Characterization and quantitative proteomics of mammospheres-forming cells of triple negative breast cancer

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Abstract

Breast cancer is a leading cause of death in women worldwide. Although several therapeutic approaches are combined to successfully cure this disease, some cancer cells can escape and are recurrent, especially in triple negative breast cancer (TNBC). Several lines of evidence suggest that cancer cells which possess stemness-related characters are contributed to drug resistance thereby leading to cancer relapse. Thus, this study aims to establish breast cancer cell – derived mammospheres to investigate its stemness properties and identify key protein changes using a label-free proteomics approach. In this study, mammospheres formation was assay using a TNBC cell line, MDA-MB231. MDA-mammospheres showed upregulated stemness-related genes. Moreover, these mammospheres demonstrated a significant resistance to paclitaxel, a first-line chemotherapy medication for patients with TNBC, compared to that of their parental cells, MDA-MB231. Therefore, protein identification and quantification were performed using a nano-LC coupled with Orbitrap mass spectrometer (LC-MS/MS) and label-free quantitative analysis (Progenesis QI), respectively. Then, almost 200 proteins were identified and compared. With a cut-off value of > 1.5 fold change, there were 37 and 10 proteins that up-regulated and down-regulated in MDA-mammosphers, respectively. Furthermore, the levels of some selected proteins were confirmed by westernblotting. Lastly, all altered proteins were subjected to protein-protein interaction network analysis by STRING database. Several altered proteins expressed in mammospheres were categorized into many biological processes including regulation of cell death, cell activation involved in immune responses, and cytoskeletal reorganization. Collectively, these proteins and predicted molecular pathways enlighten potential markers and therapeutic targets within stemness-possessed breast cancer mammospheres.This study was supported by the Chulabhorn Research Institute (Grant no. 302-2098).

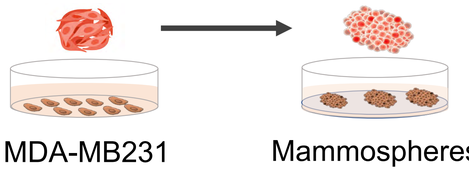
Background

Triple negative breast cancer (TNBC) is the most aggressive subtype of breast cancer and the major cause of women worldwide

Drug resistance and **cancer recurrence** were considered to associate with **stemness** properties in cancer cells which allow cells to re-propagate and survive

The aim of this study is to establish a TNBC stem-like cell line (mamospheres) and to study its molecular characterization and proteomic pattern.

Materials & Methods



2 × 10[6] MDA-MB231 cells were cultured in poly-HEMA coated 6-well plates. Mammocult medium (StemCell Tech.) were used. Cells were passaged every 7 days.

- **Stemness markers validation** using **qRT-PCR** quantitative analysis
- **Cell viability** test with Paclitaxel treatment using **MTS assay**

- **Label-free LC-MS/MS** analysis by nano-LC coupled with **Orbitrab MS**.
- Peptide ions were quantitated and identified using **Progenesis QI** and **Byonic** searched from UniProt database (Human Proteome UP0005640).
- **Protein-Protein interaction** was conducted from STRING database.

Results

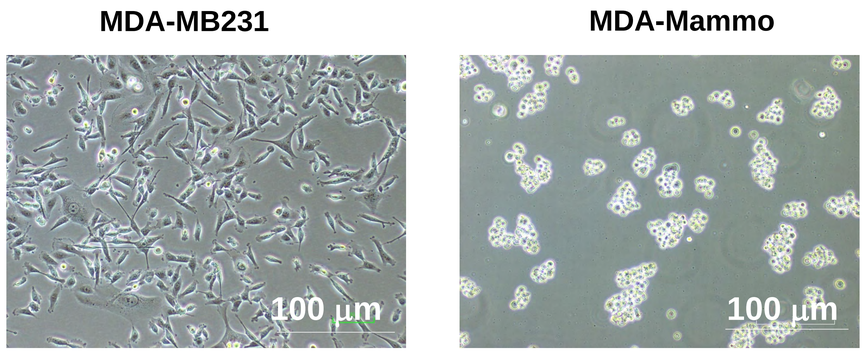


Fig. 1 Mammospheres (MDA-Mammo) derived from MDA-MB231. The spheres were cultured for 7 days and imaged using inverted microscope at 100X magnification.

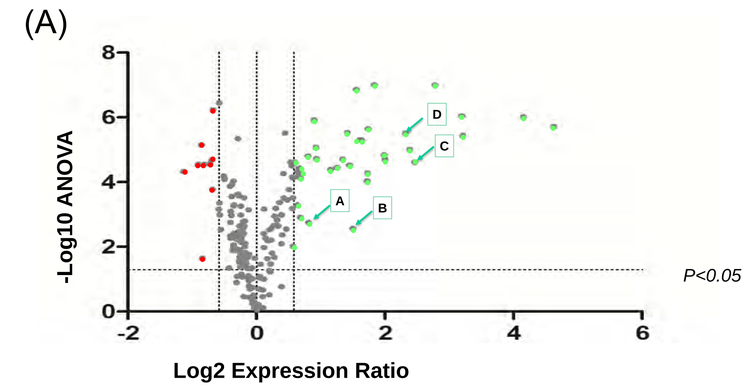


Fig.4 Volcano plot of proteins obtaining from Label-free Proteomics analysis and validation of identified proteins. (A) The differential expressions of proteins were plotted using GaphPad Prism V.5. The x-axis represents $\log_2$ fold changes of proteins and the y-axis represents $-\log_{10}$ *p-values*. The red and green dots indicate proteins with significantly different expression of +1.5 and -1.5 fold-change and *p-values* < 0.05, respectively. (B) Westernblotting of some selected proteins, **protein A, B, C, and D**. β-Actin was used as a protein loading control.

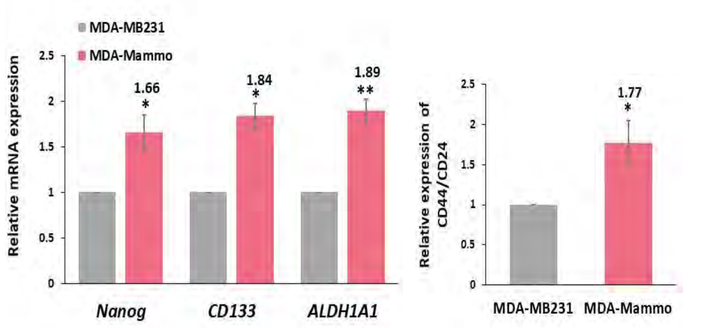


Fig. 2 mRNA expression of breast cancer stem cells and stemness markers. The relative mRNA expression of indicated genes in MDA-Mammospheres in comparison to those of MDA-MB231. All data were normalized by *RPS13* as a reference gene.
* = $p < 0.05$ and ** = $p < 0.01$.

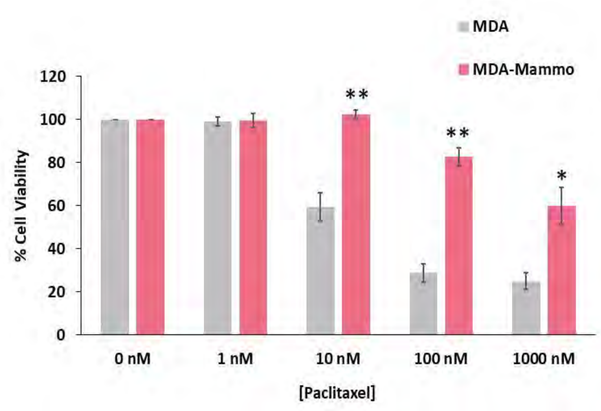


Fig.3 Cell viability test for drug resistance study with Paclitaxel treatment. MDA-mammo showed a significant resistance to Paclitaxel at high doses (>10 nM) compared to those of MDA-MB231. * = $p < 0.05$ and ** = $p < 0.01$.

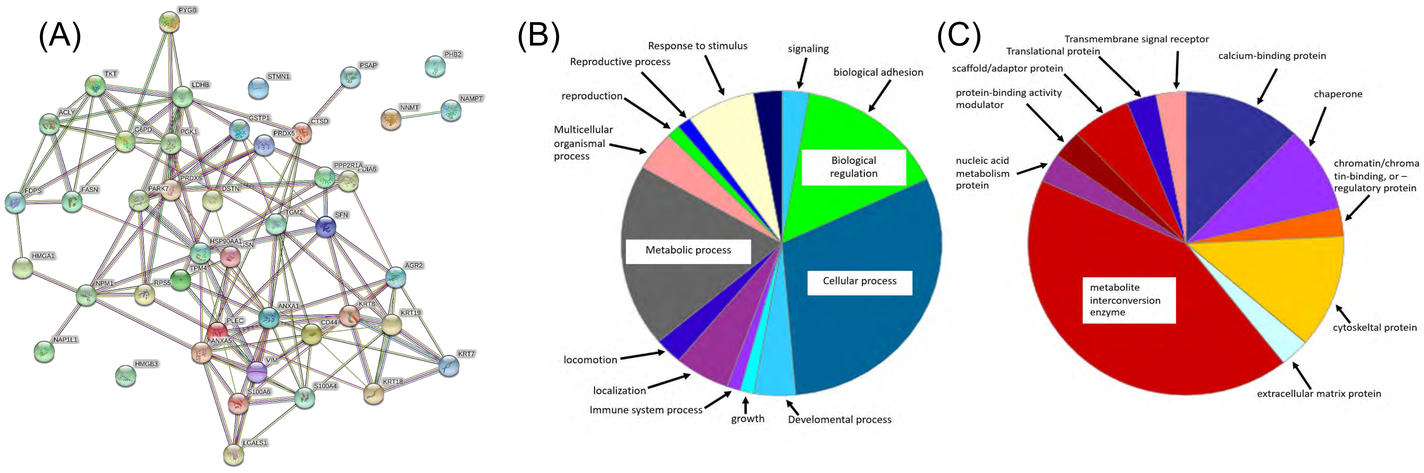


Fig.5 Protein network analysis of 44 proteins altered in MDA-Mammospheres. Proteins with their expression alterations more than 1.5 fold change were analyzed using (A) STRING and clustered according to their (B) biological processes and (C) protein classes using PANTHER Classification System.

Summaries & Conclusions

- ❑ We found that mammospheres-forming cells derived from a TNBC cell line, MDA-MB231 demonstrated stemness potency.
- ❑ We showed that stemness-related markers were significantly up-regulated in MDA-mammospheres, and the cells showed a significant resistance to paclitaxel, a first line drug for patients with TNBC.
- ❑ Label-free proteomics analysis revealed a total of 44 proteins significantly altered in MDA-mammospheres. These altered proteins were majorly involved in cellular processes, biological regulations and metabolic processes and most were reported to play important roles in cancer stem cells and relapses as well as drug resistance.
- ❑ Collectively, these data demonstrate a breast CSC model and its altered proteins which would be targets for biomarkers and treatments of breast cancer stem cells with aggressive phenotypes.