Human Autoantibodies to CA-125

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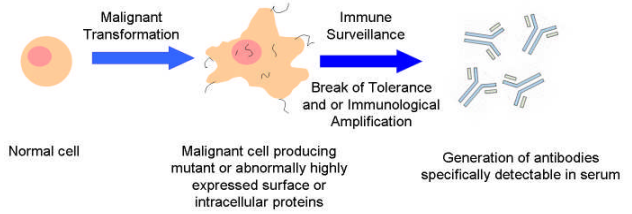
Introduction

CA-125 (MUC16) is a 200-2000kDa non-mucinoid glycoprotein normally expressed by tissues of mullerian duct origin [1]. CA-125 is an indicative tumour marker of advanced epithelial ovarian cancer patients, where serum levels are elevated in ~80% of patients [2]. CA-125 levels are also often seen raised in patients with breast cancer [3].

Tumour markers such as CA-125 are detected and measured using mouse monoclonal antibodies to monitor disease progression. These mouse antibodies however are neither sufficiently sensitive or cancer-specific for use in early diagnosis.

Autoantibodies against tumour-associated proteins such as MUC1 and p53 have been described [4, 5]. These antibodies are produced as part of an early immune response to the cancer, and may provide an *in vivo* amplification of an early carcinogenic signal.

Identification of such tumour-specific antibodies may therefore provide a method for earlier diagnosis of such cancers.



Aims

- **Purify normal and cancer-associated CA-125, and human antiCA-125 antibodies.**
- **Investigate whether there are any immunologically identifiable differences between the normal and tumour-associated forms of the antigen, by comparing antibody responses to these different CA-125 proteins.**
- **Investigate the presence of circulating autoantibodies to CA-125 in the sera of patients with primary breast cancer.**

Materials and Methods

CA-125 Antigen Purification

CA-125 preparations from normal sera (n=1) **an ovarian cancer cell line, OVCAR-433** (n=2), **and breast cancer pleural effusion** (n=2), **were affinity purified using a mouse monoclonal antibody specific for CA-125, VK-8 (kindly provided by KO Lloyd), bound to a CNBr Sepharose matrix [6].**

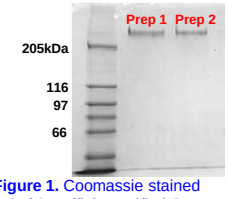


Figure 1. Coomassie stained gel of 2 x affinity purified CA-125 preparations from OVCAR-433.

Human CA-125 Autoantibody Purification

CA-125 autoantibodies were affinity purified from a breast cancer patient pleural effusion with known reactive antibodies to CA-125 (as identified by ELISA), using CA-125 (purified from OVCAR-433 cells) immobilised through oxidised sugar groups, to CarboLink™ coupling gel (Pierce).

Comparison of Human and Mouse anti CA-125 Antibodies

CA-125 antigen preparations were adsorbed on to 96-well plates overnight in sodium carbonate buffer. Plates were blocked with HSB/Tween for 1 hr before probing with biotinylated VK-8 (5ug/ml) or biotinylated human anti CA-125 (5ug/ml) for 1 hr. Bound antibody was measured by addition of Extravidin horseradish peroxidase conjugate (1hr) and TMB substrate (15 mins). Plates were read at OD 650nm.

CA-125 Autoantibody Assay

Affinity purified CA-125 (OVAR-433) was diluted 1:10 in sodium carbonate buffer and adsorbed overnight onto 96-well plates. Plates were blocked as above with HSB/Tween and samples (normal, PBC) were diluted 1:100 and controls (1:1000) in HSB, and incubated for 1.5 hrs at room temp. Plates were then probed with anti-Human Ig HRP for 1hr. Plates were developed with TMB substrate and read at OD 650nm.

Results

Affinity chromatography can be used to successfully isolate CA-125 (Figure 1). This CA-125 can be used to isolate human antigen specific antibodies.

These autoantibodies bind with higher reactivity to tumour derived CA-125 (breast or ovarian) compared to normal CA-125, whereas the mouse antibody VK-8 binds with similar reactivity to both (Figure 2).

Furthermore, the reactivity of these human autoantibodies with the tumour-associated CA-125 is much greater than that observed for the mouse monoclonal antibody VK-8.

Elevated levels of autoantibodies to CA-125 were detected in 25% of a small sample of primary breast cancer sera (Figure 3).

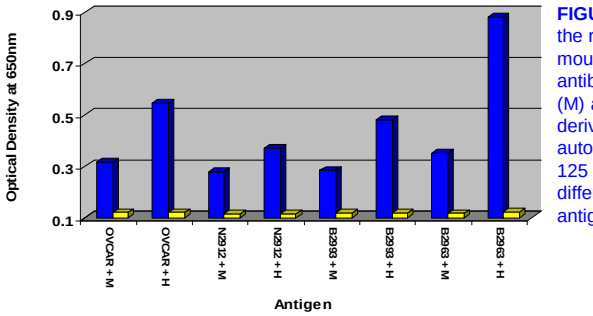


FIGURE 2. Showing the reactivity of a mouse monoclonal antibody to CA-125 (M) and patient derived human autoantibody to CA-125 (H) against different CA-125 antigen preparations.

N2912 – derived from normal serum with no history of ovarian or breast cancer; OVCAR-433 – an ovarian cancer cell line; B2993 and B2963 – purified from 2 breast cancer patient pleural effusions.

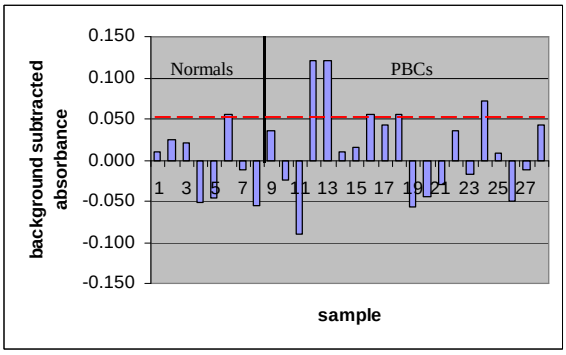


FIGURE 3. Serum samples from patients with primary breast cancer (PBC) sera (n=20) and normal sera (n=8) analysed for the presence of autoantibodies to CA-125.

----- represents cut off (mean of normal sera values +2SD = 95%CI)

Conclusions

1. Human antibodies to CA-125, purified from breast and ovarian sources show a greater specificity for tumour-derived antigen than currently available mouse antibodies.

Such discrimination suggests that there is a biological difference between cancer-associated and normal forms of the protein. Further studies are underway to investigate the biological differences between these molecules, and isolate the B-lymphocytes responsible for the production of these tumour-specific antibodies.

2. Autoantibodies to CA-125 can be detected in the serum of patients with primary breast cancer. Identification of such tumour-specific antibodies may be useful to aid early diagnosis of breast and ovarian cancer, reducing the need for surgery and radical treatment regimes.

References

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