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REVIEW ARTICLE

MUC1 ONCOGENE: CLINICAL PROSPECTS IN HUMAN BREAST CANCER

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ABSTRACT

Mucin 1 (MUC1) is large glycoprotein, expressed in most of the adenocarcinomas and involved in tumor progression, metastasis and transformation. MUC1 has large, glycosylated, extracellular domain with different number of tandem repeates (20 amino acid), transmembrane region and a cytoplasmic tail. Over expression of MUC1 gene products are reported in different carcinoma condition includes breast cancer, gastrointestinal cancer, overian cancer, pancriatic cancer, lung cancer and Lymphomas. In this study we have highlighted the involvement of MUC1 gene at different level, mechanism of action and therapeutic approach in breast cancer.

INTRODUCTION

Mucins were first described as large glycoproteins with highly glycosylation, found predominantly in the mucus secreted into the respiratory, gastrointestinal and genitourinary tracts as serve as first line of defence from external environment (1). In addition to these secretory mucins, membrane-associated mucins also have been described (2). Which are characterized as carcinoma-associated mucin, originally designated as polymorphic epithelial mucin (PEM) (3), episialin (4), epithelial membrane antigen (EMA) (5). In humans, 7 members in the family of the secreted mucins have been identified which are MUC2, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC19 (6-8). Other membrane-associated mucins are transmembrane molecules expressed by most of the glandular and ductal epithelial cells includes MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17, MUC20 and MUC21 (9-12).

Numerous alterations of mucin-associated carbohydrates have been reported in neoplastic epithelial tissues and on circulating mucins in patients with adenocarcinomas (13-15). Numerous mucin antigens are infrequently detectable in normal serum, but have been detected in the serum of patients with pancreatic, ovarian, breast, and colon carcinoma (14). Currently different diagnostic tumor markers include CA15-3 (breast cancer), CA19-9 (Gastrointestinal Cancer Antigen), CA125 (Overian cancer), SPan-1 and DuPan-2 (Pancriatic cancer), are used by clinician (16).

MUC1 gene structure

The MUC1 gene belongs to a family of genes encoding mucin glycoproteins composed of signal sequences, transmembrane domains, array of tendom repeat and cytoplasmic tail (figure 1) (1). Molecular studies have revealed several MUC1 protein isoforms includes MUC1/TM, MUC1/SEC, MUC1/Y, MUC1/X and MUC1/Z (17-19). The MUC1 isoforms demonstrate different properties and functions hence play a crucial role in many physiological and pathological processes. It involves in signal transduction, blastocyst implantation, epithelial cell morphogenesis, cellular adhesion, immune suppression of T-cell activity and cancer matastases progression (20). Expression of the MUC1/TM, MUC1/X, MUC1/Y and MUC1/Z is associated with the presence of carcinogenesis, whereas expression of MUC1/SEC is reported in non-malignant tissue (21). The cytosolic VNTR region is made up of 20 amino acid tandem repeats (TR) and the number of TR is varies, depend upon the presence of allele, and polymorphic in nature (22).

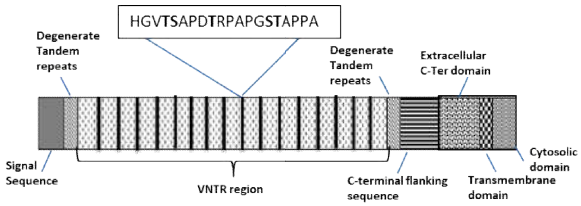

Figure 1 MUC1 core protein

MUC1 glycosylation

Mucin1 (MUC1) is glycoprotein having cytosolic variable number of tandem repeats (VNTR) region and each tandem repeats (TR) is composed of 20 amino acids. The TR fragment has several potential O-glycosylation sites and is lined at both end by degenerate tandem repeats that also have such sites (23, 24). Comparative study of human MUC1 with other species have shown that transmembrane and cytoplasmic domains have high conservation in sequence to support O-glycosylation (25). It was reported that the addition of N-acetyl galactosamine to threonine (T) and serine (S) promotes the process of O-glycosylation and a family of enzymes (polypeptide N-acetylgalactosaminyltransferases) are involved in O-glycosylation process (26, 27). Three different N-acetylgalactosaminyltransferases (GalNAcT) i.e.

GalNAcT1, GalNAcT2 and GalNAcT 3 are involved in O-glycosylation process and distributed throughout the Golgi bodies, indicating that chain initiation for O-glycosylation is not limited to the cis compartment. While passing through Golgi body, O-glycosylation of MUC1 is start by addition of sugars to specific sites. In O-glycosylation process, a polylactosamine side chain is generally added and it ended with sialic acid, galactose or fucose (28).

Glycosylation of MUC1 in breast cancer

It is reported that each tandem repeat of MUC1 having five potential sites (3xT, 2x S) for O-glycosylation means O-glycans added to each sites but in malignancies, each tandem repeat reported with 2.5 O-glycans. A studies on breast cancer cell line T47D, reported that all five sites are occupied by O-glycans. So the difference in O-glycosylation in breast cancer tissue may be due to difference degree of expression of GalNAcTs as compared to normal tissue hence less and aberrant glycosylation (29).

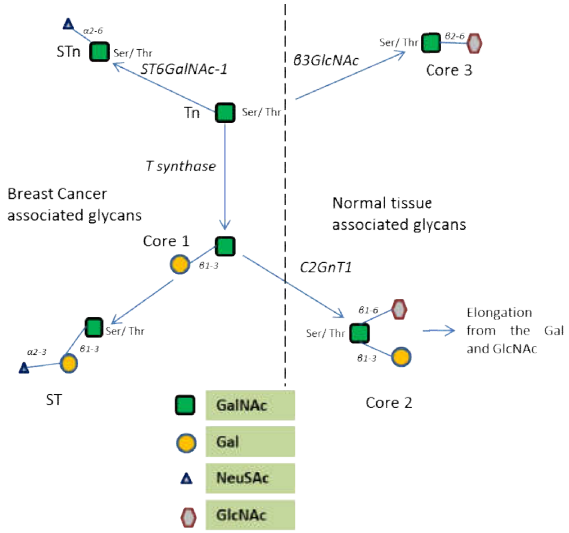

Figure 2 O-glycosylation pathways of MUC1 in breast tissue

In normal condition, the addition of GalNAc to threonine (T) or serine (S) or threonines is followed by formation of core 1 MUC1 (figure 2) by addition of galactose and this structure is acts as a substrate for C2GnT enzyme to form core 2 structure. The O-glycosylation is terminated by addition of sialic acid or fucose. So in normal condition, mammary tissues have core 2 structure (30, 31).

In breast cancer cell lines T47D and BT20, the core 1 like O-glycans was found which convert to sialyl form for T antigen (T blood group antigen) i.e. Sialyl T antigen (ST) by sialyl transferase. GalNAc (TN) antigen is also reported in breast cancer tissue in small proportion and it converts to sialyl TN antigen by sialyl transferase (32).

Detectin of cancer-antigen 15-3 immune assay (CA15.3 assay) are one of the most common tool for monitoring breast cancer disease progression and response to therapy in late stage of breast cancer (33-35). It was found that the concentration of CA 15-3 levels in serum of patients before surgery is significantly higher compared with those of CA 15-3 after surgery, demonstrated that in most of the cases CA 15-3 is abnormal with metastatic breast cancer and concentration of antigen is correlated with the clinical status of breast cancer. CA 15-3, a secreted product of MUC1 gene is a mucinous carbohydrate product of MUC1 gene originally identified by two monoclonal antibodies i.e. DF3 and 115D8 (36-39).

Mechanism of overexpression of MUC1

MUC1 in over expressed with clinical progression of from normal conditions to to metastatic stage and there are several regulation and mechanism associated with invasive and metastaic state (40). It is reported that at transcription level, MUC1 is regulated by various factors, including STATs, hypoxia, growth factors and several hormones. Several studies demonstrated that MUC1 is a transcriptional target of factor 1 α (HIF1-α) induced by hypoxia in renal cell carcinoma, and HIF1-α is reported upregulated in several metastatic cancer (41). Regulation of MUC1 expression by hypoxia is not tissue-specific which was confirmed by enhancement of MUC1 transcription by hypoxia condition is reported in cell line of human lung adenocarcinoma (42). In response to interleukin-6 and interferon gamma, transcription of MUC1 is upregulated by STATs (43).

MUC1 expression is closely associated with hypoxia (HIF-1α), glucose metabolism (Glut1), amino acid metabolism (LAT1), angiogenesis (vascular endothelial growth factor and microvessel density) and epidermal growth factor receptor (EGFR) expression (44). In several epithelial carcinomas EGFR with nuclear localization is a poor prognostic indicator. Loss of MUC1 expression cause decrease in the interaction between the CCND1 promoter and EGFR, that reason for less cyclin D1 protein expression hence regulate EGFR nuclear function. EGFR mediate by integrin αvβ5 is reported to induce the invasion of human carcinomas and metastasis which is mediated by integrin αvβ5 by MUC1 proteolytic cleavage (45, 46). Transforming growth factor alpha (TGFalpha) has been reported to be an effective inducer of cellular transformation through binding and activation of EGFR. In breast epithelial cell lines, MUC1 is reported to inhibit the ligand-stimulated degradation of EGFR and act as important modulator of tumor progression through TGF alpha (47).

In pancreatic cancer cells, MUC1 are closely correlated with EGF receptor (EGFR) and anti MUC1 antibody GP1.4 induced the internalization of EGFR and cause ERK phosphorylation inhibition by EGF stimulation (48). Androgen receptor (AR) was found to down regulate MUC1 expression through interaction with a consensus AR-element in the promoter of mucin 1 in specifically prostate cancer cell lines (49). At post-transcriptional level, the expression of mucin 1 is regulated by microRNAs. Two miRs are reported to regulate MUC1 translation, including miR-125b and miR-145. Expression of miR-125b is upregulated by miR-145 and androgen receptor. In breast cancer, miR-125b suppresses the expression of muc1 at translation stage and miR-125b is itself found downregulated (50-52). Hypoxia is typical feature of matastasis tumor which upregulate the mRNA of MUC1 in human lung adenocarcinoma cell line and expression of mRNA is found 10 times in breast carcinoma cells. Upstream of the transcription start (500 base pairs) site of MUC1 promoter have binding site for transcription factors (TF) of the STAT family which cause MUC1 over expression in breast cancer matastasis (42, 43).

MUC1 antibodies in Breast cancer

Different forms of glycosylated MUC1 is associated with mammary glands in normal and breast cancer, includes GalNAc (Tn), sialylated Tn (STn), T (Gal β1,3 GalNAcα) and sialylated T (ST). Although autoantibodies to TN and STn form of MUC1 were found in breast cancer patients but antibody response against MUC1 carrying a truncated core3 glycan (GlcNAc β 1-3GalNAcα) is more frequent. Although sialylated TN glycan (STn) is tumour specific, but expression of STn is oserved in every 3rd patient of breast cancers (26, 53-55). It is reported that ST glycoform of MUC1 is predominantly present in the serum of advanced breast cancer patients, but weaker immune responses were seen to ST MUC1, may be because of tolerance developed by immune system.[56] Autoantibodies (IgG) to MUC1 carrying core3 or STn glycans is also reported in serum of ovarian and lung cancer (54). Autoantibody against MUC1 and p53 in colorectal cancer is also reported (57). Autoantibodies against p53, c-myc, HER2, NY-ESO-1, BRCA1, BRCA2 are also reported in early stage of breast cancer. Single or combination of these antibodies can be a good tool to diagnose the breast cancer at early stage and can be used to moniter the disease progression (58).

MUC1-Based Immunotherapy

The cases and diversity in cancer is increasing day by day and development of cancer thrapy is still a great chalange for clinician and researchers (59). Different tumor associated ntigen (TAA) have been discovered includes gp100, k-ras, B-raf, p53, N-ras, HER-2/Neu, Telomerase and MUC1 (60, 61). MUC1 is overexpressed TAA with aberrant glycosylation in several cancer include breast cancer. In normal conditions, MUC1 glycoprotein expressed at the apical surface of epithelial cells and serve as first line of defence (62).

In different carcinoma, MUC1 is overexpressed with aberrant glycosylation. These differences observed between normal and aberrantly glycosylated MUC1 encouraged the research on ability to recognize humoral MUC1 epitopes by T lymphocytes. T lymphocytes generally recognize the foreign or endogenious antigen, presented by antigen presenting cells (APC) association with MHC molecule (63). It was reported that SM-3 mAb which recognized abberent glycosylated MUC1, inhibets T lymphocytes reactivity hence T cells recognized the MUC1 in cancer (64). Recent studies shown that a fragment of MUC1 (950–958 aa) peptide is presented to MUC1-specific CD8+ T cells by HLA-A 0201. It is also reported that glycosylated peptides, SAP10 [SAPDT (GalNAc) RPAPG] from the VNTR of MUC1 is generated by dendritic cells and this can be recognized by T cells (65-67).
While therapeutic approach for cancer, different strategies have been used to induce specific T-cell responses against MUC1. Researchers have used different approach to use MUC1-encoding nucleic acids or peptide vaccines for therapy. In one approach, researcher used DNA vaccine which code MUC1 and found activation of different populations of lymphocytes. Injections of MUC1 cDNA as vaccine was able to induced tumore regressins in tumor-bearing mice hence CD8+cytotoxic T cells were involved for tumor regression (68-70).

In another study, MUC1 cDNA is coupled with DNA of heat shock protein (HSP70) which stimulated the function of dendritc cells (DC) and hence caused induction of effective cytotoxic T-cell responses and inhibits growth of tumor cells (71,72). In other study, more impressive DNA vaccine of MUC1 was made with coupling with cDNA of ANT2, which caused tumor cell death and they found more effective response (73).

The BLP25 liposome vaccine (L-BLP25) contains 25-amino-acid MUC1 peptide (STAPPAHGVTSAPDTRPAPGSTAPP) within liposome particle. Although immunogenic response was not observed but this vaccine likely to enhance the patient survival for stage IIIB/IV NSCLC (74-78). Clinician and scientists are working on several startgy and which reached upto phase III and many other MUC1 vaccines are under development and validation to exploit the activation of T cell response.

CONCLUSION

MUC1 glycoprotein is generally express on apical surface of epithelial calls and in several cancers; MUC1 is highly expressed with aberrant glycosylation. MUC1 VNTR region have 5 potential sites (serine and threonine residues) for O-glycosylation which shows different glycosylation pattern in cancer including breast cancer. Various factors affecting the metastatic cascade, including cell invasion and motility, intra- and extravasation, dormancy and survival at a secondary site. MUC1 assoiciated metastasis involves different machenism at transcription and translation stage. Different therapeutic approaches have been used either upto some level or in animal model but still need to develop an effective approach which treat breast cancer in human upto some level of matastasis.

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