Analysis of the Antibody Binding of Derivative MUC1 Peptides via STD NMR

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Abstract

The binding epitope PDPFR found within the VNTR domains of MUC1 glycoprotein is recognized by the immune system and binds mucin monoclonal antibody SM2. This study analyzes the binding ability of the peptide GVTSAWD, an epitope sequence preceding PDPFR, as well as three derivative peptides against specific mono MUC1 monoclonal antibody (GVT, GVTAD, GVTASSD) by saturation transfer difference NMR (STD NMR) to determine the residues critical for antibody binding and whether analogous side chain characteristics in the derivative sequences would influence binding. The STD NMR results indicated that Pro is a critical residue for binding as it displayed greater saturation transfer effects for mono antibody than any other residue. Substituting the Pro residues with single hydrophobic or hydrophilic aliphatic residues eliminated all STD effects while substitution of Pro with single hydrophobic aromatic residues produced STD effects as the aromatic protons. Substitution at Ser position lost Asn produced STD effects that were similar in pattern and intensity to those of the native sequence. The results indicate that the Pro residue is critical for antibody binding and substitution at this position for aromatic residues conserves binding ability. This suggests that these substituted peptides may possess biological activity.

Introduction

Mucins (shown in Fig. 1) are a class of heavily glycosylated proteins, most commonly found on epithelial surfaces, which provide many protective cellular functions such as the formation of mucosal barriers. The range of human mucins (MUC) spreads from MUC1 to MUC21, however the specific mucin this study is associated with is MUC1 protein. This loss of polarity triggers an epithelial-mesenchymal transition (EMT) producing aggressive cancer cells. Additionally, transmembrane mucins have been found to be overexpressed in cancer cells, leading to profound EMT activation and acquired migratory [1,2]. Thus far it has been difficult to create an effective immunotherapy or vaccine for cancer tumours due to the highly immunosuppressive effects [3,4]. It is believed that using truncated MUC1 peptide sequences could be an effective vaccine against cancer due to the nature of the epitopes. Cancer cell-associated mucins. These mucin proteins are overexpressed and highly glycosylated, leaving short amino acid sequences exposed to the extracellular environment [4,5]. This potentially increases the efficacy of these proteins as antigens for the signaling of cytotoxic T cells if previously exposed to a vaccination of short peptide epitopes of MUC1 [4,5]. Furthermore, evidence shows that using a mimotope, a derivative sequence of the exposed peptide epitope (a substitution of 1 or 2 amino acids), may elevate the immune response when presented with a tumor cell exhibiting the natural epitope [6].

Methods

Epitope Modification via Peptide Synthesis

All peptides used in this study were manually synthesized in the solid-phase using standard Fmoc-chemistry. The focus of polarity triggers an epithelial-mesenchymal transition (EMT) producing aggressive cancer cells. Additionally, transmembrane mucins have been found to be overexpressed in cancer cells, leading to profound EMT activation and acquired migratory [1,2]. Thus far it has been difficult to create an effective immunotherapy or vaccine for cancer tumours due to the highly immunosuppressive effects [3,4]. It is believed that using truncated MUC1 peptide sequences could be an effective vaccine against cancer due to the nature of the epitopes. Cancer cell-associated mucins. These mucin proteins are overexpressed and highly glycosylated, leaving short amino acid sequences exposed to the extracellular environment [4,5]. This potentially increases the efficacy of these proteins as antigens for the signaling of cytotoxic T cells if previously exposed to a vaccination of short peptide epitopes of MUC1 [4,5]. Furthermore, evidence shows that using a mimotope, a derivative sequence of the exposed peptide epitope (a substitution of 1 or 2 amino acids), may elevate the immune response when presented with a tumor cell exhibiting the natural epitope [6].

Results

Fig. 5. HPLC chromatogram showing resolved peaks of peptide GVTSAWD.

Conclusions

The results indicate that Proline-substituted derivatives GVTSAWD and GVTASSD retain the ability to bind mAb(6A4), but to a much lesser degree. GVTSAADD and GVTASSADD do not. However, the normal epitope GVTSAWD, upstream from the main binding site in its domain, retained mAb binding ability as well and was further maintained when Ser was replaced with Asn (GVTASSADD), indicating that Pro is critical for antibody binding. This information indicates that hydrophobic ring structures such as Tyr, Phe and Trp are the Pro position are critical for the binding of antibody.

References


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Fig. 3: Panel A: Overlay of TOCSY (green) and ROESY (red) NMR spectra and spin systems of peptide epitope GVTSAWD. 

Fig. 3: Panel B: Overlay of TOCSY (green) and ROESY (red) NMR spectra and spin systems of peptide epitope GVTSAWD.