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IL-10 and CXCL10 urine quantification as useful biomarkers to predict BCG response in bladder cancer patients

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Introduction & Objectives

Identifying biomarkers to predict BCG response before beginning the treatment is an unmet need in the clinical practice for patients with high risk non-muscle invasive bladder cancer. Nowadays, there is no method to predict BCG response in our patients. In this context, protumor activity of M2 macrophages could be mediating this treatment response, so evaluation of cytokines as IL-10 and CXCL10 in urine could be an indicator of their activity on patient's urothelium. The main aim of this work is to evaluate if expression levels of IL-10 and CXCL10 in urine samples obtained before treatment are able to identify BGC responders and non-responders. We also aimed to find out the correlation of these levels in urine with the presence of M2 macrophages in tumor tissue samples obtained after transurethral resection of the bladder tumor (TURBT) and before the onset of BCG instillations.

Materials and methods

Tumor tissue samples as well as urine samples were collected from a paired sample set from patients before TURBT and BCG treatment at our Department. Response to BCG was defined as no recurrence after 2 years. Informed consent was obtained from all patients. The expression levels of IL-10 and CXCL10 were measured by ELISA and RT-qPCR. The percentages of M2 polarized or total macrophages were calculated by immunohistochemistry staining using CD163 and CD68 surface markers, respectively. Differential gene expression in immune signatures between both groups in tissue tumor samples was carried out using Nanostring platform (PanCancer IO 360).

Results

We found a good correlation between the levels of IL-10 and CXCL10 measured by ELISA in urine samples with the levels found by RT-qPCR in tumor tissue, as well as lower levels of IL-10 in urine samples from patients with recurrences (**Figure 1**). Nanostring analysis showed that tumors from non-responders contain a higher percentage of macrophages and exhausted CD T-cells, as well as a higher expression of CD68, CD163, CXCL10 and survivin (**Figure 2**). In order to define if the presence of M2 or total macrophages before BCG instillation correlates with the response to treatment, we quantified CD163 and CD68 positive cells in tissue samples from our patients (**Figure 3**). We found that patients with more CD68+ infiltrating cells have a worst prognosis after the instillations (**Figure 3**). Therefore, we evaluated whether these cytokine levels allow us predicting clinical response to this treatment. We found that the quantification of IL-10 and CXCL10 in urine samples are good indicators for BCG response (**Figure 4**).

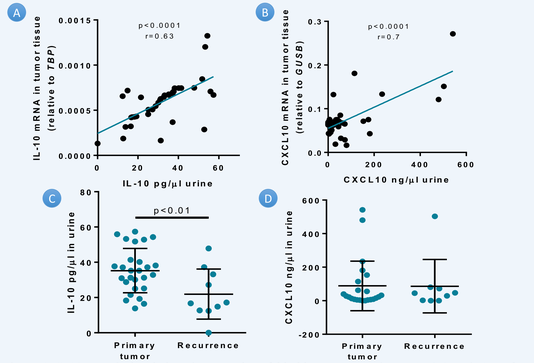


Figure 1. IL-10 protein levels in urine samples of bladder cancer patients. A-B) Correlation of mRNA expression of IL-10 (A) and CXCL10 (B) in tissue tumor samples with protein levels in urine samples of the same patients. C-D) IL-10 (C) and CXCL10 (D) protein levels in urine samples comparing primary tumors and recurrences. Mean ± SD are shown.

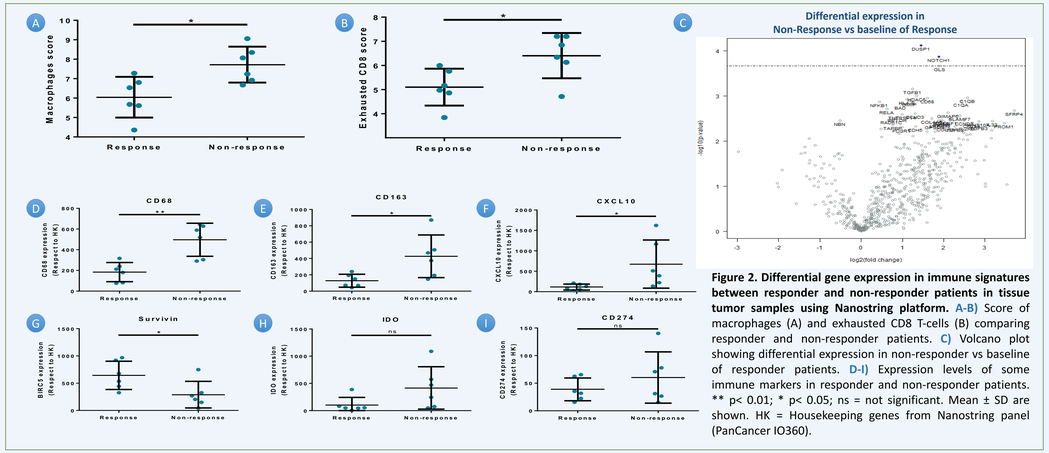
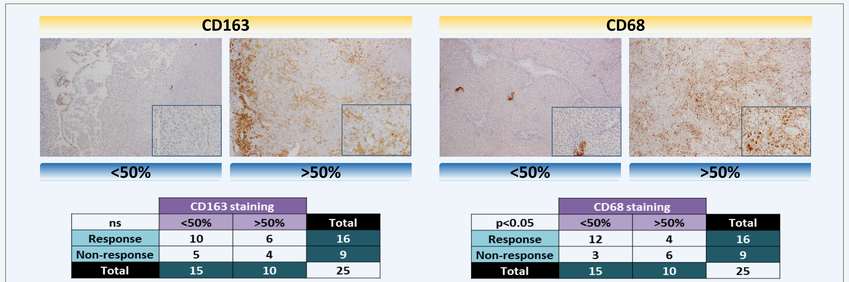


Figure 2. Differential gene expression in immune signatures between responder and non-responder patients in tissue tumor samples using Nanostring platform. A-B) Score of macrophages (A) and exhausted CD8 T-cells (B) comparing responder and non-responder patients. C) Volcano plot showing differential expression in non-responder vs baseline of responder patients. D-I) Expression levels of some immune markers in responder and non-responder patients. ** p< 0.01; * p< 0.05; ns = not significant. Mean ± SD are shown. HK = Housekeeping genes from Nanostring panel (PanCancer IO360).



CD163 staining			
ns	<50%	>50%	Total
Response	10	6	16
Non-response	5	4	9
Total	15	10	25

CD68 staining			
p<0.05	<50%	>50%	Total
Response	12	4	16
Non-response	3	6	9
Total	15	10	25

Figure 3. Immunohistochemistry analysis of CD163 and CD68 markers in bladder tumors from responder and non-responder patients to BCG treatment. Staining levels were evaluated according to the percentage of the samples with positive staining for this markers. Bar = 100 µm. Ns = Not significant.

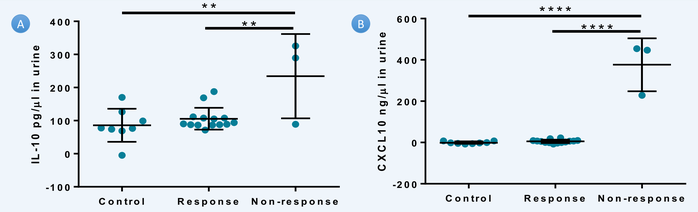


Figure 4. IL-10 and CXCL10 as BCG treatment response biomarkers in urine samples of bladder cancer patients. A-B) IL-10 (A) and CXCL10 (B) protein levels in urine samples comparing healthy donors and cystitis patients (control) with responder and non-responder patients. **** p< 0.0001; ** p< 0.01. Mean ± SD are shown.

Conclusions

The presence of M2 polarized macrophages in TURBT tissue as well as M2 markers in urine samples of bladder cancer patients appear to be predictive for BCG response. However, validation in a larger cohort is needed to confirm our data.

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