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Mucin Family of Glycoproteins (MUC-1)

Structure and Function

The mucin family of glycoproteins is classified by the presence of tandem repeat structures rich in serines, threonines, and prolines that are extensively modified by *O*-glycosylation. The human MUC family consists of 20 members, of which are classified into subcategories based on whether they are secreted or membrane bound [1]. Secreted mucins (MUC-2, 3, 5AC, 5B, and 6) form a physical gel barrier that protects epithelial cells lining the respiratory and gastrointestinal tracts and ductal surfaces of specialized organs such as the pancreas, kidney, and liver. Membrane bound mucins (MUC-1, 3, 4, 12, 13, 16, and 17) also contribute to the formation of a protective mucous gel through ectodomains of *O*-glycosylated tandem repeats that extend from the apical surface of the cell. Membrane bound mucins typically contain a sea urchin sperm protein, enterokinase and agrin (SEA) domain that resides between the glycosylated ectodomain and the transmembrane domain (Figure 1). Autoproteolysis of the MUC-1 SEA domain results in the formation of a stable non-covalent dimer, consisting of the N-terminal ectodomain and a C-terminal transmembrane subunit [2-4].

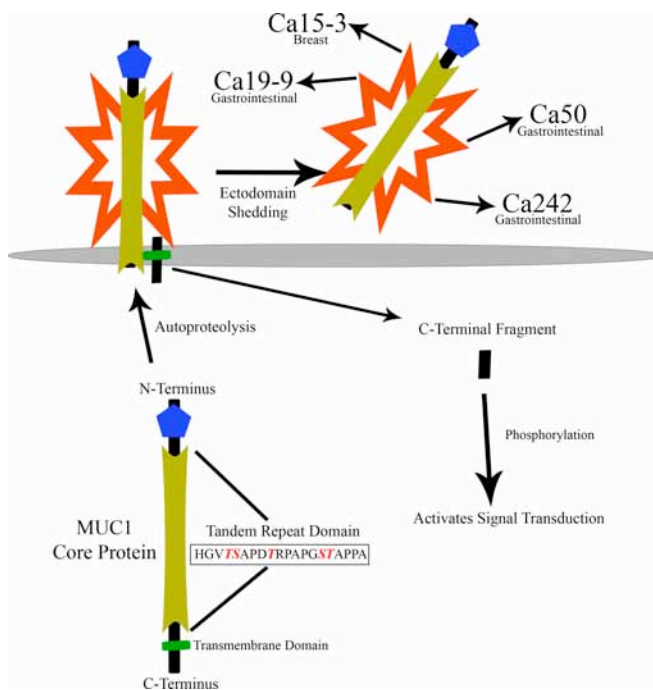


Figure 1. MUC-1 Overview

The mucin family of glycoproteins is characterized by their tandem repeat domain, which undergoes extensive *O*-linked glycosylation following translation (italicized in **RED**). MUC-1 is cleaved by autoproteolysis and forms a stable non-covalent dimer, consisting of a small cytoplasmic tail and a large N-terminal ectodomain. Following insertion into the plasma membrane, the N-terminal ectodomain is shed into circulating blood, while the C-terminal tail returns to the cytoplasm where it is phosphorylated, thus activating signal transduction pathways. At Bioprocessing, Inc., we purify carbohydrate antigens that are presented on the surface of the MUC-1 molecule. These epitopes are considered Tumor Antigens based on their increased frequency detected in cancer patients circulating fluids.

MUC-1 localizes to the apical border of normal epithelial cells [5]. Transformation and a loss of polarity in epithelial cells results in MUC-1 expression over the entire surface of the cell, as seen in Figure 2 [5].

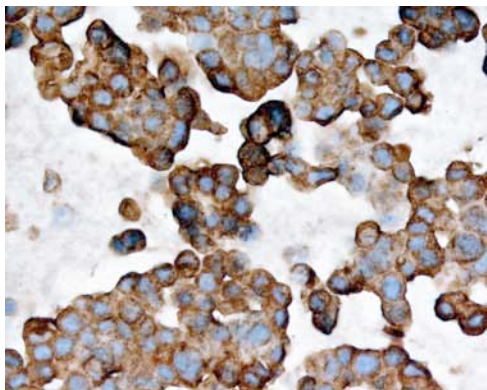


Figure 2. Immunohistochemistry

Immunohistochemistry was performed on Breast Tumor cells. Cells were washed and resuspended in 1X PBS followed by fixation in 4% paraformaldehyde. Fixed cells were permeabilized followed by staining with our MUC-1 specific monoclonal antibody (Br MoAb HP, Clone 6A4, catalogue #2250-8215) that is specific for the MUC-1 antigen Ca15-3.

Furthermore, overexpression of MUC-1 causes transformation [6] and resistance to stress induced apoptosis in normal epithelial cells [7-12]. The MUC-1 N-terminal ectodomain (MUC1-N) contains variable numbers of highly glycosylated 20 amino acid tandem repeats, that have a high density of *O*-linked oligosaccharide residues (Ser/Thr), and is shed into circulating blood. The shed form of MUC-1 has been reported to have a molecular weight of 330-400 kD [13-15].

There have been three variant isoforms of MUC-1 identified, MUC-1X, Y, or Z, that contain variable amounts of carbohydrate content ranging from 50-80% [16, 17]. MUC-1 isolated from normal epithelium contains less carbohydrate by having shorter chains of galactose N-acetyl glucosamine and N-acetyl galactosamine than in tumor epithelium. Carbohydrates are added to MUC-1 by core 2 *O*-glycans of sialyl lewis x structure (NeuAc2-3Gal β 1-4 (Fuc1-3) GlcNAc-R (SLe^x)) through post-translational modifications. In tumor cell lines and serum collected from patients with cancer, the sialylated lewis A moiety can be part of the core structure of MUC-1, which is detected by using a monoclonal antibody for sialylated lewis A (Ca19-9) [18-20]. Additionally, MUC-1 has been shown to be autoproteolytically cleaved from a 110-residue SEA transmembrane domain on tumor cell surfaces, which is also part of the Ca125 molecule expressed in ovarian cancer [4, 21, 22].

Breast Tumor Antigen (Ca15-3)

Purity Ratios

At Bioprocessing, Inc., we isolate breast tumor antigen (Ca15-3) from natural product and cell culture supernatant collected from human breast adenocarcinoma cells, obtaining average ratios of 2.5×10^4 Units/OD unit using optical density (OD) at 280 nm. Ca15-3 immunoblots at ~400 kD using a Ca27.29 monoclonal antibody (Figure 4). Additionally, we have observed that Br antigen forms aggregates that migrate at >400 kD when placed over a size exclusion column.

Currently, a conversion factor for the MUC-1 cancer epitope that will convert units/mL to mg/mL does not exist. This is primarily due to the high degree of heterogeneity that exists among the MUC-1 cancer antigen species. The MUC-1 epitope is recognized by B27.29 and DF3 monoclonal antibodies located within the 20 residue tandem repeat domain (SAPDTRPA) [23]. Within this epitope-binding site, there are 2 sites for *O*-linked glycosylation in addition to a variably number of tandem repeats within MUC-1, contributing to the inability to determine an exact unit to mass ratio. Despite the difficulty in determining

a mass for Br Ag produced at Bioprocessing, Inc., we have optimized the use of this antigen in several assays.

ELISA

The following are optimal coating concentrations for ELISA assays. These were developed internally during several projects involving testing Br Ag against B27.29:

Antigen	Product Code	[U/mL]
Br Ag HP Natural	2250-1000	1,000 – 4,000 U/mL
Br Ag PP LCR Natural	2250-1301	8,000 U/mL
Br Ag HP CC	2250-2000	1,000 – 4,000 U/mL

Immunization

Based on our experience in generating monoclonal antibodies against the Br Ag produced at Bioprocessing, Inc., we have extensive experience using the HP Ag for immunizations. For the production of monoclonal antibodies, we normally immunize and boost mice with the following concentrations:

Antigen	Product Code	[U/ Mouse]
Br Ag HP Natural	2250-1000	30,000-50,000 U/mouse
Br Ag HP CC	2250-2000	30,000- 50,000 U/mouse

PAGE

We employ PAGE analysis to determine Ca15-3 purity for both natural product and cell culture supernatant. We load 250-500 U of Br Ag on a 4-20% PAGE gel run under reducing conditions followed by staining with GelCode Blue (Thermo Fisher Scientific Inc., Rockford, IL) (Figure 3).

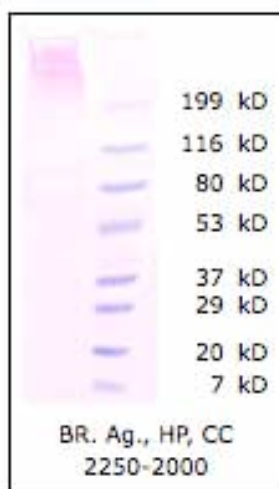


Figure 3. PAGE Analysis of Pure Br Antigen

In order to determine the purity of Ca15-3 antigen that is purified using size exclusion chromatography, we subject 200 U (Br Ag HP CC, catalogue #2250-2000) to PAGE analysis. Samples are run on a 4-20% PAGE gel under reducing conditions and stained with Pierce glycoprotein stain. Results demonstrate the relative purity of Br Antigen purified at Bioprocessing, Inc.. Ca15-3 antigen is a glycoprotein that does not stain with GelCode Blue, a coomassie stain.

Immunoblot

Immunoblotting for purified Ca15-3 (250 U) using Ca27.29 under reducing conditions on a 4-20% gradient PAGE gel (Bio-Rad, Hercules, CA) displays a double band at ~200 - 300 kD (Figure 4). As seen in Figure 4, a faint double band appears as a result of post-translational modifications of MUC-1, which is recognized by Ca27.29.

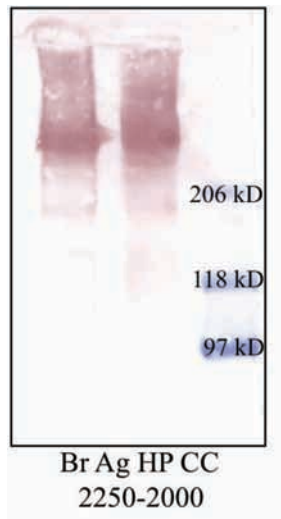


Figure 3. Western Blot of Pure Br Antigen

Purified samples of Br Antigen (250 U of Br Ag HP CC, catalogue #2250-2000) were subjected to western blotting with Ca27.29, a monoclonal antibody that is specific for Ca15-3 epitope on MUC-1. Results demonstrate the purity in addition to the degree of glycosylation, as seen by the linear staining pattern on the blot.

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