A results indicate that transduction of tat-cpg2 fusion

Business, Strategy

A significantdecrease in the cell viability of transduced cells 48 h after incubationcompared to 24 h was observed. We assume that this decrease in the cellviability at 48 h is probably due to the decrease in the levels of fusion protein as a result of degradation within48 hours, which is in agreement with the results of stability analysis. Our results show that transduction of fusion protein at the selectedconcentration does not have any toxic effect on the HepG2 cell viability. Thishas been shown in another report evaluating potentials of CPG2 in gene-directedenzyme prodrug therapy (GDEPT), where CPG2 expression did not cause any toxicityin HepG2 cells (Schepelmann et al., 2005).

We have furtherexamined the inhibitory effects of TAT-CPG2 fusion protein against MTX-inducedcell death using flow cytometry. In line with the results of cell viability analysis, flow cytometry resultsconfirmed that TAT-CPG2 in both native and denatured form can strongly inhibit theapoptosis effects of MTX on HepG2 cells. Therefore, our results indicate thattransduction of TAT-CPG2 fusion protein efficiently protects HepG2 cellsagainst cell death caused by MTX. It has been widely reported that increased oxidative stress is oneof the major mechanisms involved in MTX toxicity (Yiang et al., 2014).

Therefore, we decided to investigate the effect of some stress markers to check whether protection effects of TAT-CPG2 against MTX-inducedcell death is correlated with the inhibition of oxidativestress. We have observed thatMTX boosted intracellular ROS generation in cultured HepG2 cells. Previous reports have revealedthat oxidative damage caused by ROS generation is the major factor of MTXtissue injury (Yiang et al., 2014; Hafez

et al., 2015). However, in our study cells which were pretreated with TAT-CPG2, ROS production has been decreasedsignificantly. A significant decrease in the GSH content inMTX-treated cells compared to that of the untreated cells was observed.

Theseresults are in agreement with previous studies reporting the depletion of intracellular GSH content by MTX (Chang et al., 2013; Ewees et al., 2015). Ithas been demonstrated that GSH plays an important role in the cellularantioxidant defense, and its reduction may cause oxidative injury inhepatocytes (Mukherjee et al., 2013). However, in our study, pretreatment of HepG2cells with TAT-CPG2 ameliorated GSH content. A decrease in the CAT activity in MTX-treatedcells compared to that of the untreated cells has been observed.

MTX ratchets down the activity of the CAT as an antioxidant enzyme (Çetin et al., 2008; Chang et al., 2013). In our experiment a significant increasein the CAT activity in TAT-CPG2pretreated cells has been observed.

Therefore, transduced TAT-CPG2 prevents theaccumulation of MTX inside the cells and maintains the balance between oxidantsand antioxidants hence protects cells against the oxidative stress induced byMTX. Based on the in vitro results, and reported mechanismsfor MTX cell toxicity, also considering HepG2 as a proliferating cell line, one might conclude that in the cell death induced by MTX in HepG2 cells both mechanismsof cell cycle suppression caused by the inhibition of dihydrofolatereductase andoxidative stress caused by the accumulation of MTX are involved. Therefore, transduced TAT-

CPG2 convertsMTX into its non-toxic metabolites and prevents the accumulation of MTX in the cell and thus itstoxic effect. Delivery ofCPG2 into the cells by protein transduction is potentially valuable for a strategy known as enzyme/prodrug therapy. CPG2 is able to hydrolyzespecifically the amido, carbonyl or ureido bonds betweenL-glutamic acid and the carboxyl-, phenol or aniline-substituted aromaticrings, respectively (1, 2).

Several prodrugs such as4-(2-chloroethyl)(2-mesyloxyethyl)amino-benzoyl-L-glutamic acid (CMDA) andZD2767P have been synthesized to release potent DNA-alkylatingmustard drugs. These prodrugs are utilized in CPG2-medited strategies, such as antibody- or gene-directed enzyme prodrug therapy (ADEPTor GDEPT) (Jamin et al., 2011; Capucha et al., 2012; Karjoo et al., 2016). Because of disadvantagesof cancer gene therapy such as safety problems, it hasbeen proposed that direct delivery of proteins into the cell is an alternative to gene therapy, especially gene therapy of those type of cancers that do not require long-term sustained and regulatedexpression of the transgene (Ford et al.

, 2001). Therefore, we will propose thatTAT-CPG2 fusion protein can be used as an alternative to the GDEPT approach. 5. ConclusionWe have shown the construction, expression andpurification of CPG2 fused to HIV-1 TAT peptide (TAT–CPG2) in this study. Wehave demonstrated for the first time that TAT-CPG2 in both native and denaturedforms can be efficiently transduced into the HepG2 cells.

Also, we have providedevidences for the enzyme activity of transduced TAT-CPG2 fusion protein. Furthermore, we have shown that TAT-CPG2 fusion protein strongly protects HepG2 cells against MTX-induced cell death. We assume that transduced CPG2 converts MTX to non-toxic metabolites andprevents the accumulation of MTX in cells and therefore prevents the cell proliferation suppression and oxidative stress caused byMTX. However, further studiesare required to elucidate the involved cellular mechanisms. Our success in the protein transduction of TAT-CPG2 may provide a new strategy forprotecting against cell toxicity resulting from MTX in various organs andalso may provide an opportunity for the development of therapeutic methods forthe treatment of cancer by enzyme/prodrug strategy.