

A proteomic approach for the study of estrogen regulation in ZR-75.1 breast cancer cell line

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The most frequent cause of carcinoma related mortality in women is breast cancer and it is the first in absolute between the age of 35 and 55. Estrogen hormones and in particular estradiol (17 β -estradiol) promote the growth and the survival of the breast cancer's cells. A number of estrogen-regulated proteins with different expression pattern has been characterized by two dimensional gel electrophoresis analysis in MCF-7 cell lines. [1] The molecular basis of the mechanisms of estrogen-regulation of cell cycle progression in human breast cancer cell lines has also been investigated. [2] There are several estrogen-responsive proteins which have been identified so far such as HSP, BMPs, Cathepsin D, pS2 etc. but a more comprehensive picture which could provide new insights of the molecular mechanisms of the estrogen-regulated cell functions and signaling of the estrogen regulated proteins is still not available. Clinical significance of finding new estrogen-regulated proteins is to introduce new markers which can improve the prediction accuracy of estrogen receptor (ER) status.

Here we present our preliminary results obtained on the ZR-75.1 cell lines growth with and without estrogen (17 β -estradiol) using a general proteomic approach. Extraction of the whole protein content was carried out using standard protocols which include trypsinisation of the cell monolayer and cellular lysates using different buffers. 2-D gel electrophoresis with coomassie staining was used to separate and visualize proteins. Resulting 2-D gels were normalized and compared, providing data on matching spots, densitometry, approximate molecular weight and isoelectric point. Based on 2-D gel maps, changes in relative abundance of the proteins was monitored at selected time intervals after estrogen stimulation resulting in protein profiling curves and correlation diagrams. Spots corresponding to target proteins were selected for further mass spectrometric identification. Target proteins were analyzed in an automatic manner. Depending on the nature and the quantity of the protein, two different mass spectrometric approaches were applied in the analysis of the peptide mixtures: monoisotopic peptide mass fingerprinting by MALDI-MS and sequence-tag by micro-HPLC-ESI-MS/MS survey scans.

Acknowledgement

This work was supported by the Italian Government "Ministero dell'Istruzione, dell'Università e della Ricerca" Fondo per gli Investimenti della Ricerca di base, project RBNE0157EH.

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