Cancer Epidemiology 33 (2009) 130–136

Contents lists available at ScienceDirect

Cancer Epidemiology

The International Journal of Cancer Epidemiology, Detection, and Prevention

journal homepage: www.elsevier.com/locate/canep

Overexpression of the Interferon regulatory factor 4-binding protein in human colorectal cancer and its clinical significance

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ARTICLE INFO

Article history:
Accepted 28 May 2009

Keywords:
IBP
Antibody
Expression
Human colorectal cancer
Oncogenesis
Differentiation

ABSTRACT

Background: IFN regulatory factor 4-binding protein (IBP) is a novel type of activator of Rho GTPases. It has been linked with differentiation and apoptosis of lymphocytes, but its function in oncogenesis remains unclear. Here we studied the expression of endogenous IBP in four human colorectal cancer cell lines, normal, adenoma and tumor colorectal tissues. *Methods*: Molecular (Western blot and RT-PCR), and confocal analyses were used to investigate IBP expression in human colorectal cancer cell lines. Matched normal and tumor tissue sections of 63 patients and 15 adenoma tissue sections were analyzed for IBP expression by immunohistochemistry (IHC). *Results*: IBP was ubiquitely expressed in human colorectal cancer cell lines. The expression of IBP can be detected at both the mRNA and protein level in SW480, SW620 and HT29 cells. Clinically, IBP were elevated in human colorectal cancer specimens in comparison to normal colorectal tissues. Substantial high expression of IBP was observed in colorectal cancer tissues (67%), whereas corresponding normal tissues and 15 adenoma tissues showed consistently absent immunoreactivity of IBP. Moreover, IBP expression is correlated with the differentiation level of colorectal cancer cells ($p < 0.05$) and clinical stage of patients ($p < 0.01$). *Conclusions*: Our data show, for the first time, a dysregulated expression of IBP in human colorectal cancer, offering new perspectives for its role in cancer development and progression. IBP may be a novel tumor marker and a therapeutic target for colorectal cancer.

1. Introduction

IBP (IRF-4-binding protein, also known as: Def-6, SLAT) was identified during finding out the potential partner of the lymphoid-restricted transcription factor IRF-4 [1–3]. It is expressed in myeloid progenitors but downregulated after induction of differentiation into macrophages, granulocytes, and erythrocytes [4]. Murine IBP, which is expressed at high levels in lymphocyte, plays an important role in the development and maturation of Th2 cells [5] and it is recruited to the immunological synapse upon TCR stimulation [6]. IBP-trapped mice display an obvious defect at the earliest stages of thymocyte development [7]. It also has been found that IBP can regulate cell morphology in cooperation with activated Rac1 [8] and influence cell differentiation in cooperation with integrin [9]. Previous studies [5–9] suggest a significant physiological role for IBP; however, the biological function of IBP in mammalian cells remains largely unexplored. IBP is postulated to be directly associated with cell survival. T cells from IBP-deficient mice display a defective ability to undergo apoptosis via a cell autonomous pathway [10]. Abnormal cell survival and death have been tightly linked with oncogenesis. As yet, however, most of these studies were limited to the immune system. IBP function in tumorigenesis remains unclear, little is known about the expression or the regulation of IBP in human cancer *in vivo*. Therefore, we were interested in detecting the expression of IBP in colorectal cancer, which is the third most prevalent cancer in the world and it is an important cause of cancer-related death. In an effort to investigate the function of IBP in oncogenesis, an anti-IBP rabbit polyclonal antibody was generated and characterized by ELISA, Western blot, ICC, and confocal analysis. IBP expression was evaluated in 63 patients with colorectal cancer and 15 patients with adenoma by IHC, as well as in four human colon cancer cell lines by RT-PCR and Western blot in order to better understand its roles in human colorectal cancer biology and its potential implications for therapeutic intervention.

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doi:10.1016/j.canep.2009.05.004

2. Materials and methods

2.1. DNA constructs

The IBP expression plasmids pFLAG-CMV-2-FLAG-IBP were constructed by cloning the entire coding region of the human IBP cDNA, fused in-frame with a sequence encoding a FLAG epitope at its 5-terminus, into the pFLAG-CMV-2 mammalian cell expression vector (Sigma, St. Louis, MO, USA). An IBP expression plasmid without any epitope tag (pMIG-IBP-EGFP) was also generated by cloning the full-length coding sequence of the human IBP cDNA into the pMIG (Addgene plasmid 9044) mammalian cell expression vector. For the glutathione S-transferase (GST) and thioredoxin (Trx) fusion proteins, GST-IBP (amino acids 410–631) and Trx-IBP (amino acids 410–631) expression plasmids were constructed by cloning the corresponding coding segment of the human IBP cDNA, in-frame, into the pGEX-KG *E. coli* expression vector (Pharmacia, Piscat-away, NJ, USA) and pET-32a *E. coli* expression vector (Novagen, Darmstadt, Germany), respectively. The pGEFP-C_1-IBP (a kind gift from Dr. Alessandra B. Pernis, Columbia University, NY, USA) plasmid was used as the PCR template. Primers involved in this research were shown in Table 1. The in-frame junctions in these constructs were confirmed by DNA sequencing (Invitrogen, Shanghai, China).

2.2. Cell culture, transfection and extraction

All cell lines were obtained from the cell bank of Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). T-cell origin (Jurkat, Hut78 and H9) and B-cell origin (Raji) were grown in RPMI1640 medium (Gibco, Grand Island, NY, USA). Colon cancer cell lines (SW480, SW620, HT29, LoVo) and 293 were grown in Dulbecco's modified Eagle medium F-12 HAM (DMED/F12, Gibco). All media were supplemented with 10% fetal calf serum (Hyclone, Logan, UT, USA) and 100 U/ml penicillin and streptomycin (Gibco), and incubated at 37 °C in a humidified atmosphere with 5% CO_2. For the expression of EGFP-IBP, FLAG-IBP and IBP, 293 cells were transfected with the expression plasmids pEGFP-C1-IBP, pFLAG-IBP and pMIG-IBP by Lipofectamine 2000 (Invitrogen, CA, USA) according to the manufacturer's instructions. After 24-h incubation, the transfected cells were harvested for cell extract preparation. Protein was extracted by T-PER Tissue Protein Extraction Reagent (PIERCE, Rockford, IL, USA) supplemented with Protease Inhibitor Cocktail Tablets (Roche, Mannheim, Germany) following the manufacturer's instructions. Protein concentration was evaluated using the BCA Protein Assay kit (Beyotime Bio., Haimen, China).

2.3. Expression, purification of GST-IBP and Trx-IBP fusion proteins

The fusion proteins were expressed in *E. coli* BL21 by inducing with 1 mM IPTG for 6 h at 37 °C. His affinity purification and anion-exchange chromatography were sequentially used for the purification of Trx and Trx-IBP fusion proteins, meanwhile GST affinity purification and anion-exchange chromatography were used for GST and GST-IBP fusion proteins following the manufacturer's protocol (Amersham Biosciences, Piscataway, NJ, USA). Protein purity and integrity were confirmed by SDS-polyacrylamide gel analysis.

Table 1
Sequence of primers and products for PCR and RT-PCR.

Purpose	Sequence of primers	Product (bp)
Trx-IBP	5′-CGGAATTCATGCAGGCTGAGATGGA-3′ 5′-GACGTCGACTATTTTCTGGTGCTGGATC-3′	684
GST-IBP	5′-CGGAATTCATATGCAGGCTGAGATGGAG-3′ 5′-CCCAAGCTTATTTTCTGGTGCTGGATC-3′	685
pFLAG-IBP	5′-ATAAGCTTATGGCCCTGCGCAAGGAACT-3′ 5′-TAGGTACCTTAATTTTCTGGTGCTGGATCC-3′	1912
pMIG-IBP	5′-GAAGATCTGCCACCATGGCCCTGCGCAAG-3′ 5′-GGAATTCTTAATTTTCTGGTGCTGGATCC-3′	1917
IBP	5′-ATAAGCTTATGGCCCTGCGCAAGGAACT-3′ 5′-TAGGTACCTTAATTTTCTGGTGCTGGATCC-3′	1912
GAPDH	5′-GGAGTCCACTGGCGTCTT-3′ 5′-CCTGCTTCACCACCTTCTT-3′	505

2.4. Polyclonal antibodies

Polyclonal antiserum was raised in rabbits according to the modified method first reported by Hu [11]. Rabbits were subcutaneously injected with 300 μg of Trx-IBP in complete Freund's adjuvant twice, on days 0 and 3, and incomplete Freund's adjuvant once on day 31. The IgG fractions were purified using Protein A-Sepharose (Amersham Biosciences). To remove antibodies reacting to the Trx tag and residual bacterial protein, the antibodies eluted from the Protein A column were passed sequentially over CNBr-activated Sepharose 4 Fast Flow (GE Healthcare, Uppsala, Sweden) to which a bacterial protein, expressed from a empty pET-32a vector, had been coupled according to the instructions provided by the manufacturer.

2.5. ELISA

The reactivity of antibodies with Trx, Trx-IBP, GST and GST-IBP fusion proteins were tested by ELISA. Ninety-six well microtiter plates were coated with 0.5 μg/well antigens and then blocked with 5% skim milk in PBS. Purified anti-IBP antibodies (1:2000) were incubated for 1 h at 37 °C and washed with PBS containing 0.1% Tween-20 (PBST) for six times. A 1:4000 dilution of horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG antibody (Zhong Shan Co., Beijing, China) was added for 1 h at 37 °C. Again, the microtiter plates were washed six times in PBST. Bound antibodies were detected by incubation with substrate (o-phenylenediamine, 0.4 mg/ml). OD492 was determined by using a microplate reader (Bio-Rad model 550).

2.6. Western blotting and blocking experiment

Whole cell lysates (80 μg of total protein) were separated on 7% SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore Co., MA, USA). The membranes were blocked for 1 h at 37 °C in 5% skim milk/PBST and then incubated with primary antibodies in 5% skim milk/PBST for 1 h at 37 °C. Primary antibodies used were: anti-IBP polyclonal antibodies (1:4000) and anti-tubulin monoclonal antibodies (1:2000, Beyotime Bio). Following three washes in PBST, the membranes were then incubated for 1 h at room temperature in PBST containing a 1:10,000 dilution of the second antibody (goat anti-mouse or rabbit IgG, Zhong Shan Co.). Again, the membranes were washed five times in PBST. Bound antibodies were developed with enhanced chemiluminescence (ECL) detection system (Millipore Co., MA, USA). Signals were visualized by autoradiography.

Blocking experiment was performed as follows: anti-IBP polyclonal antibodies were first incubated with antigen GST-IBP for 1 h at 37 °C, the final concentration of antibody and antigen was 200 ng/ml and 100 μg/ml, respectively. Then, the mixture was used for primary antibody as previous description. Antibodies pre-incubated with GST were used for control.

2.7. RNA and RT-PCR

RNA were extracted and purified by the TRIzol Reagent kit (Gibco) according to the manufacturer's recommendation. Reverse transcription was performed using the ReverTra-Plus-TM (Toyobo,

Japan) according to the manufacturer's protocol. cDNA-specific IBP full-length (coding region 1–1896) sequence and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) primers were designed (Table 1) and synthesized by Invitrogen, ShangHai, China. The PCR reaction system (KOD-plus DNA polymerase PCR kit, Toyobo) consists of 5 μl 10× KOD-plus buffer, 5 μl dNTP (10 mmol/l), 4 μl Mg^{2+} (250 mmol/l), 1 μl F/R primer (10 μmol/l), 1 μl KOD-plus (5 U/μl), 1 μl cDNA, adjusted to 47 μl with distilled water and finally added with 3 μl DMSO. IBP amplification reactions consisted of 35 cycles (94 °C for 30 s, 60 °C for 30 s and 68 °C for 120 s) followed by 5 min final extension at 68 °C. The product was analyzed on 0.8% agarose gel electrophoresis.

2.8. Immunocytochemistry

Jurkat cells were arrayed on adhesive coated slides, then fixed with 10% formalin and blocked with Immunol Staining Blocking Buffer (Beyotime). IBP was detected with anti-IBP polyclonal antibodies (1:400) followed by the incubation with Streptavidin-Peroxidase ICH kit (Zhong Shan Co.) according to the instructions provided by the manufacturer. After staining with DAB kit (Zhong Shan Co.), the result was viewed microscopically. Purified IgG from normal rabbit sera was used as control.

2.9. Confocal immunofluorescence microscopy

SW480 and Jurkat cells were arrayed on adhesive coated slides. All slides were fixed with 10% formalin and blocked with Immunol Staining Blocking Buffer (Beyotime), then washed with TBSTx buffer (50 mM Tris, 0.15 M NaCl, pH 7.6 containing 0.1% Triton X-100 and Tween-20) and incubated with anti-IBP polyclonal antibodies (1:400). After washing thrice with TBSTx buffer, slides were incubated with Cy3 labeled goat anti-rabbit IgG (diluted 1:1000 in Immnol Fluoresce Staining Secondary Antibody Dilution Buffer, Beyotime). Purified IgG from normal rabbit serum was used as a control. Nuclei were stained with 0.1 μg/ml Hoechst (Sigma, St. Louis, MO, USA) in PBS for 10 min. Slides were mounted and viewed with a Leica confocal microscope (Leica, Heidelberg, Germany).

2.10. Tissue specimens

Colorectal cancer tissues and normal colorectal tissues were collected from 63 patients undergoing surgical resections of primary colorectal cancer, as well as adenoma tissues were collected form 15 patients at the Southwest Hospital affiliated to Third Military Medical University between January 2005 and 2008. None of the patients had accepted any chemotherapy or radiotherapy before the surgical resection. The tissue blocks were sectioned and stained with hematoxylin and eosin (H&E), and then graded. The study protocols were approved by the local Institutional Review Board (IRB), and informed consent was obtained from patients. Table 2 summarizes the most important clinicopathological data on the patients.

2.11. Immunohistochemical

The sections were deparaffinised in xylene, rehydrated in alcohol and water, antigen repaired and blocked (including an endogenous peroxidase blocking). Anti-IBP antibodies (1:400) were incubated overnight at 4 °C in a sealed chamber followed by the HRP-labeled polymer for 30 min (EnVision+ System, Dako Cytomation, Cambridgeshire, UK). Those sections were then stained with DAB (Dako Cytomation) for 5 min. All sections were counterstained with hematoxylin, dehydrated and mounted. Purified IgG from normal rabbit serum was used as control. Ten fields were selected for each section, and expression in 1000 tumor cells (100 cells/fields) was evaluated with high-power (400×) microscopy. The expression of IBP was evaluated semi-quantitatively as negative/low (<10% of tumor cells stained), high (>10% of tumor cells stained). The result was confirmed by two independent pathologists.

2.12. Statistical analysis

Pearson's χ^2 test was performed to evaluate the IBP expression in different colorectal specimens. All the analyses were performed with SPSS software. A value of $p < 0.05$ was regarded as statistically significant.

3. Results

3.1. Recombination protein expression, antibody generation

Amino acids 410–631 of IBP were selected as the immunogen for the generation of antibodies specific to human IBP, since this fragment is the least homologous to other molecules. GST-IBP and Trx-IBP were recovered in a soluble fraction of *E. coli* lysate and further affinity purified. The purified proteins had an apparent molecular mass of 53 kDa and 46 kDa, respectively, determined by SDS-PAGE (data not shown). Polyclonal antiserum was raised in rabbits and the IgG fractions were purified on a Protein A column. Antibodies reactive to the tags of the fusion protein were removed by affinity purification.

3.2. Antibody characterization

The antibodies showed high specificity to their recombinant antigens in ELISA (Fig. 1A). To determine the specificity of

Table 2
Patients and tumor characteristics ($N = 63$).

Characteristic	N
Age	
≤45	21
46–65	27
>65	15
Gender	
Male	29
Female	34
Pathological type	
Tubular adenocarcinoma	35
Mucinous adenocarcinoma	14
Papillary adenocarcinoma	14
Normal	63
Adenoma	15
Lymphnode metastasis	
0	40
1–3	22
>3	1
Differentiation	
Poorly/moderately	53
Well	10
Tumor size (width) (cm)	
≤3	21
3–6	37
>6	5
Clinical stage (TNM/AJCC)	
0	0
I	13
II	27
III	21
IV	2

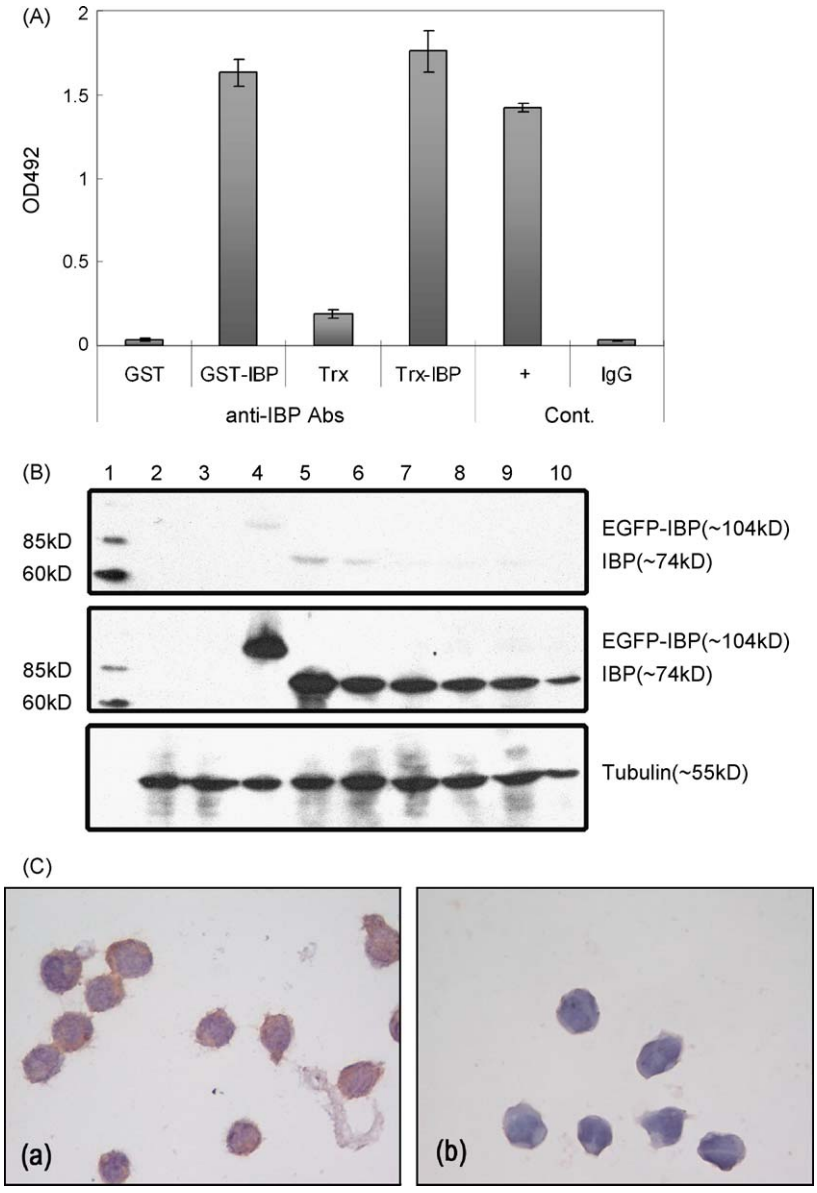

Fig. 1. Characterization of anti-IBP antibodies.
(A) ELISA analysis of anti-IBP antibodies. Anti-IBP polyclonal antibodies (1:2000) were tested by ELISA for their ability to bind recombinant proteins of Trx, Trx-IBP, GST and GST-IBP. Polyclonal antiserum without purification was used as positive control (+), while purified IgG from normal rabbit sera was used as a negative control (IgG). (B) Blocking experiment analysis of anti-IBP polyclonal antibodies. Lane 1: marker, Lane 2: wt 293, Lane 3: 293 + vector, Lane 4: 293 + EGFP-IBP, Lane 5: 293 + FLAG-IBP, Lane 6: 293 + IBP, Lane 7: Jurkat, Lane 8: Hut78, Lane 9: H9, Lane 10: Raji. Whole cell lysates were separated on 7% SDS-PAGE and analyzed by Western blotting. The anti-IBP polyclonal antibodies blocked with GST-IBP were used as primary antibody (upper panel). Anti-IBP antibodies blocked with GST were used as control (middle panel). Whole cell lysates from 293 cells transfected with EGFP-IBP expression vector, FLAG-IBP expression vector, or untagged form of full-length IBP were employed as positive control, while wt 293 and 293 cells transfected with empty vectors were used as negative control. The blot was later stripped and reprobed with an antibody against tubulin to ensure for equal loading (lower panel). (C) Immunocytochemistry analysis of IBP in Jurkat cells. Purified IgG from normal rabbit sera was used as a control. (a) Immunohistochemical staining of IBP in Jurkat cells (+). (b) Negative control (magnification 1000×).

polyclonal antibodies to IBP, blocking experiment was performed. Whole cell lysates of Jurkat, Hut78, H9 and Raji were electrophoresed on a 7% SDS-PAGE and electrotransferred on PVDF member, 293 cells transfected with EGFP epitope-tagged full-length human IBP expression vector (EGFP-IBP), or FLAG epitope-tagged human IBP expression vector (FLAG-IBP), or an untagged form of full-length IBP (IBP) were employed as positive control, while wt 293 and 293 cells transfected with empty vectors were used as negative control. After probing with anti-IBP polyclonal antibodies blocked with antigen GST-IBP, a very weak signal was detected. In contrast, after probing with anti-IBP polyclonal antibodies blocked with control antigen GST, a single band at ~74 kDa corresponding to the molecular weight of IBP was observed in the lysates of Jurkat, Hut78, H9 and Raji as previous report [1]. Meanwhile obvious band of ~104 kDa (EGFP-IBP) or ~74 kDa (FLAG-IBP and IBP) corresponding to the predicted molecular weight of fusion proteins was observed in the lysates of positive control 293 cells but none in the negative control (Fig. 1B). Moreover, the polyclonal antibodies performed well in the application of immunocytochemistry (ICC) analysis (Fig. 1C). All above confirm the high specificity and good reactivity of the rabbit polyclonal antibodies against human IBP.

3.3. Expressed of IBP in colon cancer cell lines

There is no report on the IBP expression pattern in human colon cancer cells. We examined IBP expression in four human colon cancer cell lines by RT-PCR and Western blot. High expression of

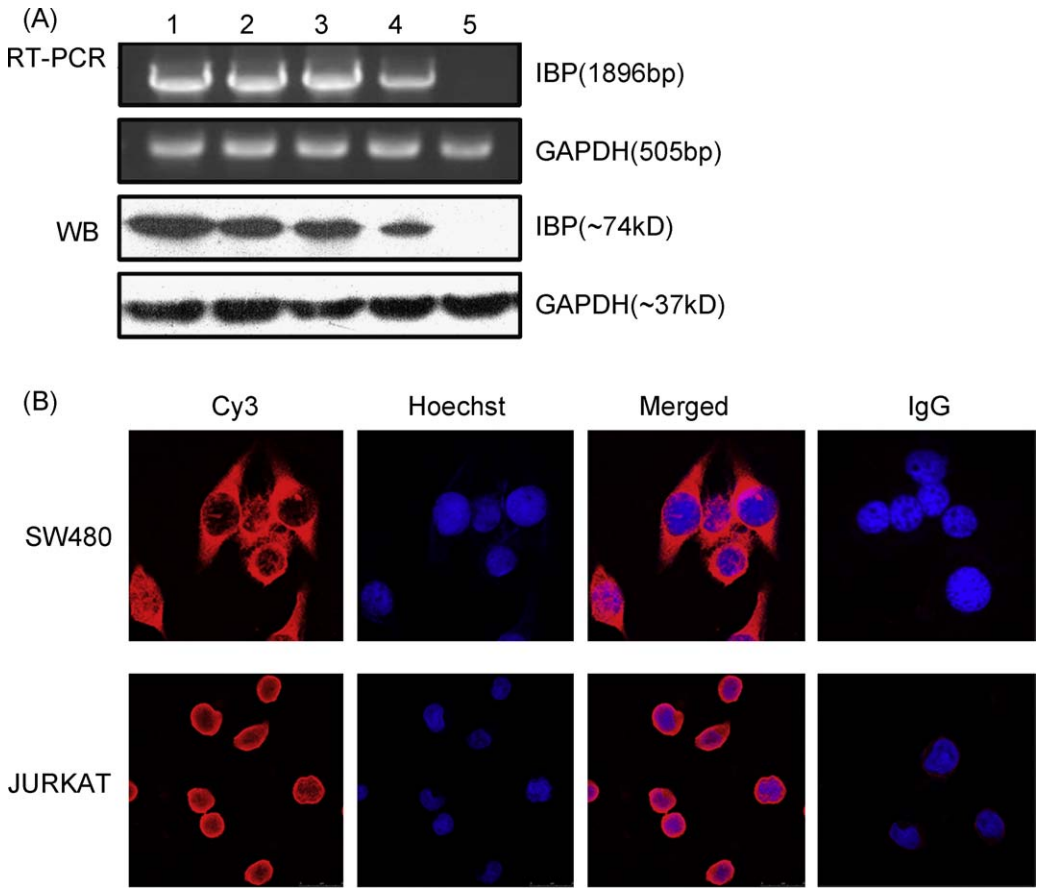

Fig. 2. Expression of IBP in colorectal cancer cell lines.
(A) RT-PCR and Western blot analysis of IBP in human colorectal cancer cell lines. GAPDH was used as an internal control. Lane 1: Jurkat, Lane 2: SW480, Lane 3: SW620, Lane 4: HT29, Lane 5: LoVo. (B) IBP localization analysis by confocal microscopy. Purified IgG from normal rabbit sera was used as a control. Scale bar represents 50 μm.

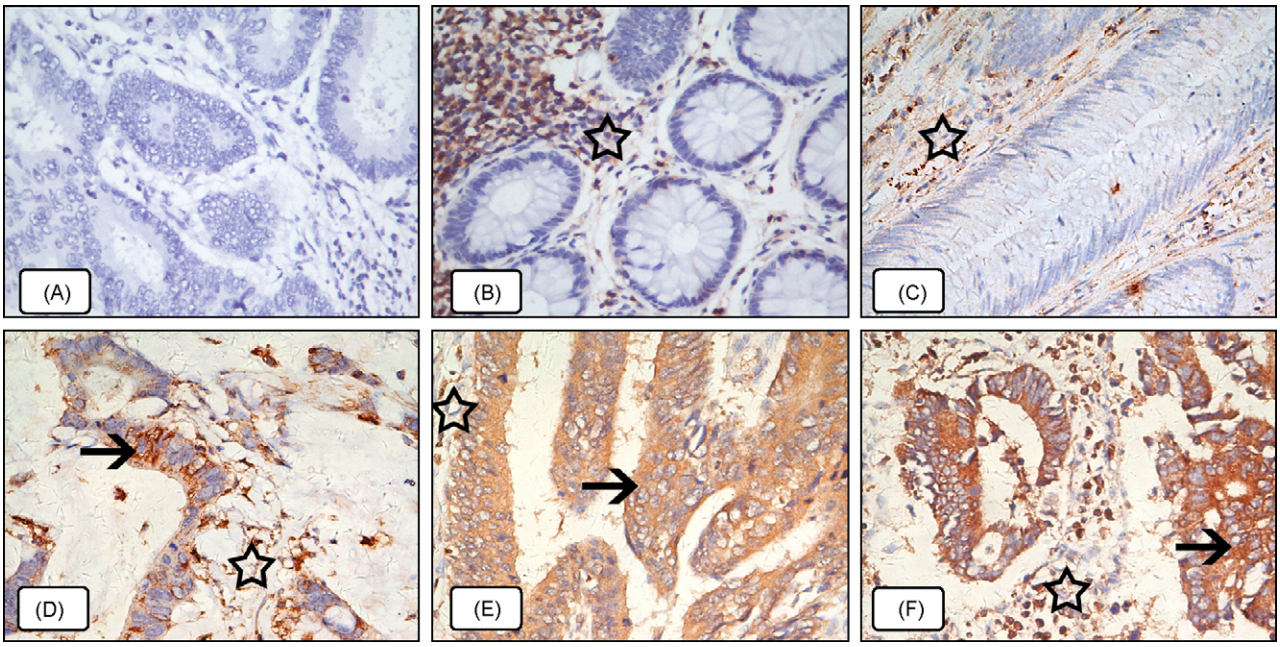

Fig. 3. (A) Negative control, (B) immunohistochemical staining of IBP in normal colorectal tissue, (C) immunohistochemical staining of IBP in colorectal adenoma, (D) immunohistochemical staining of IBP in colorectal mucinous adenocarcinoma, (E) immunohistochemical staining of IBP in colorectal papillary adenocarcinoma, (F) immunohistochemical staining of IBP in colorectal tubular adenocarcinoma. Stars indicate lymphocytes in these tissues that stained very strongly for IBP. Arrowheads indicate cancer cells in these tissues that stained very strongly for IBP (magnification 400×).

IBP can be detected at both the mRNA and protein level in SW480 and SW620 cells, lower expression can be detected in HT29 cells, but not in LoVo cells (Fig. 2A). Confocal analysis was employed to locate IBP using anti-IBP polyclonal antibodies. IBP is predominantly localized in the cytoplasm of Jurkat and SW480 cells. Immunostaining of IBP in nuclear was not observed (Fig. 2B).

3.4. Expressed of IBP in colorectal cancer tissues

IBP is highly expressed in human colon cancer cell lines. Thus, we were interested in investigating IBP expression pattern in colon tissues. 63 pairs of human colorectal cancer and the control non-cancerous tissues, as well as 15 adenoma tissues were examined by IHC. In addition to the strong staining of lymphocyte in these tissues, IBP protein was mainly localized in the cytoplasm of colorectal cancer cells (Fig. 3). The IHC results revealed that 42 IBP-positive cases were detectable in 63 colorectal cancer tissues. In contrast, none of the control non-cancerous tissues or the 15 adenoma tissues was IBP positive. There was a statistically significant difference in the expression of IBP between colorectal cancer tissues and control normal tissues ($p < 0.001$), and a statistically significant difference between colorectal cancer tissues and adenoma tissues ($p < 0.001$). Furthermore, a significant elevation could be found in the poor-moderately differentiated tumors as compared to the well differentiated tumors ($p < 0.05$), and there were also significant elevations in stages II, III and IV compared to stage I tumors ($p < 0.01$). However, there were no significant differences between levels in lymph node metastasis positive group and negative group. No statistically significant differences were found between male and female and no statistically significant differences were found among various pathologic types of tumors (Table 3).

Table 3
Immunohistochemical results for IBP.

Variable	*N*	Positive *N* (%)	Negative *N* (%)	*p*-Value
Tissue				
Normal*	63	0 (0.0)	63 (100.0)	0.000
Adenoma**	15	0 (0.0)	15 (100.0)	0.000
Colorectal cancer	63	42 (66.7)	21 (33.3)	
Gender				
Male***	29	18 (62.1)	11 (37.9)	0.475
Female	34	24 (70.6)	10 (29.4)	
Pathological type				
Tubular adenocarcinoma	35	23 (65.7)	12 (34.3)	0.907
Mucinous adenocarcinoma	14	9 (64.3)	5 (35.7)	
Papillary adenocarcinoma	14	10 (71.4)	4 (28.6)	
Lymph node metastasis				
Positive****	23	16 (69.6)	7 (30.4)	0.711
Negative	40	26 (65.0)	14 (35.0)	
Differentiation				
Poor-moderately*****	53	39 (73.6)	14 (26.4)	0.021
Well	10	3 (30.0)	7 (70.0)	
Tumor size (width) (cm)				
≤3	21	17 (81.0)	4 (19.0)	0.145
3–6	37	23 (62.2)	14 (37.8)	
>6	5	2 (40.0)	3 (60.0)	
Clinical stage (TNM/AJCC)				
I******	13	4 (30.8)	9 (69.2)	0.006
II*******	27	20 (74.1)	7 (25.9)	0.139
III	21	16 (76.2)	5 (23.8)	
IV	2	2 (100.0)	0 (0.0)	

* $p < 0.001$ vs. colorectal cancer tissue.
** $p < 0.001$ vs. colorectal cancer tissue.
*** $p > 0.05$ vs. female.
**** $p > 0.05$ vs. negative.
***** $p < 0.05$ vs. well differentiation.
****** $p < 0.01$ vs. II + III + IV stages.
******* $p > 0.05$ I + II vs. III + IV stages.

4. Discussion

IBP is a highly conserved protein in vertebrates and displays a significant similarity to SWAP-70, which was described as a novel type of Guanine nucleotide exchange factor (GEF) [1,12]. GEFs are members of the Dbl protein family and represent major regulators of the activation state of Rho GTPases [13]. Rho GTPases play fundamental roles in many biological processes including regulation of cytoskeletal organization, cell cycle progression and transduction of signals from the extra cellular environment [14–17]. They also have been shown to contribute to pathological processes such as cancer cell migration, invasion, and metastasis [18–21]. In addition, Rho GTPases have oncogenic activity and this correlates with increased expression and activity in a variety of cancers [22–24]. IBP has been shown to exhibit GEF activity for Rho-family GTPases including Rac1, Cdc42, and RhoA [25]. Previous studies indicate a significant physiological role for IBP and suggest that IBP may have an important function in tumorigenesis. However, little is known about IBP in cancer.

In this study, first, we developed rabbit anti-IBP polyclonal antibodies with the most unique portion of IBP (Amino acids 410–631) [1], and purified them by affinity chromatography. This maximally reduces the cross-reactivity of these polyclonal antibodies. These antibodies show high specificity and sensitivity against IBP in ELISA, Western blot and ICC. Consistent with previous reports, our result confirmed the high expression of IBP in immune cells [1,5]. Second, IBP expression was evaluated in four human colon cancer cell lines. We discover that expression of IBP can be detected at both mRNA and protein levels in three human colon cancer cell lines we detected. Confocal analysis indicated that IBP is predominantly located in cytoplasm of cancer cells. These data will be useful in the future study of the relevance of IBP function in colorectal cancer progression and tumor behavior. Third, the expression of IBP was evaluated in the cancer tissue compared with paired normal tissue of the same patient, as well as 15 cases of adenoma tissues. Overexpression of IBP was detected in colorectal cancer tissues and correlated strongly with the grades of differentiation of the tumors. There were stronger expressions in the poor-moderately differentiated tumors than that of the well differentiated tumors. There were also stronger expressions in the stages II, III and IV tumors than that of the stage I tumors. But the expression of IBP was undetectable in neither non-cancerous surrounding tissues nor 15 adenoma tissues. Our result showed no statistically significant difference between IBP levels in lymph node metastasis positive group and negative group or male and female groups. Also, no statistically significant differences were found among various pathologic types of tumors.

Supporting our findings, it has been reported that IBP is expressed in myeloid progenitors but downregulated after induction of differentiation [4]. It seems that IBP is expressed higher in undifferentiated and poorly differentiated cells. Analysis of more corresponding normal and cancer samples from patients are needed to confirm our result. Many studies indicate that Rho GTPases play a critical role in oncogenesis and tumor behavior. It has been reported that Rho GTPases contribute to invasion, migration and drug-resistance of colon cancer cells [26–28]. Zhang et al. found that D4-GDI, a Rho GTPase regulator, promotes breast cancer cell invasiveness [29]. Wong et al. reported that deleted in liver cancer (DLC1), a Rho GTPase activating protein, suppresses cell proliferation and invasion in hepatocellular carcinoma [30]. As a novel regulator of Rho GTPases, whether IBP is involved in these processes will be a worthwhile topic for investigation.

IBP is required for lymphocyte homeostasis and the prevention of systemic autoimmunity [10]. The difference of IBP expression between colorectal cancer and immune cells complicated our interpretation of the function of IBP in mammalian cells. Rho GTPases play a role in transduction of signals from the extra cellular environment. Our data (not shown) indicate that IBP can be detected on cell membrane, moreover, IBP contains a potential bipartite basic nuclear localization signal (amino acids KRREQ-REQRERRR) [1,31] suggesting that IBP might be able to translocate to the nucleus. Due to the apparent discrepancy IBP expression in colon cells and function of IBP in immune system, the enhanced IBP expression in colorectal cancer may be a protective response mechanism by which cells accommodates to potentially lethal stress caused by the tumor microenvironment. And cancer cells might suppress the responsibility of immune cell via an IBP dependent manner to protect them form immune surveillance. Further study is needed to confirm our hypothesis. Furthermore, the enhanced expression of IBP is one possible molecular event in the tumorgenesis of colorectal cancer and elevated expression of IBP may be a feature of human colorectal cancer.

In summary, we propose that expression of IBP may correlate to the occurrence and progress of human colorectal cancer. The biological role of IBP may not be merely limited to the immune system. The observed difference of IBP expression between normal and colorectal cancer tissues, correlation between the IBP expression and the grade of differentiation in colorectal cancer cells, as well as correlation between the IBP expression and tumor stages suggest that IBP might be a novel promising tumor marker of human colorectal cancer. The mechanisms of the overexpression of IBP in human colorectal cancer, and the biological function of IBP in human colorectal cancer or a larger variety of other human tumors need further explanation. Moreover, the novel antibodies we produced provide useful tool for both basic research and clinical studies.

Conflict of interest statement

None.

Acknowledgements

We thank Dr. Alessandra B. Pernis (Columbia University, USA) for kindly giving us plasmid pGEFP-C1-IBP. We thank Wei Sun and Liting Wang (Central Laboratories, TMMU, China) for their help with laser scanning confocal microscopy work. This project is supported by National Natural Science Foundation of China (30471577 and 30570373) and Hi-Tech Research Development Program (“863”) of China (Grant no. 2006AA02A247).

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