

Multidimensional Chromatography Coupled with Electro Spray Mass Spectrometry as an Alternative to Two-dimensional Gels for Proteomic Analysis

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ABSTRACT

The limitations of 2-D gels for proteomic analysis have encouraged the development of alternative approaches for identifying proteins in complex mixtures. In this work, we describe the application of multidimensional liquid chromatography (SCX-RPLC) coupled with electrospray quadrupole time-of-flight tandem mass spectrometry as an alternative proteomic approach analyzing a whole cell lysate from an embryonal carcinoma (EC) cell line. Through comparative studies of the cell lines NR1-0 (wild-type) and NR1-6 (mutant), we have identified 477 proteins including 53 that are down-regulated and 70 that are up-regulated in the NR1-6 mutant. Of interest, we identified a new protein (similar to the E74 like factor 1 which promotes retinoic acid metabolism) and is expressed only in parental cells. We propose that deficiency of this protein in the mutant cell line could account for its retinoid-hypersensitivity. Additional evidence will be needed to verify this finding. The method presented here will be useful for the discovery of new biomarker and drug targets (disease proteomics).

INTRODUCTION

While the human genome project close to completion, attention is now turning to the question of how to utilize this genetic data to better understand disease and more efficiently develop targeted therapeutics. Proteins, rather than genes, convey most cellular functions. Understanding protein expression and protein function is crucial to the identification of new targets for drug development. Proteomics involves the identification of proteins in the body and the determination of their role in activities in homeostasis. 2-D PAGE analysis is considered the gold standard in proteomics. However, there are numerous disadvantages associated with the 2-D gel technique, including low throughput, difficulties in analyzing proteins with extremes in pI and molecular weight, and difficulties in analyzing membrane proteins and low abundant proteins. LC-MS offers a way to overcome these limitations. Although proteins in simple mixtures can be identified by LC-MS, complex mixtures overwhelm the resolution capability of any single dimensional (1D) chromatography system (1). Orthogonal two dimensional separation methods dramatically improve the resolution of highly complex mixtures of protein and peptides compared with any 1D separation (2). The 2D LC-MS/MS uses the independent physical properties of charge (strong cation exchange, SCX chromatography) and hydrophobicity (reversed phase chromatography, RP) to resolve complex peptide mixtures derived from spectrometry (3). Retinoids, the natural and synthetic derivatives of vitamin A, play an important role in cell growth and differentiation, and have been widely used in cancer therapy. Elucidation of the exact mechanisms regulating retinoid response or resistance is crucial. Comparative proteomic analysis can be used to identify proteins that are differentially expressed in the retinoid-sensitive (NR1-6) versus resistant (NR1-0) cell line and therefore enhance our understanding of retinoid-mediated signaling. This will aid in identifying and developing novel cancer treatments. We present here a multidimensional LC-MS/MS method to identify these potential RA- target protein markers in EC cell lines.

EXPERIMENTAL

Sample Preparation

Whole cell lysates from EC parental (NR1-0) and mutant (NR1-6) cell lines were prepared as described elsewhere (4). After reduction, alkylation, and digestion with trypsin, the peptide mixture was concentrated with a C₁₈ cartridge. About 50 µg of total protein digests were injected for 2D LC-MS/MS analysis.

2D-LC conditions

The system was configured with a CapLC system, a stream selector, an ESI-Z-spray probe, and Q-ToF 2 tandem mass spectrometer. It was configured with 3 columns including polysulfated strong cation exchange (SCX) column, C18 desalting (trapping) column (300µm ID x 5mm), and analytical column (100µm ID x 110mm) packed with a BetaBasic C18 resin (ThermoHypersil_Keystone). The solvents used were (A) 3% CH3CN+ 0.4% acetic acid+0.005%HFBA, and (B) 90% CH3CN+0.4% acetic acid+0.005% HFBA. Fifteen salt steps were used for peptide fractionation: 0, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, and 1000 mM KCl. The peptides were eluted off the analytical column by the application of 65 min mobile phase B gradient (1 to 10%B in 5 min, 10 to 15%B in 25 min, 15 to 20% B in 15min, 20 to 45%B in 10min, 45 to 80% B in 5 min) at a flow rate of 200 nl/min.

MS conditions

The mass spectrometer was operated in a data dependent acquisition mode whereby following the interrogation of MS data, ions were selected for MS/MS analysis based on their intensity and charge state (+2, +3, and +4). Collision energies were chosen automatically based on the m/z and charge-state of the selected precursor ions. The tandem mass spectra were processed by Masslynx algorithm. Proteins were identified by Proteinlynx Global Sever 2.0 using an NCBI non-redundant database.

Figure 1. Flow Chart of Protein Identification by 2D LC-MS/MS with Proteinlynx Global Search

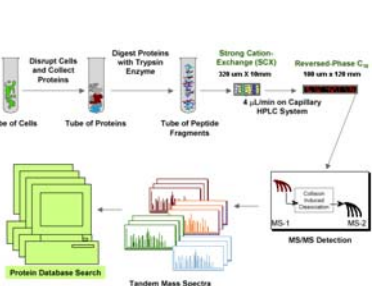


Figure 2. Instrument Configuration for 2D LC-MS/MS

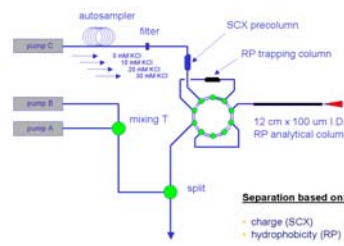


Figure 3. 2D LC-MS/MS Experiment



RESULTS

Table 1. Up-regulated Proteins in Parental Cell Line Identified by 2D LC-MS/MS

Name	mW	pI	Description	Function
NP_051121	13500	3.7	50S ribosomal protein S16	Cytosolic activity, cellular DNA repair, RNA A, B
NP_050824	26234	6.0	ribulose 1,5-bisphosphate carboxylase	cell surface adhesion, cytoskeleton
NP_277483	18081	6.2	ATP synthase H subunit	Energy
NP_050922	18956	6.0	histone H2A histone H2B interaction factor 1	Protein associated DNA from autoantigenesis
CAA52564	32068	5.7	Crystallin alpha A, Mus musculus	RNA editing
NP_060651	45539	7.9	cytoskeleton cytoskeleton	Cytoskeleton maintenance
AA185995	29668	9.7	DNF3 protein, Mus musculus	Metastatic tumor, neuroblastoma, FPKs
CAA52694	4817	4.8	thymosin binding inhibitor 1	antibiochemical neurogenesis
NP_060677	42131	5.3	DNA polymerase III beta subunit	in vivo embryonic
AA185934	101530	7.3	ERF2 protein, Mus musculus	involved in glycolysis
RNO_X285A	47974	6.2	Enzyme 2 phosphatidylinositol 3-OH kinase	Neural RFP
NP_060668	34896	9.9	neuronal arginase ANO102	Neural RFP
AA185936	21961	6.9	heat shock protein HSP70	Neural RFP
AA185937	37936	5.1	HSP70 gamma (HSP70) beta	Neural RFP
AA185938	40665	6.9	heat shock protein HSP90	involved in multiple endoplasmic reticulum chaperone systems 1
AA185939	22061	5.8	HSP90 small heat shock protein	involved in multiple endoplasmic reticulum chaperone systems 1
NP_446608	17038	9.3	myosin VIIA cytoskeletal protein	Cellular activity, cell surface signaling, signaling
RA180161	14722	6.2	myosin VIIA cytoskeletal protein	which encodes tumor
NP_051122	13500	3.7	50S ribosomal protein S16	involved in multiple endoplasmic reticulum chaperone systems 1
AA185940	28236	6.5	p27 ^{KIP1} protein, Mus musculus	binding to nuclear binding protein
AA185941	20311	4.5	p27 ^{KIP1} protein, Mus musculus	beta gene from fetal liver
AA185942	17791	3.9	PA200 cytoskeleton ATP dependent	involved in multiple endoplasmic reticulum chaperone systems 1
NP_050923	18956	6.0	histone H2A histone H2B interaction factor 1	Suppression of CD
NP_060701	38135	5.8	RBM25 (RNA binding motif 2) Mus mus	Protein with RNA binding properties
NP_050924	18956	6.0	histone H2A histone H2B interaction factor 1	involved in multiple endoplasmic reticulum chaperone systems 1
SVS_RABT	4498	4.7	Scn5a-13A cytoskeleton	From primary tumor, binding to fibroblast factor 1
NP_214612	13266	4.9	ribonucleic protein RPL10A	5' cap binding protein
NP_214613	13507	4.5	ribonucleic protein RPL10A	5' cap binding protein
NP_176272	45661	5.1	ribonucleic protein RPL10A	involved in multiple endoplasmic reticulum chaperone systems 1
NP_176273	13266	4.9	ribonucleic protein RPL10A	5' cap binding protein
NP_214614	13137	4.6	ribonucleic protein RPL10A	5' cap binding protein
NP_214615	13137	4.6	ribonucleic protein RPL10A	5' cap binding protein
NP_214616	13137	4.6	ribonucleic protein RPL10A	5' cap binding protein
NP_214617	13137	4.6	ribonucleic protein RPL10A	5' cap binding protein
NP_214618	13137	4.6	ribonucleic protein RPL10A	5' cap binding protein
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