

Label-Free Proteomics: A Versatile Tool for Differential Proteome Analysis

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Introduction

Various different gel and MS-based techniques like e.g. two-dimensional difference gel electrophoresis (2D-DIGE), ICPL, and in vivo isotope labeling have been developed and are successfully applied to elucidate biological processes. In the last years it has been shown that due to different focuses in the proteomic field no general solution exists and that most of these techniques are complementary. Thus, there is still a need for novel techniques allowing to perform quantitative proteome analysis with higher performance and throughput. Therefore, we have evaluated the MS based approach of label-free proteomics in respect to reproducibility and sensitivity by performing a comprehensive study considering multiple biological replicas. As a cell culture model we have chosen human lung carcinoma cell line A549. The treatment of these cells using TGF-beta is a model for lung fibrosis.

Methods

In order to study changes in human lung carcinoma cell line A549 caused by TGF-beta treatment via 2D-DIGE, 50 µg protein from whole cell lysate were labeled with the minimal dyes and separated by carrier ampholine based 2-DE.

The performance of label-free proteomics was investigated by a setup comprising nano-HPLC (Ultimate 3000, Dionex), ESI-QTOF-MS (micrOTOF-Q, Bruker) and MS analysis software prototype for label-free proteomics analysis. Whole cell lysates of the human lung carcinoma cell line A549 obtained from the same cell culture samples as applied in the DIGE study were digested with trypsin. 500 ng of the trypsin digest were analyzed applying a 2 hour LC gradient. The label-free workflow is summarized in Figure 1.

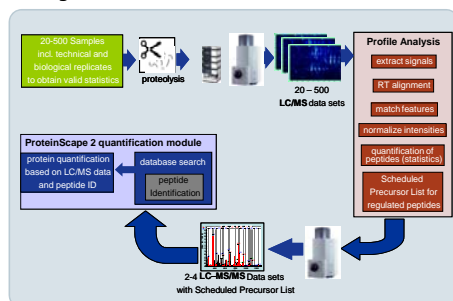


Fig. 1 Workflow for label-free quantification. Peptides showing a significant regulation are subjected to MS/MS sequencing in several LC-MS/MS runs.

Results

Considering 10 biological replicas revealed 104 differentially regulated proteins (fold change 1.5, $p < 0.05$) in the DIGE study. Until now we identified 57 of these using MALDI-TOF/TOF-MS. In comparison to this result we detected 667 differentially regulated peptides in the label-free approach. For targeted MS/MS analysis in subsequent LC-MS/MS runs the differentially determined peptides were selected to construct an inclusion list. Altogether 333 peptides were identified leading to 154 regulated proteins (Figure 2).

The comparison of the identified proteins between 2D-DIGE study and label-free proteomics results in 16 proteins found in both experiments (Table 1). However, most of the proteins were found only in one approach, indicating that a combination of results from both techniques is very promising.

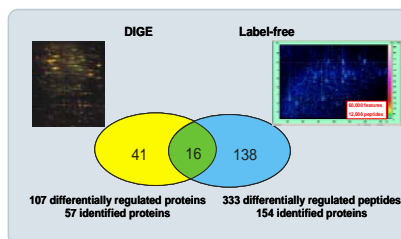


Fig. 2 DIGE study and label-free approach give complementary results.

Protein name	DIGE		Label-Free Proteomics					
	Fold Change	Change	MS	m/z	RT (min)	Fold Change		
Annexin A2	1.66	-1.96	731.43	731.42	1	45.68	-3.82	ELASALK
			745.46	745.45	1	65.95	-1.73	LMVALAK
			766.36	766.36	1	43.05	-3.39	LYDSIAK
			808.47	808.46	1	52.40	-2.98	SYPPDAK
			821.41	821.41	1	58.56	-1.79	SEVDIAK
			880.44	880.44	1	61.80	-2.04	ELYDAQK
			903.53	903.53	2	50.65	-1.47	WBSATIK
			908.84	908.77	2	50.19	-1.52	ELYDAQVQR
			908.45	908.75	2	58.99	-1.97	ATYDFEIKR
			907.60	904.30	2	84.90	-1.62	DALNETAK
			908.60	908.49	1	90.26	-1.98	ALLYVLDGSD
			111.55	556.28	2	71.72	-1.59	QDAVAVQR
			942.69	712.35	2	88.75	-1.53	ELYVYDQTK
			940.67	730.84	2	16.96	-1.85	SYVDFVLEIKR
			942.85	771.82	2	52.36	-1.79	LDVDFVYVLEIKR
			958.77	704.69	2	50.17	-2.25	DFVDFVDELEIKR
			977.99	593.33	3	92.69	-1.66	TKQVDFVYVLEIKR
			977.86	889.43	2	58.41	-2.23	GLTDFVLEIKR
			181.87	906.43	2	98.69	-1.92	YDLKDFVLEIKR
			908.88	954.94	2	90.93	-1.75	ADDSVDFVLEIKR
Profilein-1	1.82	1.80	265.07	78.03	3	97.90	-3.08	AYTFDFVLEIKR
			923.58	952.28	2	41.53	-1.60	EDVDFVLEIKR
			1079.72	690.37	2	75.05	-1.61	SYDQAPFVYVTK
Transgelin-2	1.99	1.80	820.43	487.72	2	49.33	-1.56	CPAVGLER
			986.52	496.78	2	62.85	-1.83	DAEYDFVLEIKR
			1002.63	601.82	2	76.62	-2.19	NVGLDMYVTK
			1256.69	608.35	2	59.64	-1.72	TLMLDGLVAVR
			1279.55	640.30	2	58.77	-1.74	NFSDNGLDEK
			1583.61	692.31	2	68.37	-1.79	GASDQAFVYVMPFR
16 kDa heat shock protein mitochondrial precursor	-1.67	-1.17	676.38	676.38	1	35.02	-1.86	VELVER
			737.38	717.38	1	47.30	-1.27	GDGSLK
			806.43	806.42	1	27.19	-1.80	SAEFTVTK
			844.46	844.46	1	63.25	-1.69	GDGDFVTK
			1076.60	538.80	2	74.12	-1.47	VLLPEYDQTK
			188.88	598.84	2	42.71	-1.46	GDGDFVYVTK
			929.80	765.40	2	56.55	-1.87	VLLDKDYFLR
60 kDa heat shock protein mitochondrial precursor	-1.70	-1.64	844.50	422.75	2	64.29	-1.52	PAMPAK
			925.91	456.79	2	70.83	-1.58	VLDGVAIK
			923.80	617.30	2	43.41	-1.90	VGGTDFVDEIKR
			181.69	681.35	2	35.05	-1.46	VGGTDFVDEIKR
			954.74	752.88	2	10.72	-1.86	TLNDELSEDAK
Aspartate aminotransferase mitochondrial precursor	-1.73	-2.04	1207.74	634.37	2	80.65	-2.03	AAALNTDILR
Cathepsin D precursor	-1.55	-1.89	939.82	620.31	2	107.82	-1.81	FDDGLMAYVPR
			989.02	995.00	2	10.99	-1.99	AGAVPLDQETMPCEK
Delta3-5-delta2-4-dienoyl-CoA isomerase mitochondrial precursor	-1.76	-1.61	1152.56	378.63	3	31.94	-1.49	ELKTVFYSK Phospho (ST)
			1208.64	649.83	2	81.02	-1.91	YQTFVRIER
			1483.71	630.38	2	75.71	-1.71	AGAGSATLBMAYAGAR
Keratin type II cytoskeletal 8	-1.61	-1.64	623.53	623.52	1	27.71	-1.48	HEEYTK
			870.43	436.72	2	44.85	-1.46	ISSDFSR
			1277.73	639.36	2	96.87	-1.57	LALDAIYR
Malate dehydrogenase mitochondrial precursor	1.72	-1.92	1073.58	537.29	2	46.03	-1.97	IGAGTEVVK
			116.80	658.80	2	89.21	-1.86	MEDAPFLK
			923.72	617.38	2	19.20	-2.15	FEVYLVDFR
			1483.71	727.85	2	75.71	-1.71	AGAGSATLBMAYAGAR
			1483.74	645.37	2	65.25	-1.66	DTLVDFVDEIKR
Prohibitin	1.65	-1.56	2385.24	789.08	3	193.26	-1.88	LTLYDVAHPFVVAADLSHETK
			1168.69	576.30	2	87.86	-1.65	FDGGLDFR
			1216.74	607.37	2	92.07	-1.63	VLPSTTEK
			1444.68	722.83	2	82.29	-1.57	PTDFEYDFR
			1215.62	608.11	2	41.22	-1.74	VDVSELEAK
Prohibitin-2	-1.71	-1.85	1853.99	918.67	3	104.40	-2.00	EGVVDQDLEGLDFR
Stress-70 protein mitochondrial precursor	-1.73	-1.75	1290.88	645.84	2	86.04	-1.72	VDQTVDFLFR
			1361.74	681.37	2	18.52	-1.85	ADPFGMTDLR
			945.88	623.43	2	62.48	-1.65	INPFTFALLVGLDQK
			1856.91	619.44	3	93.98	-1.84	VEAVNMAEGIDTETK

Table 1: Fold changes of proteins determined by DIGE and the label-free approach. Most protein regulations do agree well for both techniques, however some are conversely regulated. Western blot analysis is applied for further investigations on these observations.

Summary

- Results from DIGE and label-free approaches are complementary in this study
- Advantages of the label-free workflow based on the micrOTOF-Q include high selectivity and sensitivity of regulated peptides due to high mass accuracy, high resolution, and high reproducibility of LC-MS and LC-MS/MS runs
- Due to unnecessary labeling and pre-fractionation, the label-free approach requires lower sample amounts

Conclusions

- The label-free quantification workflow enabled by high resolution mass spectrometry provides high throughput protein quantification with accuracy and precision comparable to other quantification techniques
- Time required to generate reported results is in the order of days for the label-free approach with potential for further automation. The time required for the DIGE study is in the order of weeks