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日本对虾 miR-34 靶基因预测及其生物信息学分析

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[摘要]　已有研究表明日本对虾(*Marsupenaeus japonicus* shrimp)miR-34(mja-miR-34)参与调控白斑综合症病毒(white spot syndrome virus,WSSV)的感染,但其调控的宿主基因还未具体阐述. 本研究首先比对了 miR-34 在 17 个物种中的序列,并使用 TargetScan 5.1 和 miRanda 预测了 miR-34 调控的宿主基因. 结果显示,miR-34 在物种进化过程中具有高度保守性;mja-miR-34 可靶向作用于 242 个宿主编码的基因,且在病毒感染不同时间段呈现差异表达;利用 GO 注释和 KEGG 信号通路富集分析结果表明,mja-miR-34 的靶基因参与细胞代谢、细胞信号转导、免疫系统以及遗传信息的调控等过程;mja-miR-34 在对虾体内可调控靶基因(*translation initiation factor*)的表达. 结果说明 mja-miR-34 及其靶基因参与病毒感染等多个细胞进程,但还有待进一步验证. 本研究可为 mja-miR-34 靶基因的鉴定及其生物学功能的研究提供数据支持和理论指导.

[关键词]　日本对虾,mja-miR-34,靶基因,生物信息学分析

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Marsupenaeus japonicus shrimp miR-34 Target Gene Prediction and Bioinformatics Analysis

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Abstract:Previous study has shown that miR-34(mja-miR-34) of *Marsupenaeus japonicus* shrimp is involved in the regulation of white spot syndrome virus (WSSV) infection, but the host genes regulated by mja-miR-34 have not yet been elucidated. In this study,the sequences of miR-34 in 17 species were firstly compared,and the target genes of shrimp regulated by miR-34 were predicted by TargetScan 5.1 and miRanda. The results showed that miR-34 was highly conserved during species evolution;mja-miR-34 could target 242 host-encoded genes and was differentially expressed in different time periods of virus infection;the target genes of mja-miR-34 were involved in the regulation of multiple cellular process of cell metabolism,cell signal transduction,immune system and genetic information analyzed by GO annotation and KEGG signal pathway enrichment analysis. mja-miR-34 can regulate the expression of target gene *translation initiation factor* in shrimp *in vivo*. These results indicated that mja-miR-34 and its target genes were involved in multiple cellular processes such as virus infection,but further verification was required. This study can provide data support and theoretical guidance for the characterization of mja-miR-34 target genes and the investigation of their biological functions.

Key words:*Marsupenaeus japonicus* shrimp,mja-miR-34,target genes,bioinformatic analysis

MicroRNA 是一类~22 nt 的内源性非编码小 RNA,可以调控蛋白编码基因-massenger RNA(mRNA)的表达[1]. miRNA 的转录一般由 RNA 聚合酶 II(RNA Pol II)来执行,并受到 RNA Pol II 相关转录因子及表观遗传的调控[2-3]. 初级 miRNA(pirmary microRNA)在 RNA Pol II 的作用下被转录下来后,经过一系列的加工过程形成成熟的 miRNA. 产生的具有双链结构的 RNA(miRNA:miRNA*)组装到 Argonaute(Ago)蛋白上形成沉默复合体前体,并瞬时发生解链,miRNA* 链被解离下来,形成成熟的沉默复合体,发挥基因沉默的效应[4-5].

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MiRNA 在水生动物中的相关研究越来越多,鲤鱼脾脏中保守 miRNA 的靶基因预测结果显示,这些 miRNA 与免疫系统发育、先天性和获得性免疫应答及细胞因子生成等诸多免疫过程相关[6]. Dang 等[7]发现 miR-155 在鱼类细胞系中能很好地协助细胞抑制真鲷虹彩病毒的复制,且通过抗病毒因子 IFN 及相关通路来诱导抗病毒反应. 此外,研究表明,miRNA 主要通过 miRNA 的种子序列(一般从 5′端开始第 2 到第 7 个碱基)与 mRNA 的 3′UTR(3′非编码区)区域有连续的 Watson-Crick 碱基配对[8]. 而 miRNA 的种子序列可以与约 1/3 的人类基因碱基配对,说明了 miRNA 调控范围的广泛性[8]. 在乳腺癌中 miR-9 可以通过调控 6 个基因的表达来抑制乳腺癌细胞的增值、入侵,并诱导细胞发生凋亡[9];miR-125b 至少可以靶向于 10 条基因,影响人神经系统的发育[10];miR-34 可作为乳腺癌抑制因子调控多个基因的表达,引起细胞周期阻滞和削弱细胞迁移能力[11-13]. 据之前的报道,miR-34 在肿瘤细胞中可促使 Caspase-3 的裂解,诱发 Caspase 调控的凋亡途径[14],还可通过被 p53 激活,抑制肿瘤细胞的增殖与生长,促进肿瘤细胞凋亡[15]. 由此可见 miR-34 可以参与细胞凋亡、细胞增殖、细胞迁移等多个细胞过程. 此外,一些 miRNA 在进化过程中具有高度保守性. 例如,let-7 在脊椎动物、节肢动物和蛔虫中的序列都是相同的[16];在病毒与宿主的相互作用中,病毒编码的 miRNA 与人的 miRNA 具有高度同源性[17-18];有的 miRNA 在不同物种间其种子序列是一致的[19]. 已有研究表明,对虾 Argonaut(Ago)复合体中宿主及病毒编码的 miRNA、mRNA 在病毒感染不同时间段呈现差异表达,且日本对虾编码的 miRNA 在病毒感染前后靶向基因的数量发生变化[20]. 也有研究报道,日本对虾 miR-34(mja-miRNA-34)作用于白斑综合症病毒(white spot syndrome virus,WSSV)编码的基因[21],但 mja-miRNA-34 对宿主基因的调控及其参与的信号通路还有待进一步研究. 本研究根据日本对虾 miR-34 靶基因的预测结果,通过数据整合和生物信息学分析方法的应用,挖掘可能的理论结果,并对结果进行系统分析和整理. 该结果可为 mja-miR-34 靶基因的实验鉴定及其生物学功能的研究提供数据支持和理论指导.

1　材料与方法

1.1　材料

mja-miRNA-34:根据日本对虾 Ago 复合体 miRNA、mRNA 测序数据[20],获得了 mja-miR-34.

不同物种的 miR-34:搜索 miRBase(http://www.mirbase.org/)可获得不同动物的 miR-34 的成熟序列.

日本对虾基因的 3′UTR:分析获得日本对虾 Ago 复合体 mRNA 数据库,从该数据库中获得对虾基因的 3′UTR[20]. 根据对虾基因的 3′UTR 进行后续的 mja-miR-34 的靶基因预测.

1.2　方法

1.2.1　miR-34 的靶基因预测

采用 TargetScan 5.1 和 miRanda 预测 miR-34 的靶基因. TargetScan 5.1 根据 miR-34 的种子序列,搜索对虾基因 3′UTR 上的靶位点. miRanda 软件根据 miRNA 的全序列搜索可能的靶基因. miRNA 与靶基因相互作用的条件为自由能<−20 kcal/mol,分值>50. 两种预测软件获得的序列的交集,则有可能是 miR-34 的靶基因.

1.2.2　mja-miR-34 的 Northern blot 分析

按照 mirVana miRNA isolation kit 说明书(Ambion,USA),提取 RNA. 利用 NanoDrop ND−1000 spectrophotometer 进行浓度及质量鉴定. 用制备的 15%变性聚丙烯酰胺凝胶(含有 8M 尿素)进行 RNA 电泳分离. 把分离后的 RNA 转印至 Hybond-N^+膜上(Amersham Biosciences,Buckinghamshire,UK),1200 J 紫外交联 30 s. 用 Detection Buffer 稀释 NBT/BCIP 显色底物,并把配制好的显色液加入平皿中进行显色. 待目的条带清楚后,弃去显色液终止显色. mja-miR-34 地高辛标记的探针序列为 5′-ACAACCAGCTAACCACACTGCCA-3′;U6 地高辛标记的探针序列为 5′-GGGCCATGCTAATCTTCTCTGTATCGTT-3′.

1.2.3　实时荧光定量 PCR

实时荧光定量 PCR(RT-qPCR)是用特异性引物序列检测日本对虾体内 miR-34 的靶基因(*serine/threonine kinase 3*、*translation initiation factor*、*cytidine deaminase*)的表达. 本试验收集未注射 WSSV(0 h)和注射 WSSV(24 h、36 h、48 h、72 h)日本对虾的鳃、血淋巴细胞. 用 Spin Column Animal Total RNA Purification Kit(Sangon Biotech,China)提取 RNA. 以 RNA 为模板,通过 Rever Tra Ace™ qPCR RT Master

Mix with gDNA Remover(Takara,Japan)试剂盒进行反转录反应,合成 cDNA. 以日本对虾 *β-actin*(*β-actin* 正向引物:5′-CGAGCA CGGCATCGTTACTA-3′,*β-actin* 反向引物:5′-TTGTAGAAAGTGTGATGCCAGATCT-3′)作为内参基因. miR-34 靶基因的引物序列为 *serine/threonine kinase 3* 正向引物 5′-GTAAAGGCTCAAAG-GATAAC-3′,*serine/threonine kinase 3* 反向引物 5′-TGCTGTGTAACAATGGG-3′;*translation initiation factor* 正向引物 5′-CTCGGAGAAACTGCTTGA-3′,*translation initiation factor* 反向引物 5′-GGTAGAGACCCTCCTTGG-3′;*cytidine deaminase* 正向引物 5′-TGCAGAGATGGGAGAGAGGT-3′,*cytidine deaminase* 反向引物 5′-TCAGG-TAGTAGTTTGCCGACG-3′. 制备总体积为 20 μL,其中包含 10 μL 的 ChamQ Universal EYBR qPCR Master MIX(Vazyme,China),0.5 μL 的 cDNA 模板,10 μmol/L 0.4 μL 的正向和反向引物,并用无菌水加至 20 μL. 进行 PCR 扩增. 反应程序:95 ℃预变性 3 min;40 次循环:95 ℃ 20 s,60 ℃ 20 s,72 ℃ 30 s.

1.2.4　mja-miR34 在对虾体内的过表达

在对虾尾节第四节注射 WSSV(10^4 个 WSSV 粒子)和 30 μg miR-34 或 miR-34-scrambled 的模拟物. 注射 36 h 后,利用实时荧光定量 PCR 分析 miR-34 预测靶基因的表达量. miR-34 模拟物 Sense 序列 5′-UGGCAGU-GUGGUUAGCUGGUUGU-3′, Antisense 序列 5′-ACAACCAGCUAACCACACUGCCA-3′; miR-34-scrambled 的 Sense 序列 5′-AUUUGACAGAUGCCUAGUACCAG-3′,Antisense 序列 5′-CUGGUACUAGGCAUCUGUCAAAU-3′.

1.2.5　GO 分析和 KEGG 分析

TargetScan 及 miRanda 软件预测得到的靶基因与 GO 数据库进行比对,$E<1e^{-5}$. 获得匹配度最高的 GO 的 ID,并描述这些基因和基因产物的属性. 对得到的靶基因进行 KEGG Pathway 分析,利用 KAAS 预测得到对应的 KO 号,分析基因与 KEGG 中酶注释的关系文件以及映射到 pathway 的信息.

1.2.6　数据分析

使用 IBM SPSS Statistics 26 中的比较平均值,对数据进行单因素 ANONA 检验,用邓肯法进行事后多重比较,用(平均值±标准误)表示结果. 小写字母表示差异显著($P<0.05$).

2　结果与讨论

2.1　miR-34 的保守性

由表 1 可知,日本对虾编码的 miR-34(mja-miR-34)在人、鼠、果蝇等 17 个物种的成熟序列具有高度相似性,说明 mja-miR-34 在物种进化过程中具有高度保守性.

表 1　不同物种的 miR-34 的成熟序列

Table 1　Mature sequences of miR-34 in different species

序列号 Serial number	物种 Species	名称 miRNA name	保守序列 Conservative sequence
	Marsupenaeus japonicus	mja-miR-34	UGGCAGUGUGGUUAGCUGGUUGU
MIMAT0001269	*Danio rerio*	dre-miR-34	UGGCAGUGUCUUAGCUGGUUGU
MIMAT0000255	*Homo sapiens*	hsa-miR-34	UGGCAGUGUCUUAGCUGGUUGU
MIMAT0000542	*Mus musculus*	mmu-miR-34	UGGCAGUGUCUUAGCUGGUUGU
MIMAT0000350	*Drosophila melanogaster*	dme-miR-34	UGGCAGUGUGGUUAGCUGGUUGUG
MIMAT0009516	*Capitella teleta*	cte-miR-34	UGGCAGUGUGGUUAGCUGGUUGU
MIMAT0009475	*Branchiostoma floridae*	bfl-miR-34	UGGCAGUGUGGAUAGCUGGCCGUUU
MIMAT0012917	*Equus caballus*	eca-miR-34	UGGCAGUGUCUUAGCUGGUUGU
MIMAT0000005	*Caenorhabditis elegan*	cel-miR-34	AGGCAGUGUGGUUAGCUGGUUG
MIMAT0000815	*Rattus norvegicus*	rmo-miR-34	UGGCAGUGUCUUAGCUGGUUGU
MIMAT0002494	*Gorilla gorilla*	ggo-miR-34	UGCCAGUGUCUUAGCUGGUUGU
MIMAT0002495	*Ateles geoffroyi*	age-miR-34	UGGCAGUGUCUUAGCUGGUUGU
MIMAT0002496	*Pan paniscus*	ppa-miR-34	UGGCAGUGUCUUAGCUGGUUGU
MIMAT0002497	*Pongo pygmaeus*	ppy-miR-34	UGGCAGUGUCUUAGCUGGUUGU
MIMAT0003578	*Xenopus tropicalis*	xtr-miR-34a	UGGCAGUGUCUUAGCUGGUUGUU
MIMAT0002499	*Macaca mulatta*	mml-miR-34	UGGCAGUGUCUUAGCUGGUUGU
MIMAT0002500	*Saguinus labiatus*	sla-miR-34	UGGCAGUGUCUUAGCUGGUUGU

注:红色表示完全相同的碱基,下划线表示种子序列.

2.2　mja-miR-34 靶基因的预测

白斑综合症病毒感染日本对虾 0 h、24 h、48 h 后,收取对虾血淋巴细胞,提取对虾 Ago1-RNA 复合体中的 RNA,进行 RNA 高通量测序[20]. 以 RNA 高通量测序得到的转录组数据为数据库,用 TargetScan 及 miRanda 预测 mja-miR-34 的靶基因,选取两种软件靶基因预测结果的交集,共得到 242 个 mja-miR-34 的靶基因(表 2). Northern blot 结果显示 mja-miR-34 在病毒感染不同时间段呈现差异表达(图 1A);同时,转录组数据显示 mja-miR-34 的靶基因在病毒感染不同时间段呈现差异表达(图 1B-F),说明 mja-miR-34 及其靶基因可能参与病毒感染进程. 随机挑选 3 个 miR-34 的靶基因进行表达量分析,结果表明,miR-34 靶基因的差异表达情况与测序结果一致(图 1G-I).

表 2　TargetScan 及 miRanda 软件预测的 miR-34 靶基因

Table 2　miR-34 targeted genes predicted by TargetScan and miRanda software

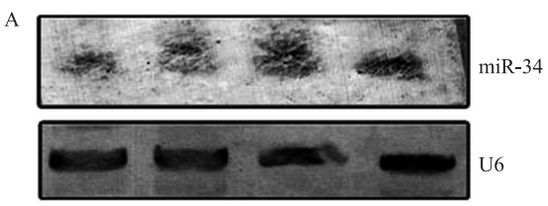
基因全称	缩写	基因全称	缩写
ATP-dependent RNA helicase DDX18/HAS1	DDX18.HAS1	CDC-like kinase	CLK
rootletin	CROCC	histone-lysine N-methyltransferase. H3 lysine-79 specific	DOT1L.DOT1
adenylate kinase	adk	dedicator of cytokinesis 1	DOCK1
bone morphogenetic protein receptor type-1. invertebrate	BMPR1N	BTB/POZ domain-containing protein 7	BTBD7
cytidine deaminase	cdd	3-hydroxybutyrate dehydrogenase	bdh
ATP-dependent RNA helicase	DDX3X.bel	CERS	CERS
bromodomain and WD repeat domain containing protein 1/3	BRWD1_3	DnaJ homolog subfamily C member 3	DNAJC3
cullin 5	CUL5	metal transporter CNNM	CNNM
sn1-specific diacylglycerol lipase	DAGL	calcium/calmodulin-dependent protein kinase kinase	CAMKK
amphiphysin	AMPH	beta-site APP-cleaving enzyme 2(memapsin 1)	BACE2
zinc finger protein ubi-d4	DPF2.REQ	dihydroxyacetone kinase	DAK1.DAK2
UDP-glucose: O-linked fucose beta-1. 3-glucosyltransferase	B3GALTL	collagen. type I/II/III/V/XI. alpha	COL1AS
chymotrypsin	CTRB	alanyl-tRNA synthetase	AARS.alaS
parafibromin	CDC73	cadherin EGF LAG seven-pass G-type receptor 1 (flamingo)	CELSR1
F-type H+-transporting ATPase subunit f	ATPeFOF.ATP5J2	histone chaperone ASF1	ASF1
DnaJ homolog subfamily C member 7	DNAJC7	MFS transporter. PAT family. beta-lactamase induction signal transducer AmpG	ampG
F-type H+-transporting ATPase subunit gamma	ATPeF1G.ATP5C1	N-acetyllactosaminide beta-1. 3-N-acetylglucosaminyltransferase	B3GNT1.B3GNT2
breast cancer 2 susceptibility protein	BRCA2.FANCD1	thymidine phosphorylase	deoA
2-oxoisovalerate dehydrogenase E2 component (dihydrolipoyl transacylase)	bkdB	nicotinic acetylcholine receptor. invertebrate	CHRNN
ubiquitin carboxyl-terminal hydrolase BAP1	BAP1. UCHL2	centrin-1	CETN1
cathepsin F	CTSF	actin beta/gamma 1	ACTB_G1
dynamin GTPase	DNM	dynein heavy chain. axonemal	DNAH
cathepsin A(carboxypeptidase C)	CTSA	cytochrome P450. family 734. subfamily A. polypeptide 1(PHYB activation tagged suppressor 1)	CYP734A1.BAS1
probable 2-oxoglutarate dehydrogenase E1 component DHKTD1	DHKTD1	cathepsin S	CTSS
ADP-ribosylation factor-like 3	ARL3	casein kinase II subunit alpha	CSNK2A
5′-nucleotidase	E3.1.3.5	human immunodeficiency virus type I enhancer-binding protein	HIVEP
Longitudinals lacking protein-like	LOLAL	aspartate beta-hydroxylase	E1.14.11.16
guanine deaminase	guaD	dihydropyrimidine dehydrogenase(NADP+)	DPYD
elongation factor 1-alpha	EEF1A	guanylate kinase	gmk
prostamide/prostaglandin F2alpha synthase	PGFS	glutamate synthase(NADPH/NADH)	GLT1
histone H3	H3	ubiquitin-conjugating enzyme(huntingtin interacting protein 2)	HIP2.UBC1
F-box and leucine-rich repeat protein 2/20	FBXL2_20	actin-binding protein IPP	IPP.KLHL27
translation initiation factor 3 subunit A	EIF3A	ketohexokinase	KHK
E3 ubiquitin-protein ligase HERC4	HERC4	translation initiation factor 4A	EIF4A

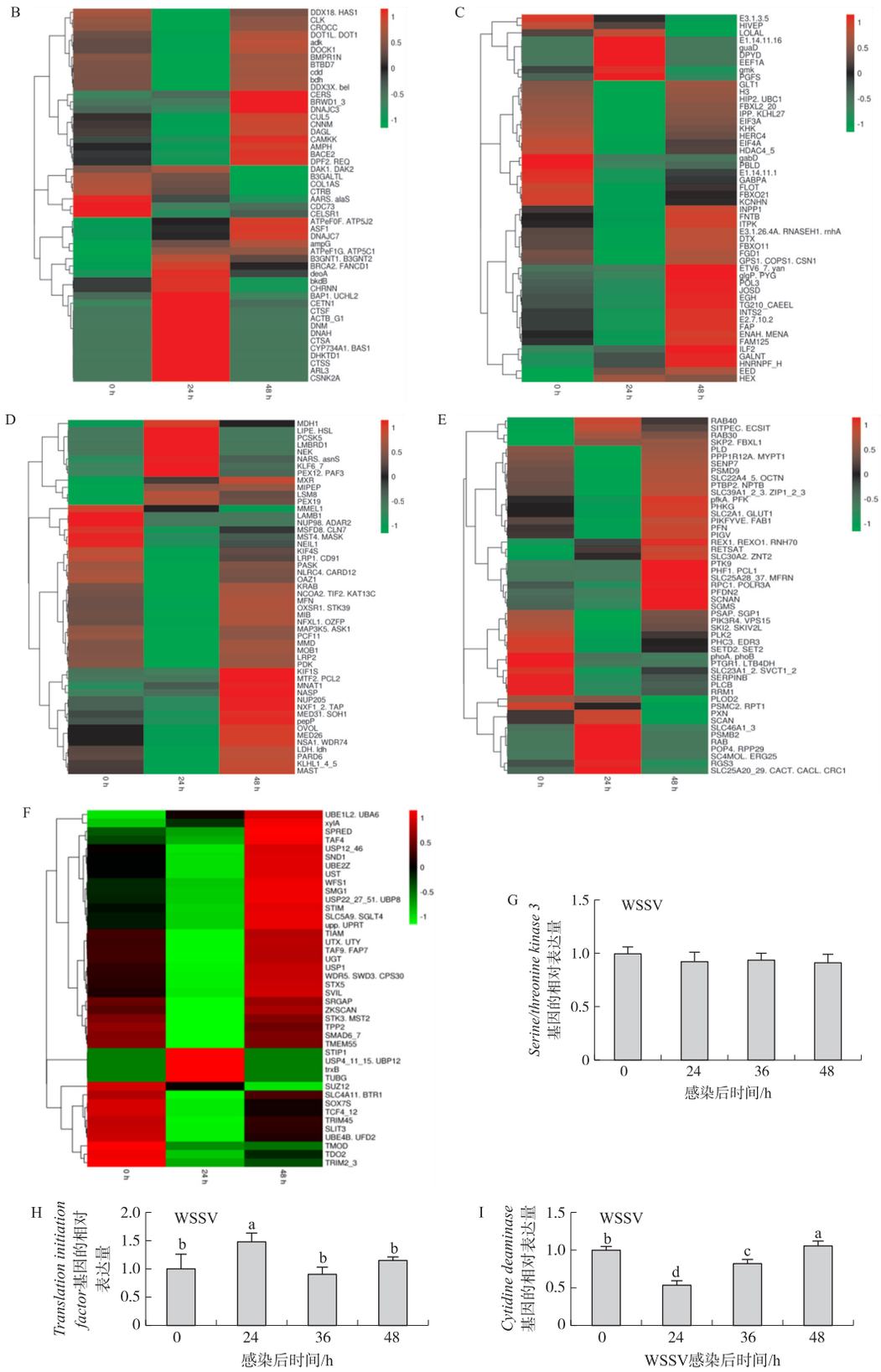
续表 2　Table 2 continued

基因全称	缩写	基因全称	缩写
histone deacetylase 4/5	HDAC4_5	succinate-semialdehyde dehydrogenase(NADP+)	gabD
Phenazine biosynthesis-like domain-containing protein	PBLD	gamma-butyrobetaine dioxygenase	E1.14.11.1
GA-binding protein transcription factor. alpha	GABPA	flotillin	FLOT
F-box protein 21	FBXO21	potassium voltage-gated channel Eag-related subfamily H. invertebrate	KCNHN
inositol polyphosphate 1-phosphatase	INPP1	protein farnesyltransferase subunit beta	FNTB
1D-myo-inositol-triphosphate 3-kinase	ITPK	ribonuclease HI	E3.1.26.4A.RNASEH1.rnhA
deltex	DTX	F-box protein 21	FBXO11
FYVE. RhoGEF and PH domain containing 1	FGD1	COP9 signalosome complex subunit 1	GPS1.COPS1.CSN1
ETS translocation variant 6/7	ETV6_7.van	starch phosphorylase	glgP. PYG
Retrovirus-related Pol polyprotein from transposon	POL3	josephin	JOSD
egghead protein(zeste-white 4 protein)	EGH	Putative GTP-binding protein tag-210	TG210_CAEEL
integrator complex subunit 2	INTS2	non-specific protein-tyrosine kinase	E2.7.10.2
fibroblast activation protein. alpha	FAP	enabled	ENAH. MENA
ESCRT-I complex subunit MVB12	FAM125	interleukin enhancer-binding factor 2	ILF2
polypeptide N-acetylgalactosaminyltransferase	GALNT	heterogeneous nuclear ribonucleoprotein F/H	HNRNPF_H
polycomb protein EED	EED	hexosaminidase	HEX
malate dehydrogenase	MDH1	hormone-sensitive lipase	LIPE.HSL
proprotein convertase subtilisin/kexin type 5	PCSK5	LMBR1 domain-containing protein 1	LMBRD1
NIMA(never in mitosis gene a)-related kinase	NEK	asparaginyl-tRNA synthetase	NARS.asnS
krueppel-like factor 6/7	KLF6_7	peroxin-12	PEX12.PAF3
metabotropic X receptor	MXR	mitochondrial intermediate peptidase	MIPEP
U6 snRNA-associated Sm-like protein LSm8	LSM8	peroxin-19	PEX19
membrane metallo-endopeptidase-like 1	MMEL1	laminin. beta 1	LAMB1
nuclear pore complex protein Nup98-Nup96	NUP98. ADAR2	MFS transporter. ceroid-lipofuscinosis neuronal protein 7	MSFD8. CLN7
serine/threonine-protein kinase MST4	MST4. MASK	endonuclease VIII-like 1	NEIL1
kinesin family member 4/7/21/27	KIF4S	low density lipoprotein-related protein 1(alpha-2-macroglobulin receptor)	LRP1.CD91
PAS domain containing serine/threonine kinase	PASK	NLR family CARD domain-containing protein 4	NLRC4. CARD12
ornithine decarboxylase antizyme 1	OAZ1	KRAB domain-containing zinc finger protein	KRAB
nuclear receptor coactivator 2	NCOA2. TIF2. KAT13C	mitofusin	MFN
serine/threonine-protein kinase OSR1/STK39	OXSR1.STK39	E3 ubiquitin-protein ligase mind-bomb	MIB
NF-X1-type zinc finger protein NFXL1	NFXL1.OZFP	mitogen-activated protein kinase kinase kinase 5	MAP3K5. ASK1
pre-mRNA cleavage complex 2 protein Pcf11	PCF11	monocyte to macrophage differentiation protein	MMD
maintenance of ploidy protein MOB1(MPS1 binder 1)	MOB1	low density lipoprotein-related protein 2	LRP2
pyruvate dehydrogenase kinase	PDK	kinesin family member 1/13/14	KIF1S
polycomb-like protein 2	MTF2. PCL2	CDK-activating kinase assembly factor MAT1	MNAT1
nuclear autoantigenic sperm protein	NASP	nuclear pore complex protein Nup205	NUP205
nuclear RNA export factor 1/2	NXF1_2.TAP	mediator of RNA polymerase II transcription subunit 31	MED31. SOH1
Xaa-Pro aminopeptidase	pepP	ovo	OVOL
mediator of RNA polymerase II transcription subunit 26	MED26	ribosome biogenesis protein NSA1	NSA1. WDR74
L-lactate dehydrogenase	LDH. Idh	partitioning defective protein 6	PARD6
kelch-like protein 1/4/5	KLHL1_4_5	microtubule-associated serine/threonine kinase	MAST
Ras-related protein Rab-40	RAB40	evolutionarily conserved signaling intermediate in Toll pathways	SITPEC.ECSIT
Ras-related protein Rab-30	RAB30	F-box and leucine-rich repeat protein 1(S-phase kinase-associated protein 2)	SKP2. FBXL1
phospholipase D	PLD	protein phosphatase 1 regulatory subunit 12A	PPP1R12A. MYPT1
sentrin-specific protease 7	SENP7	26S proteasome non-ATPase regulatory subunit 9	PSMD9
MFS transporter. OCT family. solute carrier family 22(organic cation transporter). member 4/5	SLC22A4_5.OCTN	olypyrimidine tract-binding protein 2	PTBP2.NPTB
solute carrier family 39(zinc transporter). member 1/2/3	SLC39A1_2_3.ZIP1_2_3	6-phosphofructokinase 1	pfkA.PFK
phosphorylase kinase gamma subunit	PHKG	MFS transporter. SP family. solute carrier family 2(facilitated glucose transporter). member 1	SLC2A1.GLUT1
1-phosphatidylinositol-3-phosphate 5-kinase	PIKFYVE.FAB1	profilin	PFN

续表 2　Table 2 continued

基因全称	缩写	基因全称	缩写
phosphatidylinositol glycan. class V	PIGV	RNA exonuclease 1	REX1.REXO1.RNH70
all-trans-retinol 13.14-reductase	RETSAT	solute carrier family 30(zinc transporter). member 2	SLC30A2.ZNT2
PTK9 protein tyrosine kinase 9	PTK9	polycomb-like protein 1	PHF1.PCL1
solute carrier family 25 (mitochondrial iron transporter). member 28/37	SLC25A28_37.MFRN	DNA-directed RNA polymerase III subunit RPC1	RPC1.POLR3A
prefoldin subunit 2	PFDN2	voltage-gated sodium channel alpha. invertebrate	SCNAN
shingomyelin synthase	SGMS	saposin	PSAP.SGP1
phosphoinositide-3-kinase. regulatory subunit 4. p150	PIK3R4.VPS15	antiviral helicase SKI2	SKI2.SKIV2L
polo-like kinase 2	PLK2	polyhomeotic-like protein 3	PHC3.EDR3
histone-lysine N-methyltransferase SETD2	SETD2.SET2	alkaline phosphatase	phoA.phoB
prostaglandin reductase 1	PTGR1.LTB4DH	solute carrier family 23 (nucleobase transporter). member 1/2	SLC23A1_2.SVCT1_2
serpin B	SERPINB	phosphatidylinositol phospholipase C. beta	PLCB
ribonucleoside-diphosphate reductase subunit M1	RRM1	procollagen-lysine.2-oxoglutarate 5-dioxygenase 2	PLOD2
26S proteasome regulatory subunit T1	PSMC2.RPT1	paxillin	PXN
SCAN domain-containing zinc finger protein	SCAN	MFS transporter. PCFT/HCP family. solute carrier family 46(folate transporter). member 1/3	SLC46A1_3
20S proteasome subunit beta 4	PSMB2	Rab family. other	RAB
ribonuclease P protein subunit POP4	POP4.RPP29	methylsterol monooxygenase	SC4MOL.ERG25
regulator of G-protein signalling 3	RGS3	solute carrier family 25 (mitochondrial carnitine/ acylcarnitine transporter). member 20/29	SLC25A20_29.CACT.CACL.CRC1
ubiquitin-activating enzyme E1-like protein 2	UBE1L2.UBA6	xylose isomerase	xyIA
sprouty-related. EVH1 domain containing	SPRED	transcription initiation factor TFIID subunit 4	TAF4
ubiquitin carboxyl-terminal hydrolase 12/46	USP12_46	staphylococcal nuclease domain-containing protein 1	SND1
ubiquitin-conjugating enzyme E2 Z	UBE2Z	dermatan/chondrotin sulfate uronyl 2-O-sulfo-transferase UST	UST
wolfamin	WFS1	PI-3-kinase-related kinase SMG-1	SMG1
ubiquitin carboxyl-terminal hydrolase 22/27/51	USP22_27_51.UBP8	stromal interaction molecule	STIM
solute carrier family 5 (sodium/glucose cotransporter). member 9	SLC5A9.SGLT4	uracil phosphoribosyltransferase	upp.UPRT
T-cell lymphoma invasion and metastasis	TIAM	histone demethylase	UTX.UTY
transcription initiation factor TFIID subunit 9/adenylate kinase	TAF9.FAP7	glucuronosyltransferase	UGT
ubiquitin carboxyl-terminal hydrolase 1	USP1	COMPASS component SWD3	WDR5.SWD3.CPS30
syntaxin 5	STX5	supervillin	SVIL
SLIT-ROBO Rho GTPase activating protein	SRGAP	KRAB and SCAN domains-containing zinc finger protein	ZKSCAN
serine/threonine kinase 3	STK3.MST2	tripeptidyl-peptidase II	TPP2
mothers against decapentaplegic homolog 6/7	SMAD6_7	phosphatidylinositol-4.5-bisphosphate 4-phosphatase	TMEM55
stress-induced-phosphoprotein 1	STIP1	ubiquitin carboxyl-terminal hydrolase 4/11/15	USP4_11_15.UBP12
thioredoxin reductase(NADPH)	trxB	tubulin gamma	TUBG
polycomb protein SUZ12	SUZ12	solute carrier family 4 (sodium borate transporter). member 11	SLC4A11.BTR1
transcription factor SOX7/8/9/10/18(SOX group E/F)	SOX7S	transcription factor 4/12	TCF4_12
tripartite motif-containing protein 45	TRIM45	slit 3	SLIT3
ubiquitin conjugation factor E4 B	UBE4B.UFD2	tropomodulin	TMOD
tryptophan 2.3-dioxygenase	TDO2	tripartite motif-containing protein 2/3	TRIM2_3





A:mja-miR-34 的靶基因在病毒感染不同时间段的表达量. B−F:TargetScan 及 miRanda 软件预测的靶基因及其在病毒感染不同时间段的差异表达情况. G−I:实时荧光定量 PCR 检测 miR-34 靶基因在病毒感染不同时间段(0 h、24 h、36 h、48 h)的差异表达情况. 柱形图小写字母表示差异显著(P<0.05).

图 1　TargetScan 及 miRanda 预测的靶基因及其在病毒感染不同时间段的差异表达情况

Fig. 1　Prediction of target genes by TargetScan and miRanda and differential expression map of target genes at different time course of virus infection

2.3　mja-miR-34 靶基因的 GO 分析

对 242 个靶基因进行 GO 功能富集分析,结果显示,在生物学过程层面上 mja-miR-34 靶基因显著富集在细胞过程、代谢过程、单一有机体过程、生物调节等条目中;在细胞成分层面上,靶基因显著富集在细胞、细胞部分、细胞器、膜等条目中;在分子功能层面上,靶基因显著富集在粘合物、催化活性、分子传感活性、信号转换器活性等条目中(图 2). 这些结果说明了 mja-miR-34 参与对虾体内多种生物学过程及细胞功能.

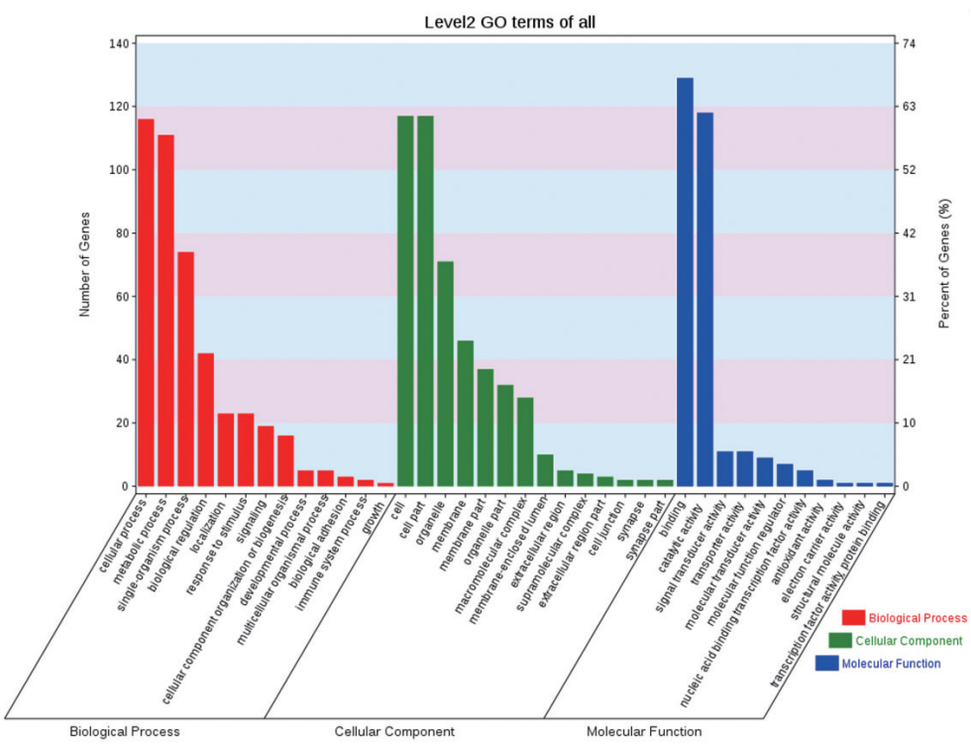


图 2　mja-miR-34 预测靶基因的 GO 注释分析

Fig. 2　GO annotation analysis of mja-miR-34 predicted target genes

2.4　mja-miR-34 靶基因的 KEGG 分析

在 GO 注释分析基础上,利用现有的生物通路数据,对基因集合中的 242 个基因进行生物通路富集分析. 结果显示,mja-miR-34 的靶基因大多参与碳水化合物代谢、细胞信号转导、运输和分解代谢等细胞过程,免疫系统、疾病感染及遗传信息的折叠、筛选、降解等通路中(图 3A). 并且富集程度排名前 20 的信号通路中显著富集在嘧啶代谢通路、赖氨酸降解通路、嘌呤代谢通路和果糖/甘露糖代谢等通路中(图 3B). KEGG 分析结果表明,mja-miR-34 参与细胞代谢、细胞信号转导、免疫系统以及遗传信息的调控等.

2.5　mja-miR-34 靶基因的初步分析

为研究 mja-miR-34 对其预测靶基因的调控作用,研究利用 mja-miR-34 的模拟物在对虾体内过表达 mja-miR-34,并利用实时荧光定量 PCR 分析 *serine/threonine kinase 3*、*translation initiation factor* 基因的表达量. 结果表明,mja-miR-34 过表达后 *serine/threonine kinase 3* 基因的表达量与 WSSV 组、WSSV+miR-34-scrambled 组无显著差异,而 *translation initiation factor* 基因的表达量显著降低(图 4),说明 *serine/threonine kinase 3* 不是 mjmiR-34 直接作用的靶基因,而 mja-miR-34 可调控 *translation initiation factor* 基因的表达.

3　结论

miRNA 种子区,也就是 miRNA 5′端中能与靶基因的 3′端 UTR 区完全互补的第 2~7 位碱基序列[8]. miRNA 在不同物种间具有高度保守性,例如 miR-150[22]、miR-504[23]、miR-652[24]、miR-497[25]等. 不同物种间 miR-34 的序列显示,miR-34 在不同物种间也具有高度保守性. “种子序列”的高度保守性可能反映了 miR-34 在物种进化过程中一直发挥着重要作用.

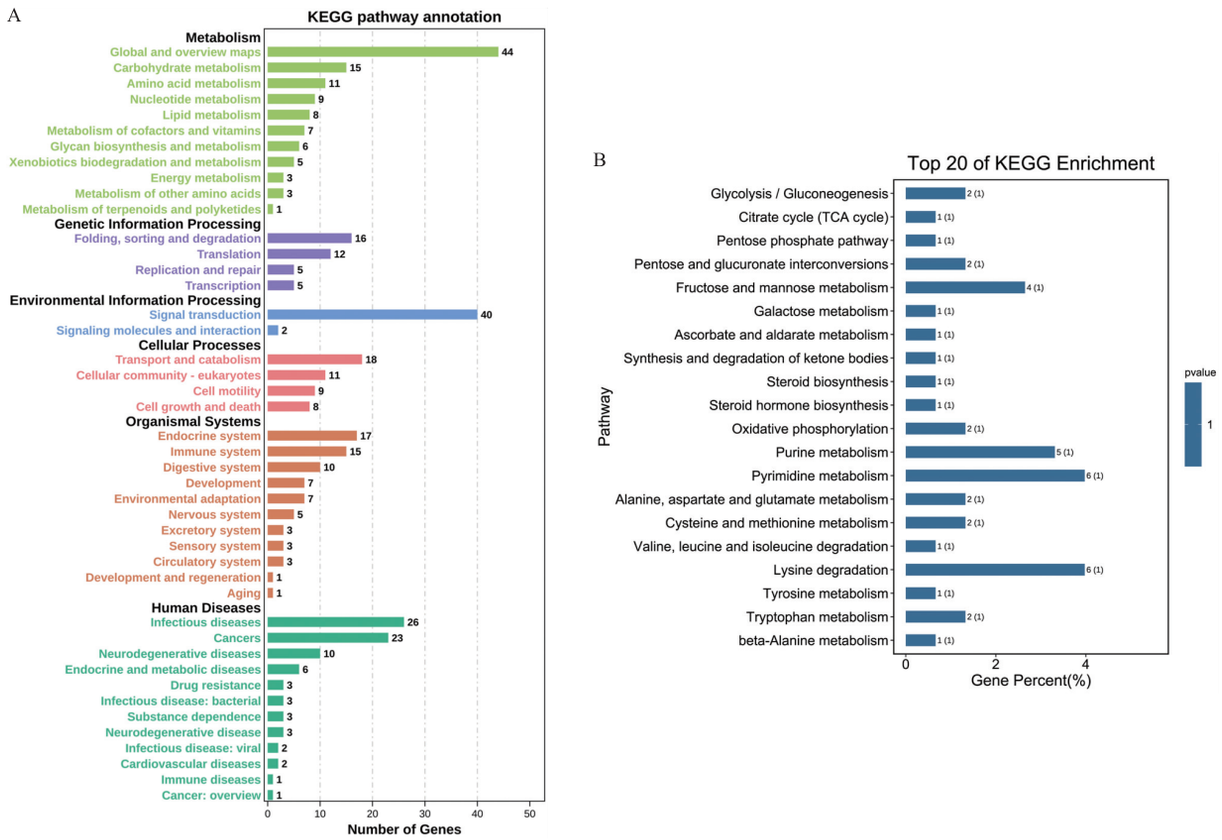
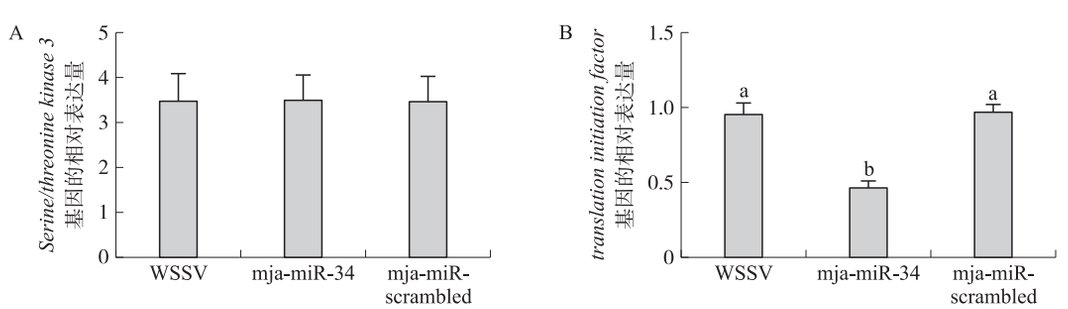


图 3　mja-miR-34 预测靶基因的通路分析

Fig. 3　Pathway analysis of predicted target genes of mja-miR-34



A:mja-miR-34 对 *serine/threonine kinase 3* 基因表达量的影响. B:mja-miR-34 对 *translation initiation factor* 基因表达量的影响. 小写字母表示差异显著(P<0.05).

图 4　mja-miR-34 过表达后,其预测靶基因的表达情况分析

Fig. 4　Expression analysis of predicted target genes of mja-miR-34 after overexpression of mja-miR-34

miRNA 作为基因表达的理想工具,可以在不同程度上同时调控多个靶基因表达. miR-155 在肿瘤中通过调控 *SK*1、*CLDN*-1、*SMAD*2 等发挥抑癌作用[26]. 在乳腺癌中 miR-132-3p 可以通过调控 *N-cadherin*、*Vimentin*、*Snail* 和 *E-cadherin* 的表达抑制乳腺癌上皮间质转化[27]. miR-1290 通过调控 *SMEK*1[28]、*NKD*1[29]和 *LHX*6[30]等基因对肿瘤起作用. Huh7.5.1 细胞感染 JFH-1 病毒 24 h 后,miR-122 的靶基因 *SREBF*1、*FASN* 和 *ACACA* 相对表达量低于未感染 JFH-1 病毒的 Huh7.5.1 细胞,而感染 48 h、72 h、96 h 后,miR-122 靶基因 *SREBF*1、*FASN* 和 *ACACA* 的相对表达量升高并且高于未感染 JFH-1 病毒的 Huh7.5.1 细胞[31]. BHK-21 细胞感染 BEFV 病毒 24 h、48 h 后,miR-3470b 的表达量上升,miR-3470b 的靶基因 *MAVS* 表达量呈负相关[32]. 本研究结果也表明,mja-miR-34 及其靶基因在病毒感染不同时间段呈现差异表达的趋势.

目前 miR-34 靶基因的研究和验证大多集中在高等动物中. 在人类中 miR-34 的靶基因有 *OCT*4[33]、*DLL*1[34]、*SATB*2[35]、*IGFBP*-3[36]等. 在褐飞虱中 miR-34 的靶基因有 *NllnR*1 和 *NllnR*2[37]. 在家蚕中 miR-34 的靶基因有 *BmE74* 和 *BmCPG4*[38]. 在小鼠中,miR-34a 可控制肥胖,主要通过代谢途径调控[39]. 关于小儿

淋巴癌病症研究发现,miR-34a 表达过量可使小儿淋巴癌症状缓解,主要与 *DKK*1 和 *WIF*-1 基因有关[40]. 本研究结果表明,mja-miR-34 可能靶向作用于对虾编码的 242 个宿主基因. mja-miR-34 的靶基因参与细胞代谢、细胞信号转导、免疫系统以及遗传信息的调控. mja-miR-34 与其预测靶基因的初步分析表明 miR-34 真正调控的靶基因还有待进一步探索. 本研究为 mja-miR-34 功能的进一步研究与完善提供了理论基础和研究方向,有助于进一步解释 mja-miR-34 在宿主病毒互作中的作用,为对虾养殖业中 WSSV 病毒的预防提供新的靶点.

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