Overcoming breast cancer resistance to tamoxifen by application of sulfotransferase SULT1A1-activated compounds

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Conclusions

1. SULT1A1 is upregulated in tamoxifen-treated breast cancer patients and cell lines.
2. Pro-drugs RITA, Aminoflavone (AF) and Oncrasin-1 (ONC) metabolised and activated by SULT1A1.
3. SULT1A1-activated compounds inhibit thioredoxin reductase 1 activity in cells and enhance ROS accumulation.
4. Low dose of RITA and aminoflavone are capable of overcoming 4-OH-TMX-resistance in cell lines and cells from patient with relapsed breast cancer.
5. SULT1A1 can be used as a clinical biomarker for identification of RITA, AF and ONC responders as well as TMX non-responders and stratification of patients.

Introduction

- Tamoxifen (TMX) anti-oestrogen therapy is widely used for treatment of breast cancer.
- However, the acquired resistance is the main hurdle for successful TMX therapy.
- Small compounds RITA, AF and ONC have anti-tumor activity in vivo and in vitro.
- Here we use primary breast cancer patient samples and breast cancer cell lines to show that upregulation of SULT1A1, a phase II detoxification enzyme, is linked to resistance to 4-hydroxy-TMX. We confirmed this phenomenon by different approaches.

Results

Figure 2: Correlation between SULT1A1 expression and small compounds anticancer activity (a) Scattered plot for RITA, AF and ONC. 50% growth inhibition concentration (GI50) and SULT1A1 mRNA expression in NCI-60 cell lines. (b) Genome wide shRNA screening in MCF7 cells after RITA treatment revealed the loss of SULT1A1 as a mechanism of resistance. (c) Protein expression of SULT1A1 in 13 cancer cell lines (d) RITA, AF and ONC-1 dose response in high SULT1A1 (black) and low (red) expressing cancer cells.

Figure 3: Antitumor cell activity of compounds is associated with ROS generation via inhibition of TrxR1 (a) 1µM RITA, 3µM AF and 5µM ONC induced ROS in HCT116 cells, but not in HCT116-SULT1A1 KO cells. The ROS induction can be rescued by 1µM Resveratol co-treatment. (b-c) Induction of p53 and inhibition of TrxR1 activity by compounds. (d-e) Whole-cell lysates from cells treated as described above in the absence or presence of resveratrol were harvested for western blot analysis of phosphorylated H2AX and PARP activation (e).

Figure 4: Tamoxifen sensitizes ER-positive breast cancer to RITA (a) Pre-treatment of 4-OH-TMX sensitizes cells to low dose of compounds. (b) Tamoxifen resistant LCC2 cells (red) showed higher sensitivity towards indicated concentration of RITA and AF. (c) Primary breast cancer cells treated with 100 nM RITA, black bar is relapse patient.

Figure 1: TMX resistant tumor exhibit upregulation of SULT1A1 (a) qRT-PCR analysis of SULT1A1 level in LCC2 (TMX) cells (b-e). Representative immunoblotting and immunostaining for the SULT1A1 level of LCC2 and wild-type MCF7 cells (d). Heat map of matched primary and metastatic tumors display upregulation of SULT1A1 (e) Representative immunostaining for SULT1A1 level after 4-OH-TMX treatment (1 µM, for 5 days in primary breast tumor) (f) qPCR expression of SULT1A1 in MCF7 cells at indicated concentration of 4-OH-TMX.

Model: Tamoxifen (4-hydroxy-tamoxifen)-treated relapsed breast tumors display upregulation of SULT1A1. This leads to efficient bioactivation RITA, AF and ONC, eliminating cancer cells by induction of reactive oxygen species (ROS).