Multiplex Analysis of Serum Biomarkers in Ovarian Cancer Patients Using Bio-Plex® Suspension Array System

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Abstract

Ovarian cancer is the fifth leading cause of cancer death among North American women. CA-125 tumor antigen has been the standard for monitoring response of ovarian cancer patients to therapy. While CA-125 levels have been studied extensively, their utility in determining early ovarian cancer has been limited by both false positive and false negative results. The analysis of novel biomarkers in combination with the established marker is a promising field to further benefit early detection, screening, and prediction of the disease. In this study, we profiled the levels of 37 potential biomarkers. These are circulating proteins in sera collected from ovarian cancer patients in different stages. The results showed that eight markers, angiotensin-1, IL-5, IL-6, IL-10, resistin, haptoglobin, and CRP were highly elevated in ovarian cancer samples as compared to age-matched healthy controls. In contrast, the levels of leptin, PECAM-1, IL-1α, and IL-1β were found to be reduced in the cancer patients.

Introduction

In the United States alone, approximately 23,000 new cases of ovarian cancer are diagnosed each year, with a mortality count of close to 13,000 per year (www.cancerresearchuk.org). Contributing to the poor prognosis in the lack of symptoms in the early stages of the disease (95% of cases). In high-risk populations, the use of annual CA-125 screening is recommended (Zuniga, 1999). Despite great attention to the use of CA-125 as a reliable biomarker for detecting ovarian cancer, many attempts are failing short of demonstrating its clinical utility as an early diagnostic marker in the high-risk patient population. In many documented cases, the level of CA-125 was found to be elevated above reference levels in only 33% of clearly detectable early stage disease (Zuniga, 2006). Ultimately, combining new biomarkers with CA-125 as a composite of markers would improve the diagnostic value of CA-125 for early detection of the disease.

In this study, we evaluated the levels of 37 serum proteins in sera collected from ovarian cancer patients in stages I-III. These markers were initially chosen to probe their clinical relevance to ovarian cancer. Targets with a high frequency of citations (Table 1) reflect the significant association of these markers to ovarian cancer. These markers covered cytokines, angiogenic factors, acute phase proteins, hormones, and other serum proteins. The levels of these markers were also measured in healthy subjects to establish baselines.

Methods

The 37 serum markers are available commercially in five separate Bio-Plex® suspension array panels. Two Bio-Plex® Pro® human acute phase panels, Bio-Plex® Pro® human angiogenic, and human diabetes panels, and the Bio-Plex® Pro® Human cytokine panel. Analysis was carried out on a Bio-Plex® suspension array system (Figure 1), which permits the simultaneous measurement of multiple serum proteins in a single well in 3 hr, using as little as 1.5 μl of serum or 50 μl of tissue culture supernatant.

Results

The serum proteins were grouped into five individual assay panels (Table 2). The design, validation, and verification of these multiple assays followed a standard workflow that addressed intra- and inter-assay %CV standard and sample recovery, the limit of detection (LOD), as well as assay range.

In the initial scan, the levels of 37 serum proteins were compared between 12 ovarian cancer patients and 12 age-matched healthy women. Age-matching was used to ensure comparable immune responses in both groups. The differences in expression between the two groups are summarized in Table 3.

Figure 2. General assay methodology. The Bio-Plex assay consists of a high throughput microplate and a high throughput array 96-well plate, in addition to the standard assay modules and reagent and diluent kits.

Figure 3. Multiplex assay workflow. The samples are analyzed using the Bio-Plex Suspension Array System. The magnetic bead-based assay and its platform integrate a series of cloning modules and reagent and diluent kits.

In this study, targets from five Bio-Plex assay panels were used to evaluate serum proteins that have potential association with ovarian cancer. The levels of eight biomarkers, IL-6, CRP, haptoglobin, angiotensin-1, IL-5, IL-10, haptoglobin, and CRP were significantly different in patients with ovarian cancer compared to healthy controls to establish baselines.

Table 3. Statistical significance of the detected marker levels evaluated using standard Student's t-test. Values of less than 0.05 were considered statistically significant. A larger sample size plus broader disease stages and grades would be required to further characterize the potential association of these markers to early detection of the disease.

Table 4. Statistical analysis of serum biomarkers in ovarian cancer patients and healthy controls. The fold change in gene expression levels between the two groups is summarized in Table 4.

Table 5. Summary of the statistical significance of the detected marker levels evaluated using standard Student's t-test. Values of less than 0.05 were considered statistically significant. A larger sample size plus broader disease stages and grades would be required to further characterize the potential association of these markers to early detection of the disease.

Table 6. Statistical analysis of serum biomarkers in ovarian cancer patients and healthy controls. The fold change in gene expression levels between the two groups is summarized in Table 6.

Conclusions

In this study, targets from five Bio-Plex assay panels were used to evaluate serum proteins that have potential association with ovarian cancer. The levels of eight biomarkers, IL-6, CRP, haptoglobin, angiotensin-1, IL-5, IL-10, haptoglobin, and CRP were significantly different in patients with ovarian cancer compared to healthy controls to establish baselines. The multiplex immunoassay platform is capable of measuring the levels of multiple targets in a single well with a 96-well microtiter. In less than 3 hr, using as little as 12.5 μl of serum, plasma, and other tissues. This significantly reduces the time and cost on preliminary screening of serum samples for biomarker profiling.

References


