COMPARATIVE MEMBRANE PROTEOME ANALYSIS OF HCC-1954 AND MCF-7 BREAST CANCER CELL LINES

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INTRODUCTION

Breast cancer aggressiveness is associated with differential protein expression resulting in poor outcome, disease recurrence and death. The molecules closely associated with these processes are predominantly present at the cell surface. The HCC1954 is a hormone receptor negative, HER2 positive, poorly differentiated cell line. MCF7 is differentiated cell line that expresses estrogen receptor and is negative for HER2 receptor. We aimed to compare and characterize the relative quantification of proteins present in both HCC1954 and MCF7 cell lines that can be involved in invasive ability and metastases.

METHODOLOGY

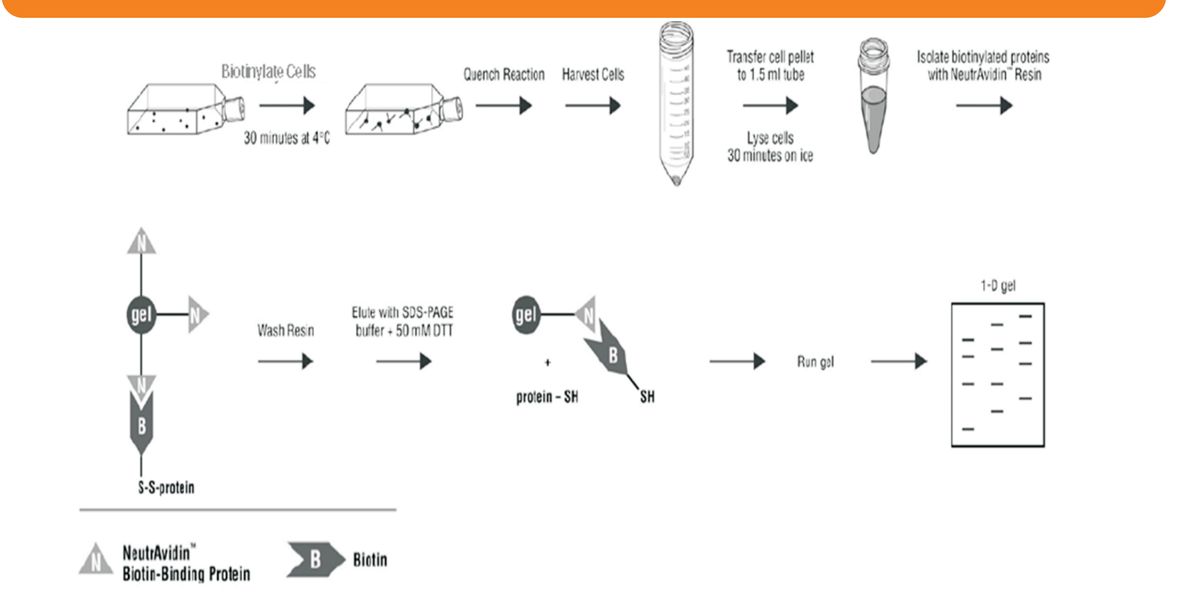


Figure 1 - Membrane proteins were biotinylated and fractionated using the cell surface protein isolation kit (Pierce®).

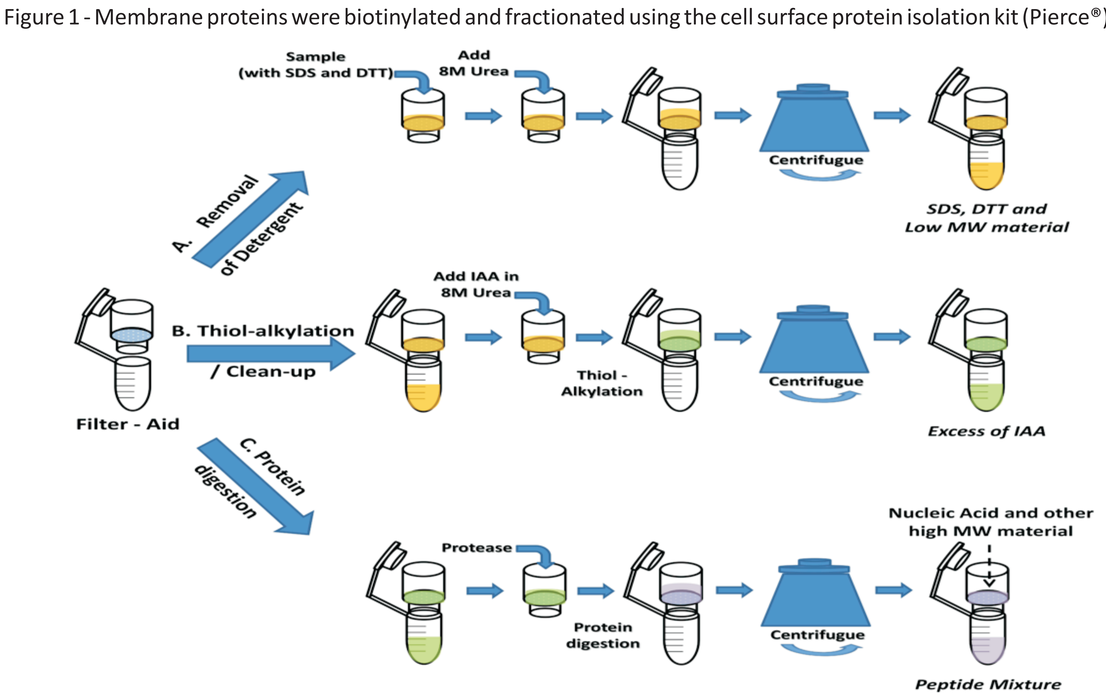


Figure 2 - Workflow of the filter aided sample preparation (FASP). (A) Removal of detergent. (B) Thiol alkylation and protein clean-up. (C) trypsin/LysC protein digestion and isolation of peptides.

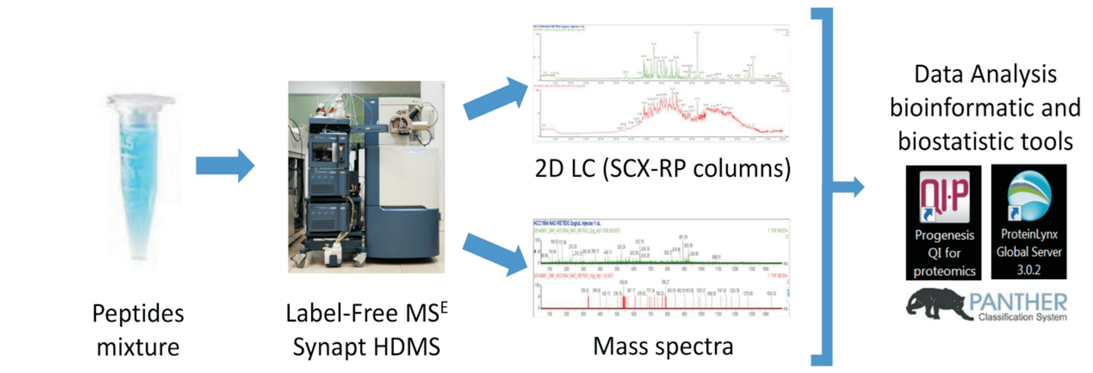


Figure 3 - The peptides were analyzed by label free LCMSE on a 2-D SCX/RP chromatography system coupled to a Synapt HDMS mass spectrometer (Waters, Farmington, MI, USA). For mass spectra processing and data analyses, ProteinLynx Global Server v.2.5 and Progenesis QI software v. 2.0 were used. Gene ontology classification was done with Panther software V 10.0 and the visual account of protein investment in cellular functions was shaped with Proteomaps software. For protein validation immunofluorescence, western blot and flow cytometry analysis were done.

CONCLUSION

Label free MS^E allowed the relative quantification of membrane proteins that was higher in HCC1954 than in MCF7 cell line. Many of these molecules, such as α-v, β-1, cadherin-1 play important roles in cell migration, cell adhesion, cell junction and cell-cell interactions. Moreover, the higher expression of α-v and β-1 integrin in HCC1954 should be investigated as this cell line share HER2 superexpression and shows resistance to trastuzumabe treatment.

RESULTS

More than 1200 proteins of the HCC1954 and MCF7 breast cancer cell lines were identified. Among them CD44, CD166, integrins α-2, α-v, β-1, cadherin-1 were more expressed in HCC1954 than in MCF7 cell line. HER2 was identified as unique protein of HCC1954 cell line as was expected, and this was confirmed by immunofluorescence and western blot. Panther gene ontology analysis showed that integrin pathway was enhanced in HCC-1954 cell line in comparison with MCF-7 cell line. The differential expression of integrins α-v and β-1 were validate by flow cytometry. HCC1954 showed a higher expression of α-v and β-1 integrins. These integrins have been related to the metastatic capacity and treatment resistance of HER2-positive tumors.

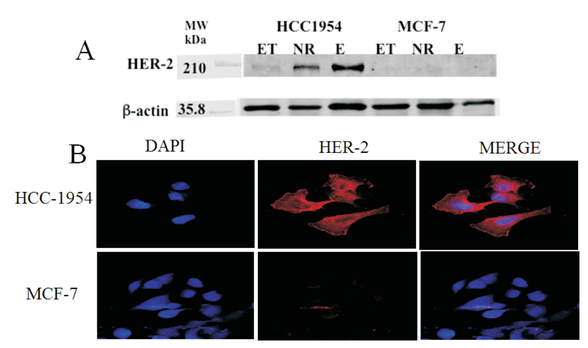


Figure 4 – HER2 analysis in HCC1954 and MCF-7 breast cancer cell lines by western blot (A) and by immunofluorescence (B).

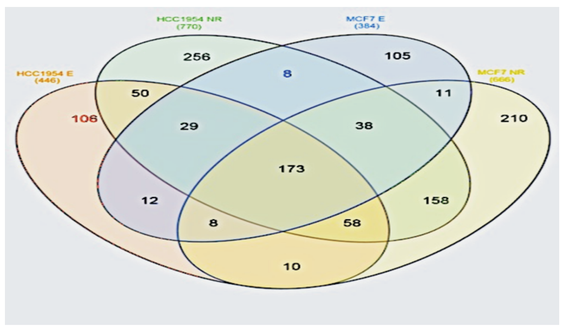
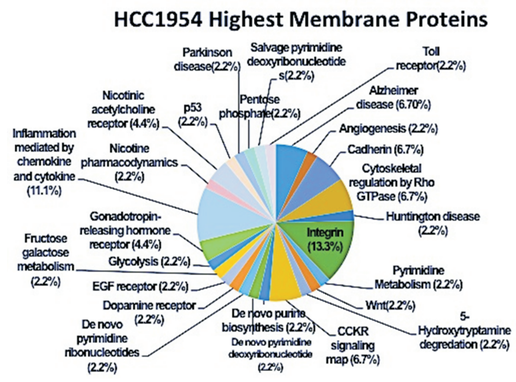


Figure5- Distribution of the proteins identified and quantified in the eluted and flow-through fractions of HCC-1954 and MCF-7 breast cancer cell lines using the cell surface protein isolation kit (Pierce®).



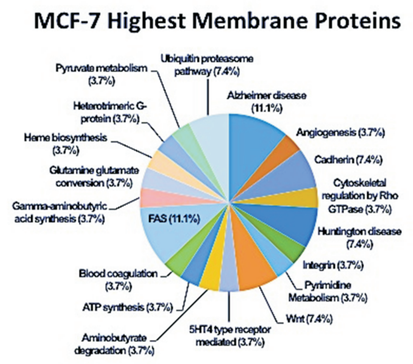


Figure 6 - Gene Onthology pathways mapping according to Panther tools (http://pantherdb.org/) of the highest membrane proteins quantified in HCC-1954 and MCF-7 breast cancer cell lines.

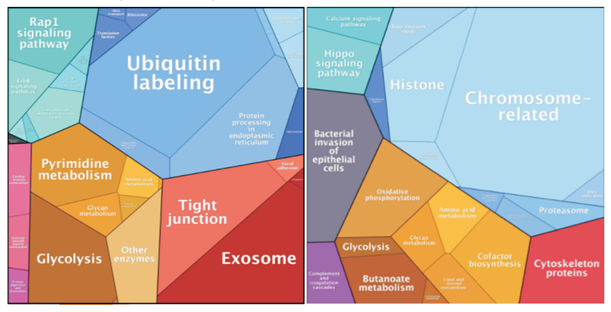


Figure 7 - Visual account of membrane protein investment in cellular functions shaped with Proteomaps software in (A) HCC-1954 and in (B) MCF-7 breast cancer cell lines. (https://www.proteomaps.net/)

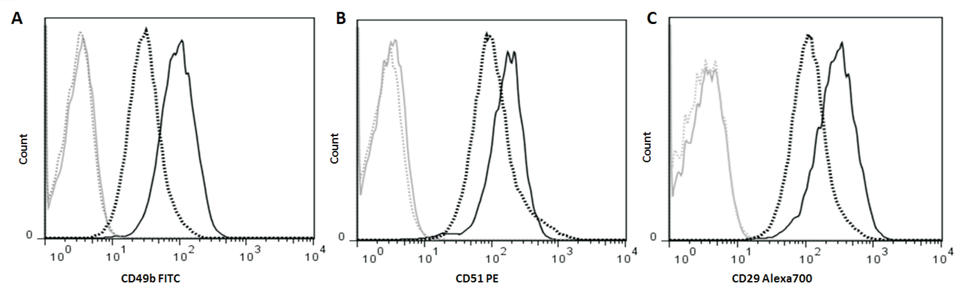


Figure 8 - Phenotype of HCC-1954 (black solid line) and MCF-7 (black dotted line) human breast cancer cell lines. (A-C) Histograms showing the expression of CD49b, CD51 and CD29 integrins.in HCC and MCF-7 cell lines. Data are representative of 3 independent experiments.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

Financial Support CNPq FAPERJ Portal do Programa de Oncobiologia