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Original Research

Increased Expression of IL-17A, IL-6, STAT3, TGF-β, and VEGF: Potential Biomarkers in Bladder Cancer?

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ABSTRACT

Aim: To explore the association of IL-17A, IL-6, STAT3, VEGF, TGF-β, and MMP-9 with the severity of bladder cancer, and analyze the pathogenesis of bladder cancer.

Methods: Immunohistochemistry (IHC) analyzed the expression and location of IL-17A, IL-6, STAT3, and VEGF in bladder cancer specimens with different degrees of malignancy (n = 80), cystitis (n = 23), and normal adjacent tissues (n = 4). ELISA measured the serum concentrations of MMP-9, TGF-β, VEGF, and IFN-γ in bladder cancer patients (n = 34) and controls (n = 50).

Results: IL-17A, IL-6, STAT3, and VEGF were significantly increased in bladder cancer tissues compared to both cystitis (P = 0.001) and normal adjacent tissues (P = 0.001), and were positively associated with the degree of malignancy. Serum concentrations of TGF-β and VEGF were significantly higher in bladder cancer patients compared with controls (P = 0.002 and P = 0.0001, respectively), while concentrations of MMP-9 and IFN-γ were not significantly different among groups. MMP-9, VEGF, and TGF-β serum concentration increased with the severity of malignancy, and the difference between low-grade and high-grade malignancy was significant (P = 0.001, P = 0.011, and P = 0.030, respectively), IFN-γ serum secretion was lower in high-grade compared to low-grade malignancy (P = 0.014).

Conclusion: Elevated expression of IL-17A, IL-6, and STAT3 in tissues and elevated serum concentration of TGF-β, and VEGF might be considered potential biomarkers of bladder carcinoma progression.

Trial registration: ISRCTN, ISRCTN2012BH006. Registered 10 January 2012.

Keywords: Bladder cancer, interleukin-17, interleukin-6, interferon-γ, vascular endothelial growth factor

INTRODUCTION

Bladder cancer is the second most common urological malignancy, accounting for 5% of all cancer-related deaths in the USA.[1] It is estimated that there were approximately 76,960 new cases of bladder cancer and 16,390 deaths in the year 2016.[2] The incidence in males is about fourfold higher than that in females, while the incidence is double in white men compared to their black counterparts. Cystitis is considered a high risk for bladder cancer.[3] It has been shown

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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, interleukin-6 (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 proinflammatory cytokines has been shown to lead to tumorigenesis, invasion, angiogenesis, and prognosis. The collecting evidence shows that proinflammatory cytokines, such as IL-17A family, tumor necrosis factor-alpha (TNF-α), IL-6, VEGF, etc. play important roles in bladder cancer. Cytokine IL-17A can activate IL-6 in inflammatory or cancerous disease, while IL-6, 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 the 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 by enhancing tumor immunogenicity.[15] Although the collecting data suggest that IL-17A, IL-6, STAT3, TGF-β, and VEGF may be critical cytokines in various malignancies, the 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 the bladder, the serum concentrations of MMP-9, TGF-β, VEGF, and IFN-γ, and their association with the severity of bladder cancer.

MATERIALS AND METHODS

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). Written consent was 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 and December 2014. All the tissue samples were identified and diagnosed by two clinical pathologists with a double-blind method. The clinical characteristics of the subjects involved in this study are summarized in Table 1, which is the same as our previously published study.[5]

Serum samples were collected from 34 preoperative patients with bladder cancer and 50 age-matched healthy volunteers. None of the bladder cancer patients received radiotherapy or chemotherapy before surgery, while the healthy volunteers had no immunological or infectious diseases of any kind. Informed consent was obtained from each participant for the use of blood samples in the present study [Table 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 mmol/L, 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 a further 30 min at room

Table 1. *Clinical characteristics of the subjects from cystitis and bladder cancer*

Condition	Age (median)	Sources	Pathological characteristics	Differentiation grade
Adjacent normal tissue n = 4	61.2 (55-73) Male 4	Resection specimens	Healthy tissue	Not applicable
Cystitis n = 23	54.3 (22-84) Male 6 Female 17	Endoscopic biopsies or resection specimens	Inflammation	Acute n = 18 Chronic n = 5
Bladder cancer n = 80	64.5 (45-84) Male 66 Female 14	Endoscopic biopsies or resection specimens	Adenocarcinoma	Low-grade n = 47 High-grade n = 33

Table 2. *Clinical characteristics of the subjects from bladder cancer and healthy volunteers*

Status	Age (median)	Sources	Pathological diagnosis	Differentiation grade
Healthy volunteers	61.3 (39-78)	Serum	Healthy serum	Not applicable
n = 50	Male 39			
	Female 11			
Bladder cancer	67.2 (48-82)	Serum	Adenocarcinoma	Low-grade *n* = 21
n = 34	Male 26			High-grade *n* = 13
	Female 8			

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 are 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 further analysis. The brown positive signals were quantified as the percentages of IL-17A, IL-6, STAT3, and VEGF immunoreactive staining of the total hematoxylin counterstaining area of the entire sections as described previously.[5]

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].

Statistical analysis

Data obtained from 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 the Kruskal-Wallis test followed by the Mann-Whitney U test. Data from ELISA assays were analyzed using the 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.

RESULTS

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 ($P = 0.001$ and $P = 0.001$, respectively) [Figure 1A and B]. In addition, immunoreactivity for IL-17A was significantly higher in tissue sections of bladder cancer than that of cystitis ($P = 0.001$). Immunoreactivity for IL-17A was mainly located in mononuclear cells, transitional epithelial cells, malignant cells, and vascular endothelial cells in bladder cancer [Figure 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 infiltrating macrophages 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 immunoreactivity for IL-6 was significantly elevated in sections of tissues of bladder cancer compared with that of cystitis and normal tissues ($P = 0.001$ and $P = 0.001$, respectively) [Figure 1C and D], while IL-6 immunoreactivity in sections of tissues of cystitis was also significantly higher in cystitis than that of normal tissues ($P = 0.024$). IL-6 immunoreactivity was predominantly located in monocytes, transitional epithelial cells, malignant cells as well as vascular endothelial cells [Figure 1C].

Table 3. *Antibodies or reagent kit used in this research*

Antibody	Item number	Isotype	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-γ Elisa 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-β Elisa kit	BMS249/4TEN	Rabbit-IgG	Already wrapped	eBioscience, Bender MedSystems GmbH, Vienna (Austria)

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 and compared them 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 (P = 0.001) [Figure 2A and B]. Although the expression of VEGF in cystitis was elevated, it did not achieve statistical significance when it was compared with that of normal tissues (P = 0.446). Immunoreactivity for VEGF was mainly located in vascular endothelial cells of tumor parenchyma compared to the stroma of tumors [Figure 2A].

STAT3 is linked to inflammation-related oncogenesis and is 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 considered to up-regulate and activate STAT3.[18,19] Again, our immunohistochemical staining analysis showed that STAT3 immunoreactivity was significantly higher in bladder cancer tissues compared with the controls (P = 0.001) [Figure 2C and D], while there was no significant difference between cystitis and normal tissues (P = 0.4172). STAT3 was mainly located in malignant cells, vascular endothelial cells, and transitional epithelial cells [Figure 2C].

Association between the expression of IL-17A, IL-6, VEGF, and STAT3 and severity of bladder cancer

Further investigation showed that tissue sections from the median and late stages (High-grade) had considerably higher levels of immunoreactivities for IL-17A, IL-6, STAT3, and VEGF than those from the early stages (Low-grade) (P = 0.012, P = 0.014, P = 0.034, P = 0.024, respectively) [Figure 3A-D].

IL-17A, IL-6, STAT3, and VEGF expression levels were correlated using the Pearson correlation method. 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 IL-6 expression in bladder cancer was associated with that of VEGF (r = 0.3968) which indicated that the IL-17A-IL-6-VEGF signal axis might participate in the occurrence and progression of bladder cancer.

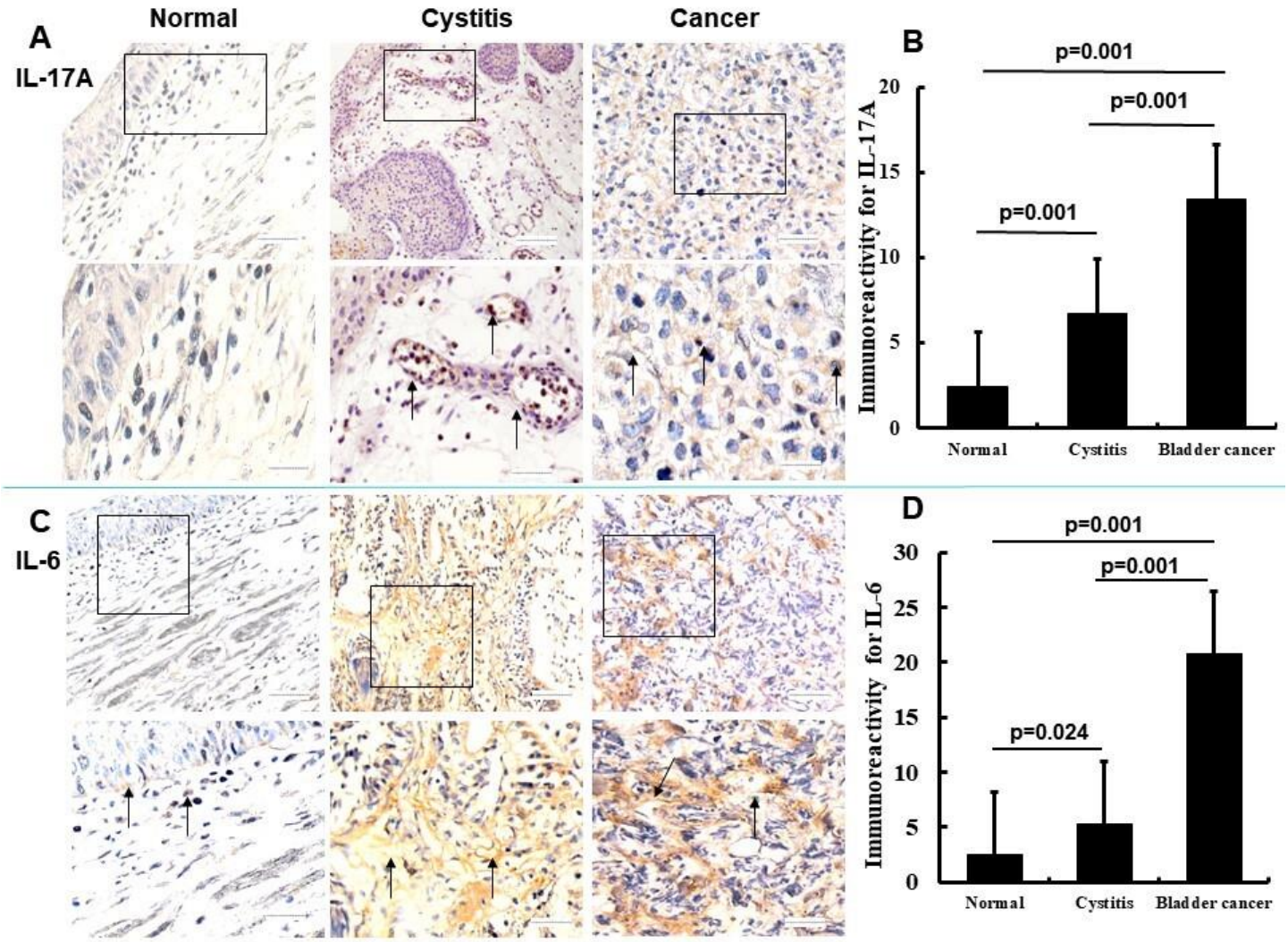

Figure 1. *Expression and location of IL-17A and IL-6 in tissue sections of cystitis and bladder cancer. A: Representative photomicrographs of tissue sections showing immunoreactivity to IL-17A in subjects with cystitis (n = 23), bladder cancer (n = 80) and adjacent normal tissues (n = 4) (original magnification × 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 cancer 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.*

Serum MMP-9

Total concentrations of MMP-9 were not significantly elevated in patients with bladder cancer compared with that of the control subjects (166.11 ± 28.66 *vs* 162.85 ± 4.25 pg/mL; $P = 0.636$) [Figure 4A]. It is interesting, however, to note that the mean concentration of serum MMP-9 of patients at the early disease stages (Low-grade) was significantly lower than that of patients at the median and late stages (High-grade) (152.15 ± 5.57 and 184.38 ± 5.57 pg/mL respectively, $P=0.001$) [Figure 4B]. There was no significant difference in concentrations of MMP-9 between male and female patients ($P > 0.05$).

Serum IFN-γ

Similarly, although there was no significant difference in the mean concentration of serum IFN-γ between patients with bladder cancer and control subjects (7.01 ± 0.61 *vs* 7.45 ± 0.62 pg/mL respectively; $P = 0.606$) [Figure 4C], the mean concentration of serum IFN-γ of patients at the median and late stages (High-grade) was significantly lower than that of early stages (Low-grade) (5.65 ± 0.87 *vs* 8.18 ± 0.12 pg/mL, $P = 0.014$) [Figure 4D]. Again, serum concentrations of IFN-γ were not significantly different between male and female patients ($P > 0.05$).

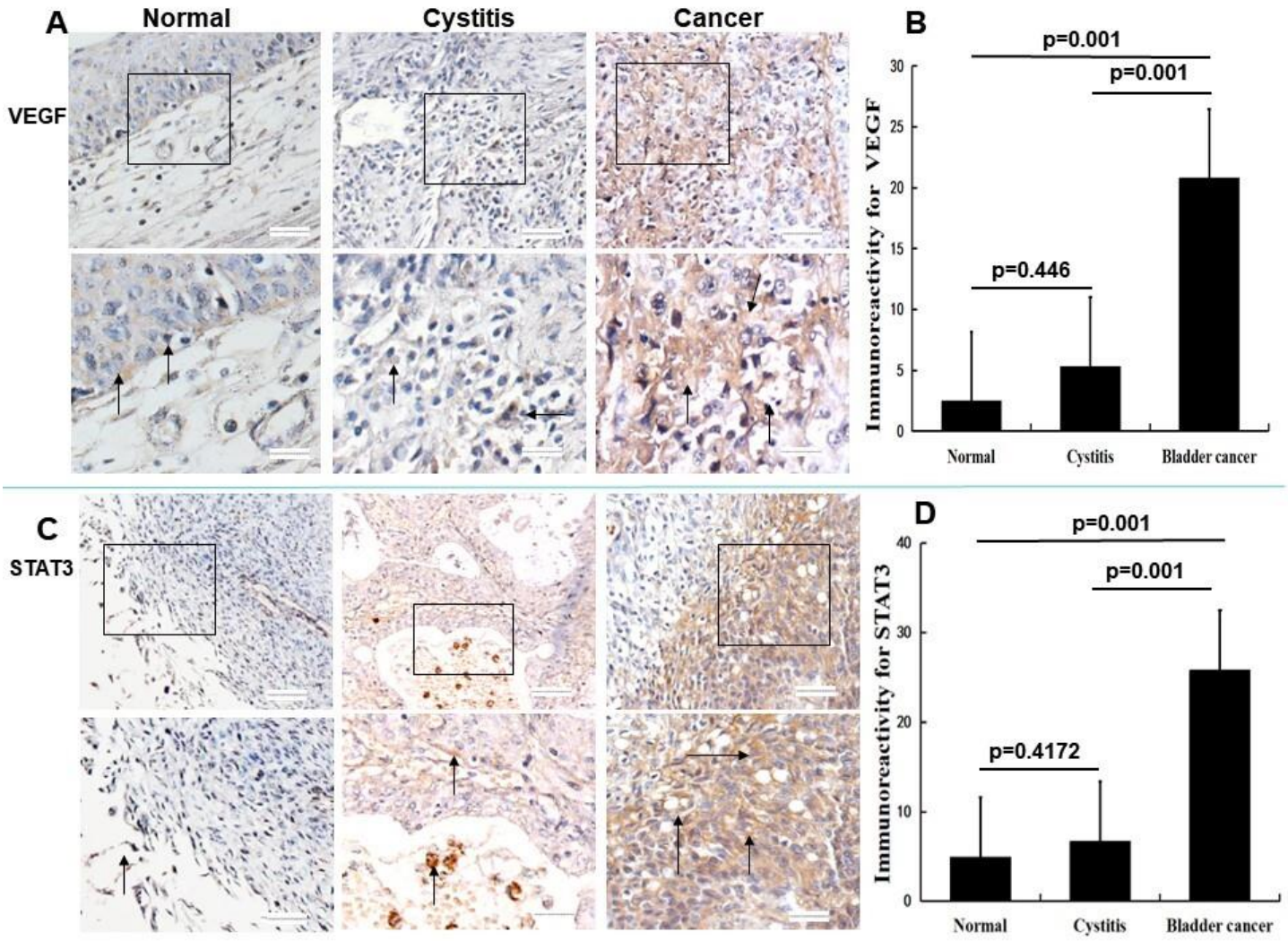

Figure 2. *Expression and location of VEGF and STAT3 in tissue sections of cystitis and bladder cancer. A: Representative photomicrographs of tissue sections showing immunoreactivity to VEGF in subjects with cystitis (n = 23), bladder cancer (n = 80) and adjacent normal tissues (n = 4) (original magnification × 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.*

Serum VEGF

Unlike MMP9 and IFN-γ, the mean concentration 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, $P = 0.0001$) [Figure 5A]. In addition, the mean concentration of serum VEGF was significantly higher in the median and late stages (High-grade) than that of early stages (Low-grade) (629.92 ± 83.75 *vs* 364.56 ± 55.35 pg/mL respectively, $P = 0.011$) [Figure 5B]. Again, there was no significant difference in concentrations of VEGF between male and female patients ($P > 0.05$).

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* 910.10 ± 65.22 pg/mL, $P = 0.002$) [Figure 5C]. In addition, serum TGF-β was significantly lower in the early stages of bladder cancer (Low-grade) than that of median and late stages (High-grade) (1088.70 ± 87.85 *vs* 1595.41 ± 227.38 pg/mL, $P = 0.030$) [Figure 5D]. Again, there was no significant difference in serum concentrations of TGF-β between male and female patients ($P > 0.05$).

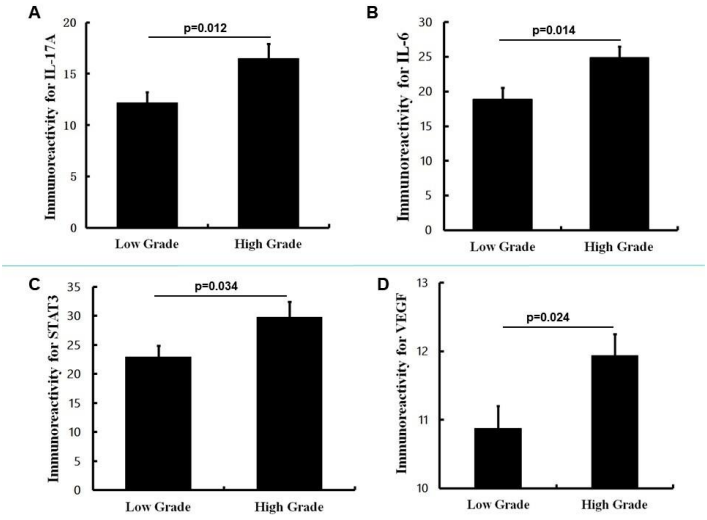

Figure 3. *The relationships between immunoreactivities for IL-17A, IL-6, STAT3 and VEGF and malignancy in bladder cancer. A: The association of immunoreactivity for IL-17A with bladder cancer malignancy. B: The association of immunoreactivity for IL-6 with bladder cancer malignancy. C: The association 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.*

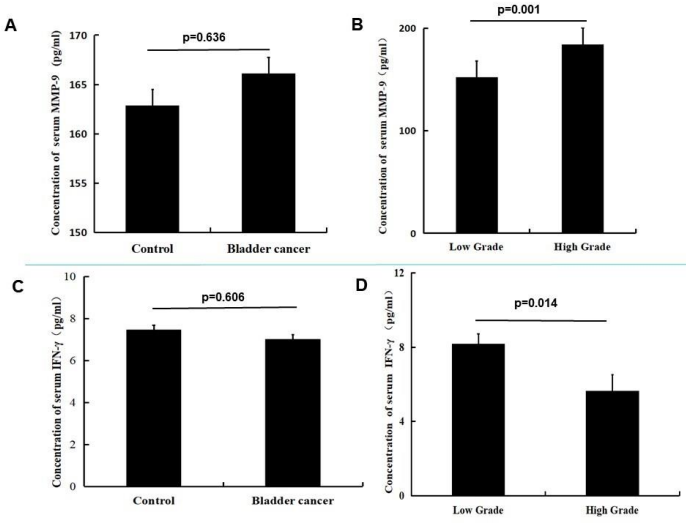

Figure 4. *Concentrations of serum MMP-9, IFN-γ in bladder cancer patients and control subjects. Concentrations of serum MMP-9 and IFN-γ were measured retrospectively in patients with bladder cancer (n = 34) and controls (n = 50) using the commercial ELISA kits. Data are expressed the mean ± SEM.*

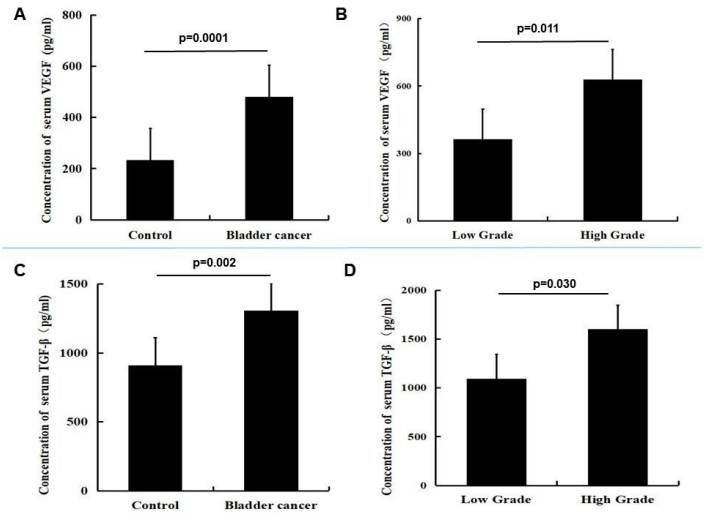

Figure 5. *Concentrations of serum VEGF and TGF-β in bladder cancer patients and control subjects. Concentrations of serum VEGF and TGF-β were measured retrospectively in patients with bladder cancer (n = 34) and controls (n = 50) using the commercial ELISA kits. Data are expressed the mean ± SEM.*

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 the 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 suggesting that this cytokine may promote the occurrence and development of malignant diseases.[5,23,24] However, the association between IL-17A and its downstream cytokines such as IL-6, VEGF, and STAT3, has not been systematically investigated. In the present study, immunoreactivities for IL-17, IL-6, VEGF, and STAT3 were significantly higher in tissues of bladder cancer than that of cystitis and controls and were also associated with malignancy of the disease. A common hallmark of cystitis and bladder cancer due to altered microbiota is the infiltration of pervasive inflammatory cells, which may generate an effective and specific inflammatory response by synthesizing and eliminating more cytokines and inflammatory markers including IL-17A and IL-6. These abnormally elevated cytokines and humoral factors may induce the production of more inflammatory cytokines leading to severe inflammatory response and tissue damage, forming a typical positive feedback process. Moreover, persistent activation of STAT3 can 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, and even metastasis through inhibiting tumor suppressor genes or activating oncogenes such as STAT3, TGF-β, and MMP-9. Our data revealed that there existed an association between the expression of these molecules and the severity of malignancy, which further suggests the importance of these molecules in the pathogenesis of the disease.

It has been shown that IL-17A can increase the growth and proliferation of cervical cancer cells via IL-6 or has the potential to act as a prognostic biomarker for the progression of colorectal cancer.[26,27] Increased IL-6 may act as the main activator of the JAK/STAT3 signaling pathway contributing to tumor proliferation, angiogenesis, and vascular modeling through VEGF and STAT3 activation.[28-30] Our immune analysis showed that an elevated IL-6 expression and serum VEGF concentration are associated with the malignancy of bladder cancer. A correlation has been shown between an elevated expression of IL-6 and decreased response to treatment, shorter survival time, and increased disease failure rates.[31-35] In addition, our research results showed that, in bladder cancer, the immunoreactivity to IL-17A was positively correlated with IL-6, further IL-17A was also associated with STAT3. Interestingly, IL-6 expression levels correlated positively with STAT3 and VEGF. All of the results indicated that IL-17A regulated the synthesis and secretion of IL-6, and IL-6 further regulated the expression of STAT3 and VEGF. Hence, the IL-17A-IL-6-VEGF axis played a vital role. Since cancer is a chronic inflammatory disease, it promotes the infiltration of inflammatory cells in the cancerous tissue. Those inflammatory cells such as lymphocytes, monocytes, phagocytes, neutrophils, etc. could release many kinds of cytokines, such as IL-17A, IL-1, TNF-α, etc. In turn, those inflammatory cytokines further activated their receptor, directly or indirectly activating the downstream corresponding elements (oncogene or the genes which promote cancer development and even metastasis).

Apart from VEGF, increased concentrations of serum TGF-β and their association with malignancy of bladder cancer were also observed in the present study. In this case, elevated TGF-β and VEGF in the 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.[11,36-38] MMP-9 is a potent proteolytic enzyme and plays a key role in the 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 MMP-9 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 inflammatory response.[39-41] Again, ELISA results showed that serum concentration of MMP-9 is associated with malignant grade of bladder cancer. This is possibly relevant to elevated expression of IL-6 because the IL-6 silencing vector decreases expressions of VEGF, MMP-9, and STAT3.[42,43]

IFN-γ, a kind of cytokine, can promote not only immunomodulation but also anticancer activity. 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 of cancer cells and infected cells.[44] The collected findings show that endogenous IFN-γ not only controls tumorigenesis and progression but also shapes the immunogenicity of tumors and promotes the growth of tumor cells with immune evolutionary properties.[45] Whether IFN-γ is anti-tumorigenic or pro-tumorigenic remains controversial.[46-48] Our research results showed that IFN-γ levels were a litter lower in serum samples of bladder cancer patients than that of healthy individuals, but its levels were significantly lower in high-grade bladder cancer than that in low-grade, which indicated that IFN-γ has an antitumor role in bladder cancer. The mechanism may involve IFN-γ secreted by natural killer (NK) cells and cytotoxic T lymphocytes, which recruit various cells of innate and adaptive immunity to tumor sites and promote their activation function. 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 the expression of tumor antigen-presenting MHC molecules to increase the immunogenicity of tumor cells and makes them more susceptible to immune recognition and destruction.[49] 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 the mediation of apoptotic cell death.[50,51] 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 the 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 MMP-9, IFN-γ, VEGF, and TGF-β in patients with bladder cancer and control subjects, the present study has obvious limitations. 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 the expression and concentrations of these molecules might not well reflect the prognosis of the diseases. Certainly, subsequent studies will be carried out; particularly more samples from subjects with low-grade, early-stage urologic diseases.

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

CONCLUSION

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

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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). Written informed consent was obtained from each subject who participated in the study. We confirmed that we have read the editorial policy and included relative ethics questions in the appropriate place in the present manuscript.

AUTHOR CONTRIBUTIONS

Yanbo Liu designed the experiments and drafted the manuscript. Zishen Xiao and Teng Zhao performed immunohistochemistral staining of IL-17A, IL-6, STAT3 and VEGF cytokines. Jiayu Lin performed the Elisa assays. Chengxia Bai and Lijuan Yang carried out the statistical analysis. Zhenjiang Wang provided the experimental place and participated in coordination and supervision. Jian Liu provided some experimental samples. Ying Sun conceived the study and modified the manuscript. All authors have read and approved the final manuscript.

REFERENCES

1. Simons MP, Nauseef WM, Griffith TS. Neutrophils and TRAIL: Insights into BCG immunotherapy for bladder cancer. *Immunol Res* 2007; 39(1-3): 79-93.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* 2016; 66(1): 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(2): 133-46.
4. Marsh GM, 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(9): 881-6.
5. Liu Y, Yang W, Zhao L, Liang Z, Shen W, Hou Q, Wang Z, Jiang J, Ying S. Immune analysis of expression of IL-17 relative ligands and their receptors in bladder cancer: comparison with polyp and cystitis. *BMC Immunol* 2016; 17(1): 36.
6. Kolls JK, Lindén A. Interleukin-17 family members and inflammation. *Immunity* 2004; 21(4): 467-76.
7. Chen MF, Lin PY, Wu CF, Chen WC, Wu CT. IL-6 expression regulates tumorigenicity and correlates with prognosis in bladder cancer. *PLoS One* 2013; 8(4): e61901.
8. Heo TH, Wahler J, Suh N. Potential therapeutic implications of IL-6/IL-6R/gp130-targeting agents in breast cancer. *Oncotarget* 2016; 7(13): 15460-73.
9. Hu Z, Luo D, Wang D, Ma L, Zhao Y, Li L. IL-17 Activates the IL-6/STAT3 Signal Pathway in the Proliferation of Hepatitis B Virus-Related Hepatocellular Carcinoma. *Cell Physiol Biochem* 2017; 43(6): 2379-90.
10. Huang Q, Duan I, Qian X, Fan J, Lv Z, Zhang X, Han J, Wu F, Guo M, Hu G, Du J, Chen C, Jin Y. 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, Han J, Wu F, Guo M, Hu G, Du J, Chen C, Jin Y. IL-17 Promotes Angiogenic Factors IL-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 S. Silencing of TGF-β1 in tumor cells impacts MMP-9 in tumor microenvironment. *Sci Rep* 2017; 7(1): 8678.
13. Shi J, Feng J, Xie J, Mei Z, Shi T, Wang S, Du Y, Yang G, Wu Y, Cheng X, Li S, Zhu L, Yang CS, Tu S, Jie Z. Targeted blockade of TGF-β and IL-6/JAK2/STAT3 pathways inhibits lung cancer growth promoted by bone marrow-derived myofibroblasts. *Sci Rep* 2017; 7(1): 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(1): 20-6.
15. Zhang L, Tai YT, Ho MZG, Qiu L, 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. Goel HL, Mercurio AM. VEGF targets the tumour cell. *Nat Rev Cancer* 2013; 13(12): 871-82.
17. Decio A, Taraboletti G, Patton V, Alzani R, Perego P, Fruscio R, Jürgensmeier JM, Giavazzi R, Belotti D. Vascular endothelial growth factor c promotes ovarian carcinoma progression through paracrine and autocrine mechanisms. *Am J Pathol* 2014; 184(4): 1050-61.
18. Crescenzo R, Abate F, Lasorsa E, Tabbo' F, Gaudiano M, Chiesa N, Di Giacomo F, Spaccarotella E, Barbarossa L, Ercole E, Todaro M, Boi M, Acquaviva A, Ficarra E, Novero D, Rinaldi A, Tousseyn T, Rosenwald A, Kenner L, Cerroni L, Tzankov A, Ponzoni M, Paulli M, Weisenburger D, Chan WC, Iqbal J, Piris MA, Zamo' A, Ciardullo C, Rossi D, Gaidano G, Pileri S, Tiacci E, Falini B, Shultz LD, Mevellec L, Vialard JE, Piva R, Bertoni F, Rabadan R, Inghirami G; European T-Cell Lymphoma Study Group, T-Cell Project: Prospective Collection of Data in Patients with Peripheral T-Cell Lymphoma and the AIRC 5xMille Consortium "Genetics-Driven Targeted Management of Lymphoid Malignancies". Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. *Cancer Cell* 2015; 27(4): 516-32.
19. Fan QW, Cheng CK, Gustafson WC, Charron E, Zipper P, Wong RA, Chen J, Lau J, Knobbe-Thomsen C, Weller M, Jura N, Reifenberger G, Shokat KM, Weiss WA. EGFR phosphorylates tumor-derived EGFRvIII driving STAT3/5 and progression in glioblastoma. *Cancer Cell* 2013; 24(4): 438-49.
20. Aben KK, Kiemeney LA. Epidemiology of bladder cancer. *Eur Urol* 1999; 36 (6): 660-72.
21. Hall MC, Chang SS, Dalbagni G, Pruthi RS, Seigne JD, Skinner EC, Wolf JS Jr, Schellhammer PF. Guideline for the management of nonmuscle invasive bladder cancer (stages Ta, T1, and Tis): 2007 update. *J Urol* 2007; 178(6): 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(3 Pt 1): 680-4.
23. Liu Y, Zhao X, Sun X, Li Y, Wang Z, Jiang J, Han H, Shen W, Corrigan CJ, Sun Y. Expression of IL-17A, E, and F and their receptors in human prostatic cancer: Comparison with benign prostatic hyperplasia. *Prostate* 2015; 75(16): 1844-56.
24. Liu Y, Sun X, Zhao X, An L, Wang Z, Jiang J, Shen W, Yang X, Sun Y. Expression and location of IL-17A, E, F and their receptors in colorectal adenocarcinoma: Comparison with benign intestinal disease. *Pathol Res Pract* 2018; 214(4): 482- 91.

25. Lee H, Herrmann A, Deng JH, Kujawski M, Niu G, Li Z, Forman S, Jove R, Pardoll DM, Yu H. Persistently activated Stat3 maintains constitutive NF-kappaB activity in tumors. *Cancer Cell* 2009; 15(4): 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(7): 1457-64.
27. Radosavljevic G, Ljujic B, Jovanovic I, Srzentic Z, Pavlovic S, Zdravkovic N, Milovanovic M, Bankovic D, Knezevic M, Acimovic LJ, Arsenijevic N. Interleukin-17 may be a valuable serum tumor marker in patients with colorectal carcinoma. *Neoplasma* 2010; 57(2): 135-44.
28. Chen MF, Lin PY, Wu CF, Chen WC, Wu CT. IL-6 expression regulates tumorigenicity and correlates with prognosis in bladder cancer. *PLoS One* 2013; 8(4): e61901.
29. Aggarwal BB, Kunnumakkara AB, Harikumar KB, Gupta SR, Tharakan ST, Koca C, Dey S, Sung B. 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-β targeted cancer therapy. *Int J Biol Sci* 2012; 8(7): 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(12): 3660-3.
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(3): 1475 - 81.
34. George DJ, Halabi S, Shepard TF, Sanford B, Vogelzang NJ, Small EJ, Kantoff PW. 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(5): 1815-20.
35. Scambia G, Testa U, Benedetti Panici P, Foti E, Martucci R, Gadducci A, Perillo A, Facchini V, Peschle C, Mancuso S. Prognostic significance of interleukin 6 serum levels in patients with ovarian cancer. *Br J Cancer* 1995; 71(2): 354-6.
36. Chen C, You F, Wu F, Luo Y, Zheng G, Xu H, Liu Y. Antiangiogenesis Efficacy of Ethanol Extract from Amomum tsaoko in Ovarian Cancer through Inducing ER Stress to Suppress p-STAT3/NF-kB/IL-6 and VEGF Loop. *Evid Based Complement Alternat Med* 2020; 2020: 2390125.
37. Yoon JH, Pham TH, Lee J, Lee J, Ryu HW, Oh SR, Oh JW, Yoon DY. 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* 2020; 30(3): 325-32.
38. Sun C, Yang J, Cheng HB, Shen WX, Jiang ZQ, Wu MJ, Li L, Li WT, Chen TT, Rao XW, Zhou JR, Wu MH. 2-Hydroxy-3-methylanthraquinone inhibits lung carcinoma cells through modulation of IL-6-induced JAK2/STAT3 pathway. *Phytomedicine* 2019; 61: 152848.
39. Offersen BV, Knap MM, Horsman MR, Verheijen J, Hanemaaijer R, Overgaard J. Matrix metalloproteinase-9 measured in urine from bladder cancer patients is an independent prognostic marker of poor survival. *Acta Oncol* 2010; 49(8): 1283-7.
40. Reis ST, Leite KR, Piovesan LF, Pontes-Junior J, Viana NI, Abe DK, Crippa A, Moura CM, Adonias SP, Srougi M, Dall'Oglio MF. Increased expression of MMP-9 and IL-8 are correlated with poor prognosis of Bladder Cancer. *BMC Urol* 2012; 12: 18.
41. Zeng FC, Cen S, Tang ZY, Kang XL. Elevated matrix metalloproteinase-9 expression may contribute to the pathogenesis of bladder cancer. *Oncol Lett* 2016; 11(3): 2213-22.
42. Bromberg J. Stat proteins and oncogenesis. *J Clin Invest* 2002; 109(9): 1139-42.
43. Oken MM. New agents for the treatment of multiple myeloma and non-Hodgkin lymphoma. *Cancer* 1992; 70(4 Suppl): 946-8.
44. Lin CF, Lin CM, Lee KY, Wu SY, Feng PH, Chen KY, Chuang HC, Chen CL, Wang YC, Tseng PC, Tsai TT. Escape from IFN-γ-dependent immunosurveillance in tumorigenesis. *J Biomed Sci* 2017; 24(1): 10.
45. Mojic M, Takeda K, Hayakawa Y. The Dark Side of IFN-γ: Its Role in Promoting Cancer Immunoevasion. *Int J Mol Sci* 2017; 19(1): 89.
46. Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. *Nat Rev Immunol* 2006; 6(11): 836-48.
47. Zaidi MR, Merlino G. The two faces of interferon-γ in cancer. *Clin Cancer Res* 2011; 17(19): 6118-24.
48. Zou Q, Jin J, Xiao Y, Zhou X, Hu H, Cheng X, Kazimi N, Ullrich SE, Sun SC. T cell intrinsic USP15 deficiency promotes excessive IFN-γ production and an immunosuppressive tumor microenvironment in MCA-induced fibrosarcoma. *Cell Rep* 2015; 13(11): 2470-9.
49. Martini M, Testi MG, Pasetto M, Picchio MC, Innamorati G, Mazzocco M, Ugel S, Cingarlini S, Bronte V, Zanovello P, Krampera M, Mosna F, Cestari T, Riviera AP, Brutti N, Barbieri O, Matera L, Tridente G, Colombatti M, Sartoris S. IFN-γ-mediated upmodulation of MHC class I expression

activates tumor-specific immune response in a mouse model of prostate cancer. *Vaccine* 2010; 28(20): 3548-57.

50. 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(17): 2111-22.
51. 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(5262): 719-22.