Poster 4188

The role of NHEJ/HR mediated DSB repair in cancer cell sensitivity to payload of T-DXd

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Objective

- To investigate the roles of DNA repair mechanisms, specifically Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR), in influencing the sensitivity of HER2-expressing breast cancer cells (MDA-MB-453) to the antibody-drug conjugate Trastuzumab deruxtecan (T-DXd) and the exatecan derivative (DXd) payload.
  - i. The study aimed to determine how the knockdown of specific proteins involved NHEJ and HR affected cancer cell response to T-DXd and DXd payload treatment *in vitro*. By manipulating the mode of DNA repair pathways as NHEJ or HR dominant, the research sought to elucidate the respective contribution of each DNA repair mode to the therapeutic efficacy of T-DXd and the DXd payload in MDA-MB-453 cells.

Conclusions

- Reduced sensitivity to T-DXd and DXd payload by suppression of TP53BP1 expression may be due to increased HR mediated DNA damage repair, whereas enhanced sensitivity to (T-)DXd by suppression of BRCA1 expression may be due to the shift to NHEJ mediated DNA damage repair.
- Increased HR via decreased NHEJ or vice versa affected the sensitivity to the payload through regulation of cell cycle progression.
- These results support that combination with DNA Damage Response inhibitors that abrogate the cell cycle checkpoint may enhance efficacy of DXd payload.

Limitations

- Current findings are limited to in vitro data with temporal KD of single genes which may not fully translate. Further investigations are warranted to elucidate the contextual factors influencing these outcomes and to validate our findings in clinical samples.

Plain language summary

Why did we perform this research?

We conducted this research to understand the role of DNA damage repair mechanisms Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR) on DNA damage induced by T-DXd and its payload (DXd).

- TP53BP1 is known to function as the main component of NHEJ mediated DNA repair.
- BRCA1 is known to function as the main component of HR mediated DNA repair.

How did we perform this research?

By using a HER2-expressing breast cancer cell line MDA-MB-453, we investigated the roles of NHEJ and HR by silencing the expression of TP53BP1 and BRCA1 using siRNA for each respective gene and observed their change in response to T-DXd and DXd.

What were the findings of this research?

NHEJ/HR balance affected the sensitivity to the DXd payload through regulation of cell cycle progression.

What are the implications of this research?

Combination of T-DXd with DNA Damage Response (DDR) inhibitors was suggested to improve drug efficacy. However, further investigations are needed to explore these results in actual patient samples.

Where can I access more information?

Information about trastuzumab deruxtecan and ongoing clinical trials can be found at ClinicalTrials.gov

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Introduction

- Trastuzumab deruxtecan (T-DXd) is an innovative HER2-targeting antibody-drug conjugate comprising a HER2-specific antibody linked to an exatecan derivative (DXd) payload[1]. This therapeutic agent has demonstrated substantial clinical efficacy across various HER2-expressing malignancies.
- The DXd payload is a topoisomerase I inhibitor, stabilizing the topoisomerase-DNA cleavage complex. This stabilization inhibits the re-ligation of DNA, leading to the accumulation of DNA double-strand breaks (DSBs) and ultimately inducing apoptotic cell death[2].
- DNA DSBs are primarily repaired through two distinct mechanisms: Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR)[3]. However the contribution of these DNA damage repair pathways to the sensitivity of cancer cells to T-DXd/DXd remain poorly understood.

Methods

siRNA Transfection

Transfection reagent mix of ON-TARGET plus siRNA duplex pool for non-targeting control, TP53BP1, or BRCA1 (final concentration: 10 nM) and Lipofectamine RNAi MAX was mixed with MDA-MB-453 cell suspension and cultured for 24 hours before performing each of the following assays.

Growth Inhibition Studies

MDA-MB-453 cells were cultured on clear bottom 96-well plates for 6 days after treating with T-DXd or DXd at the indicated doses. The amount of ATP in each well was measured by CellTiter-Glo 2.0 Cell Viability Assay. Their relative viability was calculated by the following equation.

Relative viability = T/C
T: mean luminescence intensity of un/treated samples
C: mean luminescence intensity of untreated samples

Caspase 3/7 Activity Assay

MDA-MB-453 cells were cultured on clear bottom 96-well plates for 72 hours after treating with each test substance at the indicated doses. Caspase 3/7 activity in each well was measured by Caspase-Glo 3/7 Assay System. Their relative Caspase 3/7 activity was calculated by the following equation.

Relative Caspase 3/7 Activity = $(T_{Caspase}/T_{CTG})/(C_{Caspase}/C_{CTG})$
$T_{Caspase}$: mean Caspase-glo 3/7 luminescence intensity of un/treated samples
T_{CTG}: mean CellTiter-glo luminescence intensity of un/treated samples
$C_{Caspase}$: : mean Caspase-glo 3/7 luminescence intensity of untreated samples
C_{CTG}: mean CellTiter-glo luminescence intensity of untreated samples

Western Blotting

Cells were treated with each test substance for 72 hours and were lysed with Cell Lysis Buffer supplemented with a cocktail of protease inhibitors. 5 µg of protein was loaded to each lane for SDS-PAGE and transferred to polyvinylidene difluoride membranes. Immunoblot analyses were performed for each indicated proteins and detected by LAS4000 mini.

Immunofluorescence Assay

Cells were cultured on a chamber slide and treated with DXd (10 nM) for the indicated times. Cells were then fixed and stained with automated slide stainer BOND RX for RAD51 and nucleus (DAPI). Then fluorescence was imaged through TCS-SP8 STED 3X microscope.

Live Cell Cycle Monitoring

MDA-MB-453 cells were transduced with Incucyte Cell Cycle Lentivirus Reagents and cultured with selective pressure by puromycin to obtain a stable cell line. Stable cell line was monitored every 4 hours by Confocal Quantitative Image Cytometer CQ1 over 140 hours after treatment with each drug. Dead cells were determined by DRAQ7 staining.

Cell Cycle Analysis by Flow Cytometry

BrdU (final concentration: 10 µM) was added to the culture medium for 15 minutes. Cells were fixed and permeabilized, then stained with anti-BrdU antibody and 7-AAD, which was analysed by Attune NxT Flow Cytometer.

Results

- **a,** TP53BP1 KD (green) in MDA-MB-453 cells showed reduced drug sensitivity compared to those transfected with non-targeting siRNA (blue) upon treatment with T-DXd (left panel) and DXd (right panel) at the indicated doses. In contrast, BRCA1 KD (orange) showed enhanced drug sensitivity compared to control. Plotted values are triplicate of representative experiment.
- **b,** TP53BP1 KD (green) in MDA-MB-453 cells showed reduced relative Caspase 3/7 activity when treated with T-DXd (10 µg/mL) and DXd (10 nM). In contrast, BRCA1 KD (orange) showed enhanced relative Caspase 3/7 activity. Mean ± SEM (n=5, independent experiments). P values were calculated using a two-tailed paired *t-test*. *** p<0.001, ** p<0.01, ns no significance; p>0.05
- **c,** DDR upon drug treatment was reduced by BRCA1 KD, while apoptosis was enhanced. On the other hand, DDR upon drug treatment was indifferent by TP53BP1 KD, but apoptosis was reduced compared to control. NT: non-treated.

Figure 1. KD of TP53BP1 and BRCA1 has opposing effects on in vitro activity of T-DXd and DXd payload

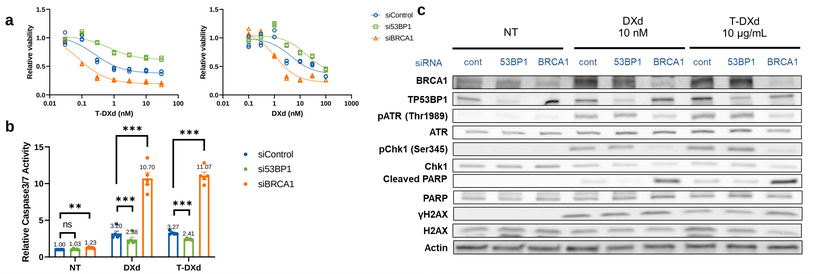
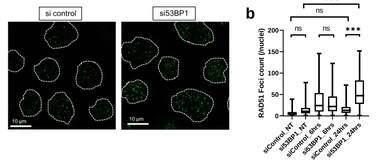
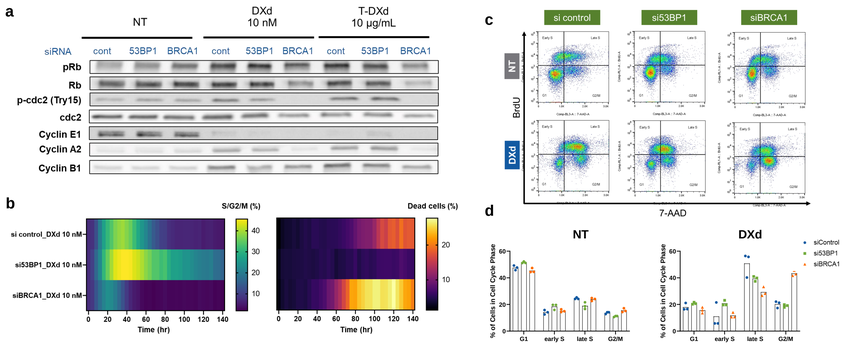


Figure 2. KD of TP53BP1 leads to increased HR



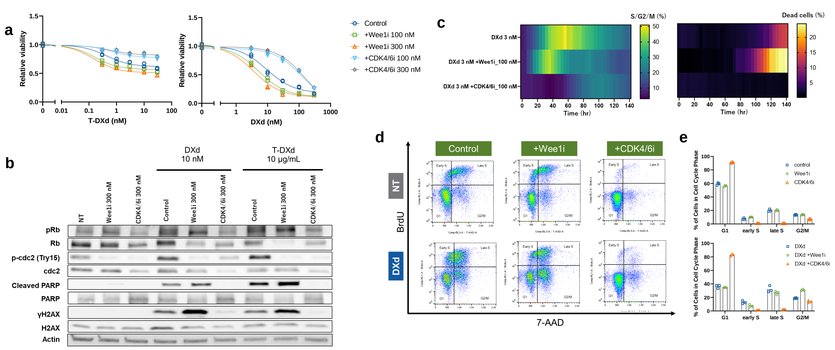
- **a,** representative micrograph of immunofluorescence of MDA-MB-453 cells treated with DXd (10 nM) for 24 hours. RAD51 (green), nucleus (dotted lines).
- **b,** TP53BP1 KD showed increased HR at 24 hrs post DXd treatment. NT: non-treated. 6hrs/24hrs: treated with DXd 10 nM for 6 or 24 hours. More than 80 cells were evaluated for each condition. P-values were calculated by Tukey's multiple comparison test. *** p<0.001 ns, no significance; p>0.05.

Figure 3. Cell cycle arrest upon DNA damage induced by T-DXd and DXd payload was reduced by BRCA1 KD



- **a,** while TP53BP1 KD and non-targeting control showed S/G2 phase arrest upon drug treatment, BRCA1 KD led to progression of cell cycle to M phase upon drug treatment. NT: non-treated. Same sample as Figure 1c was used.
- **b,** TP53BP1 KD of FUCCI-transduced MDA-MB-453 cells showed stronger and prolonged S/G2/M phase arrest (left panel) with reduced cell death upon DXd treatment (right panel), while BRCA1 KD showed rapid cell cycle progression and increase of cell death.
- **c,d,** BRCA1 KD led to increased progression to G2/M phase upon drug treatment, while TP53BP1 KD led to increased early S phase. Cells were treated with 1 nM of DXd for 24 hours. Individual values of technical triplicate are plotted, bars represent mean values. G1: $BrdU^-$ $7\text{-}AAD^-$; early S: $BrdU^+$ $7\text{-}AAD^-$; late S: $BrdU^+$ $7\text{-}AAD^+$; G2/M: $BrdU^-$ $7\text{-}AAD^+$

Figure 4. Combination with cell cycle checkpoint inhibitors affect the efficacy of T-DXd and DXd payload



- **a,** MDA-MB-453 cells treated with T-DXd (left) or DXd (right) in combination with AZD-1775 (Wee1i) showed enhanced drug sensitivity. In contrast, it showed reduced drug sensitivity when treated in combination with Palbociclib (CDK4/6i). Plotted values are duplicate of representative experiment conducted at Day 3 after drug treatment.
- **b,** MDA-MB-453 cells treated with DXd or T-DXd in combination with Wee1i showed enhanced apoptosis. In contrast, apoptosis was reduced when treated with CDK4/6i in combination with DXd or T-DXd. NT: non-treated.
- **c,** FUCCI-transduced MDA-MB-453 cells treated with DXd in combination with Wee1i led to reduced S/G2/M phase arrest (left panel) and increase of dead cells (right panel). In contrast, CDK4/6i combination did not lead to S/G2/M phase and dead cell increase.
- **d,e,** Combination of DXd with Wee1i led to increased cells in G2/M phase. In contrast, combination of DXd with CDK4/6i sustained cells in G1 phase. Cells were treated with 1 nM of DXd or in combination with Wee1i (100 nM) or CDK4/6i (100 nM) for 24 hours. Individual values of technical triplicate are plotted, bars represent mean values.

Abbreviations

7-AAD: 7-Amino-Actinomycin D, **BrdU**: Bromodeoxyuridine, **DAPI**: 4',6-diamidino-2-phenylindole, **DDR**: DNA damage response, **DSBs**: Double strand breaks, **DXd**: Exatecan derivative, **HR**: homologous recombination, **KD**: Knockdown, **NHEJ**: non-homologous end joining, **STED**: Stimulation emission depletion, **T-DXd**: trastuzumab deruxtecan

Disclosures

Len W. Katsumata, Manabu Abe, Misa Ikuta and Kumiko Koyama are employees of Daiichi Sankyo Co., Ltd.

Fumitaka Suto is an employee of Daiichi Sankyo, Inc.

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