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Identification of Ovarian Cancer-Associated Antigens Through  
the Discovery of Auto Antibody-Specific Epitopes

ABSTRACT:

The use of autoantibodies as a diagnostic tool is promising due to the ability to detect very rare antigens present at earliest stages of ovarian cancer development. In previous studies, several ovarian tumor-associated antigens (TAAs) have been reported that could potentially be exploited for diagnostic and therapeutic use. Fifty proteins have been identified in previous experiments using five different cancer serum composites. The primary goal of the project was to identify a panel of epitopes (a single antigenic site on a protein which is recognized by the immune system on which an antibody reacts) from the previously identified fifty proteins that are highly specific and reactive to autoantibodies in ovarian cancer patients. Identifying such epitopes and the presence or absence of post-translational modifications could potentially accelerate the development of an ovarian cancer-specific diagnostic assay.

Ovarian tumor lysate was first immunoprecipitated, after which the antibody was removed through size exclusion chromatography (SEC). Three different enzymes were utilized to digest the proteins present in ovarian tumor lysate, which allowed for an increase in peptide sequence coverage. Following digestion, the digested samples were pooled and immunoprecipitated a second time in order to isolate the digested peptide fragments. The resulting antibody was removed once again through SEC and the sample fractionated into three aliquots. The three aliquots were then separated by high performance liquid chromatography (HPLC), and the resulting fractions were run on a Q-TRAP (ASI 3000) mass spectrometer.

Data analysis was performed using the Sequest software, comparing the mass spectrometry data against the SwissProt database. Significant hits were then manually verified through spectra analysis and explored for relevance and function. Numerous proteins of significance were found, all of which are known to have vital roles in cancer development. These proteins were also identified previously in the composite two analysis. In future work, more composites will be analyzed in order to the most prevalent antibodies that are expressed in patients with ovarian cancer. Following the identification of such antibodies and their subsequent epitopes, a prototype diagnostic assay will be developed and tested on many patients in order to be validated.