miRNA-155在ALV-J与REV共感染中对肌动蛋白细胞骨架通路的影响

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摘要 该研究探讨了miRNA-155的表达在J亚群禽白血病病毒(subgroup J avian leucosis virus, ALV-J)和网状内皮组织增生症病毒(reticuloendotheliosis virus, REV)共感染中对肌动蛋白细胞骨架通路的影响。通过蛋白质组学和转录组学检测技术对ALV-J、REV和ALV-J+REV感染DF-1细胞分泌的外泌体进行蛋白质和miRNA定量差异表达综合分析, 筛选共感染组与单独感染组相比共同变化的因子及其参与的信号通路。结果显示, ALV-J和REV的共感染对彼此具有协同效应, REV对ALV-J的协同起主导作用; GTP结合蛋白J(GTP-binding protein J, RhoJ)、NCK相关蛋白1(NCK-association protein 1, NCKAP1)、肌动蛋白相关2/3复合体亚基5(actin-related 2/3 complex subunit 5, ARPC5)和miRNA-155的靶蛋白整合蛋白(integrin, ITG)共同参与肌动蛋白细胞骨架通路。体外转染促进或抑制miRNA-155表达, 采用RT-PCR检测其对病毒复制以及ITG、RhoJ、NCKAP1和ARPC5表达的影响。结果显示, 促进miRNA-155的表达时, 有利于病毒的复制, RhoJ和NCKAP1的表达上升, 而ITG和ARPC5表达下降; 抑制miRNA-155的表达时, 则抑制病毒复制, RhoJ和NCKAP1的表达下降, ITG和ARPC5表达上升; 且共感染组中变化更为显著, 这与前期病毒感染引起的相关蛋白变化结果相吻合。该研究结果表明, ALV-J与REV共感染时的协同促进作用可能与miRNA-155调节肌动蛋白细胞骨架通路中4种蛋白的表达变化密切相关, miRNA-155是两种病毒协同作用的关键调节因子。

关键词 miRNA-155; ALV-J; REV; 共感染; 肌动蛋白细胞骨架通路

Effects of miRNA-155 on the Cytoskeletal Pathways in REV and ALV-J Co-Infection

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Abstract This study was aimed to investigate the effects of miRNA-155 expression on actin cytoskeletal pathways in ALV-J and REV co-infection. The exosomes from ALV-J, REV and ALV-J+REV infected DF-1 cells were used to quantity the differentially expressed proteins and miRNAs by proteomic and transcriptome detection technology. Then, the common changed factor and their participation in the signal pathway in co-infected group

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compared with single infected group were screened. The results showed that ALV-J and REV had synergistic effects on each other, and REV played a leading role in synergism on ALV-J. RhoJ, NACKP1 and ARPC5, and the miRNA-155 target protein ITG were involved in the actin cytoskeleton pathway. After transfection of miRNA-155 inhibitors or mimics, RT-PCR was used to detect the effects of miRNA-155 expression on the virus replication, and the expression of ITG, RhoJ, NCKAP1 and ARPC5 as well. The results demonstrated that the virus replication and the expression of RhoJ and NCKAP1 were promoted, whereas, ITG and ARPC5 expression was decreased, when the miRNA-155 expression was increased. Inhibition of miRNA-155 expression inhibited virus replication, blocked the expression of RhoJ and NCKAP1, and increased the expression of ITG and ARPC5. These changes were more significant in the co-infected group, which was consistent with the previous results that the changes of associated proteins induced by virus infection. Our results suggested that the synergistic effect of ALV-J and REV co-infection might be associated with the miRNA-155 regulation the 4 proteins in actin cytoskeleton pathway. The miRNA-155 is a key factor regulating the synergistic effects of the two viruses.

Keywords miRNA-155; ALV-J; REV; co-infection; actin cytoskeleton signal pathway

J亚群禽白血病病毒(subgroup J avian leucosis virus, ALV-J)和网状内皮组织增生症病毒(reticuloendotheliosis virus, REV)均属于反转录病毒, 可以引起鸡群严重的免疫抑制和肿瘤形成[1-2]。近年来, ALV-J和REV的共感染在鸡群中的发生率越来越高, 且引起更严重的生长迟缓和免疫抑制、肿瘤谱的扩增[3-4]。但二者共感染引起协同致病作用的机制未明。我们在前期的实验研究中对ALV-J、REV和ALV-J+REV感染DF-1细胞分泌的外泌体进行蛋白组学和转录组学分析, 证实病毒感染细胞后分泌的外泌体与病毒感染机制密切相关[5-6]。本研究对前期获得的蛋白组学和转录组学数据进行差异比较、综合分析后, 筛选了与病毒共感染密切相关的miRNAs、蛋白质及其参与的共同信号通路; 鉴定出关键因子miRNA-155和其他4种宿主蛋白质及其共同参与的肌动蛋白细胞骨架通路; 并通过调节miRNA-155的表达, 检测其对病毒复制及参与信号通路的其他4种蛋白质表达的影响, 为进一步阐明ALV-J和REV的共感染机制提供了实验依据。

1 材料与方法

1.1 细胞与主要试剂

鸡胚成纤维细胞系DF-1购自中国科学院上海细胞库。DMEM、胎牛血清购自Gibco公司。胰蛋白酶(trypsin)购自Amresco公司。外泌体和RNA提取试剂盒、Lipofectamine 3000转染试剂盒购自Invitrogen公司。安捷伦2100 RNA Nano 6000 Assay试剂盒购自Agilent Technologies公司。其他试剂均为国产分析纯。

1.2 细胞培养及病毒感染

鸡胚成纤维细胞细胞系DF-1以6×10^5/mL的密度接种于含有100 U/mL青霉素、100 μg/mL链霉素和10%胎牛血清的DMEM完全培养基的25 cm^{2}培养瓶, 于37 ℃的5% CO_2培养箱中常规培养。细胞完全贴壁后分别接种感染复数(multiplicity of infection, MOI)值为0.1的ALV-J、REV和ALV-J+REV, 用含1%胎牛血清的DMEM培养基维持2 h, 弃上清, 更换为5%血清的完全培养基, 5天后, 收集细胞上清。以未接种病毒的DF-1细胞作为对照组。

1.3 外泌体的提取和蛋白质组学、转录组学分析

从收集的细胞上清中提取外泌体, 并对提取的外泌体进行蛋白质组和转录组的检测、分析。蛋白质组学分析和转录组学分析的步骤按照参考文献[5]完成。

1.4 miRNA-155 Minics和Inhibitor的转染

DF-1细胞接种于6孔板中常规培养至密度达到50%左右后弃上清。按照Lipofectamine 3000转染试剂盒的操作方法, 分别将20 μmol/μL的miRNA-155 Minics 6 μL或Inhibitor 5 μL加入到无血清无双抗的DMED培养基中配制转染混合液, 将混合液加入到6孔板中, 培养6 h。之后接种MOI值为0.1的ALV-J、REV和ALV-J+REV, 用含1%胎牛血清的DMEM培养基培养。以未接种病毒且直接更换维持培养基的细胞作为对照组。

1.5 实时荧光定量PCR检测

收集1.4中处理的DF-1细胞, 提取细胞总RNA, 反

表1 实时荧光定量PCR的引物

Table 1 Primers used for Real-time fluorescence quantitative PCR

目的基因 Gene target	引物序列(5′→3′) Primer sequence (5′→3′)	片段大小 (bp) Fragment size (bp)
REV (env)	Forward: TTG TTG AAG GCA AGC ATC AG Reverse: GAG GAT AGC ATC TGC CCT TT	330
ALV-J (env)	Forward: TGC GTG CGT GGT TAT TAT TTC Reverse: AAT GGT GAG GTC GCT GAC TGT	144
ITG	Forward: TAA GTT CAT AGC GAG CGA CC Reverse: TCA GCA CAG CCC CAT TCC	125
RhoJ	Forward: GAC GCC TCT AAA CTA CAT CCT Reverse: GGC TTA CAG ACC ACC ACA T	189
NCKAP1	Forward: TTG TCT TTT CGG TCG TTG Reverse: TGC CAC CTT CAT GTC AGT	126
ARPC5	Forward: TGG ACG AGT ACG ACG AGA Reverse: TGA GGA CCT TCA GGA CAA T	254
GADPH	Forward: GAA CAT CAT CCC AGC GTC CA Reverse: CGG CAG GTC AGG TCA ACA AC	132

转录成cDNA, 以甘油醛-3-磷酸脱氢酶(glyceraldehyde 3-phosphate dehydrogenase, *GAPDH*)作为内参, 进行实时荧光定量PCR, 引物序列如表1所示。以$2^{-\Delta\Delta Ct}$值表示检测基因的相对表达量, 实验重复三次, 每组取平均值分析基因相对表达水平的差异。

1.6 统计学分析

每组实验重复三次, 数据采用“平均值±标准差”表示。采用SPSS 20.0软件进行方差分析, $P<0.05$表示差异具有统计学意义。

2 结果

2.1 蛋白定量差异表达分析

符合表达差异倍数大于1.2倍(上/下调)且$P<0.05$筛选标准的蛋白质视为表达差异蛋白质。结果显示, 与ALV-J感染组相比, 共感染组表达差异的蛋白质有134个, 其中表达上调的有74个, 表达下调的有60个(表2); 与REV感染组相比, 共感染组表达差异的蛋白质共92个, 其中上调的有50个, 下调的有42个(表3); 与两个单独感染组相比, 共感染组均存在差异的蛋白质有30个, 其中表达上调的16个, 下调的14个(表2和表3中加粗标记代表感染组与两个单独感染组表达均有变化的蛋白)。由表2可以看出, 共感染组中REV的重要成分表达最高上调2倍。表3显示, 共感染组中ALV-J的重要成分最高上调约3倍多。由转录组学分析结果可以看出, 共感染组中miRNA-155的表达水平相比ALV-J组上调约50倍, 而相比REV组上调3倍多。由此可见, 共感染时, REV对ALV-J的协同促进起主导作用。

2.2 表达差异的miRNA定量分析

符合表达差异倍数大于1.2倍(上/下调)且$P<0.05$筛选标准的miRNA视为表达差异miRNA。与ALV-J感染组相比, 共感染组表达差异的miRNA有54个, 其中表达上调的有23个, 下调的有31个(表4); 与REV感染组相比, 共感染组表达差异的miRNA共16个, 其中表达上调的有7个, 下调的有9个(表5); 与两个单独感染组相比, 共感染组均存在差异的miRNA有6个, 其中表达均上调的5个, 还有1个比较特殊(miRNA-3538), 与ALV-J组相比上调, 而与REV组相比下调(表4和表5中加粗标记代表感染组与两个单独感染组表达均有变化的miRNA)。由表4和表5均可见, miRNA-155的表达差异很显著。

2.3 miRNA-155的表达对病毒复制的影响

为了明确miRNA-155的表达对病毒复制的影响, 我们通过调节miRNA-155的表达检测病毒复制量。qRT-PCR的检测结果显示, 当促进miRNA-155的表达时, ALV-J和REV的复制水平均显著上升; 当抑制miRNA-155的表达时, 病毒的复制水平显著下降; 且在miRNA-155不同表达水平下, 共感染组中病毒的复制水平均显著高于单独感染组(图1)。结果表明, miRNA-155对ALV-J和REV的复制具有促进作用, 且两种病毒共感染对彼此复制具有协同促进作用, miRNA-155在两种病毒共感染时对病毒复制的

表2 共感染组与ALV-J感染组差异表达的蛋白质

Table 2 The differentially expressed proteins between co-infection group and ALV-J infection group

序号 No.	登记号 Accession	蛋白质名称 Protein's name	变化(REV+ALV-J/ALV-J) Change (REV+ALV-J/ALV-J)	上调/下调 Up/down
1	F1NJS6	KPNA2	3.944	Up
2	F1NBD8	C3H6ORF103	4.018	Up
3	E1C6L5	SCG2	3.511	Up
4	E1BQ84	CPNE2	3.141	Up
5	F1NZF2	MYCBP2	2.814	Up
6	Q2XP49	DNASE2	3.078	Up
7	E1BYC6	GNPTAB	2.625	Up
8	A8VHZ1	IL-6	2.569	Up
9	F1NVW5	FAM38B	2.501	Up
10	F1NHI4	SOD3	2.501	Up
11	F1NK52	RhoJ	2.257	Up
12	E1BVT1	IFT172	2.486	Up
13	E1C277	POLR1D	2.534	Up
14	E1C9E0	MMP10	2.179	Up
15	E1BYG8	GSPT1	2.348	Up
16	P02701	AVD	2.130	Up
17	R4GH98	CRLF1	2.263	Up
18	Q462Q8	Avian reticuloendotheliosis virus env	2.181	Up
19	C7FGW7	Avian reticuloendotheliosis virus gag	2.118	Up
20	Q7ZZY5	DcR3	2.051	Up
21	F1P2P4	CARKD	2.050	Up
22	F1NN84	VPS37B	1.985	Up
23	Q462Q9	Avian reticuloendotheliosis virus pol	1.945	Up
24	E1C0K1	LCN8	1.939	Up
25	**Q155F6**	**TNFIP6**	**1.896**	**Up**
26	R4GHJ3	SMPD1	1.991	Up
27	**P08317**	**IL-8**	**1.733**	**Up**
28	F1NY89	RHPN1	1.689	Up
29	R4GIZ6	C1H12ORF5	1.659	Up
30	Q90784	Claustrin	1.629	Up
31	F1NGT3	MMP9	1.722	Up
32	F1NVG0	ISLR	1.607	Up
33	E1C332	C9ORF39	1.541	Up
34	E1C8V9	FAM136A	1.659	Up
35	F1NC06	BBS2	1.469	Up
36	F1N9J7	LOC425049	1.729	Up
37	**F1P4I7**	**VNN1**	**1.573**	**Up**
38	**R4GHA7**	**TRIM62**	**1.557**	**Up**
39	R4GHM4	LOC100857484	1.582	Up
40	P67881	CYC	1.486	Up
41	Q5ZJA7	OGDH	1.530	Up
42	**Q5F3X4**	**EFTUD2**	**1.709**	**Up**
43	Q5ZL58	SNRPD3	1.599	Up

续表2

序号 No.	登记号 Accession	蛋白质名称 Protein's name	变化(REV+ALV-J/ALV-J) Change (REV+ALV-J/ALV-J)	上调/下调 Up/down
44	Q9DDD3	calsyntenin-1	1.419	Up
45	E1C958	LGMN	1.491	Up
46	Q90681	Cation-independent mannose-6-phosphate receptor	1.626	Up
47	**E1C6G9**	**NCKAP1**	**1.436**	**Up**
48	Q5ZLA7	LOC100857165	1.444	Up
49	F1NRQ9	TGFB3	1.451	Up
50	F1P326	CAMKK2	1.582	Up
51	R4GIZ2	PTX3	1.395	Up
52	F1NTV6	UGGT1	1.428	Up
53	P0CB50	PRDX1	1.407	Up
54	E1BSA4	PTTG1IP	1.439	Up
55	F1NTJ1	SLC2A3	1.464	Up
56	O42388	Ubiquitin-ribosomal protein fusion protein	1.389	Up
57	P0CG62	UBB	1.450	Up
58	Q6EE33	S-(hydroxymethyl) glutathione dehydrogenase	1.370	Up
59	E1C378	HNMT	1.402	Up
60	P08267	Ferritin heavy chain	1.382	Up
61	F1NQT4	NPC1	1.405	Up
62	F1NXX9	BPI	1.365	Up
63	B5TME2	MIP1alpha	1.387	Up
64	F1NUQ4	ANGPTL4	1.383	Up
65	R4GFH1	SDPR	1.383	Up
66	**Q9DEQ5**	**P37NB**	**1.377**	**Up**
67	Q5F3C6	PPP2R5C	1.371	Up
68	**F1NUF8**	**AMBP**	**1.378**	**Up**
69	P07583	Beta-galactoside-binding lectin	1.358	Up
70	**F1N9E1**	**MASP1**	**1.419**	**Up**
71	F1P331	AKR7A2	1.374	Up
72	A0M8T8	CAV1	1.453	Up
73	**F1P1G6**	**KIAA1199**	**1.593**	**Up**
74	P18660	RPLP1	1.390	Up
75	E1C836	PDGFRL	1.468	Up
76	**P48440**	**DDOST**	**0.664**	**Down**
77	E1C5P6	FBN3	0.646	Down
78	F1NI79	COL5A1	0.661	Down
79	R4GJS6	C3	0.662	Down
80	P02457	COL1A1	0.663	Down
81	F1NA29	PCSK6	0.669	Down
82	F1NW50	ATP6V1H	0.597	Down
83	E1BRC1	CD99	0.647	Down
84	R4GM21	COL5A2	0.670	Down
85	E1BX99	FIBIN	0.664	Down
86	F1P0H9	COL1A2	0.586	Down
87	F1NJ08	VIM	0.668	Down
88	**Q98TF9**	**RPL14**	**0.623**	**Down**

续表2

序号 No.	登记号 Accession	蛋白质名称 Protein's name	变化(REV+ALV-J/ALV-J) Change (REV+ALV-J/ALV-J)	上调/下调 Up/down
89	E1C1G8	FBLN5	0.663	Down
90	E1C8N1	COMP	0.603	Down
91	**F1NU61**	**PCSK5**	**0.643**	**Down**
92	A9DAB9	MDK	0.591	Down
93	**P41125**	**RPL13**	**0.601**	**Down**
94	E1BUB7	OLFM3	0.641	Down
95	E1C4H4	PODN	0.643	Down
96	Q25C36	OLFML3	0.609	Down
97	E1BSV7	YKT6	0.596	Down
98	Q90796	Alpha-1 type XI collagen	0.671	Down
99	F1NGB1	NUCB2	0.551	Down
100	F1NX22	COL12A1	0.629	Down
101	F1NYJ1	CTSL2	0.600	Down
102	R4GME9	Gga.48096	0.619	Down
103	**E1C2U6**	**PRKAR1B**	**0.659**	**Down**
104	P51890	LUM	0.670	Down
105	Q90612	COL3A1	0.569	Down
106	E1BSP8	SEC24D	0.593	Down
107	E1BXG9	PPID	0.583	Down
108	**Q09121**	**EIF5A1**	**0.553**	**Down**
109	E1C840	STON2	0.587	Down
110	F1P4N9	POSTN	0.342	Down
111	**F1NME8**	**ZMYND8**	**0.617**	**Down**
112	F1P2R3	COL4A1	0.606	Down
113	F1NP51	LMNB2	0.553	Down
114	F1NY60	SMOC1	0.558	Down
115	R4GGQ4	TIMM13	0.663	Down
116	P35440	THBS2	0.598	Down
117	P08250	APOA1	0.534	Down
118	F1P2Q3	COL4A2	0.534	Down
119	F1NDF6	COL4A5	0.614	Down
120	**P32760**	**PTN**	**0.642**	**Down**
121	F1NSI3	CYB5B	0.521	Down
122	F1NE59	SVEP1	0.606	Down
123	E1BTM1	ETF1	0.509	Down
124	F1P4K9	COL11A1	0.556	Down
125	E1BRJ2	STC2	0.551	Down
126	O42163	COCH	0.532	Down
127	**E1C4W3**	**PITPNM2**	**0.508**	**Down**
128	F1P0A1	XPNPEP1	0.465	Down
129	Q7ZTS9	Dimethylarginine dimethylaminohydrolase I	0.479	Down
130	**C7G540**	**OCX32**	**0.415**	**Down**
131	E1BW00	WISP2	0.486	Down
132	F1NUA2	ARPC5(Arp2/3)	0.414	Down
133	E1BYD4	NDRG1	0.421	Down
134	R4GKL8	C1QTNF3	0.577	Down

表3 共感染组与REV感染组差异表达的蛋白质

Table 3 The differentially expressed proteins between co-infection group and REV infection group

序号 No.	登记号 Accession	蛋白质名称 Protein's name	变化(REV+ALV-J/REV) Change (REV+ALV-J/REV)	上调/下调 Up/down
1	Q64997	Avian leukosis virus HPRS103 env	3.699	Up
2	F1NBD8	C3H6ORF103	2.557	Up
3	F1NZ81	ACADSB	1.839	Up
4	R4GHA7	TRIM62	2.080	Up
5	F1NVN3	MFI2	1.724	Up
6	R4GH98	CRLF1	1.840	Up
7	F1NUF8	AMBP	1.905	Up
8	P00565	CKM	1.822	Up
9	F1P4I7	VNN1	1.802	Up
10	Q155F6	TNFIP6	1.797	Up
11	F1NY89	RHPN1	1.646	Up
12	E1C1V3	JUP	1.501	Up
13	F1NHI4	SOD3	1.597	Up
14	R4QXY1	Gag and reverse transcriptase polyprotein	1.803	Up
15	Q90681	Cation-independent mannose-6-phosphate receptor	1.642	Up
16	Q64996	Avian leukosis virus HPRS103 gag	1.753	Up
17	P08317	IL-8	1.514	Up
18	Q02020	FGB	1.699	Up
19	F1NK52	RHOJ	1.581	Up
20	O73840	Heparin cofactor II	1.480	Up
21	E1BUM0	PPIE	1.592	Up
22	O93532	Keratin, type II cytoskeletal cochleal	1.477	Up
23	Q5G8Y9	ApoD	1.395	Up
24	P09207	Tubulin beta-6 chain	1.410	Up
25	**E1C6G9**	**NCKAP1**	**1.415**	**Up**
26	F1NHW8	PPARD	1.469	Up
27	E1C7K8	NDUFAF4	1.406	Up
28	**A8VHZ1**	**IL-6**	**1.410**	**Up**
29	F1NV02	APOB	1.432	Up
30	**Q5F3X4**	**EFTUD2**	**1.469**	**Up**
31	F1NGF8	ASTN2	1.363	Up
32	F1N803	C1S	1.420	Up
33	B3VE14	ITIH2	1.375	Up
34	E1C262	AP3D1	1.382	Up
35	F1NNM9	LOC415932	1.385	Up
36	F1NX60	FBLN1	1.364	Up
37	**Q9DEQ5**	**P37NB**	**1.375**	**Up**
38	**P02701**	**AVD**	**1.386**	**Up**
39	**F1N9E1**	**MASP1**	**1.423**	**Up**
40	F1NBJ5	CAPS2	1.358	Up
41	E1C6L4	PROS1	1.386	Up
42	R4GIY3	TOR2A	1.364	Up
43	E1C312	CAPN6	1.385	Up
44	E1C4R5	SCN1A	2.164	Up
45	E1BQC2	TF	1.416	Up

续表3

序号 No.	登记号 Accession	蛋白质名称 Protein's name	变化(REV+ALV-J/REV) Change (REV+ALV-J/REV)	上调/下调 Up/down
46	F1NB93	PEX5	1.369	Up
47	F1NJC7	ALDH1A1	1.375	Up
48	F1P4I3	VAMP3	1.459	Up
49	E1C958	LGMN	1.355	Up
50	**F1P1G6**	**KIAA1199**	**1.413**	**Up**
51	F1NRM8	ERP29	0.696	Down
52	F1NZ86	HSPA9	0.698	Down
53	F1NYE0	NOV	0.682	Down
54	Q2XNL5	TSG-6	0.686	Down
55	P61355	RPL27	0.687	Down
56	**P48440**	**DDOST**	**0.677**	**Down**
57	Q90784	Claustrin	0.688	Down
58	Q9YHD2	URP	0.684	Down
59	**R4GKL8**	**C1QTNF3**	**0.679**	**Down**
60	F1NBW3	SERINC5	0.686	Down
61	P62801	H4-I	0.635	Down
62	**E1BW00**	**WISP2**	**0.672**	**Down**
63	**E1C4W3**	**PITPNM2**	**0.661**	**Down**
64	E1C4N0	RPS10	0.682	Down
65	**F1NUA2**	**ARPC5(Arp2/3)**	**0.668**	**Down**
66	Q6EE32	Calreticulin	0.657	Down
67	Q8UWG7	RPL6	0.611	Down
68	F1NF80	PPCS	0.623	Down
69	R4GGP6	LOC100859114	0.617	Down
70	F1NZC6	PRPSAP2	0.698	Down
71	**F1NU61**	**PCSK5**	**0.697**	**Down**
72	F1P4F4	SSR1	0.618	Down
73	P51417	RPL15	0.604	Down
74	Q03669	ATP2A2	0.617	Down
75	H9KZC8	PA2G4	0.619	Down
76	Q5ZHW8	RPS14	0.615	Down
77	**F1NME8**	**ZMYND8**	**0.656**	**Down**
78	**P41125**	**RPL13**	**0.608**	**Down**
79	E1C8Q3	ALDH18A1	0.636	Down
80	**Q09121**	**EIF5A1**	**0.608**	**Down**
81	**C7G540**	**OCX32**	**0.676**	**Down**
82	**P32760**	**PTN**	**0.572**	**Down**
83	F1P494	TGFBI	0.598	Down
84	P84229	H3-I	0.554	Down
85	F1NUH4	PFN2	0.595	Down
86	Q5ZMF5	RCJMB04	0.465	Down
87	**E1C2U6**	**PRKAR1B**	**0.589**	**Down**
88	P09987	Histone H1	0.527	Down
89	P84172	TUFM	0.589	Down
90	**Q98TF9**	**RPL14**	**0.504**	**Down**
91	P02263	Histone H2A-IV	0.453	Down
92	E1BYD4	NDRG1	0.488	Down

表4 共感染组与ALV-J感染组表达差异的miRNA

Table 4 The differentially expressed miRNAs between co-infection group and ALV-J infection group

序号 No.	miRNA名称 miRNA's name	变化倍数 Fold change	上调/下调 Up/down
1	gga-miR-3529	3.083	Up
2	gga-miR-2131-3p	2.655	Up
3	gga-miR-429-3p	86.038	Up
4	gga-miR-1416-5p	4.240	Up
5	gga-miR-375	29.179	Up
6	gga-miR-223	2.061	Up
7	gga-miR-3538	3.101	Up
8	gga-miR-1684a-3p	2.511	Up
9	gga-miR-221-3p	4.649	Up
10	gga-miR-184-3p	5.937	Up
11	gga-miR-456-3p	2.800	Up
12	gga-miR-30c-2-3p	2.045	Up
13	gga-miR-155	50.834	Up
14	gga-miR-1329-5p	2.412	Up
15	gga-miR-147	3.111	Up
16	gga-miR-222b-3p	11.771	Up
17	gga-miR-146a-5p	41.039	Up
18	gga-miR-30a-3p	3.098	Up
19	gga-miR-92-3p	2.772	Up
20	**gga-miR-146a-3p**	**47.631**	**Up**
21	gga-miR-144-3p	2.946	Up
22	gga-miR-142-5p	2.265	Up
23	gga-let-7c-5p	2.058	Up
24	gga-miR-146c-3p	0.404	Down
25	gga-miR-181a-3p	0.439	Down
26	gga-miR-16-5p	0.414	Down
27	gga-miR-190a-5p	0.267	Down
28	gga-miR-20a-3p	0.253	Down
29	gga-miR-101-2-5p	0.390	Down
30	gga-miR-460a-3p	0.382	Down
31	gga-miR-30b-5p	0.215	Down
32	gga-miR-455-3p	0.379	Down
33	gga-miR-301a-3p	0.487	Down
34	gga-miR-32-3p	0.172	Down
35	gga-miR-10b-3p	0.426	Down
36	gga-miR-2954	0.185	Down
37	gga-miR-301b-3p	0.434	Down
38	gga-miR-146b-5p	0.368	Down
39	gga-miR-455-5p	0.336	Down
40	gga-miR-219b	0.472	Down
41	gga-miR-1451-5p	0.249	Down
42	gga-miR-18a-5p	0.465	Down
43	gga-miR-146c-5p	0.317	Down
44	gga-miR-30d	0.450	Down
45	gga-miR-148a-5p	0.462	Down
46	gga-miR-26a-3p	0.199	Down

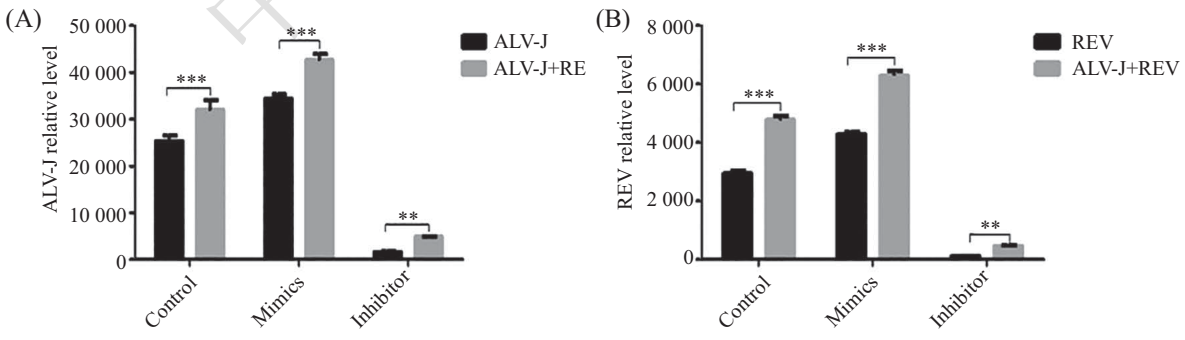
续表4

序号 No.	miRNA名称 miRNA's name	变化倍数 Fold change	上调/下调 Up/down
47	gga-miR-203a	0.090	Down
48	gga-miR-1729-5p	0.106	Down
49	gga-miR-34a-3p	0.322	Down
50	gga-miR-193b-3p	0.130	Down
51	gga-miR-30e-5p	0.305	Down
52	gga-miR-6548-5p	0.071	Down
53	gga-miR-199-5p	0.313	Down
54	gga-miR-2131-5p	0.412	Down

表5 共感染组与REV感染组差异表达的miRNA

Table 5 The differentially expressed miRNAs between co-infection group and REV infection group

序号 No.	miRNA名称 miRNA's name	变化倍数 Fold change	上调/下调 Up/Down
1	gga-miR-429-3p	2.084	Up
2	gga-miR-184-3p	3.133	Up
3	gga-miR-458a-3p	2.291	Up
4	gga-let-7a-2-3p	2.742	Up
5	gga-miR-155	3.274	Up
6	gga-miR-147	2.579	Up
7	gga-miR-146a-3p	4.183	Up
8	gga-miR-1454	0.410	Down
9	gga-miR-3538	0.416	Down
10	gga-miR-499-5p	0.194	Down
11	gga-miR-489-3p	0.239	Down
12	gga-miR-1563	0.152	Down
13	gga-miR-206	0.099	Down
14	gga-miR-133c-3p	0.319	Down
15	gga-miR-1677-3p	0.161	Down
16	gga-miR-460b-5p	0.356	Down

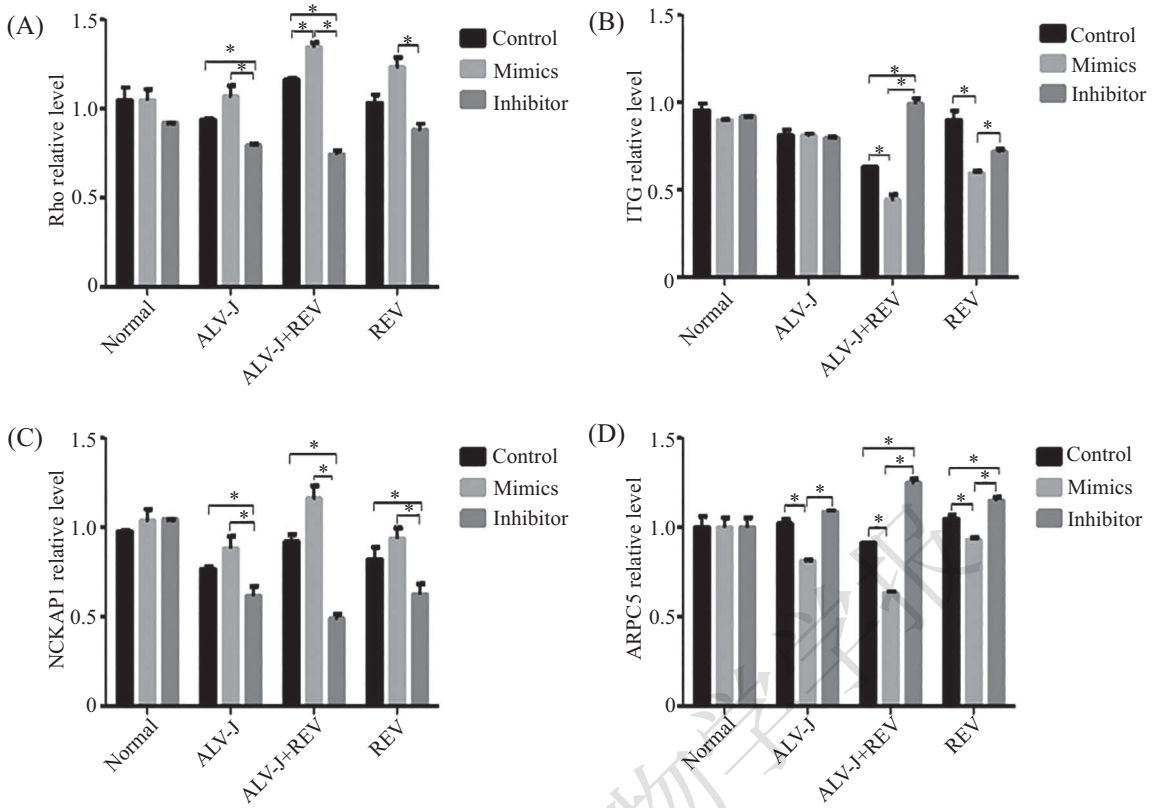


A: miRNA-155的表达对ALV-J复制的影响; B: miRNA-155的表达对REV复制的影响。Control: 未改变miRNA-155表达; Minics: 转染miRNA-155促进剂; Inhibitor: 转染miRNA-155抑制剂。ALV-J: ALV-J单独感染组; ALV-J+REV: ALV-J和REV共同感染组; REV: REV单独感染组。**P<0.01, ***P<0.001。

A: the effect of miRNA-155 expression on the replication of ALV-J; B: the effect of miRNA-155 expression on the replication of REV. Control: the expression of miRNA-155 not changed; Minics: transfection of miRNA-155 minics; Inhibitor: transfection of miRNA-155 inhibitor. ALV-J: ALV-J infection alone group; ALV-J+REV: ALV-J and REV co-infection group; REV: REV infection alone group. **P<0.01, ***P<0.001.

图1 miRNA-155的表达对病毒复制的影响

Fig.1 The effects of miRNA-155 expression on the replication of virus



A: miRNA-155的表达对Rho的影响; B: miRNA-155的表达对ITG的影响; C: miRNA-155的表达对NCKAP1的影响; D: miRNA-155的表达对ARPC5的影响。Normal: 未感染组; ALV-J: ALV-J单独感染组; ALV-J+REV: ALV-J和REV共同感染组; REV: REV单独感染组。Control: 未改变miRNA-155表达; Minics: 转染miRNA-155促进剂; Inhibitor: 转染miRNA-155抑制剂。*P<0.05。

A: the effect of miRNA-155 on Rho; B: the effect of miRNA-155 on ITG; C: the effect of miRNA-155 on NCKAP1; D: the effect of miRNA-155 on ARPC5. Normal: uninfected group; ALV-J: ALV-J infection alone group; ALV-J+REV: ALV-J and REV co-infection group; REV: REV infection alone group. Control: the expression of miRNA-155 not changed; Minics: transfection of miRNA-155 minics; Inhibitor: transfection of miRNA-155 inhibitor. *P<0.05.

图2 不同感染状态下miRNA-155对肌动蛋白细胞骨架通路相关蛋白的影响

Fig.2 The effects of miRNA-155 on the proteins in actin cytoskeletal pathways under different virus infection status

促进作用更显著。

2.4 miRNA-155的表达对肌动蛋白细胞骨架通路重要蛋白的影响

蛋白组学和转录组学的综合分析发现, 与单独感染组相比, 共感染组miRNA-155的靶蛋白ITGα1和ARPC5的表达显著下降, RhoJ和NCKAP1的表达则显著上升, 且共同参与肌动蛋白细胞骨架通路。为了进一步明确miRNA-155对该通路中4个蛋白影响, 改变miRNA-155表达后, 我们检测并分析了4种蛋白的表达情况。结果如图2所示, 与单独感染组相比, 共感染组ITG和ARPC5的表达显著下降, RhoJ、NCKAP1的表达则显著上升; 这与前期蛋白组学和转录组学的定量分析结果(表2~表5)相一致。当促进miRNA-155的表达时, ITG和ARPC5的表达下降更显著; RhoJ和NCAKP1的表达则上升更显著。反之, 当抑制miRNA-155的表达时, ITG和ARPC5的表达显著上升; RhoJ和NCAKP1的表达则显著下降。而且, 不同miRNA-155表达情况下, 与单独感染组相比, 共感染组4种蛋白的变化显著增强。

3 讨论

ALV-J和REV均属于禽反转录病毒, 都可引起禽群严重的免疫抑制和肿瘤形成, 越来越多的临床数据显示, 两种病毒混合感染使临床症状复杂多样。但是二者共感染的协同致病机制仍是未解之谜。我们在前期的研究中证实, 外泌体与ALV-J和REV感染密切相关[5-6], 为进一步探究两种病毒共感染协同致病机制, 本研究对共感染DF-1细胞来源的外泌体进行蛋白质组学分析。结果发现, 与ALV-J单独感染组相比, 共感染组携带REV的重要成分(表2中的env、

gag和pol); 与REV单独感染组相比, 共感染组携带ALV-J的重要成分(表3的env、gag和反转录酶聚合蛋白和gag)。两种病毒各自重要成分的存在可能是共感染作用加强的原因之一。共感染引起致病作用加强究竟是两种病毒致病作用的叠加还是协同呢? 我们又对表达差异的蛋白质和miRNA进行了综合分析比较, 找到了共感染组与单独感染组相比均存在差异的miRNA-155、RhoJ、NCKAP1和ARPC5。miRNA-155的靶蛋白ITG与RhoJ、NCKAP1和ARPC5共同参与肌动蛋白细胞骨架通路, 暗示筛选的4个因子和肌动蛋白细胞骨架通路可能与共感染致病机制密切相关。

miRNA-155是一种炎性相关miRNA, 可以在不同层次调节炎性和免疫细胞功能[7-8]。有人采用外泌体快速提取方法, 发现血浆中miRNA-155与外泌体密切相关, 证实了miRNA-155以无细胞的形式存在, 发挥细胞间信号的作用[9]。血浆中循环miRNA-155的过表达可以作为弥漫性B淋巴细胞瘤预后不良的标志[10]。有研究显示, B细胞来源的外泌体可以作为载体携带外源性miRNA-155的促进剂或抑制剂分别进入肝细胞或巨噬细胞[11]。本研究结果显示, 病毒感染后外泌体携带miRNA-155表达显著上调, 今后我们也可以研究血浆中miRNA-155的表达情况与禽肿瘤形成的关系或在肿瘤病早期诊断以及疾病防控方面开展一系列研究。

miRNA-155的表达不仅影响病毒复制, 也影响肌动蛋白细胞骨架通路中ITG、RhoJ、NCKAP1和ARPC5的表达, 进一步证实肌动蛋白细胞骨架通路在ALV-J和REV共感染的协同致病中具有重要作用。RhoJ是一种富含于内皮的Rho家族的成员, Rho家族是一类鸟苷酸(GTP)结合蛋白, 具有GTP酶活性。有研究显示, 在体外和肿瘤移植过程中, RhoJ以依赖于p21蛋白(Cdc42/Rac)活化酶1[p21 protein (Cdc42/Rac)-activated kinase 1, PAK-1]的方式诱导LIM蛋白激酶(LIM domain containing protein kinase, LIMK)、丝蛋白和Arp2/3复合体亚基(p41-ARPC)磷酸化, 进而改变肌动蛋白细胞骨架的动力学, 是黑色素瘤的关键决定因子[12]。RhoJ还与胃癌的转移和发展有关, 可作为胃癌存活率的预后因素[13]。而RhoJ阻断剂的使用, 可以有效抑制肿瘤血管的生成, 从而导致肿瘤血管功能衰竭, 因此血管RhoJ可作为肿瘤血管生成和血管破坏的一种有效的选择性靶点[14]。NCKAP1是WASP家族富含脯氨酸的同源蛋白(Wiskott-Aldrich syndrome proteins verprolin homologous proteins, WAVE)复合体的成员, WAVE形成复杂的信号复合物激活Arp2/3复合体并促进肌动蛋白丝的组装。研究显示, WAVE家族成员与肿瘤细胞的形成、侵袭、转移密切相关[15-18]。ARPC5是Arp2/3复合体的七个亚基中最小的亚基[19], 参与肿瘤细胞的迁移和侵袭, 研究显示, ARPC5直接受头颈部的鳞状细胞癌中的肿瘤抑制性miRNA-133a的调控[20]。

综上, ALV-J和REV可能利用miRNA-155促进RhoJ的表达, RhoJ又进一步激活NCKAP1结合到Arp2/3的亚基ARPC5上形成复合物, 促进肌动蛋白多聚体的形成, 从而形成伪足促进细胞的运动, 其具体作用方式和途径有待进一步研究确定。我们在前期的研究中发现, REV感染会引起细胞黏附性降低[21], 而细胞黏附性下降, 运动能力增强, 有利于肿瘤细胞的形成和迁移。控制肿瘤细胞转移的一个主要因素就是它们的能动性, 控制其调控的信号通路的改变可控制肿瘤细胞的侵袭和转移。本研究的结果为深入研究ALV-J和REV的协同致病机制(尤其是致瘤机制)提供了理论基础, 同时也为肿瘤形成与抑制提供了数据基础。

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