Proteomic analysis of differential proteins in colon cancer DLD-1 cells: Effects of GRP78 knock-down by stable RNA interference

Hung-Yen Wang[#], Huey-Chun Huang*

Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung, Taiwan

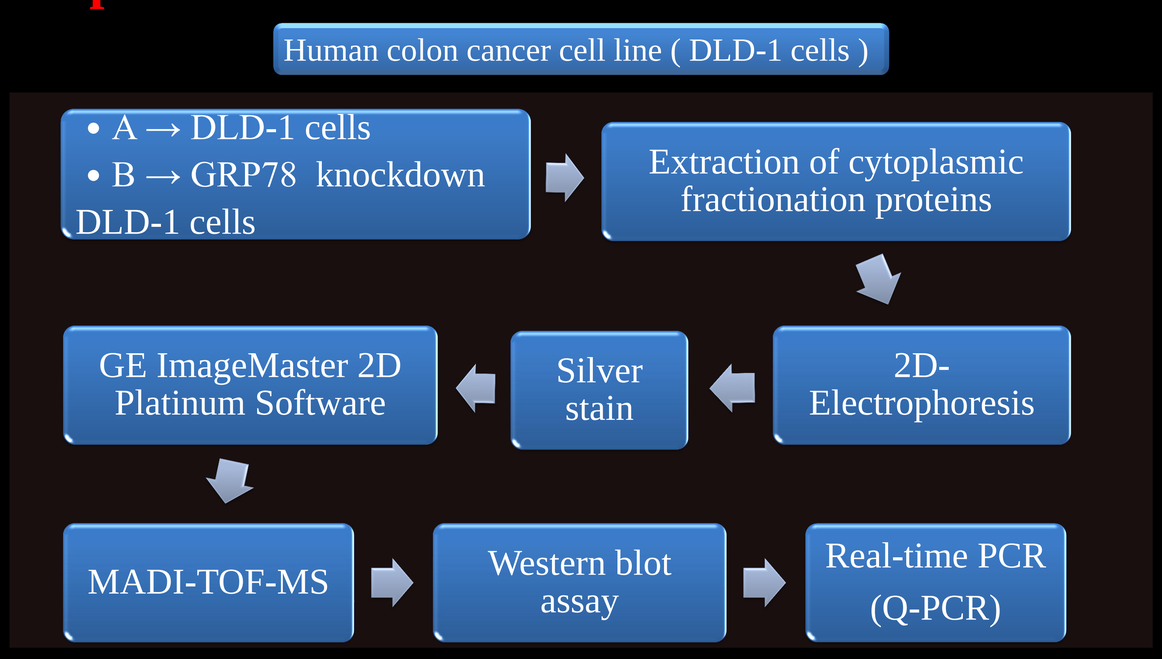
Abstract

Proteomic technology has been applied to early detect colorectal cancer, perform accurate clinical diagnosis, or in the development of novel drugs. There are many human colorectal cancer related proteomic studies in the literature. The proteomic database of human colon carcinoma cells (DLD-1) has been established in 2005 by Cancer Research Center in Japan.

Glucose-regulated protein 78 (GRP78), also known as immunoglobulin heavy chain binding protein (BiP), is an endoplasmic reticulum chaperone protein belongs to the heat shock protein 70 family. The major roles of GRP78 in the regulation of ER function include protein folding and aggregation, and targeting misfolded proteins for proteasome degradation. Moreover, GRP78 plays an important role in embryonic development and tumor progression. Many studies have shown increased levels of GRP78 expression in malignant metastasis or drug-resistant colon cancer cells. Besides, some anticancer drugs such as fluorouracil or paclitaxel which increased the oxidative stress lead to cancer cells cytotoxicity, meanwhile, these anticancer drugs would also induce a rise in GRP78 and promote cancer cells growth rapid.

In our study, the proteomic analysis of GRP78 effect on cancer cells was performed by two dimensional polyacrylamide gel electrophoresis and matrix-assisted laser desorption ionization-time of flight mass spectrometry in GRP78 knockdown DLD-1 cells. Then the differentially expression proteins of cytoplasmic fractionation will be further confirmed by real time PCR and Western blot assays.

Experimental workflow



Results

1.Representative 2-DE protein patterns of cytoplasmic fractination from DLD-1 (A) and GRP78 knockdown DLD-1 (B) cells.

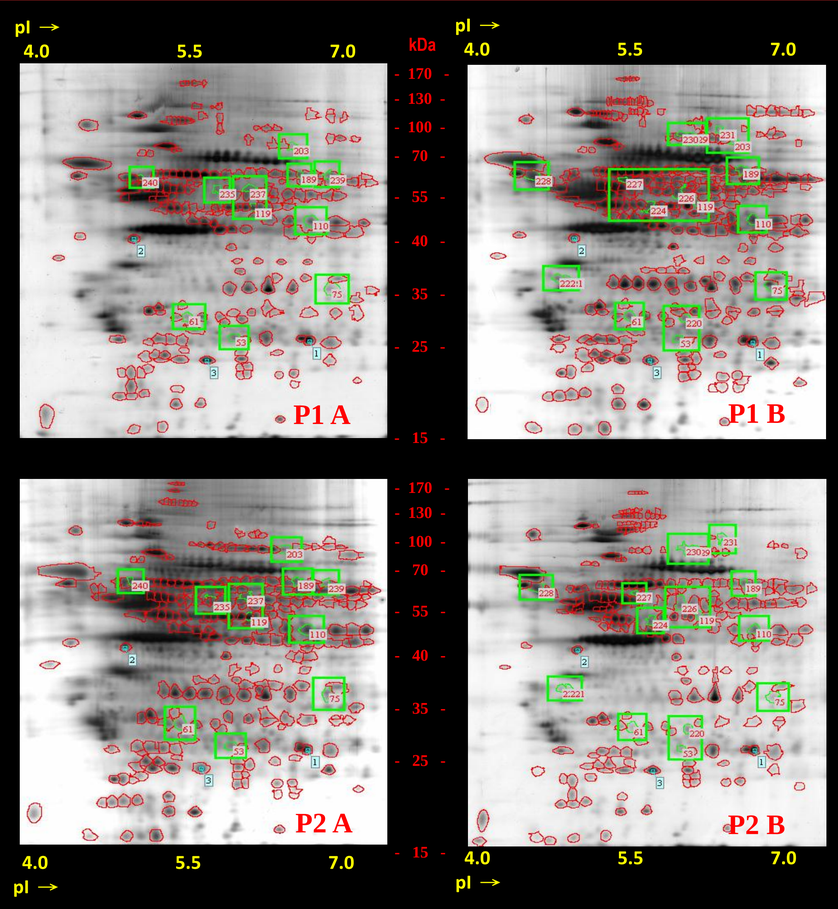


Table 1. Detection of the match proteins number was compared with reference gel.

Gel name	*Spots	Match spots number	**Match rate	Differentially expressed spots number
P1 A	227	174	77%	9
P1 B	305	232	84%	B class reference gel
P2 A	276	233	76%	A class reference gel
P2 B	255	175	69%	16

**Four gels analysis by GE ImageMaster 2D Platinum Software

**Gel match rate > 60 %

Table 2. Differentially expressed proteins in GRP78 knockdown DLD-1 cells (B) compared with DLD-1 cells (A).

Match ID	A	B	Anova (p<0.05)
119	2.77332	-2.77332	0.00529408
110	2.00932	-2.00932	0.0203333
189	-1.80814	1.80814	0.020757
53	1.95	-1.95	0.0217007
75	1.49517	-1.49517	0.02652
203	7.32523	-7.32523	0.0404752

Figure 1.
The 2D images of cytoplasmic fractionation proteins extracted from DLD-1 cells (P1 A , P2 A) and GRP78 knockdown DLD-1 cells (P1 B , P2 B) . 2-DE analysis were performed by using linear IPG strips pH 4-7, followed by 10% SDS-PAGE and were detected by silver staining. Numbered spots indicate the MADI TOF MS/MS analysed proteins as listed in Table 1. The total number of spots observed is approximately 200-300. Of the 9 differentially expressed spots in P1 A , 16 different proteins in P2 B were detected by GE ImageMaster 2D Platinum.

2. Enlarged regions of images of DLD-1 cells (left panels) and GRP78 knockdown DLD-1 cells (right panels) are shown, with the matched spots of interest green highlighted.

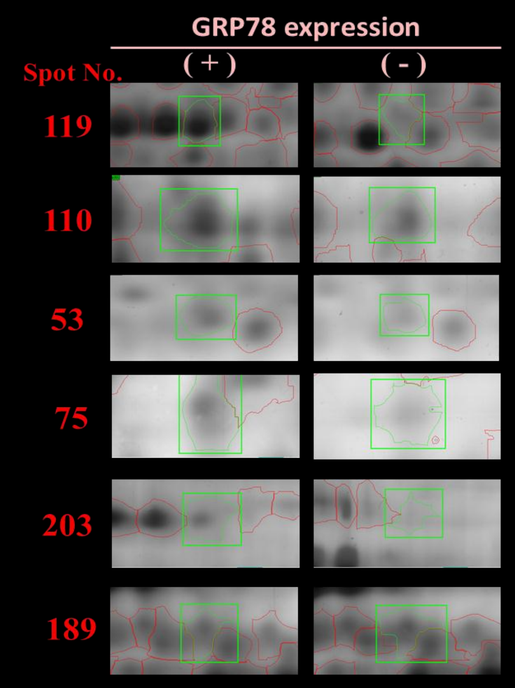


Figure 2.
The 2D images of the DLD-1 protein expression with (+) and without GRP78 expression (-) are summarized. Six protein spots were identified by GE ImageMaster 2D Platinum Software.

3. Analysis GRP78 protein expression by Western blot assay.

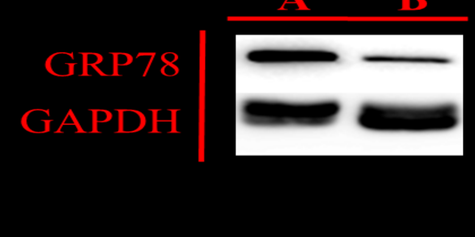


Figure 3.
Silence of GRP78 expression in DLD-1 cell line (B cells) was examined by Western blot.

Conclusion

1. We observed that 9 differentially expressed spots in DLD-1 and 16 different proteins in GRP78 knockdown DLD-1 cells .
2. Using proteomic analysis, we demonstrate quantitative differences in the expression of 6 proteins of potential interest in GRP78 knockdown DLD-1 cells compared mock-transfected control.
3. In the ongoing study , we will identify protein ID by MALDI-TOF/TOF and confirm or correlate with gene expression wherever possible using real time PCR or by Western blot.