TK1 PROTEIN LEVELS IN SERUM FROM SUBJECTS WITH SOLID TUMORS DETERMINED WITH THE AROCELL TK 210 ELISA

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Aim of the Study

- Thymidine Kinase 1 (TK1) is an ATP dependent enzyme involved in DNA precursor synthesis and its activity and protein levels increase in S phase cells. Uncontrolled cell proliferation, which is a hallmark of cancer, results in leakage of TK1 into the blood.
- The concentration of TK1 in blood is related to overall cell turnover and it has been shown to be a biomarker for prognosis, treatment monitoring and follow-up of cancer patients.
- Serum TK1 activity determinations have been used for many years as a biomarker, particularly in case of a blood malignancies. (1, 2, 3).
- The development of several TK1 peptide antibodies provides an alternative route for the development of TK1 assays, especially for patients with solid tumours where TK1 activity assays have shown low sensitivity (4,5). However, there is still no routine clinical assay for TK1 protein measurements.
- The aim of this study was to evaluate the performance of new AroCell TK 210 ELISA for measuring TK1 protein levels in sera from patients with different malignant diseases.

Introduction

- Several attempts have been made to develop ELISA assays for TK1 protein determinations but none of them have, so far, led to a clinically accepted routine method. Here we evaluate the performance of the TK 210 ELISA assay from AroCell AB, Sweden.
- The two monoclonal anti-TK1 antibodies used in this ELISA have been produced against specific exposed epitopes (XPA 210) in the C-terminal region of the human TK1 protein (6) (Fig 1).
- Pre-incubation of the samples with a special serum dilution buffer was needed to obtain consistent results. The AroCell TK 210 ELISA is a sandwich immunoassay, with a monoclonal capture antibody in the coated wells and a biotinylated monoclonal detector antibody. Recombinant TK1 diluted in a serum matrix buffer was used as calibrator (Fig. 2) (www.arocell.com)

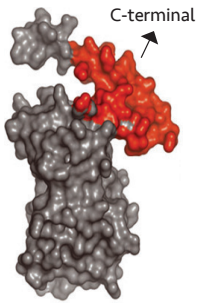

Fig 1.

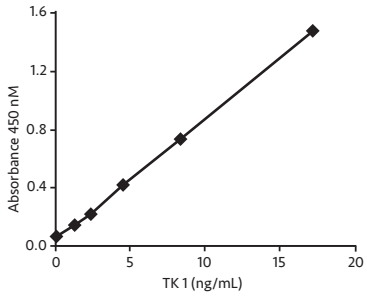

Fig 2.

Materials and Methods

Test Procedure for the AroCell TK 210 ELISA

- Calibrators, controls and serum samples were diluted 1:1 in the AroCell dilution buffer and incubated at 23-25°C for 60 min.
- Plates with coated antibody were prewashed 4 x 350 µL Wash Buffer.
- Incubation of the plate with prepared calibrators, controls and samples at 23-25°C for 2h.
- Wash 4 x 350 µL Wash Buffer.
- Addition of Biotinylated anti-TK1 antibody diluted in reagent buffer and incubation at 23-25°C for 60 min.
- Wash 4 x 350 µL Wash Buffer.
- Addition of Strep- HRP conjungate and incubation for 30 min at 23-25°C.
- Wash 4 x 350 µL Wash Buffer.
- Addition of Substrate (TMB) and Incubation for 15 min in dark.
- Addition of Stop Solution and measurement of absorbance at 450 nM.

Results

- TK1 protein levels determined with the AroCell TK 210 ELISA in sera from breast cancer patients (n=140) were significantly higher than those in sera from female blood donors (n=53) (Fig 3a). The ROC curve analysis of the same data showed a sensitivity of 0.49 and a specificity of 0.98 (Fig 3b).
- TK 210 ELISA analysis of sera from prostate cancer patients (n=70) also demonstrated significantly higher TK1 levels compared to those in sera from male blood donors (n=62) (Fig 4a), although the healthy control group in this case was not age matched. ROC curve analysis of the prostate cancer results with the AroCell TK 210 ELISA showed a sensitivity of 0.42 and a specificity of 0.97 (fig 4b).
- The TK1 activities in all the samples used in this preliminary studies were also determined using the ^{3}H-Thd phosphorylation assay (7). The ROC curve analysis of these results showed a sensitivity of 0.26 and 0.1 and a specificity of 0.98 and 0.92 with breast and prostate cancer groups, respectively.

Conclusions

- Serum TK1 levels assayed with the AroCell TK 210 ELISA differ significantly between subjects with breast or prostate cancer and healthy subjects.
- The AroCell TK 210 ELISA offers new opportunities for the study of these conditions and their therapy.

TK1 Protein Levels in Breast and Prostate Cancer Determined with the AroCell TK 210 ELISA

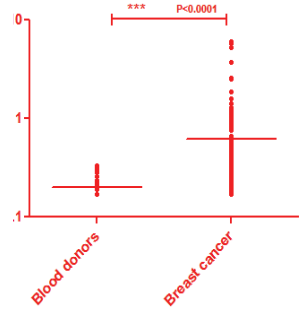

Fig 3a.

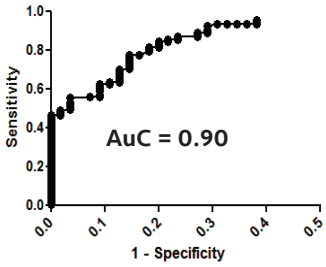

Fig 3b.

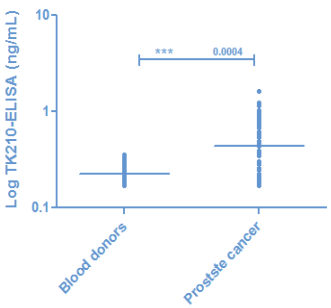

Fig 4a.

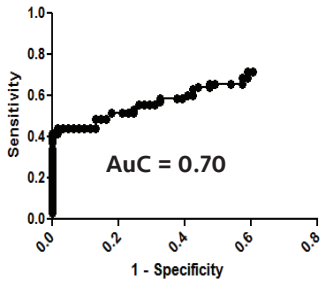

Fig 4b.

The AroCell TK 210 ELISA is for Research Use Only in the USA. Not for Use in Diagnostic Procedures.

Conflicts of Interest
Staffan Eriksson is an inventor of a TK1 patent licensed to DiaSorin Inc. and is a consultant and shareholder in AroCell AB.
KK Jagarlamudi is an employee at AroCell AB and O Nilsson is a consultant. AC Aronsson is a shareholder in AroCell AB.
The other authors have no conflicts of interest.

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