

## Sweet side of bladder cancer

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### Abstract

The malignant transformation of cells is often accompanied by deranged expression of the sugar chains, *i.e.*, glycans, attached the cancer cell surfaces or attached to secreted proteins. The aberrant expression of specific glycans in bladder cancer has also been reported by several research groups. Similarly to other cancers, glycans such as the sialyl Tn antigens have been

suggested as diagnostic and prognostic biomarkers of bladder cancer, and associated with disease progression and patient's response to treatment. At present our understandings about the role of glycans in bladder cancer is still limited, but at the same time it is now assumed that this understanding urges and it will fuel the development of novel strategies of diagnostic and therapy.

**Key words:** Bladder cancer; Glycosylation; Tumor biomarker; Sialyltransferase; Immunotherapy; Bacillus Calmette-Guérin

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**Core tip:** The deranged expression of glycans in bladder cancer has been reported, but somehow disregarded. Glycans, such as the sialyl Tn, show a very tumor specific pattern and have been suggested as diagnostic and prognostic biomarkers of bladder cancer, and associated with disease progression and patient's response to treatment. Understandings about the role of glycans in bladder cancer is still limited, but, it is now assumed that this understanding urges and it will fuel the development of novel strategies of diagnostic and therapy.

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### INTRODUCTION

#### *Sialyl Tn in bladder cancer*

Among the glycans that are known to be aberrantly expressed in bladder cancer, the sialyl Tn (STn) emerges as the most distinctive glycan due to its tumor specificity. STn is considered a pan-carcinoma antigen,

and many other epithelial cancers express it<sup>[1]</sup>. But the most interesting feature is the fact that STn is not expressed by healthy cells, guarantying specificity in any therapy against it.

STn expression in bladder cancer was first described in the nineties by Langkilde *et al*<sup>[2]</sup> that analysed 34 patients with initially noninvasive (Ta) transitional cell carcinomas. According to these authors, STn showed a very restricted pattern of expression in bladder cancer and it was not expressed by normal urothelium. No association with recurrence and progression was found. Much later, in 2014, the prognostic value of STn expression in bladder cancer was revised by Ferreira *et al*<sup>[3]</sup>, using higher number of cases and different patient stratification. We reported that the STn is expressed majorly in high grade lesions and in muscle invasive bladder tumors (around 70% of the cases). STn is expressed also in low grade lesions and carcinoma *in situ* but with less expression levels<sup>[3]</sup>. STn is expressed in tissues presenting high proliferation indexes and high risk of recurrence/progression and it is related with higher invasiveness of bladder cancer cells<sup>[3]</sup>, thus being suggested as associated with malignant profile.

In contrast to other epithelial cancers there has been very little interest in studying aberrant glycosylation in bladder cancer. A possible explanation for the dormancy of research interest in the glycosylation of bladder cancers may have to do due to the lack of interest in bladder cancer research itself, due to its low mortality rates. In addition, these studies require sufficient understanding on glycosylation and use methodologies that involve the collection and preservation of high quality tissue for immunodetection techniques, especially when using antibodies against glycans. Therefore with our report, we were able to bring to the spotlight the relevance of glycans and in particular of STn as bladder cancer biomarker. These findings now launch the question of the benefits of considering STn in diagnosis and bladder cancer therapy.

The STn antigen is a simple O-linked disaccharide constituted by N-acetylneuraminic acid linked to N-acetyl-galactosamine [Neu5Ac (2-6) GalNAc], bound to serine or threonine residues in proteins. The aberrant expression of STn in cancer results from the incomplete biosynthesis of mucin-type O-glycans, due to alterations of cellular glycosylation machinery, namely the increased activity of the sialyltransferase ST6GALNAC1<sup>[4]</sup>, which transfers N-acetylneuraminic acid to N-acetyl galactosamine linked to the protein carrier. The elevation of the expression of the sialyltransferase ST6GALNAC1 gene can actually be also considered as a transcriptional biomarker<sup>[3,5]</sup>.

### **Sialyl Tn and immune response**

Nearly after, in two other reports the expression of STn in bladder cancer was associated with immunotherapy and immune responses. In the first report, in 2013, it was described that STn can be used to predict

patients response to Bacillus Calmette-Guérin (BCG) treatment<sup>[6]</sup>. The use of STn as a predictive marker to BCG treatment response still needs to be further investigated with higher cohorts of patients. However to may be anticipated that STn identification may be useful for patient stratification and for the identification of patients that could benefit from BCG immunotherapy. Interestingly, STn expressing cancer cells internalized much better BCG and therefore experience improved BCG-induced apoptosis, when compared with STn negative cancer cells<sup>[6]</sup>. While interesting these findings it remain to be understood which is the mechanisms that leads to better internalization of BCG into STn-expressing bladder cancer cells. It may be hypothesised that BCG express receptors, such as adhesins, that bind preferentially STn-containing glycans at cell surface of bladder cancer cells. However further investigations are needed to dissect the BCG mechanism of binding to STn.

In the second report, in 2014, we showed that STn behaves as a tolerogenic molecule inducing immunosuppression in human dendritic cells (DC). DCs are one of the most important coordinators of anti-tumor immune responses involved in the activation of multiple arms against tumor cells. However their ability to elicit anti-tumoral immune responses is critically dependent on their maturation status which in turn is highly influenced by the microenvironment. We observed that STn-expressing cancer cells dampen the maturation of DCs *in vitro*. DCs when in contact with STn-expressing cancer cells show an immature phenotype, by expressing lower levels of major histocompatibility complex class II, co-stimulatory molecules and less pro-inflammatory cytokines<sup>[5]</sup>. Pro-inflammatory cytokines are also significantly decreased in STn positive bladder tumours<sup>[5]</sup>. Furthermore, DCs loaded with STn<sup>+</sup> cancer antigens induce T cells with regulatory properties<sup>[5]</sup>, suggesting that the adaptive immune response is compromised. It may be hypothesized that immunosuppressive receptors expressed by DCs recognize STn and mediate tolerization. In fact DCs express sialic acid-binding Ig-like lectins (Siglecs)<sup>[7]</sup> and the macrophage galactose type lectin (MGL)<sup>[8]</sup>, which are tolerogenic receptors described to recognize STn antigens<sup>[8]</sup>. Nevertheless, the specificity of these receptors is still debatable and validation of its capacity to recognize STn is necessary at cellular level. A better understanding of the mechanisms by which DCs are rendered tolerogenic in the presence of STn-expressing cancers is important for the development of effective immunotherapies.

### **The future of anti-sialyl Tn therapies in bladder cancer**

Immunotherapies against STn have already been developed, in breast cancer patients<sup>[9]</sup>. In fact, the biotech company Biomira (now Oncothyreon, Alberta, Canada) designed the Theratope™ vaccine that consists of a synthetic construct of STn disaccharide

conjugated to the Keyhole limpet hemocyanin<sup>[10]</sup>. Breast cancer patients that were vaccinated with STn vaccine significantly improved survival. However, the benefits of Theratope were suboptimal and the clinical trials did not pass after phase III<sup>[11]</sup>. In light of our findings showing that DCs become tolerogenic with STn, it seems that the immunosuppressive environment exerted by STn may abrogate any anti-tumor immunity against STn cancer cells, thus explaining the failure of molecular STn-based vaccines. In addition, the percentages of STn positive cases in breast cancer is less than 50% and in the Biomira trials, no patient screening has been performed, therefore the odds for successful immunization in the breast cancer patients were low. It remains to be understood the efficacy of STn vaccine in other cancers that also express STn, such as bladder cancer, where the expression of STn is much higher than breast cancer.

Interestingly, breast cancer patients receiving Theratope developed anti-STn antibodies, whose abundance was directly correlated with disease free survival<sup>[12]</sup>. Patients receiving low-dose intravenous cyclophosphamide, an inhibitor of suppressor T cells, before vaccinations showed longer survival and generated higher antibody titers than control patients<sup>[13]</sup>. These observations demonstrate the relevance of antibodies against STn and of strategies to break immune tolerance to maximize treatment. In agreement, we observed *in vitro* that the use of antibodies to block STn antigens expressed by bladder cancer cells was able to lower the induction of tolerance and DCs become more mature<sup>[5]</sup>. Thus targeted therapies based on anti-STn antibodies may provide efficient means to enhance immune responses against STn-expressing tumor cells. While several antibodies have been established against STn<sup>[14]</sup> none has actually been applied in clinics to elicit elimination of STn cancer cells. Possible limitations to its application have to do with lack of interest or low specificity and efficiency of the existing antibodies.

A better understanding of STn role in immune evasion and influence in existing immunotherapies is now deemed to boost the development of novel therapies and to better stratify patients for therapeutic regime. It has been fascinating to notice that a better understanding of the factors restricting effective immune responses launched the recent development of antibodies targeting inhibitory immune checkpoints, with an extraordinary capacity to break tumor-induced immune tolerance. In bladder cancer, the antibody MPDL3280A against the inhibitory immune checkpoints PD-L1, was already approved by Food and Drugs Administration and has shown promising results<sup>[15]</sup>. The targeting of inhibitory immune checkpoints may be considered in the future as a therapy for cancers expressing factors, such as STn, that lead to immune tolerance. An irrefutable knowledge is arising from novel therapies that will warrantee the future development of more successful treatments for bladder cancer.

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