Doxorubicin effects on leukemia and breast cancer cells in culture on the TK1 protein levels using AroCell TK 210 ELISA: a tool for drug development.

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Introduction

- Thymidine Kinase 1 (TK1) is a cytosolic enzyme involved in DNA precursor synthesis and its activity is cell cycle regulated.
- Uncontrolled cell proliferation is a major characteristic of cancer progression which involves increased DNA synthesis and up-regulation of TK1 protein (1).
- TK1 has been widely used to study cell proliferation and malignancies *in-vivo*, however, there have been few studies presented on its application to *in-vitro* cell culture methods, widely used in drug development.
- Recent development of an ELISA by AroCell for determining TK1 protein levels in serum has extended the utility of TK1 as a biomarker for monitoring therapy and detecting recurrence in various malignancies (2).
- Doxorubicin is an anti-cancer agent commonly used for treatment of a variety of cancers. Oxidation of doxorubicin results in production of reactive oxygen species which is one process that lead to DNA damage and may results in cell death.
- The aim of this study was to evaluate the effect of Doxorubicin on leukemia and breast cancer cell lines *in-vitro* using measurements of TK1 protein levels in cell extracts and culture media supernatants with the AroCell TK 210 ELISA kit.

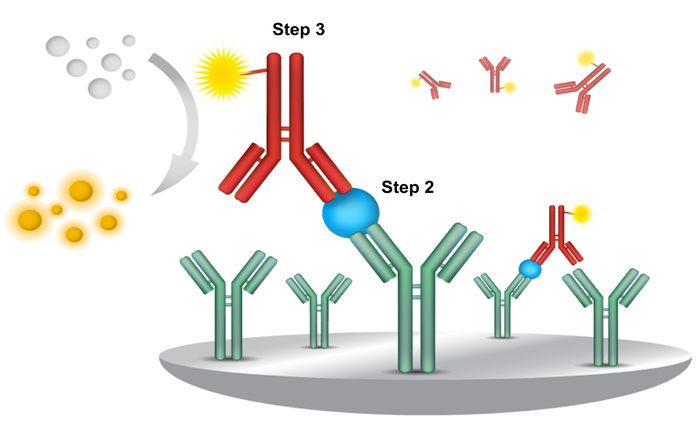
Materials and Methods

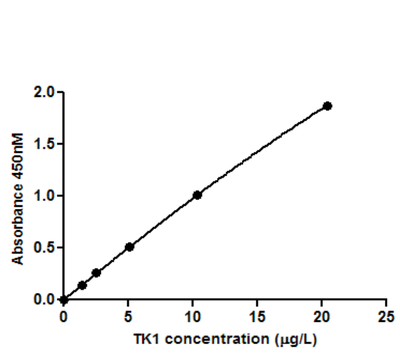
Cell culture method

- Human lymphoblastic leukemia cells (CCRF-CEM) were cultured in RPMI media with 10% fetal bovine serum and adenocarcinoma breast cancer cell lines (MDA MB-231) were cultured in DMEM media with 10% fetal bovine serum. in a humidified atmosphere containing 5% CO_2 at 37°C.
- Both cell lines were seeded in a 96-well plates at concentrations ranging from 8000 cells to 800 cells per well. Cells were exposed to 0, 0.5, 1, 5 or 10 µM doxorubicin for 24 hours.
- After 24 hours of exposure, culture media were centrifuged and the cell pellets lysed with a buffer containing 50mM Tris/HCl, pH 8.0, 150 mM NaCl, 0.5% NP-40 and 0.1% SDS.
- The TK1 protein levels in cellular extracts and culture media were determined by using the AroCell TK 210 ELISA kit as described (www.e-labeling.eu/ARO1001-15-3) Fig. 1).

AroCell TK 210 ELISA: A sensitive assay to measure the TK1 protein

- ❑ **Step-1:** Pre-incubation
- ❑ Cell extracts and culture supernatants are pre-incubated with AroCell sample dilution buffer, the buffer will expose the TK1 epitope that facilitate the binding of antibody to TK1 in samples.
- ❑ **Step-2:** The treated samples are added to a microtiter plate coated with anti-TK 210 monoclonal antibodies to which the TK1 complexes are bound.
- ❑ **Step-3:** The bound TK1 complexes are detected using a second biotinylated anti TK 210 monoclonal antibody followed by addition of a streptavidin-HRP conjugate.
- ❑ Following the addition of TMB substrate, the resulting optical density is proportional to the TK1 concentration in the samples and standards.
- ❑ The concentrations of TK1 in the samples are determined using a standard curve and a 4PL curve fit program.





Standard curve for TK 210 ELISA (absorbance of calibrators reduced from the blank)

Fig 1: Cell culture method.

Conclusions

These results demonstrate that AroCell TK 210 ELISA can be used for measuring TK1 protein as a biomarker in *in-vitro* studies, particularly with drugs targeting cell proliferation and DNA damage.

Results

- To evaluate the linearity of the AroCell TK 210 ELISA, TK1 content from unexposed cells was plotted against the cell number. A linear relationship was found for both cell lines (Fig. 2A and 2B).

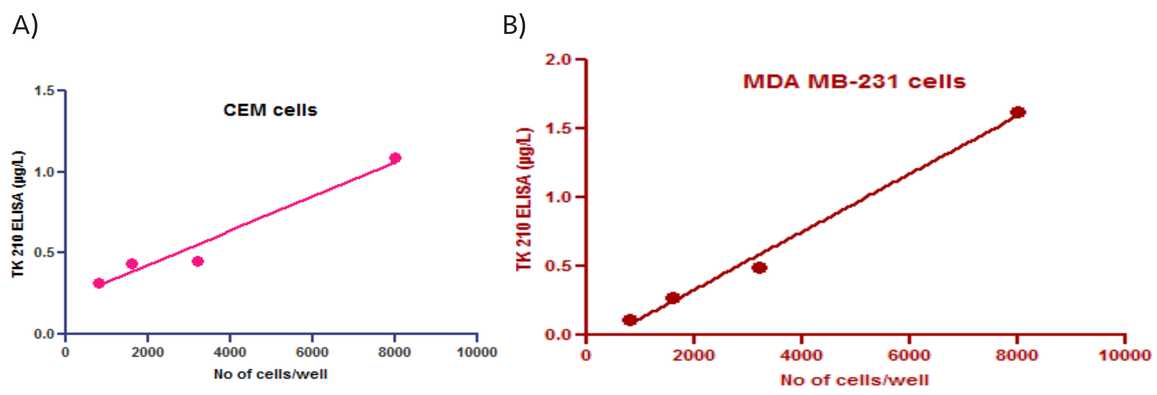


Fig 2: Relation between TK1 protein and cell number

- TK1 protein levels in CEM cell extracts increased about 2 fold with increasing concentration of doxorubicin up to 1µM. Increased TK1 protein was expressed as % change after normalization to the unexposed TK1 values as shown in Fig 3.
- However, TK1 protein levels in culture media only increased significantly with 10 µM dose when a clear decrease in intra cellular TK1 levels were also apparent (Fig. 3 A and 3B).

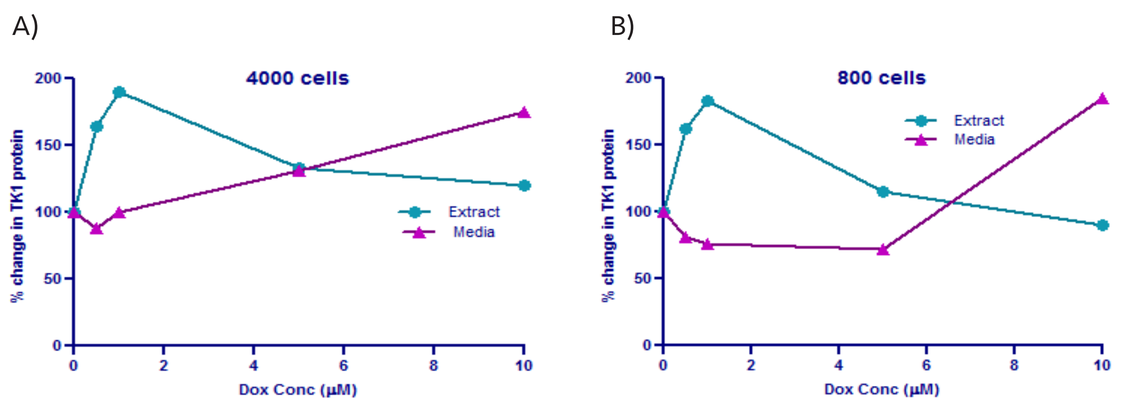


Fig 3: Percentage change in TK1 protein levels in CEM cells after doxorubicin treatment

- In MDA MB-231 cells, the intra cellular TK1 protein levels increased two fold, when the concentration of doxorubicin was increased from 0 to 10 µM.
- Whereas, the TK1 levels in culture media only showed minor change with 2-10 µM doxorubicin treatment (Fig. 4A and 4B).

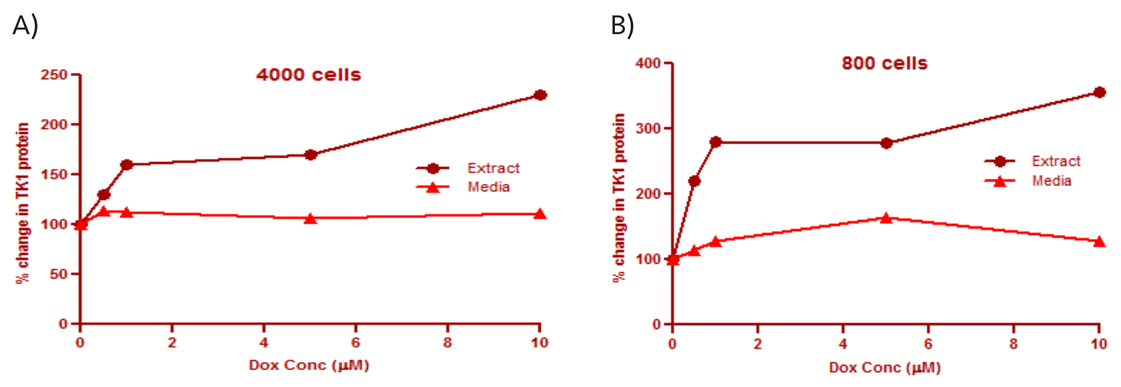


Fig 4: Percentage change in TK1 protein levels in MDA MB-231 cells after doxorubicin treatment

- These results shows that changes in intra-cellular TK1 and in the media supernatant were detectable using only 800 cells/well (both CEM and MDA MB-231).
- The changes in the TK1 protein levels in response to doxorubicin treatment indicate an induction of TK1 in cells probably due to DNA damage and a release of TK1 protein into the medium at higher doses due to cytotoxicity (3).

References
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