#328 ADAM17-induced activation of HER receptors mediates resistance to trastuzumab in a subset of HER2 moderately expressing breast cancer cells

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ABSTRACT

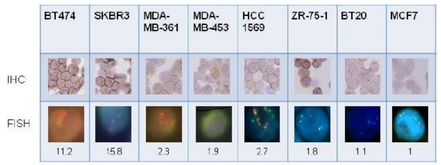
Several studies suggested that some of the HER2 negative patients may respond to anti-HER2 treatments and that moderately HER2 expressing breast tumours may respond better to tyrosine kinase inhibitors compared to trastuzumab[1-2]. The aim of this study is to understand the mechanisms of action and resistance of HER2 targeting treatments in HER2 moderate-expressing and low-expressing breast cancer cells. The HER2 expression of a panel of 8 breast cell lines was assessed by IHC, FISH, western blot, and qRT-PCR. Using cell viability studies we found that in comparison with high (IHC 3+) and low HER2 expressing (IHC 0 or 1+) breast cancer cells, moderately expressing HER2 (2+) cells showed an intermediate response to trastuzumab and neratinib. Three breast cancer cell lines, MDA-MB-361, MDAMB-453 and HCC1569 were more sensitive to neratinib than trastuzumab monotherapy. The response to trastuzumab and neratinib correlated with basal ADAM17 but not ADAM10 expression. Trastuzumab treatment led to an upregulation of ADAM17 and the shedding of HER ligands in the media, as well as the activation of HER members. ADAM17 inhibition, using a specific anti-ADAM17 antibody or knockdown of the protein, decreased activation of HER members as well as downstream markers, correlating with markedly reduced cell viability in HER2 2+ cell lines. In addition, the combination of trastuzumab with neratinib was more effective than either single agent in these cells. Thus, ADAM17 may play a key role in the resistance to trastuzumab in HER2 moderately expressing breast cancer cells. Combining trastuzumab with an ADAM17 inhibitor or a panHER inhibitor like neratinib maybe effective in a small subset of HER2 moderately expressing breast cancer cells.

OBJECTIVES AND METHODS

Objectives: Assessing 1) the effect 2) the mechanisms of action and 3) resistance of neratinib and other anti-HER2 treatments in HER2 moderately expressing breast cancer cells (IHC 2+)

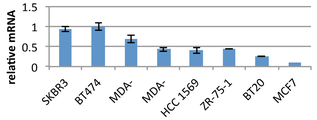
Methods: HER2 expression of a panel of eight breast cell lines was assessed by IHC, FISH, western blot, and qRT-PCR. Cell viability assays were used to assess the effect of anti-HER2 treatments. Western blot and knock down assays were used to assess the effects of drugs on various HER receptors and their downstream pathways and to determine the role of ADAM proteases in mediating resistance to trastuzumab in these breast cancer cells

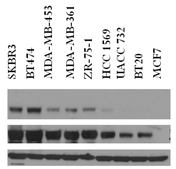
RESULTS



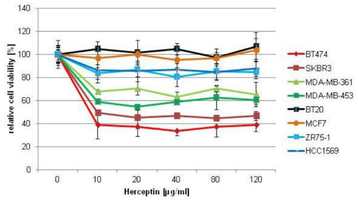
Cell lines	IHC	FISH ratio
BT474	3+	11
SKBR3	3+	16
MDA-MB361	2+	2.3
MDA-MB453	2+	1.9
HCC 1569	2+	2.7
ZR 75-1	2+	1.8
BT20	1+	1.1
MCF7	0	1

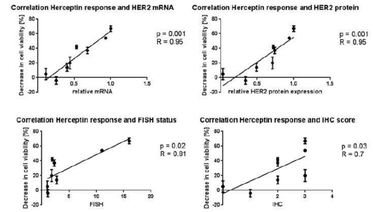
HER2 mRNA expression



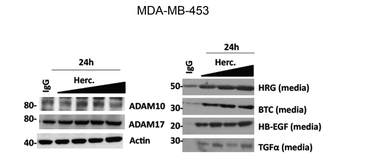


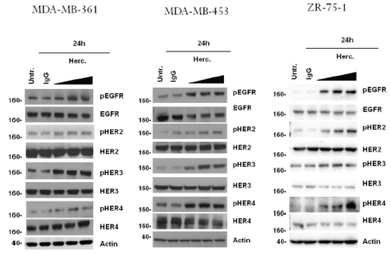
The HER2 status of a panel of breast cell lines by IHC, FISH, western blot and qPCR



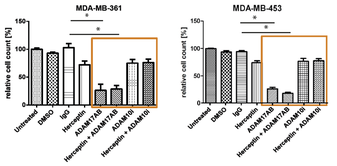


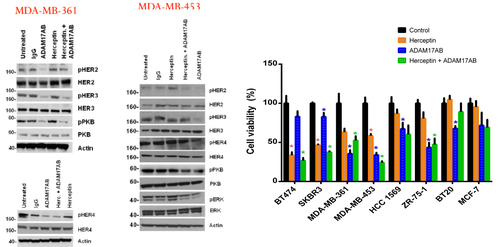
The HER2 status correlate with response to Herceptin in a panel of breast cell lines



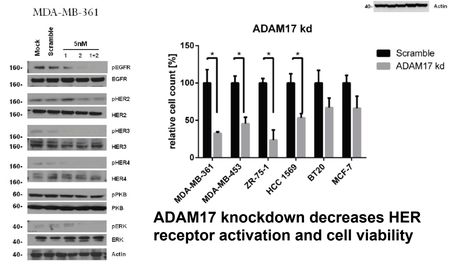


Herceptin induces upregulation of ADAM10 and 17 as well as activation of HER receptors in HER2 moderately expressing breast cancer cells

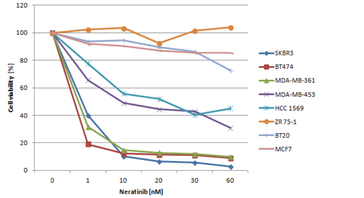




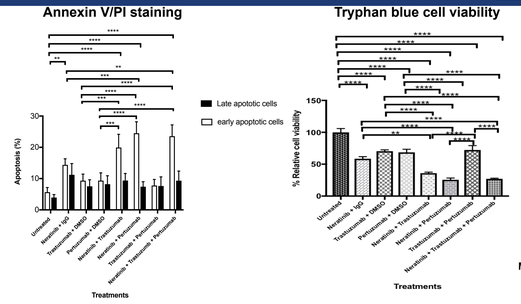
ADAM17 inhibitor[3] +/- Herceptin decreases HER receptor activation and cell viability

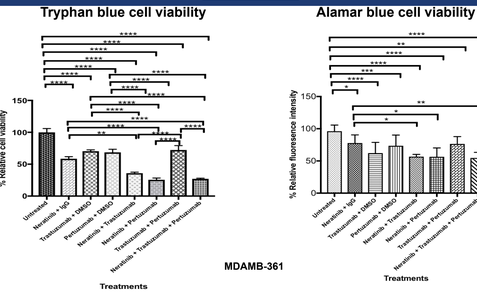


ADAM17 knockdown decreases HER receptor activation and cell viability

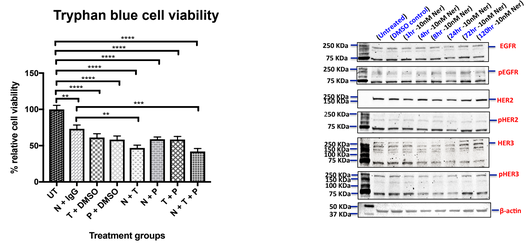


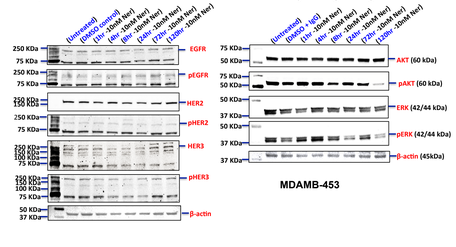
Neratinib was effective in decreasing cell viability in IHC 2+ breast cancer cells

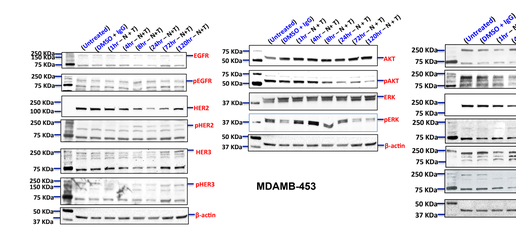




Combination of neratinib with trastuzumab and/or pertuzumab: more effective than any single agent in MDAMB-361







Combination of neratinib with trastuzumab +/- pertuzumab: more effective than neratinib alone in downregulating HER2 level and decreasing pAkt at 24h + decreasing cell viability at 5 days in MDAMB-453 cells

CONCLUSIONS

Trastuzumab induces upregulation of ADAM17 and HER ligands in IHC 2+ breast cancer cells, resulting in activation of HER receptors; trastuzumab with ADAM17 inhibition or neratinib was more effective than tratuzumab alone in reducing cell viability in IHC 2+ breast cancer cells.

Reference: 1) Paik S, et al. Benefit from adjuvant Trastuzumab may not be confined to patients with IHC 3+ and/or FISH-positive tumors: Central testing results from NSABP B-31. J Clin. Oncol. 2007. Vol 25, No. 18S (June 20 Supplement): 511. 2) Robidoux A, Tang G, Rastogi P, al. e. Evaluation of lapatinib as a component of neoadjuvant therapy for HER2+ operable breast cancer: NSABP protocol B-41. J Clin Oncol 2012; (Suppl):LBA506. 3) Tape C.J. et al. 2011. Cross-domain inhibition of TACE ectodomain. PNAS. 108 (14), p5578-5583.