

# 调控过氧化物酶体生物合成和增殖的 microRNA 的计算机分析

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**摘要** microRNA 是真核生物中一大类参与基因转录后水平调控的长度为 20~24 个核苷酸的非编码小分子 RNA, 它们在生物体的细胞增殖、细胞分化、细胞凋亡、形态建成、个体发育、激素分泌与信号转导等一系列生命活动中起重要作用。现以小鼠调控过氧化物酶体生物合成和增殖的蛋白质基因为靶基因, 采用生物信息学方法搜寻到了调控这些靶基因表达且在系统发育上保守的 microRNA, 为进一步鉴定参与过氧化物酶体生物合成和增殖的 microRNA, 阐明 microRNA 在过氧化物酶体的生物合成和增殖中的作用和调控机制提供有价值的信息。

**关键词** 过氧化物酶体; 生物合成; microRNA; 计算机分析

MicroRNA 是真核生物中一类由内源基因编码的长度为 20~24 个核苷酸的非编码单链 RNA 分子<sup>[1,2]</sup>。自 1993 年 Lee 等<sup>[3]</sup>在秀丽新小杆线虫(*C.elegans*)中发现了第一个与发育时序调控相关的 microRNA *lin-4* 以来, 人们已在动植物及病毒中发现了 8 619 个 miRNA 分子(v12.0, <http://microrna.sanger.ac.uk>)。这些 microRNA 在转录后水平上通过介导 mRNA 靶分子的切割或抑制靶分子的翻译来调节基因的表达, 从而调控生物体器官的形态建成、细胞增殖、细胞分化、细胞凋亡、个体发育、激素分泌与信号转导等一系列重要的生命活动<sup>[4]</sup>。近年来, 人们还发现 microRNA 的表达与人类某些疾病有密切联系, 例如, microRNA 在肿瘤形成<sup>[5,6]</sup>和神经疾病的发生<sup>[7,8]</sup>中都起着调控作用。因此, 揭示 microRNA 介导的调控网络将为人类疾病的诊断和治疗提供全新的视野。

在目前已发现的 8 619 个 microRNA 分子中, 人们采用生物信息学的方法预测了其中部分 microRNA 的靶基因。最近, 我们在对真核生物的一些 microRNA 的靶基因进行研究时发现, 与哺乳动物的过氧化物酶体生物合成和增殖相关的基因也是 microRNA 的靶基因。过氧化物酶体广泛存在于真核生物中, 它行使脂肪酸的  $\beta$ -氧化、分解过氧化氢等毒性物质和抗氧化胁迫等功能<sup>[9,10]</sup>。过氧化物酶体具有重要的生物学功能, 人类有些疾病, 如脑肝肾综合征和新生儿脑白质营养不良等就是由于过氧化物酶体生物合成基因(peroxisome biogenesis, PEX)的突变而导致过氧化物酶体生物合成缺陷(peroxisome biogenesis disorders,

PBDs)引起的<sup>[11,12]</sup>。此外, 过氧化物酶体增殖物激活受体(peroxisome proliferator-activated receptors, PPAR)在不饱和脂肪酸和某些药物激活下导致动物肝脏过氧化物酶体数量和体积增加。PPAR 不仅参与过氧化物酶体的增殖, 它们也参与脂类代谢、线粒体代谢、细胞分化、细胞周期和细胞凋亡的调节<sup>[11,13]</sup>, 可见过氧化物酶体的代谢与生物的其他代谢存在着密切的联系。目前, microRNA 在过氧化物酶体的生物合成和增殖中的作用和表达机制还不是很清楚, 本文以小鼠调控过氧化物酶体生物合成和增殖的蛋白质基因为靶基因, 采用生物信息学方法搜寻调控这些靶基因表达的 microRNA, 为进一步系统研究 microRNA 在过氧化物酶体的生物合成和增殖中的作用和表达机制提供依据。

## 1 材料与方法

本文所使用的 microRNA 靶位点分析软件, 其算法都是基于 miRNA 与其靶分子 3'UTR 之间存在的互补性, 重点关注 miRNA 5'端 2~8 碱基与靶位点之间的互补性, 以及 miRNA 与其靶位点之间的热力学关系。寻找到 miRNA 的候选靶位点后, 再分析靶位点在进化上的保守性。

### 1.1 microRNA、PEX 和 PPAR 基因 3'UTR 序

收稿日期: 2008-11-05 接受日期: 2009-02-03

国家自然科学基金(No.30660042)和江西省自然科学基金(No.0630137)资助项目

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**Table 1 Base-pairing of miRNAs and their targets predicted by four softwares**

microRNA:mRNA	microRNAs targets	microRNA:mRNA	microRNAs targets
GUC-UGAGGCCACCUUACUUCu 5'     :   :	miR-205	cgccUUGAAUCGGUGACACU 5'      :	miR-27a
CAGTGCTGTGATGG-ATGAAGGc 3'	PEX19	acctAAGAAATTTACTGTGAA 3'	PPARG
cgucUUGAAUCGGUGACACU 5'      :	miR-27b	uuucucuggccAAGUGACACU 5'   :	miR-128a
acctAAGAAATTTACTGTGAA 3'	PPARG	cacctaagaaaTTACTGTGA 3'	PPARG

**Table 2 Base-pairing of miRNAs and their targets predicted by three softwares**

microRNA:mRNA	microRNAs targets	microRNA:mRNA	microRNAs targets
GU-GUUUGGUA--UA-CACGACGAu 5'      :	miR-15a	gCGGUUAUAAAUGCACGACGAu 5'         :	miR-16
CAUCAGGCCUUUCUUGGUGUGCUu 3' aUUGGCUAAAGUCUACCACGAU 5'  :            :	PEX5	gGCCUUUCUUG-GUGCUGCUu 3'	PEX5
cAGGCCUUUC--UUGGUGCUG 3'	miR-29a	uugugacuAAAGUUUACCACGAU 5'          :	miR-29b
GUCGAUACGGUCGU-AGAACGGa 5'        :	PEX5	ucaggcccUUUC--UUGGUGCUG 3'	PEX5
CAG--AAGUU-GCAUUCUUGCCa 3'	miR-31	cgguuauAAAGA--CACGACGAu 5' 	miR-195
GAUGGUA-UCCAU-UUUGGUGAC 5'                  :	PEX5	caggcccUUUCUUGGUGUGCUu 3'	PEX5
CUAGCAUCAGAGCACUAACUACUG 3'	miR-140	AGUGUCAACGGUCGACUCUAu 5'  :        :	miR-216a
UCUCCGUCCGUGA--GCCCGUCUGu 5'        :	PEX7	UUACA--UG-UA-CUGAGAUu 3'	PEX7
AGAAAGAGAUACUGACUGGCAGACu 3'	miR-346	cAUGGGGACCUCU-AAGACUAUu 5'     :     :	miR-361
cGCGGGUUA-AUU--ACA-GACAACUA 5' : :	PEX7	uUACAUUUUAGGCAUCUGAUAA 3'	PEX7
aUGUACAAUAUACCACUCCUGUUGAU 3' guguuuGGUAAU-ACACGACGAU 5'      :	miR-421	cGACGGUAUAUACCCACAGUAa 5'     :	miR-489
cugugcCGAGUGUUCUGCUGCUA 3'	PEX7	uCUGAUAAAGACAUUGUGUCAu 3' ACAUUUGGUACUACACGACGAU 5'    : :	PEX7
GCGGUUAUAAAUGCACGACGAU 5' :   :  :	miR-15a	UGUGCCGAGUGUUCUGCUGCUA 3'	PEX13
UGCCGA-GUGUUC-UGCUGCUA 3'	miR-16	CGGUUAUAAAAGACACGACGAU 5'    :	miR-195
ucacuUAAGAU-GGUCACGGUAU 5' 	PEX13	GCCGAGUGUUC--UGCUGCUA 3'	PEX13
aucccAUUCAAGACUGUGCCAUa 3'	miR-183	gAUGGACGUGACAUU--CGUGAAAc 5' 	miR-17
gAUGGAC--GUGA-UAUUCGUGAAAu 5' 	PEX19	aUCCCGAAAACUAAUCUGCACUUu 3'	PPARA
aUCCCGAAAACUAAUCUGCACUUu 3'	miR-20a	gAUGGAC--GUGA-UACUCGUGAAAc 5' 	miR-20b
gAUGGAC--GUGCUU-GUCGUGAAAc 5' 	miR-93	aUCCCGAAAACUAAUCUGCACUUu 3'	PPARA
aUCCCGAAAACUAAUCUGCACUUu 3'	miR-291b-3p	gAUGGAC--GUGA-CAAUCGUGAAAc 5' 	miR-106a
ugUUUGUUUACCUACGUGAAA 5' 	PPARA	aUCCCGAAAACUAAUCUGCACUUu 3'	PPARA
gaAAAC-UAAU--CUGCACUUU 3'	miR-130b	uUUCUCUGCCAAGUGACACU 5'         :	miR-128b
uacGGGAAAGUAG-UAACGUGAc 5' :    :  :	PPARA	uAAGAAA---UUUACUGUGA 3'	PPARG
tgtTCCTTCTATTGATTGCACTa 3'	PPARG	CGAAACUGUUAUGG-UAACGUGAc 5'  :    :   :	miR-301
		GTGTTCTTCTATTGATTGCACTa 3'	PPARG

Table 3 Base-pairing of miRNAs and their targets predicted by two softwares

microRNA:mRNA	microRNAs targets	microRNA:mRNA	microRNAs targets
cGACUCUCACAUCCUACAAUgu 5' 	miR-30c	AAGUCAUAGUGUCAUGACAU 5' 	miR-101a
aCTCTGAAATTAGGATGTTTAc 3' GCGCAUG-GU-UU-UCAUUUUUAc 5' 	PEX3 miR-126-5p	UUCAUUUAGCACC UUACUGUA 3' UGUGGCCUCCUCGGUAGUACUA 5'   :      :	PEX3 miR-433
CGCUUACUCACAAUUUUAAUUu 3' UCCCUAAGG-ACCCUU-UUGACCUg 5'        :    :  :	PEX3 miR-145	ACATC-AGGA-TCAATCATGAT 3' UUGAUUUGUAGAUGAGGAGu 5'         :     :	PEX3 let-7f
AGGGATTCTGAGGGAGCAGCTGGAg 3' UGUUAGUCGAUUAAUGUGACGGA 5'          :   :	PEX5 miR-34b-5p	AAATAAAGTGTACTGCCTC 3' gUUAGUCGAUUGUGACGGA 5'        :   :	PEX6 miR-34c
AAAATAAAGTG-TATACTGCCT 3' CCUGUGUU-CUAGUACUUUCUCGU 5'      :      : :	PEX6 miR-469	aaATAAAGTG-TATACTGCCT 3' ACUUA-AUGAAACAUUUGGUGAu 5'  :	PEX6 miR-876
GGCCCTAGTGCTCAGGAGGAGGCA 3' ugUCAAG-AAGUUGACCGUCGAA 5'    :        :	PEX6 miR-22	TGGATGTACAATAT-AACCACTc 3' CUCGAUGUCACGAAGUAGAGU 5'       :     :	PEX7 miR-143
tgAGTTTGCTCAACTGCCAGTTT 3' UUUCUAGU-UGUACAUAAUa 5'   :      :	PEX12 miR-369-3p	GAGCT-TAGTGCATTATCTCA 3' gACGUCAUGACAAGGGCGACGAU 5'            :	PEX12 miR-503
ATGAATCATGCTAGGTATTATg 3' CUACCGUCACCUCUAUCACUAA 5'     :	PEX12 miR-34b-3p	gUGCCG-AGUGUU-CUGCUGCUA 3' cAGAGGUC-UGGGGAUCAAGUCC 5'     :   :   :	PEX13 miR-345-3p
GATATAGCTGATGTTAGTGATT 3' aGUCAAAACGUUAUCUAAACGUGU 5'         :	PEX19 miR-19a	tTCCCTGGAATCCTTGGTTCAGG 3' aGUCAAAACGUACC UAAACGUGU 5' 	PEX19 miR-19b
aCAGGAGAGCAGGGATTGCACA 3' AGUUGUAGUCAGACUAUUCGAU 5'   :    :  :	PPARA miR-21	aCAGGAGAGCAGGGATTGCACA 3' AAGGGGAAAUAACGUGAc 5'    :       :	PPARA miR-721
TCGAGAATAGTTTGATAAGCTA 3'	PPARA	TTCCTTCTATTGATTGCACTa 3'	PPARG

## 列的获得

从 miRBase 数据库(<http://microrna.sanger.ac.uk/cgi-bin/sequences/browse.pl>)中获得小鼠的 microRNA 前体与成熟序列。从 GenBank 数据库获得小鼠、大鼠、人、狗等 4 个物种的 PEX 和 PPAR 基因 3'UTR 序列。

### 1.2 miRNA 靶位点的确定

从 miRBase 数据库中选择在小鼠、大鼠、人、狗等 4 个物种都存在的 microRNA, 采用 miRanda<sup>[14]</sup>、TargetScan<sup>[15]</sup>、PicTar<sup>[16]</sup>和 miRBase Targets 等 4 种 microRNA 靶位点分析软件在线分析 miRNA 的靶位点。选取那些至少用两种软件中都能预测到的靶位点及其对应的 microRNA<sup>[17]</sup>, 然后分析靶位点的保守性, 筛选出在上述 4 个物种中均保守的靶序列。

## 2 结果

### 2.1 靶基因分析

采用四种 microRNA 靶位点分析软件对小鼠中影响过氧化物酶体生物合成和增殖的 11 个 PEX 基因 (PEX1、PEX2、PEX3、PEX5、PEX6、PEX7、PEX10、PEX12、PEX13、PEX16、PEX19) 和 3 个 PPAR 基因 (PPARA、PPARG、PPARD) 转录产物 mRNA 的 3'UTR 进行分析, 寻找形成 microRNA:mRNA 匹配区的区域。至少用两种软件能够同时检测到的 microRNA:mRNA 匹配区共 129 个。其中用四种软件能同时检测到的 microRNA:mRNA 匹配区有 4 个 (表 1), 三种软件都能检测到的 26 个 (表 2), 其余是用两种软件能检测到的 microRNA:mRNA 匹配区 (部分用两种软件能检测到的 microRNA 与 mRNA 形成的匹配区见表 3)。这些 miRNA 与 PEX1 形成的匹配区 4 个, 与 PEX3 形成的 18 个, 与 PEX5 形成的 22 个, 与

Table 4 Base-pairing of conserved microRNAs and their targets

Target gene	microRNA		Alignment of targets
PEX5	mmu-miR-29a	miR-29a	UUGGCUAAAGUCUACCACGAU :
		Mm	---CAU-CAGGCCUUUCU---UGGUGCUGCU
		Hs	---CAU-CAUGCCUUUCU---UGGUGCUGCU
		Rn	---CAU-CAUGCCUUUCU---UGGUGCUGCU
		Cf	CAUCAUGCCUGCCUUUCU---UGGUGCUGCU
		Consensus	*** * ***** *****
PEX5	mmu-miR-29c	miR-29c	UGGCUAAAGUUUACCACGAU :
		Mm	---CAU-CAGGCCUUUCU---UGGUGCUGCU
		Hs	---CAU-CAUGCCUUUCU---UGGUGCUGCU
		Rn	---CAU-CAUGCCUUUCU---UGGUGCUGCU
		Cf	CAUCAUGCCUGCCUUUCU---UGGUGCUGCU
		Consensus	*** * ***** *****
PEX5	mmu-miR-29b	miR-29b	UUGUGGCUAAAGUCUACCACGAU 
		Mm	---CAU-CAGGCCUUUCU---UGGUGCUGCU
		Hs	---CAU-CAUGCCUUUCU---UGGUGCUGCU
		Rn	---CAU-CAUGCCUUUCU---UGGUGCUGCU
		Cf	CAUCAUGCCUGCCUUUCU---UGGUGCUGCU
		Consensus	*** * ***** *****
PEX5	mmu-miR-195	miR-195	CGGUUAUAAAGA---CACGACGAU 
		Mm	---CAU-CAGGCCUUUCUUGGUGCUGCU
		Hs	---CAU-CAUGCCUUUCUUGGUGCUGCU
		Rn	---CAU-CAUGCCUUUCUUGGUGCUGCU
		Cf	CAUCAUGCCUGCCUUUCUUGGUGCUGCU
		Consensus	*** * *****
PEX5	mmu-miR-16	miR-16	G-CGGUUAUAAUAGC---ACGACGAU 
		Mm	---CAU-CAGGCCUUUCUUGGUGCUGCU
		Hs	---CAU-CAUGCCUUUCUUGGUGCUGCU
		Rn	---CAU-CAUGCCUUUCUUGGUGCUGCU
		Cf	CAUCAUGCCUGCCUUUCUUGGUGCUGCU
		Consensus	*** * *****
PEX5	mmu-miR-497	miR-497	AUGUUUGUGUCA-C-ACGACGAC : :     :
		Mm	---CAU-CAGGCCUUUCUUGGUGCUGCU
		Hs	---CAU-CAUGCCUUUCUUGGUGCUGCU
		Rn	---CAU-CAUGCCUUUCUUGGUGCUGCU
		Cf	CAUCAUGCCUGCCUUUCUUGGUGCUGCU
		Consensus	*** * *****
PEX5	mmu-miR-15a	miR-15a	GUGUUUGUAAUA-C-ACGACGAU     : :
		Mm	---CAUCA-GGCCUUUCUUGGUGCUGCU
		Hs	---CAU-CAUGCCUUUCUUGGUGCUGCU
		Rn	---CAU-CAUGCCUUUCUUGGUGCUGCU
		Cf	CAUCAUGCCUGCCUUUCUUGGUGCUGCU
		Consensus	*** * *****
PEX5	mmu-miR-107	miR-107	acuaUCGGGACA-UGUUACGACGA 
		Mm	---CAU-CAGGCCUUUCUUGGUGCUGCU
		Hs	---CAU-CAUGCCUUUCUUGGUGCUGCU
		Rn	---CAU-CAUGCCUUUCUUGGUGCUGCU
		Cf	CAUCAUGCCUGCCUUUCUUGGUGCUGCU
		Consensus	*** * *****
PEX5	mmu-let-7a	Let-7a	UUGAUUGUUGGA-----UGAUGGAGU   :   :     :
		Mm	CAAGCGGUACGACCUCUCAGGAGCUGCCUCA
		Hs	UAAGCGGUACGCCUUUCAGGAGCUGCCUCA
		Rn	UAAGCGGUACGACCUCUCAGGAGCUGCCUCA
		Cf	CAAGCGGUGUGCCUCUCAGGAGCUGCCUCA
		Consensus	***** * ** *****

Target gene	microRNA	Alignment of targets
PEX5	mmu-let-7b	<p>Let-7b UUGGUGUGUUGGAUGAUGGAGU            :        : :   :       </p> <p>Mm CAAGCGGUACGACCUCUCAG-GAGCUGCCUCA            Hs UAAGCGGUACGGCCUUUCAG-GAGCUGCCUCA            Rn UAAGCGGUACGACCUCUCAG-GAGCUGCCUCA            Cf CAAGCGGUGUGCCUCUCAG-GAGCUGCCUCA            Consensus ***** * *** *****</p>
PEX7	mmu-miR-542-3p	<p>miR-542-3p AAAGUCAUAGUUAGACAGUGU              :       :                </p> <p>Mm UAUUUUUUGUUAUUUUUUUGUCAUUU            Hs UAUUUUUCAUUUUUUUUUUUGUCAUUU            Rn UAUUUUUUAUCAUUUAUUUAUCAUUU            Cf CAUUUUUUUUUUUUUUUUUGUCAUUU            Consensus ***** * *****</p>
PEX13	mmu-miR-497	<p>miR-497 AUGUUUGGUGU-CAC-ACGACGAC            : :   : :                </p> <p>Mm UCGUGGCUGUGCCGAGUGUUCUGCUGCUAG            Hs UAUUGGCUAUUUCAGGUGUUUUGCUGCUAG            Rn UCAUGGCUGUCCGGGCGUUCUGCUGCUAG            Cf UAAUGGCUAUUCAGGUGUUUUGCUGCUAG            Consensus * ***** * * * * * *****</p>
PEX13	mmu-miR-15a	<p>miR-15a GUGUUUGGU-AAUACACGACGAU            : :   :                </p> <p>Mm UCGUGGCUGUGCCGAGUGUUCUGCUGCUAG            Hs UAUUGGCUAUUUCAGGUGUUUUGCUGCUAG            Rn UCAUGGCUGUCCGGGCGUUCUGCUGCUAG            Cf UAAUGGCUAUUCAGGUGUUUUGCUGCUAG            Consensus * ***** * * * * * *****</p>
PEX13	mmu-miR-15b	<p>miR-15b ACAUUUGGUAC-UAC-ACGACGAU              : :   :                </p> <p>Mm UCGUGGCUGUGCCGAGUGUUCUGCUGCUAG            Hs UAUUGGCUAUUUCAGGUGUUUUGCUGCUAG            Rn UCAUGGCUGUCCGGGCGUUCUGCUGCUAG            Cf UAAUGGCUAUUCAGGUGUUUUGCUGCUAG            Consensus * ***** * * * * * *****</p>
PEX13	mmu-miR-16	<p>miR-16 GCGUUAUAAAUGCACGACGAU            :     :                    </p> <p>Mm UCGUGGCUGUGCCGAGUGUUC-UGCUGCUAG            Hs UAUUGGCUAUUUCAGGUGUUU-UGCUGCUAG            Rn UCAUGGCUGUCCGGGCGUUC-UGCUGCUAG            Cf UAAUGGCUAUUCAGGUGUUU-UGCUGCUAG            Consensus * ***** * * * * * *****</p>
PEX13	mmu-miR-195	<p>miR-195 CGGUUAUAAAGACACGACGAU                  :                    </p> <p>Mm UCGUGGCUGUGCCGAGUGUUC-UGCUGCUAG            Hs UAUUGGCUAUUUCAGGUGUUU-UGCUGCUAG            Rn UCAUGGCUGUCCGGGCGUUC-UGCUGCUAG            Cf UAAUGGCUAUUCAGGUGUUU-UGCUGCUAG            Consensus * ***** * * * * * *****</p>
PEX13	mmu-miR-424	<p>miR-424 AGGUUUUGUACUUAACGACGAC              : : :                    </p> <p>Mm UCGUGGCUGUGCCGAGUGUUCUGCUGCUAG            Hs UAUUGGCUAUUUCAGGUGUUUUGCUGCUAG            Rn UCAUGGCUGUCCGGGCGUUCUGCUGCUAG            Cf UAAUGGCUAUUCAGGUGUUUUGCUGCUAG            Consensus * ***** * * * * * *****</p>
PEX13	mmu-miR-503	<p>miR-503 gACGUCAUGACAAGGGCAGCAU                            :              </p> <p>Mm UCGUGGCUGUGCCG-AGUGUU-CUGCUGCUAG            Hs UAUUGGCUAUUUCA-GGUGUU-UUGCUGCUAG            Rn UCAUGGCUGUCCCG-GGCGUU-CUGCUGCUAG            Cf UAAUGGCUAUCCA-GGUGUU-UUGCUGCUAG            Consensus * ***** * * * * * *****</p>

Target gene	microRNA		Alignment of targets
PEX19	mmu-miR-183	miR-183	GUCACUUAAGAU-GGUCACGGUAU       
		Mm	CCAUCCCAUUAAGACUGUGCCAUA 
		Hs	UCAUCCCAUUAAGAUUGUGCCAUA 
		Rn	ACAUCCCAUUAAGACUGUGCCAUA 
		Cf	UCAUCCAGCUGAGAUCGUGCCAUA 
		Consensus	***** ** *****
PPARA	mmu-miR-93	miR-93	GAUGGACGUGCUUGU-CGUGAAAC   :     :
		Mm	UUAAAUCCUGAAAACUAAUCUGCACUUUU     :
		Hs	AUAUAAUUCUGAAAACUAAUCAGCACUUUU     :
		Rn	UAUAAGUCUUGAAAACUAAUCUGCACUUUA     :
		Cf	GUAUCGUUUUGAAAACUAAUCAGCACUUUU     :
		Consensus	** ***** *****
PPARA	mmu-miR-19a	miR-19a	AGUCAAAACGUAUCU--AAACGUGU       :
		Mm	AUACAGGAGAGCAGGGA-UUUGCACA       :
		Hs	--ACAGGAGAGCAGGGAUUUUGCACA       :
		Rn	AUACAGGAGAGCAGGGA-UUUGCACA       :
		Cf	--CUGGAGCAAUUGCCUGCUUUGCACA       :
		Consensus	**** * *****
PPARA	mmu-miR-19b	miR-19b	AGUCAAAACGUACCU--AAACGUGU       :
		Mm	AUACAGGAGAGCAGGGA-UUUGCACA       :
		Hs	--ACAGGAGAGCAGGGAUUUUGCACA       :
		Rn	AUACAGGAGAGCAGGGA-UUUGCACA       :
		Cf	--CUGGAGCAAUUGCCUGCUUUGCACA       :
		Consensus	**** * *****
PPARG	mmu-miR-130a	miR-130a	UACGGAAAAUUG-UAACGUGAC :           :
		Mm	CGUGUCCUUCUUAUUGAUUGCACUA           :
		Hs	CAUUUCCUUCUUCAGUUGCACUA           :
		Rn	AGUGUCCUUCUUAUCGAUUGCACUA           :
		Cf	CGUUUCCUUCUUCGGAUUGCACUA           :
		Consensus	* * ***** *****
PPARG	mmu-miR-130b	miR-130b	UACGGAAAAGUAG-UAACGUGAC :           :
		Mm	CGUGUCCUUCUUAUUGAUUGCACUA           :
		Hs	CAUUUCCUUCUUCAGUUGCACUA           :
		Rn	AGUGUCCUUCUUAUCGAUUGCACUA           :
		Cf	CGUUUCCUUCUUCGGAUUGCACUA           :
		Consensus	* * ***** *****
PPARG	mmu-miR-301	miR-301	CGAAACUGUUAUGA-UAACGUGAC :           :
		Mm	CGUGUCCUUCUUAUUGAUUGCACUA           :
		Hs	CAUUUCCUUCUUCAGUUGCACUA           :
		Rn	AGUGUCCUUCUUAUCGAUUGCACUA           :
		Cf	CGUUUCCUUCUUCGGAUUGCACUA           :
		Consensus	* * ***** *****
PPARG	mmu-miR-27b	miR-27b	CGUCUUGAAUCGGUGACAUU 
		Mm	ACACCUAAGAAAAUU---ACUGUGAA 
		Hs	ACACCUAAGAAAAUU---ACUGUGAA 
		Rn	ACACCUAAGAAAAUU---ACUGUGAA 
		Cf	ACACCUAAAAUUU---ACUGUGAA 
		Consensus	***** ***** *****
PPARG	mmu-miR-27a	miR-27a	CGCCUUGAAUCGGUGACAUU           :
		Mm	ACACCUAAGAAAAUUACUGUGAA 
		Hs	ACACCUAAGAAAAUUACUGUGAA 
		Rn	ACACCUAAGAAAAUUACUGUGAA 
		Cf	ACACCUAAAAUUUACUGUGAA 
		Consensus	***** ***** *****

Mm: *M. musculus*; Rn: *R. norvegicus*; Hs: *H. sapiens*; Cf: *C. familiaris*.

Table 5 Predict base-pairing of miRNAs and their experimental verified targets by softwares used in this work

MicroRNA	Verified target gene	Pictar	Targetscan	miRanda	miRBase targets
hsa-miR-15a	DMTF1	+	+	+	+
hsa-miR-1	TRAPPC3	+	+	+	+
hsa-miR-101	MYCN	+	+	+	+
hsa-miR-101	EZH2	+	+	+	+
has- miR-23a	POU4F2	+	+	+	+
hsa-miR-34a	DLL1	+	-	+	+
hsa-miR-126	VCAM1	-	-	+	+
hsa-miR-1	ZNF264	-	+	-	-
mmu-miR-122	Cd320	+	+	+	+
mmu-miR-122	Aldoa	+	+	-	+
mmu-miR-122	Ndr3	-	-	+	+
mmu-miR-122	Bckdk	-	+	-	+
mmu-miR-1	Tmsb4x	-	-	+	+
mmu-miR-196a	Hoxd8	-	-	+	+
mmu-miR-206	Pola1	-	+	-	+

PEX6 形成的 13 个, 与 PEX7 形成的 31 个, 与 PEX12 形成的 3 个, 与 PEX13 形成的 7 个, 与 PEX19 形成的 4 个, 与 PPARA 形成的 13 个, 与 PPARG 形成的 14 个。本研究未发现 microRNA 与 PEX2、PEX10、PEX16、PPARD 形成的匹配区。

## 2.2 靶位点保守性分析

通过查询 miRBase 数据库, 我们发现大部分能够与过氧化物酶体生物合成和增殖相关基因的转录物 mRNA 3'UTR 形成匹配区的 microRNA 在进化上高度保守, 它们广泛存在于不同的动物中。如 miR15、miR16、miR19、miR29、miR31 和 miR183 就存在于 20 种不同的动物中。将保守的 microRNA 的靶位点进行同源序列比对分析, 发现其靶位点也是相当保守的(表 4)。

## 2.3 预测结果的可靠性分析

近年来, 人们建立了许多研究 microRNA 和靶基因之间相互作用的计算机分析方法。这些方法基本上都是基于 microRNA 和 mRNA 的 3'UTR 的互补原则及靶序列在不同物种之间的保守性。生物信息学预测是寻找和发现 miRNA 靶基因的一种简便方法, 但预测的结果存在一定的假阳性。为了确定本文采用的分析方法的可靠性程度, 我们从以往的研究文献中收集经过实验证实的 microRNA 及其靶分子, 然后以本文所采用的靶基因分析方法预测这些 microRNA 的靶基因(表 5), 再将预测结果与实验结果进行比较。在所分析的 15 个 miRNA 分子中, 4 个软件均能够预测到与实验结果一致的靶分子 6 个; 3 个软件能够预测到与实验结果一致的靶分子 2 个; 2 个软件能够预测到与实验结果一致的靶分子 6 个。这说明本文采

用的分析方法所得结果可靠性程度较高。

## 3 讨论

过氧化物酶体是细胞中重要的细胞器, 其生物合成缺陷会导致多种疾病。PEX 和 PPAR 基因家族在细胞中的表达水平直接影响着过氧化物酶体生物合成和增殖。因此, 对 PEX 和 PPAR 家族表达机制的研究有利于深入认识过氧化物体形成和增殖的机制。

本文通过计算机分析, 发现了一系列参与 PPAR 和 PEX 基因表达调控的保守 microRNA。这些 microRNA 的成熟序列长度为 19~23 nt, 它们的 5' 端的 2~8 个核苷酸都能与靶序列 3'UTR 区配对, 通过抑制靶分子的翻译来调节相关基因的表达。此外, 从表 1~表 3 可看出, 同一个 microRNA 分子能够与多个不同的 mRNA 靶分子结合, 如 miR-15 和 miR-16 能同时结合到 PEX5 和 PEX13 的 3'UTR 区; 另一方面, 多个不同的 microRNA 也能够与同一个 mRNA 靶分子结合, 如 miR-130a、miR-130b、miR-301、miR-27a 和 miR-27b 能够同时与 PPARG 结合, 协同调控 PPARG 的翻译。因此, 这些 microRNA 可能在细胞中形成了一个复杂的调控网络, 精确地调控着 PEX 和 PPAR 的表达水平。

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## Computational Analyses of MicroRNA Involved in Peroxisome Biogenesis and Proliferation

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**Abstract** MicroRNAs are small non-coding RNAs with 20–24 nucleotides in length and believed to be involved in a broad range of biological function, including cell proliferation, cell differentiation, cell apoptosis, development, responses to diseases and signal transduction, by base-pairing with complementary sites of target transcript to negatively regulate gene expression at the posttranscriptional level. Here we used a bioinformatics approach to identify conserved microRNAs which can complement with 3' untranslated region of target transcripts involved in peroxisome biogenesis and proliferation.

**Key words** peroxisome; biogenesis; microRNA; computational analyses

Received: November 5, 2008 Accepted: February 3, 2009

This work was supported by the National Natural Sciences Foundation of China (No.30660042) and the Natural Science Foundation of Jiangxi Province (No.0630137)

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