ABSTRACT

Breast cancer (BC) accounts for 12% of cancer incidences in 2020 and a total of 658,000 deaths worldwide presumably due to recurrence caused by BC stem cells (BCSC). BCSC presence in bulk tumor population has long been characterised by the expression of stemness biomarkers, including CD133 and ALDH1, that eventually initiate resistance and metastasis. Based on the existing problems, a search for BCSC-targeted therapy falls to a steroidal glycoalkaloid compound called α-Solanine with reported BC antiproliferation activity *in vitro* and *in vivo*. α-Solanine presents therapeutic prospects to combat BCSC by modulating the metastasis and resistance gene expressions and cellular activities. Therefore, this research aims further to identify the potential target hub-genes of α-Solanine against BCSC and analyse the cytotoxic effect of α-Solanine towards BCSC.

This research was conducted simultaneously through bioinformatic and *in vitro* studies. Initially, gene screening was performed using GeneCards, CHEMBL, SwissTargetPrediction, SEA, TargetNet, PubChem, and SuperPred, followed by acquiring intersecting genes using Interactivenn. Functional enrichment was studied through Shiny GO 0.8 while protein-protein interaction (PPI) and hub-gene network analysis were conducted using STRING and Cytoscape with Cytohubba plug-in respectively. Moreover, genetic alterations of top 10 hub-genes were further analysed using cBioportal. Following the bioinformatic study, the anticancer activity of α-Solanine against BCSC was assessed through *in vitro* assay using MCF7 mammosphere. After generating the MCF7 mammospheres (3D cell culture), BCSC characterization was conducted using RT-qPCR to compare the mRNA expression of *CD133* and *ALDH1* in 3D cell cultures relative to the 2D. Afterwards, MTT cytotoxic assay was performed to obtain IC_{50} values and % cell viability after α-Solanine treatment in both cell models. Statistical analysis was carried out using unpaired t-test to conclude the data's significances.

From the series of bioinformatic procedures, top 10 hub-genes of α-Solanine against BCSC were *STAT3*, *HSP90AA1*, *ESR1*, *TNF*, *KRAS*, *HIF1A*, *NFKB1*, *RELA*, *PTK2*, and *RAF1*. From the top 10 hub-genes, PTK2, KRAS, RAF1, and HIF1A proteins were found to be closely interacted with CD133 and ALDH1. Afterwards, BCSC were successfully enriched *in vitro* by growing MCF7 mammosphere (MS) or 3D cells in non-adherent plates. *CD133*, one of the BCSC markers, was overexpressed ($p<0.1$) in MCF7 MS relative to the MCF7 2D, indicating MCF7 MS ability to represent BCSC. Despite having IC_{50} higher in MCF7 MS, the cytotoxic effect of α-Solanine was significantly lower in MCF7 MS compared to MCF7 2D at concentrations of 10 µM ($p<0.05$) and 20 µM ($p<0.1$). Therefore, it is concluded that α-Solanine potentially inhibited BCSC in MCF7 MS.

Keywords: Anticancer, α-Solanine, Breast cancer stem cells (BCSC), Bioinformatic, Mammospheres