Chapter 4
Proteome in Leukemic vs. Differentiated Leukemia Cells

During cells response to external stimulus, signals are transmitted to the nucleus from the surface of the the cell during the processes that regulate the signalling events, during which the cascade of protekinases and proteinphosphatases are initiated and other regulatory proteins are activated (Hunter 1995; Karin et al. 1997). Long-term effects may alter the activity of receptors in the proliferating cells and effect on the differentiation or apoptosis pathways. Leukemic cell lines are applied as an *in vitro* model in leukemia, enabling the molecular mechanisms of cellular proliferation or differentiation processes to be investigated. Differentiation inducers (DMSO, ATRA, dbcAMP, etc.) can induce NB4 and HL-60 to granulocytic differentiation. The forbolmyristat acetate (PMA), vitamin D3 and sodium butyrate can induce differentiation to monocytes/marophages lineage of NB4, HL-60, and THP-1 cells (Breitman et al. 1980; Collins et al. 1977; Collins 1987). During differentiation, cell growth is stopped and specific proteins that are involved in phenotype formation are activated.

The cell differentiation process into granulocytes can be devided into several stages: the first—initiation of differentiation, the second—differentiation into granulocytes or monocytes depending on the used inducer, and the third- maturation, when the cell that has lost its ability to multiply, begins to produce the specific proteins. The inducer of granulocytic differentiation all-trans retinoic acid (ATRA) activates genes by binding to the ATRA receptors in the nuclei of cells through the all-trans retinoic acid response elements (RARE). Some of these genes' products may be involved, directly or indirectly, in the process of differentiation induction (Sporn et al. 1994; Pawson 1995), others can be expressed in the cytoplasm or the nuclei of differentiated. Matured granulocytes induced to differentiation by

R. Navakauskienė et al., *Epigenetics and Proteomics of Leukemia*,
https://doi.org/10.1007/978-3-030-68708-3_4

ATRA, similar to neutrophils, underwent the programmed cell death (apoptosis). The signalling pathways and molecular mechanisms that are involved in the granulocytic differentiation of leukemic cells that leads to apoptosis remain unclear. The mechanisms of cellular proliferation, differentiation, and apoptosis involve various protein-modifying enzymes, including protein phosphatases and protein kinases that are responsible for phosphorylation or dephosphorylation of proteins (on threonine, serine, or tyrosine residues). The phosphorylation of tyrosine residues is particularly specific at the stage of transmission of the external signal to the DNA, so it is important not only to identify newly synthesized proteins, but also to determine where and when they are modified (e.g., thyrosine phosphorylated).

Global functional proteomic analysis helps to understand the molecular mechanisms of diseases, providing new possibilities for detecting specific regulatory proteins and new targets for the rational treatment of leukemias and cancer.

Proteomic changes in leukemia cells, promoted to granulocytic differentiation by only ATRA or together with epigenetic modifiers were investigated our study (Navakauskiene et al. 2002, 2003a,b, 2004a,b, 2012, 2014; Kulyte et al. 2001, 2002; Borutinskaite et al. 2011, 2005; Borutinskaite and Navakauskiene 2015; Treigyte et al. 2000b,a, 2004; Valiuliene et al. 2015).

Methods for computerized analysis of proteome maps have been developed (Matuzevicius et al. 2008). Such proteomic analysis serves to elucidate the network of protein interactions and proteins responsible for myeloid cell development and leukemia, as well as to discover new targets for rational cancer therapy.

4.1 Proteomic Analysis of Cytoplasmic and Nuclear Proteins in Human Hematopoietic CD34+, AML Cell Line KG1 and Mature Neutrophils

Cytoplasmic and nuclear proteins we isolated from cells of different state of hematopoietic differentiation: primary hematopoietic CD34+, cancerous cells arrested at the stage of incomplete differentiation KG1 (AML cell line) and healthy mature neutrophils. The isolated cytoplasmic and nuclear proteins were fractionated in the 2DE system (Figs. 4.1 and 4.2).

After protein visualization a comparative analysis of the protein maps was performed and proteins that could serve as potential markers of leukemia were selected for mass spectrometry analysis. Proteins were selected based on changes in their expression in different states of cell differentiation. The summarized analysis data are presented in Table 4.1. The computational methods for protein expression analysis in different hematopoietic cells were applied and fold change between protein expression calculated. The network of identified cytoplasmic proteins distinctive for human hematopoietic CD34+, incompletely differentiated KG1 cells, and mature human neutrophils is presented in Fig. 4.3.

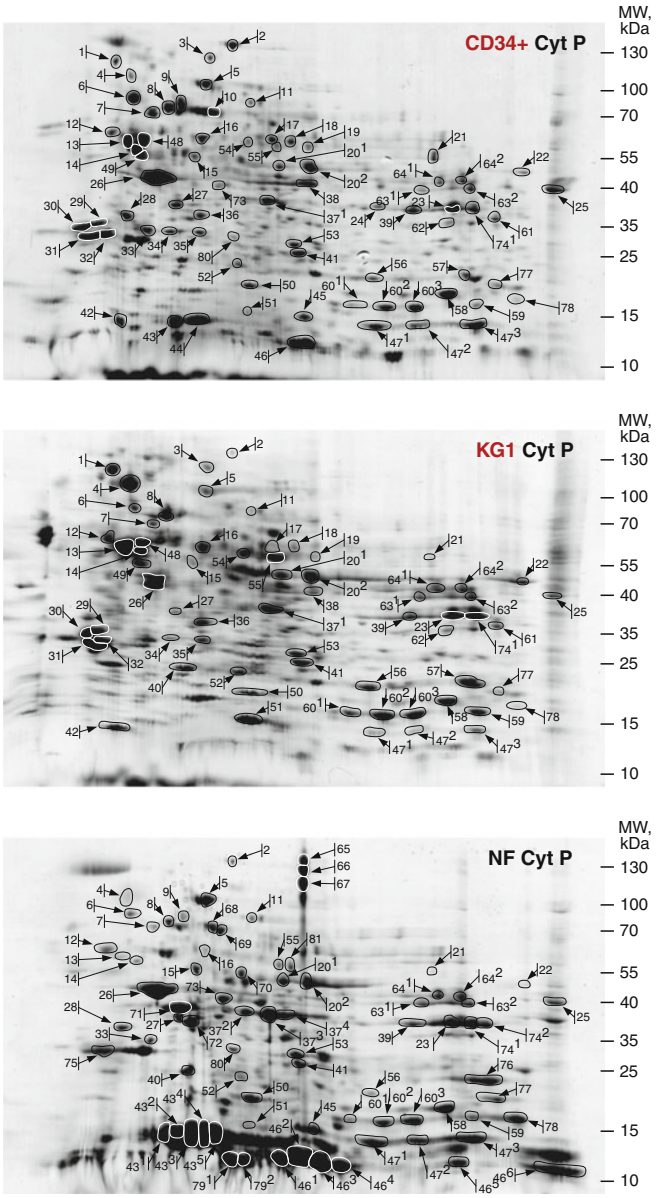

Fig. 4.1 Proteomic analysis of cytoplasmic proteins isolated from human hematopoietic CD34+, incompletely differentiated KG1 cells, and mature human neutrophils (NF). The cytoplasmic proteins (Cyt P) are isolated and fractionated in the 2DE system. After visualization of the proteins by staining with Coomassie blue, protein comparative analysis was performed to select proteins with different expression in different cell differentiation states. These proteins were prepared for mass spectrometry analysis, identified and quantified by computational analysis (Table 4.1)

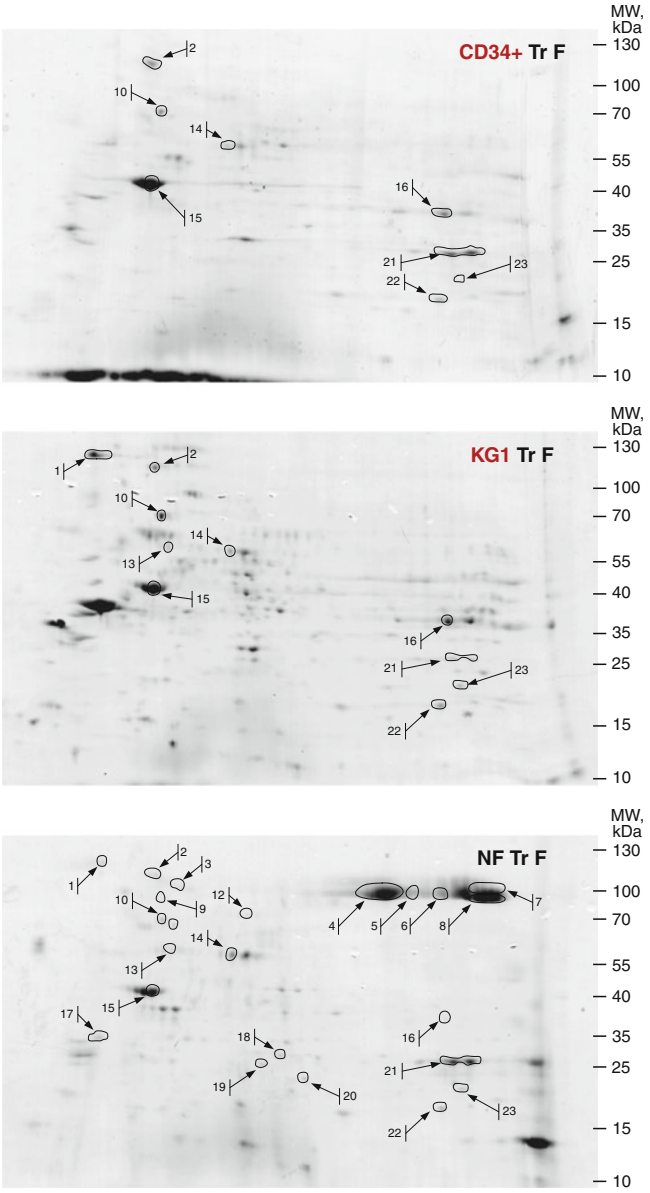

Fig. 4.2 Proteome analysis of nuclear proteins isolated from human hematopoietic CD34+, incompletely differentiated AML cell line KG1, and mature human neutrophils (NF). Nuclear proteins (Tr F) were isolated and fractionated in the 2DE system. After visualization of the proteins by staining with Coomassie blue, protein comparative analysis was performed to select proteins with different expression in different hematopoietic cell differentiation states. These proteins were analyzed by mass spectrometry, identified and quantified by computational analysis (Table 4.2)

Table 4.1 The summarized search results (by `PepIdent`, `EMBL`, `MS-Tag` software) of comparative expression analysis of cytoplasmic proteins from KG1, Neutrophils (NF) and CD34+

Spot No.	Access number	Protein name (abbreviation)	Match, %	Experimental		Modeling		Avg. vol. ratio		
				Mw, kDa	pI	Mw, kDa	pI	KG1/ CD34+	NF/ CD34+	NF/ KG1
1	Q9Y3P9	Rab GTPase-activating protein 1	13	120	4.85	121.7	5.1	3.1	-	-
	P18206	Vinkulin	23	134	5.75	123.3	5.5	−3.7	−3.0	1.2
2	Q14289	Protein-tyrosine kinase 2-beta	13	134	5.75	115.8	5.9	−3.7	−3.0	1.2
3	Q9UPQ0	LIM and calponin homology domains-containing protein 1	27	120	5.55	121.1	6.1	1.7	-	-
	O94779	Contactin-5	17	120	5.55	120.6	6.0	1.7	-	-
	Q9HBR0	Putative sodium-coupled neutral amino acid transporter 10	14	120	5.55	119.7	5.51	1.7	-	-
4	Q562E7	WD repeat-containing protein 81	6	110	5.0	95.6	5.0	7.4	1.4	−5.3
5	Q9Y6U3	Adseverin	8	108	5.6	80.4	5.5	−1.1	2.0	2.2

(continued)

Table 4.1 (continued)

Spot No.	Access number	Protein name (abbreviation)	Match, %	Experimental		Modeling		Avg. vol. ratio		
				Mw, kDa	pI	Mw, kDa	pI	KG1/ CD34+	NF/ CD34+	NF/ KG1
6	P11021	78 kDa glucose-regulated protein (GRP-78)	51	90	5.0	72.4	5.07	−2.9	−3.4	−1.2
7	P13796	Plastin-2	24	70	5.3	70.2	5.2	−2.7	−3.2	−1.2
8	P11142	HSP7C	18	80	5.35	70.8	5.37	−1.4	−2.4	−1.7
9	P08133	Annexin A6 (ANX A6)	35	85	5.4	76.1	5.42	-	−2.5	-
	P38646	Stress-70 protein	16	85	5.4	73	5.9	-	−2.5	-
10	P02768	Serum albumin (ALBU)	27	80	5.55	69.3	5.9	–	-	-
11	Q9UKJ8	Disintegrin and metalloproteinase domain-containing protein 21	14	85	5.8	80.8	6.2	−1.0	1.2	1.2
12	Q96I76	G patch domain-containing protein 3	23	63	4.85	59.3	4.9	1.6	1.3	−1.2
13	Q9H7C4	Syncoilin	14	60	4.9	55.5	4.6	2.0	−4.6	−9.1
14	P68366	Tubulin alpha-4A chain	18	55	4.95	50	4.95	−1.1	−3.2	−2.8
15	A6NHL2	Tubulin alpha chain-like 3	14	55	5.47	49.9	5.7	−1.2	1.4	1.7
16	P30101	Protein disulfide-isomerase A3	14	63	5.5	56.7	6.0	1.7	−2.3	−4.0

17	P14868	Aspartyl-tRNA synthetase (SYDC)	45	62	6.0	57.4	6.11	2.6	-	-
18	P14868	Aspartyl-tRNA synthetase (SYDC)	30	60	6.2	57.4	6.11	−1.1	-	-
19	P14868	Aspartyl-tRNA synthetase (SYDC)	31	57	6.99	57.4	6.11	−1.4	-	-
20[1]	P06733	Alpha-enolase	25	47	6.99	47.1	7.0	2.5	1.7	−1.5
20[2]	P06733	Alpha-enolase	25	47	6.99	47.1	7.0	1.3	1.5	1.1
21	Q15646	59 kDa 2'-5'-oligoadenylate synthase-like protein	17	55	8.3	59.0	8.0	−3.9	−5.9	−1.5
22	Q9H2G9	Golgin-45	38	45	9.0	45.2	8.74	1.3	−2.7	−3.4
23[1]	P04406	Glyceraldehyde-3-phosphate dehydroge-nase (GAPDH)	16	37	8.58	36.0	8.6	1.5	1.3	− 1.2
23[2]	P04406	Glyceraldehyde-3-phosphate dehydroge-nase (GAPDH)	12	39	8.58	36.0	8.6			
24	P07355	Annexin A2	48	38	7.6	38.8	7.37	-	-	-

(continued)

Table 4.1 (continued)

Spot No.	Access number	Protein name (abbreviation)	Match, %	Experimental		Modeling		Avg. vol. ratio		
				Mw, kDa	pI	Mw, kDa	pI	KG1/ CD34+	NF/ CD34+	NF/ KG1
25	Q9UBV7	Beta 1-4-galacto-syltransferase 7	20	40	10.0	37.4	9.2	−1.8	−1.4	1.3
26	Q96HG5	Actin, cytoplasmic 1, Actin, beta (ACTB)	29	43	5.3	42.0	5.29	−1.8	−1.1	1.7
27	P27216	Annexin A13 (ANX 13)	65	38.5	5.4	35.6	5.47	−2.0	1.1	2.1
28	P08758	Annexin A5 (ANX 5)	62	36.5	4.9	35.9	4.94	-	-1.1	-
29	P06753	Tropomyosin alpha-3 chain (TPM3)	55	35	4.6	32.8	4.68	1.6	-	-
30	P62258	14-3-3 protein epsilon	16	35.5	4.75	29.1	4.6	−1.0	-	-
31	P63103	14-3-3 protein zeta/delta	40	30	4.65	27.7	4.7	1.2	-	-
32	P31946	14-3-3 protein beta/alpha	36	32	4.8	28.0	4.8	−1.1	-	-
33	Q15024	Exosome complex exonu-clease (RRP42)	24	35	5.1	31.8	5.1	-	−1.9	-
34	Q9UL46	Proteasome activator complex subunit 2	28	35	5.4	27.3	5.4	−2.3	-	-

35	Q06323	Proteasome activator complex subunit 1	30	35	5.47	28.7	5.8	1.8	-	-
36	P30519	Heme oxygenase	28	36.5	5.47	36.0	5.3	1.6	-	-
37[1]	P04083	Annexin A1	59	39	6.0	38.0	6.6	1.7	2.3	1.4
37[2]	P09525	Annexin A4 (ANXA4)	83	38	5.65	36.0	5.8	-	-	-
	P04083	Annexin A1	18	38	5.65	36.0	5.8	-	-	-
37[3]	P09525	Annexin A4 (ANXA4)	60	38	6.0	36.0	5.8			
	P04083	Annexin A1	34	38	6.0	36.0	5.8			
37[4]	P09525	Annexin A4 (ANXA4)	60	38	6.15	36.0	5.8	-	-	-
	P04083	Annexin A1	43	28	6.15	36.0	5.8	-	-	
38	P63261	γ-actin, Actin, cytoplasmic 2 (ACTG)	44	41	6.99	41.7	5.29	-1.4	-	-
39	P04406	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	11	36	8.0	36.0	8.57	-1.1	1.6	1.7
40	P09211	Glutathione S-transferase P	45	25	5.45	23.3	5.4	-	-	-2.3
41	P60174	Triosephosphate isomerase	36	26	6.3	26.6	6.4	1.5	-1.1	-1.6
42	Q5TAB7	Protein ripply2	41	15	4.8	14.0	4.4	1.7	-	-
43[1]	P06702	Protein S100-A9	86	11	5.4	13.2	5.7	-	-	-
43[2]	P06702	Protein S100-A9	76	11	5.4	13.2	5.7	-	3.2	

(continued)

Table 4.1 (continued)

Spot No.	Access number	Protein name (abbreviation)	Match, %	Experimental		Modeling		Avg. vol. ratio		
				Mw, kDa	pI	Mw, kDa	pI	KG1/ CD34+	NF/ CD34+	NF/ KG1
43[3]	P06702	Protein S100-A9	92	11	5.45	13.2	5.7	-	-	-
43[4]	P06702	Protein S100-A9	72	11	5.55	13.2	5.7	-	-	-
43[5]	P06702	Protein S100-A9	86	11	5.6	13.2	5.7	-	-	-
44	Q86SG5	Protein S100-A7A (S1A7A)	62	15	5.5	11.3	6.9	-	-	-
45	Q05315	Eosinophil lysophospholipase	36	15	6.3	16.4	6.8	-	-1.5	-
46[1]	PP05109	Protein S100-A8	52	12	6.85	10.8	6.51	-	-	-
46[2]	PP05109	Protein S100-A8	60	12	6.95	10.8	6.51	-	1.8	-
46[3]	PP05109	Protein S100-A8	67	12	7.2	10.8	6.51	-	-	-
46[4]	PP05109	Protein S100-A8	52	12	7.3	10.8	6.51	-	-	-
46[5]	PP05109	Protein S100-A8	52	12	8.58	10.8	6.51	-	-	-
46[6]	PP05109	Protein S100-A8	52	12	10.0	10.8	6.51	-	-	-
47[1]	P07737	Profilin-1	37	14	7.6	15.0	8.4	-2.5	1.4	3.5
47[2]	P07737	Profilin-1	41	14	8.0	15.0	8.4	-2.2	1.2	2.6
47[3]	P07737	Profilin-1	30	14	8.6	15.0	8.4	-2.3	1.3	2.9
48	P68366	Tubulin alpha-4A chain	25	63	5.1	50.6	4.95	-4.4	-	-
49	Q15084	Protein disulfide-isomerase A6 (PDIAG)	26	50	5.1	48.4	4.95	-1.0	-	-

(continued)

50	P24666	Low molecular weight phosphotyrosine protein phosphatase, LMW-PTPase (PPAC)	68	19	5.8	18.4	6.30	2.1	2.1	1.0
51	Q01469	Fatty acid-binding protein, epidermal (FABP5)	73	14	5.85	15.4	6.6	10.1	1.0	-9.7
52	P61019	Ras-related protein Rab-2A (RAB2A)	45	22.5	5.75	23.7	6.08	2.1	1.6	-1.3
53	P18669	Phosphoglycerate mutase 1 (PGAMI)	49	26	6.2	28.9	6.67	1.5	1.6	1.0
54	P14868	Aspartyl-tRNA synthetase, cytoplasmic (SYDC)	28	58	5.8	57.4	6.11	3.2	-	-
55	P54577	Tyrosyl-tRNR synthetase, cytoplasmic (SYYC)	19	57	6.1	59.4	6.61	3.0	-1.2	-3.6
56	P30086	Phosphatedyethanolamine – b.p. 1 (PEBP-1)	33	21	7.4	21.0	7.00	2.2	-1.3	-2.9
57	Q06830	Peroxiredoxin-1	62	21	8.7	22.3	8.77	4.1	-	-
58	P23528	Coffilin 1	32	17	8.5	18.7	8.22	-1.1	-1.1	-1.0

(continued)

Table 4.1 (continued)

Spot No.	Access number	Protein name (abbreviation)	Match, %	Experimental		Modeling		Avg. vol. ratio		
				Mw, kDa	pI	Mw, kDa	pI	KG1/ CD34+	NF/ CD34+	NF/ KG1
59	O60361	Putative nucleoside diphosphate kinase (NDK8)	27	16	8.8	15.6	8.76	3.2	1.3	-2.5
60[1]	P62937	Peptidyl-prolyl cis-trans isomerase A (PPIA)	55	15	7.4	18.2	7.68	1.5	1.3	-1.2
60[2]	P62937	Peptidyl-prolyl cis-trans isomerase A (PPIA)	50	15	7.6	18.2	7.68	1.7	1.1	-1.6
60[3]	P62937	Peptidyl-prolyl cis-trans isomerase A (PPIA)	52	15	8.0	18.2	7.68	1.5	-1.5	-2.2
61	P40926	Malate dehydrogenase	25	35.5	8.9	35.0	8.9	-1.1	-	-
62	Q13155	Aminoacyl tRNA synthase complex-interacting multifunctional protein 2	16	35	8.4	35.3	8.4	1.2	-	-

63[1]	P04075	Fructose-bisphosphate aldolase A (ALDOA)	33	40	8.0	39.4	8.3	1.6	1.2	-1.3
63[2]	P04075	Fructose-bisphosphate aldolase A (ALDOA)	31	40	8.5	39.8	8.3	1.4	1.8	1.4
64[1]	Q15120	Pyruvate dehydrogenase kinase (PDH)	32	42	8.1	46.8	8.5	2.1	2.1	1.0
64[2]	Q15120	Pyruvate dehydrogenase kinase (PDH)	15	42	8.45	47.0	8.46	3.9	1.2	-3.2
65	Q86UK5	Limbin	71	140	6.95	148.8	6.5	-	-	-
66	Q9UHW9	Solute carrier family 12 member 6	12	130	6.95	128.6	6.64	-	-	-
67	P52333	Tyrosine-protein kinase JAK3	9	115	6.95	126.8	6.77	-	-	-
68	Q96T33	Merlin (MERL)	12	75	5.6	72.5	6.11	-	-	-
69	Q96T33	Merlin (MERL)	14	70	5.65	69.8	6.11	-	-	-
70	P50395	Rab GDP dissociation inhibitor beta	28	55	5.6	51.0	6.11	-	-	-

(continued)

Table 4.1 (continued)

Spot No.	Access number	Protein name (abbreviation)	Match, %	Experimental		Modeling		Avg. vol. ratio		
				Mw, kDa	pI	Mw, kDa	pI	KG1/ CD34+	NF/ CD34+	NF/ KG1
71	Q5T288	Annexin A8-like protein 1 (AXA81)	38	39	5.4	37.0	5.45	-	-	-
72	P12429	Annexin A3 (ANXA3)	57	37	5.45	36	5.6	-	-	-
73	P40121	Macrophage-capping protein (CAPG)	34	40	5.6	38.7	5.8	-	2.5	-
74[1]	P04406	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	29	36	8.58	36.2	8.5	1.8	1.3	-1.3
74[2]	P04406	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	29	36	8.7	36.2	8.5	-	-	-
75	Q04917	14-3-3 protein eta (1433F)	32	35.5	4.75	28	4.76	-	-	-
76	P80188	Neutrophil gelatinase-associated lipocalin (NGAL)	44	23	9.0	22.7	9.01	-	-	-

77	O60225	Protein SSX5 (SSX5)	44	20	9.3	21.6	9.35	1.8	3.0	5.4
78	P49913	Cathelicidin antimicrobial peptide (CAMP)	33	18	9.5	19.5	9.48	-1.0	4.5	4.5
79[1]	P80511	Protein S100-A12 (Neutrophil S100 protein)	52	13	5.6	10.5	5.83	-	-	-
79[2]	P80511	Protein S100-A12 (Neutrophil S100 protein)	48	11.5	5.65	10.5	5.83	-	-	-
80	Q96BR5	Hcp beta-lactamase-like protein C1orf163	40	26	6.1	26.4	5.66	-	1.7	-
81	P11413	Glucose-6-phosphate 1-dehydrogenase (G6PD)	17	60	5.65	59.0	6.39	-	-	-

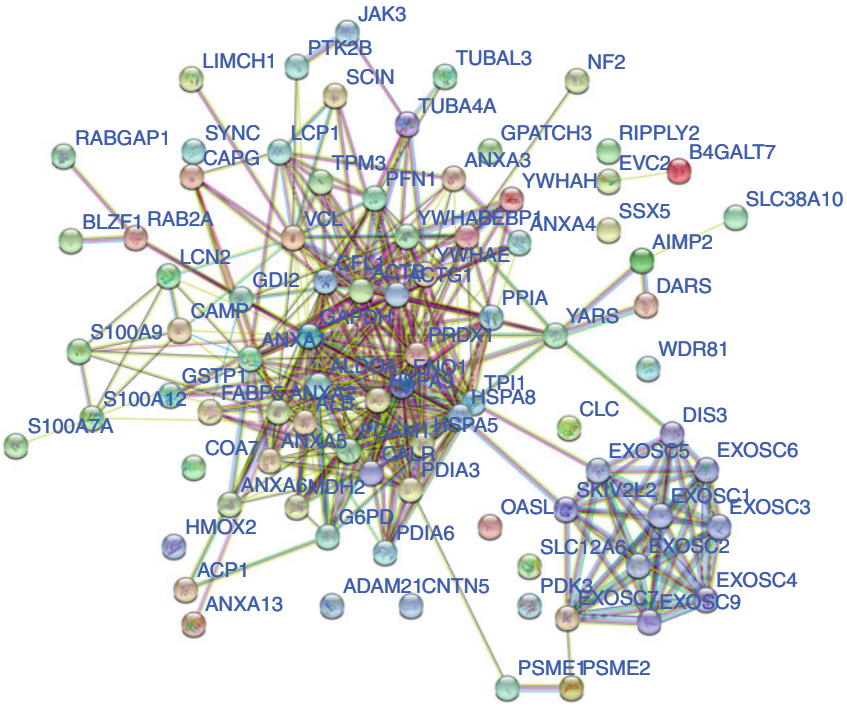

Fig. 4.3 Proteomic analysis of cytoplasmic proteins isolated from human hematopoietic CD34+, incompletely differentiated KG1 cells, and mature human neutrophils. The network of identified cytoplasmic proteins distinctive for human hematopoietic CD34+, incompletely differentiated KG1 cells, and mature human neutrophils

It is noticeable that in the cytoplasm (Figs. 4.1 and 4.3) cancerous, non-differentiated KG1 cells contain increased levels of the following proteins: Rab GTPase-activating protein 1, WD repeat-containing protein 81, Syncoilin, Aspartyl-tRNA synthetase cytoplasmic (SYDC), proteasome activator complex subunit 1, fatty acid-binding protein epidermal (FABP5), and others. Proteins with markedly reduced expression in cancer cells have also been identified: CAMP (Cathelicidin antimicrobial peptide), annexin A13, 14-3-3 protein, tubulin alfa-4A chain and several others. These proteins are related to the cellular signaling system (Rab-GTPase-activating protein, 14-3-3, etc.), to the maintenance of cell structure (sinkoiline, annexin, tubulin, etc.), are also involved in cell signaling and other cellular functions. Nuclear proteins isolated from different stages of hematopoietic differentiation were fractionated in the 2DE system (Fig. 4.2), followed by protein 2DE map comparison and mass spectrometry analysis of selected proteins. Proteins for analysis were selected on changes in their expression in different states of cell differentiation. The identified nuclear proteins are presented in Table 4.2 and the

network of identified nuclear proteins typical for human hematopoietic CD34+, incompletely differentiated AML cell line KG1, and mature human neutrophils was established (Fig. 4.4).

Table 4.2 The summarized search results (by `PepIdent`, `EMBL`, `MS-Tag` software) of comparative expression analysis of identified nuclear proteins isolated from CD34+, KG1 cells, and human neutrophils (NF)

Spot No.	Access number	Protein name (abbreviation)	Match, %	Experimental		Modeling		Avg. vol. ratio		
				Mw, kDa	pI	Mw, kDa	pI	KG1/ CD34+	NF/ CD34+	NF/ KG1
1	Q92539	Phosphatidate phosphatase (LPIN2)	20	125	4.7–4.85	100193	5.18	-	-	−6.7
	P57740	Nup107	4	125	4.7–4.85	106300	5.3	-	-	−6.7
2	Q92598	Heat shock protein 105 kDa	5	110	5.35	96866	5.3	−1.8	−2.3	−1.3
3	Q9BWU0	Kanadaptin	7	105	5.4	88815	5.1	-	-	-
4	P02788	Lactotransferrin	18	100	7.7	78183	8.5	-	-	-
	Q9P0L2	Serine/threonine-protein kinase (MARK1)	6	100	7.7	89003	9.4	-	-	-
5	P02788	Lactotransferrin	3	100	7.9	78183	8.5	-	-	-
6	P02788	Lactotransferrin	18	95	8.2	78183	8.5	-	-	-
	Q9P0L2	Serine/threonine-protein kinase (MARK1)	7	95	8.2	89003	9.4	-	-	-
7	P02788	Lactotransferrin	7	100	9.3	78183	8.5	-	-	-
	O94929	Actin-binding LIM protein 3	7	100	9.3	77802	8.9	-	-	-
8	P02788	Lactotransferrin	16	95	9.3	78183	8.5	-	-	-
	Q9P0L2	Serine/threonine-protein kinase (MARK1)	6	95	9.3	89003	9.4	-	-	-
9	Q04864	Proto-oncogene c-Rel	13	75	5.4	68520	5.6	-	-	-
10	P22460	Potassium voltage-gated channel subfamily A member 5	22	68	5.4	67228	5.7	1.3	−2.4	−3.2
11	Q8WX92	Negative elongation factor B	7	65	5.6	65698	5.8	-	-	-
12	Q86VW2	Guanine nucleotide exchange factor (GEFT)	14	70	6.0	63843	6.2	-	-	-

(continued)

Table 4.2 (continued)

Spot No.	Access number	Protein name (abbreviation)	Match, %	Experimental		Modeling		Avg. vol. ratio		
				Mw, kDa	pI	Mw, kDa	pI	KG1/ CD34+	NF/ CD34+	NF/ KG1
13	O95376	Protein ariadne-2 homolog	20	60	5.4	57819	5.4	-	-	−1.1
14	P17014	Zinc finger protein 12	10	58	5.75	58223	7.1	-	1.8	1.8
15	P60709	ACTB	18	43	5.3	42	5.29	−1.4	−2.2	−1.5
16	Q13394	Protein mab-21-like 1	15	35	8.2	40957	8.9	1.0	−6.1	−6.2
	P25105	Platelet-activating factor receptor	28	35	8.2	39204	9.2	1.0	−6.1	−6.2
17	Q9UMX6	Guanylyl cyclase-activating protein 2	22	33	4.8	23420	4.7	-	-	-
18	B5MD39	Putative γ-glutamyltransferase light chain 3	23	29	5.9	24102	5.8	-	-	-
19	Q86V88	Magnesium-dependent phosphatase 1	22	25	5.75	20109	6.0	-	–	-
20	Q5W111	Chronic lymphocytic leukemia deletion region gene 6 protein	10.	23	6.5	21666	6.2	-	-	-
	Q9Y6G5	COMM domain-containing protein 10	12	23	6.5	22967	6.1	-	-	-
	Q9BUE0	Mediator of RNA polymerase II transcription subunit 18	11	23	6.5	23663	6.1	-	-	-
21	P18847	Cyclic AMP-dependent transcription factor ATF-3	18	25	8.7	20576	8.8	−5.8	−1.0	5.7
22	Q9Y421	Protein FAM32A	32	15	8.2	13178	10	1.1	−1.5	−1.6
	P03973	Antileukoproteinase	16	15	8.2	14326	9.1	1.1	−1.5	−1.6
23	Q52LT0	Putative GTP-binding protein FLJ12595	6	21	8.5	16442	8.7	3.2	2.5	−1.3

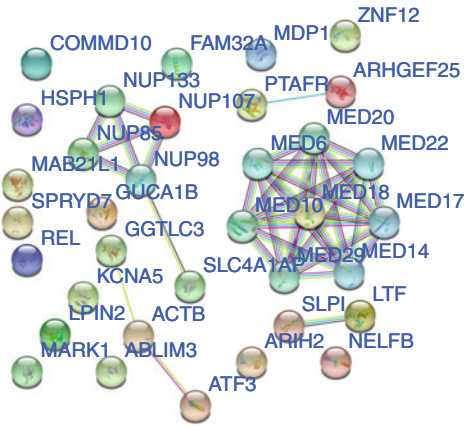

Fig. 4.4 Proteome analysis of nuclear proteins isolated from human hematopoietic CD34+, incompletely differentiated AML cell line KG1, and mature human neutrophils. The network of identified nuclear proteins typical for human hematopoietic CD34+, incompletely differentiated AML cell line KG1, and mature human neutrophils

Several specific proteins have been identified that are specific only to healthy mature neutrophils. Multiple protein expression is enhanced in KG1 cancerous non-differentiated cells: putative GTP-binding protein, Protein mab-21-like 1, etc. For example, the expression of the periodic AMP-dependent transcription factor ATF-3 is reduced in KG1 leukemic cells. Changes in the expression of these proteins could be related to blood cell differentiation.

4.2 Proteomic Analysis of APL Cells Induced to Differentiation

Our initial study (Navakauskiene et al. 2003a,b; Treigyte et al. 2000b,a, 2004; Borutinskaite et al. 2005) was concentrated on identification of proteins that can be involved in cell differentiation and proliferation in acute promyelocytic HL-60 and NB4 cells. The differentiation of leukemic cells was stimulated by using ATRA. The protein profile increase was observed afterwards.

The proteomics in NB4 cells was studied when induction of granulocytic differentiation with following growth inhibition was induced with all-trans retinoic acid and HDAC inhibitor BML-210. The alterations in protein expression at various times of treatment (2, 4, 8, 24, 48, 72, 96 h) were noticed (Fig. 4.5) (Borutinskaite et al. 2005).

Our studies demonstrated potential of combination of HDACi BML-210 with all-trans retinoic acid for differentiation induction in leukemic NB4 cells: the largest amount of differentiated cells after ATRA treatment was observed at 72–96 h (70–80%), and the combined treatment considerably improves granulocytic differentiation of NB4 cells (up to 90%).

Total soluble and less soluble proteins were isolated, fractionated, and identified by mass spectrometry. The less soluble (or insoluble) protein fraction of

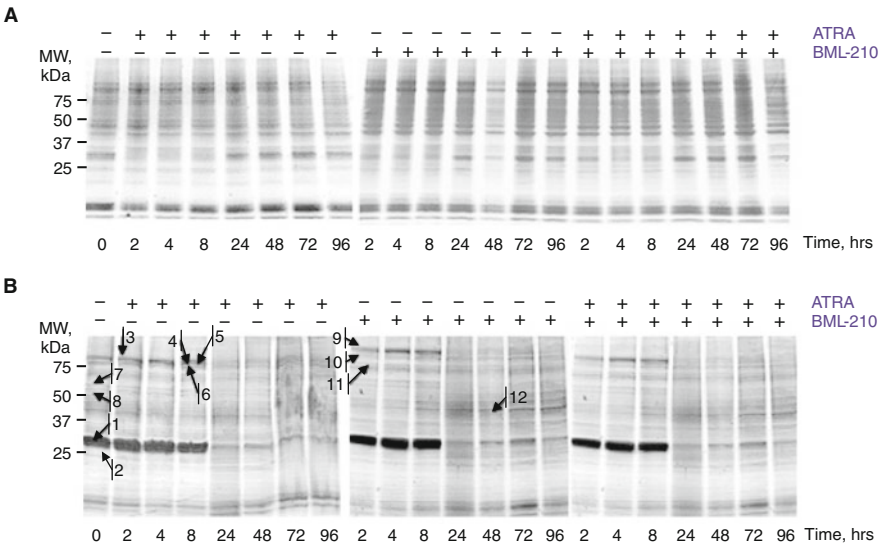

Fig. 4.5 Expression of proteins in NB4 cells treated with all-trans retinoic acid (ATRA) and histone deacetylase inhibitor BML-210. Total soluble (A) and insoluble (B) protein fractions were dissociated from control and treated with 1 μM ATRA and 10 μM BML-210 separately or in combination (1 μM ATRA and 5 μM BML-210) NB4 cells during the indicated period of time. Isolated proteins were fractionated by SDS-PAGE on an 8–16% acrylamide gradient gel and then stained with brilliant Blue G-Colloidal. Migration of the molecular size marker proteins is shown to the left (kDa values). The position of bands that were cut to MALDI-MS analysis is designated by arrows and numbers in the images. According Borutinskaite et al. (2005)

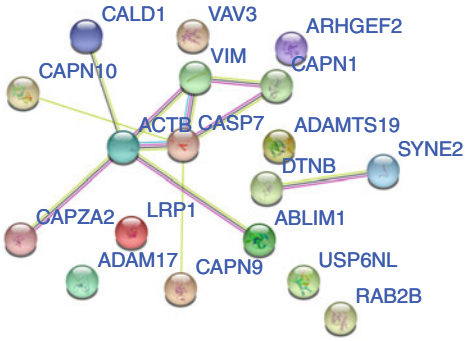

Fig. 4.6 Expression of proteins in NB4 cells treated with all-trans retinoic acid (ATRA) and histone deacetylase inhibitor BML-210. Represents the identified protein network. According Borutinskaite et al. (2005)

NB4 cells showed biggest differences in expression of proteins during treatments with ATRA and HDAC inhibitor BML-210. Using mass spectrometry, there were inspected proteins of cells with cancer and membrane or membrane-associated proteins. We identified (Figs. 4.6 and 4.7) such proteins like caspase-7, nesprin-2, RAB, USP6NL protein, Vav-3, ADAMTS-19, lipoprotein receptor-related protein, ADAM-17, actin-binding LIM protein 1, caldesmon (CDM) splice isoform 3, caldesmon, calpain 10, dystrobrevin-β, vimentin, ADAMTS-17, GEF-H1, calpain

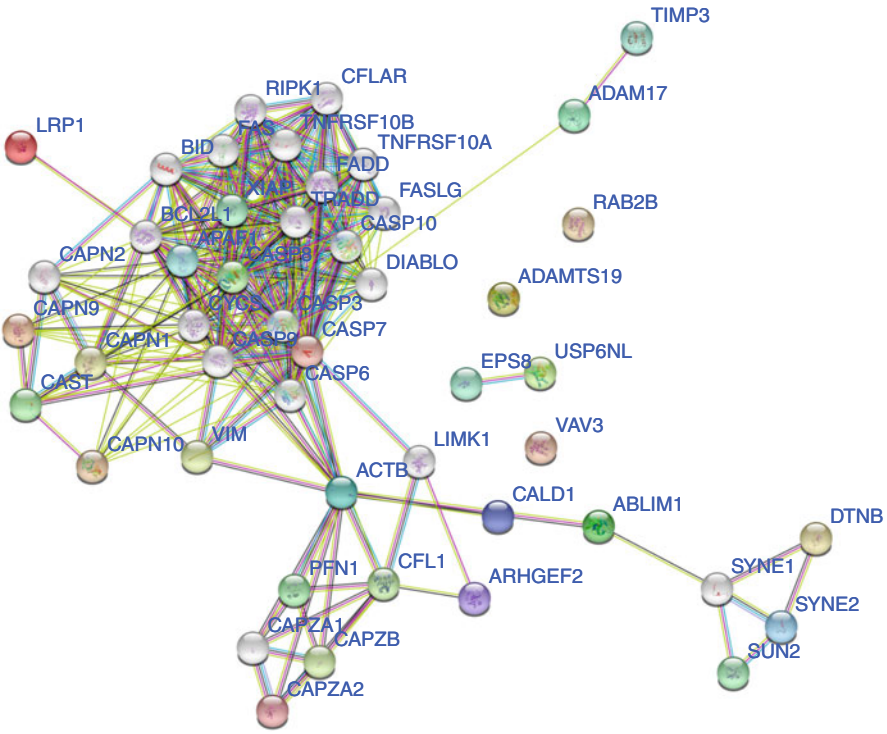

Fig. 4.7 Expression of proteins in NB4 cells treated with all-trans retinoic acid (ATRA) and histone deacetylase inhibitor BML-210. Represents the involvement of identified proteins into large scale of proteins network inclusive different cellular processes like apoptosis and cytoskeleton reorganization. According Borutinskaite et al. (2005)

9, calpain 1, actin, F-actin capping protein alfa-subunit (Borutinskaite et al. 2005). All functions of identified proteins are listed in Table 4.3.

The high expression of small GTPase, Rab2B was detected in insoluble fraction of untreated and treated cells for 8 h, and their decreased level was observed after 24 h of treatment. This indicate that Rab2B expression may be related with granulocytic differentiation or apoptosis of NB4 cells. It was shown that the main function of Rab2B protein is vesicle transport and membrane fusion regulation. Actin (ACTB) and actin-associated proteins (nesprin-2, actin-binding LIM protein, vimentin and caldesmon) were found in insoluble fraction of NB4 cells. These proteins are important for cytoskeletal reorganization during cell growth and differentiation/apoptosis. The higher expression of aforementioned proteins was in soluble fraction compared with insoluble fraction of NB4 cells.

Also we found two proteins (ADAM17 and ARHG2) that were upregulated both in control cells and induced to differentiation with following apoptosis by ATRA and HDAC inhibitor BML-210. It was showed that ADAMs have adhesive and proteolytic characteristics which result in regulation of such processes as

Table 4.3 The characteristics and functions of identified proteins in NB4 cells treated with ATRA and inhibitor BML-210. Proteins were estimated according to PeptIdent program and EXPASY database, experimental (gel position) and calculated Mw and pI, MOWSE SCORE and sequence coverage (PeptIdent) for each protein were represented: 1st column—number of band in gels (Fig. 4.5B); 2nd column—the protein Mw and pI, calculated from the amino acid sequence in the ExPASy database; 3rd column—the name of identified proteins; 4th column—general function(s) of a protein in Uniprot database. According Borutinskaite et al. (2005)

Band No.	Protein		Protein name, entry name, entry	Function
	pI	Mw, kDa		
1	8.65	28.029	Caspase-7, CASP7_HUMAN, P55210	Involved in the activation cascade of caspases responsible for apoptosis execution.
	6.5	30.269	Nesprin-2, SYNE2_HUMAN, Q8WXH0	As a component of the LINC (LInker of Nucleoskeleton and Cytoskeleton) complex participates in the connection between the nuclear lamina and the cytoskeleton.
2	4.7	26.101	Ras-related protein Rab-2B, RAB2B_HUMAN, Q8WUD1	Needed for protein transport from the endoplasmic reticulum to the Golgi complex.
	12.41	22.305	USP6 N-terminal-like protein, US6NL_HUMAN, Q92738	Acts as a GTPase-activating protein for RAB5A and RAB43. Involved in receptor trafficking.
3	6.65	97.775	Guanine nucleotide exchange factor, VAV3_HUMAN, Q9UKW4	Exchange factor for GTP-binding proteins, may be significant for integrin-mediated signaling.
4	8.13	100.198	A disintegrin and metalloproteinase with thrombospondin motifs 19, ATS19_HUMAN, Q8TE59	Uncharacterized. ADAMTS19 hypermethylation is related with transcriptional downregulation and reduces the *in vitro* migration capabilities of CRC cells[a].

(continued)

Table 4.3 (continued)

Band No.	Protein		Protein name, entry name, entry	Function
	pI	Mw, kDa		
	4.86	99.174	Prolow-density lipoprotein receptor-related protein 1, LRP1_HUMAN, Q07954	Involved in endocytosis and in phagocytosis of apoptotic cells.
5	5.6	78.542	Disintegrin and metalloproteinase domain-containing protein 17, ADA17_HUMAN, P78536	Acts as an activator of Notch, TNF-alpha and other signaling pathways.
	8.88	87.644	Actin-binding LIM protein 1, ABLM1_HUMAN, O14639	May act as scaffold protein. May be significant during the development of the retina. Has been suggested to play a role in axon guidance.
6	6.66	64.256		
	5.6	93.56	Caldesmon, CALD1_HUMAN, Q05682	Interacts with actin, myosin, two molecules of tropomyosin and with calmodulin. Also plays a significant role during cellular mitosis and receptor capping.
7	6.41	57.984	Calpain-10, CAN10_HUMAN, Q9HC96	Calcium-regulated non-lysosomal thiol-protease which catalyzes limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction.
	8.82	64.424	dystrobrevin-β, DTNB_HUMAN, O60941	Dystrobrevin-β is a component of the dystrophin-associated protein complex (DPC), which is responsible for the lateral transmission of the sarcomere-generated contractile force to the sarcolemma and the ECM.
8	5.06	53.52	Vimentin, VIME_HUMAN, P08670	Vimentins are class-III intermediate filaments, comprises the cytoskeleton.

(continued)

Table 4.3 (continued)

Band No.	Protein		Protein name, entry name, entry	Function
	pI	Mw, kDa		
9	7.77	96.496	Disintegrin and metalloproteinase domain-containing protein 17, ADA17_HUMAN, P78536	Acts as an activator of Notch, TNF-alpha and other signaling pathways.
	8.73	101.173	Rho guanine nucleotide exchange factor 2, ARHG2_HUMAN, Q92974	Activates Rho-GTPases by promoting the exchange of GDP for GTP. May be involved in epithelial barrier permeability, cell motility and polarization, dendritic spine morphology, antigen presentation, leukemic cell differentiation, cell cycle regulation, innate immune response, and cancer.
10	5.37	79.096	Calpain-9, CAN9_HUMAN, O14815	Calcium-regulated non-lysosomal thiol-protease which catalyzes limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction.
11	5.49	81.89	Calpain-1 catalytic subunit, CAN1_HUMAN, P07384	Calcium-regulated non-lysosomal thiol-protease which catalyzes limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction.
12	5.29	41.605	Actin. cytoplasmic 1, ACTB_HUMAN, P60709	Actin plays key functions, such as cell motility and contraction, regulate gene transcription and motility and repair of damaged DNA.
	7.61	35.024	F-actin capping protein alfa-subunit, CAZA2_HUMAN, P47755	Involved in actin cytoskeleton organization.

[a] https://clinicalepigeneticsjournal.biomedcentral.com/articles/10.1186/s13148-015-0158-1

cellular fate, proliferation, and growth. ADAM-17 is known as the one of the best investigated ADAM enzymes and is extensively expressed in different tissues including the brain, kidney, heart, and skeletal muscle. ADAM-17 is an important player in inflammation through TNF-alpha pathway, in the development of the nervous system, as it activates the neural cell adhesion, in carcinogenesis due to the enzyme which sheds growth factors required for tumor progression and growth. Another protein in the same band after fractionation in SDS/PAGE is ARHG2 (GEF-H1) that is involved in cellular processes, e.g., cell motility, cell-cycle regulation, polarization, epithelial barrier permeability, and cancer (Kashyap et al. 2019).

The other exciting protein that we identified—DTNB (dystrobrevin-β) protein in insoluble fraction. It was shown that dystrobrevin-β interacts with dystrophin short form DP71 and syntrophins SNTG1 and SNTG2. It was demonstrated that DTNB-knockout mice were healthy and had no abnormality, however the level of DTNB binding proteins were reduced. This leads to the conclussion that DTNB can be an anchor or scaffold for dystrophin and syntrophins/other associating proteins at the basal membranes of kidney and liver.

Also we indentified the two proteins (calpain 1 and calpain 9) that belong to the calcium ion-dependent papain-like protease (Calpain) family. It is known that these proteins are critical mediators of the action of calcium and are tightly regulated by an endogenous inhibitor, calpastatin. It was showed that calpain can regulate the dystrophin, heat shock protein (HSP90), actin, caspases, phospholipase A/B/C, and other protein functions (Patterson et al. 2011).

In conclusion, in this study we have found differently expressed proteins in the soluble and less soluble (insoluble) fraction of the NB4 cells. These changes in protein expression can be associated with early changes in chromatin structure during the process of differentiation or apoptosis after the treatment with HDAC inhibitor BML-210 and ATRA. Identification of new proteins involved in the differentiation process can be helpful in finding new therapies for APL treatment (Borutinskaite et al. 2005).

4.3 Proteome Profile in APL Cells Induced with Histone Deacetylase Inhibitor BML-210

The study was dedicated to identify proteins whose expression changed after NB4 cells treatment with HDAC inhibitor BML-210 (Borutinskaite and Navakauskiene 2015). BML-210 at concentration up to 20 μM was the reason of anti-proliferative and slight cytotoxic effects on NB4 cells in a dose- and time-dependent manner with accumulation of cells in G0/G1 cycle phase. We found out that after 20 μM BML-210 treatment apoptotic population in cell culture was about 90% after 48 h of treatment. Proliferating (control) NB4 cells and cells treated with 20 μM BML-210 for 24 h were lysed and soluble cell proteins were fractionated in 2DE gels and analysed by mass spectrometry (Fig. 4.8). Description of proteins

identified by mass spectrometry in NB4 cells treated with HDACi BML-210 and fractionated by 2DE is presented in Appendix D (Borutinskaite and Navakauskiene 2015).

We observed variations in protein expression profile while treating with BML-210. Computational analysis of protein expression changes are presented as fold change in comparisson with control cells (Table 4.4). The expression of proteins whose ratio G2/G1>0 is increased in treated (G2) cells more than in untreated (G1).

In total, 35 proteins were identified: 16 were down-regulated such as endoplasmin, ENPL; heat shock 84 kDa, HSP90B; 14-3-3 protein, 1433F; proliferating cell nuclear antigen, PCNA; calreticulin, CALR; and 19 proteins up-regulated (such as chloride intracellular channel protein 1, CLIC1; thioredoxin domain-containing protein 12, TXD12; lactoylglutathione lyase, LGUL) after treatment with BML-210. It is known that α/β-tubulin, β-actin, cofilin-1, myosin regulatory light chain 12A, tropomyosin, and gelsolin are involved in cell growth and/or homeostasis regulation. Other identified proteins participate in metabolism processes (disulphide isomerase, α-enolase), in protein folding (the heat shock proteins like endoplasmin, HSP90B, GRP75, GRP78 and CH60). Proteins of one more group, such as PCNA, nucleophosmin, 14-3-3 protein, prohibitin, guanine nucleotide-binding protein subunit α-11, chloride intracellular channel protein 1, and nucleoside diphosphate kinase A are in charge of signal transduction, apoptosis, cell differentiation and communication processes (Fig. 4.9).

One of the proteins with detected expression changes is HSP90. It was observed that HDAC inhibitors can provoke hyperacetylation of HSP90 and its inactivation, resulting to the degradation of proteins that need the chaperone function of HSP90 (including some oncoproteins) (Bali et al. 2005). Furthermore it was demonstrated that HSP90 inhibition correlated with growth arrest followed by differentiation and apoptosis (Nawarak et al. 2009; Heller et al. 2009). Calreticulin function was investigated by the other group (Sheng et al. 2014). They have found that high expression of calreticulin was positively related with tumor and metastasis and that calreticulin regulated cell proliferation, migration and invasion of pancreatic cancer cells in a MEK/ERK pathway dependent manner. 14-3-3 proteins have a significant role in a broad spectrum of vital regulatory processes, especially regarding signal transduction, apoptosis, cell cycle progression, and DNA replication. It was shown that treatment of leukemic cells with HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) provoked cofilin phosphorylation, rised of the vimentin and paxillin expression, also lowered the expression of stathmin (Grebenova et al. 2012).

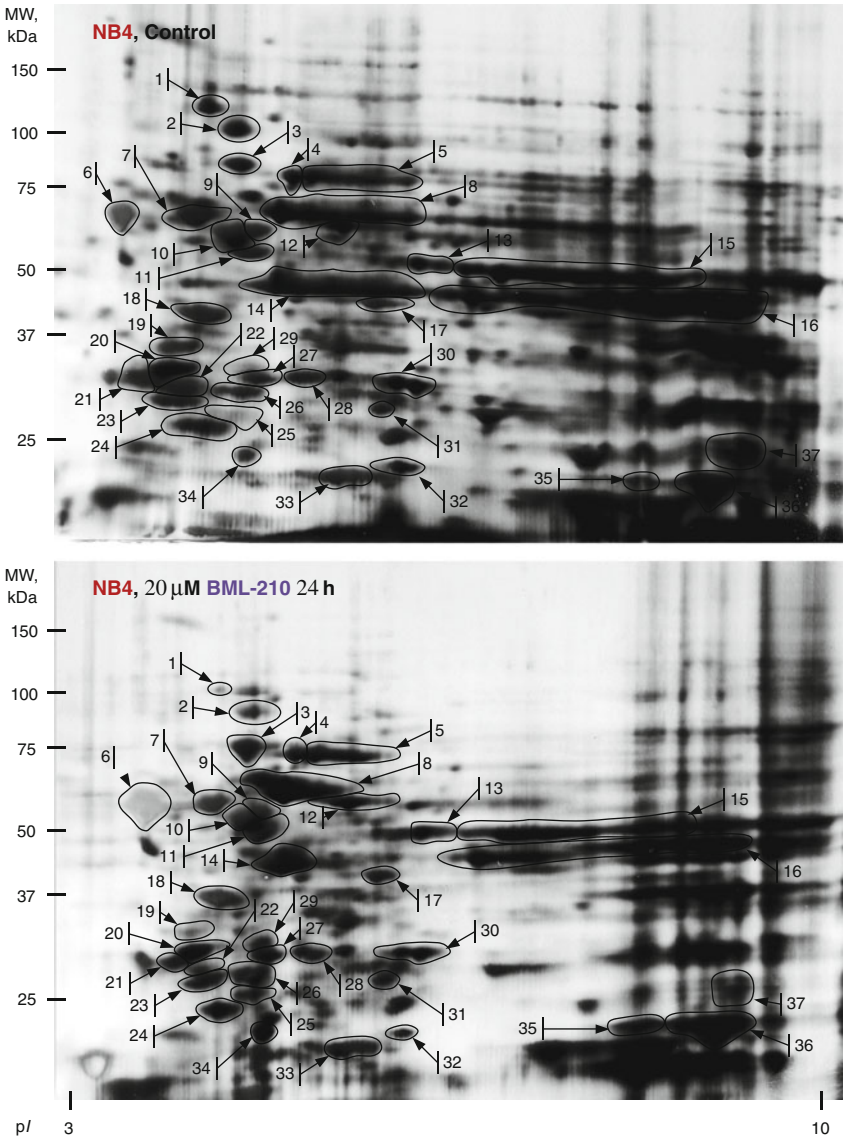

Fig. 4.8 Proteomic analysis of proteins after NB4 cell treatment with HDAC inhibitor BML-210. Proteins from untreated NB4 cells and cells treated with 20 μM BML-210 for 24 h fractionated in 2-DE system and visualized by Coomasie staining. Identified proteins are indicated in the Table 4.4. Migration of the molecular size marker proteins is shown at the center (kDa)

Table 4.4 Characterization of proteins identified in control and treated with HDAC inhibitor BML-210 NB4 cells. Computational analysis of protein expression changes are presented as fold change in comparisson with control cells (Ratio G2/G1). The expression of proteins whose Ratio G2/G1>0 is increased in treated (G2) cells than in untreated (G1)

No	Protein name	Ratio G2/G1	pI	MW	No.	Protein name	Ratio G2/G1	pI	MW
1	ENPL	−7.82	4.7	110.7	20	TPM4	−1.24	4.5	31.3
2	HSP90B	−1.30	4.9	96.9	21	1433E	−1.24	4.3	30.2
3	GRP78	1.55	4.9	78.9	22	1433F	−2.02	4.6	29.3
4	HSP72	1.17	5.2	74.9	23	1433B	1.16	4.5	28.1
5	GRP75	−1.56	5.4	74.9	24	ML12A	−1.25	4.7	25.6
6	CALR	−1.07	4.2	59.3	25	LGUL	2.40	4.9	26.8
7	PDIA1	−1.61	4.7	60.0	26	GDIR1	1.90	4.9	28.9
8	CH60	−1.15	5.2	62.0	27	EFHD2	1.40	5.0	30.4
9	TBA1B	1.25	5.0	56.7	28	PHB	1.46	5.2	30.6
10	TBB5	−1.44	4.9	54.1	29	CLIC1	2.70	5.0	31.9
11	PDIA6	2.20	5.0	51.3	30	TSG6	1.02	5.7	30.2
12	PDIA3	1.51	5.5	58.2	31	HSPB1	1.90	5.6	27.7
13	ENOA	1.27	5.7	49.4	32	COF1	−1.72	5.7	23.4
14	ACTB	−1.95	5.1	43.9	33	NDKA	1.34	5.5	22.6
15	ENOA	1.14	6.8	47.8	34	TXD12	2.26	4.9	23.7
16	NDUAA	−1.22	7.2	44.3	35	COF1	1.83	7.1	23.1
17	GNA11	1.30	5.6	41.3	36	COF1	1.76	8.4	22.7
18	NPM	1.07	4.7	38.6	37	TAGL2	−1.21	8.9	25.6
19	PCNA	−2.49	4.6	33.9					

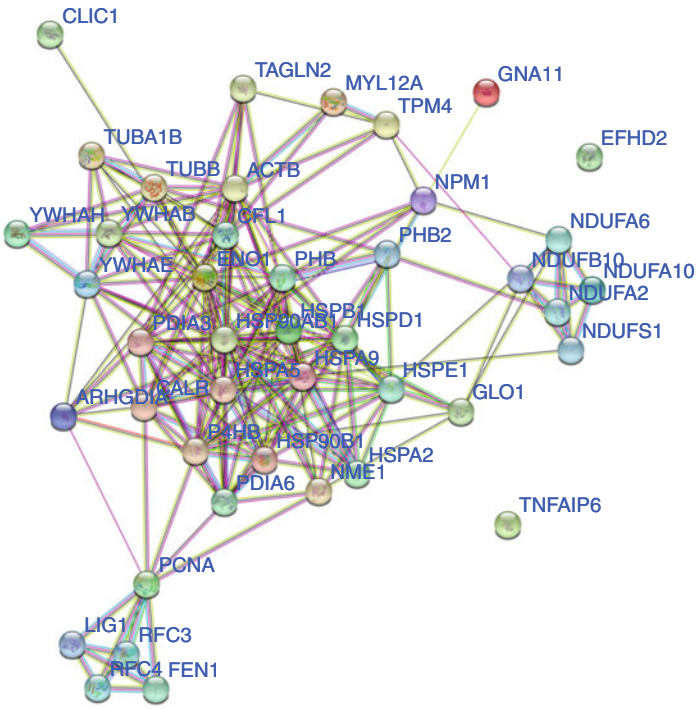

Fig. 4.9 Involvement of identified proteins in control and treated with HDAC inhibitor BML-210 NB4 cells into the cellular processes network

4.4 HDAC Inhibitor Belinostat Modulates Protein Profile in NB4 Cells

One of the most promising agents acting as epigenetic drug such as HDAC inhibitors—belinostat. It is an innovative and potent hydroxamate-type HDAC inhibitor that demonstrates potency to block enzymatic activity of 1-st and 2-nd class of HDACs (Khan et al. 2008). Belinostat exerts its anti-deacetylase action via its hydroxamic acid moiety binding to zinc ion in enzymes catalytic domain and blocking substrate access (Witter et al. 2007). In the others (Gravina et al. 2012) studies and we observed (Savickiene et al. 2014; Valiuliene et al. 2015; Valiulienė et al. 2016; Valiuliene et al. 2017; Vitkeviciene et al. 2019) its activity resulting in cell cycle arrest, apoptosis initiation, and cell proliferation inhibition. In addition, our group indicated that belinostat promotes APL granulocytic differentiation AML (Savickiene et al. 2014). Regarding belinostat's activity on APL cells, we performed proteomic analysis after chromatin immunoprecipitation with hyper-acetylated histone H4 (H4hyperAc) and established proteins being in the active complex with H4hyperAc (Valiuliene et al. 2015). Totaly we identified 68 proteins.

We found that in untreated NB4 cells hyperacetylated histone H4 (H4hyperAc) associated with 45 different proteins (Table 4.5).

The network of proteins related with hyperacetylated histone H4 in control NB4 cells is presented in Fig. 4.10 and proteins associated with hyperacetylated histone H4 in NB4 cells treated with HDAC inhibitor belinostat is presented in Fig. 4.11. Only in control cells, H4hyperAc was found related with proteins that participate in DNA replication (POLA2), transcription (GCOM1, POLR2M, NELFE, NCL), translation (RPL7) and RNA splising (SCNM1). Also it was estimated that H4hyperAc is associated with proto-oncogene SPECC1, regulator of apoptosis ADAMTSL4, together with proteins involved in various signaling cascades: NFκB, JAK2/STAT4, Ras and Hedgehog signal transduction pathways. Noteworthy, it was observed that H4hyperAc in control NB4 cells is associated with nucleophosmin (NPM), protein who is responsible for regulation of tumor suppressors TP53/p53 and ARF and is shown to be overexpressed in actively proliferating cells, like different cancer and stem cells (Lim and Wang 2006). Somewhat lesser extent of NPM was observed in complexes with H4hyperAc after treatment with 2 μM belinostat.

After 6 h treatment with 2 μM belinostat (Table 4.5, Fig. 4.11) it was estimated that H4hyperAc is associated with proteins that are pro-apoptotic and required for apoptotic response (S100A9, S100A8, LGALS7, GOLGA3, PPT1). Tumor suppressor APC was indicated in immunoprecipitated complexes, too. It should be noted that H4hyperAc is as well related with proteins that participate in the defense against oxidative stress (TXNRD2) and access of all-trans retinoic acid to the nuclear retinoic acid receptors regulation (CRABP1). We also found that after 6 h treatment of NB4 cells with 2 μM belinostat hyperacetylated histone H4 was no longer associated with proteins involved in gene transcription and/or translation. Nevereless it was found to associate with proteins, that are usually detected in cytosolic fraction as components of neutrophil extracellular traps (NETs). It is known that NETs are in association with DNA specific proteins, such as histones and antimicrobial proteins, that form an extracellular mesh able to trap and kill pathogens and they are released during a cell death that depends on ROS produced by the NADPH-oxidase complex (Valiuliene et al. 2015). After NB4 cells treatment with belinostat we identified calprotectin (S100A8 and S100A9) associated with hyperacetylted histone H4. Calprotectin is essential for the neutrophilic NADPH oxidase activation. Calprotectin is a protein complex composed of two calcium-binding proteins (S100A8 and S100A9) that are abundantly found in neutrophils cytosolic fraction and have shown to have apoptosis inducing activity. We also found a probable serine protease TMPRSS11A associated with hyperacetylated histone H4, which is in agreement with data, showing that NETs contain serine proteases, as they may execute antimicrobial functions in those structures. Taking all together, we assume that belinostat has cell death inducing activity in some manner may relate to NETs formation. Although, it is already known that belinostat triggers apoptosis in myeloid cells (Savickiene et al. 2014), not NETosis (cell death when NETs are released), the possibility that belinostat intervenes in NETs formation may not be rejected completely.

Table 4.5 Summary of identified NB4 cells proteins identified in complexes with hyperacetylated histone H4 in control (C) and belinostat (Bel) treated cells. According Valiuliene et al. (2015)

No.	Accession	Gene name	Score	C/Bel ratio	Function
1	Q5QNW6	HIST1H2AH	9505.53	0.77105	Core component of nucleosome
2	Q99878	HIST1H2AJ	8305.59	0.59452	Core component of nucleosome
3	P33778	HIST1H2BB	42815.45	1	Core component of nucleosome
4	P58876	HIST1H2BD	10401.13	0.51171	Core component of nucleosome
5	P57053	HIST2H2BF	45639.36	Bel	Core component of nucleosome
6	P84243	H3F3A	10974.41	1.10517	Core component of nucleosome
7	Q6NXT2	H3F3C	828.6	0.69768	Core component of nucleosome
8	P62805	HIST1H4A	17505.7	0.84366	Core component of nucleosome
9	P16401	HIST1H1B	615.33	1.1853	Nucleosomal condensation
10	P16403	HIST1H1C	2448.72	1.05127	Nucleosomal condensation
11	Q71UI9	H2AFV	5543.68	1.23368	Replaces conventional H2A in a subset of nucleosomes
12	P57053	H2BFS	10401.13	0.5886	Replaces conventional H2A in a subset of nucleosomes
13	P0C0S5	H2AFZ	7646.1	Bel	Replaces conventional H2A in a subset of nucleosomes
14	Q14181	POLA2	152.92	C	DNA replication
15	Q9BZD3	GCOM1	224.06	C	Component of Pol II(G) complex
16	P0CAP2	POLR2M	284.39	C	Component of Pol II(G) complex
17	P18615	NELFE	266.18	C	Represses RNA polymerase II transcript elongation
18	P51504	ZNF80	444.63	Bel	Transcriptional regulation
19	P18124	RPL7	235.63	C	Translation aparatus regulation
20	P47914	RPL29	622.4	0.92312	Translation apparatus regulation
21	Q9BWG6	SCNM1	620.47	C	RNA splicing
22	Q8WXA9	SREK1	148.46	Bel	Regulation of alternative splicing
23	P19338	NCL	132.66	C	Pre-rRNA transcription and ribosome assembly
24	P02788	LTF	275.73	C	Antimicrobial and anti-inflammatory activity
25	P61626	LYZ	751.9	3.56085	Bacteriolysis
26	P06702	S100A9	2362.67	Bel	Antimicrobial activity. Phagocyte migration promotion. Apoptosis
27	P05109	S100A8	1869.7	Bel	Antimicrobial activity. Phagocyte migration promotion. Apoptosis
28	P60709	ACTB	3806.68	1.82212	Cell motility
29	P63261	ACTG1	1713.96	0.34301	Cell motility
30	Q562R1	ACTBL2	664.65	Bel	Cell motility
31	A6NHL2	TUBAL3	110.36	C	Microtubule element
32	Q71U36	TUBA1A	452.48	C	Microtubule element

(continued)

Table 4.5 (continued)

No.	Accession	Gene name	Score	C/Bel ratio	Function
33	P07437	TUBB	620.75	2.2034	Microtubule element
34	Q9BQS8	FYCO1	61.54	C	May mediate microtubule plus end-directed vesicle transport
35	Q13326	SGCG	315.83	C	Component of sarcoglycan complex
36	Q9NY65	TUBA8	123.57	Bel	Microtubule element
37	Q9BQE3	TUBA1C	54.26	Bel	Microtubule element
38	O15144	ARPC2	666.79	Bel	Regulation of actin polimerization
39	Q96A32	MYLPF	737.84	Bel	Myosin light chain
40	Q6UY14	ADAMTSL4	247.27	C	Positive regulation of apoptosis
41	P47929	LGALS7	392.7	Bel	Apoptosis regulation. Pro-apoptotic
42	Q08378	GOLGA3	7.44	Bel	Golgi str. maintenance. Cleavage product necessary for apoptotic response
43	P50897	PPT1	124.76	Bel	Lysosomal degradation. DNA fragmentation during apoptosis
44	Q5M775	SPECC1	380.22	C	Proto-oncogene
45	P25054	APC	30.36	Bel	Tumor supressor
46	Q04760	GLO1	253.31	C	Involved in the regulation of TNF-induced transcriptional activity of NFκB
47	Q5T200	ZC3H13	22.03	C	Downregulation of NFκB pathway
48	O95989	NUDT3	215.22	C	Signal transduction. Negatively regulates ERK1/2 pathway
49	Q99665	IL12RB2	269.83	C	Signaling component coupling to the JAK2/STAT4 pathway. Promotes the proliferation of T-cells as well as NK cells
50	Q8IV04	TBC1D10C	834.75	0.8781	Ras signaling pathway inhibition
51	Q96NH3	C6orf170	1777.55	C	Controls ciliary morphology. Involved in Hedgehog signal transduction
52	P06748	NPM1	612.64	1.85893	Regulates tumor supressors TP53/p53 and ARF. Chaperone
53	Q9NNW7	TXNRD2	158.36	Bel	Implication in the defenses against oxidative stress
54	P29762	CRABP1	133.35	Bel	Regulates access of retinoic acid to the nuclear retinoic acid receptors
55	P17066	HSPA6	121.58	Bel	Chaperone
56	P48741	HSPA7	78.01	Bel	Chaperone
57	P11142	HSPA8	144.69	Bel	Chaperone. Repressor of transcriptional activation
58	P55735	SEC13	122	C	May be involved in protein transport
59	P62987	UBA52	755.27	0.77105	Proteosomal degradation, chromatin structure maintenance, gene expression regulation and stress response

(continued)

Table 4.5 (continued)

No.	Accession	Gene name	Score	C/Bel ratio	Function
60	P0CG47	UBB	241.72	C	Proteosomal degradation, chromatin structure maintenance, gene expression regulation and stress response
61	Q6ZMR5	TMPRSS11A	860.74	Bel	Pobable serine protease
62	P00738	HP	1190.67	Bel	Makes hemoglobin accessible to degradative anzymes
63	Q6S8J3	POTEE	346.61	C	Protein and ATP binding
64	A5A3E0	POTEF	369.2	2.13828	Protein and ATP binding
65	P0CG39	POTEJ	107.66	1.46228	Protein and ATP binding
66	Q9BTF0	THUMPD2	238.14	C	RNA binding. Methyltransferase activity
67	Q68CQ7	GLT8D1	206.26	C	Glycosyltransferase
68	A6NIV6	LRRIQ4	145.7	Bel	Leucine-rich repeats and IQ motif containing

Regarding effect of belinostat on cell growth, differentiation, gene and protein expression, and on epigenetic modifications, which was identified by us, belinostat could have a potential value in APL therapy.

4.5 Proteomic Maps of Leukemia Cells Induced to Granulocytic Differentiation and Apoptosis

In our study published in 2004 year (Navakauskiene et al. 2004b) we tried to evaluate protein level changes in proliferating HL-60 cells compared to cells induced for apoptosis using etoposide/Z-VAD(OH)-FMK. Programmed cell death has a significant role in the development and maintenance of homeostasis within all cells. It is widely acknowledged that the physiological form of cell death in neutrophils is apoptosis. For quite a long time it was considered that aged neutrophils die within a short period of time by spontaneous apoptosis under healthy conditions, for the sake of maintaining of homeostatic cell numbers (Geering and Simon 2011). The aim of our study was to evaluate apoptosis—associated protein patterns in HL-60 cells that were provoked to apoptosis with etoposide and also with or without the presence of the broad caspase and apoptosis inhibitor Z-VAD(OH)-FMK. Cellular cytosolic and nuclear proteins were fractionated by 2DE and changes in protein expression were detected (Fig. 4.12). In our studies that are presented in following chapters (Treigyte et al. 2000a,b; Navakauskiene et al. 2012, 2004a), we have shown that the synthesis of new proteins and protein modification takes place when HL-60 cells are induced into granulocytic differentiation. We found some quantitative and qualitative differences in the cytosolic and nuclear protein patterns of control cells, cells treated with etoposide alone or together with Z-VAD(OH)-

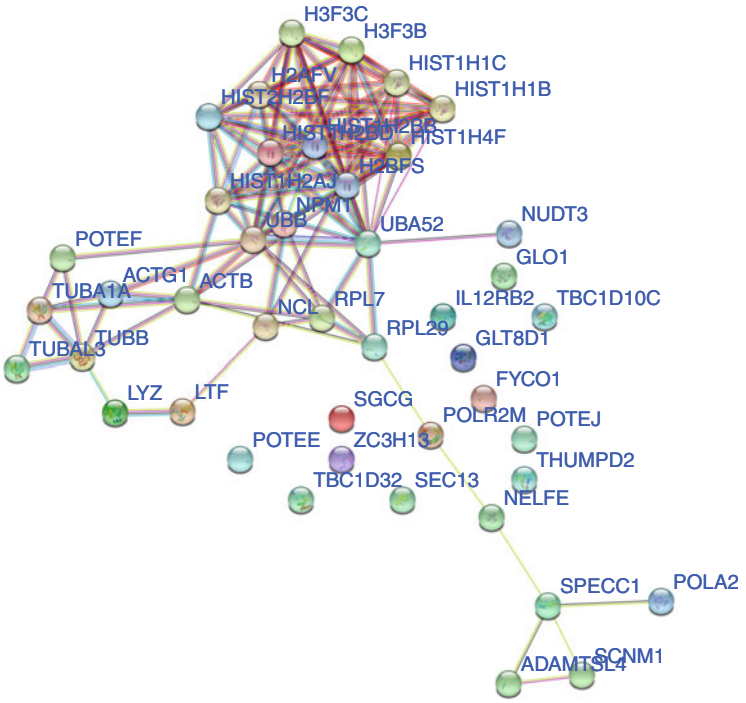

Fig. 4.10 Proteins identified in association with hyperacetylated histone H4 in control NB4 cells. Untreated NB4 cells were subjected to ChIP—MS analysis. Association network of identified proteins was studied and represented using STRING database (http://string.embl.de). According Valiuliene et al. (2015)

FMK for 6 h (Fig. 4.12). Analyses of cytosolic protein reference maps of control and drug-induced HL-60 cells revealed some new protein spots appeared, whereas the relative amount of others proteins markedly diminished or vanished after induction of apoptosis. The number of both cytosolic and nuclear polypeptides was different in HL-60 cells treated for 6 h with etoposide (60% apoptotic cells), by comparison with the control or 6 h etoposide/Z-VAD(OH)-FMK-treated cells (5–7% apoptotic cells) (Fig. 4.12). Proteins newly synthesized in HL-60 cells treated for apoptosis and absent in pan-caspase inhibitor-treated cells should have an apoptotic origin. Such proteins (marked by arrows in Fig. 4.12, 68 μM etoposide, CytP) had mostly an acidic pI 4.5–5.1, with molecular masses between 25 kDa and 150 kDa.

Some proteins with acidic and neutral pI 4.5–7.0, which were detected in the cytoplasm both etoposide and etoposide/Z-VAD(OH)-FMK treated cells were translocated exclusively into the nucleus of etoposide treated cells (Fig. 4.12, NuP, marked by arrows). These proteins could be involved in regulation of genes required in apoptosis process. We implied that variations of protein synthesis and protein modifications which were observed in differentiating HL-60 cells reflect the

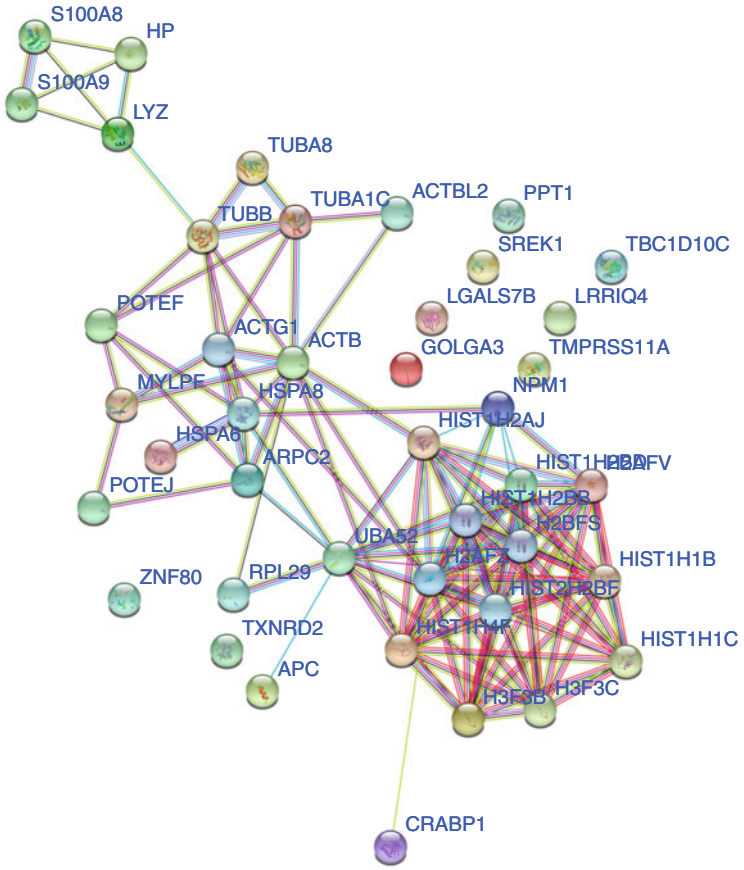

Fig. 4.11 Proteins identified in association with hyperacetylated histone H4 in belinostat treated NB4 cells. 2 μM belinostat treated NB4 cells were subjected to ChIP—MS analysis. Association network of identified proteins was studied and represented using STRING database (http://string.embl.de). According Valiuliene et al. (2015)

formation of the differentiated granulocyte phenotype and the apoptotic process. Our current research demonstrates that some proteins are highly upregulated in the cytoplasm and afterwards accumulated in the nuclei after treatment of HL-60 cells with etoposide. These proteins could participate in regulation of genes needed for apoptosis. Proteins estimated both after treatment with etoposide alone and together with pan-caspase inhibitor Z-VAD(OH)-FMK show that they do not represent a downstream event of caspase activation. We suggest that these proteins may be required for the regulation of apoptosis.

Taken together, our present study shows that some proteins are strongly upregulated in the cytoplasm and subsequently accumulated in the nuclei after treatment of HL-60 cells with etoposide. These proteins could be involved in regulation of

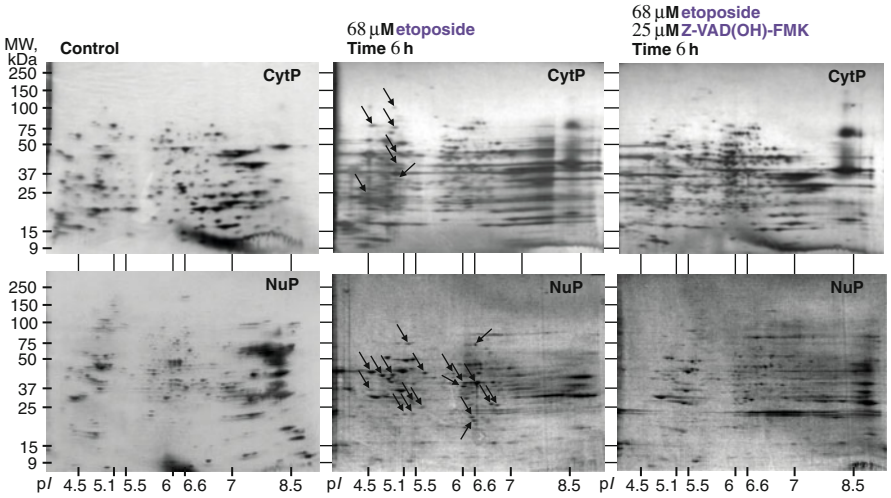

Fig. 4.12 Two-Dimensional electrophoretic maps of cytosolic and nuclear proteins of proliferating and induced to apoptosis HL-60 cells. Cytosolic (CytP) and nuclear (NuP) proteins of HL-60 cells treated for 6 h with 68 μM of etoposide alone or in the presence of 25 μM pan-caspase inhibitor Z-VAD(OH)-FMK were fractionated by 2D electrophoresis. The gels were stained by silver. Arrows show the position of proteins of apoptotic cell origin, appearing in cells treated with etoposide, but absent in broad caspase inhibitor-treated cells. According Navakauskiene et al. (2004b), License No 4796010620810

genes required for apoptosis. Proteins identified both after treatment with etoposide alone and together with pan-caspase inhibitor Z-VAD(OH)-FMK indicate that they do not represent a downstream event of caspase activation. Taken as a whole, obtained during our research results demonstrate that important alterations in both cytosolic and nuclear proteins take place in a highly regulated manner that leads to programmed cell death. Selective activation of some caspases is required to provoke apoptosis in HL-60 cells.

References

Bali P, Pranpat M, Bradner J, Balasis M, Fiskus W, Guo F, Rocha K, Kumaraswamy S, Boyapalle S, Atadja P, Seto E, Bhalla K (2005) Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90—A novel basis for antileukemia activity of histone deacetylase inhibitors. J Biol Chem 280(29):26729–26734. https://doi.org/10.1074/jbc.C500186200

Borutinskaite V, Navakauskiene R (2015) The histone deacetylase inhibitor BML-210 influences gene and protein expression in human promyelocytic leukemia NB4 cells via epigenetic reprogramming. Int J Mol Sci 16(8):18252–18269. https://doi.org/10.3390/ijms160818252

Borutinskaite V, Magnusson KE, Navakauskiene R (2005) Effects of retinoic acid and histone deacetilase inhibitor Bml-210 on protein expression in NB4 cells. Biologija 4:88–93

Borutinskaite VV, Magnusson KE, Navakauskiene R (2011) α-Dystrobrevin distribution and association with other proteins in human promyelocytic NB4 cells treated for granulocytic differentiation. Mol. Biol. Rep. 38(5):3001–3011. https://doi.org/10.1007/s11033-010-9965-9

Breitman TR, Selonick SE, Collins SJ (1980) Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. Proc Natl Acad Sci USA 77(5):2936–2940. https://doi.org/10.1073/pnas.77.5.2936

Collins SJ (1987) The HL-60 promyelocytic leukemia cell line: proliferation, differentiation, and cellular oncogene expression. Blood 70(5):1233–1244

Collins SJ, Gallo RC, Gallagher RE (1977) Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture. Nature 270(5635):347–349

Geering B, Simon HU (2011) Peculiarities of cell death mechanisms in neutrophils. Cell Death Differ 18(9, SI):1457–1469. https://doi.org/10.1038/cdd.2011.75

Gravina GL, Marampon F, Giusti I, Carosa E, Di Sante S, Ricevuto E, Dolo V, Tombolini V, Jannini EA, Festuccia C (2012) Differential effects of PXD101 (belinostat) on androgen-dependent and androgen-independent prostate cancer models. Int J Oncol 40(3):711–720. https://doi.org/10.3892/ijo.2011.1270

Grebenova D, Roeselova P, Pluskalova M, Halada P, Roesel D, Suttnar J, Brodska B, Otevrelova P, Kuzelova K (2012) Proteins implicated in the increase of adhesivity induced by suberoylanilide hydroxamic acid in leukemic cells. J Proteomics 77:406–422. https://doi.org/10.1016/j.jprot.2012.09.014

Heller T, Asif AR, Petrova DT, Doncheva Y, Wieland E, Oellerich M, Shipkova M, Armstrong VW (2009) Differential proteomic analysis of lymphocytes treated with mycophenolic acid reveals caspase 3-induced cleavage of rho GDP dissociation inhibitor 2. Ther Drug Monit 31(2):211–217. https://doi.org/10.1097/FTD.0b013e318196fb73

Hunter T (1995) Protein-kinases and phosphatases—the yin and yang of protein-phosphorylation and signaling. CELL 80(2):225–236. https://doi.org/10.1016/0092-8674(95)90405-0

Karin M, Liu ZG, Zandi E (1997) AP-1 function and regulation. Curr Opin Cell Biol 9(2):240–246. https://doi.org/10.1016/S0955-0674(97)80068-3

Kashyap AS, Fernandez-Rodriguez L, Zhao Y, Monaco G, Trefny MP, Yoshida N, Martin K, Sharma A, Olieric N, Shah P, Stanczak M, Kirchhammer N, Park SM, Wieckowski S, Laubli H, Zagani R, Kasenda B, Steinmetz MO, Reinecker HC, Zippelius A (2019) GEF-H1 signaling upon microtubule destabilization is required for dendritic cell activation and specific anti-tumor responses. Cell Rep 28(13):3367+. https://doi.org/10.1016/j.celrep.2019.08.057

Khan N, Jeffers M, Kumar S, Hackett C, Boldog F, Khramtsov N, Qian X, Mills E, Berghs SC, Carey N, etal (2008) Determination of the class and isoform selectivity of small-molecule histone deacetylase inhibitors. Biochem J 409(2):581–589

Kulyte A, Navakauskiene R, Treigyte G, Gineitis A, Magnusson KE (2001) Parallel assessment of tyrosine phosphorylation and nuclear targeting of proteins. Biotechniques 31(3):510, 512–4, 517

Kulyte A, Navakauskiene R, Treigyte G, Gineitis A, Bergman T, Magnusson KE (2002) Characterization of human alpha-dystrobrevin isoforms in HL-60 human promyelocytic leukemia cells undergoing granulocytic differentiation. Mol Biol Cell 13(12):4195–4205. https://doi.org/10.1091/mbc.E02-03-0128

Lim MJ, Wang XW (2006) Nucleophosmin and human cancer. CAncer Detect Preven 30(6):481–490. https://doi.org/10.1016/j.cdp.2006.10.008

Matuzevicius D, Zurauskas E, Navakauskiene R, Navakauskas D (2008) Improved proteomic characterization of human myocardium and heart conduction system by using computational methods. Biologija 4:283–289

Navakauskiene R, Treigyte G, Pivoriunas A, Savickiene J (2002) Cell cycle inhibitors in retinoic acid- and etoposide-mediated biological responses. Biologija 2:64–67

Navakauskiene R, Kulyte A, Treigyte G, Gineitis A, Magnusson KE (2003a) Translocation of transcription regulators into the nucleus during granulocyte commitment of HL-60 cells. Biochem Cell Biol.-Biochim et Biol Cell 81(4):285–295. https://doi.org/10.1139/O03-055

Navakauskiene R, Treigyte G, Kulyte A, Magnusson KE (2003b) Proteomic analysis by MALDI-TOf mass spectrometry and its application to HL-60 cells. Biologija 3:63–65

Navakauskiene R, Treigyte G, Gineitis A, Magnusson KE (2004a) Identification of apoptotic tyrosine-phosphorylated proteins after etoposide or retinoic acid treatment of HL-60 cells. Proteomics 4(4):1029–1041. https://doi.org/10.1002/pmic.200300671

Navakauskiene R, Treigyte G, Savickiene J, Gineitis A, Magnusson KE (2004b) Alterations in protein expression in HL-60 cells during etoposide-induced apoptosis modulated by the caspase inhibitor ZVAD.fmk. In: Diederich M (ed) Signal Transduction Pathways, Chromatin Structure, and Gene Expression Mechanisms as Therapeutic Targets, Fdn Rech Canc & Sang; Novartis Luxembourg; Q8 Petr, Annals of the New York Academy of Sciences, vol 1030, pp 393–402. https://doi.org/10.1196/annals.1329.0049

Navakauskiene R, Treigyte G, Borutinskaite VV, Matuzevicius D, Navakauskas D, Magnusson KE (2012) Alpha-dystrobrevin and its associated proteins in human promyelocytic leukemia cells induced to apoptosis. J Proteomics 75(11):3291–3303. https://doi.org/10.1016/j.jprot.2012.03.041

Navakauskiene R, Borutinskaite VV, Treigyte G, Savickiene J, Matuzevicius D, Navakauskas D, Magnusson KE (2014) Epigenetic changes during hematopoietic cell granulocytic differentiation—comparative analysis of primary CD34+cells, KG1 myeloid cells and mature neutrophils. BMC Cell Biol 15:4. https://doi.org/10.1186/1471-2121-15-4

Nawarak J, Huang-Liu R, Kao SH, Liao HH, Sinchaikul S, Chen ST, Cheng SL (2009) Proteomics analysis of A375 human malignant melanoma cells in response to arbutin treatment. Biochim Biophys Acta Proteins Proteomics 1794(2):159–167. https://doi.org/10.1016/j.bbapap.2008.09.023

Patterson C, Portbury AL, Schisler JC, Willis MS (2011) Tear me down role of calpain in the development of cardiac ventricular hypertrophy. Circ Res 109(4):453–462. https://doi.org/10.1161/CIRCRESAHA.110.239749

Pawson T (1995) Protein modules and signaling networks. Nature 373(6515):573–580. https://doi.org/10.1038/373573a0

Savickiene J, Treigyte G, Valiuliene G, Stirblyte I, Navakauskiene R (2014) Epigenetic and molecular mechanisms underlying the antileukemic activity of the histone deacetylase inhibitor belinostat in human acute promyelocytic leukemia cells. Anti-Cancer Drugs 25(8):938–949. https://doi.org/10.1097/CAD.0000000000000122

Sheng W, Chen C, Dong M, Zhou J, Liu Q, Dong Q, Li F (2014) Overexpression of calreticulin contributes to the development and progression of pancreatic cancer. J Cell Physiol 229(7):887–897. https://doi.org/10.1002/jcp.24519

Sporn MB, Roberts AB, Goodman DS (1994) The retinoids: biology, chemistry, and medicine. Raven Press, New York

Treigyte G, Navakauskiene R, Kulyte A, Gineitis A, Magnusson KE (2000a) Characteristics of cytosolic proteins and changes in their tyrosine phosphorylation during HL-60 cell differentiation. Biologija 2:32–35

Treigyte G, Navakauskiene R, Kulyte A, Gineitis A, Magnusson KE (2000b) Tyrosine phosphorylation of cytoplasmic proteins in proliferating, differentiating, apoptotic HL-60 cells and blood neutrophils. Cell Mol Life Sci 57(13–14):1997–2008. https://doi.org/10.1007/PL00000681

Treigyte G, Savickiene J, Navakauskiene R (2004) Identification of O- and N-glycosylated nuclear proteins of HL-60 cells induced to granulocytic differentiation. Biologija 2:49–51

Valiuliene G, Stirblyte I, Cicenaite D, Kaupinis A, Valius M, Navakauskiene R (2015) Belinostat, a potent HDACi, exerts antileukaemic effect in human acute promyelocytic leukaemia cells via chromatin remodelling. J Cell Mol Med 19(7):1742–1755. https://doi.org/10.1111/jcmm.12550

Valiulienė G, Treigytė G, Savickienė J, Matuzevičius D, Alksnė M, Jarašienė-Burinskaja R, Bukelskienė V, Navakauskas D, Navakauskienė R (2016) Histone modifications patterns in tissues and tumours from acute promyelocytic leukemia xenograft model in response to combined epigenetic therapy. Biomed. Pharmacother. 79:62–70. https://doi.org/10.1016/j.biopha.2016.01.044

Valiuliene G, Stirblyte I, Jasnauskaite M, Borutinskaite V, Navakauskiene R (2017) Anti-leukemic effects of HDACi belinostat and HMTi 3-Deazaneplanocin A on human acute promyelocytic leukemia cells. Eur J Pharmacol 799:143–153. https://doi.org/10.1016/j.ejphar.2017.02.014

Vitkeviciene A, Skiauteryte G, Zucenka A, Stoskus M, Gineikiene E, Borutinskaite V, Griskevicius L, Navakauskiene R (2019) HDAC and HMT inhibitors in combination with conventional therapy: a novel treatment option for acute promyelocytic leukemia. J. Oncol. 2019:11. https://doi.org/10.1155/2019/6179573

Witter DJ, Belvedere S, Chen L, Secrist JP, Mosley RT, Miller TA (2007) Benzo[b]thiophene-based histone deacetylase inhibitors. Bioorg Med Chem Lett 17(16):4562–4567. https://doi.org/10.1016/j.bmcl.2007.05.091