Characterization of three novel serum biomarkers for the early detection and prognosis of ovarian cancer


Abstract

Purpose: We have identified, through hPNA expression profiling, three novel ovarian cancer biomarkers, DD-C101, DD-P108, and DD-O110. We measured the levels of these three biomarkers in over 1200 serum samples from three independent studies, to evaluate their sensitivity and specificity for ovarian cancer detection and their efficacy in disease prognosis.

Methods: Dual recombinant antibody sandwich ELISAs for DD-C101, DD-P108, and DD-O110 have been developed. The assays were used to test serum samples from surgically healthy women and from patients with benign gynecological disease in an early ovarian cancer in an independent study. The sensitivity and specificity of each of the novel biomarkers and of CA125, and combinations of these biomarkers, were analyzed by Receiver Operating Characteristic (ROC) curves in all studies and in multivariate models. In the first study, 79 ovarian cancer patients were analyzed for the three biomarkers, and their levels were compared against CA125 values and the outcome of disease.

Results: Levels of DD-C101, DD-P108, and DD-O110 were elevated in ovarian cancer patients, compared to healthy controls in women with benign gynecological disease. In the first study, DD-C101, DD-P108, and DD-O110 had Areas Under the Curve (AUCs) of 0.73 and 0.76, respectively, in the ROC analysis. DD-C101, which had the highest AUC, showed a sensitivity of 44%, while holding specificity at 95%. In the second study, DD-C101, DD-P108, and DD-O110 yielded complete ROCs to the first study. In stages I and II ovarian cancer (20 patients), DD-C101, DD-P108, and DD-O110 showed AUCs of 0.73, 0.85, and 0.80, respectively, indicating that the biomarkers may have utility for early detection of ovarian cancer. The multivariate analysis of our biomarkers and CA125 in all stages, and in stages I + II patients, resulted in AUCs which were significantly higher than the AUCs of each biomarker alone. In the third study, correlation of the marker levels with 2 yr survival data shows that the elevation of DD-P108 and DD-O110 levels correlates with patient survival, which can be useful in prognosis and in assigning appropriate therapy for the patients.

Conclusions: These findings suggest that DD-C101, DD-P108, and DD-O110 are promising serum biomarkers for ovarian cancer that could improve the sensitivity of traditional cancer markers and may be applicable for prognosis of ovarian cancer. Additional studies are needed to confirm and expand the current findings.

Methods & Materials

ELISA

DD-C101: monoclonal antibody was used to develop mouse monoclonal antibody (mAb). A recombinant protein (aa 99-353) was developed and used to coat the 96-well plate for ELISA. A 1:100 dilution of the recombinant protein was used to coat the 96-well plates at a concentration of 1,000 ng/mL. After incubation for 2 hr at room temperature, the plates were washed and incubated with a 1:100 dilution of the dilution buffer followed by a 1:1000 dilution of a secondary antibody. After incubation for 2 hr at room temperature, the plates were washed and the antigen was detected with a mouse anti-human IgG HRP conjugate.

DD-P108: rat monoclonal antibody was used to develop monoclonal antibody (mAb). A recombinant protein (aa 99-353) was developed and used to coat the 96-well plate for ELISA. A 1:100 dilution of the recombinant protein was used to coat the 96-well plates at a concentration of 1,000 ng/mL. After incubation for 2 hr at room temperature, the plates were washed and incubated with a 1:100 dilution of the dilution buffer followed by a 1:1000 dilution of a secondary antibody. After incubation for 2 hr at room temperature, the plates were washed and the antigen was detected with a mouse anti-human IgG HRP conjugate.

DD-O110: rat monoclonal antibody was used to develop monoclonal antibody (mAb). A recombinant protein (aa 99-353) was developed and used to coat the 96-well plate for ELISA. A 1:100 dilution of the recombinant protein was used to coat the 96-well plates at a concentration of 1,000 ng/mL. After incubation for 2 hr at room temperature, the plates were washed and incubated with a 1:100 dilution of the dilution buffer followed by a 1:1000 dilution of a secondary antibody. After incubation for 2 hr at room temperature, the plates were washed and the antigen was detected with a mouse anti-human IgG HRP conjugate.

Sample Selection for Clinical Study

Serum samples were obtained from comprehensive diagnostic and oncologic sources. All samples were aliquoted upon arrival and stored at 4°C until use. Serum samples were obtained from patients with a known diagnosis of ovarian cancer using an Institutional Review Board approved study. Serum samples were obtained from healthy women with an average age of 45 years (n = 120). Serum was obtained from ovarian cancer patients with an average age of 60 years (n = 126). Serum was obtained from patients with benign gynecological disease with an average age of 45 years (n = 120)

Serum levels of DD-C101 were measured in all three models. Serum levels of DD-P108 were measured in all three models. Serum levels of DD-O110 were measured in all three models.

Diagnostic Analysis

Novel serum markers DD-C101, DD-O110, and DD-P108 showed elevation in ovarian cancer serum samples compared to normal controls and benign ovarian diseases.

ROC analyses of the markers showed that DD-C101 and DD-O110 had sensitivity and specificity comparable to CA125, and may complement CA125 in early stage ovarian cancer detection.

Correlation of the marker levels with 2-yr survival data shows that the elevation of DD-P108 and DD-O110 levels correlates with patient survival, which can be useful in prognosis and in selecting appropriate therapy for the patients.

Summary and Conclusions

• Novel serum markers DD-C101, DD-O110, and DD-P108 showed elevation in ovarian cancer serum samples compared to normal controls and benign ovarian diseases.

• ROC analyses of the markers showed that DD-C101 and DD-O110 had sensitivity and specificity comparable to CA125, and may complement CA125 in early stage ovarian cancer detection.

• Correlation of the marker levels with 2-yr survival data shows that the elevation of DD-P108 and DD-O110 levels correlates with patient survival, which can be useful in prognosis and in selecting appropriate therapy for the patients.

• Additional studies are being planned to expand and confirm the current findings.

Detection Study 2

Serum Levels of DD-C101 in Ovarian Cancer & Benign Diseases

Serum Levels of DD-P108 in Ovarian Cancer & Benign Diseases

Detection Study 1

ROC Analysis of Ovarian Cancer Markers

Multivariate ROC Analysis of Ovarian Cancer Markers