

# Differential Nuclear Proteomes in Response to *N*-Methyl-*N'*-Nitro-*N*-Nitrosoguanidine Exposure

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## ABSTRACT

Alkylating agent MNNG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) can induce DNA damages which can lead to chromosomal aberrations, mutations and cell death. Diverse cellular responses were observed to occur following MNNG treatment in our previous studies, including nontargeted mutations (NTM) at undamaged DNA bases, endoplasmic reticulum stress (ER stress) induction and the activation of several signal transduction pathways. In addition, whole cell proteome analysis also revealed that comprehensive and various changes were triggered by this mutagen. However, low abundance proteins with key functions, such as nuclear proteins, are always underrepresented in proteomic studies. To reduce the complexity of protein samples and monitor subcellular alterations in response to MNNG exposure, nuclear extracts were fractionated from MNNG-treated cells and analyzed using two-dimensional fluorescence difference gel electrophoresis (2-D DIGE). 23 differentially expressed protein spots were observed after 0.25 and 1  $\mu$ M MNNG exposure, and 17 of them were identified by MALDI-TOF MS analysis. Among them, two nuclear proteins with nucleocytoplasmic shuttling activity, 14-3-3  $\zeta$  and hnRNP K, were further demonstrated to undergo different dynamic changes in response to MNNG exposure.

## METHODS

- Cell line: Human amnion epithelial cells (FL cells);
- Doses: MNNG (0.25  $\mu$ M, 1  $\mu$ M), DMSO as control;
- Time: Treated for 2.5 h, incubated in fresh medium for another 12 h;
- Two-dimensional fluorescence difference gel electrophoresis (2-D DIGE) (24cm, pH4-7);
- Image acquisition and statistical analysis;
- In-gel digestion;
- Protein identification by MALDI-TOF mass spectrometry;
- Western blot analysis.

## RESULTS

Table 1. Identification of the Differentially Expressed Nuclear Proteins in FL Cells Exposed to 0.25 $\mu$ M MNNG Treatment												
Index no.	spot volume/total volume (%)			P-value <sup>a</sup>	q-value <sup>b</sup>	MW (kDa) <sup>c</sup>	pI <sup>d</sup>	peptide matches <sup>e</sup>	cov. (%) <sup>f</sup>	score	description (protein name)	Ref. Seq. no.
	control	treated	ratio									
310	1.067 ± 0.077	0.857 ± 0.032	-1.25	0.007	0.000	85.3	5.69	7/8	10	92	procollagen-lysin, 2-oxoglutarate 5-lyoxigenase 3	NP_001075.1
320	1.074 ± 0.012	0.854 ± 0.034	-1.24	0.004	0.000	83.2	5.55	10/23	24	109	ATP-dependent DNA helicase II	NP_005084.4
414	1.125 ± 0.129	0.842 ± 0.053	-1.34	0.017	0.000			13/19	20	137		
417	1.243 ± 0.183	0.878 ± 0.027	-1.42	0.021	0.000	66.7	5.11	15/22	29	165	lamin B1	NP_005564.1
507	0.864 ± 0.067	1.075 ± 0.116	1.24	0.028	0.078	47.8	5.46	15/24	35	166	heterogeneous nuclear ribonucleoprotein K	NP_002131.2
515	1.368 ± 0.225	0.733 ± 0.105	-1.79	0.010	0.000	58.4	6.36	9/17	22	98	chaperonin containing TCP1, subunit 6A (beta 1)	NP_001753.1
770	1.061 ± 0.059	0.889 ± 0.049	-1.23	0.002	0.000	49.5	5.13	6/9	20	77	protonome (pousoim, macropain) 28S subunit, ATPase 3	NP_002795.2
795	1.028 ± 0.050	0.811 ± 0.057	-1.27	0.001	0.000							
816	0.757 ± 0.062	1.501 ± 0.219	1.98	0.001	0.000							
856	1.028 ± 0.014	0.892 ± 0.002	-1.09	0.008	0.000	53.2	6.13	9/10	21	123	lamin A/C	NP_005563.1
924	0.880 ± 0.086	1.151 ± 0.035	1.31	0.004	0.078	32.9	4.79	7/12	26	100	ribosomal protein SA	NP_00101252.1
1281	0.941 ± 0.033	1.045 ± 0.067	1.24	0.003	0.078	29.2	4.76	11/19	42	130	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide (14-3-3 $\beta$ )	NP_001935.1
1254	0.822 ± 0.041	1.076 ± 0.022	1.31	0.002	0.078	28.5	4.80	7/12	26	84	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide (14-3-3 $\gamma$ )	NP_006011.1
1267	0.834 ± 0.029	1.084 ± 0.099	1.30	0.004	0.078	28.0	4.68	6/12	23	77	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, delta polypeptide (14-3-3 $\delta$ )	NP_006011.1
1274	0.834 ± 0.049	1.099 ± 0.087	1.21	0.018	0.078	30.1	4.72	10/20	38	115	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (14-3-3 $\zeta$ )	NP_003397.1
1277	0.856 ± 0.031	1.035 ± 0.056	1.21	0.003	0.078	23.4	5.19	5/8	17	73	Ran-binding protein 1	NP_002873.1
1281	0.941 ± 0.033	1.045 ± 0.067	1.24	0.003	0.078	29.2	4.76	11/19	42	130	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide (14-3-3 $\beta$ )	NP_001935.1
1319	0.773 ± 0.074	1.227 ± 0.323	1.59	0.008	0.078	49.4	5.79	6/9	19	76	heterogeneous nuclear ribonucleoprotein H1	NP_005111.1

Table 2. Identification of the Differentially Expressed Nuclear Proteins in FL Cells Exposed to 1 $\mu$ M MNNG Treatment												
Index no.	spot volume/total volume (%)			P-value <sup>a</sup>	q-value <sup>b</sup>	MW (kDa) <sup>c</sup>	pI <sup>d</sup>	peptide matches <sup>e</sup>	cov. (%) <sup>f</sup>	score	description (protein name)	Ref. Seq. no.
	control	treated	ratio									
455	0.910 ± 0.033	1.131 ± 0.124	1.24	0.017	0.000							
465	0.931 ± 0.082	1.186 ± 0.071	1.27	0.008	0.000							
507	0.864 ± 0.067	1.075 ± 0.116	1.24	0.019	0.000	47.8	5.46	15/24	35	166	heterogeneous nuclear ribonucleoprotein K	NP_002131.2
515	1.368 ± 0.225	0.733 ± 0.104	-2.06	0.009	0.000	58.4	6.36	9/17	22	98	chaperonin containing TCP1, subunit 6A (beta 1)	NP_001753.1
516	0.830 ± 0.034	1.040 ± 0.014	1.22	0.008	0.000	47.8	5.46	12/20	39	100	heterogeneous nuclear ribonucleoprotein K	NP_002131.2
856	1.028 ± 0.014	0.757 ± 0.126	-1.35	0.018	0.000	53.2	6.13	9/10	21	123	lamin A/C	NP_005563.1
869	1.146 ± 0.119	0.843 ± 0.042	-1.36	0.009	0.000	47.8	5.46	6/10	19	117	heterogeneous nuclear ribonucleoprotein K	NP_002131.2
1117	1.119 ± 0.106	0.829 ± 0.042	-1.25	0.006	0.000							
1267	0.834 ± 0.029	0.989 ± 0.050	1.19	0.003	0.000	28.0	4.68	6/12	23	77	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, delta polypeptide (14-3-3 $\delta$ )	NP_006011.1
1274	0.834 ± 0.049	0.979 ± 0.072	1.17	0.023	0.000	30.1	4.72	10/20	38	115	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (14-3-3 $\zeta$ )	NP_003397.1
1277	0.856 ± 0.031	1.035 ± 0.072	1.19	0.010	0.000	23.4	5.19	5/8	17	73	Ran-binding protein 1	NP_002873.1
1281	0.941 ± 0.033	1.045 ± 0.061	1.15	0.003	0.000	28.2	4.76	11/19	42	130	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide (14-3-3 $\beta$ )	NP_001935.1
1307	1.200 ± 0.240	0.841 ± 0.101	-1.53	0.030	0.000							

Table 1,2. Differentially expressed nuclear proteins in FL cells exposed to MNNG treatment.

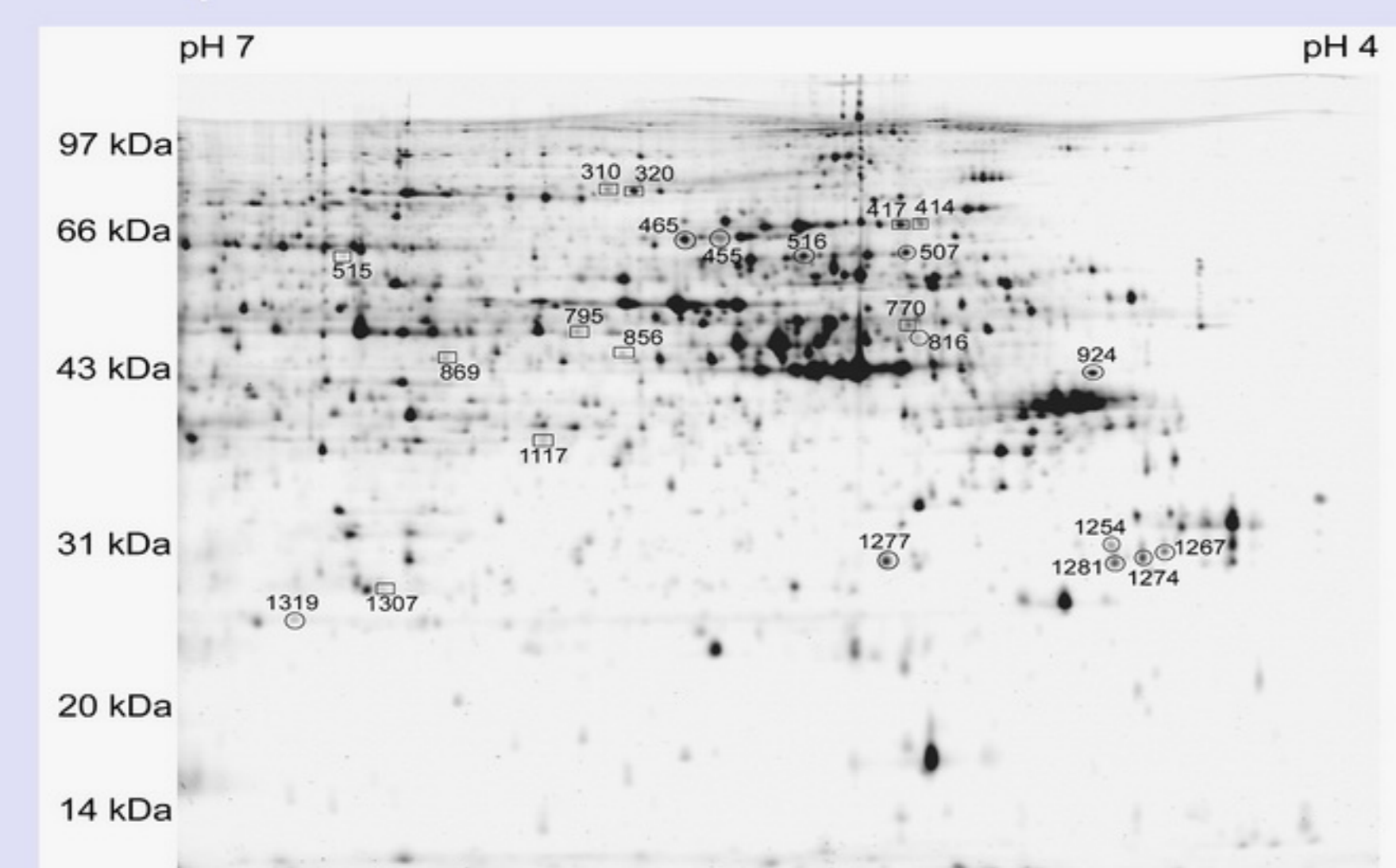


Figure 1. Representative postsilver stained 2D-DIGE gel image indicating differentially expressed nuclear protein spots identified by MS analysis after MNNG exposure. (Circles: up-regulated; rectangles: down-regulated.)

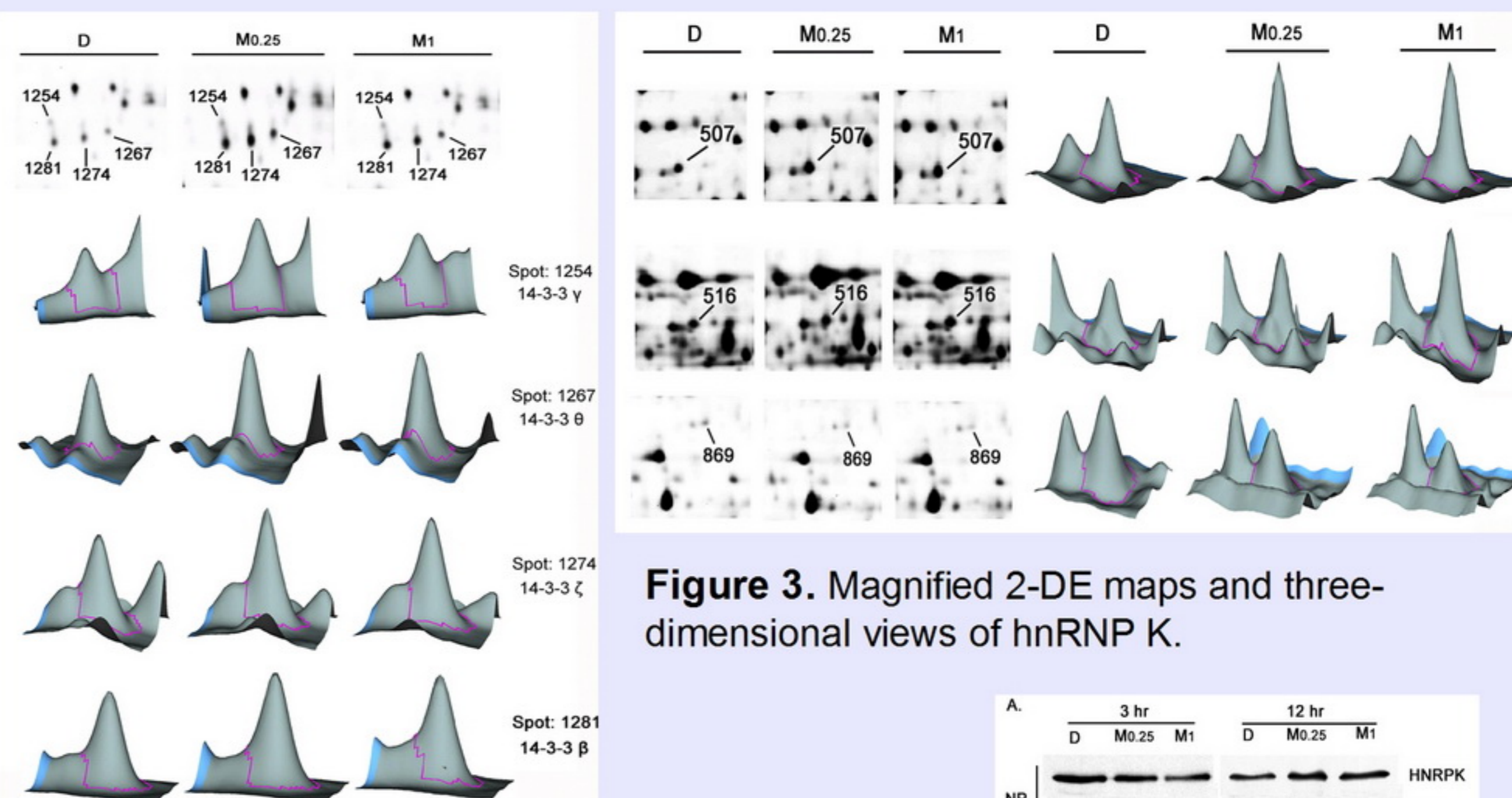


Figure 2. Magnified 2-DE maps and three-dimensional views of 14-3-3 proteins.

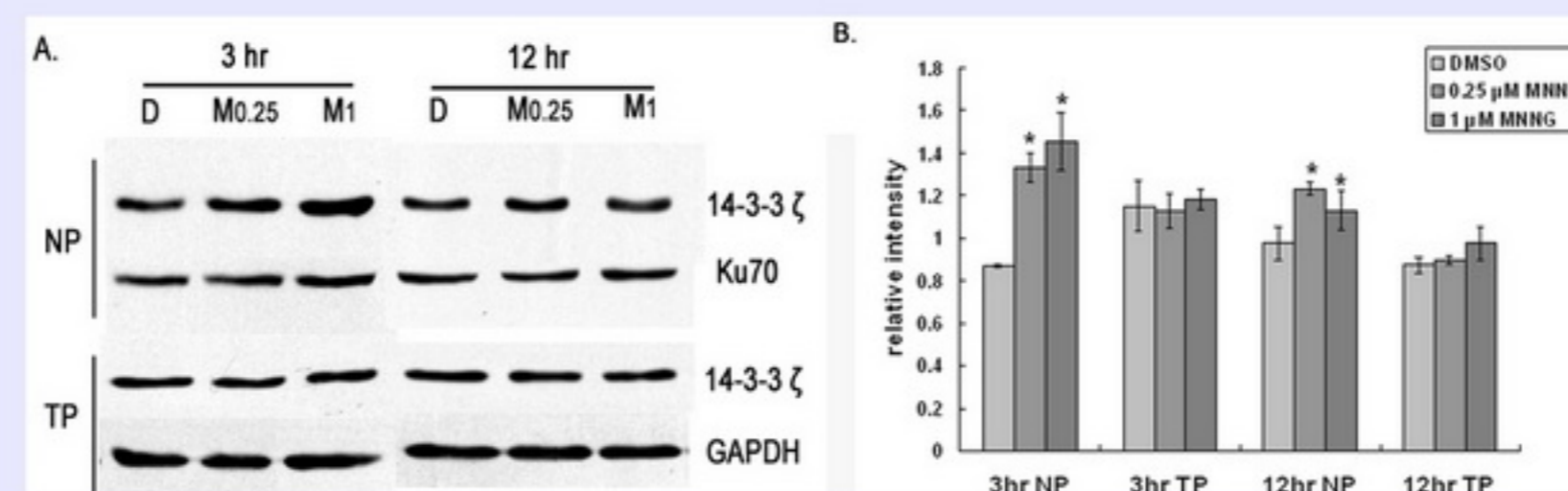


Figure 4. Western blot analysis of dynamic nuclear expression patterns of 14-3-3  $\gamma$ . NP: Nuclear proteins; TP: total proteins.

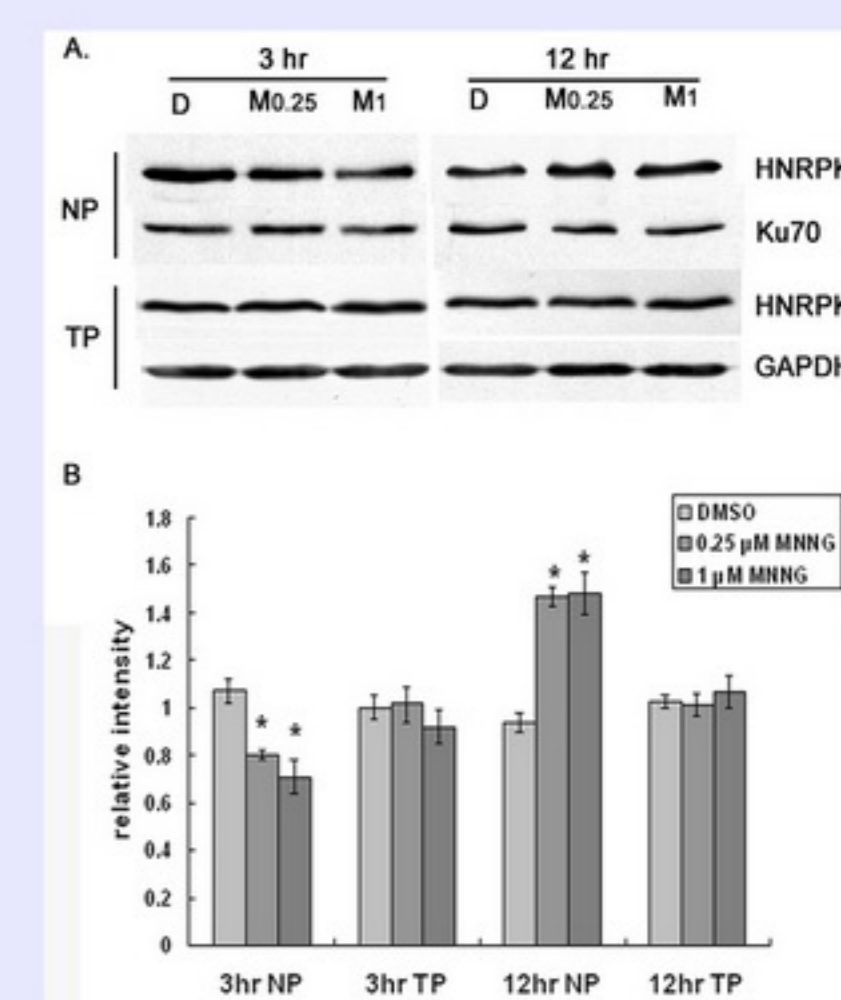


Figure 5. Western blot analysis of dynamic nuclear expression patterns of hnRNP K. NP: Nuclear proteins; TP: total proteins.

## Conclusion

- One-third of the differentially expressed nuclear proteins (8 of 23) were shared between 0.25 and 1  $\mu$ M MNNG treatment groups. The results indicated that some similar nuclear effects may exist after different concentrations of MNNG treatment.
- The nuclear level of 14-3-3  $\gamma$  elevated at 3 and 12 h after MNNG exposure, especially at 3 h after 1  $\mu$ M MNNG treatment, indicated that the nuclear accumulation of 14-3-3  $\gamma$  seemed to occur as a relatively early cellular event after MNNG exposure.
- Heterogeneous nuclear ribonucleoprotein K (hnRNP K) was also demonstrated to undergo dynamic changes in response to MNNG exposure.

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