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Increased circulating tumour-associated macrophages (IL-23R^{+} CD14^{+} subset) levels during bladder cancer progression: As accomplice in inflammation-related carcinogenesis.

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ABSTRACT

Background: Multiple studies have reported that tumour-associated-macrophages (TAMs), which are abundant in the tumour stroma, are related to poor prognosis in different tumours, such as pancreatic adenocarcinoma, gastric cancer, and bladder cancer (BC). They exert a pivotal role in modulating tumor-progression and adjusting response to immunotherapy. In this article, we strive to evaluate the significance of CD14-positive TAMs levels in relation to BC development. **Methodology**: Based on the immuno-phenotypic analysis, the counts of TAMs bearing CD14 and IL23-receptor (IL23R) fractions were determined in 15 healthy-controls and 26 BC-patients, using a panel of phycoerythrin (PE)-labelled monoclonal-antibodies. Concomitantly, serum-levels of IL23 and IL17 cytokines were identified by ELISA-technique. **Results:** Findings revealed a higher levels of CD14^{+} IL23^{+} TAMs in BC patients sera [*P*= 0.035] compared to controls, and this increment was positively associated with advancing in tumour-grade [*P*=0.001] and stage [*P*< 0.043]. In context of bladder carcinogenesis, concentration of the aforesaid TAM subpopulation correlates positively with increased levels of both IL23 [*r* = 0.66, *P*<0.01] and IL17 [*r* = 0.657, *P*<0.01]. **Conclusions**: The study suggested that CD14-positive TAMs, especially those harboring IL23R engage in inflammation-related tumourgenesis by fostering the up-regulation of the inflammatory IL23/17 immune axis, and further can be utilized as a prognostic marker for BC development and progression.

1. Introduction

Bladder cancer (BC) is regarded the 10th most pervasive malignancies all over the world, with a particularly high morbidity and mortality rates in Western Asia and Northern Africa [1-3]. Although the mortality and incidence-rates have substantially-declined across the globe in the past ten years, there are still a noticeable-increases observed in some economically vulnerable countries [4, 5]. In Egypt, owing to the differences in health-related behaviors and the consumption patterns between the sexes, chances for men to contract the BC are four times higher than women, with an average incidence-rate of 12.5 male-patients for each 100,000 people yearly [6]. Most BCs are derived histologically from the transitional epithelium layer that lines the inner surface of the urinary tract, and hence are termed as transitional-cell-carcinoma (BTCC)[7, 8]. The BC aetiology is multi-factorial process influenced by both acquired and inherited variables. Smoking in either positive or passive form is well-recognized as the best risk-factor for developing BC [9]; However, long-term-inflammation induced by renal stones, schistosomiasis eggs and chemical irritants may have a more closely related role to the development of BC [10-17]. Of note, the tumour survival is regarded as a chaotically governed process refers to the interplay between cancerous cells and the tumour micro-environment (TME), where their activities are rerouted to benefit tumour growth. TME is a multi-cellular system comprising a heterogeneous cells involving the cancerous cells and other tumor-infiltrating myeloid cells. The latter cells provide the essential "soil" for the tumour growth and progression[18]. Cancer can disarm the immune-surveillance by crippling the anti-tumoural functions of

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natural killer (NKs) and cytotoxic T-cells through production of multiple immuno-suppressive cytokines. These cytokines are secreted in large amounts in TME either by the tumour cells or by the non-cancerous cells present in the TME, especially tumor-associated macrophages (TAMs) [19-21]. Hanada and his coworkers found that in BC patients with high TAMs count, tumour distant spreading and cystectomy were significantly more frequent and a 5-year survival rates lower [22]. Following Bacillus Calmette-Guérin (BCG) Immunotherapy, a high density of TAMs was significantly correlated with worse response to the treatment [23, 24]. In cancer-related inflammation, TAMs and dendritic cells are known to be the predominant sources for the pro-tumoural cytokine IL-23 [25, 26]. The main role of IL-23 is to uphold the differentiation of naïve $CD4^+$ T-cells and subverts T-helper (Th)-1 and Th-2 differentiation for the generation of Th-17 cells. In tumourgenesis, this mechanism is believed to mediate tumour growth by over-expressing of IL-17 in TME [27-32]. Despite the clear link between IL-23 and Th-17 cells, a role for IL-17 in tumour initiation has only recently been evaluated. Emerging data suggested the involvement of IL-17 in the persistent activation of the nuclear transcription factor (NF)-κB and bring about genomic instability, resistance to apoptosis, tumour-cell survival and proliferation [33-38]. Within the tumour stroma, Th-17 cells are gradually elevated during tumour development [39] and contribute to the carcinogenesis process via directly and indirectly manners. Investigators suggest that IL-17 can induce tumourigenesis by acting directly on transformed cells. For instance, IL-17 can induce the proliferation of transformed colon enterocytes [40]. However, to indirectly promote tumour development and metastasis, IL-17 is implicated in up-regulating the production of pro-angiogenic factors [41, 42]. In this paper, we firstly strive to assess the significance of TAMs serum levels in relation to clinicopathologic features of BTCC patients. Second, we proposes a novel hypothesis that TAMs, especially those bearing IL23 receptor (IL23R), may have a potential biological impact on IL23/IL17 inflammatory immune axis, which may lead to increase the chances of inflammation-induced bladder carcinogenesis.

2.Methedology

2.1.Study population

The current research was performed at the Urology and Nephrology Center [UNC], Mansoura University, Egypt, from April 30, 2017 to December 1, 2018, on 26 BC patients who were hospitalized for a possible tumour resection. All research cases have the same ethnic history and come from the northern Egyptian province of Dakahlia. In compliance with ethical guidelines, prior to hospital admission, informed consent was regularly obtained from each patient. On the basis of reviewed and validated records received from the hospital information system, patients were pathologically diagnosed as having BTCC. The baseline features and laboratory findings of BTCC patients and controls are included in **Table**-**1**. To nominate any confounding-risk-factors, we picked 26 newly-diagnosed patients with no past-history of autoimmune disorders, free of other cancers and with no previous-chemotherapy. For this pilot-study, cases included 8 females and 17 males, with a median-age of 64 years, and none of the cases reported a past history of pro-inflammatory-drugs within two months before their admission to the hospital. The controls were mostly matched (P>0.05) with cases by age (±5 years) and sex with lacking evidence of autoimmune disorders or malignant tumours and were not genetically-linked to the cases.

2.2. Flow cytometric analysis

Five milliliters of venous-blood were withdrawn from each patient and healthy individual into a 10 ml sterile-vacutainer® tube containing EDTA as an anti-coagulant and the samples were preserved at 4˚C to be appropriate for analysis. As displayed in the **Figure 1**, the immuno-phenotypic-analysis of the IL23R-carrying TAMs relied on phycoerythrin (PE)-labelled monoclonal antibodies (MoAbs) which were conjugated with CD14 and IL23. Both MoAbs were imported from (Dickinson, CA-95131, USA). Each individual's blood sample was aseptically and aliquotted. One part was planned to determine the count of these labelled cells, although the other part was allocated to evaluating the antibody's non-specific binding. In the first tube indented to the cell-measurement, leucocytes were stained by the addition of 5µL of the fluorescence-labeled MoAbs to 100ul of the whole-blood and incubated in the dark at 22-25°C for 20 minutes. After staining, erythrocytes were utterly destroyed with the addition of 200 ul Facs Lyse-solution (Becton & Dickinson, San-Jose, CA). Thereafter, the reaction mixture was tenderly vortexed for 30 seconds, incubated again for 10 minutes in the dark at 22-25°C temperature, centrifuged at 300*g* for 5 minutes and the supernatant should be cautiously dispensed. The cell-pellets were redissolved in a solution containing 500µL phosphate-buffered-saline containing % bovine-serum-albumin and 0.09 % sodium-azide, PH 7.2, to be eligible for flow-cytometry.

2.3. Cytokine levels determination

Once again, three milliliters of blood were collected from each patient and healthy individuals into a 4 ml sterile serum vacutainer ® tube containing no anticoagulant substance and compelled to coagulate using a refrigerated-centrifuge by centrifuging for 30 minutes at 4000 rpm. Then, sera was promptly isolated and transferred into sterile-eppendorf tubes using a Pasteur pipette and held at 2-8 °C till handling. Using the Enzyme-linked immunosorbent (ELISA) kit ordered from [Shanghai Coon Koon Biotech Co., China], the serum-concentrations of IL23 and IL17 were then measured.

2.4. Statistics

The data was tabulated and analyzed using the SPSS-software [Chicago, IL, version 22] and GraphPad Prism 7.04 [GraphPad Software, Inc., California] program. As regard to demographic and clinical characteristics of the

study population, categorical variables including gender, is presented as frequencies with percentages and were compared with the chi-square χ2 test. On the other, continuous variables with normally distribution such as age and laboratory findings are compared by Student *t* test and represented as mean ± standard error (SE).To discriminate disparities in serum levels of TAMs, IL23 and IL17 within the categories of BTCC patients and healthy individuals, we adopted a non-parametric Mann-Whitney-*U* test, and data was depicted as a median and interquartile-range [IQR; 25th to 75th percentile]. Pearson-correlation coefficients (r) were calculated for the potential relationship between TAMs levels and other measured cytokines (*P*-value of <0.05 is regarded significant).

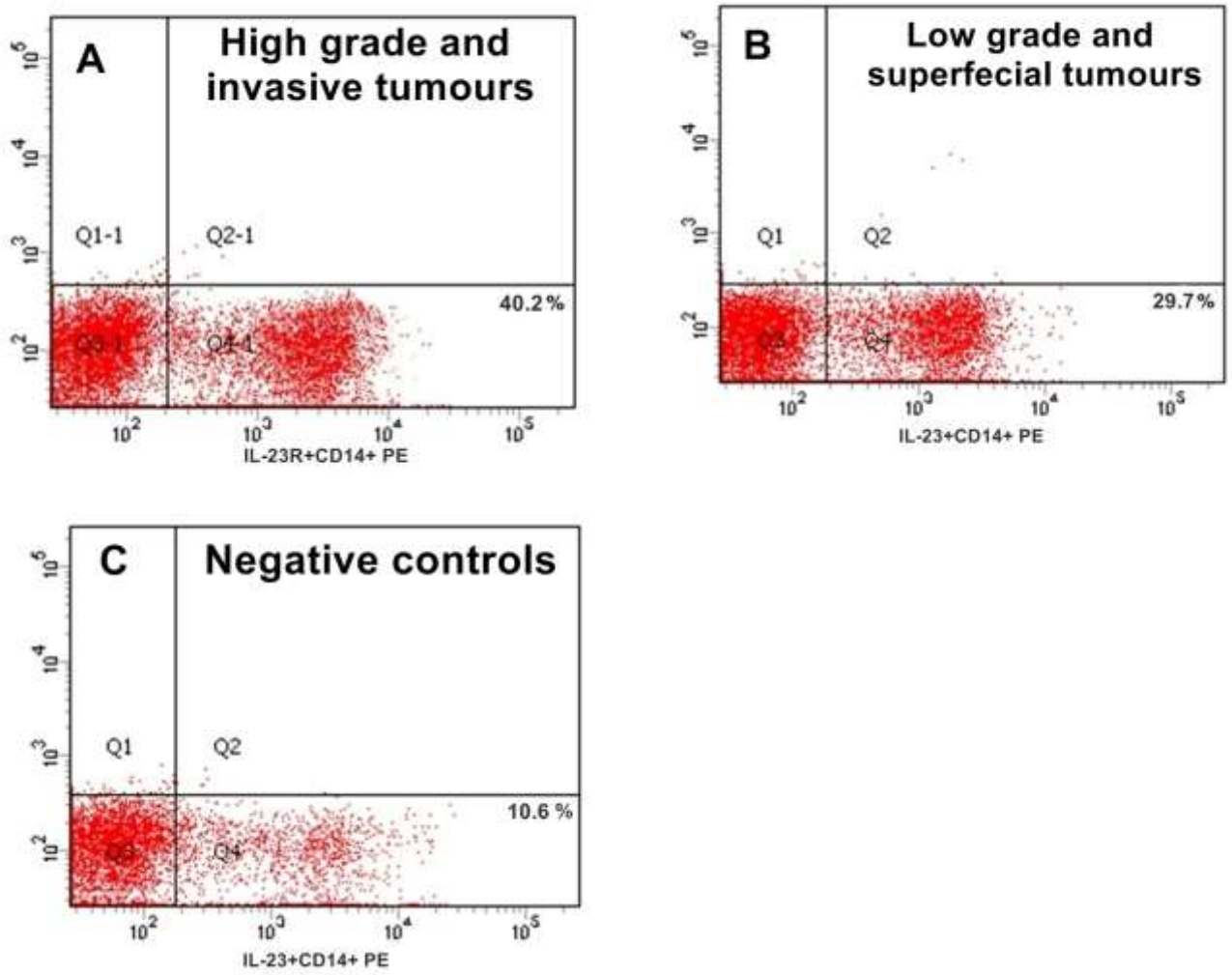

Figure 1. Flow-cytometric analysis of the frequency IL23R+ CD14+ cells in peripheral-blood obtained from A) BTCC patients with high grade and invasive tumours, B) BTCC patients with Low-grade and superficial tumours and C) healthy individuals. TAMs cells were stained with PE-conjugated anti-CD14 and anti-IL23R monoclonal antibodies.

3.Results

Patients were graded as conveyed in **Table-1** into low-grade(grade 1-2) and high-grade(grade 3-4) BTCC, using a combined-pathological-classification system for tumour tissues, according to the World-Health-Organization-guidelines (WHO) for 2014 (3rd edition). [43-46]. Further, patients were subcategorized into two major groups with regard to tumour invasion into the muscularis propria layer: muscle invasive carcinomas (stage-T2 and above) that invade the muscle layer of the bladder (lamina-muscularis-propria), while non-muscle-invasive-neoplasm contains tumours restricted to mucosa and/or invasive sub-mucosa (stages Ta, T1, and carcinoma in situ-Cis) [7]. All the study findings are compiled in **Table 2**. It was observed that the levels of TAMs-harboring IL23R were higher in the sera of BTCC patients [*P*= 0.035] than in healthy individuals, and that the increase in their levels was positively correlated with advancing in tumour-grade [*P* = 0.001] and stage (*P* < 0.043) respectively. Likewise, concentrations of IL23 [*P* < 0.001] and IL17 [*P* < 0.001] in the cancer-patients were higher than in the controls and their levels has shown a typical association with a high-stage [*P* < 0.001] and high-grade [*P* < 0.04] cancers compared to less severe types of the disease, as shown in **Table 2 and Figure 2**.

Table 1. Baseline-characteristics of the BTCC patients and Controls

Characteristics	BTCC (N= 26)	Healthy controls (N= 15)	*P*-value
Age (Years), Mean ± SE	64.04±7.4	61.33 ±1.47	0.49 [a]
Gender; Male, N(%)	17(65.4%)	11(73.3%)	0.434[b]
Female, N,(%)	9(34.6%)	4(26.6%)	
Clinicopathological features			
Grade; High grade, N(%)	14(46.2%)	-----	-----
Low grade, N(%)	12(53.8 %)	------	------
Stage; Invasive (N,%)	17(65.4 %)	------	------
Superficial (N,%)	9(34.3%)	------	------
Laboratory findings;			
Creatinine (mg/dl, mean ± SE)	1.7 ± 0.28	1.25 ± 0.26	0.215 [a]
Glucose (mg/dl, mean ± SE)	160.03 ± 22.8	117.60 ± 10.7	0.101 [a]
Uric acid (mg/dl, mean ± SE)	6.046 ± 0.58	6.36 ± 0.77	0.74 [a]
Total bilirubin (mg/dl, mean ± SE)	0.873±0.19	0.673 ± 0.07	0.45 [a]
Neutrophils ($x10^3$ cells/ µl, mean ± SE)	67.08 ± 2.3	67.47 ± 0.1	0.9 [a]
Lymphocytes ($x10^3$ cells/ µl, mean ± SE)	23.31±2.76	21.93±2.9	0.7 [a]
Monocytes ($x10^3$ cells/ µl, mean ± SE)	8.92±0.699	8.0±0.67	0.35 [a]

Abbreviations; SE; standard error; a) Student T-test b) Chi-square ($\chi 2$) test. *P*-Value <0.05 considered significant (bolded)

Table 2. Circulating Levels of IL23R+ CD14+ TAMs, IL23 and IL17 in the peripheral blood of BTCC and control groups

Study groups	IL23R+CD14+TAMs (cells/ µl)		Interleukin-23 (pg/ml)		Interleukin-17 (pg/ml)	
	Median [IQR]	*P*-value	Median [IQR]	*P*-value	Median [IQR]	*P*-value
Healthy group	1004.9 [836]	reference	101.6 [41.7]	reference	47.5 [8.0]	reference
BTCC patients	1965.29 [1352]	**0.035**[a]	213.0 [192]	**<0.001** [a]	60.82 [25.14]	**< 0.001** [a]
Superficial Tumours	1047.2 [833]	reference	123 [149]	reference	51.8 [10.95]	reference
Invasive Tumours	2084.2 [5036]	**0.043**[a]	222.9 [156.7]	**0.038**[a]	66.8 [21.6]	**0.036**[a]
Low grade Tumours	1368[921]	reference	123.5 [52.7]	reference	47.8 [15.4]	reference
High grade Tumours	2335 [40.99]	**0.001**[a]	305 [156.4]	**<0.001**[a]	70.8 [27.3]	**<0.001**[a]

Abbreviations; IQR, Interquartile range [a] Mann whitney U test; The value of $p < 0.05$ was statistically considered significant (bolded)

With respect to the cancer patients, positive correlations were evidenced between serum $IL23^+$ $CD14^+$ TAMs levels and the serum levels of IL23 (r = 0.66, P<0.01) and IL17 (r = 0.657, P<0.01), when applied a bivariate correlation analysis (**Figure 3**).

4.Disscussion

BTCC represents a growing numbers of solid-tumours accompanied by the infiltration of a significant number of a wide variety of myeloid-cells into a neoplastic lesion [47, 48]. Among the diverse circulating myeloid-cells recruited to the TME, tumor-derived macrophages or TAMs are rendered predominant elements [49] Copious evidence showed that TAMs are crucial in regulating cross-talk between TME and tumour-cells and further implicated in multiple steps of tumour progression and invasion [50]. Clinical and preclinical data suggested a close association between increased TAMs infiltration and poor prognosis in most of tumours types [49], such as BC [51], gastric cancer [52], glioblastoma [53] and pancreatic-ductal-adenocarcinoma (PDAC) [54]. On the other side, TAM penetration has also been shown to be correlated with a favorable prognosis in certain cancers, such as in colorectal cancer [55] and ovarian cancer [56]. These varying outcomes may be attributed not only the divergent cancer types, but also some intratumoural factors, including the TAM distribution location in the TME. For instance, studies conducted on non-small-cell lung-cancer (NSLSC) reported that higher TAMs infiltration in tumour-islets was correlated with a good prognosis, whereas elevated levels of circulating TAMs in the tumour stroma were observed to be linked with a poor prognosis [57, 58].

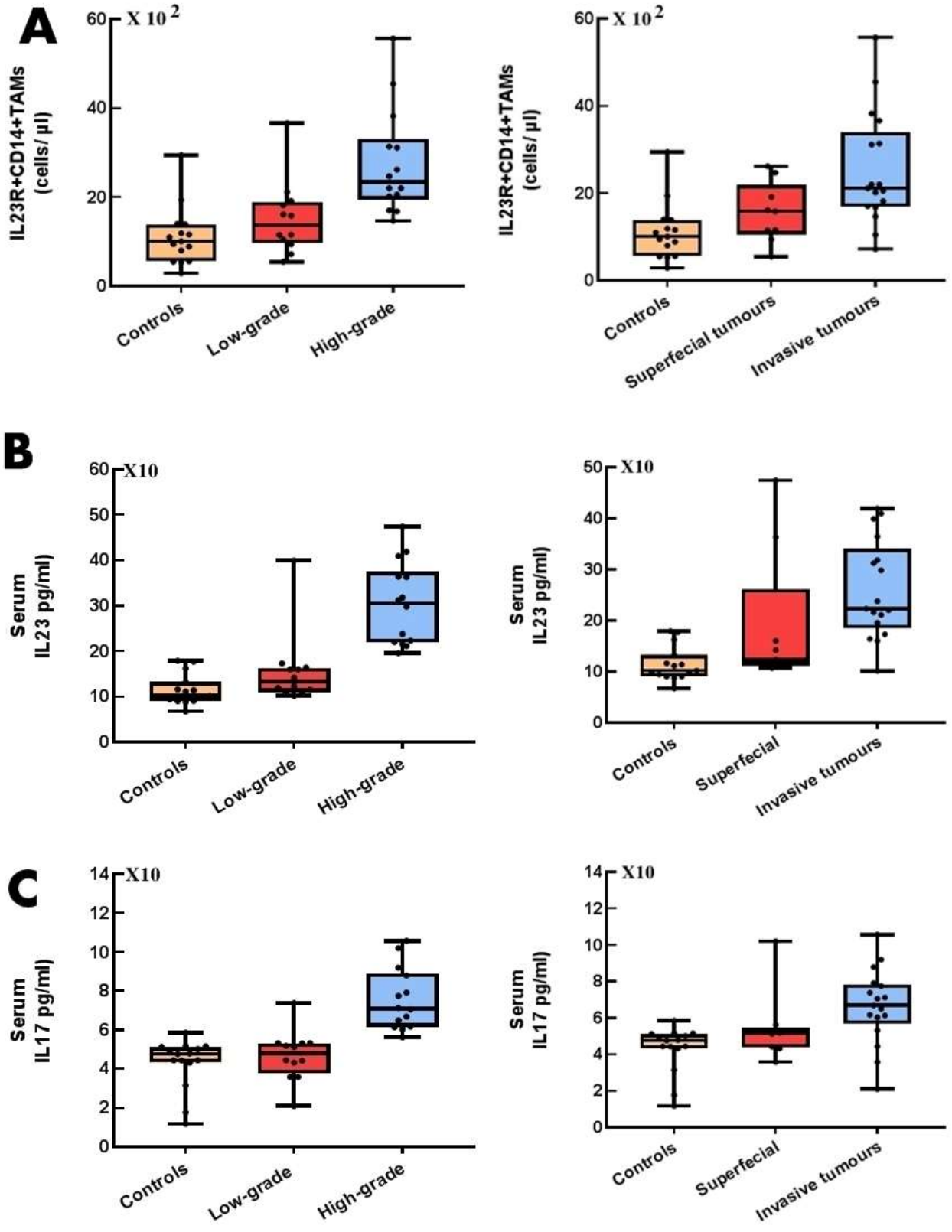

Figure 2. Box-and-whiskers diagram denotes the serum-levels of parameters including A) IL23+ CD14+ TAMs B) IL23, and C) IL17 in relation to clinicopathologic features. Based on the Mann whitney U test, the middle-line of each bar presents the median serum-levels, while the top and bottom of the boxes represents the third and first quartiles.

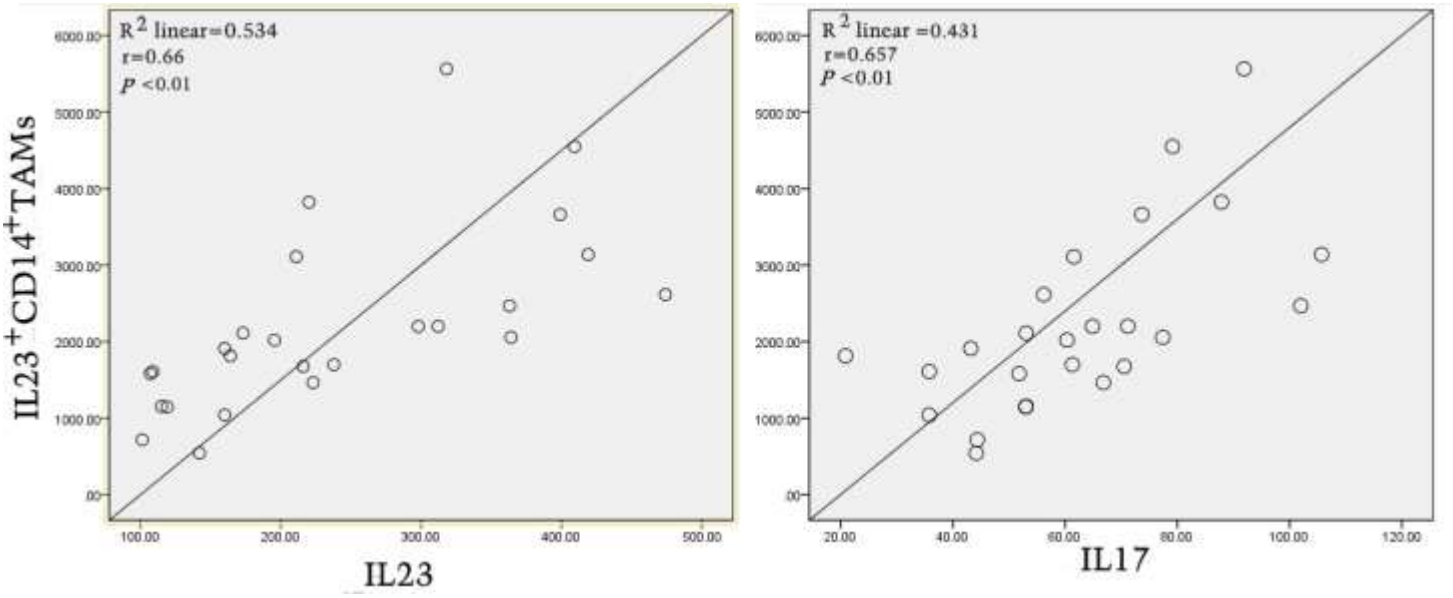

Figure 3. Correlation of CD14-positive-TAMs harboring-IL23R with the serum levels of IL23 and IL17 regarding the BTCC patients. Correlation is significant for *P*<0.05.

As regards circulating TAMs levels within BTCC patients sera, the current work revealed that high counts of CD14-positive TAMs relative to controls and that was significantly correlated with high tumour grade and increased degree of tumour invasion. Therefore, elevated levels of such TAM sub-population can be used as a possible prognostic marker for tumour progression. In support of our notion, Hanada and his coworkers reported that a high density of TAMs was correlated significantly with a poor prognosis of BC, highlighting its importance in predicting the clinical outcome [22]. To discriminate the distinct role of CD14-positive TAMs in tumour initiation or progress, we should uncover its implicit influence in creating a persistent inflammatory milieu more vulnerable to tumourgenesis [31, 59-61]. In the context of BTCC, it is urgently needed to assesses the impact of these TAMs subsets, particularly those bearing IL23R [$CD14^+$ $IL23R^+$ TAMs] on the modulation of IL23-IL17 inflammatory-immune-axis. To up-regulate of IL23R-positive TAMs in TME during tumourgenesis, IL-23 elaborated by TAMs requires to act on themselves through the IL-23R-mediated-autocrine-manner [25, 62, 63]. Consistent to this envision, our findings revealed that the increased levels of IL23R-positive TAMs in cancer patients was positively correlated with augmenting the expression of IL23 and IL17 cytokines, which in turn establishes inflammatory environment orchestrating cancer initiation and promotion of tumour growth [26, 64, 65]. Further on, much noticeable evidence suggests that the increment of IL-23 and IL17 levels in the tumour stroma is thought to be in charge of mitigating the cytotoxic effects of $CD8^+$T-cells and natural killers, thus profoundly inclining the local environment toward the pro-tumour profile [19-21, 66]. Nevertheless, further in-depth investigation on a wide-scale of volunteers is urgently warranted in terms of fully understand the distinct mechanism through which CD14-positive TAMs mediate chronic-inflammation and cancer growth.

5. conclusion

Our study defines the crucial role of a high CD14-positive TAMs serum levels as a hallmark of BTCC tumour development and progression. In this regard, our findings revealed a significant increase in their levels in cancer patients compared to the healthy volunteers, and that the increase in their levels was positively correlated with advancing in tumour-stage. In particular, $CD14^+$ TAMs subpopulation bearing IL23R might drive tumour growth by a sustained elevation of IL23 and IL17 cytokine levels. As consequence, therapeutic targeting of TAMs might represent a strategy for treating BTCC.

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References

1. Chamie K, Litwin MS, Bassett JC, Daskivich TJ, Lai J, Hanley JM, Konety BR, Saigal CS, Urologic Diseases in America P: Recurrence of high-risk bladder cancer: a population-based analysis. *Cancer* 2013, 119(17):3219-3227.

2. Fahmy O, Khairul-Asri MG, Schubert T, Renninger M, Kübler H, Stenzl A, Gakis G: Urethral recurrence after radical cystectomy for urothelial carcinoma: A systematic review and meta-analysis. *Urologic oncology* 2018, 36(2):54-59.

3. Krochmal M, van Kessel KEM, Zwarthoff EC, Belczacka I, Pejchinovski M, Vlahou A, Mischak H, Frantzi M: Urinary peptide panel for prognostic assessment of bladder cancer relapse. *Scientific reports* 2019, 9(1):7635-7635.

4. Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F: Bladder Cancer Incidence and Mortality: A Global Overview and Recent Trends. *European Urology* 2017, 71(1):96-108.

5. Wong MCS, Fung FDH, Leung C, Cheung WWL, Goggins WB, Ng CF: The global epidemiology of bladder cancer: a joinpoint regression analysis of its incidence and mortality trends and projection. *Scientific Reports* 2018, 8(1):1129.

6. Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H: Cancer incidence in Egypt: results of the national population-based cancer registry program. *Journal of cancer epidemiology* 2014, 2014.

7. Babjuk M, Böhle A, Burger M, Capoun O, Cohen D, Compérat EM, Hernández V, Kaasinen E, Palou J, Rouprêt M *et al*: EAU Guidelines on Non-Muscle-invasive Urothelial Carcinoma of the Bladder: Update 2016. *Eur Urol* 2017, 71(3):447-461.

8. Prasad SM, Decastro GJ, Steinberg GD, Medscape: Urothelial carcinoma of the bladder: definition, treatment and future efforts. *Nature reviews Urology* 2011, 8(11):631-642.

9. Chu H, Wang M, Zhang Z: Bladder cancer epidemiology and genetic susceptibility. *J Biomed Res* 2013, 27(3):170-178.

10. Wang W, Fan Y, Xiong G, Wu J: Nitrate in drinking water and bladder cancer: A meta-analysis. *Journal of Huazhong University of Science and Technology [Medical Sciences]* 2012, 32(6):912-918.

11. Koutros S, Lynch CF, Ma X, Lee WJ, Hoppin JA, Christensen CH, Andreotti G, Freeman LB, Rusiecki JA, Hou L *et al*: Heterocyclic aromatic amine pesticide use and human cancer risk: results from the U.S. Agricultural Health Study. *International journal of cancer* 2009, 124(5):1206-1212.

12. Gakis G: The Role of Inflammation in Bladder Cancer. *ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY* 2014, 816:183-196.

13. Mantovani A, Allavena P, Sica A, Balkwill F: Cancer-related inflammation. *Nature* 2008, 454:436.

14. Moussa M, Abou Chakra M: Urothelial carcinoma arising from a bladder diverticulum containing multiple stones: A case report. *Urology Case Reports* 2018, 20:80-82.

15. Stone L: Bladder cancer: Urinary tract infection increases risk. *NATURE REVIEWS UROLOGY* 2015, 12(1):4-4.

16. Vermeulen SH, Hanum N, Grotenhuis AJ, Castano-Vinyals G, van der Heijden AG, Aben KK, Mysorekar IU, Kiemeney LA: Recurrent urinary tract infection and risk of bladder cancer in the Nijmegen bladder cancer study. *BRITISH JOURNAL OF CANCER* 2015, 112(3):594-600.

17. Richards KA, Ham S, Cohn JA, Steinberg GD: Urinary tract infection-like symptom is associated with worse bladder cancer outcomes in the Medicare population: Implications for sex disparities. *INTERNATIONAL JOURNAL OF UROLOGY* 2016, 23(1):42-47.

18. Horimoto Y, Polanska UM, Takahashi Y, Orimo A: Emerging roles of the tumor-associated stroma in promoting tumor metastasis. *Cell Adh Migr* 2012, 6(3):193-202.

19. Martínez-Lostao L, Anel A, Pardo J: How do cytotoxic lymphocytes kill cancer cells? In.: AACR; 2015.

20. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M: Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *The Journal of Immunology* 1995, 155(3):1151-1164.

21. Hughes R, Qian B-Z, Rowan C, Muthana M, Keklikoglou I, Olson OC, Tazzyman S, Danson S, Addison C, Clemons M: Perivascular M2 macrophages stimulate tumor relapse after chemotherapy. *Cancer research* 2015, 75(17):3479-3491.

22. Hanada T, Nakagawa M, Emoto A, Nomura T, Nasu N, Nomura Y: Prognostic value of tumor-associated macrophage count in human bladder cancer. *International Journal of Urology* 2000, 7(7):263-269.

23. Takayama H, Nishimura K, Tsujimura A, Nakai Y, Nakayama M, Aozasa K, Okuyama A, Nonomura N: Increased infiltration of tumor associated macrophages is associated with poor prognosis of bladder carcinoma in situ after intravesical bacillus Calmette-Guerin instillation. *The Journal of urology* 2009, 181(4):1894-1900.

24. Ayari C, LaRue H, Hovington H, Caron A, Bergeron A, Têtu B, Fradet V, Fradet Y: High level of mature tumor-infiltrating dendritic cells predicts progression to muscle invasion in bladder cancer. *Human pathology* 2013, 44(8):1630-1637.

25. Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, Pflanz S, Zhang R, Singh KP, Vega F *et al*: A Receptor for the Heterodimeric Cytokine IL-23 Is Composed of IL-12Rβ1 and a Novel Cytokine Receptor Subunit, IL-23R. *The Journal of Immunology* 2002, 168(11):5699.

26. Langowski JL, Zhang X, Wu L, Mattson JD, Chen T, Smith K, Basham B, McClanahan T, Kastelein RA, Oft M: IL-23 promotes tumour incidence and growth. *Nature* 2006, 442:461.

27. Crew J, O'Brien T, Bradburn M, Fuggle S, Bicknell R, Cranston D, Harris AL: Vascular Endothelial Growth Factor is a Predictor of Relapse and Stage Progression in Superficial Bladder Cancer, vol. 57; 1998.

28. Langowski JL, Zhang X, Wu L, Mattson JD, Chen T, Smith K, Basham B, McClanahan T, Kastelein RA, Oft M: IL-23 promotes tumour incidence and growth. *Nature* 2006, 442(7101):461-465.

29. Martin-Orozco N, Dong C: The IL-17/IL-23 axis of inflammation in cancer: Friend or foe?, vol. 10; 2009.

30. Moschen AR, Tilg H, Raine T: IL-12, IL-23 and IL-17 in IBD: immunobiology and therapeutic targeting. *Nature reviews Gastroenterology & hepatology* 2019, 16(3):185-196.

31. Murugaiyan G, Saha B: Protumor vs antitumor functions of IL-17. *The Journal of Immunology* 2009, 183(7):4169-4175.

32. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B: TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 2006, 24(2):179-189.

33. Iwakura Y, Ishigame H: The IL-23/IL-17 axis in inflammation. *Journal of Clinical Investigation* 2006, 116(5):1218-1222.

34. Nakae S, Saijo S, Horai R, Sudo K, Mori S, Iwakura Y: IL-17 production from activated T cells is required for the spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. *Proceedings of the National Academy of Sciences of the United States of America* 2003, 100(10):5986-5990.

35. Nakae S, Saijo S, Horai R, Sudo K, Mori S, Iwakura Y: IL-17 production from activated T cells is required for the spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. *Proceedings of the National Academy of Sciences* 2003, 100(10):5986.

36. Yang B, Kang H, Fung A, Zhao H, Wang T, Ma D: The role of interleukin 17 in tumour proliferation, angiogenesis, and metastasis. *Mediators Inflamm* 2014, 2014:623759-623759.

37. Paunescu V, Bojin FM, Tatu CA, Gavriliuc OI, Rosca A, Gruia AT, Tanasie G, Bunu C, Crisnic D, Gherghiceanu M *et al*: Tumour-associated fibroblasts and mesenchymal stem cells: more similarities than differences. *Journal of cellular and molecular medicine* 2011, 15(3):635-646.

38. Antoniades HN, Galanopoulos T, Neville-Golden J, Kiritsy CP, Lynch SE: Injury induces in vivo expression of platelet-derived growth factor (PDGF) and PDGF receptor mRNAs in skin epithelial cells and PDGF mRNA in connective tissue fibroblasts. *Proc Natl Acad Sci U S A* 1991, 88(2):565-569.

39. Kryczek I, Wei S, Zou L, Altuwaijri S, Szeliga W, Kolls J, Chang A, Zou W: Cutting edge: Th17 and regulatory T cell dynamics and the regulation by IL-2 in the tumor microenvironment. *Journal of immunology (Baltimore, Md : 1950)* 2007, 178(11):6730-6733.

40. Wang K, Kim MK, Di Caro G, Wong J, Shalapour S, Wan J, Zhang W, Zhong Z, Sanchez-Lopez E, Wu L-W: Interleukin-17 receptor a signaling in transformed enterocytes promotes early colorectal tumorigenesis. *Immunity* 2014, 41(6):1052-1063.

41. Takahashi H, Numasaki M, Lotze MT, Sasaki H: Interleukin-17 enhances bFGF-, HGF-and VEGF-induced growth of vascular endothelial cells. *Immunology letters* 2005, 98(2):189-193.

42. Numasaki M, Fukushi J-i, Ono M, Narula SK, Zavodny PJ, Kudo T, Robbins PD, Tahara H, Lotze MT: Interleukin-17 promotes angiogenesis and tumor growth. *Blood* 2003, 101(7):2620-2627.

43. Compérat EM, Burger M, Gontero P, Mostafid AH, Palou J, Rouprêt M, van Rhijn BW, Shariat SF, Sylvester RJ, Zigeuner R: Grading of urothelial carcinoma and the new "World Health Organisation classification of Tumours of the urinary system and male genital organs 2016". *European urology focus* 2019, 5(3):457-466.

44. Ball R: Pathology and genetics of tumours of the urinary system and male genital organs. *Histopathology* 2005, 46(5):586-586.

45. Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM: The 2016 WHO classification of tumours of the urinary system and male genital organs—part A: renal, penile, and testicular tumours. *European urology* 2016, 70(1):93-105.

46. El-Hakim A, Weiss GH, Lee BR, Smith AD: Correlation of ureteroscopic appearance with histologic grade of upper tract transitional cell carcinoma. *Urology* 2004, 63(4):647-650; discussion 650.

47. Eruslanov E, Neuberger M, Daurkin I, Perrin GQ, Algood C, Dahm P, Rosser C, Vieweg J, Gilbert SM, Kusmartsev S: Circulating and tumor-infiltrating myeloid cell subsets in patients with bladder cancer. *International journal of cancer* 2012, 130(5):1109-1119.

48. Michaud DS: Chronic inflammation and bladder cancer. *Urologic oncology* 2007, 25(3):260-268.

49. Zhang Q-w, Liu L, Gong C-y, Shi H-s, Zeng Y-h, Wang X-z, Zhao Y-w, Wei Y-q: Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. *PloS one* 2012, 7(12):e50946.

50. Wu K, Lin K, Li X, Yuan X, Xu P, Ni P, Xu D: Redefining Tumor-Associated Macrophage Subpopulations and Functions in the Tumor Microenvironment. *Frontiers in Immunology* 2020, 11(1731).

51. Wu H, Zhang X, Han D, Cao J, Tian J: Tumour-associated macrophages mediate the invasion and metastasis of bladder cancer cells through CXCL8. *PeerJ* 2020, 8:e8721.

52. Räihä MR, Puolakkainen PA: Tumor-associated macrophages (TAMs) as biomarkers for gastric cancer: a review. *Chronic diseases and translational medicine* 2018, 4(3):156-163.

53. Zhang X, Chen L, Dang W-q, Cao M-f, Xiao J-f, Lv S-q, Jiang W-j, Yao X-h, Lu H-m, Miao J-y: CCL8 secreted by tumor-associated macrophages promotes invasion and stemness of glioblastoma cells via ERK1/2 signaling. *Laboratory Investigation* 2020, 100(4):619-629.

54. Huang X, He C, Lin G, Lu L, Xing K, Hua X, Sun S, Mao Y, Song Y, Wang J: Induced CD10 expression during monocyte-to-macrophage differentiation identifies a unique subset of macrophages in pancreatic ductal adenocarcinoma. *Biochemical and biophysical research communications* 2020, 524(4):1064-1071.

55. Edin S, Wikberg ML, Oldenborg P-A, Palmqvist R: Macrophages: Good guys in colorectal cancer. *Oncoimmunology* 2013, 2(2):e23038.

56. El-Arabey AA, Denizli M, Kanlikilicer P, Bayraktar R, Ivan C, Rashed M, Kabil N, Ozpolat B, Calin GA, Salama SA: GATA3 as a master regulator for interactions of tumor-associated macrophages with high-grade serous ovarian carcinoma. *Cellular Signalling* 2020, 68:109539.

57. Feng P-H, Yu C-T, Wu C-Y, Lee M-J, Lee W-H, Wang L-S, Lin S-M, Fu J-F, Lee K-Y, Yen T-H: Tumor-associated macrophages in stage IIIA pN2 non-small cell lung cancer after neoadjuvant chemotherapy and surgery. *American journal of translational research* 2014, 6(5):593.

58. Dai F, Liu L, Che G, Yu N, Pu Q, Zhang S, Ma J, Ma L, You Z: The number and microlocalization of tumor-associated immune cells are associated with patient's survival time in non-small cell lung cancer. *BMC cancer* 2010, 10(1):220.

59. Kortylewski M, Xin H, Kujawski M, Lee H, Liu Y, Harris T, Drake C, Pardoll D, Yu H: Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment. *Cancer cell* 2009, 15(2):114-123.

60. Schön MP, Erpenbeck L: The Interleukin-23/Interleukin-17 Axis Links Adaptive and Innate Immunity in Psoriasis. *Frontiers in Immunology* 2018, 9(1323).

61. El-Gedamy M, El-khayat Z, Abol-Enein H, El-said A, El-Nahrery E: Rs-1884444 G/T variant in IL-23 receptor is likely to modify risk of bladder urothelial carcinoma by regulating IL-23/IL-17 inflammatory pathway. *Cytokine* 2021, 138:155355.

62. Belladonna ML, Renauld J-C, Bianchi R, Vacca C, Fallarino F, Orabona C, Fioretti MC, Grohmann U, Puccetti P: IL-23 and IL-12 have overlapping, but distinct, effects on murine dendritic cells. *The Journal of Immunology* 2002, 168(11):5448-5454.

63. Grohmann U, Bianchi R, Belladonna ML, Vacca C, Silla S, Ayroldi E, Fioretti MC, Puccetti P: IL-12 acts selectively on CD8α− dendritic cells to enhance presentation of a tumor peptide in vivo. *The Journal of Immunology* 1999, 163(6):3100-3105.

64. Volpe E, Servant N, Zollinger R, Bogiatzi S, Hupé P, Barillot E, Soumelis V: A critical function for transforming growth factor-beta, interleukin 23 and proinflammatory cytokines in driving and modulating human T(H)-17 responses, vol. 9; 2008.

65. Chen Z, Laurence A, O'Shea JJ: Signal transduction pathways and transcriptional regulation in the control of Th17 differentiation. *Seminars in Immunology* 2007, 19(6):400-408.

66. He D, Li H, Yusuf N, Elmets CA, Li J, Mountz JD, Xu H: IL-17 Promotes Tumor Development through the Induction of Tumor Promoting Microenvironments at Tumor Sites and Myeloid-Derived Suppressor Cells. *The Journal of Immunology* 2010, 184(5):2281-2288.