MODULATION OF THE TOLL-LIKE RECEPTORS SIGNALING PATHWAY AFTER INTRAVESICAL ONCOTHERAD® IMMUNOTHERAPY ASSOCIATED TO PLATELET RICH PLASMA (PRP) FOR NON-MUSCLE INVASIVE BLADDER CANCER TREATMENT

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Introduction

There is no effective second-line therapy for non-muscle-invasive bladder cancer (NMIBC) when Bacillus Calmette-Guerin fails. In this scenario, a new perspective is represented by OncoTherad immunotherapy. OncoTherad® is a nanostructured inorganic phosphate complex associated to glycosidic protein, developed by University of Campinas/Brazil, which exhibits antitumor properties. The use of phosphate moieties can exert biological activities, since polyphosphates are secreted by activated platelets and mast cells, interfering in innate immune response. Our research group also demonstrated that Platelet Rich Plasma (PRP) acts on immune activation and exerts antitumor effects.

Objective

This study characterized the histopathological and molecular effects of the OncoTherad® associated with PRP in the treatment of NMIBC chemically induced in mice and the possible mechanisms of action of this association involving the Toll-like receptors (TLRs) 2 and 4 signaling pathways.

Methods

C57BL/6J mice were divided into 5 groups (n=7 animals/group): Control (rest); Cancer (induction using N-ethyl-N-nitrosourea carcinogen, 50mg/mL); PRP (0.1 ml); OncoTherad® (20mg/mL) and OncoTherad+PRP (1:1,10mg/mL). The intravesical doses (0.1mL) were instilled once a week for 6 consecutive weeks after induction. The urinary bladders were collected and submitted to histopathological and immunohistochemical analyzes. The diagnosis of urothelial lesions was classified according to the World Health Organization/International Society for Urological Pathology.

Results and Discussion

Our results showed flat carcinoma in situ (pTis) in 100% of the animals from Cancer. In this group, TLR2, IL-6, TLR4 and IRF-3 immunoreactivities were significantly lower in relation to Control, indicating suppression of the immune system in the tumor microenvironment. When treated intravesically with PRP only, animals showed 28,57% of tumor progression inhibition rate (Table 1). The groups that received OncoTherad® only and OncoTherad® plus PRP showed high rates tumor progression inhibition. The combined OncoTherad and PRP treatment led to distinct activation of TLRs 2 and 4-mediated innate immune system, resulting in increased interleukins (MyD88-dependent pathway) and interferons (TRIF-dependent pathway) signaling pathways in relation to isolated treatments (Figure 1; 2).

Conclusions

The combined OncoTherad® plus PRP treatment significantly promoted inhibition of tumor progression, possibly due to its immunomodulatory properties, including acting on the TLR pathway. This association can constitute a new therapeutic strategy for refractory NMIBC patients.

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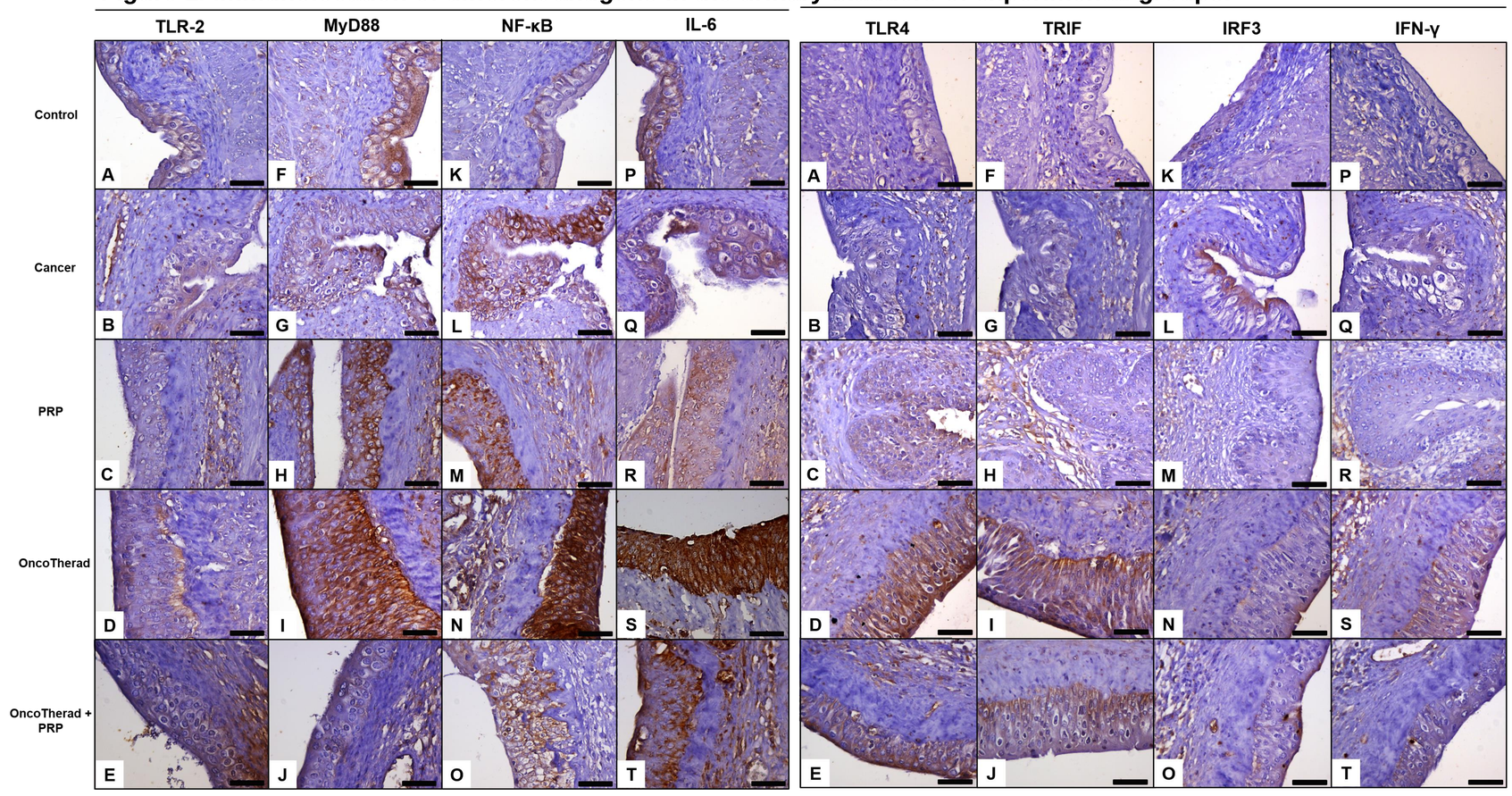
Table 1. Histopathological changes in the urinary bladder of mice and Tumor Progression Inhibition Rate (%).

	Experimental Groups (n = 7)				
Histopathology	**Control**	**Cancer**	**PRP**	**OncoTherad**	**Oncotherad+PRP**
Normal	7 (100%) *	-	-	2 (28,57%) *	-
Flat Hyperplasia	-	-	-	3 (42,85%) *	3 (42,85%) *
Low-grade Intraurothelial Neoplasia	-	-	2 (28,57%) #	1 (14,28%) *	2 (28,57%) *
Carcinoma in situ (pTis)	-	7 (100%)	1 (14,28%)	-	1 (14,28%)
Papillary Urothelial Carcinoma (pTa)	-	-	4 (57,14%)	1 (14,28%)	1 (14,28%)
Tumor Progression Inhibition	-	0% a	28,57% ac	85,71% b	71,42% bc

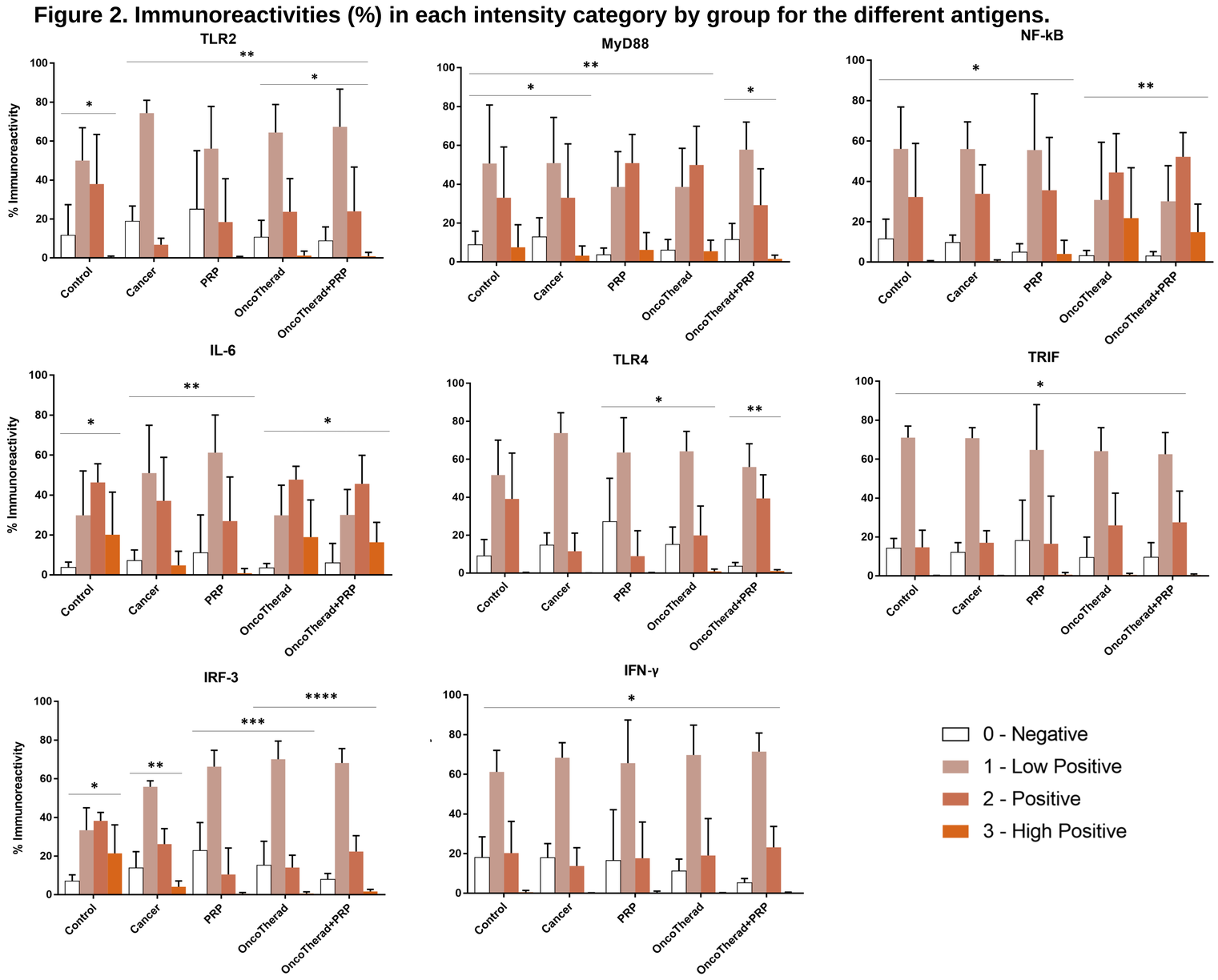
*Statistical significance compared to the Cancer Group (p<0.05). #Statistical significance in relation to the OncoTherad group (p<0.05). Tumor Progression Inhibition: Percentage of animals that did not have malignant lesions compared to the Cancer group. In the same line, values followed by different letters indicate a significant difference between groups (p<0.05). Chi square Test. **Benign lesions:** Flat hyperplasia. **Pre-Malignant Lesions:** Low-Grade Intraurothelial Neoplasia. **Malignant lesions:** pTis e pTa.

Figure 1. Immunolocation of different antigens in the urinary bladders of experimental groups.



TLR2, TLR4 (A-E); MyD88, TRIF (F-J); NF-κB, IRF3 (K-O) and IL-6, IFNγ (P-T). Control (healthy urothelium: A,F,K,P); Cancer (pTis: B,G,L,Q); PRP (pta: C,H,M,R; low grade intraurothelial neoplasia: repetiton of C,H,M,R); Oncotherad (flat hyperplasia: D,I,N,S) and OncoTherad+PRP (low grade intraurothelial neoplasia : E,J,O,T).

Figure 2. Immunoreactivities (%) in each intensity category by group for the different antigens.



*Groups with different symbols indicate significant difference (p<0.05). ANOVA, Tukey Test (n=5 sections/animal/group). Immunoreactivity was classified into four categories: Negative (0) - absence of marking; Low Positive (1) - weak immunoreaction; Positive (2) - moderate immunoreaction and High Positive (3) - strong immunoreaction.