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Hsp90 inhibitor 17-AAG reduces ErbB2 levels and inhibits proliferation of the trastuzumab resistant breast tumor cell line JIMT-1

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Abstract

ErbB2, a member of the EGF receptor family of tyrosine kinases is overexpressed on many tumor cells of epithelial origin and is the molecular target of trastuzumab (Herceptin), the first humanized antibody used in the therapy of solid tumors. Trastuzumab, which is thought to act, at least in part, by downregulating ErbB2 expression is only effective in ∼30–40% of ErbB2 positive breast tumors. Geldanamycin and its derivative 17-AAG are potential antitumor agents capable of downregulating client proteins of Hsp90, including ErbB2. To investigate the ability of 17-AAG to downregulate ErbB2 in trastuzumab resistant breast cancer cells and the possibility of 17-AAG and trastuzumab potentiating each other's effect, the recently established trastuzumab resistant breast cancer cell line, JIMT-1 was compared to the known trastuzumab sensitive SKBR-3 line. Baseline and stimulus-evoked dimerization and activation levels of ErbB2, and the effects of trastuzumab and 17-AAG alone and in combination on cell proliferation and apoptosis, as well as on ErbB2 expression and phosphorylation have been measured. Baseline activation and amenability to activation and downregulation by trastuzumab was much lower in the resistant line. However, 17-AAG enhanced ErbB2 homodimerization after 5–10 min of treatment in both cell lines, and decreased proliferation with an IC_{50} of 70 nM for SKBR-3 and 10 nM for JIMT-1. Thus, 17-AAG may be a useful drug in trastuzumab resistant ErbB2 overexpressing tumors. The antiproliferative effect of 17-AAG was positively correlated with phosphorylation and downregulation of ErbB2 and was dominated by apoptosis, although, especially at higher doses, necrosis was also present. Interestingly, IC_{50} values for ErbB2 downregulation and phosphorylation, in the 30–40 nM range, were not significantly different for the two cell lines. This observation and the negative correlation between resting ErbB2 levels and the antiproliferative effect of 17-AAG may indicate that activation of ErbB2 to some extent could counteract the overall cytostatic effect, especially at higher levels of ErbB2 expression. The usual therapeutic dose of trastuzumab did not change the IC_{50} of 17-AAG on the proliferation of either cell line, but nevertheless decreased overall ErbB2 phosphorylation and at low doses of 17-AAG further decreased cell growth in the sensitive SKBR-3, thus trastuzumab may be a good combination partner to counteract undesired activating effects of 17-AAG.

Keywords: Breast tumor immunotherapy; Trastuzumab; 17-AAG; ErbB2

1. Introduction

ErbB2 is a member of the ErbB receptor tyrosine kinase family. Although it has no soluble high-affinity ligand [1], it fulfills a central role in the ErbB signal transducing network by increasing the ligand binding spectrum and affinity of ErbB1, 3 and 4, the other members of the family [2,3]. Activation of the ErbB family members requires their homo- or heterodimerization which is followed by transphosphorylation and downstream signaling cascades leading to cell proliferation and survival [4,5]. A most mitogenic heterodimer is formed of ErbB2 and ErbB3, the latter, though kinase deficient, serving as a receptor for neuregulins [6,7]. ErbB2-containing heterodimers are internalized less efficiently [8,9] and evade lysosomal degradation [10], an effect

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which is even more pronounced upon ErbB2 overexpression [11], also leading to ligand-independent constitutive activation of ErbB2 homodimers [12,13].

ErbB2 is overexpressed in 25–30% of human breast cancers, and is associated with very poor prognosis [14]. Overexpressed membrane proteins, especially those – like ErbB2 – that are scarce in differentiated cells are ideal targets of molecular therapy. ErbB2 was the first antigen on a solid tumor to be targeted with a humanized monoclonal antibody, trastuzumab (Herceptin) [15,16]. Other molecular therapies targeting ErbB2 include the humanized 2C4 antibody (omnitarg) that inhibits ErbB2 dimerization [17,18] and the pan-ErbB kinase inhibitor CI-1033 [19], that are now investigated in several clinical trials.

While the use of trastuzumab in antibody-based cancer therapy has dynamically expanded over the past years, it also had to be realized that as single agent, it is ineffective in 60–70% of ErbB2 overexpressing breast tumors [20]. Even though trastuzumab, when combined with chemotherapy gives better initial results [21], continued administration of the antibody usually leads to secondary resistance. The molecular mechanisms of resistance to trastuzumab, similarly to its mode of action, are largely unknown and possibly include various factors. Autocrine production of EGF-like ligands [22] or overexpression of insulin-like growth factor 1 receptor (IGF1R) [23], leading to an ErbB2-independent means for the constitutive activation of the PI3K pathway, as well as blocking of trastuzumab binding by MUC4, a cell surface mucin [24,25], have been implicated in trastuzumab resistance.

Trastuzumab binds to a membrane-proximal domain of ErbB2 [26,27] and causes partial activation and internalization of the receptor [28]. Although the internalization itself may not be a definitive requirement for the antiproliferative effect of trastuzumab [29,30], decreased cell surface ErbB2 levels could well be one distinct cause of decreased cell proliferation [31]. Thus, in the clinical setting, it could be a reasonable approach to use alternative methods to decrease ErB2 levels of overexpressing, but trastuzumab resistant tumor cells.

The chaperon Hsp90, besides catalyzing the proper folding of newly synthesized client proteins into a stable tertiary conformation, has been implicated in the stabilization of a number of cellular proteins that play central roles in signal transduction processes [32]. ErbB2, alone in the ErbB family, possesses a sequence in its kinase domain that is responsible for Hsp90 binding [33,34]. Interestingly, binding of Hsp90 to ErbB2 not only serves to maintain its physiological conformation, but also to restrain ErbB2 from forming active signaling dimers [35].

Ansamycin antibiotics isolated from *Streptomyces hygroscopicus* [36], such as geldanamycin, were found to inhibit the growth of many cancer cell lines at nanomolar concentrations [37]. Geldanamycin having a very narrow therapeutic window [38], more promising analogs such as 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) were synthesized. 17-AAG exhibited decreased toxicity and enhanced stability, and even though its binding to Hsp90 was weaker than that of geldanamycin, 17-AAG displayed an antitumor effect similar to geldanamycin [39,40]. It also proved to be reasonably successful in phase I clinical trials in spite of formulation problems and side effects [41], especially in two cases of melanoma [42].

Among several signaling molecules (such as Raf-1, CDK4, Lck), 17-AAG was found to decrease ErbB2 levels in breast [39,43], prostate [44] and ovarian [45] cancer cells. However, it remains unclear to what extent downregulation of ErbB2 levels is correlated with the antiproliferative effect of 17-AAG, and whether it would also be effective in the cases where trastuzumab does not effectively decrease cell surface ErbB2 levels and proliferation. Furthermore, it is of importance to learn whether in trastuzumab sensitive tumors 17-AAG and trastuzumab could be used together to potentiate each others effect.

In this study we have exploited the recently established first trastuzumab resistant breast cancer cell line, JIMT-1, that can be passaged in vitro [46] and compared these cells to the known trastuzumab sensitive SKBR-3 line. We investigated baseline and stimulated dimerization and activation levels of ErbB2, and the effects of trastuzumab and 17-AAG alone and in combination on cell proliferation and apoptosis, as well as on ErbB2 expression and phosphorylation. Our results indicate that while baseline activation and amenability to activation and downregulation by trastuzumab is much lower in the resistant line, its proliferation is more prone to inhibition by 17-AAG. In both cell lines, the antiproliferative effect of 17-AAG was correlated with the downregulation of ErbB2. The usual therapeutic dose of trastuzumab did not change the IC_{50} of 17-AAG on the proliferation of either cell line, but nevertheless decreased overall ErbB2 phosphorylation and at low doses of 17-AAG further decreased cell growth in the sensitive SKBR-3.

2. Materials and methods

2.1. Cell cultures and reagents

SKBR-3 was obtained from the American Type Culture Collection (ATCC, Manassas, VA), whereas the JIMT-1 breast tumor cell line [46] was isolated at the University of Tampere. SKBR-3 cells were cultured in a humidified atmosphere with 5% CO_2 at 37 °C in Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal calf serum and antibiotics. JIMT-1 cells were grown in a 1:1 mixture of DMEM and Ham's F-12 similarly to SKBR-3. Cells were propagated every 3–4 days using 0.05% trypsin and 0.02% EDTA. For microscopy experiments, cells were seeded onto 12 mm diameter glass coverslips or on Lab-Tek™ II chambered coverglass (Nalge Nunc International, Rochester, NY) 2 days in advance and used at a confluence of 30–50%. For flow cytometric measurements cells were harvested with trypsinization. Before stimulation experiments, the cultures were starved in serum-free DMEM for 24 h.

Natural murine EGF was obtained from IC Chemikalien (Ismaning, Germany). 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) was a kind gift of Kosan Biosciences Inc. (Hayward, CA), and stored as a 10 mM stock solution in DMSO at −20 °C. All other chemicals, unless indicated otherwise, were from Sigma (Schnelldorf, Germany).