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Summary

Background: The humanized anti-HER2 monoclonal antibody trastuzumab (Herceptin®) is useful in the treatment of ErbB2-overexpressing breast cancers, but its efficiency is limited because development of resistance is common. In order to study the possibility of improving the efficacy of therapies directed against HER2/erbB2, we investigated the effects of co-targeting this receptor and the insulin-like growth factor 1 receptor (IGF-1R), a widely-expressed protein tyrosine kinase with important roles in suppression of apoptosis and stimulation of proliferation.

Material / Methods: The experimental strategy involved combining trastuzumab treatment and reduction of IGF-1R signaling through incremental heat-induced expression of the dominant-negative IGF-1 receptor 486/STOP under the control of the heat-sensitive Drosophila HSP70 promoter, in HER2/erbB2-overexpressing MCF7her18 breast cancer cells.

Results: Isobologram analysis of combinatorial treatment data revealed a strong synergistic interaction between trastuzumab treatment and the induction of the dominant-negative IGF-1R expression, resulting in potentiation of growth inhibition in transfected cancer cells.

Conclusions: These observations support the concept that simultaneously co-targeting tyrosine kinase receptors may be therapeutically useful, and provide a specific rationale for combining IGF-1R and HER2/erbB2 targeting strategies in anti-neoplastic approaches.

key words: IGF-1R • HER2 / ErbB2 • Trastuzumab • Herceptin • co-targeting • synergy

Abbreviations: EGFR – epidermal growth factor receptor; HER2/ErbB2 – human erbB-related/erythroblastosis virus gene B; IGF-1R – insulin-like growth factor-1 receptor; MAPK – mitogen-activated protein kinase; P1-3K – phosphatidyl-inositol 3-kinase; PTK – protein tyrosine kinase; RT – reverse transcriptase

Word count: 2112

BACKGROUND

A promising field of oncological study has been opened by the elucidation of the crucial role of protein tyrosine kinases (PTKs) in signal transduction, and by the discovery that PTK signalling is frequently abnormal in cancer. Many novel approaches in antineoplastic therapy take aim at PTK functions [1], and one receptor in particular, HER2/erbB2 (also known as EGFR2 or *neu*) is an excellent clinical target because its frequent overexpression in several types of cancer is associated with poor prognosis [2]. In contrast to other members of the EGFR family, HER2/erbB2 does not seem to possess its own high-affinity ligand, but forms with other ligand-bound other ypeeef-daD1Pve1ypeeef-dhetr G6D7KS;1ligan1poorG]erodimher G606D7C1which G606D7C1se-PSPD06D7C1grov

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mutant product capable of inhibiting the wild-type protein function, i.e. a dominant-negative. In this study, a truncated IGF-1R dominant-negative construct (486/STOP) under the control of the *Drosophila* HSP70 heat-sensitive promoter [20] was used for controlled reduction of IGF-1R signalling. Expression of the 486/STOP shortened protein has been shown to decrease autophosphorylation of endogenous IGF-1 receptors in transfected cells, and is known to cause a blockage of cell proliferation [26,27]. Heat-dependent effects on cell growth in the absence of the 486/STOP construct are negligible in the conditions used here as shown by parental cell controls, and the complete growth inhibition observed at 39.5°C in all MCF7her18-486/STOP lines tested is therefore related to IGF-1R dominant-negative interference with cell metabolism. The use of heat shocks of various durations to incrementally induce the expression of genes under the control of heat-sensitive promoters has been reported before [28,29], and this property is used here to allow the design of a combinatorial protocol for isobologram analysis [24,25] which reveals a strong synergistic interaction between trastuzumab treatment and 486/STOP expression. Other synergistic interactions with trastuzumab have been observed for drugs such as cisplatin, docetaxel, thiotepa, and etoposide [30], but this is the first report of synergistic inhibition of growth through interference with the HER2/ErbB2 and IGF-1R signaling pathways. In this type of study, a simple additivity pattern would imply that the agents are acting independently on different metabolic pathways, while synergy (potentiation of one agent on the action of another) suggests the existence of interactions between the pathways targeted. Trastuzumab allows $p27^{kip1}$ expression and facilitates its release from sequestering proteins; it also decreases the levels of cyclin E and of early and mid G1 cyclins, resulting in accumulation of cells in G1 phase [4,5]. IGF-1 has been demonstrated in MCF7 cells to increase cyclin D1 and 5 expression, and to post-transcriptionally decrease $p27^{kip1}$ levels, all of which facilitate passage from G1 to S phase [31]. Signaling pathways for HER2/ErbB2 and IGF-1R have been shown to cross-talk [32], and unpublished results from our laboratory suggest that IGF-1 antagonism to trastuzumab is effected through the targeting of $p27^{kip1}$ to the proteasome degradation machinery (Lu et al, unpublished). It is therefore likely that the synergistic action of trastuzumab and 486/STOP treatments involves potentiation of this cell cycle control step towards the significant growth inhibition observed in this study.

CONCLUSIONS

Our results represent the first report of formal isobologram analysis for evaluating antagonism, additivity or synergy between treatments targeting two tyrosine kinase receptors. The finding of synergy confirms that there are benefits to co-targeting approaches, and more specifically, that the antineoplastic effects of blocking tyrosine kinase receptors of the EGF family may be enhanced by co-targeting IGF-1R.

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