Glutamate, glutamine, cystine, glutathione, and xanthine biomarkers correlation with their genes expression in Tamoxifen-resistant MCF-7 cells

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Abstract

The development of tamoxifen resistance in breast cancer cells is a significant clinical challenge. Metabolites and amino acids play a crucial role in the progression of cancer and drug resistance. Based on a previous research, significant intracellular changes were observed in the concentration of glutamate, glutamine, cystine, glutathione and xanthine, so, in this thesis, a developed LC-MS/MS method was used to investigate the concentrations of (glutamate, glutamine, cystine, glutathione and xanthine) in the supernatant media of MCF-7 tamoxifen-resistant cells, the gene expression of the metabolites transporter was quantified by using real-time PCR, and then correlate the results of resistant cells with control cells .

For glutamate, glutamine, cystine, glutathione, and xanthine, the LC-MS/MS validation results showed very good linearity (R^2= 0.9998, 0.9998, 0.9969, 0.9989, and 0.9991) and high sensitivity with LOD values of 1, 20, 10, 0.5, and 0.2 mg/L and LOQ values of 10, 200, 100, 5 and 2 mg/L, respectively. Excellent intra-day accuracy results and percent recovery were obtained in the range of 95.40% – 104.64% and 93.2% - 98.4%, respectively. Furthermore, precision values obtained were very good and ranged between 0.174 to 1.363 %RSD.

As a result of acquiring resistance to tamoxifen on the cellular level of MCF-7 cells, there was a significant transition in morphological shape from epithelial phenotype into mesenchymal phenotype. Also, the concentrations of glutamate, glutamine, cystine, glutathione, and xanthine were altered in tamoxifen-resistant MCF-7 cells. Our study also has shown that the gene expression of SLC1A2, SLC1A1, SLC2A9, SLC7A11, ABCC1 and SLC1A5 was altered in tamoxifen-resistant MCF-7 cells. The decreased expression of SLC1A1and SLC1A2 may contribute to the altered glutamate metabolism observed in tamoxifen-resistant cells, while the decreased expression of SLC7A11 may contribute to the altered cystine and glutathione metabolism observed in these cells. The increased expression of ABCC1 may contribute to the altered glutathione metabolism observed in these cells and finally, the decreased expression of SLC2A9 may contribute to the altered xanthine metabolism observed in these cells. The data obtained in this thesis will contribute in understanding the mechanisms of tamoxifen resistance and help in finding methods to lower the breast cancer cell from acquiring the resistance.