Increase expression of IL-17A, IL6, STAT3, TGF-β and VEGF in Bladder Cancer: potential biomarkers?

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Abstract

Background: Bladder cancer is the most common urinary system malignant disease all over the world. Our former research results showed that IL-17 family relative cytokines involved in the occurrence and development of bladder cancer, moreover A variety of inflammatory cells (such as lymphocyte, macrophage, mast cell and neutrophil) infiltrated in bladder cancer tissues and were closely related to bladder cancer.

Results: Immunohistochemistry (HIS) was employed to measure expression and location of IL-17A, IL-6, STAT3 and VEGF in pathological specimens of bladder cancer patients with different degree of malignancy (n=80), cystitis (n=23) and relative normal adjacent tissues (n=4). ELISA was used for measuring concentrations of MMP-9, TGF-β, VEGF and IFN-γ in serum samples collected from patients with bladder cancer (n=34) and control subjects (n=5). Immunoreactivity for IL-17A, IL-6, STAT3 and VEGF significant increased in tissues of bladder cancer compared with that of cystitis and normal adjacent tissues (p = 0.001. respectively), which was positively associated with the degrees of malignancy of the cancer. Serum concentrations of TGF-β and VEGF were significantly higher in patients with bladder cancer compared with that of controls (p=0.002 and p=0.0001 respectively), while concentrations of MMP-9 and IFN-γ were not significantly different between the groups of subjects. In the meanwhile, serum concentrations of MMP-9, TGF-β, VEGF, but not IFN-γ were significantly higher in patients with high degree of malignancy (stage Ⅲ and Ⅳ) than that of patients with low malignancy (stage Ⅰ and Ⅱ) (p=0.001, p=0.030, and p=0.011 respectively).

Conclusion: Elevated expression of IL-17A, IL-6, STAT3 in tissues and of TGF-β, VEGF in serum might be considered as potential biomarkers for clinical stages of bladder carcinoma progress.

Trial registration: ISRCTN, ISRCTN2012BH006. Registered 10 January 2012

1. Introduction

Bladder cancer is the second most common urological malignancy and accounting for 5% of all cancer related deaths in USA [1]. It is estimated that there were approximately 76,960 new cases of bladder cancer and that there were 16,390 deaths in the year 2016 [2]. The incidence in male is about fourfold higher than that of female, while the incidence is double in white men compared to their black counterpart. Cystitis is considered as a high-risk for bladder cancer [3]. It has been shown that hyperplasia and chronic cystitis are easy to develop bladder cancer [4]. Although many factors including long term smoking, rubber, leather, dye and aluminum polluted work environment may be associated with the pathogenesis, some cytokines, such as interleukin-17 (IL-17) family, IL-6, vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β) and signal transducer and activator of transcription 3 (STAT3) are considerably involved in the occurrence and development of cystitis and bladder cancer [2, 5–6]. An increase in inflammatory mediators or proinflamatory cytokines has been shown to lead to tumorigenesis, invasion, angiogenesis and prognosis. The collecting evidences show that

proinflammatory cytokines play important roles in bladder cancer, such as IL-17A family, TNF-α, IL-6 and VEGF etc. Cytokine IL-17A can activate IL-6 in inflammatory or cancerous disease, while IL-6, as a major activator of STAT3 signaling pathways, is the most important cytokine influencing the inflammatory response in humans [7–8]. IL-17A, IL-6 and STAT3 signals have been implicated in regulation of tumor growth and metastatic spread, which are also associated with prognosis in different cancers [9–11]. Furthermore, it has been reported that the increased concentrations of matrix metalloproteinase-9 (MMP-9), TGF-β and VEGF in serum were associated with metastasis and poor prognosis of different kinds of malignant tumors [12–14]. In addition, interferon-γ (IFN-γ) may play a role in the control of tumor growth and metastasis, positively through enhancing tumor immunogenicity [15]. Although the collecting data suggest that IL-17A, IL-6, STAT3, TGF-βand VEGF may be critical cytokines in various malignancies, expression and association of these molecules with bladder cancer remain unclear. In the present study, we focused on the expression of IL-17A, IL-6, VEGF and STAT3 in tissues of bladder, the serum concentrations of MMP-9, TGF-β, VEGF and IFN-γ and association with severity of bladder cancer.

2 Materials And Methods

2.1 Patients and specimens

The present study was approved by the institution of Hospital Ethics Committees of Urinary System Diseases Prevention and Treatment Research Centre in Affiliated Hospital of Beihua University, Jilin City, Jilin Province, People's Republic of China (approval reference: 2012BH006). The written consents were obtained from the participants. The tissue specimens were collected from patients with bladder cancer (n = 80, including 4 adjacent normal tissues) and patients with cystitis (n = 23) between January 2012 to December 2014. All the tissue samples were identified and diagnosed by clinical two pathologists with double blind method. The clinical characteristics of subjects involved in this study are summarized in Table 1, which is same as our previously published study [5].

Table 1

Clinical characteristics of the subjects from cystitis and bladder cancer

Status	Age (median)	Sources	Pathological characteristics	Differentiation grade
Normal	61.2(55–73) Male 4	Resection specimens	Adjacent normal tissues	No
Cystitis n = 23	54.3(22–84) Male 6 Female 1	Endoscopic biopsies or resection specimens	Inflammation	Acute 18 Chronic 5
Bladder cancer n = 80	64.5 (45–84) Male 66 Female 14	Endoscopic biopsies or resection specimens	Adenocarcinoma	□ and □stage n = 47 □ and □ stage n = 33

Serum samples were collected from 34 preoperative patients with bladder cancer patients and 50 age-matched healthy volunteers. None of the bladder cancer patients received radiotherapy or chemotherapy before surgery, while the healthy volunteers had no any immunological or infectious diseases. Informed consent was obtained from each participant for the use of blood samples in the present study (Table 2).

Table 2

Clinical characteristics of the subjects from bladder cancer and healthy volunteers

Status	Age (median)	Sources	Pathological diagnosis	Differentiation grade
Healthy volunteers n = 50	61.3 (39–78) Male 39 Female 11	serum	No	healthy
Bladder cancer n = 34	67.2 (48–82) Male 26 Female 8	serum	Adenocarcinoma	□ and □ stage n = 21 □ and □ stage n = 13

2.2. Immunohistochemistry

Immunohistochemistry was employed to measure immunoreactivity for IL-17A, IL-6, STAT3 and VEGF following a protocol as previously described [5]. Briefly, a household pressure cooker was used to retrieve antigens at high temperature and high pressure with sodium citrate solution (0.01 mM, pH = 6.0). Endogenous peroxidase activity was inhibited using freshly prepared hydrogen peroxide in methanol (0.3%) at room temperature for 30 min. Slides were washed with PBS and then blocked with 2.5% horse serum blocking buffer (Vector Laboratories, Cat# S-2012) for 20 minutes and then with dilution buffer containing 5% goat serum for further 30 min at room temperature. Proposed immunoreactivity was

measured using antibodies against IL-17A (Novus Biological, USA, NBPI-42746, 1:100), IL-6 (Novus Biological, NBPI-42746, 1:800), VEGF (Beijing Biosynthesis Biotechnology CO, LTD,Beijing, China, bs-1141R, 1:500) and STAT3(Beijing Biosynthesis Biotechnology CO, LTD, bs-1141R, 1:500). The information of antibodies used in the present study is summarized in Table 3. DAB kit (diaminobenzidine, ZhongShan Golden Bridge Biological Company, Beijing, China) was used to detect positive signals (brown). The stained slides were observed and scanned using an Olympus microscope at 10 × magnification. These images were exported as TIFF files and uploaded into Image Pro Plus 6.0 software (Media Cybernetics, Maryland, USA) for the further analysis. The brown positive signals were quantified as the percentages of IL-17A, IL-6, STAT3 and VEGF immunoreactive staining of the total haematoxylin counterstaining area of the entire sections as described previously [5].

Table 3

Relative antibodies or reagent kit used in this research

Antibody	Item number	Isotype or	Dilution	Sources
Anti-IL-17A	NBP1-42746	Rabbit-IgG	1:300	Novus Biologicals(USA)
Anti-IL-6	NBPI-42746	Rabbit-IgG	1:800	Novus Biologicals (USA)
Anti-IL-stat3	bs-1141R	Rabbit-IgG	1:300	Beijing Biosynthesis Biotechnology CO, LTD,Beijing (China)
Anti-IL-VEGF	bs-1141R	Rabbit-IgG	1:500	Beijing Biosynthesis Biotechnology CO, LTD,Beijing (China)
MMP-9 Elisa kit	BMS2016/2TEN	Rabbit-IgG	Already wrapped	eBioscience, Bender MedSystems GmbH, Vienna(Austria)
IFN-γElisa kit	BMS228TEN	Rabbit-IgG	Already wrapped	eBioscience, Bender MedSystems GmbH, Vienna (Austria)
VEGF Elisa kit	BMS277/2TEN	Rabbit-IgG	Already wrapped	eBioscience, Bender MedSystems GmbH, Vienna (Austria)
TGF-βElisa kit	BMS249/4TEN	Rabbit-IgG	Already wrapped	eBioscience, Bender MedSystems GmbH, Vienna (Austria)

2.3 Enzyme-linked immunosorbent assay (ELISA)

ELISA kits were purchased from eBioscience, Bender MedSystems GmbH, Vienna, Austria, for measuring concentrations of MMP-9 (LOT, 98474017, sensitivity: 50 pg/mL), TGF-β (LOT, 98482009, sensitivity: 8.6 pg/mL), VEGF (LOT, 98478015, sensitivity: <5 pg/mL,) and IFN-γ (LOT, 98476080, sensitivity: <4 pg/mL) according to the manufacturer's instructions (Table 3).

2.3. Statistical analysis

Data of immunohistochemistry were analyzed with a commercially available statistical package (Minitab for Windows, Minitab Release 9.2; Minitab, Inc, State College, PA). Differences between groups were analyzed using Kruskal-Wallis test followed by the Mann-Whitney U test. Data from ELISA assay were analyzed using Student t test and one-way analysis of variance (ANOVA). Data are presented as the mean ± SEM. A p value of less than 0.05 was considered statistically significant.

3 Results

3.1 Expression and location of IL-17A and IL-6 in bladder tissues

The immunohistochemical staining analysis showed that global IL-17A immunoreactivity was significantly higher in tissue sections from bladder cancer and cystitis compared with normal tissues (Fig. 1a and b, p = 0.001 and p = 0.001, respectively). In addition, immunoreactivity for IL-17A was significant higher in tissue sections of bladder cancer than that of cystitis (p = 0.001). Immunoreactivity for IL-17A mainly located in mononuclear cells, transitional epithelial cells, malignant cells and vascular endothelial cells in bladder cancer (Fig. 1a).

IL-6, as the more important downstream cytokine of IL-17A, is the primary pro-inflammatory cytokine in humans and is produced primarily by T-lymphocytes and macrophages. We previously showed that there were more infirtrating macrophates in bladder cancer tissues [5]. So we measure the expression and location of IL-6 in tissues of patients with bladder cancer compared with that of control subjects. Immunohistochemistry revealed that immunereactivity for IL-6 was significantly elevated in sections of tissues of bladder cancer compared with that of cystitis normal tissues (Fig. 1C-D, p = 0.001, p = 0.001, respectively), while IL-6 immunoreactivity in sections of tissues of cystitis was also significantly higher in cystitis than that of normals (p = 0.024). IL-6 immnuoreactivity was predominantly located in monocytes, transitional epithelial cells, malignant cells as well as vascular endothelial cells in bladder cancer.

3.2 Expression and location of VEGF and STAT3 in bladder tissues

Previous research results have confirmed that VEGF acted as an important regulator in cell proliferation, and metastasis in many types of malignant tumors [16–17], which is upregulated by IL-17A and IL-6. In this case, we measured immunoreactivity for VEGF in tissues of bladder cancer compared with the controls. Immunohistochemical staining analysis showed that the immunoreactivity for VEGF was significantly higher in tissue sections from bladder cancer compared with the controls (Fig. 2a and b, p = 0.001 and p = 0.001, respectively). Although the expression of VEGF in cystitis was elevated, it did not achieve statistically significant when it was compared with that of normal tissues (p = 0.446). Immunoreactivity for VEGF mainly located in vascular endothelial cells of tumor parenchyma, with less location in the stroma of tumors (Fig. 2a).

STAT3 is considered to inflammation related oncogenesis and constitutively activated in various cancers. Persistent activation of STAT3 is involved in promoting tumor cell proliferation, survival, tumor invasion, angiogenesis and immunosuppression, while IL-6 is considerate to up-regulate expression and activation [18–19]. Again, our immunohistochemical staining analysis showed that STAT3 immunoreactivity was significantly higher in bladder cancer tissues compared with the controls (Fig. 2c and d, p = 0.001 and p = 0.001, respectively), while there was no significant difference between cystitis and normal tissues (p = 0.4172). STAT3 mainly located malignant cells, vascular endothelial cells and transitional epithelial cells (Fig. 2c).

3.3 Association of immunoreactivity for IL-17A, IL-6, VEGF and STAT3 in bladder cancer

Further analysis revealed that immunoreactivities for IL-17A, IL-6, STAT3 and VEGF were significantly elevated in tissue sections of median and late stages (Ⅲ and Ⅳ stage) than that of early stages (I and II stage) p = 0.012, p = 0.014, p = 0.034, p = 0.024, respectively) (Fig. 3a-3d).

The Pearson correlation method was used to determine the association between immunoreactivity for the IL-17A, IL-6, STAT3 and VEGF in bladder cancer. The obvious positive correlation was observed between IL-17A and IL-6 (r = 0.5931), IL-17A and STAT3 (r = 0.6374), IL-6 and STAT3(r = 0.3963). Furthermore, the associations between IL-6 in bladder cancer was consistent with VEGF (r = 0.3968) which indicated that IL-17A-IL-6-VEGF signal axis participated the occurrence and progression of bladder cancer.

3.4 Serum MMP-9

Total concentrations of MMP-9 were not significantly elevated patients with bladder cancer compared with that of the control subjects (166.11 ± 28.66 vs 162.85 ± 4.25 pg/ml; p = 0.636) (Fig. 3a). It is interesting, however, to note that the mean concentration of serum MMP-9 of the patients at the early disease stages (stages I and II) were significantly lower than that of patients at the median and late stages (Ⅲ and Ⅳ) (152.15 ± 5.57 and 184.38 ± 5.57 pg/ml respectively, p = 0.0014) (Fig. 3b). There was no significant difference in concentrations of MMP-9 between male and female patients (p > 0.05).

3.5 Serum IFN-γ

Similarly, although there was significant difference in the mean concentration of serum IFN-γbetween patients with bladder cancer control subjects (7.01 ± 0.61 vs 7.45 ± 0.62 pg/ml respectively; p = 0.606) (Fig. 3c), the mean concentration of serum IFN-γof patients at the median and late stages (Ⅲ and Ⅳ) was significantly lower than that of early stages (stages I and II) (5.65 ± 0.87 vs 8.18 ± 0.12 pg/ml, p = 0.014) (Fig. 3d). Again, serum concentrations of IFN-γwere not significantly different between male and female patients (p > 0.05).

3.6 Serum VEGF

Unlike MMP9 and IFN-γ, the mean of concentrations of serum VEGF of bladder cancer patients was significantly higher than that of control subjects (479.55 ± 40.38 vs. 233.15 ± 30.93 pg/ml; p = 0.0001)

(Fig. 4a). In the meanwhile, the mean concentration of serum VEGF was significantly higher in median and late stages (Ⅲ and Ⅳ stage) than that of early stages (I and II stage) (629.92 ± 83.75, 364.56 ± 55.35 pg/ml respectively; p = 0.011) (Fig. 4b). Again, there was no significant difference in concentrations of VEGF between male and female patients (p > 0.05).

3.7 Serum TGF-β

Similar to VEGF, the mean concentration of serum TGF-β was significantly elevated in patients with bladder cancer than that of control subjects (1308.28 ± 89.65 vs. 1088.70 ± 87.85 pg/ml, p = 0.002) (Fig. 4c). In addition, serum TGF-β was significantly lower in early stages of bladder (I and II stage) than that of median and late stages (Ⅲ and Ⅳ stage) (1088.70 ± 87.85, 1595.41 ± 227.38, p = 0.030). (Fig. 4d) Again, there was no significant difference in serum concentrations of serum TGF-β between male and female patients (p > 0.05).

4. Discussion

It is known that urothelial cell carcinomas constitute approximately 95% of all bladder cancer cases [20], while early diagnosis and timely treatment are very pivotal for increasing 5-year survival rate [21, 22]. Thus, any potential biomarkers for predicting the prognosis and malignancy of the disease are worth to be investigated. Our previous studies proved that elevated expression of IL-17A was observed in tissues derived from bladder cancer, prostate cancer and rectal carcinoma [5, 23, 24] suggesting that this cytokine may promote occurrence and development of malignant diseases. However, there is a lack of systematically association study of IL-17A with its downstream cytokines such as IL-6, VEGF and STAT3. In the present study, immunoreactivities for IL-17, IL-6, VEGF and STAT3 were only statistically higher in tissues of bladder cancer than that of cystitis and controls, but also associated with malignancy of disease (Fig. 1). Because of changes of microbiome, infiltration of pervasive inflammatory cells is a typical feature in cystitis and bladder cancer, which might trigger effective and specific inflammatory response through synthesizing and eliminating more cytokines and inflammatory indicators including IL-17A and IL-6. These abnormal elevated cytokines and humoral factors might make further efforts to produce more inflammatory cytokines and to cause more severe inflammation response and tissue damage,forming a typical positive feedback process. Moreover, persistent activation of STAT3 is able to maintain constitutive VEGF activity, thus providing evidence for the relation between oncology signaling pathways within the inflammatory microenvironment [25]. On the other hand, these cytokines might also promote tumorigenesis, tumor growth even metastasis through inhibiting tumor suppressor genes or activating oncogens such as STAT3, TGF-βand MMP-9. Our data revealed that there existed association between expression of these molecules and malignancy of bladder cancer, which further suggests the importance of these molecules in the pathogenesis of the disease.

It has been shown that IL-17A can increase growth and proliferation of cervical cancer cells via IL-6 or has a potential to act as a prognostic biomarker for the progression of colorectal cancer [16, 27]. Increased IL-6 may act as a main activator of the JAK/STAT3 signaling pathway contributing to tumor

proliferation, angiogenesis and vascular modeling through acting of VEGF and STAT3 [28–30]. Our immune analysis showed that increases in immunoreactivity for IL-6 and in the concentration of serum VEGF are associated with malignancy of bladder cancer (Fig. 1 and Fig. 5). It has been shown that there is a correlation of expression of IL-6 with decreased response following treatment, shorter survival and increased disease failure rates [31–35]. In addition, our research results showed that in bladder cancer, the immunoreactivity IL-17A was positive correlation with IL-6, IL-17A was also associated with STAT3. It is interesting that the expression level of IL-6 was both positive with STAT3 and VEGF. All of the results indicated that IL-17A regulated the synthesis and secretion of IL-6, IL-6 further regulated the expression of STAT3 and VEGF. So IL-17A-IL-6- VEGF axis played a vital role. Because cancer is seen as a kind of chronic inflammatory disease, which promoted the inflammatory cells infiltration in cancerous tissue. Those inflammatory cells such as lymphocytes, monocytes, phagocytes and neutrophils etc could releasing many kinds of cytokines, such as IL-17A, IL-1 and TNF-α etc. In turn, those inflammatory cytokines further activated their receptor, directly or indirectly activate the downstream corresponding elements (oncogene or the genes which promote cancer development even metastasis).

Apart of VEGF, increased concentrations of serum TGF-β and association with malignancy of bladder cancer were also observed in the present study (Fig. 5). In this case, elevated TGF-β and VEGF in late stage of cancer may imply cancer deterioration. It has been known that malignant neoplasms and T cells can produce a large amount of TGF-β at the late stages of most solid cancers [30]. Therefore, targeting cytokines IL-17A, IL-6, VEGF and TGF-β might provide some clinical benefits for patients suffering from bladder cancer. On the other hand, levels of expression of these cytokines might also be used as biomarkers for predicting tumor progression and recurrence.

It is also well known that MMP-9, VEGF and TGF-β, as downstream products of STAT3 or IL-6, can be secreted into serum or local environment to participate in invasion or metastasis of malignancy or to prevent cancer progression [36–39]. MMP-9 is a potent proteolytic enzyme and plays a key role in degradation of basal membranes and the extracellular matrix, through cleaving type IV collagen and gelatin, which are the main structural components of the basal membrane. The proteolytic activity of MMP9 is not only to induce invasion and metastasis, but also to generate matrix-bound growth factors and other signaling molecules responsible for growth signaling, angiogenesis and an inflammatory response [40–42]. Again, ELISA results showed that serum concentration of MMP-9 is associated with malignant grade of bladder cancer (Fig. 4). This is possibly relevant to elevated expression of IL-6 because IL-6 silencing vector decreases expressions of VEGF, MMP-9 and STAT3 [43–44].

Interferon–γ (IFN) as a kind of cytokines, can promote not only immunomodulation but also anticancer activity. After IFN-γ binds to its receptor and subsequently activates its downstream signaling transcriptional pathways which are principally involved in its biological activities. Regarding IFN-γ-dependent immunosurveillance, IFN-γ can directly suppress tumorigenesis and/or can modulate the immunological status in cancer cells and infected cells[45]. However, collected findings showed endogenous IFN-γ not only controls tumor initiation and progression but also shapes tumor immunogenicity and promotes the outgrowth of tumor cells with immunoevasive properties [46]. Whether

IFN-γ is anti-tumorigenic or pro-tumorigenic remains controversial[47–49]. Our research results showed that IFN-γ levels was a litter lower in serum with bladder cancer than that in healthy individuals, but its level was obvious lower in high grade bladder cancer than that in low grade (Fig. 4), which indicated that IFN-γ has antitumor role in bladder cancer. The mechanism may be related with that the secreted IFN-γ by natural killer (NK) cells, cytotoxic T lymphocytes that recruits various cells of innate and adaptive immunity to tumor sites and promote their activation and promote their roles. It is also very well known that IFN-γ enhances antigenicity of tumor cells via up-regulation of the major histocompatibility complex (MHC) class Ia membrane expression. IFN-γ could stimulate expression of tumor antigen-presenting MHC molecules to increase immunogenicity of tumor cells and makes them more susceptible to immune recognition and destruction [50]. IFN-γ also displays direct anticancer activity via inhibition of cell proliferation, e.g, by upregulation of p21 and p27 molecules to arrest the cell cycle, or through mediation of apoptotic cell death [51–52]. Moreover, by targeting non-transformed cells present in the tumor microenvironment, IFN-γ displays its indirect anti-tumor actions, acting as an antiangiogenic factor to inhibit tumor angiogenesis and/or to promote destruction of established tumor-associated blood vessels.

Although we have done a series of comparative immunoreactivities for IL-17A, IL-6, VEGF and STAT3 in bladder cancer and “normal” tissues and serum concentrations of serum MMP-9, IFN-γ, VEGF and TGF-β in patients with bladder cancer and control subjects, there exists obvious limitations in present study. Firstly, the specimens used were obtained through endoscopic biopsy or resection, which might limit our measurements. Secondly, the specimens and serum samples were not completely marched. Finally, we did not collect the follow up data, so that the expression and concentrations of these molecules might not well reflect prognosis of the diseases. Certainly, subsequent studies will be carried out; particularly more samples from subjects with low-grade, early stages of urologic diseases.

In summary, our data suggest that elevated expression of IL-17A, IL-6, STAT3, VEGF□TGF-βand MMP-9 may participate in the pathogenesis of bladder cancer. Association of these molecules with malignant grades suggests that these molecules might be considered as biomarkers, either for diagnosis and prognosis, or for the therapeutic purpose, or both. IFN-γ would be seen a kind of antitumor cytokine in bladder cancer deterioration.

Declarations

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information file.

Authors' contributions

YB designed the experiments and drafted the manuscript. ZS, LJ and JY performed immunohistochemistral staining of IL-17A, IL-6, STAT3 and VEGF cytokines. ZW and WG carried out the statistical analysis. QL and ZJ performed immunohistochemistral staining of inflammatory cells and structural cells.WG and SX performed the Elisa assays. HG provided the experimental place, participated in coordination and supervision. XS and SY conceived of the study and modified the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was approved by the Hospital Ethics Committees of Urinary System Diseases Prevention and Treatment Research Centre, the Affiliated Hospital of Beihua University (approval number: 2012BH006). The written informed consent was obtained from each subject participated in the study. We conformed that we have read the Editorial policy and included relative ethics questions in appropriate place in the present manuscript.

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References

1.
Simons MP, Nauseef WM, Griffith TS. Neutrophils and TRAIL: Insights into BCG immunotherapy for bladder cancer. Immunol Res. 2007;39:79–93.
2.
Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66:7–30.
3.
Young RH. Pseudoneoplastic lesions of the urinary bladder and urethra: a selective review with emphasis on recent information. Semin Diagn Pathol. 1997;14:133–46.
4.
Marsh GM1, Callahan C, Pavlock D, Leviton LC, Talbott EO, Hemstreet G. A protocol for bladder cancer screening and medical surveillance among high-risk groups: the Drake Health Registry experience. J Occup Med. 1990;32:881–6.
5.
Yanbo Liu W, Yang L, Zhao Z, Liang W, Shen Q, Hou, et al. Immune analysis of expression of IL-17 relative ligands and their receptors in bladder cancer: comparison with polyp and cystitis. BMC Immunology. 2016;17:36.
6.
Kolls JK, Linden A. Interleukin-17 family members and inflammation. Immunity. 2004;21:467–76.
7.
Chen M-F, Lin P-Y, Wu C-F, Chen W-C, Wu C-T. IL-6 Expression Regulates Tumorigenicity and Correlates with Prognosis in Bladder Cancer. PLoS One. 2013;8:e61901.
8.
Heo T-H, Wahler J, Suh N. Potential therapeutic implications of IL-6/IL-6R/gp130-targeting agents in breast cancer. Oncotarget. 2016;7:15460 – 73.
9.

Hu Z, Luo D, Wang D, Ma L, Zhao Y, Li L. IL-17Activates the IL-6/STAT3 Signal Pathway in the Proliferation of Hepatitis B Virus-Related Hepatocellular Carcinoma. Cell Physiol Biochem. 2017;43:2379–90.
10.
Huang Q, Duan I, Qian X, Fan J, Lv Z, Zhang X, et al. Corrigendum: IL-17 Promotes Angiogenic Factors IL-6, IL-8, and VEGF Production via Stat1 in Lung Adenocarcinoma. Sci Rep. 2017;7:39566.
11.
Huang Q, Duan L, Qian X, Fan J, Lv Z, Zhang X, et al. IL-17 Promotes Angiogenic FactorsIL-6, IL-8, and Vegf Production via Stat1 in Lung Adenocarcinoma. Sci Rep. 2016;6:36551.
12.
Moore-Smith LD, Isayeva T, Lee JH, Frost A. Ponnazhagan. Silencing of TGF-β1 in tumor cells impacts MMP-9 in tumor microenvironment. Sci Rep. 2017;7:8678.
13.
Shi J, Feng J, Xie J, Mei Z, Shi T, Wang S, et al. Targeted blockade of TGF-β and IL-6/JAK2/STAT3 pathways inhibits lung cancergrowth promoted by bone marrow-derived myofibroblasts. Sci Rep. 2017;7:8660.
14.
Riemensberger J, Böhle A, Brandau S. IFN-gamma and IL-12 but not IL-10 are required for local tumour surveillance in a syngeneic model of orthotopic bladder cancer. Clin Exp Immunol. 2002;127:20–6.
15.
Li Zhang Y-T, Tai MZhiG, Ho L, Qiu, Anderson KC. Interferon-alpha-based immunotherapies in the treatment of B cell-derived hematologic neoplasms in today's treat-to-target era. Exp Hematol Oncol. 2017;6:20.
16.
Hira Lal Goel and. Arthur M. Mercurio. VEGF targets the tumour cell. Nat Rev Cancer, 2013; 13: 871–82.
17.
Decio A, Taraboletti G, Patton V, Alzani R, Perego P, Fruscio R, et al. Vascular endothelial growth factor c promotes ovarian carcinoma progression through paracrine and autocrine mechanisms. Am J Pathol. 2014;184:1050–61.
18.
Crescenzo R, Abate F, Lasorsa E, Tabbo F, Gaudiano M, Chiesa N, et al. Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. Cancer Cell. 2015;27:516–32.
19.
Fan QW, Cheng CK, Gustafson WC, Charron E, Zipper P, Wong RA, et al. EGFR phosphorylates tumor-derived EGFR vIII driving STAT3/5 and progression in glioblastoma. Cancer Cell. 2013;24:438–49.
20.
Aben KK, Kiemeney LA. Epidemiology of bladder cancer. Eur Urol. 1999;366:660–72.
21.

Hall MC, Chang SS, Dalbagni G, Pruthi RS, Seigne JD, Skinner EC, et al. Guideline for the management of nonmuscle invasive bladder cancer (stages Ta, T1, and Tis): 2007 update. J Urol. 2007;1786:2314–30.
22.
Millán-Rodríguez F, Chéchile-Toniolo G, Salvador-Bayarri J, Palou J, Algaba F, Vicente-Rodríguez J. Primary superficial bladder cancer risk groups according to progression, mortality and recurrence. J Urol. 2000;164:680–4.
23.
Liu Y, Zhao X, SunX, Li Y, Wang Z, Jiang J, et al. Expression of IL-17A, E, and F and their receptors in human prostatic cancer: Comparison with benign prostatic hyperplasia. Prostate. 2015;7516:1844–56.
24.
LiuY, Sun X, Zhao X, An L, Wang Z, Jiang J, et al. Expression and location of IL-17A, E, F and their receptors in colorectal adenocarcinoma: Comparison with benign intestinal disease. Pathol Res Pract. 2018;214:482–91.
25.
Lee H, Herrmann A, Deng JH, Kujawski M, Niu G, Li Z, et al. Persistently activated Stat3 maintains constitutive NF-kappaB activity in tumors. Cancer Cell. 2009;15:283–93.
26.
Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H. IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway. J Exp Med. 2009;206:1457–64.
27.
Radosavljevic G, Ljujic B, Jovanovic I, Srzentic Z, Pavlovic S, Zdravkovic N, et al. Interleukin-17 may be a valuable serum tumor marker in patients with colorectal carcinoma. Neoplasma. 2010;57:135–44.
28.
Chen M-F, Lin P-Y, Wu C-F, Chen W-C, Wu C-T. IL-6 expression regulates tumorigenicity and correlates with prognosis in bladder cancer. PLoS One. 2013;8:e61901.
29.
Aggarwal BB, Kunnumakkara AB, Harikumar KB, Gupta SR, Tharakan ST, Koca C, et al. Signal transducer and activator of transcription-3, inflammation, and cancer: how intimate is the relationship? Ann N Y Acad Sci. 2009;1171:59–76.
30.
Connolly EC, Freimuth J, Akhurst RJ. Complexities of TGF-beta Targeted Cancer Therapy. Int J Biol Sci. 2012;8:964–78.
31.
Kishimoto T. Interleukin-6: from basic science to medicine–40 years in immunology. Annu Rev Immunol. 2005;23:1–21.
32.
Schafer ZT, Brugge JS. IL-6 involvement in epithelial cancers. J Clin Invest. 2007;117:3660–63.
33.
Andrews B, Shariat SF, Kim JH, Wheeler TM, Slawin KM, Lerner SP. Preoperative plasma levels of interleukin-6 and its soluble receptor predict disease recurrence and survival of patients with bladder

cancer. J Urol. 2002;167:1475–81.
34.
George DJ, Halabi S, Shepard TF, Sanford B, Vogelzang NJ, Small EJ, et al. The prognostic significance of plasma interleukin-6 levels in patients with metastatic hormone-refractory prostate cancer: results from cancer and leukemia group B 9480. Clin Cancer Res. 2005;11:1815–20.
35.
Scambia G, Testa U, Benedetti Panici P, Foti E, Martucci R, Gadducci A, et al. Prognostic significance of interleukin 6 serum levels in patients with ovarian cancer. Br J Cancer. 1995;71:354–6.
36.
Qi Huang limin, Duan X, Qian J, Lv FZ, Zhang X, et al. IL-17 Promotes Angiogenic Factors IL-6, IL-8, and Vegf Production via Stat1 in Lung Adenocarcinoma. Sci Rep. 2016;6:36551.
37.
Chen C, You F, Wu F, Luo Y, Zheng G, Xu H, et al. Antiangiogenesis Efficacy of Ethanol Extract from Amomum tsaoko in Ovarian Cancer through Inducing ER Stress to Suppress p-STAT3/NF-kB/IL-6 and VEGFLoop. Evid Based Complement Alternat Med. 2020;2020:2390125.
38.
Yoon JH, Pham TH, Lee J, Lee J, Ryu HW, Oh SR, et al. Methyl linderone suppresses TPA-stimulated IL-8 and MMP-9 expression via the ERK/STAT3 pathway in MCF-7 breast cancer cells. J Microbiol Biotechnol. 2019; (Epub ahead of print).
39.
Sun C, Yang J, Cheng HB, Shen WX, Jiang ZQ, Wu MJ, et al. 2-Hydroxy-3-methylanthraquinone inhibits lung carcinoma cells through modulation of IL-6-induced JAK2/STAT3 pathway. Phytomedicine. 2019;61:152848.
40.
Offersen BV, Knap MM, Horsman MR, Verheijen J, Hanemaaijer R, Overgaard J. Matrix metalloproteinase9 measured in urine from bladder cancer patients is an independent prognostic marker of poor survival. Acta Oncol. 2010;49:128387.
41.
Reis ST, Leite KR, Piovesan LF, Pontes-Junior J, Viana NI, Abe DK, et al. Increased expression of MMP-9 and IL-8 are correlated with poor prognosis of Bladder Cancer. BMC Urology. 2012;12:18.
42.
Zeng FC, Cen S, Tang ZY, Kang XL. Elevated matrix metalloproteinase9 expression may contribute to the pathogenesis of bladder cancer. Oncol Let. 2016;11:2213–22.
43.
Bromberg J. Stat proteins and oncogenesis. J Clin Invest. 2002;109:1139–42.
44.
Oken MM. New agents for the treatment of multiple myeloma and non-Hodgkin lymphoma. Cancer. 1992;70:946–8.
45.

Lin C-F, Lin C-M, Lee K-Y, Wu S-Y, Feng P-H, Chen K-Y, et al. Escape from IFN-γ-dependent immunosurveillance in tumorigenesis. J Biomed Sci. 2017;24:10.
46.
Marija Mojic K, Takeda. Yoshihiro Hayakaw. The Dark Side of IFN-γ: Its Role in Promoting Cancer Immunoevasion. Int J Mol Sci, 2018; 19: 89.
47.
Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. Nat Rev Immunol. 2006;6:836–48.
48.
Zaidi MR, Merlino G. The two faces of interferon-γ in cancer. Clin Cancer Res. 2011;17:6118–24.
49.
Zou Q, Jin J, Xiao Y, Zhou X, Hu H, Cheng X, et al. T cell intrinsic USP15 deficiency promotes excessive IFN-γ production and an immunosuppressive tumor microenvironment in MCA-induced fibrosarcoma. Cell Rep. 2015;13:2470–79.
50.
Martini M, Testi MG, Pasetto M, Picchio MC, Innamorati G, Mazzocco M, et al. IFN-γ-mediated upmodulation of MHC class I expression activates tumor-specific immune response in a mouse model of prostate cancer. Vaccine. 2010;28:3548–57.
51.
Harvat BL, Seth P, Jetten AM. The role of p27Kip1 in γ interferon-mediated growth arrest of mammary epithelial cells and related defects in mammary carcinoma cells. Oncogene. 1997;14:2111–22.
52.
Chin YE, Kitagawa M, Su WC, You ZH, Iwamoto Y, Fu XY. Cell growth arrest and induction of cyclin-dependent kinase inhibitor p21 WAF1/CIP1 mediated by STAT1. Science. 1996;272:719–22.

Figures

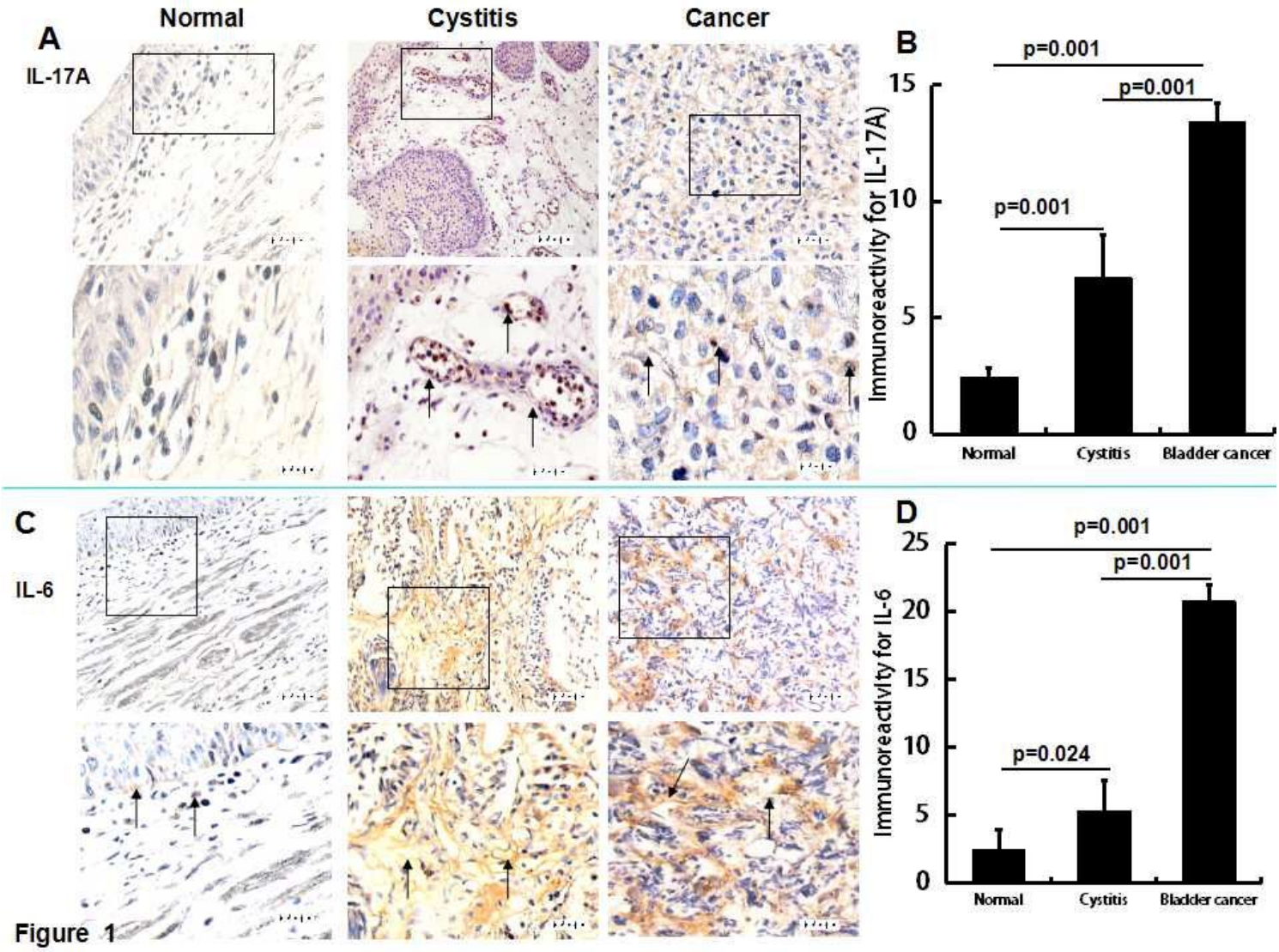

Figure 1

Expression and location of IL-17A and IL-6 in tissue sections of cystitis and bladder cancer. A: Representative photomicrographs of immunoreactivity for IL-17A in tissue sections of subjects with cystitis (n = 23) and bladder cancer (n = 80) and adjacent normal tissues (n=4) (original magnification × 10 and 20). B: Quantitative analysis of immunoreactive area of IL-17A in tissue sections (% of whole sections). C: Representative photomicrographs of immunoreactivity for IL-6 in tissue sections of subjects with cystitis, bladder and adjacent normal tissues (original magnification × 10 and 20). D: Quantitative analysis of immunoreactive area of IL-6 in tissue sections (% of whole sections). Data are expressed as the mean ± SEM. Arrows show examples of positively stained cells. The scale bar means 100 μm.

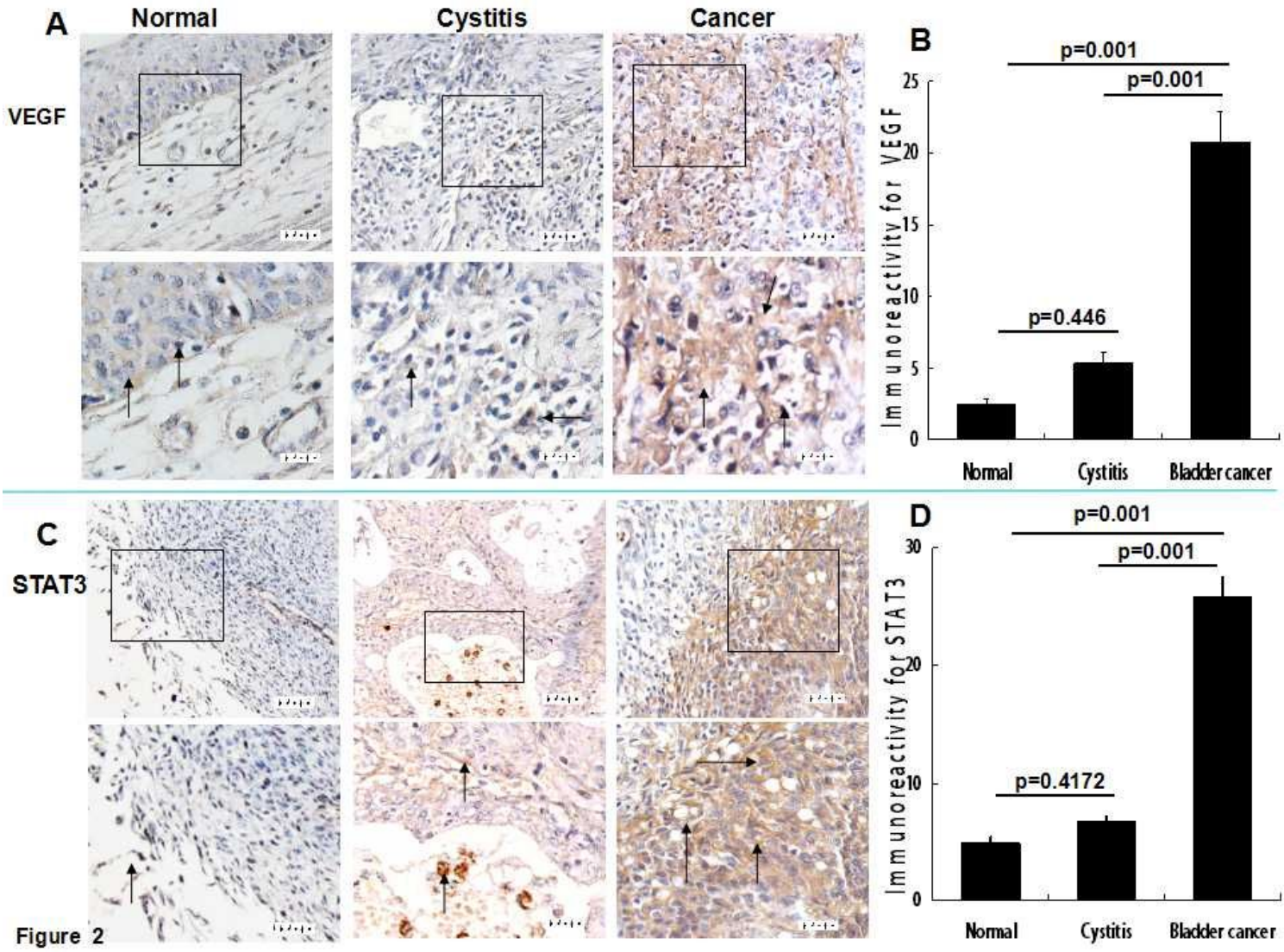

Figure 2

Expression and location of VEGF and STAT3 in tissue sections of cystitis and bladder cancer. A: Representative photomicrographs of immunoreactivity for VEGF in tissue sections of subjects with cystitis (n = 23) and bladder cancer (n = 80) and adjacent normal tissues (n=4) (original magnification × 10 and 20). B: Quantitative analysis of immunoreactive area of VEGF in tissue sections (% of whole sections). C: Representative photomicrographs of immunoreactivity for STAT3 in tissue sections of subjects with cystitis, bladder cancer and adjacent normal tissues (original magnification × 10 and 20). D: Quantitative analysis of immunoreactive area of STAT3 in tissue sections (% of whole sections). Data are expressed as the mean ± SEM. Arrows show examples of positively stained cells. The scale bar means 100 μm.

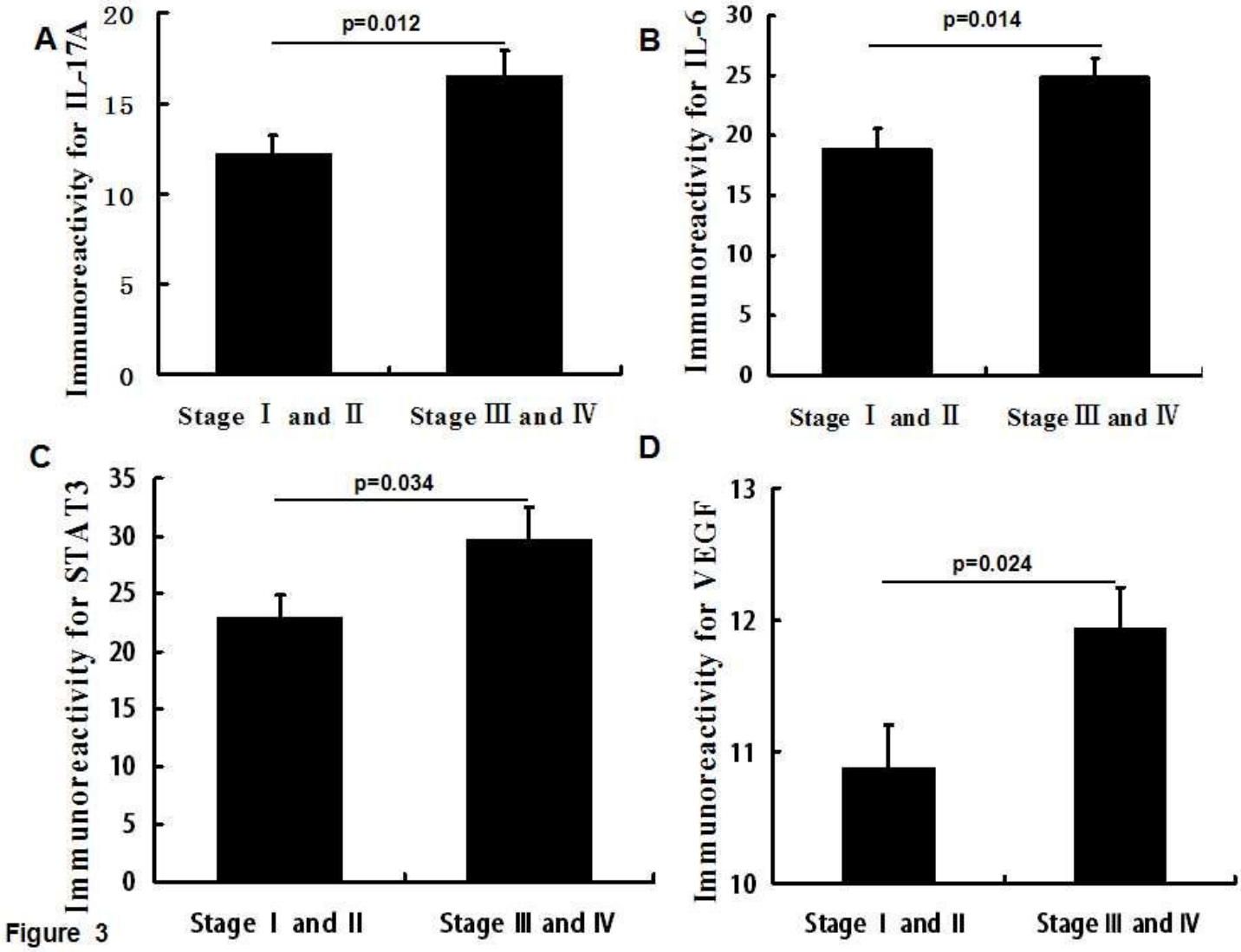

Figure 3

Figure 3

The relationships between immunoreactivities for IL-17A, IL-6, VEGF and STAT3 and malignancy in bladder cancer. A: The association of immunoreactivity for IL-17A with bladder cancer malignancy. B: The association of immunoreactivity for IL-6 with bladder cancer malignancy. C: The association of immunoreactivity for STAT3 with bladder cancer malignancy. D: The association of immunoreactivity for VEGF with bladder cancer malignancy. Data are expressed as the mean ± SEM.

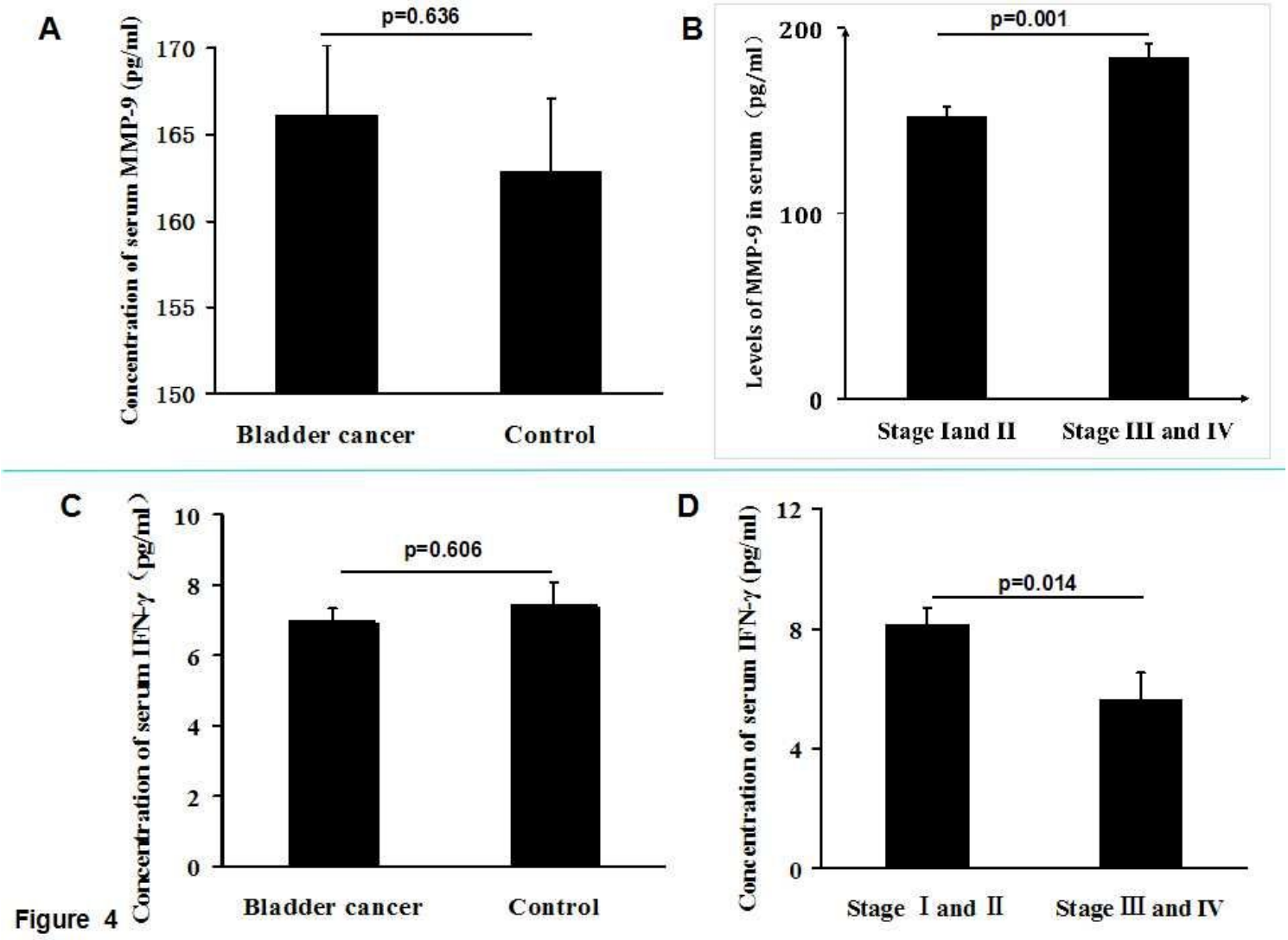

Figure 4

Figure 4

Concentrations of serum MMP-9 and IFN-γin bladder cancer patients and control subjects. Concentrations of serum MMP-9 and IFN-γwere measured retrospectively in patients with bladder cancer (n = 34) and controls (n = 50) using the commercial ELISA kits. Data are expressed the mean ± SEM.

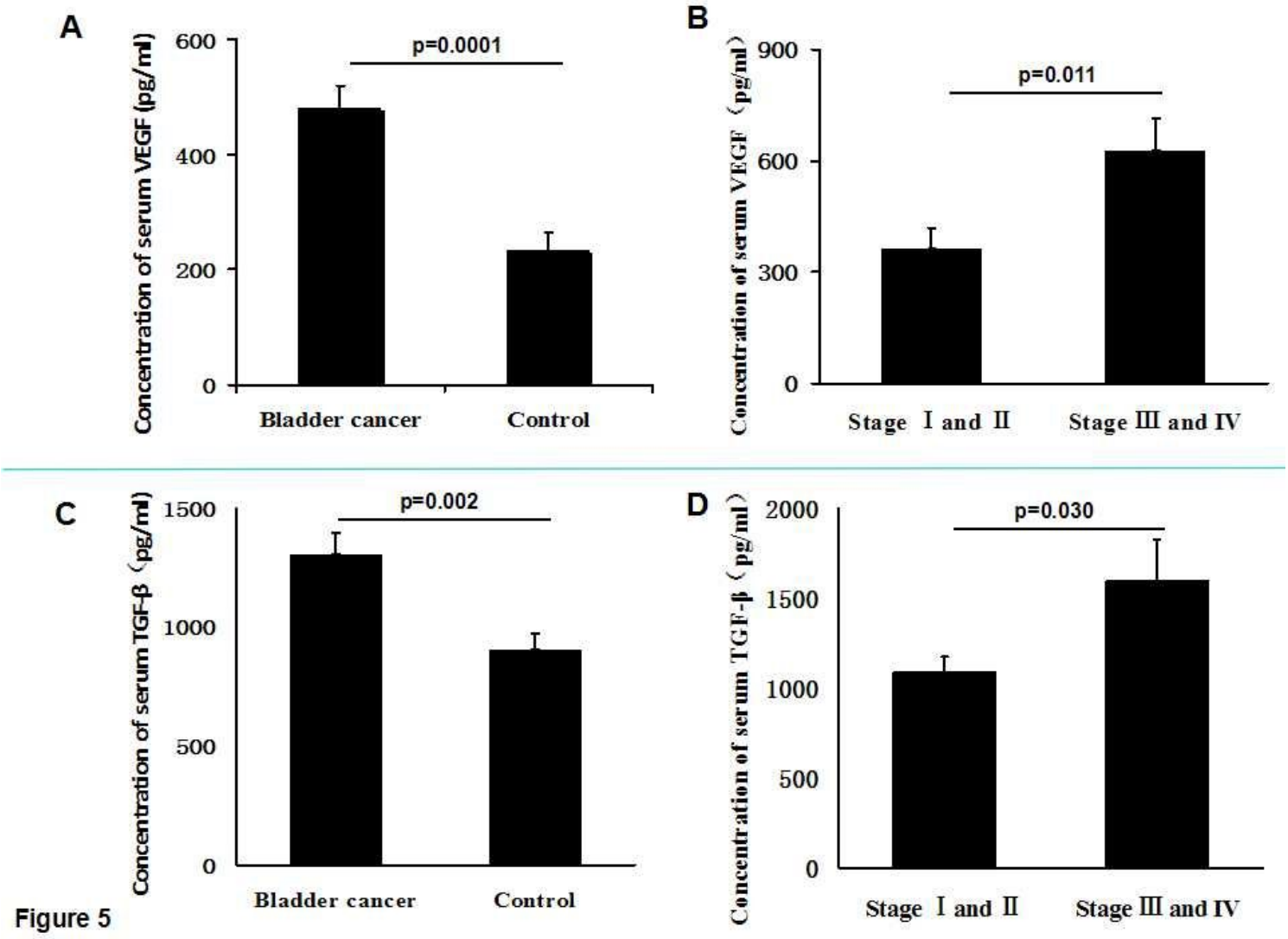

Figure 5

Figure 5

Concentrations of serum VEGF and TGF-βin bladder cancer patients and control subjects. Concentrations of serum VEGF and TGF-β were measured retrospectively in patients with bladder cancer (n = 34) and controls (n = 50) using the commercial ELISA kits. Data are expressed the mean ± SEM.