The Combinatory Effects of Lapatinib and SSI-4 on HER2+ Breast Cancer

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INTRODUCTION

HER2+ Breast Cancer is an extremely aggressive form of cancer caused by the overexpression of the HER2 oncogene, which codes for the protein receptor, classified as a membrane receptor tyrosine kinase (RTK). These RTKs homo and hetero-dimerize through transphosphorylation, upregulating and activating various metabolic, antiapoptotic and proliferative signaling pathways/cascades known to be linked to cancer.

These pathways have been exploited by using Lapatinib, a small molecule dual tyrosine kinase inhibitor, targeting not only HER2, but also its dimerization partners such as human epidermal growth factor receptors 1 and 2, as well as other members of the HER family. It binds to the receptor's intracellular domain, blocking autophosphorylation. This is especially effective as any dimerization containing HER2 proves to be especially potent.

Stearoyl-CoA desaturase 1 (SCD1) is an important enzyme in the de-novo lipogenesis pathway (formation of new lipids) that catalyzes the conversion of saturated fatty acids (SFAs) into monounsaturated fatty acids, or MUFAs. SCD-1 overexpression is being discovered in a growing number of cancers, leading to a growing interest in the SCD-1 gene as an agent in the development, maintenance, and progression of cancer. Particularly in breast cancer, SCD-1 overexpression is associated with a worse prognosis and ultimately, poor survival. Out of all the breast cancer subtypes, HER2+ has been shown to have the highest SCD-1 expression. We predict that blocking SCD1 will have antitumor activity, as the overexpression of SCD1 mRNA has been correlated with a poor prognosis in patients with HER2+ Breast Cancer.

We have developed a SCD1 inhibitor, SSI-4, which has been shown to have an excellent binding affinity for SCD-1, and has been shown to induce biological stress in various other cancers known to overexpress SCD-1. In addition, SCD-1-induced changes in plasma membrane lipid domains favor the activation of tyrosine-kinase receptor signaling platforms, an example being the HER2 receptor. This has given us strong cause to suspect drug synergy between the associated pathways of HER2 and SCD1, potentially producing a novel molecular targeted therapy that could ultimately prolong a patient's life one day.

Research Questions/Experimental Aims

Is the SCD-1 gene expressed and active in HER2+ Breast Cancer?
What is the antitumor effect (proliferation and/or cell death) effect of molecular targeted therapy of the SSI-4 inhibitor on HER2+ Breast Cancer cell lines expressing the active SCD-1 gene?
When SSI-4 and Lapatinib inhibitor molecular targeted therapies are combined, will antitumor synergy occur?
What are the precise mechanisms of cell death that result from SSI-4/Lapatinib-induced inhibiton?

Hypothesis

Combining the SSI-4 inhibitor and Lapatinib dual inhibitor into a novel molecular targeted therapy will produce synergistic antitumor effects in human and mice cell lines containing HER2+ breast cancer cells, through blockade of their respective and combined oncogenic signaling pathways.

Mechanisms of Cell Death

In addition to figuring out the biological effect of SCD-1 induced inhibition, this experiment seeks to elucidate the precise mechanism of cell death exerted on the cell when SCD-1 inhibition is induced. So far, this is predicted to be through Endoplasmic Reticulum (ER) stress.

Overview of Experiments/Methodology

The experiment was divided into three sub-experiments, as follows:

Experiment 1: Measure protein expression of HER2, SCD1, EGFR, an pEGFR in HER2+ cell lines

Immunocytochemistry (ICC) for cell lines was performed at 75% confluence in 4-well Lab-Tek chamber slides coated with poly-D-lysine (Nunc, Rochester, NY). Fixation was done with 2% paraformaldehyde. Immunostaining was done for 1 hour with anti-HER2 at 1:800 (Cell Signaling), anti-SCD1 at 1:1000 (Abcam), EGFR at 1:75 (rabbit Abcam) and anti pEGFR at 1:2000 (rabbit Abcam). Normal tissues from various areas of the breast were used as a positive control. For detection, the Envision Dual Labeled Polymer kit (Dako) was used for 30 minutes for anti-species secondary antibody, stained with DAB Chromagen for 5 minutes and then, lightly counterstained with Gill I hematoxylin (Sigma-Aldrich) for 30 seconds before dehydration and mounting. Images were obtained at 20X using an Aperio AT2 ScanScope (Leica Biosystems, Buffalo Grove, IL).

Experiment 2: IC50 values for SSI-4 and Lapatinib were determined though Cell Proliferation Treatment Assays.

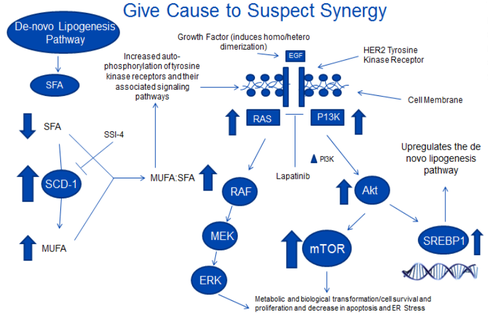
A cell proliferation dose out assay was performed in the identified HER-2 positive breast cancer cell lines with SSI-4 and Lapatinib to determine IC50 values.

When the cell lines were deemed confluent, they were plated at 30,000 cells in 12-well plates in triplicate. SSI-4 was diluted in dimethyl sulfoxide (DSMO) and Lapatinib was diluted in 0.9% saline via serial dilution and treated with their respective treatments. After 96 hours, the cells were trypsinized and counted using a Coulter particle counter. IC50 values of the responsive HER2+ breast cancer cell lines were determined by dosing out Lapatinib and SSI-4 in independent setups. Dose ranges for each drug/compound were 0.001 – 10, 000 nanomolar (nM).

Experiment 3: Demonstrate antitumor synergy with combined SSI-4 and Lapatinib in HER2+ breast cancer cell lines

The Chou-Talalay method was utilized for determining drug combination synergy. It was based upon IC50 concentration quantities using fixed ratios to determine the combination index. (CI< 1=synergistic, CI>1= antagonistic, CI=1= additive). For dose outs, cells were seeded at 30,000 cells/ well in 12-well plates in triplicate. SSI-4 and Lapatinib were independently diluted in dimethyl sulfoxide (DMSO). Cells were independently treated for the IC50 values of each drug, then with the combined drugs for their respective IC50s. Drug treatment was applied at 1:1000. After 96 hours, the cells were trypsinized and counted using a Coulter particle counter. Determination of synergy, additivity or antagonism was based on the multiple drug effect equation of Chou and Talalay and was quantified by the combination index (CI). CI = 1 indicates an additive effect, < 1 is synergy and > 1 is antagonism (Chou and Talalay 1984).

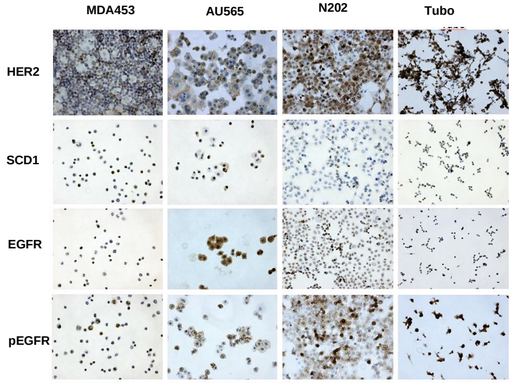
Intertwined HER2 and SCD-1 Signaling Pathways Give Cause to Suspect Synergy



Rationale: SCD-1-induced changes in plasma membrane lipid domains through accelerated MUFA production favor the activation of tyrosine-kinase receptor signaling platforms, an example being the HER2 receptor. HER2 homo/hetero-dimerizes with various HER/EGFR pathways via ligand binding, initiating various signaling pathways that control key cell processes such as angiogenesis, cell proliferation and the cell cycle. In addition, an experiment inhibiting Fatty Acid Synthase (FASN), which is immediately upstream SCD1 in the de-novo lipogenesis pathway, and HER2 demonstrates antitumor synergy in HER2+ Breast Cancer.

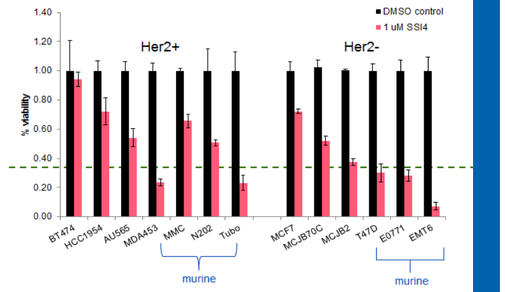
Immunocytochemistry Results

Immunocytochemistry was done to assess the presence of HER2, SCD1, EGFR and pEGFR (phosphorylated EGFR). HER2+ Breast Cancer cell lines were not only selected for the presence, but also the amount of HER2, SCD1, EGFR and pEGFR protein expression as this increased the chance for a targeted molecular therapy to inhibit multiple pathways, primarily the de-novo lipogenesis pathway and the Ras-Raf-MEK-MAPK signaling cascades. These pathways are associated with an increase in proliferation and a decrease in apoptotic mechanisms such as ER Stress, particularly in HER2+ Breast Cancer cells. Eventually, 4 cell lines were selected: 2 human cell lines (MDA453 and AU565) and 2 murine cell lines (N202 and Tubo)



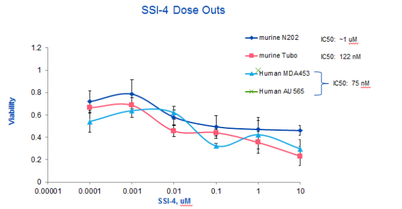
Identification of SSI-4 sensitive cell lines

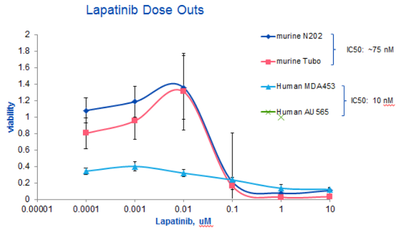
To get an accurate understanding of how the interaction between various metabolic pathways associated with HER2 and SSI-4 manifested from cell line to cell line, HER2 cells for both human and murine cell lines were screened for SSI-4 responsiveness. Cell lines that were HER2+ and responded more than 50% to 1uM of SSI4 would be selected for combination therapy. HER2- cell lines were also screened to compare their SSI-4 responsiveness against HER2+ cell lines.



IC50 determination of Responsive HER2+ Cell Lines

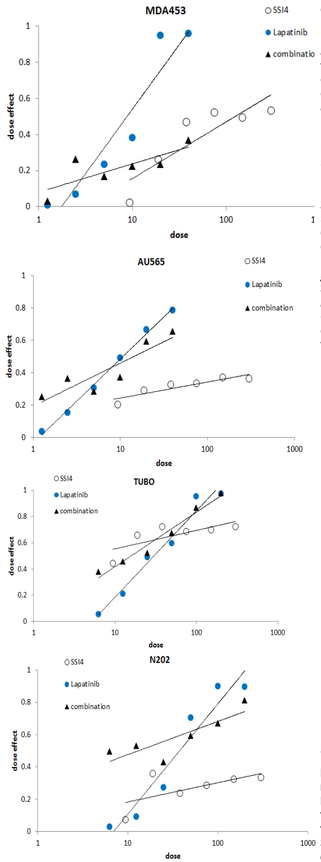
The IC50 values for SSI-4 (SCD1 inhibitor) and Lapatinib (HER2/EGFR inhibitor) were determined by dosing out each drug for each cell line (MDA453, AU656, Tubo, and N202) on a logarithmic scale from 10 to 0.00001.





Drug Combination Therapy to Demonstrate Synergy

Dose response curves were graphed for MDA453, AU565,
N202 and Tubo: these were HER2+ cell lines with the highest responsiveness to SSI-4.

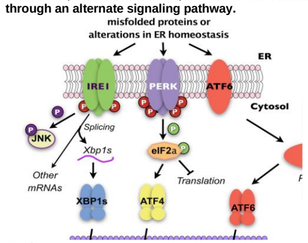


Conclusion/Overarching Implications

Cell lines demonstrated an overall lack of synergy in most cases, with the CI index coming out to be greater than 1, meaning that the combined effect of SSI-4 and Lapatinib induced inhibition turned out to be antagonistic. However, synergistic combinations of Lapatinib and SSi-4 did appear in some combinations. This happened most notably in drug dose outs where drugs were at concentrations, for both drugs, were below 20 nM. However, even though there was an overall lack of synergy, SCD-1 activation was clearly present in HER2+ Breast Cancer, as cancer cells clearly responded to SSI-4 induced inhibition. This is significant as this experiment is added to a growing list of evidence implicating SCD-1's role in the upregulation of various metabolic pathways related to cancer proliferation and survival, and its role in the downregulation of signaling pathways associated with apoptosis.

Future Directions

1) Western blot analysis will be performed to examine ER stress induced proteins indicative of apoptotic cell death. Below, a schematic representation of ER Stress-induced proteins
2) Trastuzumab, a monoclonal antibody that targets specifically HER2, binds to domain IV of the extracellular domain of the HER2/neu receptor, and will be tested in combination with SSI-4 for anti-tumor synergy. If negative like Lapatinib, I will conclude that no synergy occurs in blocking solely SCD1 and HER2 pathways in combination.
3) A replication of the study *Dual FASN and HER2 signaling Blockade Shows Marked Antitumor Activity against Breast Cancer Models Resistant to Anti-HER2 Drugs* will be conducted, instead with SSI-4, to learn if SSI-4 inhibited SCD1 through an alternate signaling pathway.



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