TWO-DIMENSIONAL MOLECULAR PROFILING OF MULTIPLE MYELOMA

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BACKGROUND

Multiple myeloma (MM) is a B-cell malignancy characterized by proliferation and accumulation of B lymphocytes and plasma cells, secreting monoclonal immunoglobulins, in the bone marrow and, less frequently, at extramedullary sites.

Proteome analysis of clinical samples aims at characterizing disease-specific changes in the protein expression profile of an affected cell. Such changes could serve as potential diagnostic markers.

Two-dimensional polyacrylamide gel electrophoresis (2-DE) is widely used in comparative studies of protein expression levels. The potential of this method is strongly dependent on good sample preparation, in order to obtain proteomic maps with good reproducibility and resolution.

AIMS

The aim of this study was to optimize two-dimensional electrophoresis (2-DE) conditions for separation of human myeloma proteins.

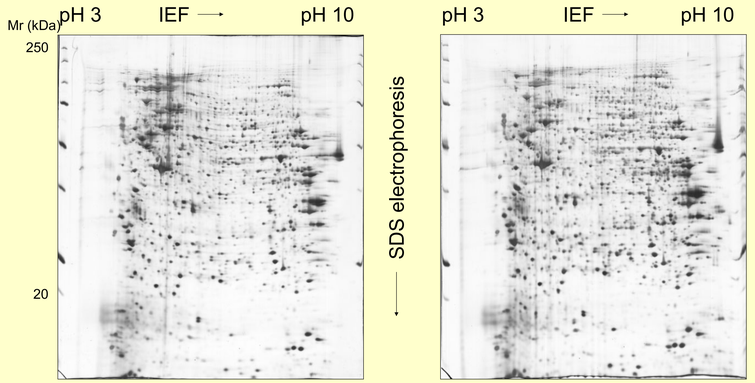
METHODS

We have compared two different solubilization buffers, we also evaluated protein precipitation with ethanol (EtOH) and optimized 2-DE conditions for human myeloma proteins. Myeloma cell line ARH-77 was used to in the pilot study. Proteins from myeloma cell line ARH-77 were separated by 2-DE using a 17 cm pH 3-10 nonlinear immobilized pH gradient strips in the first dimension. Separation in the second-dimension was carried out on the Protean II Cell (Bio-Rad) using a 12% SDS-polyacrylamide gel. Separated proteins were stained with ProteoSilver Plus (Sigma). Spot detection, quantification, and alignment were performed with the PDQuest software (version 7.3.0., Bio-Rad), (Figure 1).

Figure 1: Processing scheme

Examined samples
(myeloma cell line ARH-77)
↓
Protein lysis of sample
(2-DE buffers containing two concentrations of detergent CHAPS, protein precipitation with ethanol)
↓
2-DE
↓
Image analysis (PDQuest software)

Figure 2: A representative 2-DE maps of proteins from myeloma cell line ARH-77. Proteins (150 µg) extracted from cell line ARH-77 were separated on nelinear pH 3-10 IPG strips in the first dimension and by 12% acrylamide SDS-PAGE in second dimension. The resulting gels was stained with silver.



lysis buffer containing 2% CHAPS

lysis buffer containing 4% CHAPS

Table 1: Number of protein spots detected on the silver (A) without EtOH precipitation (B) with EtOH protein precipitation

Lysis buffer containing CHAPS	2%	4%
Gels (A)	1359	1448
Gels (B)	1149	1242
Loss of detected spots	210	206

RESULTS

Two concentrations of the detergent CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) in the solubilization buffer were tested, 2% and 4%. Higher concentration of CHAPS showed a greater ability to solubilize proteins (Figure 2). The most efficient solubilization was obtained with lysis buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, 60 mM DTT (dithiothreitol), 0.8% carrier ampholytes and 0.003% BPB (bromphenol blue). About 1448 spots were visualized in the gel using this lysis buffer. Many proteins (206 spots) were lost after the EtOH precipitation, though (Table 1).

CONCLUSIONS

Initial extraction and solubilization are key factors of proteomic analysis. The successful solubilization and 2-DE separation of proteins will be valuable tool for further study of the mechanisms of therapy resistance in purified plasma cells of patients with MM and further study of the biomarkers for prediction of progression of MGUS to overt myeloma.

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