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RIVIEW ARTICLE

MUC1 from the Mucin Family as Potential Tools in Breast Cancer Immunotherapy

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Many breast cancer patients develop minimal residual disease that becomes resistance to treatments, and finally are faced with relapse and progression of disease. Currently, immunotherapy has become a potential therapy in treating minimal residual disease and preventing cancer occurrence. Cancer vaccines provide a unique therapeutic modality in that they initiate a dynamic process of activating the host's own immune system. A lot of tumor specific antigens as a target of immune system were identified and some have been applied for cancer vaccine. Mucin 1 (MUC1) oncoprotein, which is overexpressed in breast cancer in contrast with normal mammary tissue, is one of the first tumor antigens shown to be a target for human tumor-specific T cells and thus a valid target for immunotherapy. MUC1 is a high-molecular-weight glycoprotein rich in serine and threonine residues that are O-glycosylated. MUC1 is expressed on glandular epithelia and on epithelial tumors. But, tumor MUC1 differs from normal MUC1 by modified glycan side chains. Over-expression and aberrant glycosylation of MUC1 antigen by epithelial tumors results in endogenous antibody responses in cancer patients to MUC1 antigen. This finding has led to the identification of MUC1 derived peptide epitopes that induce T-cell responses. MUC1 based clinical trials have used peptides, protein, DNA, pulsed dendritic cells, or glycopeptide. This review will summarize the potential utility of breast cancer immunotherapy of MUC1, as well as the structure and function.

Key Words: *Breast neoplasms, Cancer vaccine, Immunotherapy, MUC-1*

INTRODUCTION

Breast cancer is the most prevalent cancer in woman worldwide. In Korea, breast cancer accounts for 37.3% of all cancers in women and is 6th as a cause of cancer-related death. The incidence rate represents an upward trend that has continued to increase by more than 6.8% per year since 1999.*(1)* This trend is expected to progress due to early detection and westernized lifestyles. Unfortunately, the severe morbidity of these cancers, reflected in the poor 5-yr relative survival rate, has not been improved by current treatments that include surgery, radiotherapy, hormone therapy, and adjuvant chemotherapies.*(2)* Although breast cancer research has developed at a rapid pace over the last decade, the curative potential of currently available therapies remains disappointing. Thus, there is a need for ongoing research into the development of new breast cancer therapeutic approaches.

The immune system is capable of recognizing and rejecting autologous tumor cells, as suggested by cases of spontaneous remission of various cancers,*(3)* and the presence of infiltrating leukocytes, the majority of which consist of T cells.*(4)* Furthermore, there is a direct correlation between immunosuppresion and increased incidence of certain malignancies, e.g., Epstein-Barr virus (EBV)-associated lymphomas, Kaposi's sarcoma, and cervical cancer.*(5)* However, the very existence of cancer and its inevitable progression without treatment demonstrate the inefficiency of natural defense against tumors, and the ability of neoplastic cells to evade immune-surveillance. Thus, the major objective of cancer immunotherapeutic approaches is to augment the effectiveness and efficiency of the immune response against malignant

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tissues.

Cancer vaccines provide a unique therapeutic modality in that they initiate a dynamic process of activating the host's own immune system. This process has the potential to change the patient's initial responses, and responses to subsequent therapies after vaccination, are evaluated. Recent preclinical and clinical findings have shown that well-designed clinical trials with appropriate endpoints, as well as administration of vaccines in new paradigms for combination therapies, may ultimately lead to the use of cancer vaccines for treatment of many types of cancer. Mucin 1 (MUC1) is one of the first tumor antigens shown to be a target for human tumor-specific T cells and thus a valid target for immunotherapy. MUC1 is a member of the mucin family of molecules.

Mucins encompass a family of high molecular weight, heavily O-glycosylated proteins that are normally expressed by epithelial, endothelial, and other cell types to contribute to lubrication of surfaces and to serve as a barrier to physical and biological assaults.*(6)* Mucins are often aberrantly expressed by human tumors and are thought to contribute to tumor progression by altering the surface properties of tumor cells. Several membrane mucins, members of the family that possess transmembrane and cytosolic domains, have additionally been shown to engage intracellular signaling pathways to elicit a variety of cellular responses.*(7)* Until now, more than 15 different mucins have been identified and are being partially characterized with respect to their repeat domains and chromosomal localizations. Mucins have been grouped on the basis of their availability in the transmembrane region or their presence as soluble (gel forming) proteins. The transmembrane glycoprotein group includes MUC1, MUC3, MUC4, MUC10-18, whereas MUC2, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC9, and MUC19 form the group of soluble (gel forming) glycoproteins.*(8)* The most well-characterized signaling membrane mucin is MUC1, the cytosolic domain of which has been shown to interact with a wide range of signaling proteins that promote cellular growth properties.*(9)*

MUC1 was first isolated from the human breast milk and has been localized to the apical surface of normal epithelial cells of the breast, salivary glands, and lung, as well as in circulating cells, e.g., activated T cells and activated dendritic cells.*(10-12)* An underglycosylated form of MUC1 unique to tumor cells is over-expressed in all invasive breast carcinomas,*(13-15)* making MUC1 a prime candidate for several promising therapeutic vaccine strategies*(16-19)* and a potential marker for prognosis.*(13-15)* A lot of articles relation between MUC1 and the breast cancer have been published. Here we review the structure and function of MUC1 and how its altered state in malignancy can be exploited for breast cancer immunotherapy.

STRUCTURE OF MUC1

MUC1, in common with all mucins, consists of a protein backbone containing highly glycosylated and unglycosylated regions.*(20)* The MUC1 protein core consists of the variable number of tandem repeats (VNTR) of 20 amino acids sequences.*(21)* Carbohydrate side-chains in mucins are attached by an α 1,3 linkage between N-acetylgalactosamine and the oxygen atom of serine or threonine. This gives five potential sites for glycosylation in each repeat, although the average number of O-glycans added to each tandem repeat is around 2.5.*(22)*

MUC1 is up-regulated and secreted in larger quantities from a variety of malignant tumors. An abnormal number of shorter carbohydrate side-chains in cancer-associated MUC1 distinguish it from MUC1 in normal tissues. Novel regions of the protein core in MUC1 are consequently exposed, and cancer-associated MUC1 is thereby made antigenically distinct from MUC1 found in normal epithelia.

Exposure of the peptide core in malignancy is a result of abnormal glycosylation during biosynthesis, and follows altered expression of glycosyl- and sialyltransferase. These enzymes are responsible for termination of side-chain extensions, and therefore elevated levels may lead to truncated side-chains and greater exposure of the protein core. Elevated serum levels of certain sialyltransferase enzymes have been reported in primary breast carcinoma.*(23)*

PHYSIOLOGY OF MUC1

MUC1 mucin is a transmembrane glycoprotein found on the luminal surface of the normal glandular epithelium. It is a long, rigid, and negatively-charged structure extruding from the cell membrane. The combination of physical characteristics and charge repulsion between negatively charged sialic acid residues is important in fetal development and subsequent maintenance of the lumen of tubular structures.*(24)* The ability of MUC1 to alter cell morphology occurs not only during development of normal tissues, but may also be important in carcinogenesis.*(25)*

Like other mucins, MUC1 aids the lubrication of epithelial surfaces, protects against dehydration, and constitutes a barrier to infection.*(10)* Through the sugar residues it is able to bind bacteria (e.g., *Staphylococcus* and *Pseudomonas)* and viruses.

Research has recently focused on the role of MUC1 in transduction of extracellular signals to the nucleus.*(26, 27)* It is known to be closely associated with the erbB family of receptors, including epidermal growth factor receptor, at the cell membrane.*(28,29)* Activation of such a receptor results in phosphorylation of tyrosine residues in the cytoplasmic tail of both the erbB receptor and MUC1 molecule. This phosphorylation leads to recruitment of effector proteins, including ERK1/2, which promote the transcription of factors responsible for cell growth, differentiation, and apoptosis.*(30)* Tyrosine phosphorylation also leads to binding of β-catenin to the cytoplasmic tail of MUC1. β-catenin plays a dual role in both mediating cell-cell adhesion via E-cadherin and mitosis through cyclin D1 expression.*(31-33)* Increased MUC1 expression results in increased binding of the β-catenin, and, hence, both diminish cell-cell adhesion while promoting cell division.

PATHOPHYSIOLOGICAL ROLE

MUC1 appears to play a role in cell adhesion. The extracellular domain of the molecule consists largely of the highly glycosylated protein backbone which towers 200-500 nm above the cell membrane*(34)* and other cell surface molecules. Transfection of cell lines with DNA encoding MUC1 causes over-expression of MUC1 on cell surfaces. This is analogous to the situation in malignancy, where high levels of MUC1 on surfaces have been shown to suppress cellular aggregation.*(35)* This may partly be a result of the negative charge of its numerous sialic acid residues, but more so to its large, rigid structure, causing steric hindrance.*(36)*

MUC1 may also influence tumor progression through suppression of E-cadherin and other adhesion molecules.*(37)* On the contrary, there is evidence that mucins may be important in invasion of basement membranes by modulating the cell-matrix attachment. Cancer cells that produce higher levels of MUC1 adhere more readily to basement membrane proteins such as laminin, type IV collagen, and fibronectin. Tumor-associated membrane-bound MUC1 is a ligand for the intercellular adhesion molecule, ICAM-1.*(38)* This interaction between MUC1 and ICAM-1 is inhibited by the addition of antibodies that bind to the protein core backbone of MUC1, suggesting that the ICAM-1 binding site on MUC1 resides within the protein core, which is exposed by under-glycosylation associated with malignancy. The interaction between MUC1 and ICAM-1 may be important in the attachment of circulating tumor cells to vascular endothelium during the process of metastasis. In malignancy, MUC1 can be shed from tumor cells and is thus elevated in the serum. This soluble MUC1 can inhibit the interaction between membrane bound MUC1 and ICAM-1.*(39)*

MUC1 is also thought to have an immunosuppressive effect. There is evidence that over-expression of MUC1 may inhibit cell lysis by cytotoxic T lymphocytes (CTL) and enable tumor cells to escape from immune surveillance.*(40)* There is also evidence that cancer-associated MUC1 and synthetic tandem repeats of the MUC1 polypeptide core can specifically inhibit human T-cell proliferative responses to polyclonal stimuli, resulting in T-cell anergy*(41)* and secondarily, recruitment of inflammatory cells to the tumor site. It is possible that MUC1 can induce this state by occupying ICAM-1 receptors on CTL.

While much of the above work has been conducted in

vitro, there are several cancers in which altered MUC1 expression may have a role in tumor progression. High levels of MUC1 expression have been shown to be associated with a poor prognosis in breast cancer.*(42)* In animal studies where mammary tumors are induced, wild-type mice have been shown to develop larger tumors that metastasize more readily than tumors induced in MUC1-null mice.*(43)* In colorectal malignancy, Patients with MUC1-positive at the deepest invasive margin showed a poorer prognosis than those with MUC1-negative.*(44, 45)* Other malignancies where MUC1 is thought to relate to prognosis include ovary*(46)* and lung.*(47)*

MUC1 IN BREAST CANCER

MUC1 is a serum marker useful for detecting recurrence or prognosis in breast cancer patients as serum levels increase.*(48,49)* Studies show that MUC1 serum levels increased in breast cancer patients with distant metastasis, while it was not significantly elevated in breast cancer patients without metastasis.*(50,51)* This phenomenon may be due to circulating MUC1 expressing tumor cells that break off from the primary site and travel to distant sites. In addition, MUC1 can provide an indication of recurrence even before diagnosis by conventional, clinical, or radiological diagnosis in 41-54% of treated patients,*(52,53)* thereby enabling earlier diagnosis and treatment decisions, and resulting in a cost savings of at least 50% when compared to the cost of diagnosis using expensive imaging techniques.*(54)* Hence, MUC1 is useful as a marker to monitor patients for early detection of recurrence and metastasis following treatment of primary breast cancer.

CA15-3 (MUC1 mucin glycoprotein) is used only for monitoring patients in advanced stages of disease, as it lacks sensitivity for early stage disease. Concentrations of CA15-3 are elevated in ~10% of patients in stage I, 20% in stage II, 40% in stage III, and 75% in stage IV. A 5-10 fold increase in CA15-3 indicated the presence of metastasis disease. However, increased CA15-3 can be found in a small percentage of healthy people and in patients with benign disease, such as chronic hepatitis, liver cirrhosis, and sarcoidosis.*(55)* Hence, CA15-3 may not be suitable for early diagnosis and early prognosis of breast cancer.

Since MUC1 is over-expressed in breast cancer and absent or weakly expressed in the apical surface of healthy mammary glands, anti-MUC1 antibodies are valuable immunohisochemical markers in the diagnosis of breast cancer. There are a number of anti-MUC1 antibodies against different regions of MUC1, such as VNTR and cytoplasmic tail. Studies demonstrate that the anti-MUC1 cytoplasmic tail monoclonal antibodies (anti-MUC1 CT) are better than antibodies against the MUC1 VNTR (anti-MUC1 VNTR) for diagnosis of metastatic adenocarcinomas. CT33 and CT2 (anti-MUC1 CT) have higher percentages of positive staining in malignant carcinoma, 90% and 93% respectively, compared with C595 (anti-MUC1 VNTR), 73.5%.*(56)* This observable fact may be due to MUC1 VNTR cleaved from the cell, and released into the serum. In addition, anti-MUC1 VNTR may bind to multiple VNTR sites within the same MUC1 molecule, and thereby amplify staining; whereas each anti-MUC1 CT can only bind to one MUC1 molecule, and therefore give a quantitative staining pattern. Moreover, MUC1 expression may have a prognostic value in predicting patient outcome, as shorter survival time is related to aberrantly located MUC1 in the tumor cell cytoplasm and the non-apical membrane.*(42)*

Additionally, MUC1 antibodies may be able to differentiate different types of breast cancer using immunohisochemistry. Li et al.*(57)* has shown that MUC1 antibodies can differentiate between invasive micropapillary carcinoma (IMPC) and invasive ductal carcinoma (IDC) of the breast. IMPC (a subtype of IDC) is associated with lymphatic invasion and lymph node metastasis and a poor prognosis. IMPC showed a reversed apical membrane pattern of MUC1 expression in neoplastic cell clusters; whereas MUC1 expression in pseudo-IMPC was present in the whole cytoplasmic membrane and/or cytoplasm.

IMMUNOTHERAPEUTIC APPLICATIONS WITH MUC1

In patients with cancer hypoglycosylation of the MUC1

protein core exposes an immunodominant repetitive amino acid sequence that is masked in healthy individuals.*(14)* Cellular immune responses to MUC1 have been documented in malignant disease. MUC1-specific CTLs have been detected in the tumor-draining lymph nodes of patients with breast cancer. These T-cells specifically recognize both breast and pancreatic tumor cells, and recognition is MHC-unrestricted due to cross-linkage to under-glycosylated MUC1 tandem repeats.*(58,59)* In addition, humoral responses to MUC1 in malignant disease have been documented, and the presence of a natural humoral immune response to MUC1 has been correlated with outcome in patients with breast cancer.*(60,61)*

The abnormal intracellular distribution of MUC1 seen in cancer cells, as opposed to the apical distribution seen in normal epithelial cells, also makes MUC1 a potential target molecule for therapy. Such observations have generated considerable interest in evaluating antibodies to MUC1, and immunogens based on MUC1, as immunotherapy for patients with cancer, as the induction of an anti-MUC1 response has potential benefits in treating tumors expressing this antigen.*(62)*

MUC1-BASED CANCER VACCINES

Because MUC1 is highly over-expressed and aberrantly O-glycosylated in most adenocarcinomas, including breast, ovarian, and pancreatic cancers, it has long been considered a prime target for immunotherapeutic and immunodiagnostic measures. Using various recombinant N-acetylgalactosamine-transferases and other glycosyltransferases, MUC1 glycopeptides with a well-defined cancer-associated glycosylation pattern can be chemoenzymatically synthesized and applied by conjugation to carrier molecules in cancer vaccines.

PEPTIDE-BASED VACCINES

Vaccines based on synthetic peptides have the advantage of being readily available, although they require identification of the exact epitope recognized by T or B cells. Most peptide vaccines have been tested for their ability to elicit strong CTL responses; however, optimal vaccine formulations should also include one or more antigen-specific T helper epitope. Helper responses to MUC1 have not been detected to date in cancer patients. Therefore, identification of MUC1-derived helper epitopes and testing such epitopes in vivo are of crucial importance and need to be further addressed.

A vaccine consisting of a single tandem-repeat peptide of MUC1 (20 amino-acids) conjugated to diphtheria toxoid has been assessed for safety in a phase I trial.*(63)* No MUC1-related toxicity was noted in this study, although the preparation did not appear to be highly immunogenic. In particular, very weak T-cell proliferative responses were detected in treated patients.*(64)* In other studies, mice immunized with conjugates of a short MUC1 peptide and mannan, a carbohydrate polymer, responded with a CTL response that resulted in tumor cell lysis in vitro.*(65)*

Low levels of MUC1-specific antibodies were seen in some patients, but it is unknown whether these antibodies reacted with cancer-associated MUC1. In clinical trials performed by the group of Philip Livingston, it was found that unglycosylated KLH-conjugated peptide induced high IgM and IgG titers against the O-glycosylation region in synthetic MUC1, but only weak IgG reactivity with MUC1 positive breast cancer cells.*(66)*

FUSION PROTEIN-BASED VACCINES

In a recent pilot phase III study of early-stage breast cancer patients using oxidized mannan-MUC1,*(67)* recurrences were observed in 4 of 15 patients receiving placebo, whereas none of the 16 patients immunized with MUC1 vaccine had recurrences. No response to MUC1 was seen in patients treated with placebo, whereas 9 of 13 patients immunized with MUC1 developed MUC1-specific antibodies, and 4 of 10 patients had MUC1-specific T cell responses.

Clinical results obtained with this strategy are very promising, although the antibodies generated react with unglycosylated MUC1, and not MUC1 as it is presented by cancer cells. Directing humoral and cellular responses to cancer-associated glycoforms might further improve

the effect of the vaccine.

DNA-BASED VACCINES

The concept of DNA-based vaccine is the cellular uptake of cDNA, which results in antigen expression followed by processing and presentation by MHC Class I and/or Class II molecules. Accordingly, the glycosylation status of MUC1 is determined by antigen-presenting cells and dependent on the chosen cDNA vehicle. MUC1 cDNA vaccines have been tested with either plasmid or virus as the vehicle. After immunization with MUC1 cDNA plasmid, Graham et al.*(68)* observed tumor protection in 80% of C57 wild type mice challenged with syngeneic tumor cells.

As an alternative to plasmids for encoding MUC1, viral vectors have proven effective. In a small clinical study, patients with advanced breast cancer were vaccinated with recombinant vaccinia virus expressing MUC1 and IL-2 genes. Cellular responses were detected in a few patients, but no increase in MUC1-specific antibodies was detected.*(69)* The company Transgene (Strasbourg, France) has in phase I and II trials tested vaccines where MUC1 is encoded by vaccinia virus or modified vaccinia Ankara co-expressed with IL-2.*(70)*

CELL-BASED VACCINES

Dendritic cell are also currently being investigated in clinical trials for their role in stimulating the immune system. The dendritic cell-based approach in which dendritic cells are fused with tumor cells, pulsed with purified tumor-associated antigen, or genetically modified to express tumor antigens have also showed significant progress.*(71)*

In a clinical pilot study, vaccination with MUC1 peptide-pulsed dendritic cells has been shown to induce MUC1-specific CTL responses in patients with advanced breast and ovarian cancer.*(72)* Subsequently, in a phase I trial executed by the same group, MUC1 peptide-specific T cell responses and stable disease were induced in some renal cell carcinoma (RCC) patients vaccinated with MUC1 peptide-pulsed dendritic cells. Following immunization with five dendritic cell injections, a subset of patients showed good tolerance and tumor regression. MUC1 peptide-specific T-cell responses in vivo were detected in peripheral blood mononuclear cells. This study showed that MUC1 peptide-pulsed dendritic cells can induce clinical and immunologic responses in patients with RCC.*(73)*

Although cell-based strategies may hold promise for cancer immunotherapy, a major downside, except for limited control with O-glycosylation, is the requirement of individual vaccines for each cancer patient.

GLYCOPEPTIDE-BASED VACCINES

Since MUC1 is highly over-expressed and presents tumor-associated carbohydrate antigens in high amounts, many attempts to target the carbohydrate antigens have been carried out in the past. Survival in patients with advanced breast carcinoma vaccinated with T and Tn antigens derived from group O red blood cell membranes following conventional therapy appeared to be significantly improved.*(74)* The aim of the vaccine called Theratope® (Biomira Inc., Edmonton, Canada) STn-KLH is to target the STn glycans that are over-expressed in mucins. Following a short period of animal studies, clinical studies enrolling hundreds of cancer patients were carried out.*(75-77)* Although a correlation between high levels of anti-STn antibodies and survival was observed in a subgroup of patients, all clinical results are inconclusive.

CONCLUSION

The mucin family has considerable potential importance in the biology, diagnosis, and treatment of malignancy. In particular, MUC1 can act as a target and as a tumor marker in breast cancer management. This article has discussed progress in development of a MUC1 based vaccine for breast cancer. Development of new molecular biology techniques will enable researchers to more accurately determine disease progression and response to therapy. By genetically modulating the degree of MUC1 expression in early cancer, it may be possible to influence

cell adhesion and polarity, thereby reducing the risk of metastasis. Our understanding of cancer antigen-directed immune response at the cellular and molecular level continues to grow, which should lead to further development of cancer immunotherapy.

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