Analysis of protein expression regulated by lumican in PANC-1 cells using shotgun proteomics

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Abstract. Lumican, a member of the class II small leucine-rich proteoglycan family, regulates the assembly and diameter of collagen fibers in the extracellular matrix of various tissues. We previously reported that lumican expression in the stromal tissues of pancreatic ductal adenocarcinoma (PDAC) correlates with tumor invasion, and tends to correlate with poor prognosis. Lumican stimulates growth and inhibits the invasion of a PDAC cell line. In the present study, we performed a global shotgun proteomic analysis using lumican-overexpressing PANC-1 cells and lumican downregulated PANC-1 cells to identify candidate proteins that are regulated by lumican and related to cell growth and invasion in PDAC cells. A total of 448 proteins were identified from lumican-overexpressing PANC-1 and control cells. Additionally, 451 proteins were identified from lumican-downregulated PANC-1 cells and control cells. As a result of semi-quantification based on spectral counting, 174 differentially expressed proteins were identified by lumican upregulation, and 143 differentially expressed proteins were identified by lumican downregulation. The expression levels of 24 proteins, including apoptosis- and invasion-related proteins correlated with lumican expression levels. It is likely that the expression of these proteins is regulated by lumican, and that they are involved in apoptosis and invasion in PDAC. These findings suggest that lumican may be involved in cell growth and invasion through the regulation of these 24 proteins expressed in PDAC.

Introduction

Lumican is a member of the class II small leucine-rich proteoglycan (SLRP) family. Members of this family have relatively small molecular sizes, with core proteins of approximately 40 kDa, and possess 6-10 leucine-rich repeat units in the core protein (1,2). Amino acid sequencing indicates that lumican has 4 potential sites for *N*-linked keratan sulfate (KS) or oligosaccharides (3,4). Therefore, lumican includes a core protein, glycoprotein and proteoglycan forms due to glycosylation (5). Lumican is a secreted collagen-binding extracellular matrix protein of the cornea, dermis and tendon stroma, arterial wall, and intestinal submucosa (6-9). Corneal opacity, as well as skin and tendon fragility due to disorganized and loosely packed collagen fibers in lumican-null mice suggest that lumican plays an important role in collagen fibrillogenesis (10,11).

Lumican was first reported as one of the major KS proteoglycans in the chicken cornea (12). In addition to the cornea, lumican expression has been reported in various human tissues, including malignant tumor tissues (5,13-27). Among the clinicopathological characteristics of pancreatic ductal adenocarcinoma (PDAC), the localization of lumican in the stromal tissue adjacent to cancer cells correlates with advanced cancer stage, retroperitoneal and duodenal invasion, and residual tumor, and tends to correlate with shorter survival (21). These reports suggest that lumican localized in the stromal tissue is secreted from cancer cells and affects cancer cells through an autocrine and paracrine mechanism. We previously reported that PANC-1 cells, one of the PDAC cell lines, secrete only 70-kDa glycosylated lumican into the extracellular space. We also demonstrated that the secreted lumican stimulated cell growth through ERK activation and inhibited cell invasion and matrix metalloproteinase (MMP)-9 activation using lumican-overexpressing PANC-1 cells and lumican-downregulated PANC-1 cells (28). However, the mechanism of how lumican affects cell growth and invasion remains unclear.

In the present study, we performed shotgun liquid chromatography (LC)/mass spectrometry (MS)-based global proteomic analysis using protein from lumican-overexpressing PANC-1 cells and lumican-downregulated PANC-1 cells to examine how lumican regulates cell growth and invasion in PDAC cells. We identified 24 candidate proteins that may play an important role in cell growth and invasion and could be regulated by lumican.

Materials and methods

Materials. The following materials were purchased from Wako Pure Chemical Industries (Osaka, Japan): urea, 3-(3-cholami-

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dopropyl) dimethylammonio-1-propanesulphonate (CHAPS), dithiothreitol (DTT), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and iodoacetamide (IAA); Amicon Ultra 0.5-ml 3K was from Millipore (Tokyo, Japan), and thiourea from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals and reagents were purchased from Sigma Chemical Corp. (St. Louis, MO, USA).

PDAC cell line. PANC-1 cells were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan).

Protein extraction of lumican-regulated PANC-1 cells. The lumican-overexpressing PANC-1 cells, lumican-downregulated PANC-1 cells, and control cell lines (Mock and NC, respectively) were prepared as previously described (28). The lumican-regulated PANC-1 cells were cultured at a density of $5x10^5$ cells in a 100-mm dish in RPMI-1640 medium with 10% fetal bovine serum (FBS) for 72 h. Then, cells were solubilized in urea lysis buffer (7 M urea, 2 M thiourea, 5% CHAPS, 1% Triton X-100). Protein concentration was measured using the Bradford method.

In-solution trypsin digestion. A gel-free digestion approach was performed in accordance with the protocol described by Bluemlein and Ralser (29). In brief, 10 μg of protein extract from each sample was reduced by addition of 45 mM DTT and 20 mM TCEP and was then alkylated using 100 mM IAA. Following alkylation, samples were digested with Proteomics Grade Trypsin (Agilent Technologies, Inc., Santa Clara, CA, USA) at 37˚C for 24 h. Next, digests were evaporated in a vacuum concentrator centrifuge and the residue was resuspended in 0.1% trifluoroacetic acid/5% acetonitrile. The digests were filtered through Amicon Ultra 0.5-ml 3K to remove undigested proteins and the flow-through was used in the following analyses.

LC-MS/MS analysis of protein identification. Approximately 2-μg peptide samples were injected into a peptide L-trap column (Chemicals Evaluation and Research Institute, Tokyo, Japan) using an HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland) and further separated through an Advance-nano UHPLC using a Reverse-Phase C18-column (Zaplous column α, 3-μm diameter gel particles and 100 Å pore size, 0.1x150 mm; both from AMR, Inc., Tokyo, Japan). The mobile phase consisted of solution A (0.1% formic acid in water) and solution B (acetonitrile). The column was developed at a flow rate of 500 nl/min with a concentration gradient of acetonitrile from 5 to 45% B over 120 min. Gradient-eluted peptides were analyzed using an amaZon ETD ion-trap mass spectrometer (Bruker Daltonics, Billerica, MA, USA). The results were acquired in a data-dependent manner in which MS/MS fragmentation was performed on the 10 most intense peaks of every full MS scan.

All MS/MS spectra data were searched against the SwissProt *Homo sapiens* database using Mascot (v2.3.01; Matrix Science, London, UK). The search criteria was set as follows: enzyme, trypsin; allowance of up to two missed cleavage peptides; mass tolerance ± 0.5 Da and MS/MS tolerance ± 0.5 Da; and modifications of cysteine carbamidomethylation and methionine oxidation.

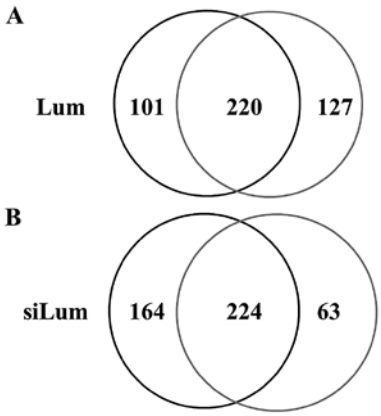

Figure 1. The Venn map of proteins identified from lumican-regulated PANC-1 cells. (A) In lumican-overexpressing PANC-1 cells (Lum), 321 proteins were identified and 347 were identified in control cells (Mock). (B) In lumican-downregulated PANC-1 cells (siLum), 388 proteins were identified and 287 were identifed in control cells (NC).

Semi-quantitative analysis of identified proteins. The fold-changes in expressed proteins on the base 2 logarithmic scale were calculated using Rsc based upon spectral counting (30). Relative amounts of identified proteins were also calculated using the normalized spectral abundance factor (NSAF) (31). Differentially expressed proteins were chosen so that their Rsc satisfy >1 or <–1, which correspond to fold-changes of >2 or <0.5.

Bioinformatics. Functional annotations for the identified proteins whose expression level was regulated by lumican were processed using the Database for Annotation, Visualization and Integrated Discovery (DAVID), v6.7 (http://david.abcc.ncifcrf.gov/home.jsp) (32-34).

Results

Protein identification and profile in lumican-regulated PANC-1 cells. To examine the effect of lumican on cell growth and invasion of PDAC cells, we created two types of PANC-1 cells whose lumican expression level was regulated: lumican-overexpressing PANC-1 cells and lumican-downregulated PANC-1 cells (28). We then investigated the molecular profile of proteins whose expression level was regulated by lumican using shotgun proteomics. Fig. 1 shows the Venn map for the identified proteins in lumican-regulated PANC-1 cells. In lumican-overexpressing PANC-1 cells (Lum), 321 proteins were identified, and 347 were identified in control cells (Mock) under the search parameter settings used (Fig. 1A). On the other hand, 388 proteins were identified in lumican-downregulated PANC-1 cells (siLum) and 287 in control cells (NC) (Fig. 1B). Among the 448 proteins identified from lumican upregulated cells, 220 (49.1%) proteins were identified in both cell lines, while 101 (22.6%) and 127 (28.3%) proteins were unique to Lum and Mock, respectively (Fig. 1A). Of the 451 total proteins identified from lumican downregulated

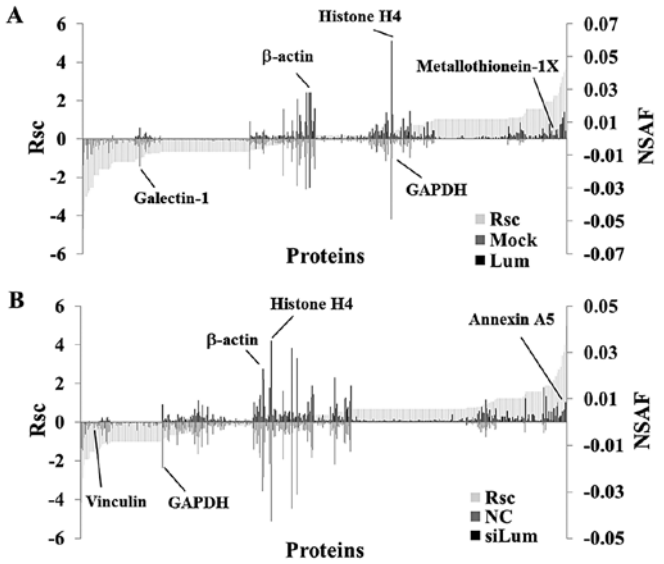

Figure 2. Semi-quantitative comparisons of the proteins identified in lumican-regulated PANC-1 cells. Rsc and normalized spectral abundance factor (NSAF) values were calculated for the proteins identified (X-axis). Protein expression was compared between (A) Lum vs. Mock and (B) siLum vs. control cells (NC). Proteins highly expressed in either Lum or Mock cells were to the right or left sides of the X-axis. Housekeeping proteins were located near the center along the X-axis.

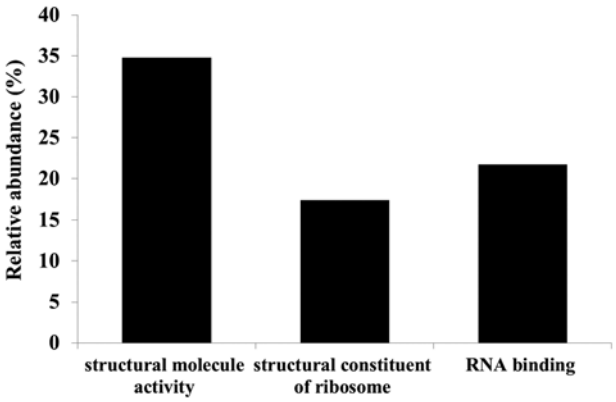

Figure 3. Analysis of identified proteins by gene ontology (GO) molecular function. Proteins assigned to a GO molecular function category; only significant categories (P<0.05) are shown.

cells, 224 (49.7%) proteins were identified in both cell lines, whereas 164 (36.3%) and 63 (14.0%) proteins were unique to siLum and NC, respectively (Fig. 1B).

Semi-quantitative comparison of identified proteins in lumican-regulated PANC-1 cells. Next, we performed a label-free semi-quantitative method based on spectral counting, as described in the Materials and methods section, to find proteins whose expression levels were regulated by lumican. The Rsc value was plotted against the corresponding protein (X-axis) from left to right for proteins identified in the Lum and Mock groups (Fig. 2A). The positive and negative Rsc values indicate increased and decreased expression, respectively, in the Lum group. The NSAF value (bar) plotted against the corresponding protein (X-axis), NSAF of Lum (black bar) and Mock (gray bar) proteins are indicated above and below the X-axis, respectively (Fig. 2A). Proteins with either a high positive or negative Rsc value were considered candidate proteins whose expression level was regulated by lumican. Fig. 2B shows the Rsc and NSAF values of the siLum and NC groups as described above. In the lumican upregulated cells, the Rsc of metallothionein (MT)-1X was positive, and the Rsc of galectin-1 was negative (Fig. 2A). In the lumican downregulated cells, the Rsc of Annexin A5 was positive and the Rsc of vinculin was negative (Fig. 2B). Housekeeping proteins such as β-actin, histone H4 and GAPDH were located near the center of the X-axis (Fig. 2).

As a result of semi-quantification, 174 differentially expressed proteins were identified in lumican upregulated PANC-1 cells (Table I), and a total of 143 differentially expressed proteins were identified in lumican downregulated PANC-1 cells (Table II). However, the expression level of housekeeping proteins such as β-actin, GAPDH and histone H4 was not changed in lumican upregulated cells or lumican downregulated cells. In order to identify proteins whose expression levels were regulated by lumican, we selected proteins whose Rsc value was inversely associated between lumican upregulated cells and lumican downregulated cells. Twenty four proteins were identified as candidates (Table III).

Functional annotation of proteins whose expression level is regulated by lumican. Gene ontology (GO) analyses were performed using the identified candidate proteins for each molecular function (Fig. 3), biological process (Fig. 4) and cellular component (Fig. 5) using DAVID. We also analyzed pathway terms, but no significant category was found. Functional annotations were counted by normalizing to the total number of proteins identified. Since a multifunctional protein yields more than one annotation and some proteins are not defined by GO terms yet, the total number of classified proteins resulted in more or less than 100% (Fig. 3). Major GO molecular function categories of the identified proteins were 34.8% structural molecule activity, 17.4% structural constituents of ribosomes, and 21.7% RNA binding proteins (Fig. 3).

Discussion

In the present study, we used a gel-free LC-MS-based proteomics approach to examine the effect of lumican on cell growth and invasion. Using semi-quantitative methods based on spectral counting, we successfully identified several proteins whose expression levels were altered more than 2-fold in both lumican upregulated PANC-1 cells and lumican downregulated PANC-1 cells. A limitation of spectral counting is in its accurate quantitative capacity (35). Therefore, we selected candidate proteins whose expression level was regulated by lumican using the analysis results of two types of cells; lumican upregulated cells and lumican downregulated cells. Thus, we identified 24 proteins whose Rsc values were inversely correlated among differentially expressed proteins in lumican upregulated and downregulated cells as candidate proteins (Fig. 3).

Table I. Differentially expressed proteins in lumican upregulated PANC-1 cells.

No.	ID	Accession no. and description	No. of amino acids	Spectral counting		
				Mock	Lum	Fold-change (Rsc)
1	ACTH_HUMAN	(P63267) Actin, γ-enteric smooth muscle	376	35	0	-4.69652
2	ACTC_HUMAN	(P68032) Actin, α cardiac muscle 1	377	18	0	-3.7706617
3	TBB2A_HUMAN	(Q13885) Tubulin β-2A chain	445	10	0	-2.9897736
4	H2B1H_HUMAN	(Q93079) Histone H2B type 1-H	126	9	0	-2.8547299
5	HS904_HUMAN	(Q58FG1) Putative heat shock protein HSP 90-α A4	418	8	0	-2.7058891
6	TBA4A_HUMAN	(P68366) Tubulin α-4A chain	448	8	0	-2.7058891
7	H2AJ_HUMAN	(Q9BTM1) Histone H2A.J	129	7	0	-2.540088
8	H2B3B_HUMAN	(Q8N257) Histone H2B type 3-B	126	7	0	-2.540088
9	RLA0_HUMAN	(P05388) 60S acidic ribosomal protein P0	317	7	0	-2.540088
10	H2B1O_HUMAN	(P23527) Histone H2B type 1-O	126	4	0	-1.885788
11	K2C71_HUMAN	(Q3SY84) Keratin, type II cytoskeletal 71	523	4	0	-1.885788
12	NDKB_HUMAN	(P22392) Nucleoside diphosphate kinase B	152	4	0	-1.885788
13	RA1L2_HUMAN	(Q32P51) Heterogeneous nuclear ribonucleoprotein A1-like 2	320	4	0	-1.885788
14	CH10_HUMAN	(P61604) 10 kDa heat shock protein, mitochondrial	102	4	0	-1.885788
15	H2A1H_HUMAN	(Q96KK5) Histone H2A type 1-H	128	3	0	-1.5801931
16	HNRPK_HUMAN	(P61978) Heterogeneous nuclear ribonucleoprotein K	463	3	0	-1.5801931
17	K1C25_HUMAN	(Q7Z3Z0) Keratin, type I cytoskeletal 25	450	3	0	-1.5801931
18	K22O_HUMAN	(Q01546) Keratin, type II cytoskeletal 2 oral	638	3	0	-1.5801931
19	MYL6_HUMAN	(P60660) Myosin light polypeptide 6	151	3	0	-1.5801931
20	NFH_HUMAN	(P12036) Neurofilament heavy polypeptide	1026	3	0	-1.5801931
21	TAGL3_HUMAN	(Q9UI15) Transgelin-3	199	3	0	-1.5801931
22	YI016_HUMAN	(A6NKZ8) Putative tubulin β chain-like protein	372	3	0	-1.5801931
23	ANXA5_HUMAN	(P08758) Annexin A5	320	3	0	-1.5801931
24	MT1G_HUMAN	(P13640) Metallothionein-1G	62	3	0	-1.5801931
25	K1C14_HUMAN	(P02533) Keratin, type I cytoskeletal 14	472	6	1	-1.5040946
26	K2C80_HUMAN	(Q6KB66) Keratin, type II cytoskeletal 80	452	5	1	-1.2892288
27	ACTBL_HUMAN	(Q562R1) β-actin-like protein 2	376	13	4	-1.259281
28	1433B_HUMAN	(P31946) 14-3-3 protein β/α	246	2	0	-1.1924301
29	AINX_HUMAN	(Q16352) α-internexin	499	2	0	-1.1924301
30	H2AZ_HUMAN	(H2AZ_HUMAN) Histone H2A.Z	128	2	0	-1.1924301
31	H2B1D_HUMAN	(P58876) Histone H2B type 1-D	126	2	0	-1.1924301
32	H2B1J_HUMAN	(P06899) Histone H2B type 1-J	126	2	0	-1.1924301
33	K1C13_HUMAN	(P13646) Keratin, type I cytoskeletal 13	458	2	0	-1.1924301
34	K2C6C_HUMAN	(P48668) Keratin, type II cytoskeletal 6C	564	2	0	-1.1924301
35	KRT85_HUMAN	(P78386) Keratin, type II cuticular Hb5	507	2	0	-1.1924301
36	MLL3_HUMAN	(Q8NEZ4) Histone-lysine N-methyltransferase MLL3	4911	2	0	-1.1924301
37	NDK8_HUMAN	(O60361) Putative nucleoside diphosphate kinase	137	2	0	-1.1924301
38	NFM_HUMAN	(P07197) Neurofilament medium polypeptide	916	2	0	-1.1924301
39	SERPH_HUMAN	(P50454) Serpin H1	418	2	0	-1.1924301
40	U17L5_HUMAN	(A8MUK1) Ubiquitin carboxyl-terminal hydrolase 17-like protein 5	530	2	0	-1.1924301
41	URFB1_HUMAN	(Q6BDS2) UHRF1-binding protein 1	1440	2	0	-1.1924301
42	MDHM_HUMAN	(P40926) Malate dehydrogenase, mitochondrial	338	2	0	-1.1924301
43	IF5A1_HUMAN	(P63241) Eukaryotic translation initiation factor 5A-1	154	2	0	-1.1924301
44	SFPQ_HUMAN	(P23246) Splicing factor, proline- and glutamine-rich	707	2	0	-1.1924301
45	NACA_HUMAN	(Q13765) Nascent polypeptide-associated complex subunit α	215	2	0	-1.1924301
46	EF1B_HUMAN	(P24534) Elongation factor 1-β	225	2	0	-1.1924301
47	RS4X_HUMAN	(P62701) 40S ribosomal protein S4, X isoform	263	2	0	-1.1924301
48	IF4A2_HUMAN	(Q14240) Eukaryotic initiation factor 4A-II	407	9	3	-1.0866682
49	EF2_HUMAN	(P13639) Elongation factor 2	858	16	6	-1.0697973
50	HSP72_HUMAN	(P54652) Heat shock-related 70 kDa protein 2	639	11	4	-1.0396137

Table I. Continued.

No.	ID	Accession no. and description	No. of amino acids	Spectral counting		
				Mock	Lum	Fold-change (Rsc)
51	DESM_HUMAN	(P17661) Desmin	470	13	5	-1.0068984
52	LEG1_HUMAN	(P09382) Galectin-1	135	13	5	-1.0068984
53	CC113_HUMAN	(Q9H0I3) Coiled-coil domain-containing protein 113	377	0	1	1.03639903
54	H11_HUMAN	(Q02539) Histone H1.1	215	0	1	1.03639903
55	UBA1_HUMAN	(P22314) Ubiquitin-like modifier-activating enzyme 1	1058	0	1	1.03639903
56	TCPH_HUMAN	(Q99832) T-complex protein 1 subunit eta	543	0	1	1.03639903
57	RL31_HUMAN	(P62899) 60S ribosomal protein L31	125	0	1	1.03639903
58	ADT4_HUMAN	(Q9H0C2) ADP/ATP translocase 4	315	0	1	1.03639903
59	BRE1B_HUMAN	(O75150) E3 ubiquitin-protein ligase BRE1B	1001	0	1	1.03639903
60	CALX_HUMAN	(P27824) Calnexin	592	0	1	1.03639903
61	CAP1_HUMAN	(Q01518) Adenylyl cyclase-associated protein 1	475	0	1	1.03639903
62	CAZA1_HUMAN	(P52907) F-actin-capping protein subunit α-1	286	0	1	1.03639903
63	CCNT1_HUMAN	(O60563) Cyclin-T1	726	0	1	1.03639903
64	CGNL1_HUMAN	(Q0VF96) Cingulin-like protein 1	1302	0	1	1.03639903
65	CISY_HUMAN	(O75390) Citrate synthase, mitochondrial	466	0	1	1.03639903
66	CLIP1_HUMAN	(P30622) CAP-Gly domain-containing linker protein 1	1438	0	1	1.03639903
67	CN37_HUMAN	(P09543) 2',3'-cyclic-nucleotide 3'-phosphodiesterase	421	0	1	1.03639903
68	CTSR1_HUMAN	(Q8NEC5) Cation channel sperm-associated protein 1	780	0	1	1.03639903
69	CX057_HUMAN	(Q6NSI4) Uncharacterized protein CXorf57	855	0	1	1.03639903
70	DDX21_HUMAN	(Q9NR30) Nucleolar RNA helicase 2	783	0	1	1.03639903
71	DYXC1_HUMAN	(Q8WXU2) Dyslexia susceptibility 1 candidate gene 1 protein	420	0	1	1.03639903
72	EFTU_HUMAN	(P49411) Elongation factor Tu, mitochondrial	452	0	1	1.03639903
73	EHD1_HUMAN	(Q9H4M9) EH domain-containing protein 1	534	0	1	1.03639903
74	EIF3H_HUMAN	(O15372) Eukaryotic translation initiation factor 3 subunit H	352	0	1	1.03639903
75	EHD1_HUMAN	(Q9H4M9) EH domain-containing protein 1	534	0	1	1.03639903
76	FETUA_HUMAN	(P02765) α-2-HS-glycoprotein	367	0	1	1.03639903
77	HIP1R_HUMAN	(O75146) Huntingtin-interacting protein 1-related protein	1068	0	1	1.03639903
78	HMGN1_HUMAN	(P05114) Non-histone chromosomal protein HMG-14	100	0	1	1.03639903
79	HMGN4_HUMAN	(O00479) High mobility group nucleosome-binding domain-containing protein 4	90	0	1	1.03639903
80	HNRH3_HUMAN	(P31942) Heterogeneous nuclear ribonucleoprotein H3	346	0	1	1.03639903
81	IDE_HUMAN	(P14735) Insulin-degrading enzyme	1019	0	1	1.03639903
82	IMA2_HUMAN	(P52292) Importin subunit α-2	529	0	1	1.03639903
83	IMDH2_HUMAN	(P12268) Inosine-5'-monophosphate dehydrogenase 2	514	0	1	1.03639903
84	K1C26_HUMAN	(Q7Z3Y9) Keratin, type I cytoskeletal 26	468	0	1	1.03639903
85	K6PL_HUMAN	(P17858) 6-phosphofructokinase, liver type	780	0	1	1.03639903
86	MYH11_HUMAN	(P35749) Myosin-11	1972	0	1	1.03639903
87	MYH14_HUMAN	(Q7Z406) Myosin-14	1995	0	1	1.03639903
88	PAR10_HUMAN	(Q53GL7) Poly [ADP-ribose] polymerase 10	1025	0	1	1.03639903
89	PI3R4_HUMAN	(Q99570) Phosphoinositide 3-kinase regulatory subunit 4	1358	0	1	1.03639903
90	PRDX4_HUMAN	(Q13162) Peroxiredoxin-4	271	0	1	1.03639903
91	PRS6A_HUMAN	(P17980) 26S protease regulatory subunit 6A	439	0	1	1.03639903
92	PSB3_HUMAN	(P49720) Proteasome subunit β type-3	205	0	1	1.03639903
93	PTN22_HUMAN	(Q9Y2R2) Tyrosine-protein phosphatase non-receptor type 22	807	0	1	1.03639903
94	RAB10_HUMAN	(P61026) Ras-related protein Rab-10	200	0	1	1.03639903
95	RBBP7_HUMAN	(Q16576) Histone-binding protein RBBP7	425	0	1	1.03639903
96	RL18A_HUMAN	(Q02543) 60S ribosomal protein L18a	176	0	1	1.03639903
97	RL27A_HUMAN	(P46776) 60S ribosomal protein L27a	148	0	1	1.03639903
98	RL34_HUMAN	(P49207) 60S ribosomal protein L34	117	0	1	1.03639903
99	RL3L_HUMAN	(Q92901) 60S ribosomal protein L3-like	407	0	1	1.03639903
100	RL40_HUMAN	(P62987) Ubiquitin-60S ribosomal protein L40	128	0	1	1.03639903

Table I. Continued.

No.	ID	Accession no. and description	No. of amino acids	Spectral counting		
				Mock	Lum	Fold-change (Rsc)
101	ROAA_HUMAN	(Q99729) Heterogeneous nuclear ribonucleoprotein A/B	332	0	1	1.03639903
102	SET_HUMAN	(Q01105) Protein SET	290	0	1	1.03639903
103	SODC_HUMAN	(P00441) Superoxide dismutase [Cu-Zn]	154	0	1	1.03639903
104	SPSY_HUMAN	(P52788) Spermine synthase	366	0	1	1.03639903
105	SYRC_HUMAN	(P54136) Arginyl-tRNA synthetase, cytoplasmic	660	0	1	1.03639903
106	TADBP_HUMAN	(Q13148) TAR DNA-binding protein 43	414	0	1	1.03639903
107	TALDO_HUMAN	(P37837) Transaldolase	337	0	1	1.03639903
108	TECT2_HUMAN	(Q96GX1) Tectonic-2	697	0	1	1.03639903
109	TEBP_HUMAN	(Q15185) Prostaglandin E synthase 3	160	0	1	1.03639903
110	TPD54_HUMAN	(O43399) Tumor protein D54	206	0	1	1.03639903
111	UGPA_HUMAN	(Q16851) UTP - glucose-1-phosphate uridylyltransferase	508	0	1	1.03639903
112	WDR53_HUMAN	(Q7Z5U6) WD repeat-containing protein 53	358	0	1	1.03639903
113	ZC12A_HUMAN	(Q5D1E8) Ribonuclease ZC3H12A	599	0	1	1.03639903
114	RL22_HUMAN	(P35268) 60S ribosomal protein L22	128	0	1	1.03639903
115	RL13_HUMAN	(P26373) 60S ribosomal protein L13	211	0	1	1.03639903
116	RL23_HUMAN	(P62829) 60S ribosomal protein L23	140	0	1	1.03639903
117	RS11_HUMAN	(P62280) 40S ribosomal protein S11	158	0	1	1.03639903
118	SUMO2_HUMAN	(P61956) Small ubiquitin-related modifier 2	95	0	1	1.03639903
119	TFR1_HUMAN	(P02786) Transferrin receptor protein 1	760	0	1	1.03639903
120	RL11_HUMAN	(P62913) 60S ribosomal protein L11	178	0	1	1.03639903
121	EF1A2_HUMAN	(Q05639) Elongation factor 1-α 2	463	10	19	1.04422777
122	CBX1_HUMAN	(P83916) Chromobox protein homolog 1	185	1	3	1.10688593
123	RL12_HUMAN	(P30050) 60S ribosomal protein L12	165	1	3	1.10688593
124	RAN_HUMAN	(P62826) GTP-binding nuclear protein Ran	216	1	3	1.10688593
125	PDIA6_HUMAN	(Q15084) Protein disulfide-isomerase A6	440	1	3	1.10688593
126	RL10L_HUMAN	(Q96L21) 60S ribosomal protein L10-like	214	1	3	1.10688593
127	SRC8_HUMAN	(Q14247) Src substrate cortactin	550	1	3	1.10688593
128	RL8_HUMAN	(P62917) 60S ribosomal protein L8	257	1	3	1.10688593
129	RS23_HUMAN	(P62266) 40S ribosomal protein S23	143	1	3	1.10688593
130	RS13_HUMAN	(P62277) 40S ribosomal protein S13	151	1	3	1.10688593
131	RL24_HUMAN	(P83731) 60S ribosomal protein L24	157	1	3	1.10688593
132	RL37A_HUMAN	(P61513) 60S ribosomal protein L37a	92	2	5	1.13371212
133	PAL4A_HUMAN	(Q9Y536) Peptidylprolyl cis-trans isomerase A-like 4A/B/C	164	2	5	1.13371212
134	RL18_HUMAN	(Q07020) 60S ribosomal protein L18	188	2	5	1.13371212
135	K1C9_HUMAN	(P35527) Keratin, type I cytoskeletal 9	623	8	17	1.17638585
136	TBB1_HUMAN	(Q9H4B7) Tubulin β-1 chain	451	3	8	1.31408061
137	PAIRB_HUMAN	(Q8NC51) Plasminogen activator inhibitor 1 RNA-binding protein	408	2	6	1.34868122
138	CATD_HUMAN	(P07339) Cathepsin D	412	0	2	1.56775608
139	FLNB_HUMAN	(O75369) Filamin-B	2602	0	2	1.56775608
140	MYL6B_HUMAN	(P14649) Myosin light chain 6B	208	0	2	1.56775608
141	PSB2_HUMAN	(P49721) Proteasome subunit β type-2	201	0	2	1.56775608
142	RS7_HUMAN	(P62081) 40S ribosomal protein S7	194	0	2	1.56775608
143	IQGA1_HUMAN	(P46940) Ras GTPase-activating-like protein IQGAP1	1657	0	2	1.56775608
144	DX39B_HUMAN	(Q13838) Spliceosome RNA helicase DDX39B	428	0	2	1.56775608
145	GANAB_HUMAN	(Q9BS14)Neutral α-glucosidase AB	944	0	2	1.56775608
146	H13_HUMAN	(P16402) Histone H1.3	221	0	2	1.56775608
147	H14_HUMAN	(P10412) Histone H1.4	219	0	2	1.56775608
148	HNRH2_HUMAN	(P55795) Heterogeneous nuclear ribonucleoprotein H2	449	0	2	1.56775608
149	ATPA_HUMAN	(P25705) ATP synthase subunit α, mitochondrial	553	0	2	1.56775608
150	H31T_HUMAN	(Q16695) Histone H3.1t	136	0	2	1.56775608

Table I. Continued.

No.	ID	Accession no. and description	No. of amino acids	Spectral counting		
				Mock	Lum	Fold-change (Rsc)
151	RL10A_HUMAN	(P62906) 60S ribosomal protein L10a	217	0	2	1.56775608
152	H12_HUMAN	(P16403) Histone H1.2	213	0	2	1.56775608
153	TBB8_HUMAN	(Q3ZCM7) Tubulin β-8 chain	444	5	15	1.57505162
154	EF1D_HUMAN	(P29692) Elongation factor 1-δ	281	1	5	1.6649664
155	RLA0L_HUMAN	(Q8NHW5) 60S acidic ribosomal protein P0-like	317	1	6	1.8799355
156	RS8_HUMAN	(P62241) 40S ribosomal protein S8	208	0	3	1.95562202
157	CCD50_HUMAN	(Q8IVM0) Coiled-coil domain-containing protein 50	306	0	3	1.95562202
158	K2C6A_HUMAN	(P02538) Keratin, type II cytoskeletal 6A	564	0	3	1.95562202
159	MT1X_HUMAN	(P80297) Metallothionein-1X	61	0	3	1.95562202
160	RL17_HUMAN	(P18621) 60S ribosomal protein L17	184	0	3	1.95562202
161	RS26_HUMAN	(P62854) 40S ribosomal protein S26	115	0	3	1.95562202
162	H15_HUMAN	(P16401) Histone H1.5	226	0	3	1.95562202
163	ATPB_HUMAN	(P06576) ATP synthase subunit β, mitochondrial	529	1	7	2.06719342
164	ADT1_HUMAN	(P12235) ADP/ATP translocase 1	298	0	4	2.26131991
165	H2A1_HUMAN	(P0C0S8) Histone H2A type 1	130	0	4	2.26131991
166	RS6_HUMAN	(P62753) 40S ribosomal protein S6	249	0	4	2.26131991
167	H2B1B_HUMAN	(P33778) Histone H2B type 1-B	126	0	4	2.26131991
168	FLNA_HUMAN	(P21333) Filamin-A	2647	1	9	2.38204237
169	H2B1M_HUMAN	(Q99879) Histone H2B type 1-M	126	0	6	2.72867159
170	H2AX_HUMAN	(P16104) Histone H2A.x	143	0	7	2.9159295
171	H2A1B_HUMAN	(P04908) Histone H2A type 1-B/E	130	0	9	3.23077846
172	H2A1A_HUMAN	(Q96QV6) Histone H2A type 1-A	131	0	9	3.23077846
173	H2B1C_HUMAN	(P62807) Histone H2B type 1-C/E/F/G/I	126	0	11	3.48962935
174	ACTA_HUMAN	(P62736) Actin, aortic smooth muscle	377	0	15	3.90067941

The expression levels of the 174 proteins were altered more than 2-fold as a result of lumican upregulation.

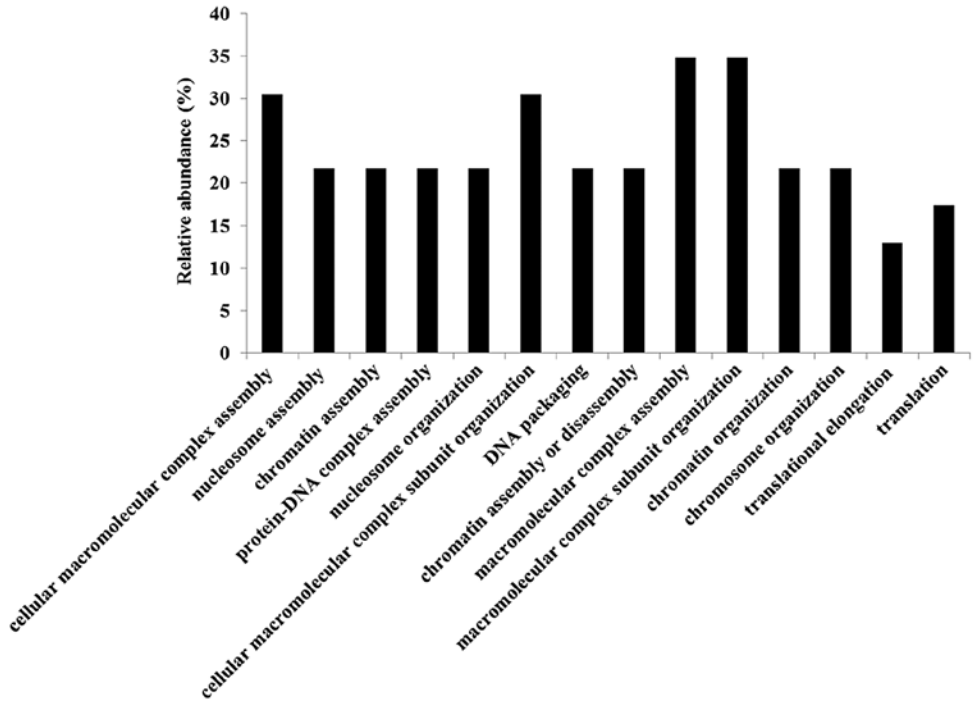

Figure 4. Analysis of identified proteins in the gene ontology biological process category. Only significant categories (P<0.05) are shown.

Table II. Differentially expressed proteins in lumican downregulated PANC-1 cells.

No.	ID	Accession no. and description	No. of amino acids	Spectral counting		
				NC	siLum	Fold-change (Rsc)
1	ACTG_HUMAN	(P63261) Actin, cytoplasmic 2	375	19	0	-4.1933172
2	H2B1D_HUMAN	(P58876) Histone H2B type 1-D	126	7	0	-2.8944219
3	H2A2A_HUMAN	(Q6FI13) Histone H2A type 2-A	130	4	0	-2.2414896
4	HNRPU_HUMAN	(Q00839) Heterogeneous nuclear ribonucleoprotein U	825	3	0	-1.9363502
5	H2B1M_HUMAN	(Q99879) Histone H2B type 1-M	126	3	0	-1.9363502
6	KRT81_HUMAN	(Q14533) Keratin, type II cuticular Hb1	505	3	0	-1.9363502
7	RL10_HUMAN	(P27635) 60S ribosomal protein L10	214	3	0	-1.9363502
8	K2C6B_HUMAN	(P04259) Keratin, type II cytoskeletal 6B	564	6	1	-1.8595343
9	CAPZB_HUMAN	(P47756) F-actin-capping protein subunit β	277	2	0	-1.5490424
10	FUBP2_HUMAN	(Q92945) Far upstream element-binding protein 2	711	2	0	-1.5490424
11	IPO7_HUMAN	(O95373) Importin-7	1038	2	0	-1.5490424
12	K2C6C_HUMAN	(P48668) Keratin, type II cytoskeletal 6C	564	2	0	-1.5490424
13	ML12A_HUMAN	(P19105) Myosin regulatory light chain 12A	171	2	0	-1.5490424
14	NUCB1_HUMAN	(Q02818) Nucleobindin-1	461	2	0	-1.5490424
15	EF1B_HUMAN	(P24534) Elongation factor 1-β	225	2	0	-1.5490424
16	NDKA_HUMAN	(P15531) Nucleoside diphosphate kinase A	152	2	0	-1.5490424
17	VINC_HUMAN	(P18206) Vinculin	1134	7	2	-1.5155258
18	PDIA3_HUMAN	(P30101) Protein disulfide-isomerase A3	505	4	1	-1.3933005
19	K2C5_HUMAN	(P13647) Keratin, type II cytoskeletal 5	590	8	3	-1.2936552
20	K1C19_HUMAN	(P08727) Keratin, type I cytoskeletal 19	400	11	5	-1.1429912
21	ECH1_HUMAN	(Q13011) δ(3,5)-δ(2,4)-dienoyl-CoA isomerase, mitochondrial	328	5	2	-1.1144173
22	PAIRB_HUMAN	(Q8NC51) Plasminogen activator inhibitor 1 RNA-binding protein	408	5	2	-1.1144173
23	RS3_HUMAN	(P23396) 40S ribosomal protein S3	243	3	1	-1.0881611
24	CRIP1_HUMAN	(P50238) Cysteine-rich protein 1	77	3	1	-1.0881611
25	RS28_HUMAN	(P62857) 40S ribosomal protein S28	69	3	1	-1.0881611
26	RS13_HUMAN	(P62277) 40S ribosomal protein S13	151	3	1	-1.0881611
27	RS27A_HUMAN	(P62979) Ubiquitin-40S ribosomal protein S27a	156	3	1	-1.0881611
28	CAPG_HUMAN	(P40121) Macrophage-capping protein	348	1	0	-1.0182433
29	CDC42_HUMAN	(P60953) Cell division control protein 42 homolog	191	1	0	-1.0182433
30	CX6B1_HUMAN	(P14854) Cytochrome *c* oxidase subunit 6B1	86	1	0	-1.0182433
31	GDIA_HUMAN	(P31150) Rab GDP dissociation inhibitor α	447	1	0	-1.0182433
32	IQGA1_HUMAN	(P46940) Ras GTPase-activating-like protein IQGAP1	1657	1	0	-1.0182433
33	RL12_HUMAN	(P30050) 60S ribosomal protein L12	165	1	0	-1.0182433
34	RL35A_HUMAN	(P18077) 60S ribosomal protein L35a	110	1	0	-1.0182433
35	SQSTM_HUMAN	(Q13501) Sequestosome-1	440	1	0	-1.0182433
36	RL10L_HUMAN	(Q96L21) 60S ribosomal protein L10-like	214	1	0	-1.0182433
37	RS14_HUMAN	(P62263) 40S ribosomal protein S14	151	1	0	-1.0182433
38	AGR3_HUMAN	(Q8TD06) Anterior gradient protein 3 homolog	166	1	0	-1.0182433
39	ALMS1_HUMAN	(Q8TCU4) Alstrom syndrome protein 1	4167	1	0	-1.0182433
40	AP3D1_HUMAN	(O14617) AP-3 complex subunit δ-1	1153	1	0	-1.0182433
41	BI2L2_HUMAN	(Q6UXY1) Brain-specific angiogenesis inhibitor 1-associated protein 2-like protein 2	529	1	0	-1.0182433
42	CH041_HUMAN	(Q6NXR4) Uncharacterized protein C8orf41	508	1	0	-1.0182433
43	CORO7_HUMAN	(P57737) Coronin-7	925	1	0	-1.0182433
44	CSPG4_HUMAN	(Q6UVK1) Chondroitin sulfate proteoglycan 4	2322	1	0	-1.0182433
45	CXL14_HUMAN	(O95715) C-X-C motif chemokine 14	111	1	0	-1.0182433
46	DDX17_HUMAN	(Q92841) Probable ATP-dependent RNA helicase DDX17	650	1	0	-1.0182433
47	DNJB1_HUMAN	(P25685) DnaJ homolog subfamily B member 1	340	1	0	-1.0182433
48	EIF3C_HUMAN	(Q99613) Eukaryotic translation initiation factor 3 subunit C	913	1	0	-1.0182433
49	FER_HUMAN	(P16591) Tyrosine-protein kinase Fer	822	1	0	-1.0182433

Table II. Continued.

No.	ID	Accession no. and description	No. of amino acids	Spectral counting		
				NC	siLum	Fold-change (Rsc)
50	GELS_HUMAN	(P06396) Gelsolin	782	1	0	-1.0182433
51	INSRR_HUMAN	(P14616) Insulin receptor-related protein	1297	1	0	-1.0182433
52	KRT85_HUMAN	(P78386) Keratin, type II cuticular Hb5	507	1	0	-1.0182433
53	MT1X_HUMAN	(P80297) MT-1X	61	1	0	-1.0182433
54	NALP6_HUMAN	(P59044) NACHT, LRR and PYD domains-containing protein 6	892	1	0	-1.0182433
55	PLEC_HUMAN	(Q15149) Plectin	4684	1	0	-1.0182433
56	PRS8_HUMAN	(P62195) 26S protease regulatory subunit 8	406	1	0	-1.0182433
57	PUR2_HUMAN	(P22102) Trifunctional purine biosynthetic protein adenosine-3	1010	1	0	-1.0182433
58	RFX6_HUMAN	(Q8HWS3) DNA-binding protein RFX6	928	1	0	-1.0182433
59	RS17_HUMAN	(P08708) 40S ribosomal protein S17	135	1	0	-1.0182433
60	RUNX1_HUMAN	(Q01196) Runt-related transcription factor 1	453	1	0	-1.0182433
61	SEPT9_HUMAN	(Q9UHD8) Septin-9	586	1	0	-1.0182433
62	SRSF4_HUMAN	(Q08170) Serine/arginine-rich splicing factor 4	494	1	0	-1.0182433
63	SUMO3_HUMAN	(P55854) Small ubiquitin-related modifier 3	103	1	0	-1.0182433
64	TIM13_HUMAN	(Q9Y5L4) Mitochondrial import inner membrane translocase subunit Tim13	95	1	0	-1.0182433
65	UGDH_HUMAN	(O60701) UDP-glucose 6-dehydrogenase	494	1	0	-1.0182433
66	WDHD1_HUMAN	(O75717) WD repeat and HMG-box DNA-binding protein 1	1129	1	0	-1.0182433
67	ZZEF1_HUMAN	(O43149) Zinc finger ZZ-type and EF-hand domain-containing protein 1	2961	1	0	-1.0182433
68	RL22_HUMAN	(P35268) 60S ribosomal protein L22	128	1	0	-1.0182433
69	BASP1_HUMAN	(P80723) Brain acid soluble protein 1	227	1	0	-1.0182433
70	SPSY_HUMAN	(P52788) Spermine synthase	366	1	0	-1.0182433
71	RS25_HUMAN	(P62851) 40S ribosomal protein S25	125	1	0	-1.0182433
72	RS9_HUMAN	(P46781) 40S ribosomal protein S9	194	1	0	-1.0182433
73	HIP1R_HUMAN	(O75146) Huntingtin-interacting protein 1-related protein	1068	1	0	-1.0182433
74	RL15_HUMAN	(P61313) 60S ribosomal protein L15	204	1	0	-1.0182433
75	G6PI_HUMAN	(P06744) Glucose-6-phosphate isomerase	558	1	0	-1.0182433
76	TERA_HUMAN	(P55072) Transitional endoplasmic reticulum ATPase	806	1	4	1.05291534
77	RS5_HUMAN	(P46782) 40S ribosomal protein S5	204	1	4	1.05291534
78	PCBP3_HUMAN	(P57721) Poly(rC)-binding protein 3	371	1	4	1.05291534
79	K2C74_HUMAN	(Q7RTS7) Keratin, type II cytoskeletal 74	529	4	11	1.0534109
80	CH60_HUMAN	(P10809) 60 kDa heat shock protein, mitochondrial	573	7	20	1.19690092
81	RAN_HUMAN	(P62826) GTP-binding nuclear protein Ran	216	0	2	1.20893401
82	ADT2_HUMAN	(P05141) ADP/ATP translocase 2	298	0	2	1.20893401
83	CAZA1_HUMAN	(P52907) F-actin-capping protein subunit α-1	286	0	2	1.20893401
84	G6PD_HUMAN	(P11413) Glucose-6-phosphate 1-dehydrogenase	515	0	2	1.20893401
85	H2AV_HUMAN	(Q71UI9) Histone H2A.V	128	0	2	1.20893401
86	H2B1H_HUMAN	(Q93079) Histone H2B type 1-H	126	0	2	1.20893401
87	H2B1J_HUMAN	(P06899) Histone H2B type 1-J	126	0	2	1.20893401
88	H2B1K_HUMAN	(O60814) Histone H2B type 1-K	126	0	2	1.20893401
89	HCD2_HUMAN	(Q99714) 3-hydroxyacyl-CoA dehydrogenase type-2	261	0	2	1.20893401
90	MT1A_HUMAN	(P04731) Metallothionein-1A	61	0	2	1.20893401
91	RL18A_HUMAN	(Q02543) 60S ribosomal protein L18a	176	0	2	1.20893401
92	RL21_HUMAN	(P46778) 60S ribosomal protein L21	160	0	2	1.20893401
93	RS7_HUMAN	(P62081) 40S ribosomal protein S7	194	0	2	1.20893401
94	SPT6H_HUMAN	(Q7KZ85) Transcription elongation factor SPT6	1726	0	2	1.20893401
95	SRP14_HUMAN	(P37108) Signal recognition particle 14 kDa protein	136	0	2	1.20893401
96	TBA4B_HUMAN	(Q9H853) Putative tubulin-like protein α-4B	241	0	2	1.20893401
97	TCTP_HUMAN	(P13693) Translationally-controlled tumor protein	172	0	2	1.20893401

Table II. Continued.

No.	ID	Accession no. and description	No. of amino acids	Spectral counting		
				NC	siLum	Fold-change (Rsc)
98	UBB_HUMAN	(P0CG47) Polyubiquitin-B	229	0	2	1.20893401
99	RL19_HUMAN	(P84098) 60S ribosomal protein L19	196	0	2	1.20893401
100	FKB1A_HUMAN	(P62942) Peptidyl-prolyl cis-trans isomerase FKBP1A	108	0	2	1.20893401
101	MDHM_HUMAN	(P40926) Malate dehydrogenase, mitochondrial	338	0	2	1.20893401
102	NDE1_HUMAN	(Q9NXR1) Nuclear distribution protein nudE homolog 1	346	0	2	1.20893401
103	PSA5_HUMAN	(P28066) Proteasome subunit α type-5	241	0	2	1.20893401
104	H2B1B_HUMAN	(P33778) Histone H2B type 1-B	126	0	2	1.20893401
105	HNRH2_HUMAN	(P55795) Heterogeneous nuclear ribonucleoprotein H2	449	1	5	1.30464663
106	K2C1_HUMAN	(P04264) Keratin, type II cytoskeletal 1	644	2	8	1.34002273
107	K1C9_HUMAN	(P35527) Keratin, type I cytoskeletal 9	623	0	3	1.59614949
108	RLA0L_HUMAN	(Q8NHW5) 60S acidic ribosomal protein P0-like	317	0	3	1.59614949
109	H2A1B_HUMAN	(P04908) Histone H2A type 1-B/E	130	0	3	1.59614949
110	SRC8_HUMAN	(Q14247) Src substrate cortactin	550	0	3	1.59614949
111	UBA1_HUMAN	(P22314) Ubiquitin-like modifier-activating enzyme 1	1058	0	3	1.59614949
112	H2A1H_HUMAN	(Q96KK5) Histone H2A type 1-H	128	0	3	1.59614949
113	H2A2B_HUMAN	(Q8IUE6) Histone H2A type 2-B	130	0	3	1.59614949
114	HN1_HUMAN	(Q9UK76) Hematological and neurological expressed 1 protein	154	0	3	1.59614949
115	HNRPK_HUMAN	(P61978) Heterogeneous nuclear ribonucleoprotein K	463	0	3	1.59614949
116	K1C14_HUMAN	(P02533) Keratin, type I cytoskeletal 14	472	0	3	1.59614949
117	K22O_HUMAN	(Q01546) Keratin, type II cytoskeletal 2 oral	638	0	3	1.59614949
118	K6PP_HUMAN	(Q01813) 6-phosphofructokinase type C	784	0	3	1.59614949
119	POTEJ_HUMAN	(P0CG39) POTE ankyrin domain family member J	1038	0	3	1.59614949
120	RADI_HUMAN	(P35241) Radixin	583	0	3	1.59614949
121	HS904_HUMAN	(Q58FG1) Putative heat shock protein HSP 90-α A4	418	2	10	1.62280828
122	PROF1_HUMAN	(P07737) Profilin-1	140	3	14	1.67515744
123	H90B3_HUMAN	(Q58FF7) Putative heat shock protein HSP 90-β-3	597	1	7	1.70556987
124	TBB2A_HUMAN	(Q13885) Tubulin β-2A chain	445	7	33	1.88806959
125	TIM50_HUMAN	(Q3ZCQ8) Mitochondrial import inner membrane translocase subunit TIM50	353	0	4	1.90119651
126	RL4_HUMAN	(P36578) 60S ribosomal protein L4	427	0	4	1.90119651
127	H2A1C_HUMAN	(Q93077) Histone H2A type 1-C	130	0	4	1.90119651
128	K2C6A_HUMAN	(P02538) Keratin, type II cytoskeletal 6A	564	0	4	1.90119651
129	NDK8_HUMAN	(O60361) Putative nucleoside diphosphate kinase	137	0	4	1.90119651
130	TKT_HUMAN	(P29401) Transketolase	623	0	4	1.90119651
131	H2B1O_HUMAN	(P23527) Histone H2B type 1-O	126	0	5	2.15292781
132	H2B2F_HUMAN	(Q5QNW6) Histone H2B type 2-F	126	0	5	2.15292781
133	ANXA5_HUMAN	(P08758) Annexin A5	320	0	6	2.36724523
134	H2A2C_HUMAN	(Q16777) Histone H2A type 2-C	129	0	6	2.36724523
135	H2B1L_HUMAN	(Q99880) Histone H2B type 1-L	126	0	7	2.55385105
136	HSP76_HUMAN	(P17066) Heat shock 70 kDa protein 6	643	0	8	2.71910308
137	TBA3E_HUMAN	(Q6PEY2) Tubulin α-3E chain	450	0	8	2.71910308
138	TBA3C_HUMAN	(Q13748) Tubulin α-3C/D chain	450	0	9	2.86739459
139	TBA4A_HUMAN	(P68366) Tubulin α-4A chain	448	0	14	3.44154556
140	TBA1A_HUMAN	(Q71U36) Tubulin α-1A chain	451	0	14	3.44154556
141	TBB8_HUMAN	(Q3ZCM7) Tubulin β-8 chain	444	0	16	3.61971953
142	ACTC_HUMAN	(P68032) Actin, α cardiac muscle 1	377	0	21	3.98789716
143	ACTS_HUMAN	(P68133) Actin, α skeletal muscle	377	0	42	4.95088405

The expression levels of the 143 proteins were altered more than 2-fold as a result of lumican downregulation.

Table III. Correlation between lumican expression level and identified protein expression level.

ID	Accession no. and description	Fold-change (Rsc)	
		Lumican upregulation	Lumican downregulation
ACTC_HUMAN	(P68032) Actin, α cardiac muscle 1	-3.770661694	3.987897158
TBB2A_HUMAN	(Q13885) Tubulin β-2A chain	-2.989773612	1.888069587
H2B1H_HUMAN	(Q93079) Histone H2B type 1-H	-2.854729916	1.208934009
HS904_HUMAN	(Q58FG1) Putative heat shock protein HSP 90-α A4	-2.705889054	1.622808285
TBA4A_HUMAN	(P68366) Tubulin α-4A chain	-2.705889054	3.441545557
H2B1O_HUMAN	(P23527) Histone H2B type 1-O	-1.885788034	2.152927808
ANXA5_HUMAN	(P08758) Annexin A5	-1.580193134	2.367245226
H2A1H_HUMAN	(Q96KK5) Histone H2A type 1-H	-1.580193134	1.596149489
HNRPK_HUMAN	(P61978) Heterogeneous nuclear ribonucleoprotein K	-1.580193134	1.596149489
K22O_HUMAN	(Q01546) Keratin, type II cytoskeletal 2 oral	-1.580193134	1.596149489
K1C14_HUMAN	(P02533) Keratin, type I cytoskeletal 14	-1.504094641	1.596149489
H2B1J_HUMAN	(P06899) Histone H2B type 1-J	-1.192430072	1.208934009
MDHM_HUMAN	(P40926) Malate dehydrogenase, mitochondrial	-1.192430072	1.208934009
NDK8_HUMAN	(O60361) Putative nucleoside diphosphate kinase	-1.192430072	1.901196513
HIP1R_HUMAN	(O75146) Huntingtin-interacting protein 1-related protein	1.036399032	-1.018243251
RL22_HUMAN	(P35268) 60S ribosomal protein L22	1.036399032	-1.018243251
SPSY_HUMAN	(P52788) Spermine synthase	1.036399032	-1.018243251
RL10L_HUMAN	(Q96L21) 60S ribosomal protein L10-like	1.106885931	-1.018243251
RL12_HUMAN	(P30050) 60S ribosomal protein L12	1.106885931	-1.018243251
RS13_HUMAN	(P62277) 40S ribosomal protein S13	1.106885931	-1.088161097
PAIRB_HUMAN	(Q8NC51) Plasminogen activator inhibitor 1 RNA-binding protein	1.348681223	-1.114417304
IQGA1_HUMAN	(P46940) Ras GTPase-activating-like protein IQGAP1	1.567756075	-1.018243251
MT1X_HUMAN	(P80297) Metallothionein-1X	1.955622017	-1.018243251
H2B1M_HUMAN	(Q99879) Histone H2B type 1-M	2.728671586	-1.936350191

Twenty-four proteins showed correlation between lumican expression level and their expression level.

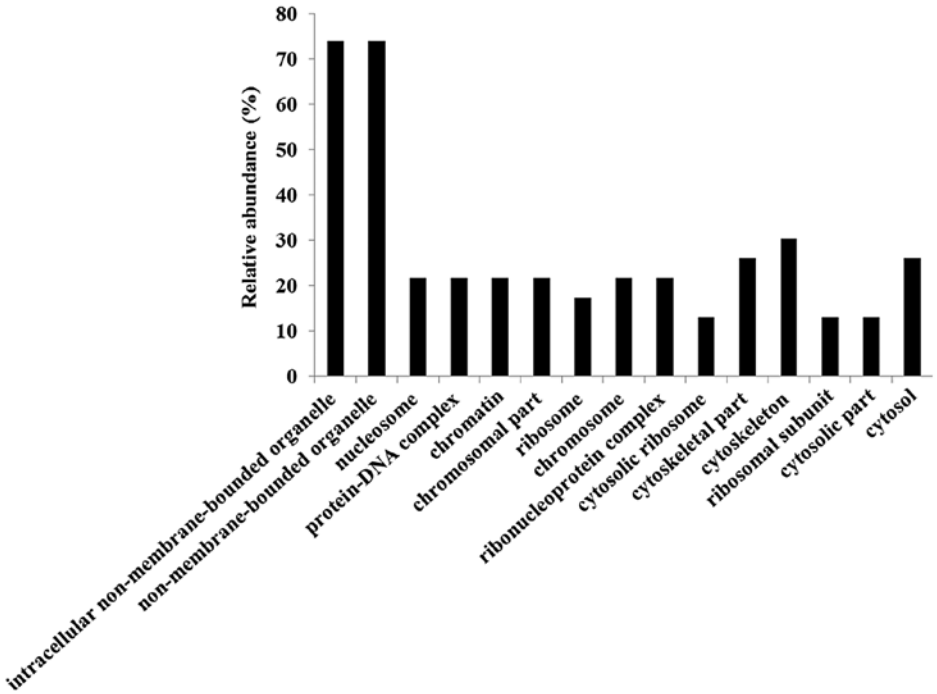

Figure 5. Analysis of identified proteins in the gene ontology cellular component. Only significant categories (P<0.05) are shown.

To examine the role of these candidate proteins in the effect of lumican on cell growth and invasion, we performed a functional classification of the candidate proteins by GO analysis. Although the GO terms for molecular function, biological processes, and cellular components were examined, we focused on molecular function. The molecular functions of candidate proteins were mainly classified in the 'structural molecule activity' category. Since structural molecule activity proteins contribute to the formation of complexes within or outside of the cell, such candidate proteins may be related to cell invasion regulated by lumican.

Annexin A5 expression may be regulated by lumican since Annexin A5 expression was negatively correlated with the lumican expression level. Annexin A5, also known as Annexin V, is widely known as a marker of early stage apoptosis (36,37). We previously demonstrated that the PDAC cell line secretes 70-kDa glycosylated lumican and that this secreted lumican stimulates cell growth (28). Thus, the induction of cell growth by lumican may be related to an inhibition of apoptosis through Annexin A5 expression. However, previous reports suggest that lumican plays an important role in apoptosis induction (22,38-40). This discrepancy may be derived from the differences between glycosylated lumican secreted from the PDAC cell line and other cells.

Furthermore, MT-1X expression levels positively correlated with the lumican expression level. MT family proteins are encoded by 10 functional isoforms (MT-1A, MT-1B, MT-1E, MT-1F, MT-1G, MT-1H, MT-1X, MT-2A, MT-3 and MT-4), and seven non-functional isoforms (MT-1C, MT-1D, MT-1I, MT-1J, MT-1K, MT-L and MT-2B). MT family proteins are involved in essential metal homeostasis, cellular free radical scavenging, cell proliferation, apoptosis, and metal detoxification. MT-1X and MT-2A transcripts were significantly upregulated under hypoxia in human prostate cancer cell lines, and siRNA-MT-2A treatment inhibited cell growth and induction of apoptosis, but an effect of MT-1X on cell growth and apoptosis was not demonstrated (41). Zinc is an abundant metal in the human prostate, and zinc inhibits cell growth and induces apoptosis in human prostate cancer cell lines (42,43). These findings suggest that MT-2A may play an important role in cell growth and apoptosis in prostate cancer through intracellular zinc homeostasis. MT-1X function in cancer cells, particularly PDAC, is not well understood. MT-1X is a known zinc-binding protein, and MT-1X mRNA expression was induced, as well as MT-2A, under hypoxic conditions (41). Thus, MT-1X may inhibit apoptosis as well as MT-2A. Furthermore, Ryu *et al* (44) suggested that MT-1E could enhance the migration and invasion of human glioma cells by inducing MMP-9 activation. MT-1X may have functions resembling MT-1E, since MMP-9 is a zinc-requiring enzyme, and MT-1X is classified into isoforms such as MT-1E. As mentioned above, it may be postulated that MT-1X plays an important role in cell growth and invasion by lumican. Further study is required to validate the expression levels of these candidate proteins, and to clarify the effect of candidate proteins, including MT-1X, on cell growth and invasion that are affected by lumican.

In conclusion, we identified more than 400 proteins from both lumican upregulated and lumican downregulated cells using global shotgun proteomics. A label-free semi-quantitative method based on spectral counting led to 24 candidate proteins whose expression was regulated by lumican. These candidate proteins included apoptosis-related and invasion-related proteins. Therefore, lumican may be involved in cell growth and invasion by altering the expression of these proteins.

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