Emerging Roles of T-Cell CX3CR1, Toll-Like Receptor 4 Signaling Pathway and Immune Checkpoints in Non-Muscle Invasive Bladder Cancer

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BACKGROUND

Immunotherapies have revolutionized treatment for various cancers, including urothelial carcinoma. Due to the inherent complexity of the immune response, patient selection and the development of biomarkers to guide the identification of patients who will derive the greatest benefit from a given immunotherapy remain critical. Most immunotherapies work by enhancing the antitumor responses of $CD8^+$ T cells, which can be severely limited by the immunosuppressive tumor microenvironment (TME). Tumor-associated macrophages (TAMs), a key component of the immunosuppressive TME, decrease anti-tumor T cell reactivity in most solid tumors. The lack of robust predictive biomarker for response is a major obstacle of immunotherapies. CX3C chemokine receptor 1 (CX3CR1) was found to be a marker of T-cell differentiation, where $CX3CR1^+CD8^+$ T cells were the progeny of $CX3CR1^-CD8^+$ T cells, and exhibited robust cytotoxicity. The selection of CX3CR1 as a potential marker to identify a subset of immunotherapy–responsive $CD8^+$ T cells is of particular importance.

This study characterized and compared the molecular profiles of CX3C chemokine receptor 1 (CX3CR1, a marker of T-cell differentiation), Toll-like receptor 4 (TLR4)-mediated interferon signaling pathway and immune checkpoints in the different histological stages of non-muscle invasive bladder cancer (NMIBC), aiming the adaptation of these biomarkers as a criterion of clinical response to immunotherapy.

RESULTS

- pTis group showed the lowest activation of TLR4-mediated IFN-γ signaling pathway when compared ($p<0.01$) to high-grade pTa and pT1 groups.
- Both the immunoreaction intensity and positive cells percentage were lower ($p<0.01$) for TLR4, TRIF, IRF-3 and IFN-γ in the pTis group with respect to other groups.
- No statistical differences were found between high-grade pTa and pT1 groups for these biomarkers.
- Likewise, CX3CR1 immunoreactivities were remarkably lower ($p<0.01$) in the pTis group in relation to high-grade pTa and pT1 groups, which did not show statistical differences between them.
- Furthermore, immune checkpoints (PD-1/PD-L1 and CTLA4) and FOXP3+ Treg cells immunoreactivities were significantly higher ($p<0.01$) in the high-grade pTa and pT1 in relation to pTis group.

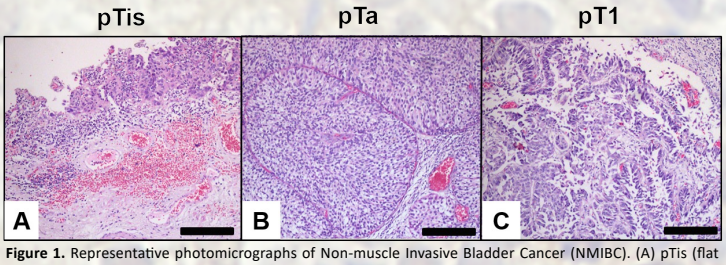

Figure 1. Representative photomicrographs of Non-muscle Invasive Bladder Cancer (NMIBC). (A) pTis (flat carcinoma *in situ*); (B) pTa high-grade (non-invasive papillary carcinoma) and; (C) pT1 high-grade (tumor invades lamina própria). Bars = 50 µm.

Tabel 2. Total immunoreactivity (%) for TLR4-mediated IFN-γ production signaling pathway, $CX3CR1^+CD8^+$ T-cells, immune checkpoints and regulatory T cells antigens in the pTis, pTa high-grade and pT1 groups.

	Groups		
Antigens	**pTis**	**pTa**	**pT1**
TLR4	76.20 ± 2.6 ab	79.64 ± 5.6 a	71.15 ± 5.1 b
TRIF	70.17 ± 3.2 a	76.96 ± 3.4 b	75.33 ± 4.0 b
TBK1	65.93 ± 4.1 a	71.96 ± 4.7 a	66.82 ± 13.2 a
IRF-3	66.56 ± 5.2 a	82.88 ± 6.7 b	76.25 ± 5.3 b
IFN-γ	61.17 ± 15.6 a	77.74 ± 10.7 b	76.64 ± 11.7 b
CX3CR1	45.59 ± 15.3 a	74.25 ± 1.9 b	78.30 ± 7.7 b
FOXP3	71.79 ± 3.6 a	74.03 ± 2.5 a	80.80 ± 4.5 b
PD-1/PD-L1	51.84 ± 10.5 a	77.55 ± 3.4 b	72.78 ± 10.6 b
CTLA4	49.98 ± 8.4 a	79.80 ± 3.5 b	78.51 ± 7.4 b

Values equivalent to the means of the percentage of positive urothelial cells cells for each specific antigen. ANOVA Kruskal-Wallis, Student-Newman-Keuls test. On the same line, values followed by different letters indicate the difference between the groups ($p < 0.01$).

Acknowledgements and Funding

METHODS

- Thirty formalin-fixed paraffin-embedded samples of bladder were obtained from 34 to 96-year-old patients (mean 65 years) with NMIBC diagnosis in University of Campinas (UNICAMP) and Paulinia Municipal Hospital/ Brazil.
- Subsequently, the samples were divided into 3 groups (n= 10 samples per group): pTis group, pTa high-grade group, and pT1 group; and submitted to immunohistochemistry analysis: TLR4-mediated IFN-γ production signaling pathway (TRIF, TBK1, IRF-3, IFN-γ), CX3CR1+CD8+ T-cells, immune checkpoints (PD-1/PD-L1 CTLA-4) and regulatory T (Treg) cells (FOXP3).
- The retrospective anonymous study was approved by the local ethics committee (Clinical Trial: RBR-6swqd2).

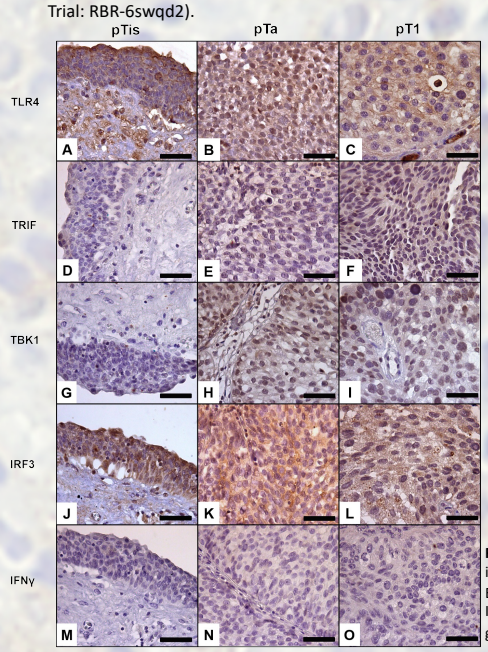

Figure 2. Immunolabelled antigen intensities for TLR-4 (A, B, C), TRIF (D, E, F), TBK1 (G, H, I), IRF-3 (J, K, L) and IFN-γ (M, N, O) in the pTis, pTa high-grade and pT1. Bars = 50 µm.

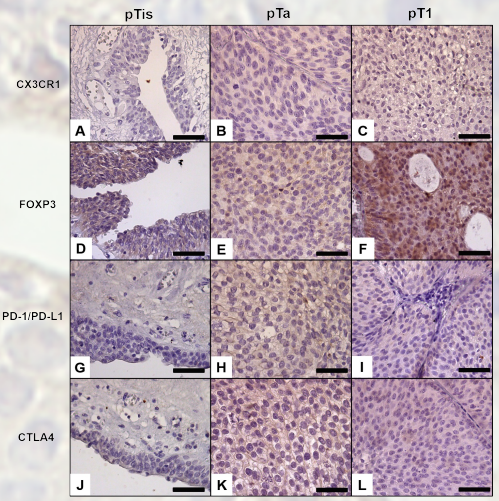

Figure 3. Immunolabelled antigen intensities for CX3CR1 (A, B, C), FOXP3 (D, E, F), PD-1/ PD-L1 (G, H, I) and CTLA4 (J, K, L) in the pTis, pTa high-grade and pT1. Bars = 50 µm.

Conclusions

Our data demonstrated that pTis stage was characterized by an immunosuppressive microenvironment in relation to pTa and pT1 stages, showing decreased TLR4-mediated interferon signaling pathway and low activation of $CX3CR1^+CD8^+$ T-cells, and therefore, resulting in low sensitivity to immunotherapy. The larger number of $FOXP3^+$ Treg cells in pTa and pT1 was correlated with intensified immune checkpoints immunoreactivities, indicating high sensitivity to immunotherapy. Finally, these biomarkers may be useful in the clinical management of patients with NMIBC.