

Drug Sensitivity Profiling of BT-474 Breast Cancer Cell Line for Identification of Novel Therapies Targeting HER2-positive Breast Cancer

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Introduction

Targeted therapy for breast cancer expressing hormone receptors such as estrogen receptor (ER) and progesterone receptor (PR) as well as human epidermal growth factor receptor 2 (HER2) is essential. In spite of current targeted therapies, breast cancer has high susceptibility to spread and metastases often turn resistant to the used treatment. Therefore, new therapies are needed especially when cancer becomes resistant to hormonal therapy.

The aim of the study was to investigate drug sensitivity and resistance of BT-474 breast cancer cell line and to recognize possible vulnerabilities especially in HER2+ breast cancer.

Materials and Methods

BT-474 cell line (ER+, PR+, HER2+) is originally derived from human solid, invasive ductal carcinoma of the breast. The cells were cultured in DMEM/Nutrient Mixture F12 Ham supplemented with 10% FBS and penicillin-streptomycin at 37 °C with 5% CO₂. HER2 expression was verified by IHC staining (SP3 clone, obtained from Spring Bio). The drug sensitivity of the cell line was assessed by applying a large panel of drugs covering cancer chemotherapeutics and clinically available and emerging drugs including e.g. conventional chemotherapy and kinase inhibitors such as HER2, pan-HER2 and EGFR-HER2 inhibitors. A panel of altogether 525 compounds was tested in five concentrations covering a 10,000-fold drug-relevant concentration range in 384-well format. Cells were seeded to pre-drugged plates, followed by cell viability measurements (CellTiter-Glo) after 72 hours. Maximal and minimal responses to drugs were analyzed, the EC₅₀ values were calculated, and a Drug Sensitivity Score (DSS) was calculated for each drug as a measure of reduced viability (1-3). A selective Drug Sensitivity Score (sDSS) was calculated to identify the selective drug response pattern (1-3).

References

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Results

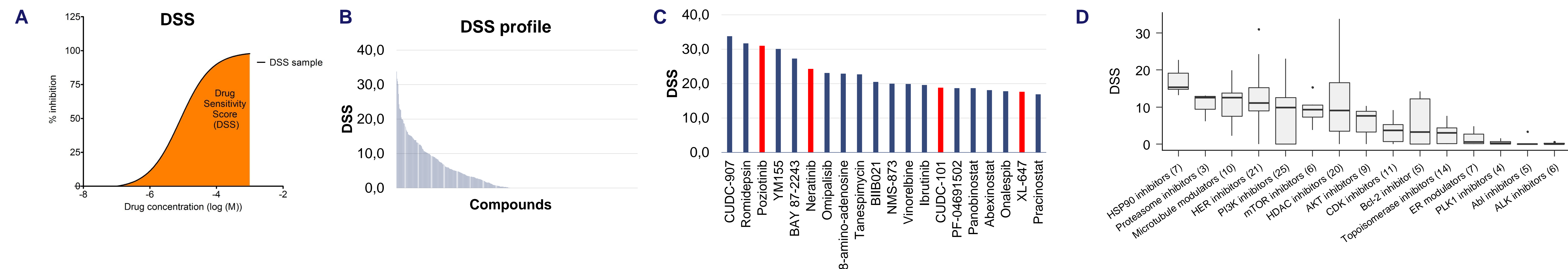


FIGURE 1. A) Illustration of determining DSS. B) DSS profile of BT-474 cells. C) Top 20 compounds in the BT-474 DSS profile. Red bars indicate HER2 inhibitors. D) DSS of selected compound groups. A panel of 525 compounds was tested. Cells were seeded to pre-drugged 384-well-plates followed by cell viability measurements (CellTiter-Glo) after 72 hours.

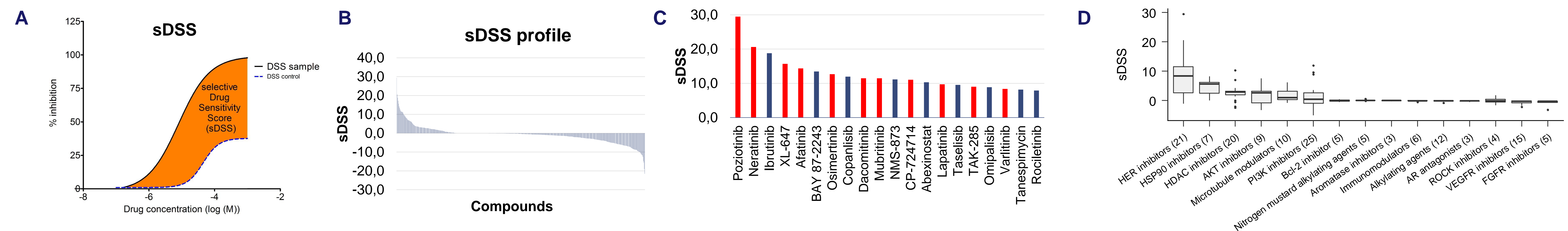


FIGURE 2. A) Illustration of determining sDSS. B) sDSS profile of BT-474 cells. C) Top 20 compounds in the BT-474 sDSS profile. Red bars indicate HER2 inhibitors. D) sDSS of selected compound groups. A panel of 525 compounds was tested. For sDSS determination, DSS was compared to a reference panel consisting of 6 breast cancer cell lines (sDSS = DSS of BT-474 – average DSS of the breast cancer reference panel). sDSS = 0 depicts inactivity in cells, sDSS = 0-5 depicts low activity, sDSS = 5-10 depicts semi-activity, sDSS = 10-20 depicts activity and sDSS >20 depicts high activity of the compounds in cells.

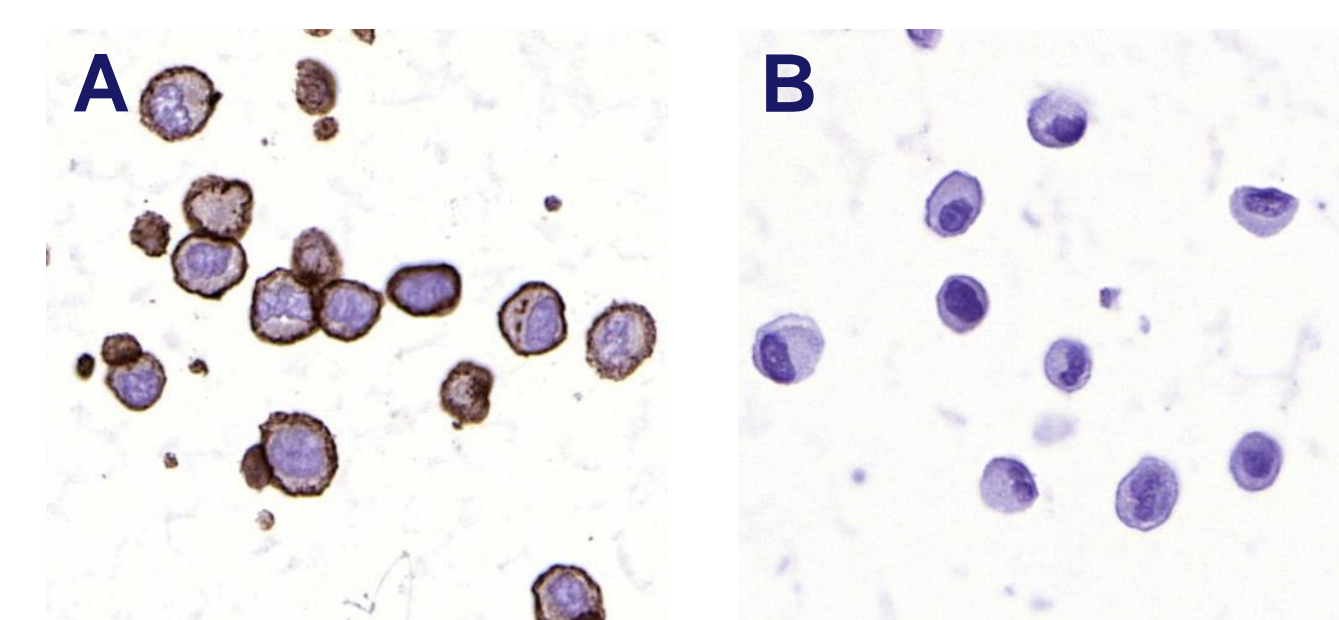


FIGURE 3. BT-474 cells stained for HER2 (A) vs control (B).

Conclusions

DSS analysis of BT-474 cell line showed sensitivity to conventional chemotherapy including paclitaxel and docetaxel and to kinase inhibitors such as EGFR and HER2 inhibitors neratinib, afatinib, mubritinib, poziotinib, dacomitinib and lapatinib with EC₅₀ range from 2 to 98 nM. Furthermore, the BT-474 cells showed sensitivity to PI3K and mTOR inhibitors, such as copanlisib and omipalisib, and to HSP90 and HDAC inhibitors, such as tanespimycin and abexinostat.

Screening of large compound libraries combined with DSS and sDSS analysis enables drug sensitivity profiling of HER2+ BT-474 breast cancer cells for identification of novel anti-cancer compounds against HER+ breast cancer. Moreover, the assay enables repurposing of existing drugs to new indications, identification of vulnerabilities in different types of cancer cells, and functional investigation of cellular pathways behind drug sensitivity or resistance.