



Identification of Antibacterial Components in Human Hair Shafts*

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Antimicrobial peptides (AMPs) are evolutionarily old components of innate immunity. AMPs identified in human skin include defensins, cathelicidin, dermcidin, psoriasin, and RNase7 (1, 2). Some AMPs, such as the human cathelicidin peptide LL-37, are upregulated during inflammation in skin (1). Various microbes colonize the hair follicular canal, and various AMPs may modulate their population and composition (3, 4). It is not known whether the hair shaft *per se* contains functionally active antimicrobials.

In a recent study, we explored different extraction procedures to determine the complete proteome of human hair shafts. That work focused on establishing potential biomarkers for hair with importance for evaluating factors, such as ageing, infection, and potentially underlying follicular disorders. A multitude of potential antimicrobial peptides and proteins were observed, including lysozyme, various S100 proteins, and histones (5). This report focuses on the identification of hair-derived molecules with potential antimicrobial activity. Hair shafts were subjected to an acid-based extraction method (Appendix S1¹). The extracted material was fractionated using reverse-phase high-pressure liquid chromatography (RP-HPLC), and each fraction was analysed for antimicrobial activity by radial diffusion assay. Potential AMPs were identified by Western blotting combined with high-throughput mass spectrometry coupled with liquid chromatography.

RESULTS AND DISCUSSION

Hair shaft extraction using a buffer containing 8 *M. urea* (see Appendix S1¹), followed by mass spectrometric analysis of this extract revealed the presence of multiple proteins (Table SI¹). Next, we attempted to detect possible antibacterial activity derived from hair shafts. Using RP-HPLC, a major protein peak was observed (Fig. 1A, peak at 18.44, fractions 17–23). The eluent was characterized using SDS-PAGE analysis (Fig. 1A insert) and antibacterial activity was analysed using a radial diffusion assay (RDA) by collecting and analysing

fractions eluted between 17 and 23 min (corresponding to 23–40% acetonitrile) (Fig. 1B). The results indicated that the antibacterial activity was related to proteins with molecular masses of 4–15 kDa.

The RDA analysis demonstrated that fractions 19 and 20 showed zones of inhibition against *Escherichia coli*, indicating the presence of antimicrobials (Fig. 1B). The fractions showing antimicrobial activity were selected for further analysis by mass spectrometry. The fractions were found to contain multiple protein sequences (Table SII¹), in particular those linked to host defence, including multiple histone sequences. The gene ontology analysis is shown in Fig. 1C. One example of an identified histone sequence, H2A, is shown in Fig. 2A.

To confirm the mass spectrometry results, RP-HPLC fractions 18–23 were further analysed by Western blot using specific rabbit polyclonal antibodies against histones. The results are presented in Fig. 2B and demonstrate proteins of 24, 17 and 14 kDa, which correspond to histones H1, H2B and H3 and H2A, respectively. To illustrate the presence of one such histone variant, immunohistochemistry analysis using an antibody against H3 was performed. The results indicated that this histone is localized in the cortex of hair shafts (Fig. 2C).

Antimicrobial activity of histones was first reported in 1958 by Hirsch (6), who demonstrated that arginine-rich preparations (later denoted as histones) isolated from calf thymus exerted potent bactericidal activity. Subsequent reports showed that histones H1, H2A and H2B isolated from different species can act as potent antimicrobial agents (7–9). Recombinant human histone H1.2 shows activity against both Gram-positive and Gram-negative bacteria, including drug-resistant strains, such as multidrug-resistant *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) (10). Histone H2B is found in human placenta and colonic epithelial cells, but also in the skin of tree frogs (11). Chicken H2B (12) displays antimicrobial activity against both Gram-positive and Gram-negative bacteria. Histones H4 and H3 obtained from calf thymus are active against *E. coli*, and histone H4 also exerts antimicrobial activity (13). Recombinant histone H4 from human sebocytes inhibited the growth of *S. aureus* and *Propionibacterium acnes*. Antimicrobial histones are also major components of neutrophil extracellular traps

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¹<https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-2952>

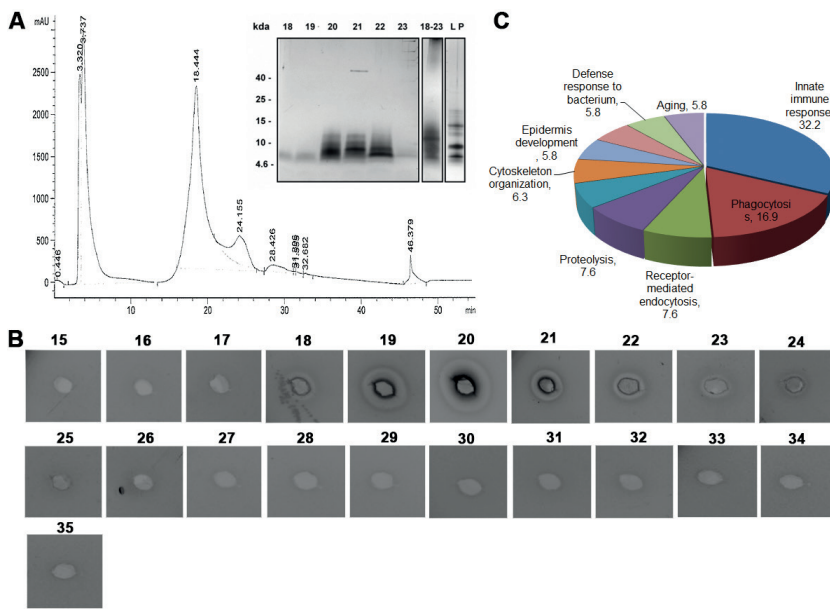


Fig. 1. Profiling and characterization of the low-molecular-weight (LMW) fraction of the human hair proteome. (a) Reverse-phase high-pressure liquid chromatography (RP-HPLC) elution profile of the LMW hair proteome (*inset*: sodium dodecyl sulphate-polyacrylamide gel electrophoresis of LMW protein extract and individual fractions eluted from minutes 15 to 35). (b) Antimicrobial activity (visualized as bacterial inhibition zones around the 3-mm wells) of individual HPLC fractions as determined by radial diffusion assay (RDA) using *Escherichia coli*. (c) Gene ontology analysis of the proteins identified in fractions 18–23 (employing PANTHER, Appendix S1¹).

histones, like many cationic polymeric hair conditioners (15), can contribute to a potential cationic charge density at the hair shaft surface. Finally, our study also shows that human hair shafts could be a new source for the purification of functionally active histones.

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(14). Hence, given the identification of multiple histones in the antibacterial fractions of the hair extracts makes it plausible that these well-known antimicrobials contribute to the observed activity. However, since other components were also identified, it cannot be excluded that the observed bacterial inhibition is due to contributions from other AMPs, or even non-peptide molecules, such as lipids, possibly co-migrating in the RP-HPLC analysis. The latter possibility is unlikely, as possible antibacterial sebum components should be washed away during the initial washes of the hair shafts with 70% ethanol (see Appendix S1¹).

In conclusion, the present study demonstrates, for the first time, methods to detect and purify antimicrobial factors, such as histones, from hair shafts. Further studies are warranted to explore whether histones or other AMPs of hair shafts confer a resistance to infection on hair *in vivo*. Furthermore, due to the cationic nature of histones, the results should encourage further research into whether

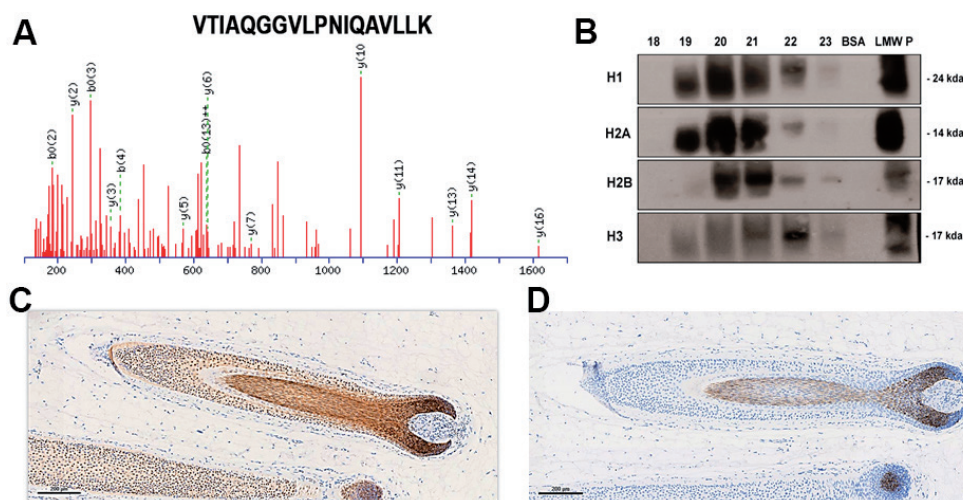


Fig. 2. Identification of histones in fractions separated by high-pressure liquid chromatography (HPLC). (a) Spectrum of histone H2A identified in the combined HPLC fractions 18–23. (b) Western blot analysis of histone isoforms in individual HPLC fractions displaying antimicrobial activity. Antibodies against H1, H2A, H2B, and H3 were used. Bovine serum albumin (BSA) was used as a negative control, and a low-molecular-weight protein (LMW P) extract from human hair shafts was used as a positive control. (c) Immunohistochemical detection of histone H3 in human hair shafts of a formalin-fixed paraffin-embedded skin section using a rabbit polyclonal anti-histone H3 (tri-methyl K9) antibody. (d) Negative control.

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Since this is a very extensive table, the format and content has not been edited by ActaDV.

Table SI. Proteins identified in the material extracted using Urea buffer (8 M urea, 25mM Tris-HCl (pH 9.5), 25% ethanol, 200 mM DTT)

Accession	GS	Name	Pro_M ass	Urea Set1_Pro tein Hit	Urea Set2- Protein hit	Urea Set1_Pr otein Score	Urea Set2_Prote in Score	Urea Set1_e mPAI	Urea Set2_em PAI	Urea Set1_Pe ptide Number	Urea Set2_Pep tide Number
sp O43790 KRT86_HUMAN	KRT86	Keratin, type II cuticular Hb6	55120	1	1	12371	12579	831,22	1111,02	534	568
tr AOA087X106 AOA087X106_HUMAN	KRT81	Keratin, type II cuticular Hb1	54850	2	2	10093	10135	429,31	609,57	462	495
sp P78385 KRT83_HUMAN	KRT83	Keratin, type II cuticular Hb3	55928	3	3	9934	9849	602,96	537,7	454	474
sp P78386 KRT85_HUMAN	KRT85	Keratin, type II cuticular Hb5	57306	4	4	9256	8943	651,23	651,23	457	468
sp Q15323 K1H1_HUMAN	KRT31	Keratin, type I cuticular Ha1	48633	5	5	8907	8803	418,06	343,16	375	382
sp O76009 KT33A_HUMAN	KRT33A	Keratin, type I cuticular Ha3-I	47166	6	6	7674	7502	207,59	181,22	312	314
sp Q14525 KT33B_HUMAN	KRT33B	Keratin, type I cuticular Ha3-II	47325	7	7	7103	7074	167,3	119,13	308	309
sp A6NCN2 KR87P_HUMAN	KRT87P	Putative keratin-87 protein	29555	8	8	6138	6468	1150	1424,1	297	307
sp P02768 ALBU_HUMAN	ALB	Serum albumin	71317	9	9	4711	4740	37,41	34,1	243	250
sp O76011 KRT34_HUMAN	KRT34	Keratin, type I cuticular Ha4	50818	10	10	3855	3940	25,22	23,62	189	190
sp Q92764 KRT35_HUMAN	KRT35	Keratin, type I cuticular Ha5	51640	11	11	3000	3184	6,71	5,81	128	134
sp P04264 K2C1_HUMAN	KRT1	Keratin, type II cytoskeletal 1	66170	12	12	1994	1607	3,72	3,08	74	69
sp P13645 K1C10_HUMAN	KRT10	Keratin, type I cytoskeletal 10	59020	14	13	1584	1463	2,88	2,3	75	74
sp Q14532 K1H2_HUMAN	KRT32	Keratin, type I cuticular Ha2	51793	13	14	1734	1405	1,68	1,52	64	67
sp P08729 K2C7_HUMAN	KRT7	Keratin, type II cytoskeletal 7	51411	15	15	1020	1035	0,86	0,98	37	43
sp Q9BYR8 KRA31_HUMAN	KRTAP3-1	Keratin-associated protein 3-1	11558	17	16	874	860	1,19	1,19	20	22
sp O95678 K2C75_HUMAN	KRT75	Keratin, type II cytoskeletal 75	59809	16	17	966	850	0,62	0,53	37	42
sp Q8IUUC1 KR111_HUMAN	KRTAP11-1	Keratin-associated protein 11-1	18385	24	18	613	690	2,24	2,24	19	25
tr K7EMD9 K7EMD9_HUMAN	KRT13	Keratin, type I cytoskeletal 13 (Fragment)	19202	21	19	718	684	2,1	1,64	49	46
sp Q9NSB2 KRT84_HUMAN	KRT84	Keratin, type II cuticular Hb4	65942	19	20	785	679	0,41	0,48	28	34
sp P35908 K22E_HUMAN	KRT2	Keratin, type II cytoskeletal 2 epidermal	65678	20	21	770	677	0,98	0,8	34	31
sp O76013-2 KRT36_HUMAN	KRT36	Isoform 2 of Keratin, type I cuticular Ha6	48424	23	22	689	671	0,69	0,59	41	41
sp Q52LG2 KR132_HUMAN	KRTAP13-2	Keratin-associated protein 13-2	19912	26	23	501	594	1,19	1,56	19	21
sp P02533 K1C14_HUMAN	KRT14	Keratin, type I cytoskeletal 14	51872	18	24	829	557	1,09	0,85	40	40
sp P04259 K2C6B_HUMAN	KRT6B	Keratin, type II cytoskeletal 6B	60315	32	25	397	543	0,53	0,79	21	25
sp Q9NSB4 KRT82_HUMAN	KRT82	Keratin, type II cuticular Hb2	57985	25	26	585	518	0,84	0,94	41	38
sp P02538 K2C6A_HUMAN	KRT6A	Keratin, type II cytoskeletal 6A	60293	28	27	426	502	0,7	0,79	23	25

sp Q9BYR6 KRA33_HUMAN	KRTAP3-3	Keratin-associated protein 3-3	11441	39	28	334	492	0,69	0,69	9	11
sp Q14CN4-2 K2C72_HUMAN	KRT72	Isoform 2 of Keratin, type II cytoskeletal 72	56342	38	29	338	462	0,33	0,41	19	19
sp P08779 K1C16_HUMAN	KRT16	Keratin, type I cytoskeletal 16	51578	22	30	708	451	1,24	0,75	41	38
sp Q3LI72 KR195_HUMAN	KRTAP19-5	Keratin-associated protein 19-5	7847	27	31	455	428	3,39	3,39	10	8
sp Q9BYR7 KRA32_HUMAN	KRTAP3-2	Keratin-associated protein 3-2	11483	30	32	414	401	0,69	0,69	10	10
sp P08727 K1C19_HUMAN	KRT19	Keratin, type I cytoskeletal 19	44079		33		372		0,44		34
sp P19012 K1C15_HUMAN	KRT15	Keratin, type I cytoskeletal 15	49409	29	34	418	352	0,38	0,29	33	33
tr G3V1C2 G3V1C2_HUMAN	KRT40	Keratin, type I cytoskeletal 40	27344	45	35	246	346	0,78	1	20	23
sp O76014 KRT37_HUMAN	KRT37	Keratin, type I cuticular Ha7	51084	33	36	381	327	0,37	0,21	27	30
sp O76015 KRT38_HUMAN	KRT38	Keratin, type I cuticular Ha8	52044	37	37	339	316	0,28	0,2	25	23
sp Q8IUC0 KR131_HUMAN	KRTAP13-1	Keratin-associated protein 13-1	19505	42	38	260	298	0,61	0,61	7	8
sp Q9BYR3 KRA44_HUMAN	KRTAP4-4	Keratin-associated protein 4-4	21487	40	39	292	277	0,78	0,78	10	8
sp P35527 K1C9_HUMAN	KRT9	Keratin, type I cytoskeletal 9	62255	34	40	378	264	0,85	0,59	25	21
sp P13647 K2C5_HUMAN	KRT5	Keratin, type II cytoskeletal 5	62568	35	41	370	264	0,59	0,51	22	17
sp P0C7H8 KRA23_HUMAN	KRTAP2-3	Keratin-associated protein 2-3	15465	52	42	200	255	0,22	0,49	5	5
tr A0A087WV47 A0A087WV47_HUMAN	IGHG1	Ig gamma-1 chain C region	51863	43	43	255	240	0,36	0,54	17	18
sp Q6A163 K1C39_HUMAN	KRT39	Keratin, type I cytoskeletal 39	57155	63	44	135	220	0,32	0,48	9	14
sp Q5XKE5 K2C79_HUMAN	KRT79	Keratin, type II cytoskeletal 79	58085	48	45	231	189	0,18	0,18	9	9
tr A0A075B6N8 A0A075B6N8_HUMAN	IGHG3	Ig gamma-3 chain C region (Fragment)	42327	59	46	146	173	0,46	0,83	17	21
sp P01876 IGHA1_HUMAN	IGHA1	Ig alpha-1 chain C region	38486	44	47	254	171	0,78	0,51	18	14
sp Q08188 TGM3_HUMAN	TGM3	Protein-glutamine gamma-glutamyltransferase E	76926	41	48	271	165	0,09	0,09	7	6
sp P0CG05 LAC2_HUMAN	IGLC2	Ig lambda-2 chain C regions	11458	47	49	233	158	1,2	1,2	8	6
sp Q9BYQ5 KRA46_HUMAN	KRTAP4-6	Keratin-associated protein 4-6	26141	54	50	180	157	0,43	0,43	10	7
sp Q8IUC3 KRA71_HUMAN	KRTAP7-1	Keratin-associated protein 7-1	9624	65	51	119	156	0,85	0,85	5	5
sp Q5VU13 VSIG8_HUMAN	VSIG8	V-set and immunoglobulin domain-containing protein 8	44946	64	52	126	153	0,24	0,33	4	4
tr A0A075B6Z2 A0A075B6Z2_HUMAN	TRAJ56	Protein TRAJ56 (Fragment)	2220	58	53	161	149	1,81	1,81	12	11
sp Q9BYR4 KRA43_HUMAN	KRTAP4-3	Keratin-associated protein 4-3	24536	57	54	165	142	0,67	0,47	10	6
sp P01620 KV302_HUMAN	sp P01620 KV302_HUMAN	Ig kappa chain V-III region SIE	11882	66	55	118	140	0,29	0,29	3	3
sp P02787 TRFE_HUMAN	TF	Serotransferrin	79294	56	56	172	138	0,38	0,33	14	16
tr H0YH81 H0YH81_HUMAN	ATP5B	ATP synthase subunit beta (Fragment)	38226	70	57	103	136	0,09	0,09	3	3

sp P01009-2 A1AT_HUMAN	SERPINA1	Isoform 2 of Alpha-1-antitrypsin	40409	89	58	54	133	0,27	0,08	4	4
tr A0A087WTX5 A0A087WTX5_HUMAN	IGKC	Ig kappa chain C region	25950	46	59	234	130	0,62	0,27	12	9
sp Q7Z794 K2C1B_HUMAN	KRT77	Keratin, type II cytoskeletal 1b	62149	60	60	145	126	0,17	0,11	6	5
tr F8W696 F8W696_HUMAN	APOA1	Apolipoprotein A-I	28005	53	61	189	122	0,12	0,12	7	5
sp O14556 G3PT_HUMAN	GAPDHS	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific	44815	72	62	92	121	0,24	0,24	7	7
sp P01023 A2MG_HUMAN	A2M	Alpha-2-macroglobulin	164613	87	63	58	119	0,04	0,04	5	4
sp Q9BYQ6 KR411_HUMAN	KRTAP4-11	Keratin-associated protein 4-11	24844	84	64	66	112	0,29	0,29	5	5
tr A0A087WU08 A0A087WU08_HUMAN	HP	Haptoglobin	31673	50	65	210	110	0,82	0,49	14	10
sp Q86SJ6-2 DSG4_HUMAN	DSG4	Isoform 2 of Desmoglein-4	117088	49	66	224	106	0,12	0,15	9	9
sp Q9BYQ3 KRA93_HUMAN	KRTAP9-3	Keratin-associated protein 9-3	19805	80	67	79	102	1,57	1,2	7	10
tr A0A087WW89 A0A087WW89_HUMAN	IGHV3-72	Protein IGHV3-72	11274	62	69	136	94	0,3	0,3	3	2
sp Q01546 K22O_HUMAN	KRT76	Keratin, type II cytoskeletal 2 oral	66370	74	68	90	94	0,1	0,16	10	10
sp P25705-2 ATPA_HUMAN	ATP5A1	Isoform 2 of ATP synthase subunit alpha, mitochondrial	54574	69	70	106	80	0,19	0,12	4	4
sp P00739-2 HPTR_HUMAN	HPR	Isoform 2 of Haptoglobin-related protein	44076	88	71	54	79	0,07	0,24	4	5
sp P31947-2 1433S_HUMAN	SFN	Isoform 2 of 14-3-3 protein sigma	24378	73	72	90	77	0,14	0,14	1	1
sp P27482 CALL3_HUMAN	CALML3	Calmodulin-like protein 3	16937	67	73	113	73	0,44	0,44	3	3
tr Q9BS19 Q9BS19_HUMAN	HPX	HPX protein	29068	78	74	83	66	0,24	0,11	4	2
tr F2Z2W8 F2Z2W8_HUMAN	SELENBP1	Selenium-binding protein 1	13968	81	75	78	63	0,24	0,24	2	3
sp Q96HU8 DIRA2_HUMAN	DIRAS2	GTP-binding protein Di-Ras2	22813	91	76	49	60	0,15	0,15	3	3
sp P02763 A1AG1_HUMAN	ORM1	Alpha-1-acid glycoprotein 1	23725	103	77	37	59	0,14	0,14	1	2
tr A0A087WU60 A0A087WU60_HUMAN	KRTAP9-2	Keratin-associated protein 9-2	20290	77	78	88	58	0,85	0,58	7	6
sp P01859 IGHG2_HUMAN	IGHG2	Ig gamma-2 chain C region	36505	68	79	112	53	0,3	0,54	13	13
sp P60413 KR10C_HUMAN	KRTAP10-12	Keratin-associated protein 10-12	28680	83	80	67	51	0,12	0,12	3	1
tr A0A087X0W2 A0A087X0W2_HUMAN	NEFL	Neurofilament light polypeptide	32621	95	81	42	48	0,11	0,11	2	2
sp A8MTY7 KRA97_HUMAN	KRTAP9-7	Keratin-associated protein 9-7	20915	117	83	31	46	0,35	0,16	2	3
tr J3QLI0 J3QLI0_HUMAN	APOH	Beta-2-glycoprotein 1 (Fragment)	22960	140	82		46	0,15	0,15	1	1
sp Q14134-2 TRI29_HUMAN	TRIM29	Isoform Beta of Tripartite motif-containing protein 29	64488	94	84	43	43	0,1	0,1	5	3
tr A0A087WUA0 A0A087WUA0_HUMAN	FGA	Fibrinogen alpha chain	33440		85		43		0,1		3

sp Q8IU81 KRA13_HUMAN	KRTAP1-3	Keratin-associated protein 1-3	20850	90	86	50	42	0,16	0,16	10	12
sp P05109 S10A8_HUMAN	S100A8	Protein S100-A8	10885	85	88	62	41	0,32	0,32	1	2
sp P04792 HSPB1_HUMAN	HSPB1	Heat shock protein beta-1	22826		87		41		0,15		2
sp B5MCY1 TDR15_HUMAN	TDRD15	Tudor domain-containing protein 15	224488	86	89	59	40	0,01	0,01	3	3
sp Q3LI83 KR241_HUMAN	KRTAP24-1	Keratin-associated protein 24-1	29012		90		39		0,11		1
sp P01024 CO3_HUMAN	C3	Complement C3	188569	96	94	42	38	0,02	0,02	1	2
sp Q9UDY8-2 MALT1_HUMAN	MALT1	Isoform 2 of Mucosa-associated lymphoid tissue lymphoma translocation protein 1	92505		93		38		0,04		3
tr A0A087WWG1 A0A087WWG1_HUMAN	SSH3	Protein phosphatase Slingshot homolog 3 (Fragment)	36130		92		38		0,09		3
tr A0A087WVV2 A0A087WVV2_HUMAN	RRBP1	Ribosome-binding protein 1	103112		91		38		0,03		3
tr G3V3R6 G3V3R6_HUMAN	LGALS3	Galectin	24128	93	96	44	36	0,3	0,14	2	1
sp Q9BYP9-2 KRA99_HUMAN	KRTAP9-9	Isoform 2 of Keratin-associated protein 9-9	21512		95		36		0,34		3
sp P20042 IF2B_HUMAN	EIF2S2	Eukaryotic translation initiation factor 2 subunit 2	38706	105	97	35	35	0,09	0,09	3	3
sp P05165-2 PCCA_HUMAN	PCCA	Isoform 2 of Propionyl-CoA carboxylase alpha chain, mitochondrial	77569		98		34		0,04		1
sp Q96PN6-2 ADCYA_HUMAN	ADCY10	Isoform 2 of Adenylate cyclase type 10	179041	113	99	32	33	0,02	0,02	3	3
sp Q5BKY1 LRC10_HUMAN	LRRC10	Leucine-rich repeat-containing protein 10	32078	116	101	32	32	0,1	0,1	1	1
tr J3KNM9 J3KNM9_HUMAN	FBXL7	F-box/LRR-repeat protein 7	52039		100		32		0,06		2
tr Q3KNR6 Q3KNR6_HUMAN	ST13	Hsc70-interacting protein	24010		102		31		0,14		4
sp P30049 ATPD_HUMAN	ATP5D	ATP synthase subunit delta, mitochondrial	17479		104		30		0,19		3
sp P0C0S5 H2AZ_HUMAN	H2AFZ	Histone H2A.Z	13545		107		30		0,25		1
sp O95072-2 REC8_HUMAN	REC8	Isoform 2 of Meiotic recombination protein REC8 homolog	61077		105		30		0,05		3
sp Q8WVE6-2 TM171_HUMAN	TMEM171	Isoform 2 of Transmembrane protein 171	35208		103		30		0,09		2
tr J3KRP8 J3KRP8_HUMAN	MICAL3	Protein-methionine sulfoxide oxidase MICAL3 (Fragment)	20026		106		30		0,17		3

sp P36957-2 ODO2_HUMAN	DLST	Isoform 2 of Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	39585	97	111	40	0,08	0,08	1	1
tr D6RD49 D6RD49_HUMAN	DDIT4L	DNA damage-inducible transcript 4-like protein (Fragment)	14728	109	119	34	0,23	0,23	10	8
sp Q9BYR5 KRA42_HUMAN	KRTAP4-2	Keratin-associated protein 4-2	17130	92	124	46	0,43	0,2	2	1
sp P02675 FIBB_HUMAN	FGB	Fibrinogen beta chain	56577	115	125	32	0,06	0,06	3	4
tr C9JC84 C9JC84_HUMAN	FGG	Fibrinogen gamma chain	52932	79	134	82	0,27	0,06	7	2
sp P48668 K2C6C_HUMAN	KRT6C	Keratin, type II cytoskeletal 6C	60273	31		406	0,61		24	
tr C9JM50 C9JM50_HUMAN	KRT19	Keratin, type I cytoskeletal 19 (Fragment)	20831	36		351	0,82		29	
sp P01861 IGHG4_HUMAN	IGHG4	Ig gamma-4 chain C region	36431	51		204	0,42		16	
tr U3KQK0 U3KQK0_HUMAN	HIST1H2BN	Histone 1, H2bn, isoform CRA_b	18792	55		176	0,18		3	
sp Q9BQ66 KR412_HUMAN	KRTAP4-12	Keratin-associated protein 4-12	25609	61		142	0,28		9	
tr F8W6P5 F8W6P5_HUMAN	HBB	Hemoglobin subunit beta (Fragment)	9664	71		93	0,85		3	
sp Q86Y46-2 K2C73_HUMAN	KRT73	Isoform 2 of Keratin, type II cytoskeletal 73	42244	76		90	0,16		4	
sp Q7RTS7 K2C74_HUMAN	KRT74	Keratin, type II cytoskeletal 74	58229	75		90	0,12		4	
tr A0A0B4J259 A0A0B4J259_HUMAN	LYZ	Lysozyme C	15661	82		77	0,22		1	
tr B4DEB1 B4DEB1_HUMAN	H3F3A	Histone H3.3	14158	98		40	0,24		2	
sp Q17RB8-2 LONF1_HUMAN	LONRF1	Isoform 2 of LON peptidase N-terminal domain and RING finger protein 1	87186	99		40	0,04		2	
sp P01008 ANT3_HUMAN	SERPINC1	Antithrombin-III	53025	100		38	0,06		1	
sp P55265-4 DSRAD_HUMAN	ADAR	Isoform 4 of Double-stranded RNA-specific adenosine deaminase	142061	102		38	0,02		3	
sp P15924-2 DESP_HUMAN	DSP	Isoform DPII of Desmoplakin	262237	101		38	0,01		1	
sp Q5JSJ4 DX26B_HUMAN	DDX26B	Protein DDX26B	97239	104		37	0,03		1	
sp P09848 LPH_HUMAN	LCT	Lactase-phlorizin hydrolase	219533	107		34	0,02		2	
tr E9PP40 E9PP40_HUMAN	LRRC14	Leucine-rich repeat-containing protein 14 (Fragment)	34154	110		34	0,1		1	
tr K7EPH2 K7EPH2_HUMAN	FARSA	Phenylalanine--tRNA ligase alpha subunit (Fragment)	43015	108		34	0,08		2	
tr Q5JNW7 Q5JNW7_HUMAN	PSMB8	Proteasome subunit beta type-8	28117	106		34	0,12		2	

sp Q7Z5M8-2 AB12B_HUMAN	ABHD12B	Isoform 2 of Abhydrolase domain-containing protein 12B	32263	111	33	0,1	2
tr A0A087X1A2 A0A087X1A2_HUMAN	KRTAP19-7	Keratin-associated protein 19-7	7984	112	33	0,44	1
tr E7ETB0 E7ETB0_HUMAN	C21orf91	Protein EURL homolog (Fragment)	28214	114	32	0,12	1
tr A0A087X1H6 A0A087X1H6_HUMAN	ST13	Hsc70-interacting protein	28334	119	30	0,12	2
tr A0A0A0MSY6 A0A0A0MSY6_HUMAN	IQCA1	IQ and AAA domain-containing protein 1	96507	118	30	0,03	1
sp Q8IWG1-2 WDR63_HUMAN	WDR63	Isoform 2 of WD repeat-containing protein 63	99322	120	30	0,03	1

Since this is a very extensive table, the format and content has not been edited by ActaDV

Table SII. Proteins identified in the HPLC fraction 18-23 by LC-MS/MS

Accession	GS	Name	Pro_Mass	Protein Score			emPAI			Peptide number		
				F18_F23SetA	F18_F23SetB	F18_F23SetC	F18_F23SetA	F18_F23SetB	F18_F23SetC	F18_F23SetA	F18_F23SetB	F18_F23SetC
sp P13645	KRT10	Keratin, type I cytoskeletal 10	59020	7043	4611	2462	18,72	17,68	5,32	178	113	65
sp P04264	KRT1	Keratin, type II cytoskeletal 1	66170	6367	4521	1877	7,04	7,04	2,05	139	92	47
sp P35527	KRT9	Keratin, type I cytoskeletal 9	62255	4086	3010	1108	6,44	6,06	1,28	114	83	31
sp P35908	KRT2	Keratin, type II cytoskeletal 2 epidermal	65678	3913	2571	1372	3,32	2,92	1,53	96	60	36
sp P01023	A2M	Alpha-2-macroglobulin	164613	2453	1732	751	1,15	1,07	0,42	132	85	47
sp O43790	KRT86	Keratin, type II cuticular Hb6	55120	2350	1315	1068	0,89	0,68	0,89	65	30	35
sp P02768	ALB	Serum albumin	71317	10818	5910	4941	32,55	16,86	23,48	384	178	206
tr A0A087X	IGKC	Ig kappa chain C region	25406	2266		678	7,16		1,37	64		23
sp P01024	C3	Complement C3	188569	2263	1468	823	0,67	0,62	0,36	86	55	31
sp Q52LG2	KRTAP13-2	Keratin-associated protein 13-2	19912	1916	985	963	7,93	4,59	4,59	43	21	22
tr A0A087W	IGHG1	Ig gamma-1 chain C region	51863	1879	1278	632	2,22	2,22	1,37	103	58	45
sp Q8IU00	KRTAP13-1	Keratin-associated protein 13-1	19505	1576	1011	597	5,74	3,9	2,57	30	14	16
sp Q9BYR3	KRTAP4-4	Keratin-associated protein 4-4	21487	1485	753	748	1,76	1,06	1,06	31	13	18
sp Q9BYQ3	KRTAP9-3	Keratin-associated protein 9-3	19805	1423	1131	311	26,18	15,96	4,64	60	33	27
sp P02787	TF	Serotransferrin	79294	1348	845	534	1,65	1,34	0,84	81	43	38

sp Q9BYQ6	KRTAP4-11	Keratin-associated protein 4-11	24844	1335	687	666	1,42	1,13	1,13	35	16	19
sp P01861	IGHG4	Ig gamma-4 chain C region	36431	1322	889	463	2,1	2,1	1,61	53	31	22
sp P02533	KRT14	Keratin, type I cytoskeletal 14	51872	1167	785	412	2,03	2,03	0,54	40	27	13
sp P08779	KRT16	Keratin, type I cytoskeletal 16	51578	1131	778	381	1,1	1,1	0,28	34	22	12
sp P02679-	FGG	Isoform Gamma-A of Fibrinogen gamma chain	50092	1097	874	258	1,02	1,02	0,47	42	30	12
tr J3QR68	HP	Haptoglobin (Fragment)	45697	1041		368	1,15		0,42	37		15
sp P01876	IGHA1	Ig alpha-1 chain C region	38486	987	543	473	1,28	0,93	0,78	39	20	19
tr A0A087W	KRTAP9-8	Keratin-associated protein 9-8	20295	978	709	288	6,32	2,97	5,28	54	25	29
sp Q9BYQ5	KRTAP4-6	Keratin-associated protein 4-6	26141	956	661	318	1,05	1,05	0,82	24	14	10
tr A0A075B	IGHG3	Ig gamma-3 chain C region (Fragment)	42327	936	681	288	0,97	0,97	0,69	44	26	18
sp P0C0L4-	C4A	Isoform 2 of Complement C4-A	189125	892	533	386	0,19	0,19	0,05	29	19	10
sp Q9BYR0	KRTAP4-7	Keratin-associated protein 4-7	26850	853	487	390	1,27	0,8	0,8	22	13	9
tr A0A087W	KRTAP9-2	Keratin-associated protein 9-2	20290	793	490	321	5,28	4,39	1,92	38	21	17
sp Q9BQ66	KRTAP4-12	Keratin-associated protein 4-12	25609	784	577	230	0,63	0,63	0,63	23	13	10

tr F5H1T9	KRTAP2-1	Keratin-associated protein 2-1	14926	748	580	194	3,23	2,44	1,8	33	16	17
sp P01859	IGHG2	Ig gamma-2 chain C region	36505	724	454	305	1,38	1,38	1	54	31	23
sp POC7H8	KRTAP2-3	Keratin-associated protein 2-3	15465	718	486	258	3,02	2,3	1,7	33	14	19
sp A8MTY7	KRTAP9-7	Keratin-associated protein 9-7	20915	715	582	153	7,08	4,99	2,3	34	18	16
sp P01620	sp P01620 KV302_HUMAN	Ig kappa chain V-III region SIE	11882	705	593	136	2,56	2,56	0,66	14	11	3
sp P02671-	FGA	Isoform 2 of Fibrinogen alpha chain	70227	696	589	142	0,51	0,51	0,2	29	19	10
sp P04259	KRT6B	Keratin, type II cytoskeletal 6B	60315	692	457	263	0,89	0,79	0,45	32	18	14
sp P13647	KRT5	Keratin, type II cytoskeletal 5	62568	688	412	304	0,85	0,76	0,36	29	17	12
sp Q8IUC1	KRTAP11-1	Keratin-associated protein 11-1	18385	676	532	166	2,84	2,84	0,66	21	16	5
sp P48668	KRT6C	Keratin, type II cytoskeletal 6C	60273	669	468		1	0,89		31	20	
sp P68871	HBB	Hemoglobin subunit beta	16102	620	485		3,61	3,61		24	17	
tr K7EMD9	KRT13	Keratin, type I cytoskeletal 13 (Fragment)	19202	618	385	256	1,24	1,24	0,62	18	11	7
sp P00739-	HPR	Isoform 2 of Haptoglobin-related protein	44076	594	428	199	0,54	0,54	0,16	20	14	6
sp P78386	KRT85	Keratin, type II cuticular Hb5	57306	590	461	162	0,32	0,32	0,18	16	10	6
sp Q7Z794	KRT77	Keratin, type II cytoskeletal 1b	62149	571	361	237	0,36	0,36	0,23	23	14	9

sp Q9BYR5	KRTAP4-2	Keratin-associated protein 4-2	17130	569	368	218	1,06	1,06	1,06	15	7	8
sp P01619	sp P01619 KV301_HUMAN	Ig kappa chain V-III region B6	11742	563	452		1,16	1,16		9	6	
sp Q9BYR4	KRTAP4-3	Keratin-associated protein 4-3	24536	549	359	205	0,89	0,67	0,47	18	11	7
sp P19012	KRT15	Keratin, type I cytoskeletal 15	49409	534	279	281	0,47	0,38	0,29	17	10	7
sp P02647	APOA1	Apolipoprotein A-I	30759	534	331	230	1,52	1,27	0,51	25	18	7
sp Q9BYR2	KRTAP4-5	Keratin-associated protein 4-5	23110	503	362	166	0,72	0,5	0,72	19	11	8
sp Q9BYP9-	KRTAP9-9	Isoform 2 of Keratin-associated protein 9-9	21512	500	292	224	5,57	4,68	1,38	27	15	12
sp P01009-	SERPINA1	Isoform 2 of Alpha-1-antitrypsin	40409	438	360	108	0,48	0,48	0,37	19	9	10
sp P02675	FGB	Fibrinogen beta chain	56577	410	321	123	0,57	0,4	0,33	29	16	13
tr C9JM50	KRT19	Keratin, type I cytoskeletal 19 (Fragment)	20831	382	201	210	1,11	0,82	0,56	12	7	5
sp Q9BYR6	KRTAP3-3	Keratin-associated protein 3-3	11441	361	163	234	0,69	0,69	0,69	10	6	4
sp P69905	HBA1	Hemoglobin subunit alpha	15305	355	263	118	1,72	1,72	0,82	13	9	4
sp P0CG05	IGLC2	Ig lambda-2 chain C regions	11458	342	258		1,2	1,2		12	10	
sp P05787-	KRT8	Isoform 2 of Keratin, type II cytoskeletal 8	56573	337	195		0,33	0,25		13	7	

sp P78385	KRT83	Keratin, type II cuticular Hb3	55928	334		176	0,26		0,26	16		10
sp Q3LI72	KRTAP19-5	Keratin-associated protein 19-5	7847	312	168	172	1,09	1,09	1,09	7	4	3
sp P01779	sp P01779 HV318_HUMAN	Ig heavy chain V-III region TUR	12537	270		107	0,27		0,27	4		2
tr A0A087X	KRTAP4-9	Keratin-associated protein 4-9	23399	263	201	84	1,23	0,95	0,49	19	9	10
sp Q9NSB2	KRT84	Keratin, type II cuticular Hb4	65942	254	114		0,21	0,16		12	6	
sp Q7RTS7	KRT74	Keratin, type II cytoskeletal 74	58229	254		169	0,25		0,18	11		6
sp Q86Y46-	KRT73	Isoform 2 of Keratin, type II cytoskeletal 73	42244	254		169	0,35		0,25	11		6
sp Q9BYR8	KRTAP3-1	Keratin-associated protein 3-1	11558	233	159	108	0,69	0,3	0,69	8	3	5
tr Q5T985	ITIH2	Inter-alpha-trypsin inhibitor heavy chain H2	105606	218	163		0,03	0,03		3	2	
tr A0A087W	IGHV3-72	Protein IGHV3-72	11274	217	182	69	0,3	0,3	0,3	4	2	2
sp P60413	KRTAP10-12	Keratin-associated protein 10-12	28680	216	154	91	0,25	0,25	0,25	11	7	4
sp Q15323	KRT31	Keratin, type I cuticular Ha1	48633	194	165	54	0,3	0,22	0,14	8	5	3
sp P02743	APCS	Serum amyloid P-component	25485	192	141	87	0,28	0,28	0,28	9	5	4
sp Q9NSB4	KRT82	Keratin, type II cuticular Hb2	57985	185	109	104	0,18	0,18	0,12	9	5	4
sp Q9BYR7	KRTAP3-2	Keratin-associated protein 3-2	11483	184	93	126	0,69	0,3	0,69	5	2	3

tr A0A075B	IGHM	Ig mu chain C region (Fragment)	50093	159	83	110	0,56	0,21	0,29	15	7	8
tr Q5T3N1	ANXA1	Annexin (Fragment)	22741	156		156	0,15		0,15	2		2
tr H7BXR9	KRTAP4-8	Keratin-associated protein 4-8	19570	155	143	39	0,37	0,37	0,17	9	4	5
sp P01762	sp P01762 HV301_HUMAN	Ig heavy chain V-III region TRO	13578	137	80	91	0,57	0,25	0,57	3	1	2
sp O14556	GAPDHS	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific	44815	136	79	78	0,15	0,15	0,07	5	3	2
sp P19827-	ITIH1	Isoform 2 of Inter-alpha-trypsin inhibitor heavy chain H1	86480	135		92	0,08		0,04	3		2
tr F5H5D3	TUBA1C	Tubulin alpha-1C chain	58606	133		101	0,06		0,06	3		2
sp Q9BYS1	KRTAP1-5	Keratin-associated protein 1-5	20392	133		133	0,84		0,84	9		8
tr B4E1Z4	tr B4E1Z4 B4E1Z4_HUMAN	Uncharacterized protein	143191	132	106	43	0,07	0,05	0,05	4	2	2
sp P36957-	DLST	Isoform 2 of Dihydrolipoyllysine-succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	39585	125	70	78	0,08	0,08	0,08	4	2	2

sp Q969S9-	GFM2	Isoform 2 of Ribosome-releasing factor 2, mitochondrial	81891	121	121		0,04	0,04		2	2	
sp P05109	S100A8	Protein S100-A8	10885	116	116		0,32	0,32		4	3	
sp P81605-	DCD	Isoform 2 of Dermcidin	12520	114	81	55	0,27	0,27	0,27	4	2	2
sp P01616	sp P01616	Ig kappa chain V-II region MIL	12162	92	70	60	0,64	0,64	0,64	7	4	3
	KV203_HUMAN											
tr A0A075B	IGKV2D-28	Protein IGKV2-28 (Fragment)	13062	91	70	58	0,59	0,59	0,26	7	4	3
tr A0A087W	IGKV1D-33	Protein IGKV1-33	11949	86	86		0,29	0,29		1	1	
sp P01593	sp P01593	Ig kappa chain V-I region AG	12099	86	86		0,28	0,28		1	1	
	KV101_HUMAN											
sp Q86SS6	SYT9	Synaptotagmin-9	56894	84	54	59	0,06	0,06	0,06	4	2	2
sp Q8IUG1	KRTAP1-3	Keratin-associated protein 1-3	20850	81		81	0,35		0,35	14		12
sp P02790	HPX	Hemopexin	52385	81	74		0,13	0,13		4	2	
tr H0YKS4	ANXA2	Annexin (Fragment)	19632	79		79	0,17		0,17	1		1
sp P25705-	ATP5A1	Isoform 2 of ATP synthase subunit alpha, mitochondrial	54574	76	55	59	0,06	0,06	0,06	4	2	2
tr J3QRE9	KRT39	Keratin, type I cytoskeletal 39	21416	75	61	51	0,16	0,16	0,16	4	2	2
sp P60709	ACTB	Actin, cytoplasmic 1	42052	74			0,25			7		
tr H0YH81	ATP5B	ATP synthase subunit beta (Fragment)	38226	73	60	42	0,09	0,09	0,09	2	1	1
tr G3V3A0	SERPINA3	Alpha-1-antichymotrypsin	23448	73	73		0,14	0,14		1	1	

sp Q8N1N4-	KRT78	Isoform 2 of Keratin, type II cytoskeletal 78	45560	72	60	30	0,07	0,07	0,07	2	1	1
sp Q5D862	FLG2	Filaggrin-2	249296	71	71		0,01	0,01		2	1	
sp Q8IUC3	KRTAP7-1	Keratin-associated protein 7-1	9624	71		71	0,36		0,36	1		1
sp Q9Y5I4-	PCDHAC2	Isoform Short of Protocadherin alpha-C2	96821	70	57	47	0,03	0,03	0,03	3	2	1
sp O76009	KRT33A	Keratin, type I cuticular Ha3-I	47166	70	70		0,14	0,14		3	3	
tr D6RBJ7	GC	Vitamin D-binding protein	39969	69	69		0,17	0,17		3	2	
tr C4AM86	KRT35	Keratin, type I cuticular Ha5	48816	68	59	46	0,14	0,14	0,07	6	4	2
tr G5E9F8	PROS1	Protein S (Alpha), isoform CRA_b	61914	68		68	0,05		0,05	1		1
tr J3QLI0	APOH	Beta-2-glycoprotein 1 (Fragment)	22960	57		57	0,15		0,15	1		1
sp Q9BV73-	CEP250	Isoform 2 of Centrosome-associated protein CEP250	275069	53	53		0,01	0,01		2	2	
sp P04004	VTN	Vitronectin	55069	52		52	0,06		0,06	2		2
tr H7C5R1	CP	Ceruloplasmin (Fragment)	98335	52		39	0,07		0,03	4		2
tr J3KNF5	CEP290	Centrosomal protein of 290 kDa	291050	52	51	24	0,01	0,01	0,01	3	2	1
tr AOA0A0M	FBXL13	F-box/LRR-repeat protein 13	80219	52	40	39	0,04	0,04	0,04	4	2	2
sp P01625	sp P01625 KV402_HUMAN	Ig kappa chain V-IV region Len	12746	51	51		0,27	0,27		2	1	

sp POC0S8	HIST1H2AG	Histone H2A type 1	14083	50		40	0,54		0,54	4		3
tr A0A075B	IGLV1-47	Protein IGLV1-47 (Fragment)	12447	49	49		0,28	0,28		1	1	
sp P15924-	DSP	Isoform DPII of Desmoplakin	262237	47	47		0,01	0,01		1	1	
sp Q9UKU6	TRHDE	Thyrotropin-releasing hormone-degrading ectoenzyme	117439	46	46		0,03	0,03		1	1	
sp Q96HU8	DIRAS2	GTP-binding protein Di-Ras2	22813	40	31	29	0,15	0,15	0,15	3	2	1
tr A0A087X	KRTAP19-7	Keratin-associated protein 19-7	7984	39		39	0,44		0,44	1		1
tr F5H274	NLRC5	Protein NLRC5 (Fragment)	8699	38	38		0,4	0,4		1	1	
tr A0A087W	GALC	Galactocerebrosidase (Fragment)	45641	35	35		0,07	0,07		4	2	
tr S4R407	TRIM45	Tripartite motif-containing protein 45 (Fragment)	35304	34	27	22	0,09	0,09	0,09	4	2	2
tr M0QWZ7	SARS2	Serine--tRNA ligase, mitochondrial	58602	34	26	25	0,12	0,06	0,06	4	3	1
sp Q5JXC2-	MIIP	Isoform 2 of Migration and invasion-inhibitory protein	40123	32	32		0,08	0,08		1	1	

tr I3L3K4	KCTD13	BTB/POZ domain- containing adapter for CUL3- mediated RhoA degradation protein 1 (Fragment)	9314	32	32	0,37	0,37	1	1
sp P01008	SERPINC1	Antithrombin-III	53025	30	30	0,06	0,06	1	1
sp P04003	C4BPA	C4b-binding protein alpha chain	69042	30	30	0,05	0,05	1	1
sp Q9P219	CCDC88C	Protein Daple	229231	30	30	0,01	0,01	3	2
sp P01765	sp P01765 HV304_HUM AN	Ig heavy chain V- III region TIL	12462		194		0,28		2
tr F8VQQ4	TUBA1A	Tubulin alpha-1A chain (Fragment)	24377		50		0,14		1
sp POC0S5	H2AFZ	Histone H2A.Z	13545		32		0,25		1
tr I3L1U9	ACTG1	Actin, cytoplasmic 2, N-terminally processed (Fragment)	23887		29		0,14		3
sp A0M8Q6	IGLC7	Ig lambda-7 chain C region	11467		121		0,3		2
sp A5A3E0	POTEF	POTE ankyrin domain family member F	123020		73		0,05		4
tr HOYFX9	H2AFJ	Histone H2A (Fragment)	9970		43		0,35		1

Appendix S1

SUPPLEMENTARY METHODS

Hair samples and extraction of low-molecular-weight proteins

Human hair shafts derived from the distal parts (usually representing 10–30% of total hair length) used in this study were obtained from 5 healthy donors (mean \pm standard deviation age: 24.2 ± 0.44 years). The collected hair was free of dandruff, scalp diseases and without prior chemical treatments, such as hair dyes, bleach, or perms. Written informed consent was taken from donors to collect hair samples for analysis. This study was approved by Institutional Review Board of Nanyang Technological University (IRB-2016-11-042). This study followed the principles of the Declaration of Helsinki. Before extraction, the human hair shafts were washed twice with 70% ethanol, followed by 3 washes with MilliQ water for 2 min. The hair shafts were then vacuum-dried and low-molecular-weight (LMW) proteins were extracted from hair as described earlier (S1, 5). In brief, human hair (100 mg) was cut into fragments and incubated in 3 ml of "Shindai solution" consisting of 25 mM Tris-HCl (pH 9.5), 25% ethanol, 200 mM dithiothreitol and 8 M urea for 72 h at 50°C. The solution was centrifuged at $17,000 \times g$ for 15 min at 25°C and the supernatant comprising of LMW proteins was stored at -80°C until further use.

Separation of antimicrobial proteins on reverse-phase high-performance liquid chromatography

Antimicrobial proteins in the LMW extracts were separated by reverse-phase high-performance liquid chromatography (RP-HPLC), as described earlier, with slight modifications (S2). Ten μl LMW protein extract was loaded onto a C18 column (Delta PAK WAT011793 Waters column, 5 μm , 150×3.9 mm) and separated using an RP-HPLC system (Agilent Technologies, 1290 Infinity Series) at 24°C after column equilibration in 0.1% trifluoroacetic acid at a flow rate of 0.5 ml/min, using a gradient of acetonitrile in 0.1% formic acid. Protein separation was monitored at wavelengths of 214, 254 and 280 nm. The eluted fractions were collected at 1-min intervals, frozen and then lyophilized. Protein concentration was determined using the Micro BCA protein Assay Kit (23235#, Pierce Company).

Antimicrobial activity assay of high-performance liquid chromatography fractions

For determination of potential antimicrobial activity, a radial diffusion assay (RDA) method was used essentially as described previously (S3), with minor modifications. Lyophilized fractions were re-suspended in distilled water and tested against *Escherichia coli*. In brief, *E. coli* was grown overnight at 37°C in 20 ml full-strength trypticase (3% w/v) soy broth (TSB). To obtain mid-log bacteria, 20 μl culture was inoculated into 20 ml fresh TSB and incubated until OD 600 of 0.5 was reached. Bacterial CFU (4×10^6) was added to 10 ml sterile 1% (w/v) low electroendosmosis (EEO) agarose gel containing 1% (v/v) TSB, 10 mM Tris Buffer (pH 7.4) and 0.02% (v/v) Tween-20 in a Petri dish. Samples were added to punched 3-mm wells. The plate was incubated for 3 h at 37°C to enable diffusion and was subsequently overlaid with 10 ml sterile 1% (w/v) low EEO agarose gel containing 6% (w/v) TSB. The diameter of the bacterial inhibition zone was measured after 18 h of incubation at 37°C.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis analysis

Dialysed LMW protein extract and HPLC fractions were analysed for protein/peptides by sodium dodecyl sulphate-polyacrylamide

gel electrophoresis. Ten μg of samples were separated on 10–20% Tris-tricine gels (Novex™ EC6625, Novex Life Technologies) for 2 h at 90 V. Protein bands were visualized using a silver staining kit (Thermo Fisher Scientific, Cat#24612) according to the manufacturer's instructions.

Mass spectrometry analyses

HPLC fractions (18–23) were lyophilized and 40 μg protein were reduced with dithiothreitol (10 mM), alkylated using iodoacetamide (55 mM) and then subjected to overnight digestion in sequencing-grade modified trypsin at 37°C. The resultant peptides were separated and analysed on a Dionex Ultimate 3000 RSLC nanoLC system coupled to a Q-Exactive apparatus (Thermo Fisher, Waltham, MA, USA). Approximately 5 μl sample was injected into an acclaim peptide trap column via the autosampler of the Dionex RSLC nano LC system. The flow rate was set at 300 nl/min. Mobile phase A (0.1% formic acid in 5% acetonitrile) and mobile phase B (0.1% formic acid in acetonitrile) were used to establish a 60-min gradient. Peptides were then analysed on a Dionex EASY-spray column (PepMap® C18, 3 μm , 100 A) using an EASY nanospray source at an electrospray potential of 1.5 kV. A full MS scan (350–1,600 m/z range) was acquired at a resolution of 70,000 at m/z 200, with a maximum ion accumulation time of 100 ms. Dynamic exclusion was set to 30 s. Resolution for MS/MS spectra was set to 35,000 at m/z 200. The AGC setting was 1E6 for the full MS scan and 2E5 for the MS2 scan. The 10 most intense ions above a 1000-count threshold were selected for high-energy collision dissociation (HCD) fragmentation, with a maximum ion accumulation time of 120 ms. An isolation width of 2 Da was used for the MS2 scan. Single and unassigned charged ions were excluded from MS/MS. For HCD, normalized collision energy was set to 28%. The under-fill ratio was defined as 0.1%.

Data analysis

Raw data files were converted into the mascot generic file (mgf) format using Proteome Discoverer version 1.4 (Thermo Electron, Bremen, Germany) with the MS2 spectrum processor for de-isotoping the MS/MS spectra. The concatenated target-decoy UniProt human database (sequence 92867, downloaded on 25 July 2016) was used for data searches. In-house Mascot server (version 2.4.1, Matrix Science, Boston, MA, USA) with MS tolerance of 5.1 ppm and MS/MS tolerance of 0.02 Da was used for database search. Two missed trypsin cleavage sites per peptide were tolerated. Carbamidomethylation (C) was set as a fixed modification, while oxidation (M) and deamidation (N and Q) were variable modifications. The peptide/protein lists obtained were exported to Microsoft Excel and analysed further. Gene ontological analysis was performed by adopting the online tool PANTHER (Protein ANalysis THrough Evolutionary Relationships) classification system (<http://www.pantherdb.org/>).

Western blot analysis

Rabbit polyclonal antibodies against histone H1.0 (Cat#ab83058, Abcam, Cambridge, UK), histone H2A (Cat# 2578S, Cell Signalling Technology, Danvers, MA, USA), histone H2B (Cat#ab1790, Abcam), histone H3 (Cat#9715S, Cell Signalling Technology) and histone H4 (Cat#2592S, Cell Signalling Technology) were used. Twenty μg samples from the LMW protein extract and HPLC fractions were loaded on a 4–20% Tris-Glycine gel (Mini-PROTEAN® TGX™, Cat#4561091). Electrophoresis was carried out as described above and gels were transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore, Danvers, MA, USA). The membrane was blocked with 5% BSA dissolved in Tris-buffered

saline containing 0.1% Tween 20 (Tween 20) and incubated with primary antibodies (1:1000) overnight at 4°C. The membranes were probed with a secondary antibody (Polyclonal Goat Anti-Rabbit Immunoglobulins, Cat#D0487, Dako) and the bands were detected by the Chemiluminescence method (SuperSignal® West Dura Extended Duration substrate, Cat #34076, Thermo Scientific, Waltham, MA, USA).

Immunohistochemistry

Formalin-fixed, wax-embedded sections of hair biopsies were stained by immunoperoxidase after epitope heat retrieval at pH 6. After 1 h incubation with a primary antibody (Histone 3, ab8898 Abcam), sections were washed in tap water and incubated for 30 min with HRP-labelled polymer conjugated to goat anti-rabbit immunoglobulins (Dako). Sections were developed with 3,3'-Diaminobenzidine substrate (Abcam) and counterstained with haematoxylin before dehydrating and mounting in a mixture of distyrene plasticiser xylene (CellPath). Photographs were taken

with a microscope (Axio Imager Z1; Carl Zeiss) using a 40×, NA 0.60 Ph2 Corr long distance Plan-Neofluar objective (S4).

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