MUC1 BASED BREAST CANCER VACCINES: ROLE OF POST TRANSLATIONAL MODIFICATIONS

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Background: Vaccine development is one of the most promising fields in cancer research. After autologous transplantation, due to low tumour burden, patients are more likely to respond immunologically to a cancer vaccine[7]. MUC1 with its adhesive and antiadhesive functions, immunostimulatory and immunosuppressive activities, is therefore a good candidate for breast cancer vaccine. A structure-based insight into the immunogenicity of natural MUC1 glycoforms, of its sub-domains, motifs and post translational modification like glycosylation and myriostoylation may aid the design of tumour vaccines. **Methods:** Primary sequences of human MUC1 were retrieved from the SWISSPROT data bank. Protein pattern search: The primary sequence of Human MUC1 was searched at PROSITE (a dictionary of protein sites and patterns) database. **Results:** Our study observes that post-translational modifications play an important role in presenting MUC1 as a candidate for breast cancer vaccine. **Conclusion:** It is found that the phosphrylation and glycosylation of important functional motifs of MUC1 may take part in the production of cytokines that may provide immunization.

Keywords: Vaccine, MUC1, glycosylation

INTRODUCTION

The CA15-3/MUC1 is a large transmembrane glycoprotein which is encoded by the mucine gene present on chromosome 1q21. It is a large, heavily glycosylated mucin expressed on the apical surfaces of most simple, secretory epithelia including the mammary gland, gastrointestinal, respiratory, urinary and reproductive tracts.[1,2] The expression of MUC1in human breast cancer cells is altered by factors affecting cell proliferation.[3] It consists of the large extracellular domain that is primarily composed of a variable number of highly conserved 20 amino acid tandem repeats (AHGVTSAPESRPAPGSTAPA).[5] The conserved 20 amino acids of tandem repeat motif rich in serine and threonine residues are linked via O-glycosidic linkages to sugar residues.[6] The increase in level of expression of MUC1 in breast cancer is accompanied by changes in the profile of glycosyl transferases involved in the synthesis of the O-glycan attached to MUC1 core protein.[8] It is also reported that MUC1 is over expressed in tumours as a hypoglycosylated form.[3] Glycoproteins on epithelial tumour cells often exhibit aberrant glycosylation profiles.[4]

Of the 8 human epithelial mucins known, MUC1 has had the most attention for immunotherapeutic applications. In breast cancer MUC1 is up-regulated and as a result of changes in glycosyl transferases, the complex carbohydrate side-chains are shortened leading to the exposure of novel peptide and carbohydrate epitopes. Cellular and humoral immune responses to MUC1 have been documented in benign and malignant breast disease and in some circumstances, T-cell responses to MUC1 may not depend on presentation by the major histocompatibility complex.[1] These observations have led to the development of several different approaches to immunize against breast cancer using synthetic carbohydrates or peptides conjugated to carriers and given together with a variety of adjuvant to elicit the appropriate immune response.[9]

METHODS

Sequence Retrieval:

Primary sequences of human MUC1 were retrieved from the SWISSPROT data bank.[10]

Sequence Analysis:

Protein pattern search: The primary sequence of Human MUC1 was searched at PROSITE database.[11] PROSITE (a dictionary of protein sites and patterns) is a method of determining the function of uncharacterised proteins translated from genomic or cDNA sequences. It consists of a database of biologically significant sites and patterns formulated in such a way that with appropriate computational tools it can rapidly and reliably identify to which known family of protein the new sequence belongs.

Tandem repeat Analysis:

The 20 amino acid tandem repeat sequence, SAPDNKPAPGSTAPPAHGVT, of Human MUC1 was submitted to the BLAST server and searched for short sequence match against PDB.[12]

Secondary structure prediction of human MUC1 was carried out by the SABLE server, which provides accurate sequence-based prediction of relative Solvent Accessibilities, secondary structures and trans-membrane domains for proteins of unknown structure.[21]

RESULTS AND DISCUSSION

The role MUC1 plays in cancer progression has two aspects. On the one hand, loss of polarity and over-expression of MUC1 in cancer cells interferes with cell adhesion and shields the tumour cell from immune recognition by the cellular arm of the immune system, thus favouring metastases. On the other hand, MUC1, which is a self-antigen, is displaced and altered in malignancy and induces immune responses. Tumour-associated MUC1 has short carbohydrate side chains and exposed epitopes on its peptide core. It gains access to the circulation and comes into contact with the immune system provoking humoral and cellular immune responses. Natural antibodies to MUC1 present in the circulation of cancer patients may be beneficial to the patient.[13]

MUC1 has a rigid rod like structure protruding from the cell surface.[14] Like other trans-membrane proteins MUC1 also has 3 domains: a) externally located domain comprising 1–1161 amino acid residues that bears carbohydrate chains, b) middle membrane spanning region comprising 1162–1179 residues consisting mainly of hydrophobic residues and c) a cytoplasmic domain comprising 1180–1255 amino acid residues that has a high proportion of charged and polar residues.[15] According to the secondary structure prediction, human MUC1/CA 15-3 consists of ~5.0% helix, ~2.6% strands and ~92.3% loops or turns. Amino acid composition shows that the protein consists of high percentage of alanine, proline, serine, tyrosine, valine, and glycine.

Post translational modification (glycosylation) is mainly observed in the extracellular sub unit. Due to these modifications, the protein CA 15-3/MUC1 performs different functions. However, according to our PROSITE search results, only one serine residue has glycosaminoglycan attachment site, showing O-linked glycosylataion at the position of 1152. This shows that MUC1 having O-linked glycosylation at its domain has carbohydrate linked charged and polar amino acid residues. It has been observed that glycosylation not only introduces novel potential epitopes into a protein, but also causes a series of restrictions due to sterical hindrance by the bulky carbohydrate chains. The O-linked glycans are known to protect peptide backbones of proteins from ready proteolytic digestion.[16] Another study found that the side chain of serine and thronine are in an appropriate environment on the protein surface to serve as acceptor for the GalNAc-transferase that attaches N-acetyl galactosamine to the protein.[7] The increase in level of expression of MUC1 in breast cancer is accompanied by changes in the profile of glycosyl transferases involved in the synthesis of the O-glycan attached to MUC1 core protein[8]. It is therefore possible that due to the malfunctioning of the enzyme GalNAc-transferase, the side chain of Ser residue present at position 1152 does not recognize the enzyme. This may effect the attachment of N-galactosamine to the signalling protein which may enable immune response to it.

Beside O-linked glycosylation, the PROSITE search results also show the N-linked glycosylation which is mainly observed on positions 957, 975, 1029, 1055 and 1133 of MUC1(Table-1). It is observed that these are the tandem repeat region of MUC1.

Table-1: PROSITE motif search of CA 15-3 (MUC1)

ID: ASN_GLYCOSYLATION PS00001 PDOC00001
DE: N-glycosylation site
957 NVTS NGTS NHST
1055 NSSL
1133 NLTI
ID: GLYCOSAMINOGLYCAN PS00002
DE: Glycosaminoglycan attachment site
1152 SGAG
ID: PKC_PHOSPH_SITE PS00005 PDOC00005
DE: Protein kinase C phosphorylation site
40 TQR 49 TEK 978 SAR 1009 STK 1224 TDR
ID: CK2_PHOSPHO_SITE PS00005 PDOC00006
DE: Casein kinase C phosphorylation site
47 SSTE 120 SAPD 140 SAPD 160 SAPD 180 SAPD 200 SAPD
220 SAPE 240 SAPD 260 SAPD 280 SAPD 300 SAPD 320 SAPD 340 SAPD 360 SAPD 380 SAPD 400 SAPD
420 SAPD 440 SAPD 460 SAPD 480 SAPD 500 SAPD 520 SAPD 540 SAPD 560 SAPD 580 SAPD 600 SAPD
620 SAPD 640 SAPD 660 SAPD 680 SAPD 700 SAPD 720 SAPD 740 SAPD 760 SAPD 780 SAPD 800 SAPD
820 SAPD 840 SAPD 860 SAPD 880 SAPD 990 SAPD 920 SAPD 1010 TKTD 1056 SSLE 1135 TISD 1140 SVSD
1222 SSTD 1227 SPYE
ID: MYRISTOYLATION PS00008 PDOC00008
DE: N-myristoylation site
23 GSGHAS 67 GSGSST 75 GQDVTL 89 GSAATW 95 GQDVTS
963 GSASGS 1084 GGFLGL 1235 GNGGSS

A number of clinical trials have proposed to evaluate the use of tandem repeat region of MUC1 as vaccine for breast cancer. It is proposed by one study that peptide and glycopeptide with the immunodominant DTR (Asp-Thr-Arg) of tandem array, when mutated to ESR (Glu-Ser-Arg) motif, are evaluated as potential targets in multiepitope adjuvant based vaccine strategies, for their capacity to induce cytotoxic T cell response.[25] Another study reported that in cancer samples, frequency of preferential binding shifts towards the DTR motif, could be linked to tumour-associated O-glycosylation with shortened chains and may enable immune responses to it.[18]

In the predicted structure of MUC1, we observed that the immunodominant DTR is present in Domain 1 of MUC1 comprising 1–1161 amino acids. It is observed that domain 1 is rich in the DTR region and the last DTR motif is present at position 922 of the domain. It may be possible that in the folding structure, the DTR motif region may form H-bonds with five of the N-glycosylation sites and one of the O-linked glycosylated Ser and the mutation (causes conformational change) of DTR may affect both O and N-glycosylation process of MUC1. This in turn may enable the immune response in humans.

Our study observed that next to most of the DTRP regions is a site highly phosphorylated by casein kinase C and phosphokinase C (Table-1). The main function of the phsophorylation is activation of functional sites of protein and enzyme. It is therefore possible that due to the phsophorylation, a number of serine and threonine residues that may be present in the core or functional site of MUC1 are activated and may react with monoclonal antibodies made against breast cancer and used as vaccine.

Single molecules of glycolipids (tetrasaccharides, pentasaccharides, or hexasaccharides) and MUC1 peptides (containing between one and five MUC1 tandem repeats) conjugated to keyhole limpet hemocyanin or KLH (antibody against cancer antigen) have proven sufficient for antibody recognition and vaccine construction.[19] Our study observed possible myristoylation sites at positions 23, 67, 75, 89, 95, 963, 1084 and 1235. Though these myristoylation sites are spread out in whole molecule of MUC1, most of them are present near the N-terminal region of MUC1 so it may be possible that in the folding form of protein (3 dimensional structure) these may be near to the O and N-linked glycsoylation sites of MUC1. There, they may bind with a specific antibody like KLH and behave like vaccine.[9]

Lately it has been demonstrated that cultures of peripheral blood mononuclear cells from patients with adenocarcinomas, when stimulated with peptide 610 (GSTAPPAHGVTSAPDTRPAP) showed the highest specific killing of the MUC1-expressing breast cancer MCF-7 cells. Peptide 610 was also found superior to the other peptides in inducing better production of the type 1 cytokines, tissue necrosis factor alpha and interferon gamma[20]. Our study observed that in peptide 620 a small motif of SAPD was phosphorylated with casein kinase C (Table-1). It is therefore possible that this phosphorylated motif, that is superior to other peptides in inducing the production of cytokines, may provide immunization.

CONCLUSION

Due to the malfunctioning of the enzyme GalNAc-transferase, the side chain of Ser residue present at position 1152 does not recognize the enzyme. This may effect the attachment of N-galactosamine to signalling protein which may enable immune response to it. In predicted structure of MUC1, it is observed that the immunodominant DTR is well-heeled in Domain 1. The last DTR motif is present at the 922 position of domain. It may be possible that in folding structure, the DTR motif region may form H-bonds with five of the N-glycosylation sites and one of the O-linked glycosylated Ser and the mutation (causes conformational change) of DTR may affect both the O and N-glycosylation process of MUC1. This in turn may enable the immune response in humans. Next to the DTRP region is the site which is highly phosphorylated by casein kinase C and phosphokinase C. It is therefore probable that due to phsophorylation, a number of serine and threonine residues, that may be present in the core or functional site of MUC1, are activated and may react with monoclonal antibodies made against breast cancer and be used as vaccine. Myristoylation sites are spread out in the MUC1 molecule but most of these are present near the N-terminal region of MUC1, so it may be possible that in the folding form of the protein (3 dimensional structure) these may be near the O and N-linked glycsoylation sites of MUC1, where these bind with specific antibodies like keyhole limpet haemocyanin or mannan (protein carrier for eliciting a cellular or humoral immune response) and behave like vaccines. In peptide 620 a small motif of SAPD was phosphorylated with casein kinase C. It is possible that this phosphorylated motif may provide immunization.

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