Proteomic out-line of Breast Cancer cell line HCC1954: What changes upon Trastuzumab Treatment?

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Background

Breast cancer is the most common type of disease among women, accounting for about 30% of new cases each year in Brazil. HER2 amplification in breast cancer is observed in 20% of these new cases and is associated with aggressive and invasive behavior of tumors. HER-2 belongs to the family of human epidermal growth factor receptors (EGFR or HER-1) which include HER-2, HER-3 and HER-4. Several studies imply that cooperative signaling between EGFR and integrin proteins is a common feature of invasive cancer cells. Integrin proteins are a large family of heterodimers that orchestrate extracellular matrix-cell and cell-cell interactions critical for cell adhesion, polarity and migration. Treatment with Trastuzumab, a target humanized monoclonal antibody specific for the HER-2 protein extracellular domain, has been approved for treatment of HER2+ breast cancer patients however, 70% of them acquire resistance. Proteomic analysis of HCC-1954 a cell line resistant to Trastuzumab treatment can provide an overview of the mechanisms involved in these biological processes. In-depth knowledge of the mechanisms involved in the resistance may enable the development of new therapies and more effective drugs for the treatment of this type of tumor. This work aims out-line the differences between proteomic profile of HCC-1954 treated and untreated with Trastuzumab.

Methods

1-D gel electrophoresis of protein extracts from HCC-1954 treated with 20µg/mL during 72 hours and HCC-1954 untreated cells were prepared. After in gel digestion of protein bands with trypsin, the peptides were analyzed in a synapt-G1 mass spectrometer (Waters, Farmington, MI, USA). Proteins identifications were carried out against the Swiss-Prot databank in the MASCOT search machine (http://www.matrixscience.com). Gene Ontology analyses were done with PANTHER (http://www.pantherdb.org/).

Results

Proteomic analyses rendered a total of 854 proteins identified, 161 unique proteins were from untreated cells and 105 unique proteins were from treated HCC1954 cells. Gene Ontology classification of these unique proteins showed some differences related to HCC-1954 treated and untreated cell line. After trastuzumab effect HCC1954 showed unique proteins that function as translation activity regulators and that participate in biological process of adhesion and cell junction.

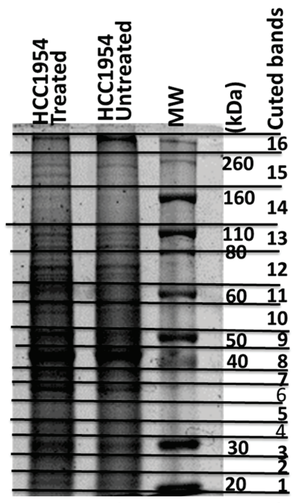

Figure 1-Total protein extract (30µg) from HCC1954 untreated and treated cell line were applied in a 10% SDS-PAGE

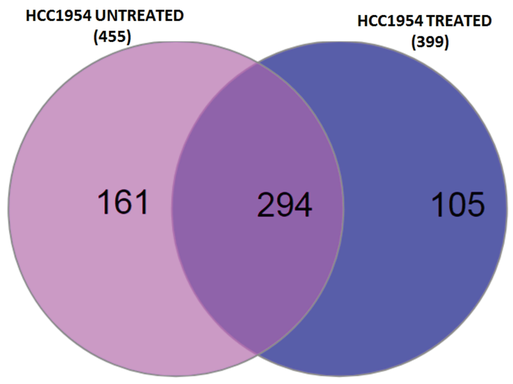

Figure 2- Venn diagram showing distribution of total proteins identified by Proteomic analyses

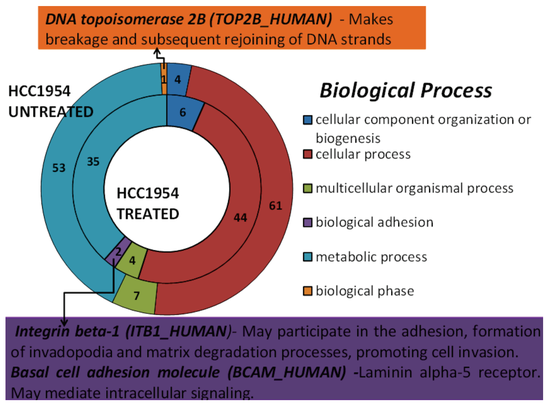

Figure 3 - Gene Ontology Classification in Biological Processes of the unique proteins identified in each group, HCC1954 Untreated or Treated cell line.

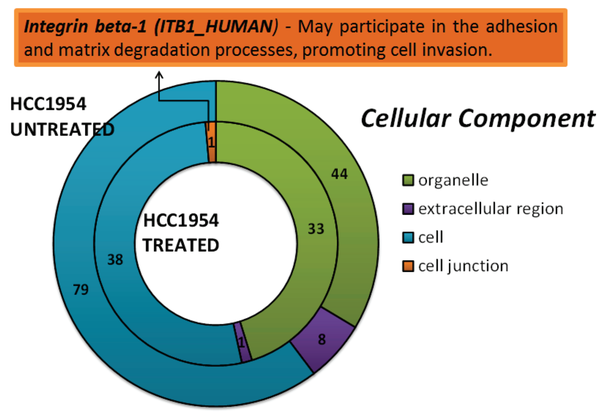

Figure 4 - Gene Ontology Classification in Cellular component of the unique proteins identified in each group, HCC1954 Untreated or Treated cell line.

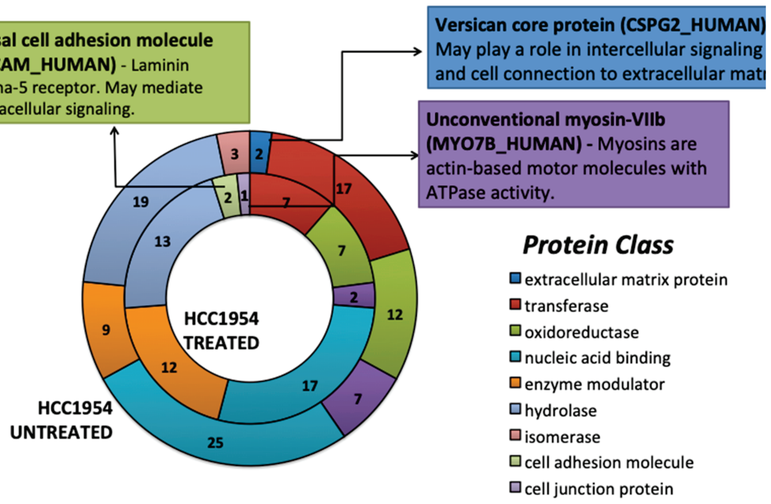

Figure 5 - Gene Ontology Classification in Protein Class of the unique proteins identified in each group, HCC1954 Untreated or Treated cell line.

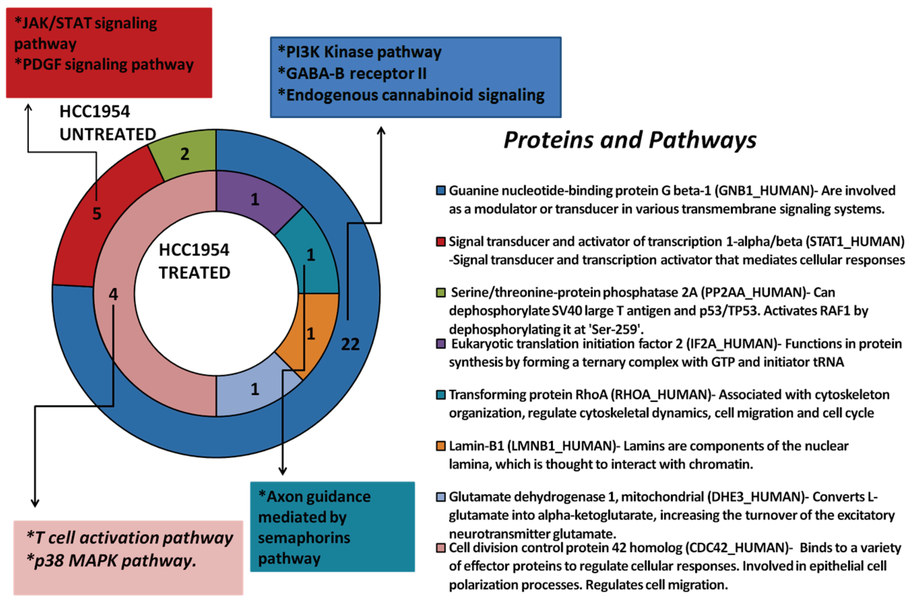

Figure 6 - Gene Ontology Classification in Pathways of the unique proteins identified in each group, HCC1954 Untreated or Treated cell line.

Conclusion

These preliminary proteomic study based on 1D-gel comparing the HCC-1954 cell line treated and untreated with trastuzumab suggested that more protein alterations should be found with other approaches. Further proteomic investigations should be done in order to better understand these mechanisms of resistance.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

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