RESEARCH ARTICLE

Overexpression of immunoglobulin G prompts cell proliferation and inhibits cell apoptosis in human urothelial carcinoma

Pei-Yu Liang · Hao-Yong Li · Zhi-Yan Zhou · Ying-Xia Jin · Sheng-Xing Wang · Xiao-Hui Peng · Shan-Ji Ou

Abstract Only B lymphocytes can express immunoglobulins according to the traditional immunological theories, and the expression of immunoglobulin G (IgG) messenger RNA (mRNA) and protein was found in certain human cancer cells recently. However, the expression pattern of IgG and its possible role in human urothelial carcinoma are still elusive. In this study, we investigated the expression of IgG in two human urothelial carcinoma cell lines, T24 and BIU-87, and in 56 cases of clinical urothelial carcinoma tissues. The mRNA of IgG was positively detected by in situ hybridization and reverse transcription PCR; furthermore, IgG protein was also positively detected by immunohistochemistry and Western blot. Moreover, blockade of tumor-derived IgG by either antihuman IgG antibody or antisense oligonucleotides increased cell apoptosis and inhibited cell growth in bladder cancer cell lines in vitro, and antihuman IgG antibody could suppress the growth of xenotransplant tumor in vivo. In addition, either antihuman IgG antibody or antisense oligonucleotides enhanced the sensitivity to mitomycin C in bladder cancer cell line T24. Furthermore, blockade of IgG in bladder cancer cell T24 resulted in upregulation of cleaved caspase-3 and cleaved poly(ADP-ribose) polymerase. Our results indicated that bladder cancer cells were capable of expressing IgG, and blockade of IgG expression induced cell apoptosis through activation of caspase-dependent pathway. A novel potential targeted therapy for bladder cancer will be possibly developed based on these data.

Abbreviation

IgG	Immunoglobin G
ASODN	Antisense oligodeoxynucleotides
MMC	Mitomycin C
UC	Urothelial carcinoma

Introduction

Immunoglobulins are some certain proteins produced by plasma cells and lymphocytes and characteristic of these types of cells. Immunoglobulins play an essential role in the body's immune system, and immunoglobulins are composed of four polypeptide chains: two "light" chains (lambda or kappa) and two "heavy" chains (alpha, delta, gamma, epsilon, or mu). The type of heavy chain determines the immunoglobulin isotype (IgA, IgD, IgG, IgE, and IgM, respectively) [1].

However, some recent findings have reported that immunoglobulins were found to be expressed in multiple malignant epithelium tumors and activated in hyperplasia epithelium, but it was negatively expressed in normal epithelial cells [2, 3]. Heavy and light chain constant regions of immunoglobulin was found in many human epithelial carcinoma cell lines, such as MCF-7 (human breast carcinoma cell line), SW480 (human colon carcinoma cell line), MGC (human gastric carcinoma cell line), HeLa (human cervical carcinoma cell line), and HNE2 (human nasopharyngeal

P.-Y. Liang · H.-Y. Li (✉) · Z.-Y. Zhou · Y.-X. Jin · S.-X. Wang · X.-H. Peng · S.-J. Ou
Department of Urology, The Affiliated Hospital of Medical College of Hainan, NO.31 Longhua Road, Meilan District, Haikou 570102 Hainan Province, China
e-mail: haoyli5118@yahoo.com.cn

carcinoma cell line) [4]. Furthermore, some human cancer cell lines could express and secret IgG [5, 6], and IgG and IgA were also found to be expressed in oral epithelial tumors by immunohistochemical analysis [7].

Tumor-derived IgG was found to be involved in the survival and growth of epithelial tumor cells, and the cancer-derived IgG stimulated cell growth in an autocrine/paracrine fashion [8]. This study indicated that tumor-derived IgG contributed to cancer initiation in the precancerous stage when the epithelial cells were actively proliferating, and selective blockade of tumor-derived IgG thus may initiate a new approach for cancer therapy and prevention.

However, the expression pattern of IgG in human bladder cancer has not been investigated. In the present study, we reported the expression pattern of IgG in urinary bladder transitional epithelium cancer, and then, we further investigated the biological effect of tumor-derived IgG in human bladder cancer.

Materials and methods

Clinical tissue samples

For this retrospective study, archival formalin-fixed, paraffin-embedded tissue specimens from 56 bladder cancer patients, who underwent radical cystectomy or transurethral resection at Department of Urology, the affiliated hospital of Hainan Medical College from July 2005 to June 2006, were recruited. The 56 patients include 47 men and 9 women, with a mean age of 60 years (range, 22–86). Meanwhile, the normal control bladder epitheliums were obtained from 11 cases among those 56 bladder cancer patients, and the normal epitheliums were taken from the area more than 2.0 cm away from the margin of cancer tissue. These bladder cancer patients were graded according to the WHO grading system (G1, 19 cases; G2, 21 cases; G3, 16 cases) and were staged according to the TNM classification (superficial tumor Tis, Ta, T1: 42 cases; advanced tumor T2, T3, T4: 14 cases). All procedures were performed with the approval of the Institutional Review Board.

Cell culture

The human bladder carcinoma cell lines T24 and BIU-87 were cultured in Dulbecco's modified eagle's medium (DMEM, Life Technologies, Inc.) with 10 % fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin. All the cell lines were grown in a humidified incubator at 37 °C with 5 % CO_2.

Immunohistochemistry

Primary antibodies against IgG (IR512, Dako, Denmark) and CD20 (CD20cy, clone: L26, Dako, Denmark) were used in this study. Briefly, tissue sections were dewaxed, incubated with hydrogen peroxide for 10 min, incubated in retrieval buffer solution for antigen recovery, blocked with normal serum for 10 min, and incubated with a primary antibody for 60 min, followed by detection using a Catalyzed Signal Amplification Kit (DAKO, USA); signal was visualized using diaminobenzidine. Nonimmune goat or rabbit serum was substituted for the primary antibody as a negative control. The immunohistochemistry results were evaluated and classified as − (positive cell under 10 %), + (positive cells 10–30 %), ++ (positive cell under 30–50 %), +++ (positive cells 50–70 %), and ++++ (positive cells 70–100 %) by one senior pathologist without knowledge of the clinicopathological outcomes of the patients.

In situ hybridization

Paraformaldehyde-fixed and paraffin-embedded tissue sections were deparaffinized, dehydrated, and incubated in perforating buffer (0.1 M citric acid buffer with 0.5 % Triton X-100) for 10 min, and then incubated with 3 % hydrogen peroxide for 20 min. The sections were treated with proteinase K and hybridized overnight at 37 °C with the probe against human IgG1 CH1~CH3 (GenBank ID AY623427.1). After hybridization, the sections were incubated with antidigoxigenin antibody conjugated with biotin for 60 min at 37 °C, and then incubated for 50 min with highly sensitive streptavidin peroxidase (SP) at 37 °C. After extensive washing, the bound antibody was detected using DAB for color reaction.

Reverse transcription PCR

Total RNA was extracted from cell lines using TRIzol reagent (Invitrogen). After reverse transcription of the total RNA, the first-strand complementary DNA was then used as templates for detection of IgG and β-actin expression. β-actin was used as internal control. The primers were IgG (184 bp) (forward, ACT ACA AGA CCA CGC CTC C and reverse, CGT CGC ACT CAT TTA CCC), β-actin (569 bp) (forward, ACT ACC TCA TGA AGA TCC TC and reverse, CTA AAG ATT GCG TGG CGA GG). PCR products were electrophoresed on 1.5 % agarose gels containing 0.5 μg/ml ethidium bromide.

Western blotting analysis

Cells were harvested and lysed with radioimmunoprecipitation assay buffer (Upstate, USA). Equal amounts of denatured protein sample were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and were then transferred electrophoretically to polyvinylidene fluoride

membranes (Pall, USA) for immunoblot analysis. Antibodies used for immunoblot analysis were against human IgG (IR512, Dako, Denmark), Caspase-3 (1:500 dilution, sc-7272, Santa Cruz, USA), poly(ADP-ribose) polymerase (PARP) (1:1,000 dilution, sc-1562, Santa Cruz, USA), GAPDH (1:3,000 dilution, sc-32233, Santa Cruz, USA). All protein bands were detected using an enhanced chemiluminescent western blot kit (Cell Signaling Technology, USA).

Flow cytometry analysis

Cells were collected, washed twice with ice-cold phosphate-buffered saline (PBS), resuspended in cold PBS, and fixed with 70 % ethanol. After fixation overnight and subsequent rehydration in PBS for 30 min at 4 °C, the samples were stained for 30 min in dark with 50 μg/ml propidium iodide (Sigma) containing 125 U/ml protease-free RNase, both diluted in PBS in a FACS flow cytometer(Beckman-Coulter ELITE, USA).

MTT assay

The logarithmically growing T24 and BIU-87 cells were seeded in 96-well plate at a density of 2×10^3 cells/well. After an overnight growth, cells were treated with goat antihuman IgG antibody (10 μg/ml; Sigma) or 0.8 μg/ml concentration of mitomycin C (MMC) alone or in combination. After indicated time duration, 10 μl of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added into each well and incubated for an additional 4 h. Supernatants were removed, and 200 μl of dimethyl sulfoxide was added. After crystals were dissolved, absorbance at 450 nm was measured in a microplate reader.

Anti-IgG antibody treatment

Cells were seeded in a 24-well plate at 6×10^4 cells/ml, incubated overnight at 37 °C in DMEM with 10 % FCS. The supernatant was discarded, and goat antihuman IgG antibody (25 μg/ml; Sigma) was added to the culture for additional 48 h incubation. Apoptosis of cancer cells was assessed by flow cytometry analysis as described above. Normal nonspecific goat IgG (25 μg/ml) was used as control. In cell proliferation assay, cancer cells were seeded in 96-well plate at 2×10^3 cells/ml and incubated overnight in DMEM with 10 % FCS. The supernatant was discarded, and the cancer cells were subsequently treated with goat antihuman IgG antibody (10 μg/ml; Sigma) and 0.8 μg/ml concentration of MMC, alone and combined. Cell proliferation was determined by MTT.

ASODN transfection

The 15-mer phosphoro-thioIate oligodeoxynucleotides were synthesized by Invitrogen. IgG antisense oligodeoxynucleotides (ASODN, 5′-ACA CGG CTG TGT ATT-3′) were complementary to human IgG1 CHI-CH3 mRNA, and the random nonsense oligodeoxynucleotides (RODN, 5′-CGG GTA TGC TTC GCC-3′) were used as controls. ASODN and RODN were separately transfected into cancer cells using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions. Transfected cells were grown at 37 °C for 6 h, followed by incubation with complete medium for an additional 66 h. When transient transfection was performed for 72 h, cells were harvested and followed by the further assay.

In vivo Studies

Totally, 25 nude mice (BALB/c) were used for in vivo studies, and each group had five nude mice. The human bladder carcinoma cell line T24 cells (5×10^6) in the logarithmic growth phase were injected into right armpit of nude mice (BALB/c). When the tumor became palpable, polyclonal antibody of goat antihuman IgG, which was purified by protein A-Sepharose 4B column and purchaseed from Chinese academy of military medical sciences, laboratory of immunology, Beijing, China, was injected 1.5 mg/tumor intratumorally for five times with a 3-day interval, along or combined with intraperitoneal injection of MMC 1.5 mg/kg. The growth of the tumor was monitored every 3 days. On day 25 after tumor cell inoculation, the tumors were excised and histologically
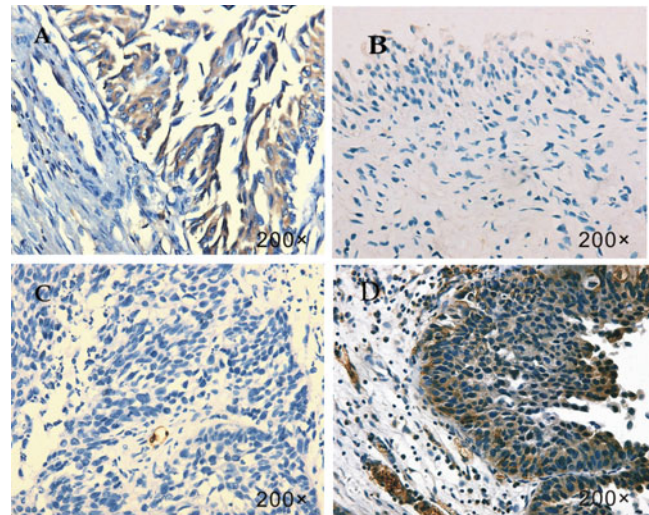

Fig. 1 IgG expression in clinical human bladder cancer tissues. **a** IgG was strongly expressed in bladder cancer by immunohistochemisty. **b** IgG was weakly or negatively expressed in normal bladder transitional epithelial cells by immunohistochemisty. **c** CD20 expression was positive in tumor-infiltrating B lymphocytes, while CD20 protein was negatively expressed in bladder cancer tissues. **d** IgG mRNA was strongly expressed in bladder cancer tissues by in situ hybridization

Table 1 IgG expression in human urothelial carcinoma tissues and normal tissues

Group	Case	Positive degree (n/%)				
		−	+	++	+++	++++
UC	56	5 (9)	4 (7)	13 (23)	24 (42)	10 (18)
Normal	11	6 (55)	3 (27)	2 (18)		

examined. Nude mice with T24 xenografts treated with normal goat IgG, and PBS was used as controls. All procedures were performed with the approval of the Institutional Review Board.

Statistical analysis

Data was analyzed using SPSS12.0 software. Data were expressed as mean ± standard deviation (SD), and t test was used to determine the significance of differences between two groups. Differences were considered significant at $p < 0.05$.

Results

IgG expression in clinical human bladder tumor tissues

For immunostaining study, the positive signal of IgG was predominantly shown in the cytoplasma of human bladder tumor cells (Fig. 1a), and negative signal of IgG was found

in normal epitheliums (Fig. 1b). CD20 staining was performed to rule out contamination of infiltrating B cells in cancer tissues. The tumor-infiltrating B cell was shown as CD20 positive expression, while the CD20 expression in the bladder cancer cells was negative (Fig. 1c). Furthermore, the mRNA level of IgG was positively detected in bladder tumor tissues by in situ hybridization (Fig. 1d).

The expression of IgG was positively detected in 91.1 % (51/56) clinical human bladder tumor tissues by immunohistochemistry, while the positive IgG expression was found only in 45.4 % (5/11) normal epitheliums. The difference of IgG expression was statistically significant between bladder tumor tissues and normal epitheliums ($p < 0.05$) (Table 1).

Moreover, the positive expression of IgG was found in 84.2 % (16/19) in grade G1, 90.5 % (19/21) in grade G2 and 100 % (16/16) in grade G3, respectively. However, the IgG expression was not correlated with tumor grade and stage ($p > 0.05$).

IgG expression in human bladder cancer cell lines T24 and BIU-87

To completely obviate contamination of infiltrating B cells in tumor tissues, the expression of IgG was further examined in two human bladder cancer cell lines T24 and BIU-87 using four different methods. Immunohistochemistry was performed to detect IgG expression in two human bladder cancer cell lines T24 and BIU-87, and positive signal was found in both of these two cell lines (Fig. 2a, b). The mRNA
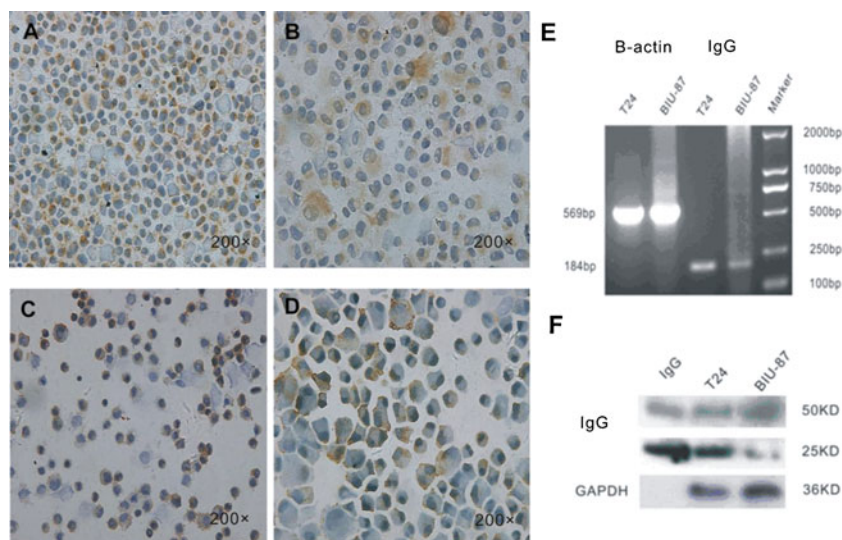

Fig. 2 IgG expression in human bladder cancer cell Lines T24 and BIU-87. The expression of IgG protein was detected by immunohistochemistry in human bladder cancer cell lines T24 (**a**) and BIU-87 (**b**). The expression of IgG mRNA was detected by in situ hybridization in human bladder cancer cell lines T24 (**c**) and BIU-87 (**d**). The

expression of IgG mRNA (184 bp products) in human bladder cancer cell lines T24 and BIU-87 was detected by RT-PCR (**e**). **f** Two splitted subunits of cancer-derived IgG (25 and 50 kDa) was detected by Western blot in human bladder cancer cell lines T24 and BIU-87. Human serum IgG was used as positive control

level of IgG was also found to be positive in T24 and BIU-87 cell lines by in situ hybridization (Fig. 2c, d). Furthermore, these results were further confirmed by RT-PCR (Fig. 2e) and Western blot (Fig. 2f).

### Effect of blockade of IgG on the proliferation and apoptosis of bladder cancer cells in vitro

The human bladder cancer cell lines T24 and BIU-87 were treated with 25 µg/ml concentrations of the goat nonspecific IgG or anti-IgG antibody, respectively, and the cell growth was assessed by MTT assay. The inhibition ratio of cell growth in T24 and BIU-87 treated with goat nonspecific IgG or anti-IgG antibody were $(4.73\pm3.73)\%$ vs $(24.98\pm3.81)\%$ and $(5.36\pm1.53)\%$ vs $(22.7\pm3.72)\%$, respectively. Hence, anti-IgG antibody could inhibited cell growth in human bladder cancer cell lines T24 and BIU-87 ($p<0.05$). In addition, the inhibitory rate of T24 cells treated with anti-IgG antibody, MMC, or anti-IgG antibody +

MMC was $(25.02\pm6.71)\%$, $(32.31\pm6.46)\%$, and $(73.66\pm5.81)\%$, respectively ($p<0.05$).

Flow cytometry (FCM) shows the percentages of apoptotic cells in T24 cells treated with PBS (Fig. 3a), goat nonspecific IgG (Fig. 3b), anti-IgG antibody (Fig. 3c), MMC (Fig. 3d), MMC + nonspecific IgG (Fig. 3e), and anti-IgG Ab + MMC (Fig. 3f) were 1.1, 2.3, 20.7, 22.4, 28.3, and 53.8 %, respectively. Furthermore, caspase-3 and PARP were cleaved in T24 cell during the course of apoptosis induced by anti-IgG Ab and MMC (Fig. 3g) by Western blot. Moreover, the percentages of apoptotic cells in BIU-87 the goat nonspecific IgG and anti-IgG antibody were 1.3 vs 15.3 %, respectively (Suppl. Fig 1). These results indicated that anti-IgG antibody could induced bladder cancer cell apoptosis and enhanced MMC-induced cytotoxicity in vitro, which was correlated with the activation of caspase-3 and PARP.

To further investigate the function of cancer derived IgG in bladder cancer, we transfected a 15-mer S-oligos of

Fig. 3 Antibody against IgG-induced cell apoptosis in human bladder cancer cell line T24. The percentage of apoptosis cells was tested by FCM after the treatment with PBS (**a**), 25 µg/ml goat IgG (**b**), goat antihuman IgG (**c**), MMC (**d**), goat IgG + MMC (**e**), and goat antihuman IgG + MMC (**f**) for 72 h. **g** The caspase-3 and PARP were assessed by Western blot in the T24 cells treated with goat antihuman IgG and/or MMC
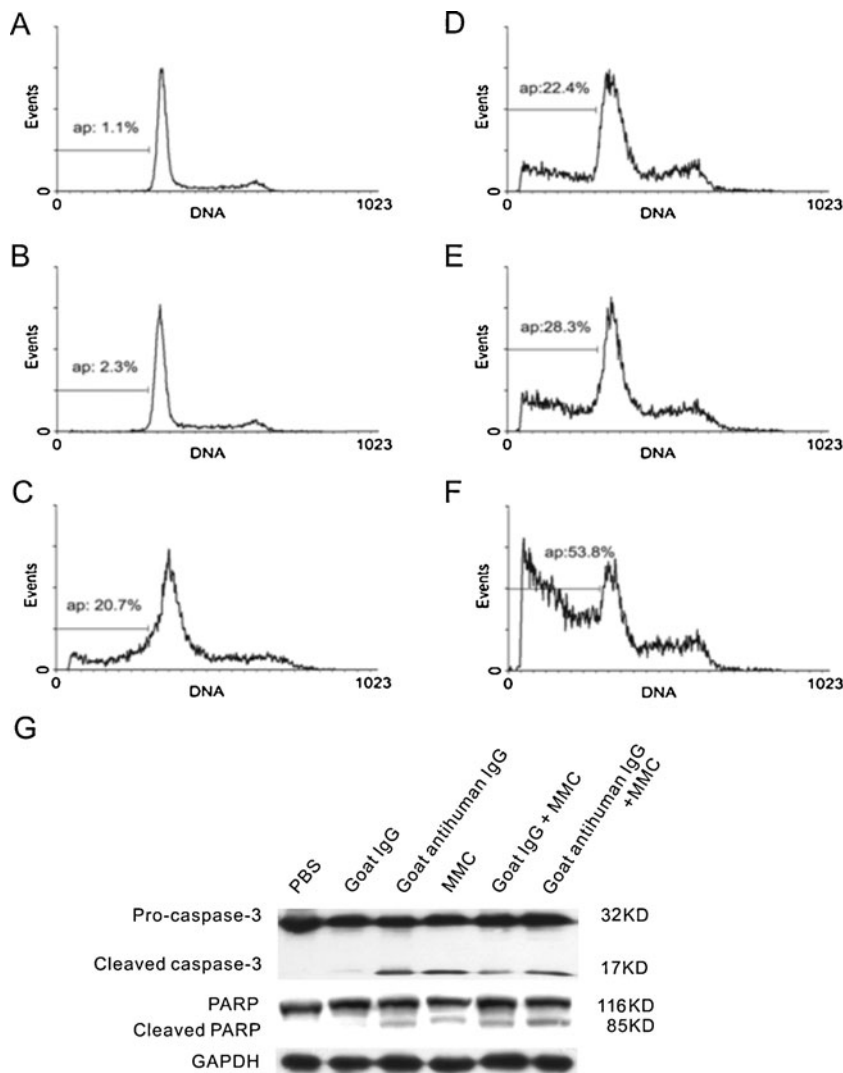
ASODN into T24 cells to block the expression of IgG. The inhibitory rate of cell growth in T24 cells treated with ASODN, MMC, MMC + RODN, and ASODN + MMC were (21.49±9.20)%, (34.26±6.62)%, (39.22±6.20)%, and (61.44±5.94)%, respectively ($p<0.05$). FCM showed the percentages of apoptotic cells in T24 cells treated with PBS (Fig. 4a), RODN (Fig. 4b), 2.5 μM ASODN (Fig. 4c), 5 μM ASODN (Fig. 4d), 10 μM ASODN (Fig. 4e), MMC (Fig. 4f), MMC + RODN (Fig. 4g), and MMC + ASODN (Fig. 4h) were 2.7, 5.2, 13.0, 16.2, 17.7, 22.8, 28.5, and 47.4 %, respectively. Western blot showed caspase-3 and

PARP were cleaved in T24 cell during the course of apoptosis induced by ASODN (Fig. 4i) and indicated that cell apoptosis was associated with caspase-3 and PARP activation.

Effect of ASODN against IgG on the proliferation and apoptosis of bladder cancer cells in vivo

Under the treatment of normal goat IgG, anti-IgG antibody, MMC, and MMC + anti-IgG antibody, the inhibitory rate of T24 xenografts in BALB/c nude mice were 2.31, 12.73,

Fig. 4 ASODN against IgG-induced cell apoptosis in human bladder cancer cell line T24. Apoptosis cell percentage of T24 cell were tested by FCM after the treatment with PBS (**a**), 10 μM RODN (**b**), 2.5 μM ASODN (**c**), 5.0 μM ASODN (**d**), 10.0 μM ASODN (**e**), 2.0 μg/ml MMC (**f**), 10.0 μM RODN+2.0 μg/ml MMC (**g**), and 10.0 μM ASODN+2.0 μg/ml MMC (**h**) for 72 h. **g** The caspase-3 and PARP were assessed by Western blot in T24 cells treated with ASODN for 72 h
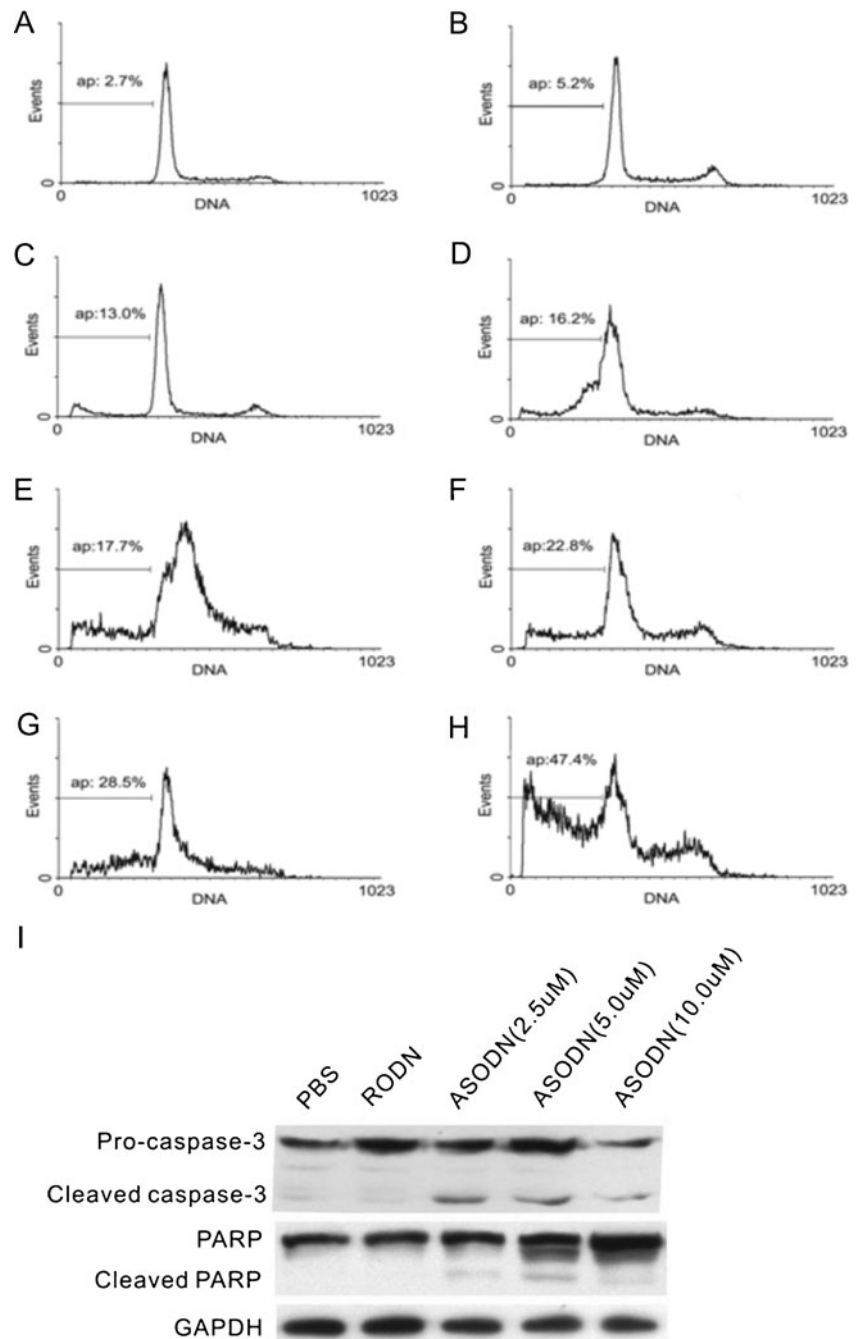
36.81, and 50.51 %, respectively (Fig. 5). Histological examination demonstrated significant necrosis and apoptosis in the mice treated with alone MMC or anti-IgG antibody, while necrosis and apoptosis was seldom shown in control goat IgG or PBS. In addition, more extensive necrosis and apoptosis was found in the mice with MMC + anti-IgG antibody (Fig. 6). These results indicated that anti-IgG antibody could induced bladder cancer cell apoptosis and enhanced MMC-induced cytotoxicity in vivo.

Discussion

Recently, many publications have shown that human cancer cell lines could express IgG [5, 6]. In the present study, we positively detected IgG expression in two human urothelial carcinoma cell lines T24 and BIU-87 and 56 cases of bladder tumor tissues by immunostaining and Western blot. Because it is possible that IgA and IgG are transferred into epithelial cells via poly-IgA/G receptor on plasma membrane [9], we further investigated the mRNA level of IgG expression using in situ hybridization and RT-PCR essentially to rule out this possibility. Taken together, all of these results indicated that IgG was expressed in human bladder cancer.

Many studies reported that the growth of tumor grafts could be enhanced by immune reaction, including antibody production [10–12]. Furthermore, several studies found the presence of circulating IgG reactive with specific tumor-associated antigens and its association with poor prognosis, and demonstrated the presence of an aberrantly glycosylated IgG population in

Fig. 6 The tumor of T24 xenografts were observed histologically in nude mice. The tumor of T24 xenografts were observed histologically in nude mice treated with PBS (a), goat non-sepcific IgG (b), goat antihuman IgG (c), MMC (d), and MMC + goat antihuman IgG (e) after 25 days treatment (H&E staining, ×400)

cancer patients [13, 14]. Carbohydrate reactive antibodies might be particularly participating in that the antigen (Ag) reactive IgGs upon tumor antigen immunization, which were observed to correlate with tumor growth in experimental mice models of human melanoma [15]. Such analogical observations were found in human colon cancer cell later [16]. Induction of cancer cell apoptosis and inhibition of cancer growth by blocking tumor-derived IgG, using either antisense oligodeoxynucleotide (ASODN) or anti-human Ig, confirmed that IgG secreted by epithelial cancers had some unidentified capacity to promote the growth and survival of tumor cells [8]. In order to explore the biological effects of tumor-derived IgG in human bladder cancer, we blocked the expression of tumor-derived IgG by either anti-human IgG antibody or antisense oligonucleotides in vitro. Functional studies showed that after blockage of IgG in bladder cancer, the cell growth was inhibited and cell apoptosis was increased. Furthermore, administration of anti-human IgG antibody could suppress the growth of bladder cancer cells in immunodeficient nude mice xenotransplant tumor. In addition, combined antihuman IgG antibody or antisense oligonucleotides with mitomycin C could enhance its sensitivity to mitomycin C in bladder cancer cell T24. These data indicated that bladder cancer-derived IgG might involved in the survival and growth of cancer cells.
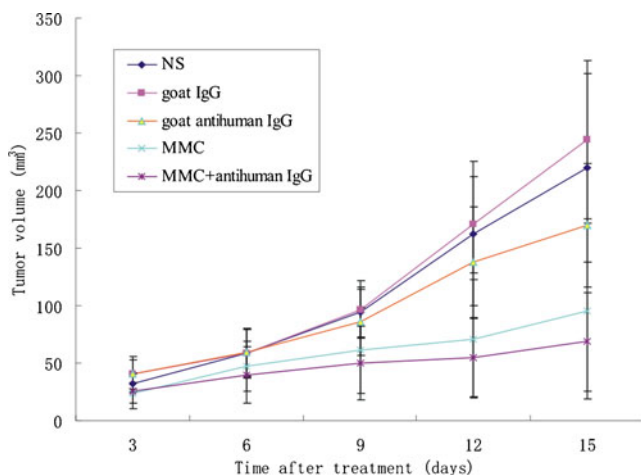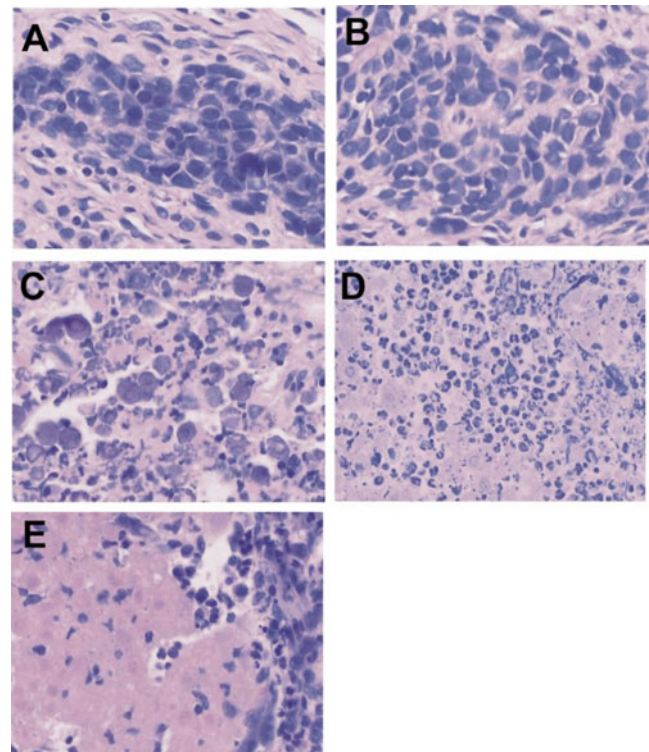

Fig. 5 Inhibitory effect of goat antihuman IgG and MMC on growth of human bladder cancer T24 cell xenografts in BALB/c nude mice. After tumor formation, antihuman IgG was injected intratumorally, and along or combined with intraperitoneal injection of MMC, once every 3 days in nude mice. The growth of the tumor was monitored every 3 days. On day 25 after treatment, the mice were killed and the tumors observed histologically (H&E staining)

Such antigen-driven antibodies can functionally mimic growth promoting agents much like siglecs, which are sialic acid-binding immunoglobulin-like lectins involved in cell–cell interactions and signalling functions in the hemopoietic, immune, and nervous systems [17]. However, the biological effects of antibodies cannot be assessed from their antigen binding properties. Tumor cells secreting antigenic secreted/shed tumor glycoproteins can induce a host IgG immune response that can promote invasion and metastasis by inducing tumor infiltrating stromal cells to release proinflammatory cytokines and VEGF [18]. Although the exact mechanism of antibody enhancement of tumor growth remains unclear, it was hypothesized that antibodies may do so by blocking target epitopes on the cancer cells [19]. Further study confirmed that antibodies may enhance tumor cell proliferation by immune complex binding and crosslinking of Fc-receptors expressed either on tumor cells or immune effect cells augmenting tumor growth and a metastatic phenotype during transition to malignancy [20]. Human IgG antigen binding fragment (Fab) in patients with malignancies might activate ERK-signaling pathways, leading to tumor cell proliferation and presumably disease progression [21]. In this study, we also investigated the mechanism that blockade of tumor-derived IgG by either antihuman IgG antibody or antisense oligonucleotides increased cell apoptosis and inhibited cell growth. Our finding suggested that antihuman IgG antibody or antisense oligonucleotides might regulate cell apoptosis through caspase-dependent pathways.

Although the exact mechanism of IgG expression in malignant epithelial cells remains unclear, several possible hypotheses may explain rearranged immunoglobulin variable (VH) region gene transcripts in cancer cells. Either de novo rearrangement or modification of VH genes in epithelial tumor cells or assimilation of lymphocyte-derived chromatin should be considered. Constitutive cytidine deaminase activation in malignant epithelial cells further raises a potential for inducing aberrant mutational activity [22]. Some observations suggested a potential for such rearrangement and modification. Endothelial cells lying adjacent to lymphoma or multiple myeloma cells have revealed genetic aberrations that are identical to the tumor clone, including entire IgH chromosomal translocations. Furthermore, the genetic aberrations acquired by endothelial cells in these diseases were maintained following several cycles of cell culture. Although the underlying mechanisms for acquisition and maintenance of aberrant genes in endothelial cells are not clear, several possibilities exist, one of which suggests gene transfer of apoptotic bodies from tumor cells [23].

Taken together, our data indicate that bladder tumor-derived IgG may contribute in cancer development. Development of methods aiming at selective blockade of tumor-derived IgG

thus may constitute a new approach for cancer therapy and prevention. While the production of IgG by cancer cells derived from epithelium and other proliferating cells has been established, its function and clinical significance in cancer development remains to be explored.

Conflicts of interest None

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