Detection of prostate specific antigen (PSA) in the serum of radical prostatectomy patients at femtogram per milliliter levels using digital ELISA (AccuPSATM) based on single molecular arrays (SiMoA)

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Objective

The aim of this study was to detect prostate specific antigen (PSA) in the serum of patients who had undergone radical prostatectomy. If we could achieve this objective, the most sensitive ELISA for PSA (AccuPSATM) based on single molecule detection was developed and evaluated.

Relevance

We have developed a method for detecting single immunocomplexes formed in the enzyme-linked immunosorbent assay (ELISA) using single molecular arrays (SiMoA). This digital ELISA for PSA (AccuPSATM) method is based on isolating single immunocomplexes labeled with an enzyme in arrays of femtogram wells, sealing the arrays in the presence of the enzyme-substrate, and fluorometrically imaging the array. Fluorescent probe molecules of the enzyme-substrate reaction are confined in the femtoliter volume, giving rise to a local high concentration that can be easily detected using a standard fluorescence microscope. By using high density arrays of femtogram wells, hundreds to thousands of single immunocomplexes can be detected simultaneously. Isolation of single immunocomplexes using SiMoA gives rise to a dramatic increase in sensitivity over bulk ensemble detection methods. The most sensitive digital ELISA for detecting PSA was developed that has a limit of detection (LOD) of 8 fg/mL (200 aM) in serum. This assay was used to measure PSA in the sera of thirty RP patients.

Results

We developed AccuPSATM based on detection of single enzyme labels using SiMoA. Figure 3 shows data from AccuPSATM. The human form of PSA was spotted into 200 base line serum to be representative of clinical test samples. Using AccuPSATM to detect PSA in 200 serum, an LOD was 18 fg/mL determined from this experiment. Figure 4 illustrates the predicted CV profile, which shows an LOD of 18 fg/mL. For comparison, a leading commercial PSA assay (ADVIA Centaur, Siemens) reports an LOD of 3.3 pg/mL (0.1 ng/mL) in human serum, and the most sensitive previously reported assay for PSA has an LOD of 18 fg/mL (3). The single molecule assay reported here is, therefore, more sensitive than the commercial assay by a factor of at least 10,000, and more sensitive than other ultra-sensitive method by a factor of at least 50.

Objective Results

(A) Capturing and labeling

Objective

We have developed a method for detecting single immunocomplexes formed in the enzyme-linked immunosorbent assay (ELISA) using single molecular arrays (SiMoA). This digital ELISA for PSA (AccuPSATM) method is based on isolating single immunocomplexes labeled with an enzyme in arrays of femtogram wells, sealing the arrays in the presence of the enzyme-substrate, and fluorometrically imaging the array. Fluorescent probe molecules of the enzyme-substrate reaction are confined in the femtoliter volume, giving rise to a local high concentration that can be easily detected using a standard fluorescence microscope. By using high density arrays of femtogram wells, hundreds to thousands of single immunocomplexes can be detected simultaneously. Isolation of single immunocomplexes using SiMoA gives rise to a dramatic increase in sensitivity over bulk ensemble detection methods. The most sensitive digital ELISA for detecting PSA was developed that has a limit of detection (LOD) of 8 fg/mL (200 aM) in serum. This assay was used to measure PSA in the sera of thirty RP patients.

References


Conclusions

PSA was successfully detected by AccuPSATM in sera from all thirty RP patients. No previously reported assay for PSA has successfully detected this protein in all RP patient sera; the most sensitive assay reported previously (SiMoA in the range 0.3 to 1 pg/mL) would have failed to detect PSA in 30-40% of the samples tested in this study. The lowest concentration detected by AccuPSATM in the serum of an RP patient was 14 fg/mL (44 aM), and the PSA concentrations ranged from 14 fg/mL to 9.8 pg/mL. The mean concentration of PSA in the sera of these patients was 1.5 ng/mL. These results suggest that digital ELISA using SiMoA has the potential to provide a more favorable prognosis for men with the lowest measurable nadir values, and to detect biochemical recurrence earlier or years earlier than conventional test methods.

Figure 3. Comparison of AccuPSATM and commercial Immunoscan 12S assay (Siemens) for levels of immunoassayable PSA in radical prostatectomy patients. The concentration of PSA was determined using digital ELISA in serum samples from 30 RP patients (age 60-89) whose blood had undergone radical prostatectomy. The concentrations of PSA were determined using digital ELISA in serum samples from 30 RP patients (age 60-89) whose blood had undergone radical prostatectomy. The concentrations of PSA were determined using digital ELISA in serum samples from 30 RP patients (age 60-89) whose blood had undergone radical prostatectomy.

Figure 4. Digital detection of PSA in serum samples of patients who had undergone radical prostatectomy. (A) Capturing and labeling using SiMoA. Figure 3 shows data from AccuPSATM. The human form of PSA was spotted into 200 base line serum to be representative of clinical test samples. Using AccuPSATM to detect PSA in 200 serum, an LOD was 18 fg/mL determined from this experiment. Figure 4 illustrates the predicted CV profile, which shows an LOD of 18 fg/mL. For comparison, a leading commercial PSA assay (ADVIA Centaur, Siemens) reports an LOD of 3.3 pg/mL (0.1 ng/mL) in human serum, and the most sensitive previously reported assay for PSA has an LOD of 18 fg/mL (3). The single molecule assay reported here is, therefore, more sensitive than the commercial assay by a factor of at least 10,000, and more sensitive than other ultra-sensitive method by a factor of at least 50.

Figure 5. Digital detection of PSA in the sera of radical prostatectomy patients using AccuPSATM. The human form of PSA was spotted into 200 base line serum to be representative of clinical test samples. Using AccuPSATM to detect PSA in 200 serum, an LOD was 18 fg/mL determined from this experiment. Figure 4 illustrates the predicted CV profile, which shows an LOD of 18 fg/mL. For comparison, a leading commercial PSA assay (ADVIA Centaur, Siemens) reports an LOD of 3.3 pg/mL (0.1 ng/mL) in human serum, and the most sensitive previously reported assay for PSA has an LOD of 18 fg/mL (3). The single molecule assay reported here is, therefore, more sensitive than the commercial assay by a factor of at least 10,000, and more sensitive than other ultra-sensitive method by a factor of at least 50.

Figure 6. Digital detection of PSA in sera from all thirty RP patients. No previously reported assay for PSA has successfully detected this protein in all RP patient sera; the most sensitive assay reported previously (SiMoA in the range 0.3 to 1 pg/mL) would have failed to detect PSA in 30-40% of the samples tested in this study. The lowest concentration detected by AccuPSATM in the serum of an RP patient was 14 fg/mL (44 aM), and the PSA concentrations ranged from 14 fg/mL to 9.8 pg/mL. The mean concentration of PSA in the sera of these patients was 1.5 ng/mL. These results suggest that digital ELISA using SiMoA has the potential to provide a more favorable prognosis for men with the lowest measurable nadir values, and to detect biochemical recurrence earlier or years earlier than conventional test methods.

Figure 7. Graph illustrates the predicted CV profile, which shows the LOD of 18 fg/mL. For comparison, a leading commercial PSA assay (ADVIA Centaur, Siemens) reports an LOD of 3.3 pg/mL (0.1 ng/mL) in human serum, and the most sensitive previously reported assay for PSA has an LOD of 18 fg/mL (3). The single molecule assay reported here is, therefore, more sensitive than the commercial assay by a factor of at least 10,000, and more sensitive than other ultra-sensitive method by a factor of at least 50.