

Ov Antigen (Ca125)

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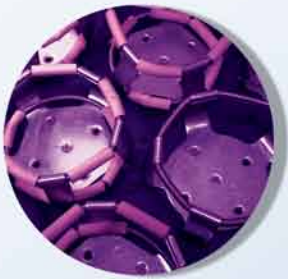


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Mucin Family of Glycoproteins (MUC16)

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 that 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 that line 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, in particular MUC1, typically contain a sea urchin sperm protein, enterokinase and agrin (SEA) domain that resides between the glycosylated ectodomain and the transmembrane domain. Autoproteolysis of the MUC1 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). MUC16 contains multiple SEA domains and a transmembrane region, but lacks epidermal growth factor (EGF) repeats (Figure 1) (5). Despite the rise in MUC16 expression in ovarian cancer patients, little is known about its function.

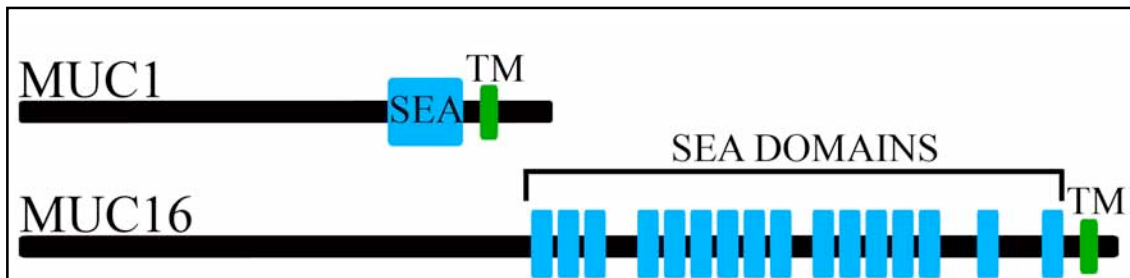


Figure 1. Comparison of MUC1 and MUC16 Protein Structure

Domain structures of two essential secreted mucin family members that are overexpressed in several different cancer types. MUC1 function has been extensively studied and used as a tumor antigen, in particular, epitopes Ca15-3 and Ca19-9. MUC16 was discovered to contain the ovarian cancer marker Ca125. SEA, sea urchin sperm protein-enterokinase-agrin domain, TM, transmembrane domain.

OV Antigen (Ca125)

This large glycoprotein is defined by a carbohydrate epitope OC125 located on the protein core of MUC16 (6). It is expressed by ovarian carcinomas and was first identified as a serum marker in women with ovarian cancer (7). Ca125 mucin circulates in the blood and accumulates in ascites or pleural fluid of patients with OV cancer. Circulating Ca125 has been reported to exist as an aggregate with an average molecular

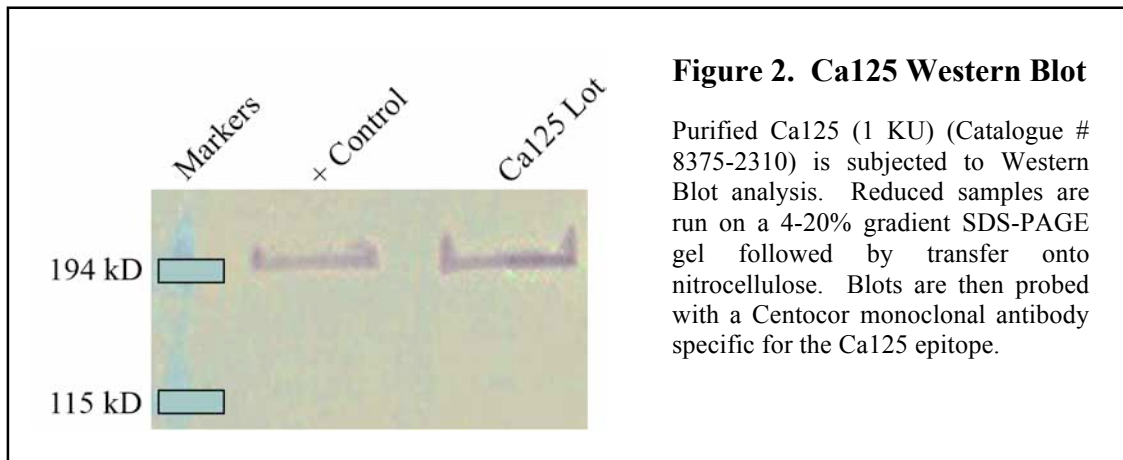
weight of 1400 kD. These aggregates are composed of various numbers of core protein units of 200 kD that can also be attached to other glycoproteins. The SEA domain is reported to be repeated 7,12, or 60 times per splicing variant per Ca125 molecule (8).

Purity Ratios

Purified Ca125 antigen from ascites yields average ratios of 1.0×10^5 units/ml based on OD 280 nm protein measurement, which is approximately $1/35^{\text{th}}$ that obtained for Ca19-9. Because the unit/mass ratio of Ca125 is unknown, we use the known ratio of Ca19-9 to estimate. We estimate there are 17 ngs of Ca125 reactive antibody determinants per 1000 units (U) or 1 kilounit (KU). Based on OD 280 nm, 1 mg of total protein of purified Ca125 by size exclusion chromatography is approximately 100 KU that equals $\sim 1.7 \mu\text{gs}$ of reactive antibody determinants.

Analysis

We employ PAGE analysis to determine Ca125 purity for both natural product and cell culture supernatant. Initially, 1-2 KU ($0.5\text{-}1 \mu\text{g}$) of Ca125 is loaded, followed by staining with GelCode Blue™ (Thermo Fisher Scientific Inc., Rockford, IL). It is difficult to detect Ca125 due to the aggregates that it forms when in a concentrated solution. Western blot analysis using an OC125 monoclonal antibody indicates a banding at slightly above 200 K Daltons when reduced and run on a 4-20% gel (Figure 2).



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