栖息于青藏高原不同海拔环境的两种草原毛虫 miRNA 转录组的比较分析

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摘要 有关动物适应异质性海拔环境的研究, 已在表型、解剖学、生理学、基因组学和转录组学等方面广泛开展, 但是很少关注小RNA (microRNAs或miRNAs)的生态适应性功能. 草原毛虫是青藏高原高寒草地的重要害虫, 并对不同海拔环境产生了良好的适应性. 为此, 本研究以两种栖息于青藏高原不同海拔环境的黄斑草原毛虫和门源草原毛虫为研究对象, 基于Illumina HiSeq技术对这两种草原毛虫的miRNA转录组进行了高通量测序. 结果表明, 两种草原毛虫中共鉴定到109个成熟miRNAs, 其中47个为保守miRNAs. 在所有miRNAs种, 共有9个miRNAs的表达水平在两种草原毛虫间存在显著差异性. 对这9个差异性表达的miRNAs采用实时荧光定量PCR进行验证, 发现8个基因的表达水平与高通量测序结果完全一致. 功能分析表明, 差异性表达的miRNAs共预测到93个靶基因. 这些靶基因主要富集到脂、核酸和维生素等代谢或衰老相关的GO条目, 并与代谢、抗寒和低氧等KEGG通路相关(如AMPK信号通路、Wnt信号通路和mTOR信号通路). 这些研究表明, miRNAs在草原毛虫对异质性海拔环境的适应性进化过程中具有重要作用. 该研究加深了对青藏高原特有昆虫适应高海拔环境的遗传基础的理解.

关键词 毒蛾亚科, 小RNA, 基因表达, 青藏高原, 高海拔适应

由不同海拔环境造成的生态因子差异, 是动物生态种的重要动力^[1,2]. 动物对不同海拔环境的适应性进化, 不仅表现在编码RNA的序列差异和表达差异上, 也表现在非编码RNA上, 如长链非编码RNA (long

non-coding RNAs, lncRNAs)^[3,4]和小RNA (microRNAs, miRNAs)^[5]. 随着新一代高通量测序技术的发展和日趋成熟, 对多种动物的高海拔适应性进化机制进行了广泛开展, 极大加深了对动物适应分歧海拔环境遗传

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学基础的理解。青藏高原拥有独特的生态和地理特征, 低氧、高寒和强紫外线是其最为显著的生态地理特征; 随着海拔的升高, 生态压力也逐渐增加, 生存在更高海拔环境的动物面临更严峻的生态挑战。为了适应不同海拔的生态环境, 青藏高原土著动物对其栖息地环境, 在解剖学^[6]、表型^[7]、生理^[8]和基因组^[9,10]等方面均表现出一系列适应性特征。近年来, 有关青藏高原不同海拔环境的适应性进化研究, 已在鸟类^[11]、两栖类^[12]、哺乳动物^[13,14]、鱼类^[15]和人类^[16,17]等许多脊椎动物中广泛开展, 而对具有最高物种多样性的昆虫而言还非常少, 仅见飞蝗和草原毛虫的高海拔适应研究^[18-22]。

草原毛虫别名红头黑毛虫、草原毒蛾, 属鳞翅目(Lepidoptera)、毒蛾科(Lymantriidae)、草原毛虫属(*Gynaephora*)。全世界文献记载的草原毛虫属昆虫共15种, 其中分布于我国的草原毛虫共8种, 且全部为青藏高原的特有种。草原毛虫作为我国青藏高原高寒草地的主要害虫之一, 不仅取食优良牧草, 造成家畜食物短缺, 破坏草地植物群落结构, 加剧草地退化和草地生态环境恶化, 而且还会导致家畜中毒, 严重阻碍了青藏高原畜牧业的健康发展。近年来, 草原毛虫在青藏高原频繁爆发成灾, 显示出了其对青藏高原缺氧、严寒、强紫外辐射等极端环境下的良好适应。草原毛虫能够在这种极端生态环境中长期生存, 并成为高寒草地的优势类群, 必定具有适应于栖息地环境的遗传学基础, 而这也是剖析草原毛虫生态适应机制及爆发成灾规律的关键所在^[23,24]。最近, 作者从生理、线粒体基因组和转录组等方面研究了草原毛虫高海拔适应性进化, 且发现基因的序列变异及表达水平与草原毛虫对不同海拔环境的适应性进化密切相关^[18,19,22]。然而, 栖息于不同海拔环境的草原毛虫, 其基因表达水平的变化是否受到miRNAs的调控还不清楚。

miRNAs是一类内源性、长度约为22 nt的非编码RNA, 通过介导靶基因的mRNA降解及抑制翻译, 从而调控动植物的基因表达^[25], 与胚胎发育、组织分化、增殖、神经发生、代谢及细胞凋亡等重要生物学过程密切相关^[26]。目前, 有关miRNAs的研究在动植物中广泛开展, 但主要集中在人类(*Homo sapiens*)^[27,28]、小鼠(*Mus musculus*)^[29-32]、果蝇(*Drosophila melanogaster*)^[33-35]、线虫(*Caenorhabditis elegans*)^[36-39]和拟南芥(*Arabidopsis thaliana*)^[40,41]等模式生物。近年来, 研究者

主要关注miRNA在人类疾病(如肿瘤^[42,43]、心血管疾病^[44]和糖尿病^[45,46]等)、生长发育^[47-51]、生物节律^[34,35]及免疫调节^[52,53]等方面, 而有关miRNA在物种生态适应性方面的研究还很少^[54-56]。对昆虫而言, miRNAs在昆虫生长发育的各个方面发挥重要作用, 如形态发育^[57]、节律行为^[35,58]、嗅觉行为^[59]、生态适应^[54]及抵抗疾病^[60,61]等。最近, 研究者鉴定到了一批低氧诱导相关的miRNAs, 它们广泛参与细胞凋亡^[62]、DNA修复^[63,64]、细胞循环^[65]、血管生成^[66]和代谢调控^[67]。因此, miRNAs极有可能介导草原毛虫对不同海拔环境的生态适应性。

本研究以栖息于青藏高原不同海拔环境的黄斑草原毛虫(*G. alpherakii*, 海拔4800 m)和门源草原毛虫(*G. menyuanensis*, 海拔3000 m)作为研究对象, 运用Illumina HiSeq测序技术对这两种草原毛虫的miRNAs转录组进行高通量测序, 采用生物信息学方法对靶基因进行预测及功能分析, 并对显著差异性表达的miRNAs采用实时荧光定量PCR (real-time quantitative PCR, qRT-PCR)进行验证。研究结果将加深对草原毛虫高海拔适应性进化机制的理解, 也为草原毛虫的有效防控提供理论依据。

1 材料与方法

1.1 研究材料

黄斑草原毛虫和门源草原毛虫分别采自西藏自治区那曲县那曲镇(海拔4800 m, 92°04'E, 31°48'N)和青海省门源县皇城乡(海拔3000 m, 101°19'E, 37°62'N)。为了避免局部环境的差异, 每种草原毛虫的每一虫态分别采集于相同海拔的3个点, 每个点相距至少70 km以上。每种草原毛虫分别采集卵、幼虫(1~6龄)、蛹和成虫等4个虫态, 幼虫龄期的判定采用严林^[68]的头宽比方法。所有虫态经液氮速冻后, 于-80℃保存待用。

1.2 总RNA提取

总RNA的提取用RNA提取试剂盒Rneasy Mini Kit (天根, 北京)提取, 提取流程参照说明书。对两种草原毛虫的不同虫态分别进行总RNA的提取(单头提取), 除卵采用约200粒外, 其余虫态的样本量均为10头。利用核酸浓度测定仪NanoDrop®ND1000 (NanoDrop Technologies, 美国)来检测所提取RNA的纯度和浓度,

并用1%琼脂糖凝胶电泳和Agilent 2100 (安捷伦科技, 美国)生物分析仪来进行RNA的完整性检测; 然后分别将两种草原毛虫各个虫态的总RNA等比例混合为一个混池。

1.3 miRNA文库的构建及测序

两种草原毛虫miRNAs的高通量测序由北京百迈克生物科技有限公司完成。首先, 使用small RNA sample Preparation Kit (Illumina, 美国)构建文库, 利用miRNAs的3'及5'端的特殊结构(5'端有完整的磷酸基团, 3'端有羟基), 以总RNA为起始样品, miRNAs的3'端加上接头, 添加反转录引物, 然后加5'端接头, 反转录合成cDNA。经PCR扩增及8%的PAGE胶电泳(100 V, 80 min), 对长度为140~160 bp (小RNA序列与3'端及5'端接头)的DNA片段进行切胶回收, 从而获得cDNA文库。对草原毛虫的两个miRNA文库, 采用HiSeq 2000平台进行高通量测序。

1.4 miRNA预测及分析

原始reads经低质量序列及接头过滤后, 保留长度为18~30 nt的序列, 从而获得clean reads。将clean reads分别与GenBank和Rfam^[69]数据库进行比对, 获得miRNAs之外的非编码RNA注释信息。排除非miRNAs (如rRNAs, tRNAs, snoRNAs等)的reads, 将剩余reads在miRBase 21.0^[70]中进行序列比对(最多允许两个错配), 预测保守与非保守的miRNAs。Clean reads若在miRBase 21.0能比对上, 则其为保守miRNAs; 其余reads进一步输入到miRDeep2^[71]软件, 采用默认参数预测新miRNAs。

为了筛选出两种草原毛虫差异性表达的miRNAs, 首先对已鉴定到的两种草原毛虫的miRNAs, 基于reads数目对其进行标准化(transcripts per kilobase million, TPM)。由于测序样品未设置生物学重复, 故选择IDEG6^[72]进行差异性表达分析。在鉴定显著差异性表达的miRNAs时, 设置阈值为FDR<0.05且FC (fold change)≥2。

1.5 差异miRNA靶基因的预测及功能分析

目前尚无草原毛虫近缘物种全基因组序列数据, 故利用门源草原毛虫和黄斑草原毛虫混合clean reads (NCBI中的SRA登录号: SRR4242137-38)组装的转录

组^[18]作为预测miRNAs靶基因的参考基因集。使用miRanda软件(适用于预测动物miRNA靶基因)将miRNAs与mRNA的3'端进行比对, 通过计算比对得分和最低自由能进行靶基因的预测(阈值: 分数≥160, 自由能≤-25 kcal/mol)。然后使用BLAST将预测到的靶基因序列和转录组分别与NR, Swiss-Prot, GO, COG和KEGG数据库进行比对, 获得靶基因序列和整个转录组的注释信息。靶基因的GO富集分析采用Blast2GO软件^[73]进行($E\text{-value}=10^{-5}$), 而KEGG富集分析通过KOBAS 2.0^[74]完成。所有富集条目的显著性都进一步通过错误发现率(the false discovery rate, FDR)进行多重矫正, 阈值为FDR<0.05。

1.6 差异性表达miRNA的实时荧光定量PCR验证

为了证实miRNA转录组高通量测序结果的可靠性, 采用qRT-PCR对所有鉴定到的显著差异性表达的miRNAs进行验证。在qRT-PCR分析中, 两种草原毛虫所用RNA与Illumina高通量测序的相同。草原毛虫cDNA的合成采用M-MLV RTase cDNA合成试剂盒 (TaKaRa, 日本)进行, 并根据说明书要求和方法操作。在ABI 7500 Real Time PCR仪(Applied Biosystems, 美国)中使用SYBR Green试剂进行所有qRT-PCR反应。反应体系共10 μL , 包括1.5 μL 的稀释cDNA、5 μL 2 $\times$ SYBR Premix ExTaq II (TaKaRa)、0.2 μL 的ROX参照染料、2.5 μL RNase-free无菌水以及0.4 μL 的上下游引物。所有qRT-PCR引物见表1。反应条件如下: (i) 95 $^{\circ}\text{C}$ 预变性30 s; (ii) 95 $^{\circ}\text{C}$ 变性5 s, 并在60 $^{\circ}\text{C}$ 下退火延伸34 s, 此过程共40个循环; (iii) 在65 $^{\circ}\text{C}$ 下加热PCR产物到95 $^{\circ}\text{C}$, 得到熔解曲线。为了确认扩增的特异性, 每个反应均得到平滑单峰的解离曲线。设置无cDNA模板的反应体系作为阴性对照。qRT-PCR验证miRNAs时, 设置3个生物学重复和3个技术重复。采用 $2^{-\Delta\Delta C_t}$ 方法, 以U6作为内参基因, 计算每一目标基因的相对表达水平。

2 结果与分析

2.1 测序结果概述

黄斑草原毛虫与门源草原毛虫测序分别得到13969120条与15516938条原始标签序列(raw tags)。质量控制后, 黄斑草原毛虫与门源草原毛虫分别得到

表 1 本研究qRT-PCR所用引物信息^{a)}

miRNAs ID	引物名称	引物序列(5'→3')
*gga-miR-1800	Stemloop primer	CTCACAGTACGTTGGTATCCTTGTGATGTTTCGATGCCATATTGTACTGT-GAGTGTCAACA
	Forward primer	ACACTCCAGCTGGGCGTGTTCGATCTG
*hsa-miR-4643	Stemloop primer	CTCACAGTACGTTGGTATCCTTGTGATGTTTCGATGCCATATTGTACTGTGAG-TAAGCAGT
	Forward primer	ACACTCCAGCTGGGTTTGATAAATGACCAGAAC
*hsa-miR-4703-5p	Stemloop primer	CTCACAGTACGTTGGTATCCTTGTGATGTTTCGATGCCATATTGTACTGTGAGCA-GACGCA
	Forward primer	ACACTCCAGCTGGG AGATATTTGTACTG
*dps-miR-2524	Stemloop primer	CTACCAGTACGTTGGTATCCTTGTGATGTTTCGATGCCATATTGTACTGT-GAGTCCCAGAC
	Forward primer	ACACTCCAGCTGGGTACTTGTACTGCGT
*T1_Unigene_BMK.32239_161242	Stemloop primer	CTCACAGTACGTTGGTATCCTTGTGATGTTTCGATGCCATATTGTACTGTGAGA-CACTACC
	Forward primer	ACACTCCAGCTGGGTCGTGGCGTGTCTGG
*CL11922Contig1_116698	Stemloop primer	CTCACAGTACGTTGGTATCCTTGTGATGTTTCGATGCCATATTGTACTGTGAG-CAACAGCC
	Forward primer	ACACTCCAGCTGGGCCAATTACCTGGG
*T2_Unigene_BMK.16405_179238	Stemloop primer	CTCACAGTACGTTGGTATCCTTGTGATGTTTCGATGCCATATTGTACTGT-GAGGTTCTCTCA
	Forward primer	ACACTCCAGCTGGGATCGTGTCTGTGCTTAGTG
*CL16755Contig1_19516	Stemloop primer	CTCACAGTACGTTGGTATCCTTGTGATGTTTCGATGCCATATTGTACTGTGA-GACTGCACA
	Forward primer	ACACTCCAGCTGGGTAGTTCGTGAAAACTG
*CL15826 Contig1_120501	Stemloop primer	CTCACAGTACGTTGGTATCCTTGTGATGTTTCGATGCCATATTGTACTGTGAGA-CACTTCC
	Forward primer	ACACTCCAGCTGGGTTATGCGAATGTAAAAATGG
**U6	Stemloop primer	CTCACAGTACGTTGGTATCCTTGTGTGTGGAACGCTTCACGAT
	Forward primer	AAATTGGAACGATACAGAGAAGATTAGCAT
**Universal reverse primer		CTCACAGTACGTTGGTATCCTTGTG

a) *: 来自于本研究; **: 来自于[75]

10537669条与12037082条高质量标签, 称为clean tags. 将clean tags分别与GenBank和核糖体Rfam数据库比对, 其中rRNA比例低于40%, 表明经过质量控制后的数据适合进行下游分析(具体注释信息见网络版附表1). 在两个样品所有序列种类中, 黄斑草原毛虫和门源草原毛虫共享的序列占58.32%, 门源草原毛虫特有的序列种类比黄斑草原毛虫特有的多5.72% (图1A). miRNAs的长度范围为18~30 nt, 其中最丰富的长度集中在20和22 nt (图1B), 这与其他昆虫的miRNAs长度一致^[76,77].

2.2 保守的与新的miRNAs鉴定及核苷酸偏好分析

共鉴定到109个具有稳定茎环结构的miRNAs, 其中黄斑草原毛虫有97个, 门源草原毛虫有95个; 83个miRNAs为两种草原毛虫共有. 在这109个miRNAs当中, 保守miRNAs为47个, 非保守miRNAs为62个(网络版附表2). 比较所有保守miRNAs发现, 两种草原毛虫中表达量最高的是bmo-miR-750-3p, 其次为bmo-miR-1175, dps-miR-2524, hsa-miR-3122和hsa-miR-4703-5p. 对miRNAs成熟序列的核苷酸偏好性分析发现, 胞嘧啶

图 1 测序结果统计(网络版彩图)

A: 黄斑草原毛虫和门源草原毛虫所有序列中公共及特有序列韦恩图; B: 黄斑草原毛虫和门源草原毛虫两个文库中miRNA长度分布统计

(C)在第一位置的出现频率最高, 其次是尿嘧啶(U), 而腺嘌呤(A)与鸟嘌呤(G)出现的频率极低(网络版附图1). 对于尿嘧啶(U)而言, 最显著的偏差发生在第10, 12, 20和30位的核苷酸(网络版附图1). 这些位点的U偏倚, 可能有助于miRNAs发挥调控作用^[78].

2.3 差异表达miRNAs的鉴定及其qRT-PCR验证

通过比较两种草原毛虫miRNAs文库的测序结果, 并对miRNAs的表达量进行标准化统计分析, 发现共有9个miRNAs呈现显著差异性表达(表2和图2). 与门源草原毛虫相比, 有6个miRNAs在黄斑草原毛虫中表达上调, 而3个表达下调(表2). 这9个差异表达miRNAs中, 除一个miRNA (has-miR-2524)外, 其余8个miRNAs的qRT-PCR结果与高通量测序结果完全一致(图2), 表明miRNA高通量测序结果是可靠的.

2.4 差异miRNAs靶基因预测与功能分析

为了明确miRNAs的功能, 根据两种草原毛虫的转录组, 采用miRanda软件预测miRNAs的候选靶基因. 结果表明, 在所有109个miRNAs中, 有98个miRNAs能够预测到靶基因, 共计2433个靶基因. 在9个显著差异性表达的miRNAs中, 3个miRNAs (CL16755Contig1_19516, CL15826Contig1_120501和CL11922Contig1_116698)没有预测到相应的靶基因. 对显著差异性表达miRNAs的93个靶基因进行功能分析, 发现这些靶基因显著富集到24个GO条目(13个生物学过程, 2个细胞组分和9个分子功能; 表3)和6个KEGG通路(表4).

3 讨论

近几年来, 关于动物对不同海拔适应机制的研究

图 2 差异表达miRNAs qRT-PCR验证结果
黑色表示基因表达上调, 灰色表示基因表达下调

表 2 黄斑草原毛虫和门源草原毛虫差异性表达的miRNAs

miRNA ID	黄斑草原毛虫 reads数	门源草原毛虫 reads数	错误发现率 (FDR)	差异倍数 (log ₂ FC)	表达模式
T1_Unigene_BMK.32239_161241	287107	139862	0	1.0375	Up
dps-miR-2524	1229720	567956	0	1.1144	Up
hsa-miR-4703-5p	480819	236254	0	1.0251	Up
CL15826Contig1_120501	27673	3780	5.51×10 ⁻⁵	2.8719	Up
CL11922Contig1_116698	18205	2984	0.013	2.6089	Up
gga-miR-1800	13836	2835	0.040	2.2870	Up
T2_Unigene_BMK.16405_179238	4150	40068	0	-3.2709	Down
CL16755Contig1_19516	12031	48483	8.68×10 ⁻⁵	-2.0106	Down
hsa-miR-4643	2767	17388	0.010	-2.6515	Down

吸引了不同领域研究者的广泛关注. 目前, 在包括人类在内的许多脊椎动物中已开展了大量且深入的高海拔适应遗传基础研究, 涉及不同的组学水平和尺度. 作者前面的研究表明, 青藏高原草原毛虫的成种过程与高原隆升及其引起的气候环境变化密切相关^[24]. 黄斑草原毛虫和门源草原毛虫亲缘关系较近, 其物种分化于330万年前, 且为青藏高原草原毛虫属中分布在最高

及最低海拔环境的两个物种. 近期, 作者针对这两种草原毛虫的线粒体基因组及转录组分别进行了比较分析, 从分子水平揭示了草原毛虫适对氧含量改变、温度变化及紫外辐射变化等生态环境因子适应的遗传基础^[18,22]. 已有研究表明, miRNAs对动物生态可塑性的形成具有重要作用^[22,79-82], 但是这些研究大多聚焦于脊椎动物, 而对无脊椎动物、特别是昆虫miRNAs的

表 3 差异性表达miRNAs靶基因的GO富集结果

GO_ID	GO类别	GO注释	FDR值
GO:0008150	Biological process	Purine nucleotide biosynthetic process	0.001
GO:0008150	Biological process	Dephosphorylation	0.003
GO:0008150	Biological process	Growth of a germarium-derived egg chamber	0.006
GO:0008150	Biological process	Transport	0.01
GO:0008150	Biological process	Protein oligomerization	0.015
GO:0008150	Biological process	Regulation of synaptic growth at neuromuscular junction	0.015
GO:0008150	Biological process	Homophilic cell adhesion	0.027
GO:0008150	Biological process	Behavioral response to ether	0.034
GO:0008150	Biological process	Phosphorelay signal transduction system	0.034
GO:0008150	Biological process	Negative regulation of hemocyte proliferation	0.034
GO:0008150	Biological process	Learning	0.034
GO:0008150	Biological process	Phosphatidylinositol biosynthetic process	0.034
GO:0008150	Biological process	Wound healing	0.042
GO:0005575	Cellular component	Cullin-RING ubiquitin ligase complex	0.007
GO:0005575	Cellular component	Integral component of membrane	0.047
GO:0005575	Molecular function	Phosphoribosylaminoimidazolecarboxamide formyltransferase activity	0.001
GO:0005575	Molecular function	IMP cyclohydrolase activity	0.001
GO:0005575	Molecular function	Phosphatase activity	0.006
GO:0005575	Molecular function	Ubiquitin protein ligase binding	0.01
GO:0003674	Molecular function	Steroid hormone receptor activity	0.02
GO:0003674	Molecular function	1-phosphatidylinositol 4-kinase activity	0.033
GO:0003674	Molecular function	Galactosyltransferase activity	0.033
GO:0003674	Molecular function	Hydrolase activity, acting on acid anhydrides	0.033
GO:0003674	Molecular function	Phosphorelay sensor kinase activity	0.033

表 4 差异性表达miRNAs靶基因的KEGG富集结果

KEGG通路	KEGG_ID	FDR值
One carbon pool by folate	ko00670	0.005
Inositol phosphate metabolism	ko00562	0.041
Phosphatidylinositol signaling system	ko04070	0.041
Wnt signaling pathway	ko04310	0.046
mTOR signaling pathway	ko04150	0.048
AMPK signaling pathway	ko04152	0.049

生态适应性研究还很少。本研究对两种草原毛虫的miRNAs进行了测序, 鉴定到了109个miRNAs, 这与Liang等人^[83]在小菜蛾中鉴定到的miRNAs数量类似。本研究鉴定出的非保守miRNAs的比例为56.9%, 一方面可能源于目前已知昆虫miRNAs还较为缺乏, 另一方

面也表明昆虫miRNAs具有较高的物种特异性。本研究对9个显著差异性表达的miRNAs进行了qRT-PCR验证, 结果支持高通量测序结果的准确性。

低温、缺氧、强紫外线辐射是青藏高原动物面临的主要生态挑战。为了适应高海拔极端环境, 青藏高原

土著动物在能量代谢、抗紫外辐射、DNA修复和低氧适应等方面表现出了适应性进化特征^[84,85]。在9个显著差异表达的miRNAs中, 仅两个miRNA (gga-miR-1800和T2_Unigene_BMK.16405_179238)在两种草原毛虫中具有较高的差异表达倍数, 且其靶基因具有功能注释。T2_Unigene_BMK.16405_179238在黄斑草原毛虫中下调表达, 且在下调miRNAs中所对应的靶基因最多。根据两种草原毛虫mRNA转录组数据(表5)^[18], 发现miRNA T2_Unigene_BMK.16405_179238及其靶基因的表达量在黄斑草原毛虫中均显著下调, 仅两个靶基因(CL12661Contig1与CL15031Contig1)为双向表达。GO富集分析发现, T2_Unigene_BMK.16405_179238的靶基因, 主要与能量代谢及信号转导相关性; 例如, “Rab GTPase activator activity”, “ATP binding”和“Signal transducer activity”等。对草原毛虫及其他青藏高原动物的高海拔适应性研究, 筛选到的目标基因也富集得到了相同的GO条目^[15,18,85]。值得注意的是, 靶基因T1_Unigene_BMK.21587和T2_Unigene_BMK.21675的GO功能均与泛素-蛋白连接酶相关。泛素-蛋白连接酶又称E3泛素连接酶, 能够将泛素分子连接到目的蛋白质并使其降解, 对细胞周期调控、信

号转导、DNA修复和胞吞作用等过程有重要作用^[68]。研究发现, smurf2作为一种泛素连接酶可控制组织的纤维化, 其中miR-132能调控cAMP-PKA-CREB这一信号上调, 使得smurf2与磷酸二酯酶4B相互作用激活该通路, 促进其纤维化组织降解^[86]。因此, 推测该miRNA (T2_Unigene_BMK.16405_179238)可能调控着与草原毛虫差异性海拔适应相关的一系列关键基因。

在黄斑草原毛虫中呈上调表达的一个miRNA (gga-miR-1800), 其靶基因主要涉及酶活性的改变, 生物学过程主要涉及蛋白修饰。这表明, 酶活和蛋白修饰的改变是可能是草原毛虫适应异质性高海拔环境的遗传基础。

GO富集分析发现, 差异表达miRNAs靶标基因主要涉及生物功能活性方面, 如催化活性、运输活性、信号转导活性等, 表明这些生物功能与草原毛虫适应差异性高海拔环境有密切关系。此外, 其靶基因参与的信号通路主要是蛋白的修饰、翻译以及信号转导方面。推测这些信号通路可能是草原毛虫高海拔适应的关键, 也是今后解析其信号通路适应信号的关键备选。KEGG富集分析发现, 差异表达miRNA靶基因显著富集到一些已知典型的能量代谢及低氧适应相关信号通

表 5 一个非保守miRNA (T2_Unigene_BMK.16405_179238)靶基因注释

靶基因ID	GO注释	FDR值	表达模式
CL12661Contig1	Molecular function: structural constituent of ribosome (GO:0003735)	0	Up, Down
CL15031Contig1	Molecular function: signal transducer activity (GO:0004871)	0	Up, Down
T1_Unigene_BMK.4958	Molecular function: Rab GTPase activator activity (GO:0005097)	0	Down
T1_Unigene_BMK.21587	Molecular function: ubiquitin protein ligase binding (GO:0031625)	0	Down
T2_Unigene_BMK.21675	Molecular function: ubiquitin protein ligase binding (GO:0031625)	0	Down
T2_Unigene_BMK.11658	Molecular function: phosphatase activity (GO:0016791)	0	Down
T2_Unigene_BMK.16405	Molecular function: calmodulin-dependent protein kinase activity (GO:0004683)	0	Down
CL1327Contig1	Molecular function: ATP binding (GO:0005524)	0	Down
CL31Contig4	Molecular function: protein tyrosine kinase activity (GO:0004713)	0	Down

表 6 一个保守miRNA(gga-miR-1800)靶基因的功能注释

靶基因ID	GO注释	FDR值	表达模式
CL260Contig1	Molecular function: metalloendopeptidase activity (GO:0004222)	0.040	Up
	Biological process: membrane protein ectodomain proteolysis (GO:0006509)		
	Molecular function: identical protein binding (GO:0042802)		
	Biological process: protein oligomerization (GO:0051259)		

路. mTOR作为细胞营养感应和能量调节因子,通过调控其下游的信号通路从而调节很多生理过程,且mTOR与AMPK可在细胞内交互调控能量平衡,维持细胞的正常生理活动^[87]. 缺氧诱导因子1 (hypoxia inducible factor-1, HIF-1)是一种哺乳动物细胞中广泛表达的调控因子,可调节细胞对低氧或缺氧状态的适应. Dolt等人^[88]对牦牛高海拔适应的研究中,克隆了HIF-1基因并用反转录PCR技术验证了该基因在牦牛适应高海拔低氧环境中的重要作用. 研究表明,MAPK, mTOR及P13K-akt等信号通路能调节HIF-1及血管内皮生长因子的表达,间接调节缺氧诱导的血管生成、红细胞生成及血管舒张,从而促进东喜马拉雅大山雀对高海拔低氧环境的适应^[89]. AMPK,即AMP依赖的蛋白激酶,是生物能量代谢调节的关键分子^[90]. 缺氧、葡萄糖缺乏等条件下生物体内的ATP的水平降低,AMP与ATP的比值增加可激活AMP,从而关闭消耗ATP合成途径并启动产生ATP的分解代谢途径. 特别

是,缺氧时快速激活AMPK,从而促进HIF-1的表达,启动缺氧的代谢适应^[91]. Wnt通路的关键组成Wnt/ β -Catenin,广泛参与低氧诱导的一些疾病的发生^[92]. 在前面作者对草原毛虫的高海拔适应性研究中,也鉴定到了mTOR通路^[18]. 本研究从miRNA的角度同样发现该通路,表明mTOR通路在草原毛虫异质性高海拔环境的生态适应中发挥重要作用. 综上所述,miRNAs通过调控Wnt, AMPK及mTOR等能量代谢及低氧适应相关的信号通路,从而介导了草原毛虫对异质性海拔环境的生态适应性.

本研究发现了一些与草原毛虫适应分歧海拔环境的miRNA,但其生理生态功能仍有待进一步研究. 今后,可基于降解组测序技术,对miRNA的靶标基因进行证实,以明确mRNA-miRNA之间的关系;结合不同层次的高通量测序结果,利用正反向遗传学手段,过表达或沉默miRNAs,进一步明确miRNAs在草原毛虫生长发育及生态适应中的作用.

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Comparative analysis of the microRNA transcriptomes between two *Gynaephora* species (Lepidoptera: Lymantriinae) inhabiting different high altitudes of the Tibetan Plateau

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Divergent-altitude adaptation in animals has been extensively investigated at various levels, such as morphology, anatomy, physiology, genomics and transcriptomics, but few studies focused on the roles of microRNA (miRNA) in ecological adaptation. Grassland caterpillars (*Gynaephora*) endemic to the Tibet Plateau (TP) are important insect pests of alpine grasslands and have well adapted to different altitude environments. In this study, we used Illumina HiSeq technique to sequence miRNA transcriptomes of *Gynaephora menyuanensis* and *Gynaephora alpherakii*. These two *Gynaephora* species are locally distributed in different altitudes. A total of 109 mature miRNAs were identified in the two species, of which 47 conserved miRNAs were determined. Nine miRNAs with significantly differentiated expression were presented between the two *Gynaephora* species, among which eight miRNAs showed the same expression patterns between the Illumina HiSeq and quantitative real-time PCR results. Functional analyses for 93 target genes of differentially expressed miRNAs revealed that these targets were enriched to GO terms involving metabolism of lipid, nucleic acid, vitamin and aging, and several KEGG associated with metabolic, cold- and hypoxia-related pathways, such as the AMPK signaling pathway, the Wnt signaling pathway and the mTOR signaling pathway. These results indicated the important roles of miRNAs in the adaptive evolution of *Gynaephora* species to different altitudes and provide insights into the genetic basis of high-altitude adaptation of insects endemic to the TP.

Lymantriinae, microRNAs, gene expression, Tibetan Plateau, high-altitude adaptation

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