experienced pathologists. Tumor staging was established according to 7th edition of the Cancer Staging Manual of the American Joint Committee on Cancer (AJCC) (Gunderson et al., 2010). The 66, and 35 repectively CRC patients included 36 males and 31 females with a median age of 59 years (range, 30-85 years). The patients were followed up annually with a median follow-up time of 70 months. Informed consent was obtained from all subjects, and this study was approved by the Institutional Ethics Committee.

Immunohistochemistry (IHC) detection

Formalin-fixed and paraffin-embedded (FFPE) specimens were cut into 4-μm sections, mounted onto the polylysine-coated slides, deparaffinized in xylene, and rehydrated in a graded ethanol series. The sections were quenched for endogenous peroxidase with 3% hydrogen peroxide, and then boiled in EDTA (1 mmol/L; pH8.0) for 15 min in a microwave oven for antigen retrieval. The sections were incubated with 10% normal goat serum in phosphate-buffered saline (PBS) at room temperature for 1 hour to block nonspecific protein binding. After removing the serum, they were incubated at 37 °C for another 1 hour with rabbit anti-ATF1 polyclonal antibody (Abcam Ltd., Hong Kong) diluted 1:200 in blocking solution. After washing three times in PBS, HRP-conjugated secondary antibody (ChemMate Envision Detection Kit, Dako) was applied onto the sections and incubated for 0.5 hour at room temperature according to the manufacturer's instruction. After additional washes, bound antibodies were visualized with 3, 3'-diaminobenzidine tetrahydrochloride (DAB), and then specimens were counterstained with hematoxylin. For negative controls, tissue sections were incubated under the same experimental conditions without anti-ATF1 antibody. ATF1 staining was scored according to its intensity (0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining) and the percentage of positive cells (0, < 5% positive cells; 1, 5%- 25% positive cells; 2, 25-50% positive cells; 3, > 50% positive cells). The final expression score was calculated from 'intensity score' multiplied by 'percentage': '-' stands for scores 0-1, '+' for scores 2-3, '++' for scores 4-6 and '+++' for scores >6. For statistical analysis, we combined the cases scored as '-' and '+' (low score) to compare with the cases with scored as '++' and '+++' (high score).

Statistical analysis

The chi-square test or Fisher's exact test were used to analyze the correlations between clinicopathological features with the ATF1 expressions. The overall survival and progress free survival were estimated using the Kaplan-Meier analysis. The log-rank test was used to evaluate the statistical significance of the differences. The prognostic significance of each clinicopathological feature was determined using the univariate Cox regression analysis. The parameters that were significantly related to survival in the univariate analysis were entered into the multivariate analysis. In a multivariate Cox proportional hazard model, the independent prognostic factors were identified from the significant predictors in univariate analysis with an enter mode. The SPSS version 16.0 software package and GraphPad Prism were used for the statistical analysis and data plotting.

Table 1. Clinicopathologic Correlation with ATF1 Expression

Parameter		ATF1		P value
		Low	High	
Gender	Female	16	15	0.632
	Male	16	19	
Age(years)	<50	11	9	0.485
	>=50	21	25	
Depth of invasion	T1+T2	2	4	0.436
	T3+T4	30	30	
Lymph node status	Positive	13	9	0.223
	Negative	19	25	
Metastasis	No	32	32	0.164
	Yes	0	2	
Pathological stage	I+II	19	24	0.339
	III+IV	13	10	
Vascular tumoral emboli	No	31	32	0.591
	Yes	1	2	
Peritumoral deposits	Negative	24	27	0.669
	Positive	8	7	
Adjuvant chemotherapy	No	10	7	0.322
	Yes	22	27	
Original tumor site	Colon	8	12	0.363
	Rectum	24	22	

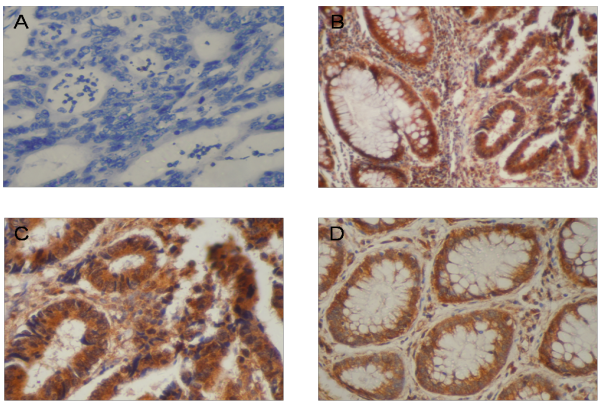

Figure 1. Representative Immunohistochemistry Staining of ATF1 (200×). A, Cancer tissues incubated without primary antibody were used as negative controls. B, Positive brown staining in CRC and corresponding adjacent normal tissues. C, Positive staining of ATF1 in colorectal cancer epithelial cells. D, Positive staining of ATF1 in adjacent normal colorectal tissues

Table 2. Univariate and Multivariate Analyses of Individual Parameters for Correlations with Overall Survival Rate: Cox Proportional Hazards Model

Parameters	Univariate analysis			Multivariate analysis		
	HR	CI(95%)	P-value	HR	CI(95%)	P-value
ATF1	0.427	0.197-0.926	0.031*	0.312	0.132-0.738	0.008*
Gender	0.912	0.434-1.913	0.807			
Age(years)	2.372	0.901-6.245	0.080			
Pathological stage	2.314	1.366-3.921	0.002*	1.402	0.374-5.258	0.616
Depth of invasion	3.105	1.380-6.989	0.006*	3.128	1.133-8.637	0.028*
Lymph node status	2.122	1.324-3.399	0.002*	1.709	0.589-4.955	0.324
Metastasis	1.543	0.209-11.376	0.670			
Vascular tumoral emboli	0.779	0.287-2.113	0.623			
Peritumoral deposits	2.393	1.102-5.197	0.028*	0.811	0.277-2.377	0.703
Original site	0.752	0.319-1.769	0.513			
Chemotherapy	0.774	0.341-1.759	0.542			

*P < 0.05

Table 3. Univariate and Multivariate Analyses of Individual Parameters for Correlations with Progress Free Survival Rate: Cox Proportional Hazards Model

Parameters	Univariate analysis			Multivariate analysis		
	HR	CI(95%)	P-value	HR	CI(95%)	P-value
ATF1	0.357	0.161-0.790	0.011*	0.239	0.096-0.593	0.002*
Gender	0.877	0.418-1.841	0.729			
Age(years)	1.724	0.699-4.255	0.237			
Pathological stage	2.392	1.419-4.032	0.001*	1.951	0.501-7.599	0.335
Depth of invasion	4.071	1.741-9.515	0.001*	5.074	1.648-15.619	0.005*
Lymph node status	2.053	1.298-3.247	0.002*	1.260	0.427-3.720	0.676
Metastasis	1.567	0.213-11.552	0.659			
Vascular tumoral emboli	0.910	0.335-2.468	0.852			
Peritumoral deposits	2.469	1.136-5.369	0.023*	0.760	0.254-2.276	0.624
Original site	0.591	0.239-1.457	0.253			
Chemotherapy	0.989	0.421-2.328	0.981			

*P < 0.05

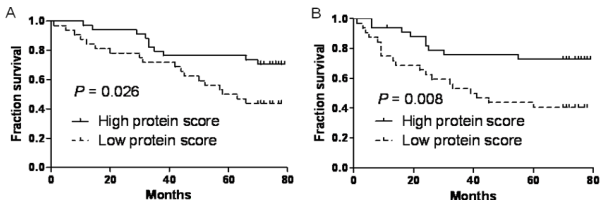

Figure 2. Kaplan-Meier Survival Analysis of CRC Patients According to ATF1 Expression. A, overall survival, log rank test P = 0.026. B, progress free survival, log rank test P = 0.008

Results

ATF1 expression and clinicopathological associations

The protein expression of ATF1 in the colorectal cancer tissues and corresponding adjacent normal counterparts were examined using immunohistochemistry staining. The representative results are shown in Figure 1. Various levels of immunoreactivity for ATF1 were found in cancer and adjacent non-tumor regions. The ATF1 protein expression was significantly lower in tumor tissues than corresponding normal tissues (51.5% and 71.1%, respectively, P = 0.038). The associations between ATF1 expression and clinicopathological parameters were assessed using chi-square test or Fisher's exact test when appropriate. However, no correlation was found between ATF1 expression and the investigated clinicopathological parameters, including gender, age, depth of invasion, lymph node status, metastasis, pathological stage, vascular tumoral emboli, peritumoral deposits, chemotherapy and original tumor site (all with P > 0.05, Table 1).

Associations between ATF1 expression and patient survival

Kaplan-Meier analysis and the log-rank test were performed to analyze the correlation between ATF1 expression and patient survival. According to the Kaplan-Meier survival curves, the lower expression of ATF1 is a significant prognostic factor for poor overall survival and progress free survival in CRC patients. Patients with higher ATF1 expression levels have a significantly higher survival rate than that with lower expression (70.6% and 43.8%, respectively, P = 0.026 for overall survival, Figure 2A; 73.5% and 40.6%, respectively, P = 0.008 for progress free survival, Figure 2B).

To identify the ATF1 expression and other variables of potential prognostic significance in all of the patients with CRC, univariate and multivariate Cox regression models were performed. Univariate Cox regression analysis showed that the ATF1 expression was significantly correlated with overall survival and progress free survival (P = 0.031 for overall survival, Table 2; and P = 0.011 for progress free survival, Table 3). The univariate Cox regression analysis also indicated that clinical variables including pathological stage, depth of invasion, lymph node status, and peritumoral deposits were significantly

associated with overall survival (P = 0.002, P = 0.006, P = 0.002 and P = 0.028, respectively, Table 2) and with progress free survival (P = 0.001, P = 0.001, P = 0.002 and P = 0.023, respectively, Table 3). Furthermore, multivariate Cox egression analyses were performed to evaluate the potential of ATF1 expression as an independent predictor for overall survival or progress free survival of CRC. Multivariate Cox regression model revealed that ATF1 expression and depth of invasion were the predictors of the overall survival (P = 0.008 and P = 0.028, Table 2) and progress free survival (P = 0.002 and P = 0.005, Table 3) in CRC.

Discussion

ATF1 is over-expressed in several cancer types and functioned as a tumor promoter. Hepatocyte growth factor (HGF) plays a major role in the pathogenesis of human epithelial tumors. ATF1 is a mediator of HGF-induced down-regulation of thrombospondin-1 expression, leading to thyroid cancer cell invasion (Ghoneim et al., 2007). Expression of t-Darpp, a cancer-related truncated isoform of Darpp-32, leads to an increase in Bcl-2 protein levels and resistance to ceramide-induced apoptosis. CREB and ATF1 transcription factors are required for t-Darpp up-regulating Bcl-2 levels (Belkhiri et al., 2008).

In this study, the ATF1 protein showed a significantly lower expression in tumor tissues than corresponding normal tissues in CRC. The down-expression of ATF1 in tumor exhibited a controversial result with that ATF1 is over-expressed in lymphoma (Hsueh and Lai, 1995) and nasopharyngeal carcinoma (Su et al., 2011). In fact, the expression of ATF1 in many tumor types is not reported before. In CRC, no data concerning ATF1 is shown. Therefore, we should acknowledge the specific lower expression of ATF1 in CRC.

The prognostic value of ATF1 in CRC was determined with our data. Patients with higher ATF1 expression levels have a significantly higher survival rate than that with lower expression. Multivariate Cox regression model revealed that ATF1 expression and depth of invasion were the predictors of the overall survival and progress free survival in CRC. This function of ATF1 is more like a tumor suppressor in CRC. The results are consistent in our study, but seemed to be contradicted with others.

The function of a transcription factor is dependent on the target protein it regulated. In interleukin 2-stimulated T lymphocytes, promoter activity of the proliferating-cell nuclear antigen gene is associated with inducible CRE-binding proteins, such as ATF1 and CREB (Huang et al., 1994). In the same model, ATF1/CREB is suggested to modulating specific gene expression at G1/S during cell cycle progression (Feuerstein et al., 1995). During myogenic differentiation of C2 cells, RB promoter activity is stimulated partially due to the dissociation of ATF-1, which suppresses the activity of the RB promoter (Okuyama et al., 1996). ATF1 regulates promoter activity of transforming growth factor-beta (TGF-β) 2 and the type II TGF-β receptor in undifferentiated and differentiated embryonal carcinoma cells respectively (Kelly et al., 1995; Kelly et al., 1998).

A dimeric complex that contains members of the JUN, FOS, ATF and MAF protein families is called the AP-1 transcription factor. AP-1 has a double-edged activity -- by inducing apoptosis, it can be tumor suppressing; by signaling cell survival, it can be tumor promoting. The final outcome of AP-1 activity in tumors depends on AP-1 dimer composition itself, as well as the cell type and its differentiation state, tumor stage and genetic background (Eferl and Wagner, 2003). ATF2 is a typical AP-1 molecule with a double-edged activity in a cell- and tissue-dependent context (Lopez-Bergami et al., 2010). In mouse skin tumors, overexpression of ATF2 is required for tumor growth and progression (Papassava et al., 2004). Inhibition of ATF2 in concert with increased JNK/Jun and JunD activities sensitizes melanoma cells to apoptosis and inhibits their tumorigenicity (Bhoumik et al., 2004). Furthermore, ATF2 confers radiation and drug resistance to human melanoma cells (Ronai et al., 1998). By contrast, a suppressor activity of ATF2 is indicated in skin tumor. Loss of ATF2 transcriptional activity promotes skin tumor formation (Bhoumik et al., 2008). The apoptosis-regulated gene GADD45alpha and the breast cancer suppressor gene Maspin are downregulated in the mammary tumors arisen in heterozygous Atf2 mutant (Atf2+/-) mice. By controlling the transcription of Maspin and GADD45 alpha genes, ATF2 is suggested to suppress mammary tumors (Maekawa et al., 2008). These results indicate that ATF2 possesses different function depending on the cellular context.

As a family member of ATF, we believe the function of ATF1 also depends on the cellular and genetic context. In CRC, our data suggested that higher ATF1 expression is a predictor of a favorable outcome for the overall survival and progress free survival. The function of ATF1 in CRC awaits further study.

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