64P Tyrosine kinase signaling profile in osteosarcomas – potential therapeutic target for sensitization to doxorubicin

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Introduction

Metastatic and recurrent forms of osteosarcoma (OS) are characterized by a malignant course and an extremely poor prognosis. Therefore, the identification of specific markers capable of predicting the course of the disease, allowing the selection of the optimal therapeutic approach, and predicting its effectiveness, is a priority area of molecular genetic research in modern oncology.

Methods and Materials

The expression of phosphorylated and common forms of AKT, MAPK, c-Met, FGFR of HT1080 fibrosarcoma cells, and U2OS, and Saos-2 OS cell lines was determined by western blotting. Cellular viability was analyzed using the MTS-based assay. The synergy between selective inhibitors of the corresponding kinases - c-Met, FGFR, Akt, and MAPK (Crizotinib, BGJ398, MK2206, and U0126, respectively) and doxorubicin (Dox) was determined in R software (the SynergyFinder package). Apoptotic markers were analyzed by FACs analysis (numbers of hypodiploid and Annexin-V-positive cells).

Figure 1: (A) Expression of phosphorylated and total forms of fibroblast growth factor receptor (FGFR), c-Met, Akt, MAPK and FGF-2 in fibrosarcoma (HT1080) and osteosarcoma (U2OS, Saos-2) cell lines. Actin stain is a loading control; (B) Quantification by mean pixel density in the phosphorylated forms of c-Met, FGFR, Akt and MAPK kinases in HT1080, U2OS and Saos-2 cells; (C) The histogram shows the average synergy of doxorubicin with crizotinib (c-Met inhibitor), BGJ398 (FGFR inhibitor), MK2206 (Akt inhibitor), U0126 (MAPK inhibitor) on HT1080, U2OS and Saos-2 cell lines. Synergy Finder was used to evaluate the synergism of doxorubicin with various selective inhibitors.

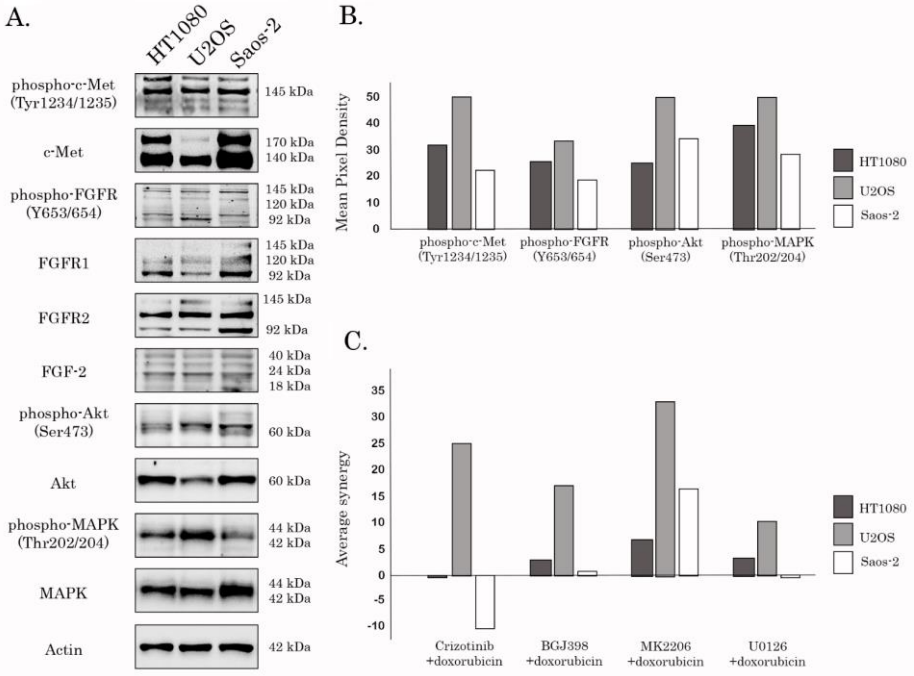


Figure 2: Synergy of Dox and AKT inhibitor in HT-1080 fibrosarcoma and U2-OS, Saos-2 osteosarcoma cells. Dox combination with MK-2206 illustrates synergy against HT-1080 (A) U2-OS (B) and Saos-2 (C). To determine the optimal synergy, a colorimetric cytotoxicity assay (MTT test) was performed with 5 doxorubicin multiples of 2 (0.0625-1 μM) and 4 levels of MK-2206 multiples of 2 (1.25-10 μM). 2D and 3D plots (surface plot) show the most pronounced cytotoxic effect when using the following concentrations of doxorubicin and MK2206: 0.0625 μM and 5 μM for fibrosarcoma cells (HT1080), 0.125 μM and 10 μM for osteosarcoma cells (U2OS, Saos-2) , respectively.

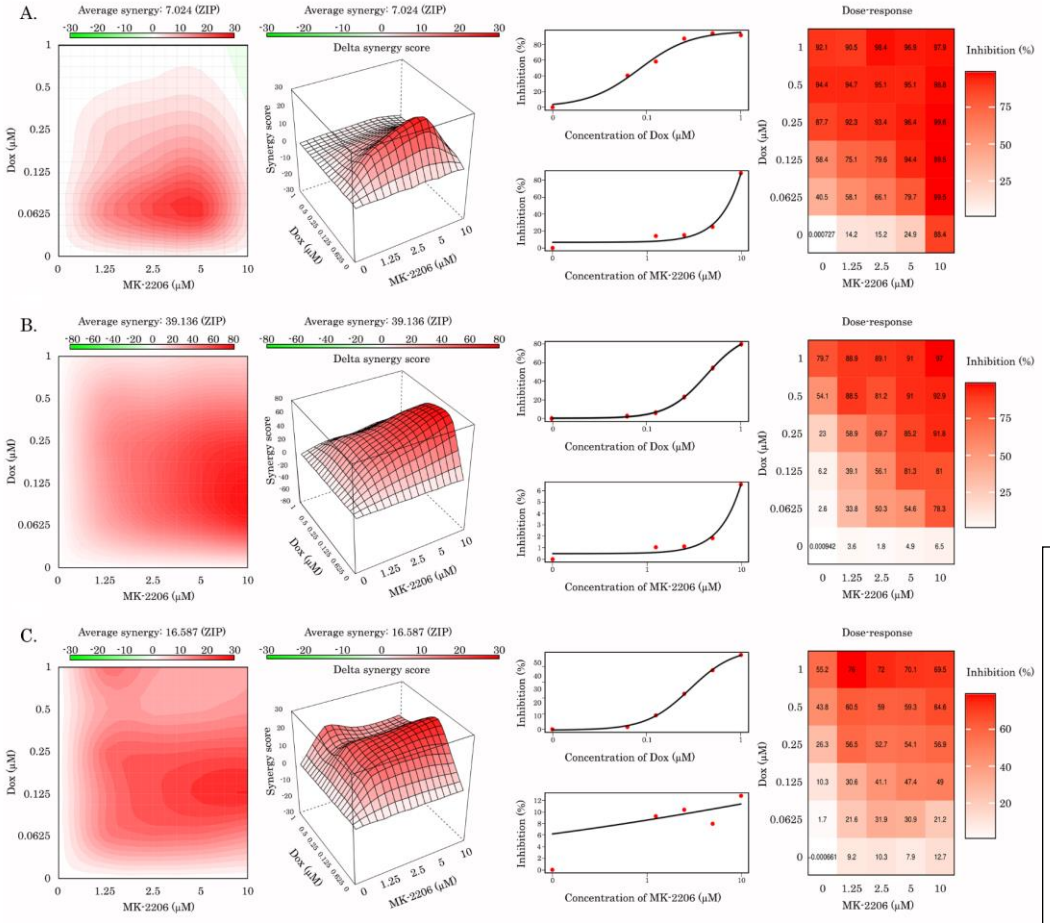
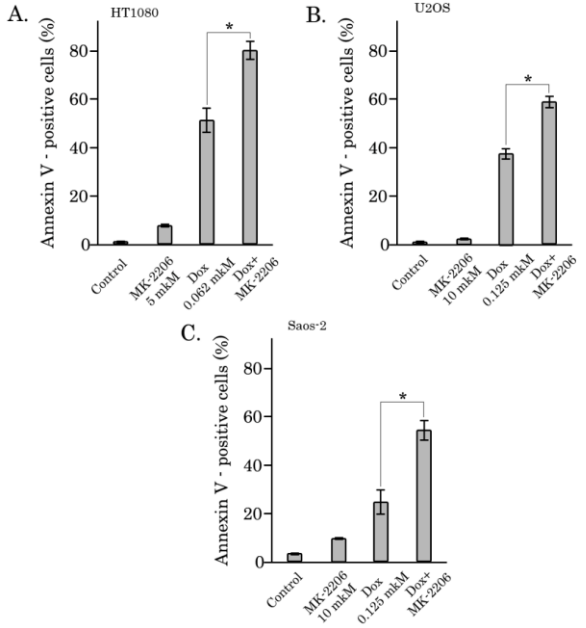


Figure 3: Inhibition of AKT-signaling potentiates pro-apoptotic effect of Dox in HT-1080 fibrosarcoma (A) and U2-OS, Saos-2 osteosarcoma (B,C) cells. Quantitative analysis of early apoptotic (annexin V-positive) cells after treatment with combination of MK-2206 and Dox for 6 h. * $p < 0.05$.



Results

At the initial stage, we studied the background expression of kinases that play an important role in cell proliferation - c-Met, FGFR, Akt, MAPK, in HT1080, U2OS, and Saos-2 cell lines (Fig.1A). It was found that the level of expression of phosphorylated forms of these kinases positively correlated with the synergism of the action of Dox and selective inhibitors of the corresponding kinases (Crizotinib, BGJ398, MK2206, and U0126). For example, the background expression of the phosphorylated form of Akt in the Saos-2 cells was less pronounced than in the U2OS cells but exceeded that in the HT1080 cells (Fig.1B,C). The average synergy score of Dox with the selective inhibitor of Akt, MK2206, showed a similar trend for HT1080, U2OS, and Saos-2 cell lines (7.024; 39.136; 16.587, respectively) (Fig.2). These results correlated with the number of Annexin-V-positive HT1080, U2OS, and Saos-2 cells cultured in the presence of Dox and MK2206 (Fig.3).

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Conclusion:
Thus, inhibition of the Akt signaling pathway sensitizes OS cells to doxorubicin.