1162 UPREGULATION OF METABOLISM ENZYMES MEDIATESADC RESISTANCE IN A HER2-POSITIVE CANCER CELL LINE

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Background The receptor tyrosine kinase HER2 exhibits overexpression in approximately 20% of breast cancer cases, and its amplification has been correlated with poor patient survival. Enhertu, also known as DS8201a, is a HER2-targeting ADC drug consisting of anti-human HER2 mAb, trastuzumab, and a derivative of DX-8951 (DXd), which are bound together by a maleimide glycynglycyn-phenylalanyn-glycyn (GGFG) peptide linker. Currently, Enhertu has received approval for the treatment of patients with HER2-positive breast cancer, gastric cancer, or HER2-mutant non-small cell lung cancer. However, acquired resistance has been considered as a major obstacle to the development of ADC therapy, and the underlying mechanisms remain incompletely elucidated.

Methods In this study, the Cell Titer-Glo assay was employed to assess the viability of tumor cells after treatment with different compounds, and the binding intensity of Enhertu with tumor cells was determined by flow cytometry. Meanwhile, the expression levels of HER2 in tumor cells were examined using flow cytometry analysis. Phosphorylation of Chk1, which is a DNA damage marker, was evaluated by western blot. To investigate the disparity of gene expression patterns between drug-resistant tumor cells and parental groups, RNA-seq was performed. qPCR was utilized to confirm the results of RNA-seq. The in vivo efficacy of Enhertu was evaluated in sub-cutaneous N87 xenograft models following intravenous injection.

Results We established Enhertu resistant N87 cells (N87-R cells) in vitro and confirmed drug resistance in vivo. In order to verify the resistant mechanism of N87-R cells, we evaluated HER2 expression and Enhertu binding of tumor cells and found no difference between N87 and N87-R cells. Then we turned to sensitivity of other approved HER2 targeted ADCs and their payloads. CTG results elucidated that the sensitivity to these payloads and ADCs were consistent in N87-R cells and N87 cells. To investigate the mechanism of drug resistance, we conducted RNA-seq assays of tumor cells which were shown as the elevated expression of drug metabolism enzymes in N87-R cell. Gene expression difference was further confirmed by qPCR. Furthermore, Enhertu combined with inhibitor of AKR1C reversed the resistance of tumor cells.

Conclusions In this study, we established an Enhertu-resistant N87 cell line. We found the HER2 expression and Enhertu binding capability didn't change significantly in N87-R cells. We performed RNA seq and our results demonstrated that drug metabolism enzymes increased in N87-R cells. In detail, AKR1C, ALDH3A1 and UGT1A6 genes were upregulated significantly in N87-R cells. Furthermore, Enhertu combined with inhibitor of AKR1C successfully reversed the resistance of tumor cells. We supposed that the upregulation of these enzymes decrease the cytotoxic effect of payload and lead to resistance of Enhertu.

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