PROTEOMIC PROFILES OF BREAST TUMORS SENSITIVE AND RESISTANT TO TREATMENT WITH TRASTUZUMAB

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INTRODUCTION

Overexpression of human epidermal growth factor receptor 2 (HER-2) accounts for about 25% of breast cancers and is associated with the aggressive and invasive behavior of tumors. Treatment with Trastuzumab, a target humanized monoclonal antibody specific for HER-2 extracellular domain, was approved for this type of tumor in 1998, unfortunately 70% of patients acquire antibody resistance throughout the treatment. In-depth knowledge of the mechanisms of drug resistance and action may allow the development of new therapies and more effective drugs for the treatment of this type of tumor.

METHODOLOGY

The metabolic viability assays in cell lines were measured using the MTT (3- (4,5-dimethylthiazolyl-2) -2,5-diphenyltetrazolium bromide) (5mg/mL), 10 µL/100 µL of culture medium during 2 hours. The reaction was stopped with DMSO and absorbance was measured at 538 nm. The cell metabolic viability test with trastuzumab treatment was done at concentrations of 0, 10, 20 and 50 µg/ml at time intervals of 24, 48, 72, 96 and 168 hours (7 days). The immunofluorescence assays were performed using anti-ErB2 (ab2428, Santa Cruz Biotechnology) and anti E-cadherin (610182, BD Biosciences) primary antibodies. Alexafluor 488 anti-rabbit (A-11034, Molecular Probes) was used as secondary antibody.

RESULTS

The MTT assay showed good correlation between the number of cells and the absorbance measured, so we decided to use this assay to measure the effect of trastuzumab treatment over the cells (Figure 1). The breast cancer cell lines HCC-1954, MCF-7 and SUM-149 PT proliferate independent of the concentration of the drug, especially in the time of 7 days, which agrees with the non-effectiveness of the trastuzumab in these cells. Although the cell line BT-474 had very little proliferation showing a decrease in cellular metabolic viability especially in 3 days treated with 20 µg/mL of trastuzumab (Figure 2). The immunofluorescence assays of HER-2 receptor showed a higher abundance in the cell membrane of HCC-1954 and BT-474 cells and a smaller in SUM-149 PT and MCF-7 as were expected (Figure 3). All the cells tested were positive for E-cadherin (Figure 4).

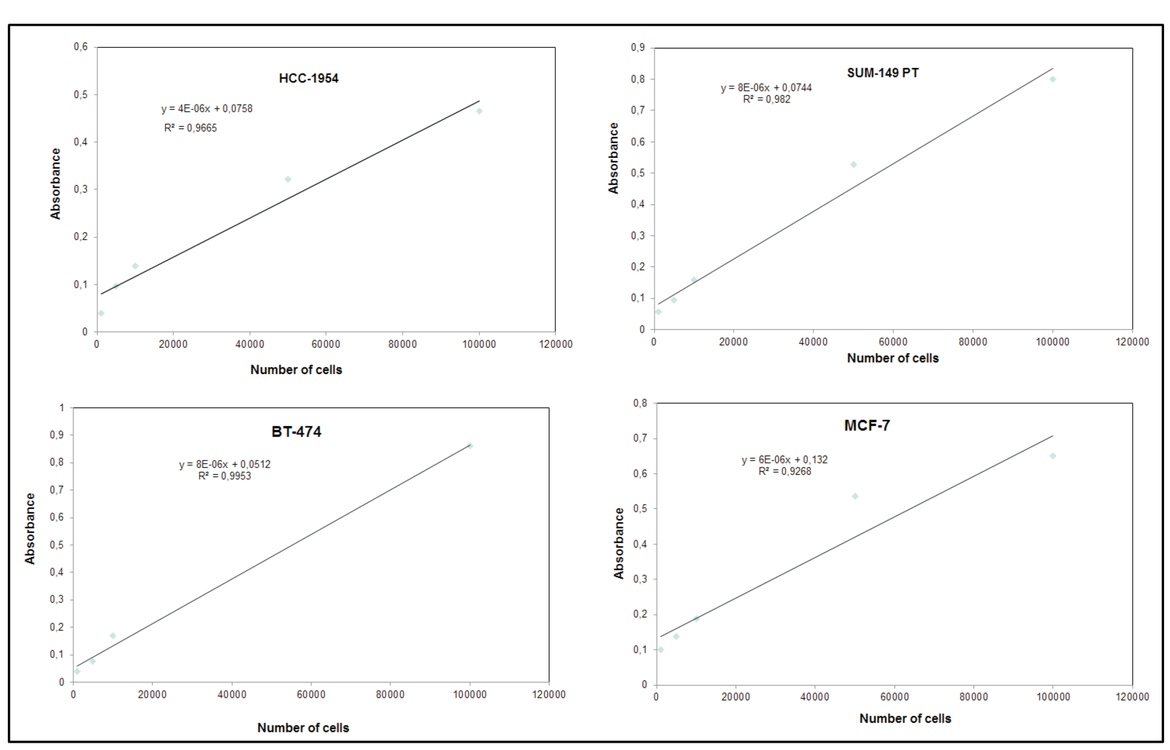

Figure 1- Viability metabolic assay of HCC-1954, BT-474, SUM-149PT and MCF-7 cells, measured with MTT (3- (4,5-dimethylthiazolyl-2) -2,5-diphenyltetrazolium bromide) (5mg/mL) during 2 hours stopped with DMSO and absorbance measured at 538 nm.

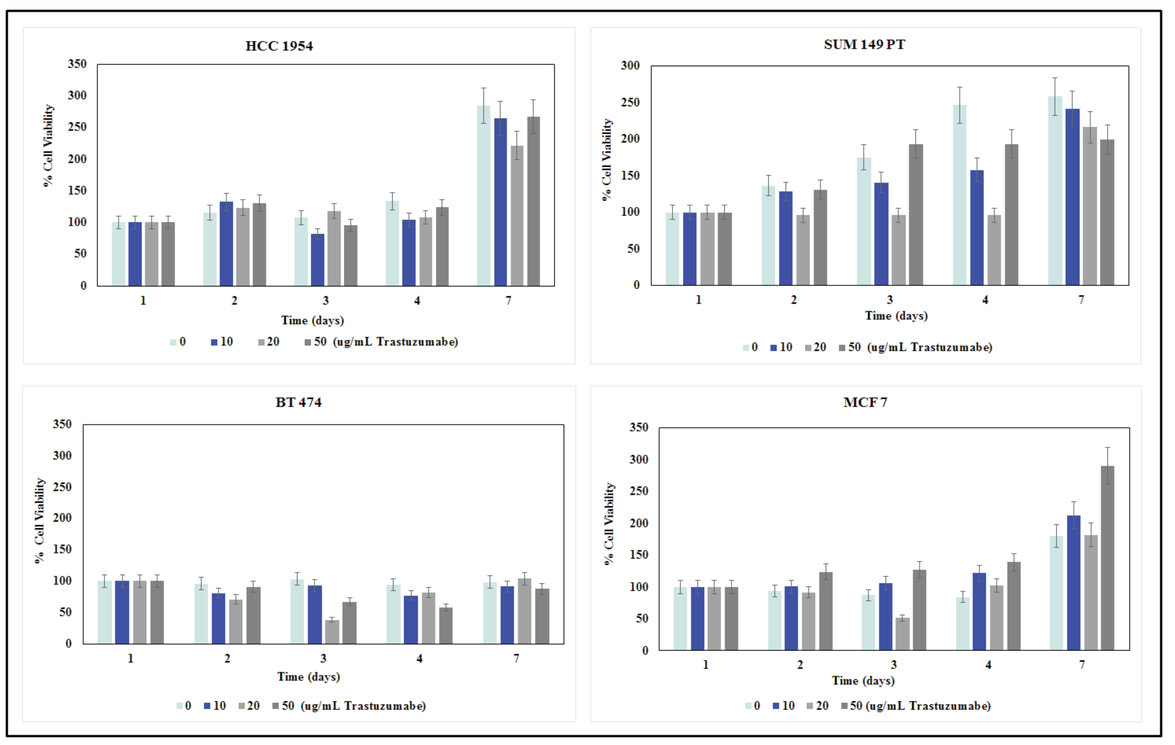

Figure 2 - Viability metabolic assay of cells treated with trastuzumab. The cells were incubated with trastuzumab in concentration of 0, 10, 20 and 50 µg/mL in time intervals of 1, 2, 3, 4 and 7 days.

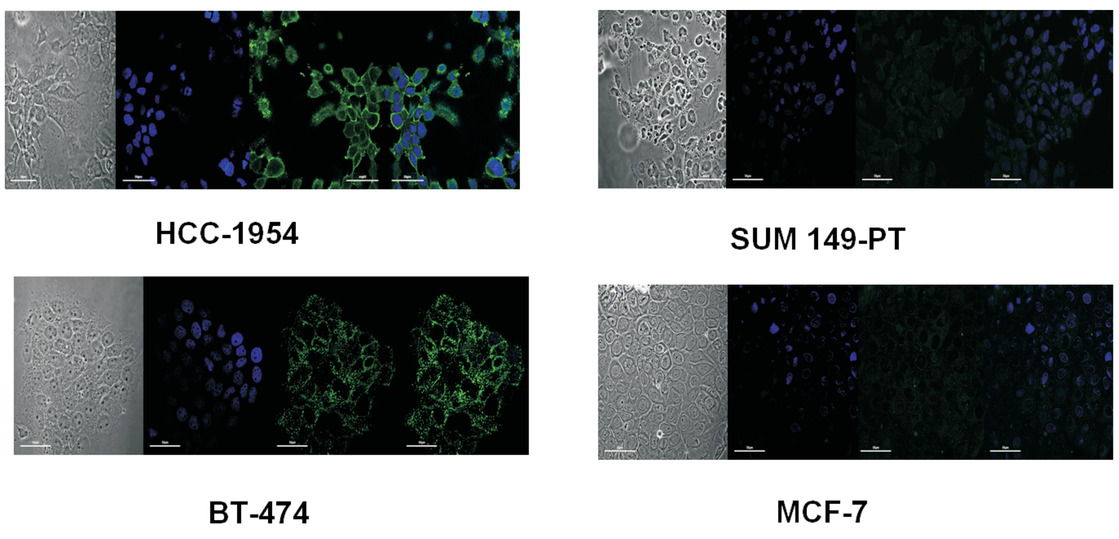

Figure 3- HER2 analysis in HCC1954,BT-474, SUM 149-PT and MCF-7 breast cancer cell lines by immunofluorescence.

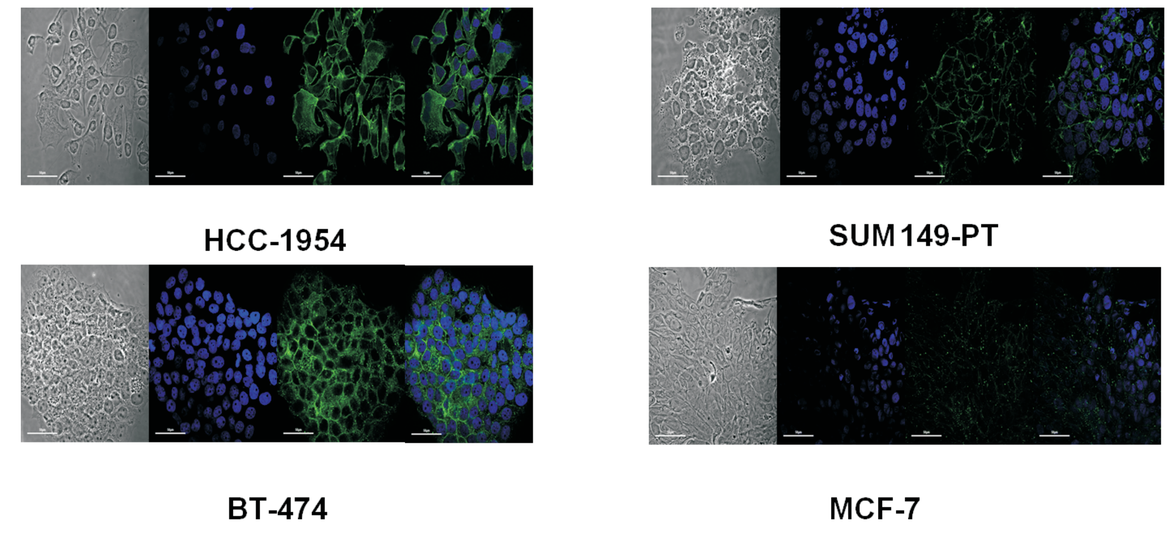

Figure 4- E-cadherin analysis in HCC1954, BT-474,SUM 149-PT and MCF-7 breast cancer cell lines by immunofluorescence.

CONCLUSION

From these preliminary results, we aimed to evaluate the profile of proteins expressed in cell lines resistant (HCC-1954) and sensitive (BT-474) with and without Trastuzumab treatment by proteomic methodologies.

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