Rapid determination of multidrug resistance-associated protein in cancer cells by capillary electrophoresis immunoassay

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Abstract

The adenosine triphosphate (ATP) binding-cassette (ABC) transporters are a superfamily of cellular proteins that have been partly implicated as a cause of multidrug resistance (MDR) in cancer cells. The ABC superfamily consists of P-glycoprotein, multidrug resistance-associated proteins (MRP) and breast cancer-related proteins, of which MRP is of particular interest because of its ability to efflux a broader range of substrates. Since MRP1 is the most prominent member of the MRP family, a simple technique is needed for its quantification. We developed a simple, fast (total analysis time of 3 h) capillary electrophoresis immunoassay (CEIA) for the quantification of MRP1 in cancer cells. MRP1 antibody was labeled with fluorescein isothiocyanate. The labeled antibody was incubated with the cell lysate for a fixed interval (1 h), after which the cell lysate mixture was directly injected into the capillary to separate the complex of MRP1 and its antibody from free antibody. The noncompetitive CEIA method had a limit of detection of 0.2 nM and a good linear range (1.7–14.9 × 10^4 cells), and was fairly reproducible (RSD < 10%). The results showed that two cell lines, A549 and RDES, expressed MRP1 in the absence of doxorubicin (DOX), with A549 registering a higher expression. Compared to DOX-free cancer cells, there was an acceleration of MRP1 expression during the 12 h-exposure to DOX, after which the level of expression remained nearly constant as the intracellular accumulation of DOX decreased. The results obtained in this work indicate that the developed CEIA method is useful for relative quantification of MRPs in cancer cells.

Keywords

- Capillary electrophoresis immunoassay;
- Multidrug resistance-associated protein;
- Cancer cell;
- Laser-induced fluorescence;
- Doxorubicin