63P Inhibition of AKT-signalling sensitizes A673 Ewing sarcoma cell line to doxorubicin

[1]Galembikova Aigul, [1] [2]Boichuk Sergei
[1] Department of Pathology, Kazan State Medical University, Kazan, 420012, Russia;
[2] Central Research Laboratory, Kazan State Medical University, Kazan, 420012, Russia

Introduction

Ewing's sarcoma is a malignant mesenchymal tumor of bone and soft tissues. Ewing's sarcoma is one of the most aggressive malignant tumors. more than 20% of patients at the time of diagnosis already have metastases, and many more patients have micrometastases that are not detected by routine diagnostic methods. The insulin-like growth factor 1 receptor (IGF-1R) has been implicated in the genesis, growth, proliferation, and the development of metastatic disease in Ewing's sarcoma. IGF-1R activates alternative pathways for protection from apoptosis, cell proliferation, and differentiation. One of these pathways leads to the activation of PI3K-AKT-mTOR, while another pathway results in MAPKs (mitogen-activated protein kinases) activation. All these pathways, however, result in maintenance of cell survival by antagonizing the processes and proteins involved in apoptosis.

Thus, the aim of this study was to identify the decisive role of AKT kinase in maintaining cell proliferation in A673 cells and the mechanism of sensitization of A673 cells to doxorubicin using an appropriate inhibitor.

Figure 1: (A) Expression of phosphorylated and total forms of AKT, MAPK in BJ tert fibroblasts and A673 Ewing sarcoma cell line. Actin stain is a loading control; (B) Quantification by mean pixel density in the phosphorylated forms of AKT and MAPK kinases in BJ tert vs. A673 cells.

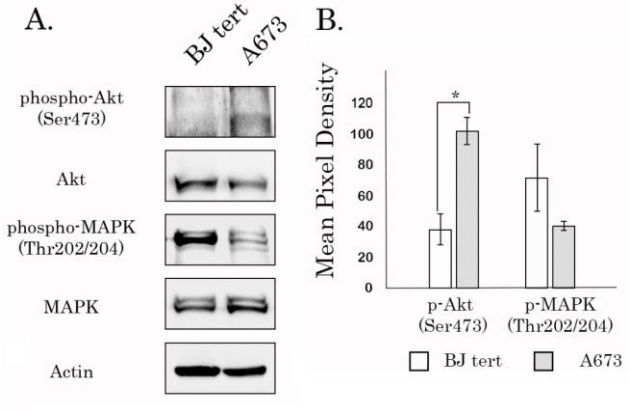
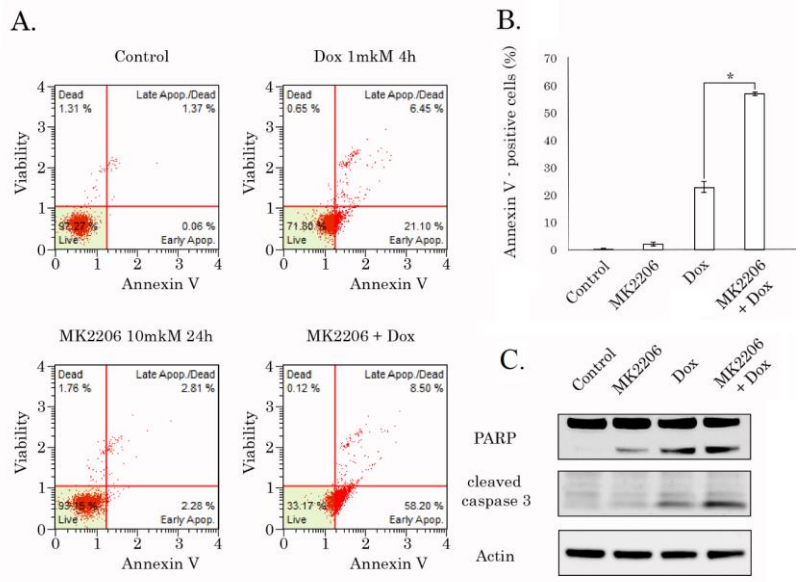


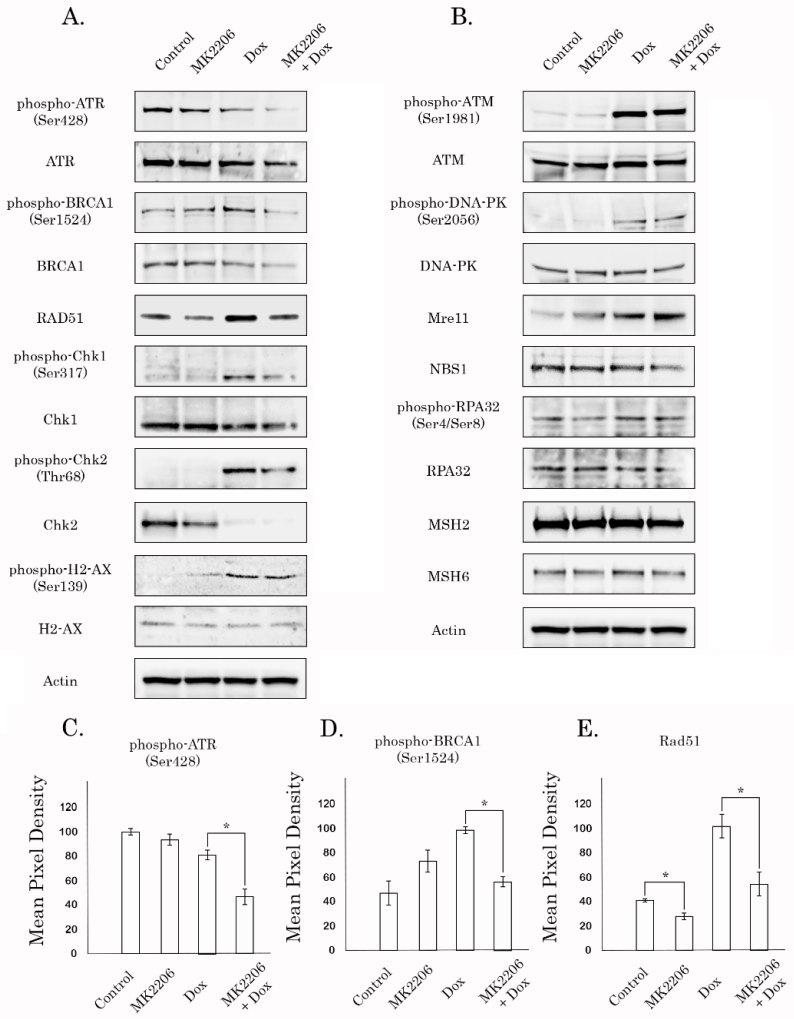
Figure 2: Inhibition of AKT-signaling potentiates pro-apoptotic effect of Dox in A673 cells. (A) Representative dot-plots histograms illustrating an increase of early (annexin V-positive/propidium iodide (PI)-negative) and late (double-positive) apoptotic cells in A673 cells treated with combination of MK2206 and Dox (lower right dot-plot), when compared to the cells treated with Dox alone. No increase of apoptotic cells was detected in MK2206-treated samples. (B) Quantitative analysis of early apoptotic cells after treatment with combination of MK-2206 and Dox for 6 h. * $p < 0.05$. (C) Expression of apoptotic markers (cleaved PARP and caspase-3) in A673 cells treated with MK-2206, and Dox alone or in combination. Actin staining was used as a loading control.



Methods and Materials

A673 cell line were maintained in culture medium (RPMI1640 or DMEM), supplemented with 10% FBS, 1% L-glutamine, antibiotics, and cultured in a humidified atmosphere of 5% CO2 at 37° C. Cellular viability and growth kinetics were analyzed using the MTS-based assay and iCELLigence system, respectively. Synergy between MK2206 and doxorubicin was determined in R software (the synergyfinder package) using the Zero Interaction Potency (ZIP) synergy assessment model. Apoptotic markers were analyzed by western blotting (cl. caspase-3, PARP) and FACs analysis (numbers of hypodiploid and Annexin V-positive cells).The expression of phosphorylated and general forms of AKT, MAPK, H2AX, Rad51, ATR, BRCA1, Chk1, Chk2, as well as actin was also determined by Western blotting.

Figure 3: (A-B) Expression of DDR markers in A673 cells treated with MK-2206, and Dox alone or in combination. Actin staining was used as a loading control. (C) Quantification by mean pixel density in phosphorylated form of ATR in A673 cells treated as indicated above; (D) Quantification by mean pixel density in the phosphorylated form of BRCA1 in A673 cells treated as indicated above; (E) Quantification by mean pixel density in Rad51 in A673 cells as indicated above. Values are means ±SD, N=3. ∗p<0.05



Results

The results obtained indicated an increase in the expression of the phosphorylated form of Akt kinase in the A673 Ewing sarcoma cell line compared to non-transformed BJ tert fibroblasts (Fig.1). MK2206, a known Akt inhibitor, synergistically increases doxorubicin-induced apoptosis (Fig.2). The average synergy for A673 cells was 15.236, whereas for BJ tert fibroblasts was 1.295 (no synergy). Inhibition of Akt signaling in doxorubicin-treated A673 cells was due to decreased expression of Rad51 recombinase, suggesting unsuccessful homology-mediated repair of doxorubicin-induced DNA double-strand breaks (Fig.3). There was also a decrease in the expression levels of DNA damage repair proteins (DDR) (eg, phosphorylated forms of ATR, BRCA1, Chk1 and Chk2) in A673 cells treated with the combination of MK2206 and doxorubicin. At the same time, the expression of the phosphorylated form of H2AX was increased, thereby revealing incurable DNA damage in Akt-inhibited cells.

Conclusion:

Thus, inhibition of Akt signaling sensitizes Ewing's sarcoma A673 cells to doxorubicin and may serve as a putative molecular target in Ewing's sarcoma to enhance the cytotoxic effects of DNA topoisomerase II inhibitors.

Corresponding author - Boichuk Sergei, Dr. Sci., Professor,
Head of Department of Pathology,
Kazan State Medical University, Kazan, Russia,
e-mail: boichuksergei@mail.ru
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