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


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Abstract

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 BCG responders and non-responders. We also aimed to find out the correlation of these levels in urine with the presence of M2 macrophages in tissue samples obtained after transurethral resection of the bladder tumor (TURBT) and before the onset of BCG instillations.

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 worse 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).

We hope to validate in a larger cohort is needed to confirm our data.

Conclusion

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 ID 360).

Figure 1. IL-10 protein levels in urine samples of bladder cancer patients. (A) Correlation of relative expression of IL-10 (A) and CXCL10 (B) in tumor tissue samples with protein levels in urine samples of the same patients. (C) IL-10 (C) and CXCL10 (D) protein levels in urine samples comparing primary tumors and metastases. * = p<0.05 and ** = p<0.01.

Figure 2. Differential gene expression in immune signatures between responder and non-responder patients in tissue tumor samples using Nanostring platform. (A) Score of macrophages and exhausted CD T-cells in tumor samples comparing responder and non-responder patients. (B) Tumor polarized differentiated monocytes/T-cells in tissue samples comparing responder and non-responder patients. (C) Tumor polarized differentiated monocytes/T-cells in tissue samples comparing responder and non-responder patients. * = p<0.05, ** = p<0.01. Mean ± SD in columns. SCAT = Staining control. * = Significant difference between responders and non-responders.

Figure 3. Quantification of CD163 and CD68+ macrophages in tissue samples from patients before (A) and after (B) the instillation of BCG. CD163 (red) and CD68 (green) immunostains were carried out in tissue sections. The number of positive cells was determined using the ImageJ software. * = p<0.05, ** = p<0.01.

Figure 4. Urine levels of IL-10 and CXCL10 in responders and non-responders to BCG treatment. (A) IL-10 levels in urine samples of responders and non-responders. (B) CXCL10 levels in urine samples of responders and non-responders. * = p<0.05, ** = p<0.01, *** = p<0.001. Mean ± SD in columns.

Figure 5. CD163 and CD68 protein levels in responders and non-responders to BCG treatment in tissue sections of bladder cancer patients. (A) CD163 (red) and CD68 (green) immunostains were carried out in tissue sections. The number of positive cells was determined using the ImageJ software. * = p<0.05, ** = p<0.01. Mean ± SD in columns.