# Serum MUC1 O-glycans from cancer patients contain the Sialyl Tn antigen

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## Summary

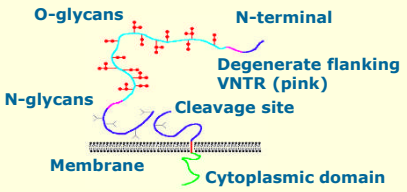
**The analysis of O-linked glycosylation present on MUC1 purified from advanced breast cancer patient's serum/pleural effusions and healthy control serum was performed. Results highlighted the presence of the Sialyl Tn sugar epitope on MUC1 from a patient serum.**

## Introduction

MUC1 is a high molecular weight glycoprotein that is aberrantly expressed and glycosylated in breast cancer. The core protein is composed of a variable number of tandem repeats (VNTR) each twenty amino acids in length. The number of repeats is highly polymorphic and is not thought to be critical for function. Of the twenty amino acids of the tandem repeat five are sites for possible O-linked glycosylation. Serum MUC1 levels are used as a tumour marker for Breast Cancer progression via the CA15.3 assay[3].

**Figure 1**

**Schematic diagram of MUC1 showing major protein regions (adapted from Dekker *et al.*, 2002).**




The Sialyl Tn sugar epitope is an important cancer marker and immunohistochemistry has demonstrated its presence in up to 84% of invasive ductal breast carcinomas[4]. It results from the expression of the STn antigen that arises from sialylation of the core monosaccharide GalNAc.

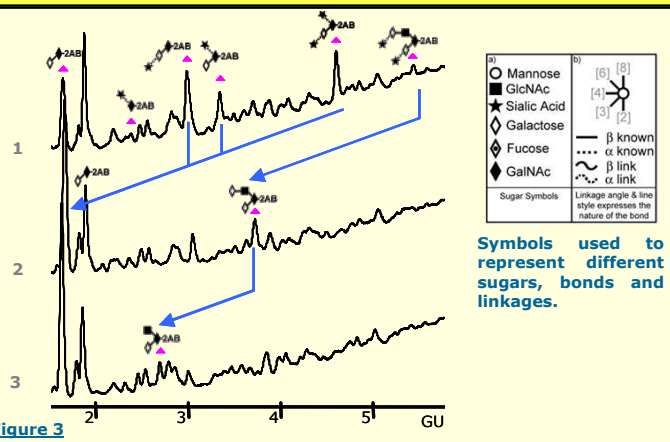

**Figure 2**

**Schematic representation of the Sialyl Tn antigen. 2AB represents the fluorescent label**

## Methods

MUC1 has been purified from 6 sources; including breast cancer patients serum/pleural effusion and healthy control serum, using affinity chromatography with NCRC-11. Purified samples were subject to IgA radial immunodiffusion and SDS gels stained by silver to show minimal non-IgA contaminants. O-glycans were released by hydrazinolysis and fluorescently labeled. Sugars were identified by a combination of Normal Phase HPLC, exoglycosidase digestions and LC- Mass spectroscopy. Glycan profiles were generated from 4 of 6 MUC1 samples.

## Results




**Symbols used to represent different sugars, bonds and linkages.**

**Figure 3**

**Figure 3 shows sequential exoglycosidase digestions of MUC1 purified from the serum of a patient with advanced breast cancer. Profile 1 shows all peaks visible prior to digestion. Profile 2 follows sialidase digestion. Profile 3 is where sialidase and beta-galactosidase digestions have been performed. GU are glucose units, a standardised value obtained from HPLC retention time.**

The Sialyl Tn (STn) sugar epitope has been identified on MUC1 purified from a breast cancer patient serum (29MS). STn represented approximately 3.8% of total assigned sugars. The most common sugar was the sialylated core 1 structure NeuNAcα2-3Galβ1-3GalNAc (29.1%), and 1.9% of assigned sugars were core 2 based. Three other MUC1 samples (one from the same patient pleural effusion, one patients serum and pleural effusion MUC1 and a healthy control serum) did not contain the STn epitope.

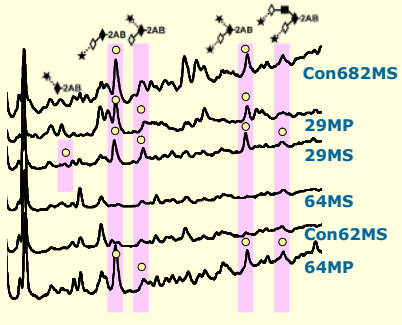



**Figure 4**

**The undigested HPLC profiles of all MUC1 samples. Yellow spots show peaks confirmed by exoglycosidase digestion and LC-MS. Notice STn present in 29MS only. Pink stripes show major glycans. MS denotes MUC1 from serum, MP from pleural effusion and con denotes healthy control sample**

MUC1 and STn are important cancer markers and a correlation between their expression in cancer has previously been reported[4]. Analysis of MUC1 glycans from MCF-7 and T47D breast cancer cell lines has not confirmed the presence of STn[2]. The STn epitope demonstrates aberrant glycosylation that arises from sialylation of the core GalNAc. Many factors can alter glycan processing, including upregulation of ST6GalNAc I, -II.1

## References and Acknowledgements

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