

55.Validation of Protein Microarray for Diagnosis of Mesothelin in asbestos exposed workers

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Background

Mesothelin is a tumor differentiation antigen that is normally present on the mesothelial cell lining the pleura, peritoneum and pericardium. Mesothelin is over expressed in several cancers; the protein could be exploited as tumor marker. Identification of tumor markers for mesothelioma might prove useful diagnosis as well as for monitoring tumor in response to therapy and for screening at high risk individuals.

Objective

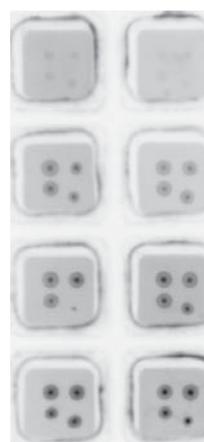
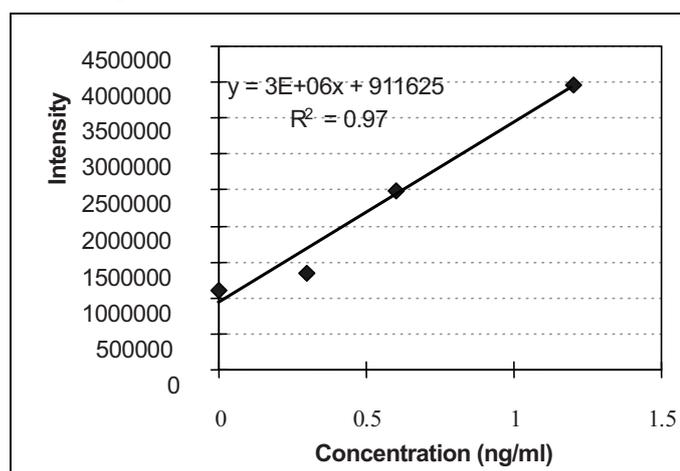
In the present research study we aimed to measure N-ERC mesothelin by protein microarray.

Methods

After the initial development and validation of protein array, the plasma samples were collected from asbestos exposed workers. The samples were printed onto nitrocellulose membrane glass plate using micro caster arrayer system and incubated at 37°C for overnight. After incubation, samples were blocked with protein array blocking buffer and incubate at 25 °C for 2 hours with gentle agitation insuring that mixing occurred. The plates were placed into zip-lock bags to avoid evaporation. Each slide was washed five times with washing buffer in the chamber with gentle agitation, incubated for five minutes, and completely dried with nitrogen gas. HRP conjugated antibody was applied on dried slides and incubated for 2 hours at 25 °C .The slides were washed again five times and dried completely with nitrogen gas. Finally, substrate solution was applied on each slide and signals were detected with CCD camera. The protocols and procedure were kept constant in each case.

Results

The results showed that a high correlation exist between concentration and intensity. The graph shows correlation



between different concentrations of mesothelin plasma samples and the intensity of signals and figure shows the spots of plasma samples.