Research Article

Quantitative iTRAQ LC-MS/MS Proteomics Reveals the Proteome Profiles of DF-1 Cells after Infection with Subgroup J Avian Leukosis Virus

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Avian leukosis virus subgroup J (ALV-J) is an avian oncogenic retrovirus that can induce various clinical tumors and has caused severe economic losses in China. To improve our understanding of the host cellular responses to virus infection and the pathogenesis of ALV-J infection, we applied isobaric tags for relative and absolute quantification (iTRAQ) labeling coupled with multidimensional liquid chromatography-tandem mass spectrometry to detect the protein changes in DF-1 cells infected and mock-infected with ALV-J. A total of 75 cellular proteins were significantly changed, including 33 upregulated proteins and 42 downregulated proteins. The reliability of iTRAQ-LC MS/MS was confirmed via real-time PCR. Most of these proteins were related to the physiological functions of metabolic processes, biosynthetic processes, responses to stimuli, protein binding, signal transduction, cell cytoskeleton, and so forth. We also found some proteins that play important roles in apoptosis and oncogenicity. The differentially expressed proteins identified may provide valuable information to elucidate the pathogenesis of virus infection and virus-host interactions.

1. Introduction

The J subgroup of avian leukosis virus (ALV-J), which belongs to the Retroviridae family, was first isolated from whitemeat-type chickens in the United Kingdom in 1988 [1]. It can predominantly lead to myeloid leukosis (ML) and immunosuppression effects in both naturally and experimentally infected chickens [2, 3]. In China, ALV-J-associated myeloid leukosis in chickens was first reported in 1999 [4]. ALV-J can induce various tumors, growth retardation, and production problems. In addition, in recent years, it has become widespread in many parts of our country and leads to severe economic losses in the poultry industry.

The pathogenesis of virus infection and the mechanism through which the virus interacts with host cells remain

unclear. During virus infection, the proteins of host cells may be significantly changed. It is now possible to use proteomic techniques to identify the changes in protein abundance that indicate host cellular responses to virus infection and provide useful information to obtain a better understanding of the pathogenesis of virus infection [5–8]. Kvaratskhelia et al. [9] applied enzymatic digestion coupled with mass spectrometry (MS) to detect the sites of glycosylation on the surface of avian leukosis virus subgroup A (ALV-A) and found that carbohydrates may play an important role in receptor binding.

To explore the possible mechanisms of virus infection, we used isobaric tags for relative and absolute quantification (iTRAQ) combined with multidimensional liquid chromatography (LC) and tandem MS analysis to perform a quantitative proteomic analysis of DF-1 cells infected with ALV-J [10]. To the best of our knowledge, no previous study had used the iTRAQ LC-MS/MS proteomics strategy to investigate the differently expressed proteins in ALV-J-infected DF-1 cells. The iTRAQ labeling technology could greatly increase the identification sensitivity and quantitation accuracy of proteomic analyses through a multiplexed quantitation strategy [11]. The results showed that 75 proteins were significantly changed after ALV-J infection. These changed proteins may provide valuable information to study the molecular mechanisms underlying ALV-J pathogenesis.

2. Materials and Methods

2.1. Reagents. The iTRAQ Reagent Multi-Plex Kit was acquired from Applied Biosystems (Foster City, CA, USA). A multidimensional liquid chromatographer (RIGOL 3220) was purchased from RIGOL, and the chromatographic column (Agela, Cl8 chromatographic column, 250 × 4.6 mm i.d., filler particles diameter: 5μ m) was acquired from Agela Co., Ltd. (Tianjin, China). The LC-MS/MS instrument (Q-Exactive) was obtained from Thermo Fisher Scientific.

2.2. Cell Culture and Virus Infection. DF-1 cells (ATCC accession number: CRL-12203) were cultured in Dulbecco's modified Eagle medium (DMEM; HyClone, Beijing, China) supplemented with 10% fetal bovine serum (FBS) and 100 μ g/mL streptomycin and penicillin at 37°C in a 5% CO₂ atmosphere. ALV-J strain HPRS-103 (GenBank: Z46390) was kindly provided by Professor Venugopal Nair. DF-1 cells cultured in flasks to approximately 80% confluence were infected with 0.5 mL of 10^{3.5}/mL 50% tissue culture infectious doses (TCID50) of ALV-J for 144 h. Uninfected DF-1 cells served as mock-infected cells.

2.3. Indirect Immune Fluorescence Assay (IFA). At 144 h after infection, the infected DF-1 cells were washed twice with PBS and fixed with anhydrous ethanol for 20 min. The fixed cells were then incubated with mouse anti-P27 monoclonal antibody (prepared in our lab) at 37°C for 60 min. After washing three times with PBST (0.01 M PBS, pH 7.2, 0.05% Tween 20), the cells were incubated with goat anti-mouse IgG conjugated to FITC (Sigma, USA) at 37°C for another 60 min. Finally, the cells were observed under a Carl Zeiss Vision microscope (ZEISS Axio Observer D1) after three washes with PBST.

2.4. Protein Extraction, Digestion, and Labeling with iTRAQ Reagents. Infected and mock-infected DF-1 cells were washed twice with PBS. The cells were lysed in a lysis buffer (9 M urea, 4% CHAPS, 1% DTT, and 1% IPG buffer). The mixtures were centrifuged at 15,000 g and 4°C for 15 min. The supernatant was collected, and the protein concentration was determined using the Bradford protein assay [12] (Bio-Rad Laboratories). Then, 100 μ g of protein was mixed overnight with four volumes of cold (-20°C) acetone and then dissolved using the dissolution buffer. After being reduced, alkylated, and digested with trypsin, the samples were labeled following the manufacturer's instructions

TABLE 1

		1110			
Time	0	24	30	31	38
В%	3	16	30	90	90

described in the iTRAQ protocol. The labeled samples were pooled for further analysis.

2.5. LC-MS/MS and Database Searches. The iTRAQ-labeled sample mixtures were then fractionated by strong cation exchange (SCX) chromatography on a high-performance liquid chromatography (HPLC) system (RIGOL 3220; Beijing, China) using a chromatographic column (Agela, C18 chromatographic column, 250 × 4.6 mm i.d., filler particles diameter: 5μ m; Tianjin, China). Mobile phase A consisted of 2% ACN-98% H₂O (pH 10.0), and mobile phase B consisted of 98% ACN-2% H₂O (pH 10.0). The solvent gradient was as follows: 5%–8% B for 1 min, 8%–32% B for 24 min, 32%–95% B for 2 min, 95% for 4 min, and 95%–5% B for 1 min. The column temperature was 45°C, the flow rate was 0.7 mL/min, and the detection wavelength was 214 nm. Peptides were collected every minute within the effective gradient from 8% to 32%. A total of 27 fractions were collected and then dried.

The dried fractions were dissolved in 1.9% ACN/98% $H_2O/0.1\%$ FA aqueous solution and combined into nine samples. The samples were centrifuged at 12,000 ×r for 3 min, and the supernatant was collected. The supernatant was then analyzed using the EASY-nLC-1000 liquid phase interfaced with a Q Exactive mass spectrometer (Thermo Fisher). The chromatographic conditions are as follows: liquid phase, EASY-nLC-1000; enriching column, C18, 5 μ m, ID100 μ m, 20 mm in length; separation column, C18, 3 μ m, ID75 μ m, 120 mm in length; mobile phase A, 1.9% ACN + 98% H₂O + 0.1% FA; and flow rate, 450 nl/min.

Elution Conditions. See Table 1.

The data were acquired at 38 min. The spray voltage was 2.0 KV, the capillary temperature was 320°C, the collision energy was 30, and the acquisition quality range was 300–1400 da.

The relative quantification and protein identification were performed with the Protein Discoverer software (version 1.2) using the built-in mascot as the search engine.

2.6. Real-Time PCR. The primers (Table 2) were synthesized by BoShi Biotechnology Company (Harbin, China). The gene was amplified from the genomic DNA of DF-1 cells by polymerase chain reaction (PCR). The PCR-amplified products were separated in a 2% agarose gel and then purified using a DNA gel extraction kit (Axygen Biotechnology Limited, Hangzhou City, China). The products were then ligated into the pZeroBack/blunt vector (Tiangen Biotech Co., Ltd., Beijing, China), and the sequence was verified. The plasmid DNA was used as the standard to construct the standard curve via SYBR Green real-time PCR. The total cellular RNA of the infected or mock-infected DF-1 cells was extracted using the RNeasy Mini Kit (QIAGEN, China)

TABLE 2: Primer sequences for real-time PCR.

Gene	Sequence	Size
BLOC185	F-TATATGAGCGGGGGCAGGCCCT	150 bp
DLOCISS	R-TTCCCCGACATCCTTGAT	150 UP
Koratin	F-ATGTCCCGCTCCGTCAGCTTC	150 bp
Relatin	R-AGAGCCCAGGTTGTAGAGGCT	150 UP
HMC14	F-ATGCCGAAGAGAAAGGTG	140 bp
11101014	R-TCAGATTTATCCTTAGCCGCC	140 Up
AACS	F-ATGTCCCGCGAGCCCGAGATT	150 bp
AACS	R-CACTGACCACTGGTATAAGTC	150 UP

according to the manufacturer's protocol. Reverse transcription was performed using a PrimeScript II First-Strand cDNA Synthesis Kit (TaKaRa, China) as described in the protocol. The real-time PCR was performed using the Roche LightCycler 480 real-time PCR System.

2.7. Bioinformatics Analysis. The functional annotation of the 75 proteins in DF-1 cells that were significantly changed after infection with ALV-J was performed using the GOSlimViewer tool of the AgBase database (http://www.agbase.msstate.edu/) [13]. In addition, we aimed to determine how ALV-J interacts with the host cellular proteins and how it affects the function of host cells. The identified proteins were inputted into the STRING database to obtain the protein-protein interaction network [14, 15] (http://string.embl.de/).

3. Results

3.1. Confirmation of ALV-J Infection in DF-1 Cells by IFA. To confirm that the DF-1 cells were infected by ALV-J, IFA was used to detect the viral P27 antigen. The results showed clear green fluorescence in ALV-J-infected DF-1 cells 144 h after infection, whereas the uninfected DF-1 cells exhibited no green fluorescence (Figure 1).

3.2. Protein Profile Obtained by iTRAQ LC-MS/MS Analysis. To explore the differences in the protein expression levels after virus infection, the total proteins of ALV-J-infected and mock-infected DF-1 cells were extracted for iTRAQ-LC-MS/MS analysis. A total of 1091 proteins were detected, including 75 proteins in DF-1 cells that were significantly changed infection with ALV-J for 144 h (Table 3). These differently expressed proteins were divided into two clusters: upregulated and downregulated. The number of upregulated proteins was 33, whereas the number of downregulated proteins was 42.

3.3. Functional Classifications of the Identified Proteins. To annotate the functions of the 75 significantly changed proteins identified in our study, the proteins were submitted to GORetriever (http://www.agbase.msstate.edu/) for analysis. Three types of annotations were obtained using the website: molecular functions, biological processes, and cellular components.

The biological process annotation revealed that the significantly changed proteins were involved in metabolic process (19%), macromolecule metabolic process (12%), regulation of biological process (11%), biosynthetic processes (10%), nucleobase-containing compound metabolic process (10%), response to stimulus (7%), and various other activities (31%) (Figure 2, biological process).

The molecular function annotation revealed that these differently expressed proteins were involved in protein binding (30%), nucleic acid binding (21%), hydrolase activity (11%), transferase activity (5%), receptor activity (5%), oxidoreductase activity (4%), and various other activities (24%) (Figure 2, molecular function).

The cellular component annotation revealed that the altered proteins were associated with the following cellular components: intracellular (28%), cytoplasm (24%), nucleus (17%), membrane (15%), extracellular region (5%), chromosome (3%), and various others (8%) (Figure 2, cellular component).

3.4. Validation of the iTRAQ Data by Real-Time PCR. To confirm the results of the differentially expressed proteins identified by iTRAQ LC-MS/MS analysis, real-time PCR was performed to detect the transcript expression levels of the genes after ALV-J infection. We generated four standard curves to determine the gene expression of BLOC1S5, keratin, HMG14, and AACS in ALV-J-infected and mock-infected DF-1 cells. The results showed that HMG14 was upregulated (Figure 3), whereas BLOC1S5, AACS, and keratin were down-regulated (Figure 3). The RT-PCR results were consistent with the results of the iTRAQ LC-MS/MS analysis (Table 3), confirming that the iTRAQ data were reliable.

3.5. Protein-Protein Interaction Analysis. The mechanism through which the virus interacts with host cells remains unclear, and oncogenicity is an important index of the pathogenicity of ALV-J. During virus infection, some proteins of host cells may be significantly changed. As a result, the functions of the changed proteins will also be altered. In our study, we aimed to determine whether the significantly changed proteins that were identified have some relationship with apoptosis or ALV-J-induced oncogenicity. We searched the STRING database to analyze the protein-protein interactions between the differently expressed proteins and PARK7, PTENP1, AKT1, PIK3CA (PI3K), and VDAC (Figure 4). These proteins are known to have some relationship with tumor-associated process and apoptosis. The protein-protein interaction networks may provide valuable information to further investigate the possible mechanism of ALV-J-induced oncogenicity.

4. Discussion

Proteomics is a relatively novel technology that has been used for the detection of the host cellular proteins response to virus infection [16, 17]. Isobaric tags for relative and absolute quantification (iTRAQ) combined with multidimensional liquid chromatography (LC) and tandem MS analysis are a powerful tool for quantitative proteomic analysis that has

Accession number	Protein name	Protein score	Fold change in expression	Protein MW	Protein PI
	Cluster 1: tendency for upregulatic	on (33)	a		
O73612	Ephrin-Bl GN=EFNB1	34.98	1.667	36.8	8.87
FIP187	Gephyrin (fragment) GN=GPHN	0.00	1.560	77.4	5.38
P12274	Nonhistone chromosomal protein HMG-14B GN=HMG14	0.00	1.473	11.2	9.63
EIBTX9	Serine/threonine-protein phosphatase	39.24	1.429	73.4	8.34
P08286	Histone H1.10	476.50	1.315	22.0	11.18
E1C281	PHD finger protein 6 GN=PHF6	0.00	1.281	41.0	8.62
Q5ZJ02	DBIRD complex subunit ZNF326 GN=ZNF326	45.04	1.280	63.5	5.78
Q5ZIK4	Protein yippee-like GN=YPEL5	0.00	1.276	13.8	7.31
Q5F3J5	Proteasome activator complex subunit 3 GN=PSME3	143.05	1.274	29.5	6.19
Q5F3Z5	DnaJ homolog subfamily B member 6 GN=DNAJB6	0.00	1.263	36.7	8.84
FINB51	Zinc finger E-box-binding homeobox 1 GN=ZEB1	41.75	1.258	123.1	5.02
FINLA7	Zinc finger CCCH domain-containing protein 11A GN=ZC3H11A	43.83	1.254	79.0	8.16
F1P5W3	Ephrin-B1 (Fragment) GN=EFNB1	34.98	1.249	32.7	8.46
FINXG2	WW domain-binding protein 4 (Fragment) GN=WBP4	0.00	1.235	45.2	5.73
P08267	Ferritin heavy chain GN=FTH	65.41	1.226	21.1	6.21
Q6K1L7	Probable RNA-binding protein EIFIAD GN=eiflad	0.00	1.210	21.2	4.79
FINEYO	Syndecan (Fragment) GN=CPQ	45.65	1.208	19.9	4.70
FINMD7	Pre-mRNA-splicing factor RBM22 GN=RBM22	21.07	1.205	46.7	8.54
O93481	Chromobox protein (CHCB2) GN=CBX3	0.00	1.190	19.8	5.12
FINFJO	DNA replication licensing factor MCM3 GN=MCM3	46.00	1.188	91.3	5.74
EIC9E9	DCNI-like protein GN=DCUN1D5	0.00	1.183	27.2	5.77
FINAQI	Vascular endothelial growth factor A GN=VEGFA	0.00	1.179	25.1	9.10
FINLU6	Enhancer of mRNA-decapping protein 3 GN=EDC3	49.54	1.175	56.0	7.17
P16527	Myristoylated alanine-rich C-kinase substrate GN=MARCKS	30.67	1.174	27.7	4.44
Q5ZMC9	Nuclear distribution protein nudE homolog 1 GN=NDE1	0.00	1.173	39.5	5.11
Q5Z116	Protein kish-A GN=TMEM167A	27.01	1.173	8.0	8.92
Q5Z1L9	KIF1-binding protein homolog GN=kbp	0.00	1.167	69.0	5.21
FINFP5	Arginine-tRNA ligase, cytoplasmic GN=RARS	184.95	1.158	75.4	6.98
EIC4V1	ATP synthase-coupling factor 6, mitochondrial GN=ATP5J	111.05	1.157	12.5	9.33
Q90595	Transcription factor MafF GN=MAFF	37.09	1.155	16.6	9.74
R4GJF8	TAR DNA-binding protein 43 GN=TARDBP	131.97	1.155	42.2	6.19
Q6B7Z6	Polymyositis/scleroderma autoantigen 1 GN=EXOSC9	0.00	1.151	49.3	5.54
E1C7X8	S-adenosylmethionine synthase GN=LOC427292	14.44	1.150	43.2	6.62
	Cluster 2: Tendency to down-regula	tion (42)			
R4GKA6	Collagen alpha-2(VI) chain GN=COL6A2	181.18	0.850	102.4	5.48
E1BXS2	Guanine nucleotide-binding protein G(i) subunit alpha-1 GN=GNAI1	147.69	0.850	40.4	5.97
Q90927	Nuclear factor 1 GN=cNF1-A4	0.00	0.850	54.6	8.31
EIBUI0	tRNA pseudouridine synthase (Fragment) GN=PUSL1	55.04	0.849	33.7	9.64
Q90617-3	Isoform LAMP-2C of Lysosome-associated membrane glycoprotein 2 GN=LAMP2	149.29	0.849	46.4	6.43
Q90733	COUP transcription factor 2 GN=NR2F2	0.00	0.848	45.4	8.28
P12957-2	Isoform Brain l-cad of Caldesmon GN=CALD1	0.00	0.847	58.8	8.44

on number	Protein name	Protein score	Fold change in expression	Protein MW	
	Cluster 1: tendency for upregulatic	1 (33)			1
	Ephrin-BI GN=EFNB1	34.98	1.667	36.8	
	Genhvrin (fræment) GN=GPHN	0.00	1.560	77.4	
	Nonhistone chromosomal protein HMG-14B GN=HMG14	0.00	1.473	11.2	
6	Serine/threonine-protein phosphatase	39.24	1.429	73.4	
	Histone H1.10	476.50	1.315	22.0	
	PHD finger protein 6 GN=PHF6	0.00	1.281	41.0	
	DBIRD complex subunit ZNF326 GN=ZNF326	45.04	1.280	63.5	
4	Protein vippee-like GN=YPEL5	0.00	1.276	13.8	
	Proteasome activator complex subunit 3 GN=PSME3	143.05	1.274	29.5	
10	Dnal homolog subfamily B member 6 GN=DNAIB6	0.00	1.263	36.7	
	Zinc finger E-box-binding homeobox 1 GN=ZEB1	41.75	1.258	123.1	
	Zinc finger CCCH domain-containing protein 11A GN=ZC3H11A	43.83	1.254	79.0	
3	Ephrin-B1 (Fragment) GN=EFNB1	34.98	1.249	32.7	
5	WW domain-binding protein 4 (Fragment) GN=WBP4	0.00	1.235	45.2	
	Ferritin heavy chain GN=FTH	65.41	1.226	21.1	
7	Probable RNA-binding protein EIFIAD GN=eiflad	0.00	1.210	21.2	
0	Syndecan (Fragment) GN=CPQ	45.65	1.208	19.9	
70	Pre-mRNA-splicing factor RBM22 GN=RBM22	21.07	1.205	46.7	
	Chromobox protein (CHCB2) GN=CBX3	0.00	1.190	19.8	
	DNA replication licensing factor MCM3 GN=MCM3	46.00	1.188	91.3	
-	DCNI-like protein GN=DCUN1D5	0.00	1.183	27.2	
1	Vascular endothelial growth factor A GN=VEGFA	0.00	1.179	25.1	
9	Enhancer of mRNA-decapping protein 3 GN=EDC3	49.54	1.175	56.0	
	Myristoylated alanine-rich C-kinase substrate GN=MARCKS	30.67	1.174	27.7	
60	Nuclear distribution protein nudE homolog 1 GN=NDE1	0.00	1.173	39.5	
	Protein kish-A GN=TMEM167A	27.01	1.173	8.0	
¢	KIF1-binding protein homolog GN=kbp	0.00	1.167	69.0	
	Arginine-tRNA ligase. cytoplasmic GN=RARS	184.95	1.158	75.4	
	ATP svnthase-counting factor 6. mitochondrial GN=ATP51	111 05	1 157	12.5	
	Transcription factor Maff GN=MAFF	37.09	1.155	16.6	
~	TAR DNA-binding protein 43 GN=TARDBP	131.97	1.155	42.2	
6	Polymyositis/scleroderma autoantigen 1 GN=EXOSC9	0.00	1.151	49.3	
~	S-adenosylmethionine synthase GN=LOC427292	14.44	1.150	43.2	
	Cluster 2: Tendency to down-regula	on (42)			
16	Collagen alpha-2(VI) chain GN=COL6A2	181.18	0.850	102.4	
	Guanine nucleotide-binding protein G(i) subunit alpha-1 GN=GNAI1	147.69	0.850	40.4	
4	Nuclear factor 1 GN=cNFI-A4	0.00	0.850	54.6	
	tRNA pseudouridine synthase (Fragment) GN=PUSL1	55.04	0.849	33.7	
-3	Isoform LAMP-2C of Lysosome-associated membrane glycoprotein 2 GN=LAMP2	149.29	0.849	46.4	
	COUP transcription factor 2 GN=NR2F2	0.00	0.848	45.4	
-2	Isoform Brain I-cad of Caldesmon GN=CALD1	0.00	0.847	58.8	

FN908 Calteryain B GN=CTSB FN908 Calteryain B GN=CTSB FN908 FN33.09 0.847 AGCTPO Gatathione S-transferase 00033 00034 00346 BN965 Firzaled 7 GN=TCD7 0000 0342 0342 EC317 Disploy toxidase homolog 2 GN+LOXL2 0000 0342 EDBRJ Diversition S-transferase 0000 0342 EC317 Disploy toxidase homolog 2 GN+LOXL2 000 0342 FINCX1 Diversition S-transferase 0.00 0342 FINCX1 Diversition S-transferase 0.00 0.842 0.843 FINCX1 Diversition S-transferase 0.00 0.843 0.843 Structure Procollagen-lysine 2-oxoglutante S-dioxygenase I GN=DDD1 198.96 0.853 0.844 0.844 PINRE2 Procollagen-lysine 2-GOLA3 BCACINA 0.00 0.834 0.844 PINRE2 PROSPERATION Disployedute phosphalace GN=DD1A3 Disployedute phosphalace GN=DD1A3 Disployedute Phosphalace GN=DNP12 Disployedute phosphalace GN=DD1A3 Disployedute PhosP	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	37.6 25.7 25.3 62.7 86.9 114.3 1127.4 39.9 84.3 339.4 1135 119.5 71.6	5.86 8.27 8.88 7.99
AddTP0Galetin66.440.846 000032 Fitzzled-T GNE FZD70.000.845FIN365Fitzzled-T GNE FZD70.000.842FIN365Fitzzled-T GNE SCIEDXL20.000.842FIN365Fitzzled-T GNE SCIEDXL20.000.842FIN367Integrin alpha-V GNETGAV0.000.842FIN367Integrin alpha-V GNETGAN0.000.843FIN367Integrin alpha-V GNETGAN0.000.843FIN367DNA-directed RNA polymease GN=POLR3B0.000.843FIN367FIN3675FIN36720.000.843FIN367Collagen alpha-3(V) chain GN=COLA398.560.863FIN367Collagen alpha-3(V) chain GN=-COLA398.560.00FIN367FIN367FIN36798.560.833FIN367Pinphhmide Biosynthesis protein I GN=FRP10.000.834FIN367Pinphinh decistrin domain-containing protein I GN=FRP10.000.834FIN367Pinphinhinde Biosy	66.44 0.846 108.74 0.846 0.00 0.842 0.00 0.842 0.172.35 0.841 0.00 0.842 0.00 0.843 172.35 0.841 0.00 0.842 0.00 0.843 98.96 0.837 98.96 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.233 0.834 39.98 0.833	25.7 25.3 62.7 86.9 84.3 339.4 1127.4 339.4 1135.5 71.6 71.6	8.27 8.88 7.99
(0632) Glutathione Stransferase (0674) (0684) FINGXI Privatione Stransferase (000) <td>(08.74 0.846 0.00 0.842 0.00 0.842 0.172.35 0.841 0.00 0.841 0.00 0.841 0.010 0.840 0.855 0.840 0.877 0.833 98.96 0.835 98.96 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.833 20.32 0.833</td> <td>25.3 62.7 86.9 114.3 339.4 339.4 119.5 71.6 71.6</td> <td>8.88 7.99</td>	(08.74 0.846 0.00 0.842 0.00 0.842 0.172.35 0.841 0.00 0.841 0.00 0.841 0.010 0.840 0.855 0.840 0.877 0.833 98.96 0.835 98.96 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.833 20.32 0.833	25.3 62.7 86.9 114.3 339.4 339.4 119.5 71.6 71.6	8.88 7.99
FIN965 FIN264 Color 0.00 0.842 FIN264 Frizale4 7.05 \pm TGAV 0.00 0.842 FINCAU Jysyl oxidase homolog 2 GN=LOXL2 0.00 0.842 FINCAU Jingyl oxidase homolog 2 GN=LOXL3 0.00 0.843 FINCAU Jingyl oxidase homolog 2 GN=POLR3B 0.00 0.843 FINAF Dive directed NNA polymerase GN=POLR3B 0.00 0.843 FINAF Collagen Jysine 2-collayanase I GN=POL 0.00 0.845 FINAF Collagen Jysine 2-collayanase I GN=POL 0.00 0.835 FINAF Collagen Jysine 2-colladeA3 0.00 0.835 FINAF Collagen Jysine 2-COLEA3 0.00 0.835 FINAF Collagen Jysine 2-COLEA3 0.00 0.835 FINAF Collagen Jysine 2-COLEA3 0.42.57 0.835 FINAF Phomologicalte phosphatase GN=ACTNA 51.54 51.52 0.835 FINAF Phomologicalte phosphatase GN=PMPP2 51.52 0.835 0.835 FINAF Phomologi GN=FINA 51.52	0.00 0.842 0.00 0.842 0.172.35 0.841 0.00 0.841 0.00 0.841 0.00 0.840 0.855 0.840 0.839 0.833 98.96 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.339 0.835 0.00 0.835 0.339 0.833 0.339 0.833	62.7 86.9 114.3 127.4 39.9 84.3 339.4 119.5 71.6 71.6	7.99
EIC3U7 Itysy toxidase homolog 2 GN=LOXL2 0.00 0.842 FIRCX1 Inegrin alpha-V GN=TGAV 0.00 0.843 FIRS1 DNA-directed RNA polymerase GN=POLR3B 0.00 0.844 $0.8AXV1$ Endophilin AI GN=SH3GL2 0.800 0.845 $0.8AXV1$ Pandophilin AI GN=SH3GL2 0.800 0.845 $0.8AXV1$ Pandophilin AI GN=SH3GL2 0.803 0.845 $0.8AXV1$ Pandophilin AI GN=SH3GL2 0.803 0.845 $0.8AXV1$ Pandophilin AI GN=SH3GL2 0.803 0.835 $0.8AXV1$ Pandopharase GN=POLR3B 0.00 0.835 $0.8AGFN0$ Diphtamatic bioxynthesis protein 1 GN=FLRM 0.00 0.835 $0.8G73$ Phosphogycolate phosphatase GN=PDF3 0.00 0.835 $0.8G613$ Phutary protein Phosphatase GN=PDF3 0.00 0.825 $0.8G613$ Phutary protein Phosphatase GN=PDF3 0.00 0.825 $0.8G613$ Phutary protein Protein CAPACINA 0.00 0.825 $0.8G613$ Phutary prouebox ICAPA 0.826 <t< td=""><td>0.00 0.842 172.35 0.841 0.00 0.841 0.01 0.840 0.02 0.840 0.855 0.840 0.856 0.837 0.837 0.833 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.339 0.835 0.339 0.833 0.339 0.833</td><td>86.9 114.3 127.4 39.9 84.3 339.4 119.5 71.6 71.6</td><td></td></t<>	0.00 0.842 172.35 0.841 0.00 0.841 0.01 0.840 0.02 0.840 0.855 0.840 0.856 0.837 0.837 0.833 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.00 0.835 0.339 0.835 0.339 0.833 0.339 0.833	86.9 114.3 127.4 39.9 84.3 339.4 119.5 71.6 71.6	
FINGX1Integrin alpha-V GN=ITGAV 72.35 0.841 EIBR1DNA-directed RNA polymerase GN=POLR3B 0.00 0.800 EIBR1DNA-directed RNA polymerase GN=POLR3B 0.000 0.830 $0.8XYY1$ Endophilin AI GN=STR3G1.2 0.835 0.835 FINMF6Procollagen lysine.2-oxoglutarate 5-dioxygenase I GN=PLD1 $1.98.96$ 0.835 $0.8X64$ Diphthamide biosynthesis protein I GN=FRARP1 0.000 0.836 0.8764 Diphthamide biosynthesis protein Containing protein I GN=FRAP1 0.000 0.834 0.9011 Diphthamide biosynthesis protein Containing protein I GN=FRAP1 0.000 0.834 0.9011 Diphthamide biosynthesis protein Containing protein I GN=FRA 0.000 0.834 0.9011 Diphthamide biosynthesis protein Containing protein I GN=FRA 0.000 0.834 0.9011 Diphthamide biosynthesis protein CN=PCP 0.812 0.825 0.9011 Disphogiptise GN=MMP2 0.812 0.825 0.9011 Disphogiptise GN=MMP2 0.826 0.833 0.9011 Disphogiptise GN=MMP2 0.826 0.833 0.9011 Disphogiptise GN=MMP2 0.812 0.825 0.9011 Disphositise GN=MP2 0.812 0.826 0.9011 Disphositise GN=MMP2 0.826 0.833 0.9011 Disphositise GN=MP2 0.826 0.833 0.9011 Disphositise GN=MP2 0.816 0.926 0.9011 Disphositise GN=MP2 0.816 0.926 0.90121	172.35 0.841 0.00 0.840 65.55 0.839 98.96 0.837 94.2.87 0.835 0.00 0.835 0.00 0.835 0.00 0.835 39.98 0.833 20.32 0.833	114.3 127.4 39.9 84.3 339.4 119.5 52.1 71.6	6.49
IBIRI4 DNA-directed RNA polymerase GN=POLR3B 000 0.849 QAXY1 Endophilin-AI (CN=H3G1G) 66255 0.89 FIP2F0 FIP2F0 6755 0.89 FIP2F0 FIP2F0 642.87 0.835 FIP2F0 Collagen alpha-3(V1) chain GN=COL6A3 642.87 0.835 FIP2F0 FIP2F0 FIP2F0 642.87 0.835 FIP3C4 Diphthanide biosynthesis protein 2 GN=DPH12 0.00 0.835 FINSG4 Diphthanide biosynthesis protein 2 GN=DPH12 0.00 0.833 FINSG4 Diphthanide biosynthesis protein 2 GN=DPH12 0.00 0.833 FINSG4 Diphthanide biosynthesis protein 2 GN=DPH12 0.00 0.833 P56673 Proteing FIC GN 0.00 0.833 Q90611 T2 Lba type IV collagenase GN=MDM2 0.00 0.835 FINMI2 Integrin beta GN=TICH35 0.00 0.826 FINMI2 Proteologene for Song Lattes GN=MDM2 0.00 0.825 FINMI2 Entegrin beta GN=DI/M 0.00 0.00 0.826 <td>0.00 0.840 65.55 0.839 98.96 0.837 942.87 0.835 0.00 0.835 0.00 0.835 0.835 0.835 0.835 0.833 0.834 0.833 0.833 0.833 0.833</td> <td>127.4 39.9 84.3 339.4 119.5 52.1 71.6</td> <td>5.58</td>	0.00 0.840 65.55 0.839 98.96 0.837 942.87 0.835 0.00 0.835 0.00 0.835 0.835 0.835 0.835 0.833 0.834 0.833 0.833 0.833 0.833	127.4 39.9 84.3 339.4 119.5 52.1 71.6	5.58
RealReal 6.55 0.839 ReactionFIP2F0Find final GN=COL6.3 6.55 0.837 FIP2F0FIP2F0Find final GN=COL6.3 19.836 0.337 FIP2F0FIP2F0FIRAR, RhoGFF and pleckstrin domain-containing protein I GN=FILPL 19.836 0.334 FINSG4Diphthanide biosynthesis protein 2 GN=DPH2 0.00 0.334 FINSG4Diphthanide biosynthesis protein 2 GN=DPH2 0.00 0.334 FINSG4Diphthanide biosynthesis protein 2 GN=DPH2 0.00 0.334 FINSG4Diphthanide biosynthesis of N=ACTN4 3.55673 0.000 0.334 FINSC5T2 Lba type IV Collagenase GN=PPF3CA 0.000 0.333 Pistoriar phosphoates GN=MNP2 0.000 0.332 0.032 FINNE2FINNE2Serine/thronine protein phosphatase GN=PPF3CA 0.000 0.325 FINNE2Serine/thronine protein phosphatase GN=PPF3CA 0.000 0.321 Pistoriar GN=UUMAcctoacety-CoA synthesise GN=AACS 0.000 0.321 FINNE2Serine/thronine protein phosphatase GN=FPF3CA 0.000 0.321 Pistoriar GN=UUMAcctoacety-CoA synthesise GN=AACS 0.000 0.321 Pistoriar GN=UUMGSTLC0Acctoacety-CoA synthesise GN=FNCAS 0.000 0.000 Pistoriar GN=UUMGSTLC0Acctoacety-CoA synthesise GN=FNCAS 0.000 0.000 Pistoriar GN=UUMGN=NUL4 0.000 0.000 0.000 Pistoriar GN=NUCAPistoriar GN=CUCAAA 0.000 0.000	65.55 0.839 98.96 0.837 94.2.87 0.835 0.00 0.835 0.00 0.835 0.834 0.00 0.834 0.834 0.833 20.32 0.833	39.9 84.3 339.4 119.5 71.6 33.0	8.54
FINMF6Procollagen-lysine,2-oxoglutarate 5-dioxygenase I GN=PLDD1198.960.837F127P0Collagera byha-3(1) chain GN=COL6A3642.870.836F14GFM0FERM, RhoGFF and plecktrint domain-containing protein I GN=FARP10.000.834H910H3Alpha-actinin - (Fragment) GN=ACTN40.000.834F18GADiphthamide biosynthesis protein 2 GN=DP120.000.8345673Phosphogycolate phosphatase GN=PGP0.000.8335673Phiniary homeoby colate phosphatase GN=PGP0.000.8335673Phiniary homeoby colate phosphatase GN=PGP0.000.8335673Phiniary homeoby colate phosphatase GN=PGP0.000.8335673Phiniary homeoby colate phosphatase GN=PGP0.000.8339061172 kDa type IV collagenase GN=PGP0.000.83372 kDa type IV collagenase GN=PGP0.000.825FINNEZSerine/Hurconine-protein phosphatase GN=PPGA0.000.825515800Innician GN=LUM0.000.82472 kDa type IV collagenase GN=PACS1.000.824711D4Collagen apha-1(VI) chain GN=COL6A11.01550.007251G0Acetoacety-1CoA synthetase GN=ACS1.89470.007251G0NPILA7 GN=PNDLA70.000.8247251G0NPILA7 GN=PNDLA70.000.8247251G0NPILA7 GN=PNDLA70.000.8247251G0NPILA7 GN=PNDLA70.000.8247251G0NPILA7 GN=PNDLA70.000.82472	98.96 0.837 942.87 0.835 0.00 0.835 0.00 0.834 0.00 0.834 0.834 0.833 39.98 0.833 20.32 0.833	84.3 339.4 119.5 52.1 71.6 33.0	5.47
FIP2F0Collagen alpha-3(VI) chain GN=COL6A3642.870.836F4GFM0FERM, RobCFF and Deckerin domain-containing protein I GN=FARP10.000.835F1NG4Diphthanide biosynthesis protein 2 GN=DPH20.000.834F1NG4Diphthanide biosynthesis protein 2 GN=DPH20.000.834F1NG4Diphthanide biosynthesis protein 2 GN=DFP120.000.834O5F4B1Diphthanide biosynthesis protein 2 GN=DFP20.000.834TS6673Diphthanide biosynthase GN=PCP0.000.833TS1D2Alpha-actinin-4 (Fragment) GN=ACTNA0.000.833TS1D3T2 kDa type IV collagentase GN=PPD3CA20.320.825FINME2Strine/Intreonine-protein phosphatase GN=PPD3CA0.000.825FINB27Strine/Intreonine-protein phosphatase GN=PPD3CA44.330.826TS1D3TAB type IV collagentase GN=AMCS0.000.827FINB27Strine/Intreonine-protein phosphatase GN=PPD3CA44.330.826TS1D3TAB type IV collagentase GN=AMCS0.000.827TS1D4Inhibitor of nuclear factor kappa B kinase subunit alpha GN=CHUK0.000.828TND4Inhibitor of nuclear factor kappa B kinase subunit alpha GN=CHUK10.000.817TS1C6Acttaacetyl-CoA synthetase GN=CNEA34.960.800TS1D4Stathmin-3 GN=NPC2189.470.803TS1D4Stathmin-3 GN=NPC2189.470.9000.812TND4Inhibitor of nuclear factor kappa - Riversa34.960.800TND	42.87 0.836 0.00 0.835 0.00 0.834 515.20 0.833 39.98 0.833 20.32 0.833	339.4 119.5 52.1 71.6	6.74
R4GFM0FERM, RhoGEF and pleckstrin domain-containing protein I GN=EARP10.000.835FN8G4Diphthamide biosynthesis protein CATN4515.200.833FN8G4Diphthamide biosynthesis protein CATN4515.200.83365673Phosphogycolare phosphatase GN=CPP515.200.833756673Phosphogycolare phosphatase GN=PCP515.200.833756673Phosphogycolare phosphatase GN=PCP515.200.833756673Phosphogycolare phosphatase GN=PCP515.900.833756673Phote GN=TCR32057.3320.320.823756673Protein Phosphotase GN=PAPP3CA20.320.825726Da type TV collagenase GN=MPP2510.40.8250.8257126Da type TV collagenase GN=PPP3CA267.530.8250.825751890Lumican GN=LUM0.000.8210.825837TZCIPNPLA7 GN=PNPLA70.000.8210.825837TZCIPNPLA7 GN=PNPLA70.000.000.821837TZCIPNPLA7 GN=PNPLA70.000.000.821837TZCIPNPLA7 GN=PNPLA70.000.000.821837TZCIPNPLA7 GN=PNPLA70.000.000.821837TZCIPNPLA7 GN=PNPLA70.000.000.821837TZCIPNPLA7 GN=PNPLA70.000.000.821837TZCIPNPLA7 GN=PNPLA70.000.000.821837TCIPNPLA7 GN=PNPLA70.000.000.821837TCIPNPLA7 GN=PUPC20.000.9	0.00 0.835 0.00 0.834 515.20 0.834 39.98 0.833 20.32 0.833	119.5 52.1 71.6	6.68
FINSG4Diphthamide biosynthesis protein 2 GN=DPH2 0.00 0.034 H910H3Alpha-actinn 4 (Fragment) GN=ACTN4 515.20 0.00 0.833 D5F4B1Phosphogycolate phosphatase GN=PGP 90611 721 kDa type IV collagenase GN=PGP 0.00 0.833 P56673Pituitary homeobox I GN=PTX1 39.98 0.833 0.825 P5673Pituitary homeobox I GN=PTX1 30.98 0.833 P5673Pituitary homeobox I GN=PTX1 30.98 0.825 P1NBZ7Strink threonine-protein phosphatase GN=PACA 0.00 0.825 P1NBZ7Strink threonine-protein phosphatase GN=AACS 0.00 0.00 0.825 P1NBZ7Strink threonine-protein for Strink threonine-protein fragment) GN=CHUK 0.00 0.825 P1NFE0Collagen apha-I(VI) chain GN=COL6AI 10.00 0.817 P1NFE0Collagen apha-I(VI) chain GN=COL6AI 10.00 0.805 P1NFE0Collagen apha-I(VI) chain GN=COL6AI 10.00 0.00 0.00 P1NFE0Collagen apha-I(VI) chain GN=COL6AI 10.00 0.00 0.805 P1NFE0Collagen apha-I(VI) chain GN=COL6AI 10.00 0.00 0.00 P1NFE0Collagen apha-I(VI) 0.00 0.00 0.907	0.00 0.834 515.20 0.834 39.98 0.833 20.32 0.833	52.1 71.6 33.0	8.15
H910H3 Alpha-actinin-4 (Fragment) GN=ACTN4 515.20 0.834 95F4B1 Phosphoglycolate phosphatase GN=PGP 0.833 39.98 0.833 356733 Pituitary homeox I GN=PTX1 20.32 0.833 39.98 0.833 306611 72 kbp a type IV collagenase GN=PGP 26753 0.825 0.825 FINME2 Finitary homeox I GN=PTR1 26753 0.825 0.825 FINBZ7 Integrin beta GN=ITGB 51.04 0.825 0.825 FINBZ7 Serine/threonine-protein phosphatase GN=PPF3CA 26753 0.825 0.825 FINBZ7 Serine/threonine-protein phosphatase GN=PPF3CA 44.33 0.825 0.825 FINBZ7 Pinpitor of nuclear factor kappa-B kinase subunit alpha GN=CHUK 0.00 0.817 0.00 0.817 OSZLG0 Acetoacety/CoA synthetase GN=ACS Inhibitor of nuclear factor kappa-B kinase subunit alpha GN=CHUK 0.00 0.817 OSZLG0 Acetoacety/CoA synthetase GN=ACS Inhibitor of nuclear factor kappa-B kinase subunit alpha GN=CHUK 0.00 0.818 FINDA Shinbitor of nuclear factor kappa-B	515.20 0.834 39.98 0.833 20.32 0.833	71.6 33.0	5.54
Q5F4B1Phosphoglycolate phosphatase GN=PGP0.833P56673Pituitary homeokox I GN=PITXI39.980.833P56673Pituitary homeokox I GN=TITXI20.320.833P56673Pituitary homeokox I GN=TITXI20.320.823P10061172 kDa type IV collagenase GN=MPP2267.530.823P1001272 kDa type IV collagenase GN=TPP3CA267.530.825P1127Lumican GN=LUM0.000.827P51890NPILA7 GN=PNPLA70.000.821P51800NPLA7 GN=PNPLA70.000.821P11124Inhibitor of nuclear factor kappa B kinase subunit alpha GN=CHUK0.000.821PNN124Inhibitor of nuclear factor kappa B kinase subunit alpha GN=CHUK0.000.812PNN125Stathmin-3 GN=NPC20.000.8120.000.812PNN125Stathmin-3 GN=NPC2110.520.000.812PNN125Stathmin-3 GN=NPC234.960.8030.000.812PNN125Stathmin-3 GN=NPC20.000.8120.000.812PNN25Stathmin-3 GN=NPC20.000.000.8120.00PNN25Stathmin-3 GN=NPC20.000.8120.000.812PNN3Stathmin-3 GN=NPC20.000.000.812PNN3Stathmin-3 GN=NPC20.000.000.802PNN3Stathmin-3 GN=NPC20.000.000.802PNN3Stathmin-3 GN=NPC20.000.000.802PNN3Stathmin-3 GN=NPC2 <td< td=""><td>39.98 0.833 20.32 0.833</td><td>33.0</td><td>6.09</td></td<>	39.98 0.833 20.32 0.833	33.0	6.09
P56673 Pituitary homeobox I GN=PITX1 20.32 0.833 72 kDa type IV collagenase GN=MMP2 26.753 0.829 RNME2 Integrin beta GN=ITGB5 51.04 0.829 FINBZ7 72 kDa type IV collagenase GN=MMP2 51.04 0.829 FINBZ7 Serine/threonine-protein phosphatase GN=PP3CA 0.00 0.825 FINBZ7 Serine/threonine-protein phosphatase GN=PP3CA 0.00 0.826 FSIXC1 NPLA7 GN=ENPLA7 0.00 0.827 B3TZC1 PNPLA7 GN=ENPLA7 0.00 0.826 G5ZLG0 Acetoacetyl-CoA synthetase GN=AACS 0.00 0.817 G5ZLG0 PNPLA7 GN=ENPLA7 0.00 0.816 G5ZLG0 Acetoacetyl-CoA synthetase GN=AACS 0.00 0.817 FINLD4 Inhibitor of nuclear factor kapa-B kinase subunit alpha GN=CHUK 0.00 0.817 G5ZLG0 Acetoacetyl-CoA synthetase GN=CALAS 0.00 0.817 FIND124 Inhibitor of nuclear factor kapa-B kinase subunit alpha GN=CHUK 0.00 0.812 G5ZLG0 Acetoacetyl-CoA Synthetase GN=COL6A1	20.32 0.833	0.00	5.73
Q9061 72 kDa type IV collagenase GN=MMP2 26753 0.829 FINME2 Integrin beta GN=ITGB5 51.04 0.825 FINME2 Integrin beta GN=ITGB5 0.00 0.825 FINME2 Serine(threonine-protein phosphatase GN=PP3CA 0.00 0.825 BSI7ZCI Serine(threonine-protein phosphatase GN=PP3CA 0.00 0.825 BSITZCI Serine(threonine-protein phosphatase GN=AACS 0.00 0.816 BSITZCI Acetoacetyl-CoA synthetase GN=AACS 0.00 0.817 FINID4 Inhibitor of nuclear factor kappa-B kinase subunit alpha GN=CHUK 0.00 0.817 FINID4 Inhibitor of nuclear factor kappa-B kinase subunit alpha GN=CHUK 0.00 0.817 FINID4 Inhibitor of nuclear factor kappa-B kinase subunit alpha GN=CHUK 0.00 0.817 FINID4 Inhibitor of nuclear factor kappa-B kinase subunit alpha GN=CHK 0.00 0.817 FINID4 Inhibitor of nuclear factor kappa-B kinase subunit alpha GN=CHK 0.00 0.817 FINID3 Stathmin-3 GN=NC2 Stathmin-3 GN=COL6A1 0.00 0.817 FINPX5 <t< td=""><td></td><td>34.5</td><td>9.11</td></t<>		34.5	9.11
FINME2Integrin beta GN=ITGB551.040.828FINME2Integrin beta GN=ITGB50.000.827FINBZ7Serine/threonine-protein phosphatase GN=PP3CA0.000.827P51890Lumican GN=LUM0.000.821B3TZC1PNPLA7 GN=PNPLA70.000.821B3TZC1PNPLA7 GN=PNPLA70.000.821B3TZC1Acetoacety1-CoA synthetase GN=AACS0.000.821FINLD4Inhibitor of nuclear factor kappa-B kinase subunit alpha GN=CHUK0.000.817FINSE0Collagen alpha-1(VI) chain GN=COL6A1110.520.812FINSP3SH3 domain-Jionding glutamic acid-rich-like protein (Fragment) GN=SH3BGRL110.520.802P10038Cystatin0.000.783GU35-2Isoform 2 of Zinc finger protein 622 GN=ZNF62234.230.769Q0035-2Isoform 2 of Zinc finger protein 622 GN=ZNF62237.750.761Q0035-2Biogenesis of lysosome-related organelles complex 1 subunit 5 GN=BLOCIS534.230.759FINMZ3FIP0D2FIP0D2FIP0D2137.3060.753FIP0D2FIP0D2FIP0D2FIP0D2137.3060.758FIP0D2FIP0D2FIP0D2FIP0D2747.590.761FIP0D2FIP0D2FIP0D2FIP0D2747.590.759FIP0D2FIP0D2FIP0D2FIP0D2747.590.759FIP0D2FIP0D2FIP0D2FIP0D2747.590.759FIP0D2FIP0D2FIP0D2FIP0D2747.59	267.53 0.829	74.9	5.49
FINBZ7Serine/threonine-protein phosphatase GN=PPP3CA0.000.827P51890Lumican GN=LUM0.000.821P51890NPLA7 GN=PNPLA70.000.821B3TZC1NPLA7 GN=PNPLA70.000.821B3TZC2Acetoacetyl-CoA synthetase GN=AACS189.470.000.817FINLD4Inhibitor of nuclear factor kappa B kinase subunit alpha GN=CHUK110.520.000.817FINLD4Inhibitor of nuclear factor kappa B kinase subunit alpha GN=CHUK110.520.000.812FIND34Stathmin-3 GN=NPC234.960.8000.812FINPX5SH3 domain-binding glutamic acid-rich-like protein (Fragment) GN=SH3BGRL110.520.802P01038Cystatin34.960.000.786Q90735-2Isoform 2 of Zinc finger protein 622 GN=ZNF62234.050.000.786Q90735-2Isoform 2 of Zinc finger protein 622 GN=ZNF62234.230.7610.766Q90735-2Biogenesis of lysosome-related organelles complex 1 subunit 5 GN=BLOCIS534.230.7660.758FIND2FIPDD2FIPDD2FIPDD2FIPDD21373.060.758FIPDD2FIPDD2FIPDD2FIPDD2FIPDD21373.060.758FINT4FIPDD2FIPDD2FIPDD21373.060.758FIPDD2FIPDD2FIPDD2FIPDD21373.060.758FIPDD2FIPDD2FIPDD2FIPDD21373.060.758FINT4FIPDD2FIPDD2FIPDD21373.060.758 </td <td>51.04 0.828</td> <td>88.4</td> <td>6.71</td>	51.04 0.828	88.4	6.71
P51890Lumican GN=LUM44.330.826B3TZCIPNPLA7 GN=PNPLA70.000.821B3TZCIPNPLA7 GN=PNPLA70.000.821B3TZCIPNPLA7 GN=PNPLA70.000.821G5ZLG0Acetoacetyl-CoA synthetase GN=AACS189.470.815FINLD4Inhibitor of nuclear factor kappa-B kinase subunit alpha GN=CHUK110.520.000.817FINLD4Inhibitor of nuclear factor kappa-B kinase subunit alpha GN=CHUK0.000.8170.00FINE0Collagen alpha-1(V1) chain GN=COL6A134.960.8080.808FINPX5SH3 domain-binding glutamic acid-rich-like protein (Fragment) GN=SH3BGRL110.520.802P01038Cystatin0.0034.960.799Q8QG94Suppressor of fused GN=SUFU39.090.7990.766Q90Y35-2Isoform 2 of Zinc finger protein 622 GN=ZNF62237.750.000.785PINMZ3Hemoglobin subunit epsilon GN=HBE900337.750.000.783Q90Y35-2Biogenesis of lysosome-related organelles complex 1 subunit 5 GN=BLOCIS534.230.7690.758FIND2Biogenesis of lysosome-related organelles complex 1 subunit 5 GN=BLOCIS534.230.7590.759FIPD2Biogenesis for lowes for end for	0.00 0.827	60.6	5.83
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Q155F6 Tumor necrosis factor-inducible protein 6 GN=TNFIP6 44.59 0.747	373.06 0.752	259.0	6.11
	44.59 0.747	30.7	6.02
FIN/13 FIDFORECTIN GN=FINI U./2/	373.06 0.727	273.1	5.64
093532 Keratin, type II cytoskeletal cochlear 95.26 0.693	95.26 0.693	53.8	6.10



FIGURE 1: Identification of DF-1 cells infected with ALV-J by IFA. (a) DF1 cells infected with ALV-B. (b) Normal uninfected DF1 cells.

been widely applied in many studies [18–20]. In this study, we first applied the iTRAQ approach to identify the differential protein expression profiles of DF-1 cells infected with ALV-J. Using the iTRAQ LC-MS/MS technology, the significantly changed proteins were mostly associated with metabolic process, signal transducer activity, cell cytoskeleton, oxidoreductase activity, response to stimulus, and immune responses. In addition, some apoptosis and tumor-associated proteins (VEGF-A, ACTN4, and METAP2) were also identified by the iTRAQ LC-MS/MS technology.

4.1. Alterations of Tumor-Associated Proteins. Vascular endothelial growth factor A (VEGF-A) is an important inducer of angiogenesis [21]. As has been shown in many reports, upregulated VEGF-A can induce tumor formation via some unique signaling pathways [22, 23]. In addition, VEGF-A, which is known as a positive regulator, contributes to tumor growth and promotes tumor formation [24, 25]. Previous studies described a threshold level of proteins to promote tumorigenesis, which indicated that the expression level of one protein needs to reach the threshold level before promoting tumorigenesis [26, 27]. Studies in our lab showed that the increased replication of ALV-J increased the expression of VEGF-A, indicating an increased opportunity for ALV-J to push the expression level of VEGF-A to reach the threshold level to promote tumorigenesis [27]. In this study, we found that VEGF-A is overexpressed in DF-1 cells after infection with ALV-J. The results further suggested that VEGF-A is closely associated with ALV-J-induced tumorigenesis and may also suggest a novel molecular mechanism for better understanding of the higher oncogenicity of ALV-J.

Alpha-actinins (ACTNs) were classified into cytoskeleton proteins, while ACTN4 has some other unique functions, such as signal transduction, protein expression regulation, and nuclear transport. Histological analyses of cancer tissues showed a strong correlation between ACTN4 expression and tumorigenesis in several types of cancers [28–30]. Furthermore, upregulated ACTN4 in cancer cells has been suggested as a biomarker for drug resistance and malignant cell invasion [31–34]. Previous studies showed that ALV-J infection in DF-1 cells led to rapid increase in Akt phosphorylation and the phosphorylation of Akt was PI3K-dependent [35]. PI3K/Akt pathway also regulates viral replication of ALV-J [35]. Furthermore, AKT interacts with ACTN4 and ACTN4 is a functional partner of AKT [36]. Therefore, the upregulated ACTN4 observed in this study may be associated with tumorigenesis induced by ALV-J through PI3K/Akt pathway. This may provide useful information to elucidate the mechanism of ALV-J induced tumorigenesis and may also become potential therapeutic targets to control ALV-J infections.

METAP2 was considered to have some relationship with angiogenesis inhibition [37]. In addition, METAP2 can block B cell differentiation into plasma cells [38]. Some viruses, whose primary target cells are B cells, can clinically induce tumor formation. Therefore, downregulation of METAP2 in this study may influence the function of B cells, which may provide evidence to explain why ALV-J infection can result in immune suppression and tumorigenesis.

4.2. Redox Regulation. Peroxiredoxins (PRDXs), a family of peroxidases as antioxidant enzymes, can support tumor maintenance and survival through protecting cells from apoptosis induced by oxidative stress [39–41]. A previous study indicated that liver cells transfected with PRDX6 siRNA resulted in an increase in peroxide-induced cytotoxicity by apoptosis, which implies that decrease of PRDX6 promotes apoptosis [42]. Therefore, downregulated PRDX6 in this study suggests that ALV-J infection may weaken the anti-apoptotic function of PRDX6.

In addition, PRDX1 was found to be upregulated in this study. A previous study indicated that the mice lacking



FIGURE 2: Functional annotation of the differently expressed proteins according to their biological process, molecular function, and cellular component.



FIGURE 3: Transcriptional profiles of the significantly changed proteins in ALV-J-infected DF-1 cells. The error bars represent the standard deviations.

PRDX1 have several malignant cancers, including sarcomas, carcinomas, and lymphomas [43]. These malignancies are associated with low expression of PRDX1, which suggests that PRDX1 may function as a tumor suppressor [43]. Studies also indicated that PRDX1 interacts with the c-Myc oncogene and can inhibit its transcriptional activity [44] and high expression of PRDX1 appears to be associated with less aggressive breast cancers [45]. Therefore, upregulation of PRDX1 in this study may result from the defense of host cells responses to the ALV-J infection.



FIGURE 4: The protein-protein interaction between the identified proteins and the tumor- or apoptosis-associated proteins analyzed by the STRING software. An edge was drawn with up to seven differently colored lines, representing the existence of the seven types of evidence used for predicting the associations: a red line indicates the presence of fusion evidence; a yellow line indicates text mining evidence; a purple line indicates experimental evidence; a blue line indicates cooccurrence evidence; a light blue line indicates database evidence; a green line indicates neighborhood evidence; a black line indicates coexpression evidence.

4.3. Cytoskeleton Proteins and ALV-J Infection. Cytoskeleton proteins are involved in the maintenance of cell morphology, regulation of protein synthesis, endocytosis, cell movement, and cell-to-cell attachment [46, 47]. As determined through iTRAQ LC-MS/MS analysis, some cytoskeleton proteins were identified to be significantly changed in DF-1 cells after infection with ALV-J. Isoform 2 of the F-actin-capping protein subunit beta isoforms 1 and 2 (CAPZB) can regulate the growth of actin filaments, and actin filaments play a vital role in the maintenance of cell morphology [48]. Furthermore, actin-related protein 3 (ACTR3) and actin-related protein 5 (ACTR5) were also found to be changed. The low expression of these proteins revealed that the cytoskeletal proteins were disrupted during infection with ALV-J. In addition, the differential expression of these proteins may be due to

the interaction between the virus and host cellular proteins after infection with ALV-J.

It has been reported that keratins have become the standard detection marker for tumor cells and were also the most common marker to identify tumor cells [49]. Previous studies showed that before tumor cells got the ability to migrate and invade the host, they need to undergo epithelial-mesenchymal transition, during which the cytoskeletons are rearranged and epithelial markers, such as keratins, claudins, and E-cadherin, are observed to be downregulated [49–52]. Immunohistochemical analysis showed that low expression of keratin was associated with a higher tumor grade in breast cancer [52]. Previous study indicated that acetoacetyl-CoA synthetase (AACS) was found in tumor tissues and plays important roles in metabolic processes of tumors [53].

Whether or not the downregulated keratin and AACS in this study were associated with tumorigenesis induced by ALV-J infection needs to be further investigated.

5. Conclusions

In summary, our study was the first to use iTRAQ LC-MS/MS to detect cellular responses to ALV-J infection in DF-1 cells. A total of 75 significantly changed proteins were identified. These differently expressed proteins may provide useful information for elucidating the molecular mechanism underlying the interaction between ALV-J and DF-1 cells and will also facilitate our understanding of the pathogenesis of ALV-J infection.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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