New therapeutic approaches in HER2-driven breast cancer: role of the chaperonin hsp90 in response to pharmacological treatments.

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Abstract

Background: With a mortality rate of 17%, breast cancer is the first cause of death in the female population. It represents 30% of invasive tumors in women and 16% of all female tumors. Approximately, 15-20% of all breast cancer (BC) cases, are characterized by amplification of the HER2 receptor that is involved in the activation of many pathways; its function is regulated by HSP90. Thus, HSP90 co-targeting is emerging as a potential molecular target for HER2-directed breast cancer therapy.
Methods: We analyzed HER2 and HSP90 expression in a panel of breast cancer cell lines, with or without HER2 amplification, including MCF7 cells stably transfected with a constitutively active HER2. HER2/HSP90 expression and growth inhibition were monitored over time upon exposure to trastuzumab (T) and docetaxel (D), in the presence or absence of HSP90 silencing. We also retrospectively evaluated a series of 24 locally advanced/operable breast cancer patients who underwent neoadjuvant (T+D) for HSP90 expression and correlated it with pathological complete response (pCR).
Results: In the breast cancer cell lines analyzed there was no clear-cut correlation between HSP90 and HER2 expression, but HER2 transfection into MCF7 cells increased HSP90 mRNA and protein expression; however, treatment with T further increased HSP90 levels. Conversely D increased HER2, but did not affect HSP90, expression. In HER2+ breast cancer cell lines, simultaneous T+D combination resulted in synergistic growth inhibition in vitro, while their staggered combination, particularly T followed by D, did not afford synergistic effects. Effects of simultaneous and staggered treatments on HSP90 and HER2 expression were analyzed by WB: HER2 expression decreased in the simultaneous and staggered combination (D followed by T), while HSP90 expression did not change upon combined treatment. The effects of HSP90 silencing and overexepression on functional response to T+D are being analyzed in HER2+ breast cancer models: preliminary results indicate that HSP90 silencing in HER2+ breast cancer decreases the therapeutic synergism of the simultaneous T+D combination. Accordingly, in locally advanced/operable pts undergoing neoadjuvant T+D, pCR occurred more frequently in pts with a baseline HSP90 score of 3+, as compared to 2+ and 1+ (50.0% vs. 14.3% vs. none, p=0.05). These results suggest the possibility to classify HER2-positive pts into HSP90 defined subgroups and elaborate specific therapeutic strategies.
Conclusions: Preclinical data indicate that constitutive HER2 activation induces HSP90 expression and HSP90 modulation influences the functional response to combined treatment. Baseline HSP90 expression may potentially represent a pre-requisite of pharmacological response in HER2-addicted BC.

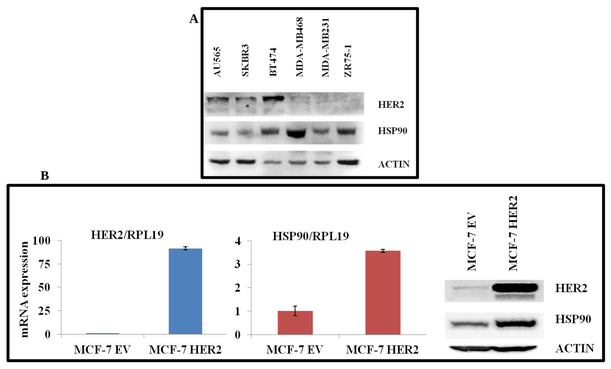
Background

The combination of Trastuzumab and Docetaxel is considered the standard of care for patients with locally advanced/operable human epidermal growth factor receptor 2 (HER2)-positive breast cancer. In the context of HER2-positive disease the achievement of a pathologic complete response (pCR) after neoadjuvant treatment represents a prerequisite for a longer progression-free survival. Recent clinical evidence have suggested that HSP90 overexpression may influence clinical response to Trastuzumab and Docetaxel in early breast cancer patients: in fact, in HER2-driven molecular context, the combination of the two drugs, achieves better results in patients with HSP90 overexpression. The pCR was significantly higher in patients with HSP90 score 3+, in comparison with score 2+ and score 1+ (50.0% vs. 14.3% vs. none, P = .05); after treatment, a statistically significant lower Ki67 staining (30.0% vs. 17.5%, P = .005) and a trend towards decreased expression of high (score 3+) and moderate (score 2+) HSP90 immunostaining (McNemar P = .25, Wilcoxon-Mann-Whitney P = .08) were found (Bria E. et al. 2014). These results suggest the possibility of further classifying HER2-positive patients into HSP90 defined subgroups. This, in turn, could translate into more specific and effective therapeutic strategies for HER2-positive breast cancer patients.

Aims

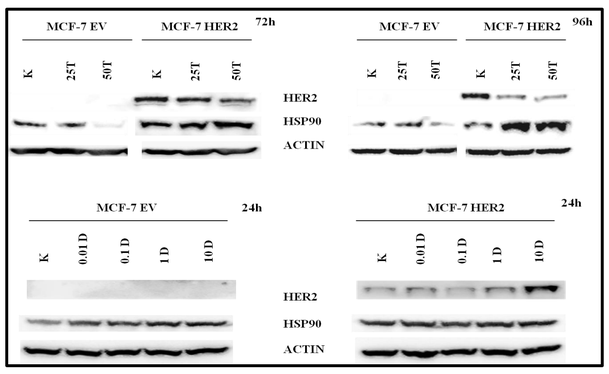
✓To find and explain a possible relationship between HER2 and HSP90 in breast cancer at the pre-clinical and clinical level.
✓ To examine the possible role played by HSP90 in the pharmacological response to the combination of Trastuzumab and Docetaxel.
✓ To evaluate the molecular mechanisms by which HSP90 determines a different response to the combination in different molecular contexts (HER2 amplified or not).

HSP90 expression, in a panel of BC Cell Lines, is not correlated with HER2 status.. but in MCF-7, stably trasfected with HER2, its expression significantly increase



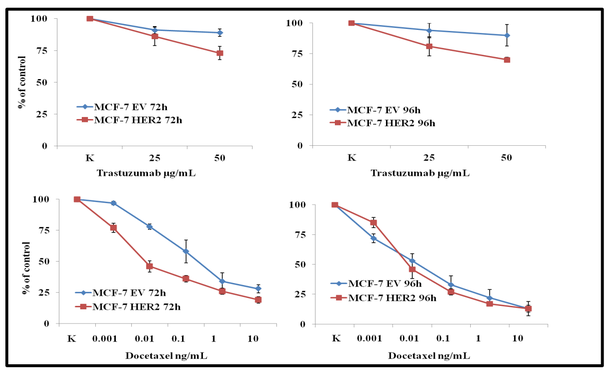
A. Different BC with HER2 amplified or not were lysed and analyzed by Western Blotting using antibodies specific for HER2 and HSP90. Western blot with antibodies specific for β-actin are shown as protein loading and blotting control. **B**. MCF-7 cells were stably trasfected with both a plasmid Empty Vector (MCF-7 EV) and a plasmid encoding costitutively active HER2 (MCF-7 HER2). The activation of HER2 induced HSP90 expression at the mRNA and protein levels.

Trastuzumab treatment modulates HSP90 protein expression



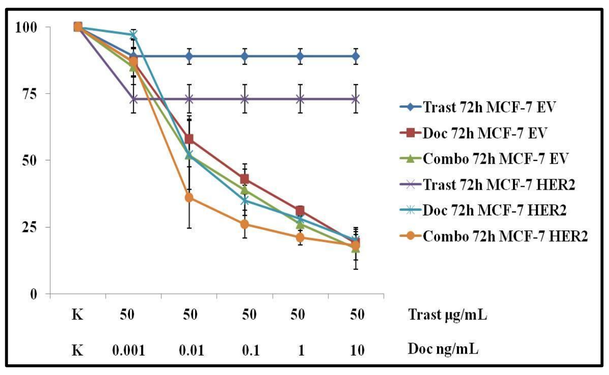
MCF-7 EV and MCF-7 HER2 cells were treated with Trastuzumab for 72-96 hours and with Docetaxel for 24 hours at indicated concentrations. The cells were lysed and analyzed by Western Blotting using antibodies specific for HER2 and HSP90. Western blot with antibodies specific for β-actin are shown as protein loading and blotting control. The Trastuzumab treatment, in HER2 amplified cells, induced the protein expression of HSP90.

MCF-7 EV and HER2 amplified: different sensitivity to Trastuzumab and Docetaxel treatments



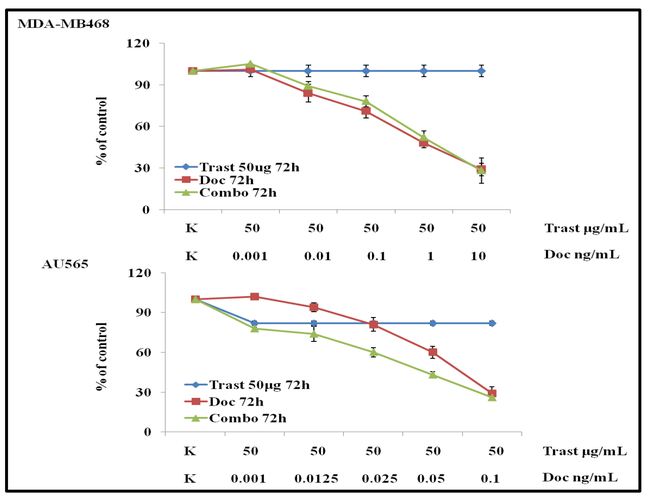
MCF-7 EV and MCF-7 HER2 cells were treated with Trastuzumab and Docetaxel at the indicated increasing concentrations. Cell growth was assessed by Crystal Violet assay after 72-96hours.

MCF-7 HER2 cells are more sensitive to combination treatment respect to MCF-7 EV



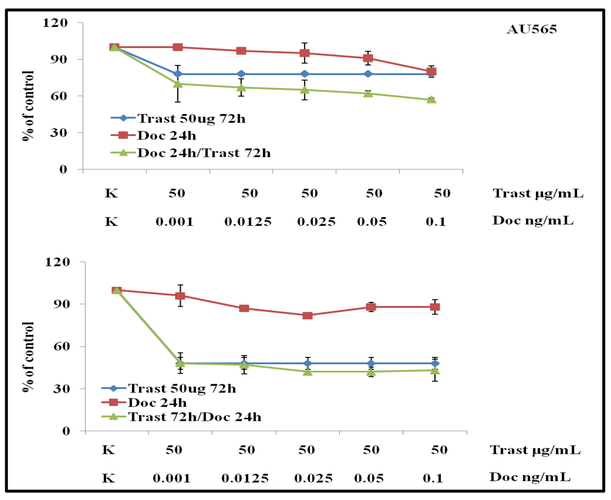
MCF-7 EV and MCF-7 HER2 cells were treated with Trastuzumab and Docetaxel, alone and in combination, at the indicated increasing concentrations. Cell growth was assessed by Crystal Violet assay after 72 hours. The combination resulted sinergistic only in MCF-7 trasfected with HER2 amplified

Simultaneous combination resulted synergistic in a context of HER2 amplified



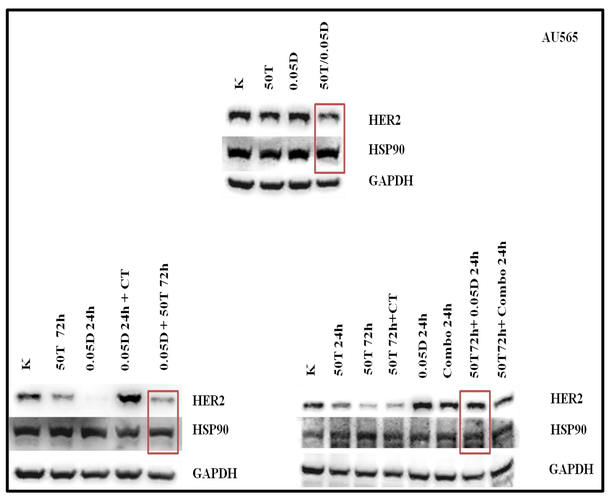
MDA-MB468 and AU565 BC cells were treated with Trastuzumab and with Docetaxel, alone and in combination, at the indicated increasing concentrations. Cell growth was assessed by Crystal Violet assay after 72 hours. The combination resulted sinergistic only in HER2 amplified context.

In HER2 amplified Breast Cancer Cell lines, sequential Trastuzumab followed by Docetaxel did not result in synergistic growth inhibition



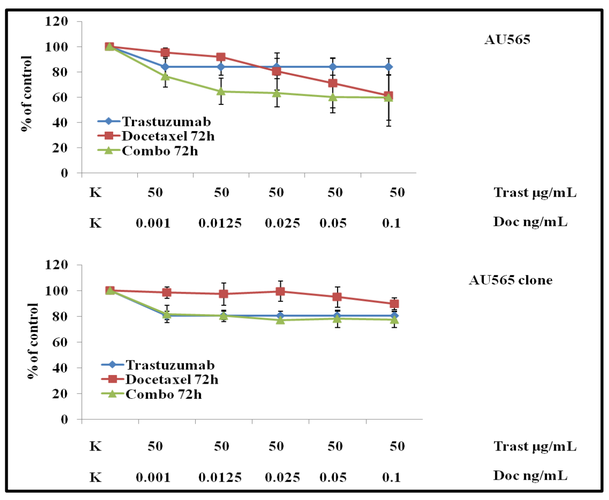
AU565 BC cells were treated with Trastuzumab and Docetaxel, alone and in combination, for different periods of time and using staggered starting times of the two treatments. Cell growth was assessed by Crystal Violet assay. The combination resulted sinergistic only in a context in which the cells were treated simultaneously or with Docetaxel followed by Trastuzumab.

Combination treatment effects on HER2 and HSP90 expression



AU565 BC cells were treated with Trastuzumab and Docetaxel, alone and in combination, for different periods of time and using staggered starting times of the two treatments. The cells were lysed and analyzed by Western Blotting using antibodies specific for HER2 and HSP90. Western blot with antibodies specific for GAPDH are shown as protein loading and blotting control. HER2 expression decreased only in the simultaneous combination and in condition of Docetaxel followed by Trastuzumab.

HSP90 silencing in HER2 amplified BC: preliminary data of response to combination treatment



AU565 BC cells stably trasfected with ShRNA for HSP90. The cells were treated with Trastuzumab and Docetaxel, alone and in combination and cell growth was assessed by Crystal Violet Assay after 72hours. The trasfected cells were less sensitive to combination treatment.

CONCLUSIONS

Although there is no correlation between the expression of HER2 and HSP90 in different breast cancer cell lines, the transfection of costitutively active HER2 induce, in MCF-7 cells, HSP90 expression at the mRNA and protein levels. HER2 transfection determined a better response of cells to Trastuzumab and Docetaxel treatments and this cells became more sensitive to combination of the two drugs. In a HER2 amplified context, simultaneous Trastuzumab/Docetaxel combination resulted sinergistic and this effect was maintained when Docetaxel treatment preceded Trastuzumab, but was completely lost with the opposite sequence (Trastuzumab followed by Docetaxel). Preliminary data, obtained on HER2 breast cancer cells trasfected with ShRNA for HSP90, show a decreased sensitivity to the pharmacological combination.

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