·基础研究·

低压缺氧对大鼠脑皮质基因表达谱及其TAC1和MT1A变化的影响*

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摘要

目的:研究低压缺氧对大鼠脑皮质基因表达谱及其TAC1和MT1A变化的影响。

方法:将24只Wistar大鼠随机分为对照组、3000m缺氧组和7000m缺氧组,每组8只。采用低压缺氧舱建立急性高原缺氧模型,应用基因芯片检测缺氧24h大鼠脑皮质差异表达基因,应用qRT-PCR定量检测TAC1和MT1A基因表达水平。

结果:3000m缺氧组共有已知差异表达基因215个,其中29个上调,186个下调。上调基因主要有TAC1、Rgs9、Serpinb6a、Adora2a、Penk1,下调基因主要有Siah1a、Acvr1、Btbd1、Cir、Abi1,差异表达最明显的基因是TAC1。7000m缺氧组共有已知差异表达基因205个,其中21个上调,184个下调。上调基因主要有MT1A、Cml3、Wfdc1、Tfpi、Vwf,下调基因主要有Zfp238、Atad1、Leprotl1、Tpm4、Fxr1,差异表达最明显的基因是MT1A。两组差异表达基因中共表达基因有120个,其中7个上调,113个下调。上调基因主要有TAC1、Serpinb6a、Ephx1、Cml3、Olr606,下调基因主要有Acvr1、Btbd1、Leprotl1、Unc119、Canx。qRT-PCR实验证实低压缺氧脑皮质TAC1和MT1A上调。

结论:低压缺氧程度不同,脑皮质基因表达谱亦不同。TAC1对缺氧较为敏感,轻、重度高原缺氧表达均上调;MT1A对缺氧反应较为迟钝,轻度缺氧下MT1A基因表达水平不变甚至下降,而重度缺氧时MT1A基因显著上调。

关键词 低压缺氧;脑皮质;基因表达谱

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Abstract

Objective: To study the effects of hypobaric hypoxia on the changes of gene expression profile and TAC1 and MT1A in rats' cerebral cortex.

Method: Twenty-four rats were randomly divided into control group, 3000m hypoxia group and 7000m hypoxia group(8 rats in each group). The acute plateau hypoxia model was established in hypobaric hypoxia cabin, the differential expression genes were detected by chip technology in cerebral cortex of rats with 24h hypoxia, and the expression levels of TAC1 and MT1A were measured quantitatively by qRT-PCR.

Result: In the 3000m hypoxia group,215 differential expression genes were found, 29 of which were up-regulated and 186 down-regulated. The up-regulated genes were mainly TAC1,Rgs9,Serpinb6a, Adora2a and Penk1. The down-regulated genes were mainly Siah1a,Acvr1,Btbd1,Cir and Abi1,and the most obvious expression gene was TAC1. In the 7000m hypoxia group, 205 differentially expression genes were found, 21 of which were expressed and 184 down-regulated. Up-regulated genes were mainly MT1A,Cml3,Wfdc1,Tfpi and Vwf. Down-reg

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ulated genes were mainly Zfp238, Atad1, Leprotl1, Tpm4 and Fxr1, and the most obvious expressed gene was MT1A. In the two sets of differential expression genes, 119 genes were co-expressed, 6 genes were up-regulated and 113 were down-regulated. The up-regulated genes were mainly TAC1, Ephx1, Cml3, Olr606 and Dusp7. The down-regulated genes were mainly Acvr1, Btbd1, Leprotl1, Unc119 and Canx. TAC1 and MT1A in hypobaric hypoxia cerebral cortex were up-regulated, which were confirmed by qRT-PCR experiments.

Conclusion: The difference of cerebral cortical gene expression profiling were according to the changes of hypobaric hypoxia, meanwhile. TAC1 seemed to be more sensitive to hypoxia, of which the expression were up-regulated in either the mild plateau hypoxia model or the severe one. The responses of MT1A to hypoxia were relatively blunted, and in the mild hypoxia model, the expression levels of gene MT1A were stable or even reduced, but in the severe hypoxia model, it were significantly up-regulated.

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Key word hypobaric hypoxia; cerebral cortex; gene expression profile

人体暴露于急性缺氧环境会发生缺氧应激反应，过强的缺氧应激反应可导致机体各种功能出现衰竭，机体的神经系统、呼吸系统、循环系统等会受到不同程度的损伤，从而引起机体一系列功能、代谢和结构的病理生理学改变，最终导致心、脑等重要脏器由于能量供应不足而死亡[1]。因此，对急性高原缺氧的防治是近些年来面临的一大难题，目前，对急性高原缺氧发病机制主要从形态、生理、生化和相关分子生物学方面加以探讨，而基因表达谱变化的探讨报道甚少，尤其是TAC1和MT1A对低压缺氧的报道更不多见。因此，本文采用基因芯片技术，筛选差异表达基因，获得急性高原缺氧基因表达谱变化的研究数据，为深入分析缺氧病理机制提供RNA水平的证据。同时，证实的差异表达基因可作为潜在的急性高原缺氧诊断生物标志物，为预防和实时监控高原缺氧的发生奠定研究基础。

1 材料与方法

1.1 实验动物及样本准备

选用SPF级健康雌性Wistar大鼠24只（购自解放军军事医学科学院实验动物中心，质量合格证书编号：0024638），约6—7周龄，质量为180—200g，自由饮水，动物适应性饲养1周后，按随机数字表分为正常对照组、急性高原海拔3000m缺氧组和急性高原海拔7000m缺氧组，共3组，每组8只。

将大鼠放置于低压缺氧舱，抽气减压至相当于3000m和7000m海拔高度，连续低压缺氧24h，控制舱内温度（20 ± 3℃）、湿度（65% ± 5%）、气流量（0.09—0.10m^3/h）及氧含量（21% ± 2%）。对照组大鼠于相同湿温度条件下舱外饲养。

1.2 石蜡切片的制备及染色

缺氧24h结束后处死大鼠，取大脑皮质，部分组织置于10%中性缓冲福尔马林固定24h，将载有组织的玻片取出放入4%多聚甲醛中固定1h左右，后取出用PBS冲洗1—2次，石蜡切片制备完成；然后加入提前预热的1%甲苯胺蓝溶液，在50℃左右的水浴中染色20—30min；用梯度酒精依次脱水，分别为75%、90%、100%；在镜下控制分色效果；其余部分置于-80℃冰箱中备用。

1.3 总RNA抽提

每50—100mg组织加入1ml TRIzol（Invitrogen Biotechnology CO,Ltd,USA）试剂，用研钵和液氮匀浆处理，样本体积不应超过TRIzol体积的1/10，通过纯化柱进行纯化，提取大鼠脑组织全部的RNA。

1.4 基因表达谱芯片杂交及数据处理

1.4.1 基因芯片杂交：将上步中提取的RNA逆转录为cDNA（Ambion Illumina RNA Amplification Kit, USA），再与杂交混合液混合后，加到Illumina RatRef-12（Illumina inc.,USA）芯片上，55℃杂交反应16—22h；封闭缓冲液进行封闭；cy3荧光进行标记染色，清洗芯片，离心甩干，进行后续杂交结果的检测与分析。

1.4.2 杂交结果检测：通过芯片扫描软件Bead station对芯片灰度扫描，对扫描结果使用illumina软件Genome studio V2011进行分析，得到芯片上每个基因点的原始信号值。实验组信号值与对照组比值称为ratio，实验组与对照组中任何一组检测值$P < 0.01$为有效基因，且ratio值符合一定范围即为差异基

因。在3000m与7000m缺氧的差异表达基因筛选中选择ratio值>2.0为上调基因,ratio值<0.5为下调基因。

1.4.3 差异基因的生物信息学分析:通过DAVID(The Database for Annotation, Visualization and Integrated Discovery,DAVID)生物信息学分析工具(http://david.abcc.ncifcrf.gov/),将差异基因依据GO(Gene Ontology,GO)数据库提供的3种本体(生物学过程、分子功能、细胞组分)进行GO分类[2],并按照功能进行生物信息学归类分析。

1.5 实时荧光定量PCR

采用Oligo软件设计引物[3],并由北京鼎国昌盛生物技术有限责任公司合成(表1)。取2μg RNA按First Strand cDNA Synthesis Kit提供的方法合成了cDNA,以样本的cDNA为模板采用SYBR Green方法使用AB steponeplus PCR仪对差异表达基因进行实时荧光定量PCR验证。反应条件:95℃预变性10min,95℃变性10s,MT1A 63℃、TAC1 65℃退火30s,72℃扩增25s,共40个循环[4]利用2-△△Ct相对定量法分析[△CT(待测组)=待测组目的平均CT值-待测组管家基因平均CT值。△CT(对照组)=对照组目的平均CT值-对照组管家基因平均CT值。△△CT=CT(待测组)-△CT(对照组)。F=2-{△△CT}],以管家基因β-actin为参照基因。以正常组为校准样本检测样品中TAC1和MT1A基因的表达水平,比较实验组和对照组样本间的表达差异,每个样品平行3次实验并取均值进行计算。

表1 MT1A、TAC1基因和内参照β-肌动蛋白基因引物序列

	向前引物	逆向引物
MT1A	GTCGCTTAC ACCGTTGCT	CACTTGTCC GAGGCACC
TAC1	ACAACACAGGA AACATGCTGCT	AGTTGTACAAG TTTGCCAGCGA
β-肌动蛋白	CACCCGCGAGT ACAACCTTC	CCCATACCCAC CATCACACC

1.6 统计学分析

应用SPSS18统计学软件进行数据分析,3个样本均数间差异用方差分析检验,其中$P<0.05$,判定差异有显著性意义。

2 结果

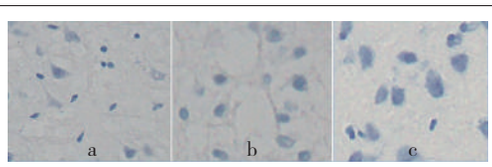
2.1 大鼠脑皮质形态学变化

应用甲苯胺蓝染色大鼠脑皮质后显示,正常对照组,神经元排列规则,胞浆均匀淡染,核仁清晰可见;3000m缺氧组,神经元排列较为规则,部分轴突断裂或消失;7000m缺氧组,神经元排列散乱,细胞固缩变形,胞浆深染,轴突断裂或消失,见图1。

2.2 总RNA提取结果

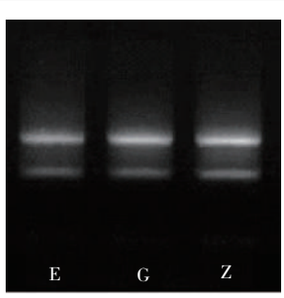
提取的RNA吸光度A260nm/A280nm比值均在1.8—2.0之间。经琼酯糖凝胶电泳质量检测,18S和28S电泳条带清晰,28S条带无明显降解,显示获得高质量的总RNA,见图2。

图1 大鼠脑皮质甲苯胺蓝染色 (×40)



正常对照组 3000m缺氧组 7000m缺氧组

图2 总RNA琼脂糖凝胶电泳检测



E组正常组 G组3000m缺氧组 Z组7000m缺氧组

注:经琼酯糖凝胶电泳质量检测,28S和18S核糖体RNA条带非常亮而浓,28S的密度大约是18S的2倍,显示获得高质量的总RNA。

2.3 各组差异表达基因

3000m缺氧组差异表达基因215个,其中29个上调基因,主要有TAC1、Rgs9、Serpinb6a、Adora2a、Penk1、Ephx1、Rxrg、Gng7、Gpr88、Accn4;186个下调基因,主要有Siah1a、Acvr1、Btbd1、Cir、Abi1(表2)。7000m缺氧组差异表达基因205个,其中21个上调基因,主要有MT1A、Cml3、Wfdc1、Tfpi、Vwf;184个下调基因,主要有Zfp238、Atad1、Leprotl1、Tpm4、Fxr1(表3)。两组差异表达基因中共表达基因有

120个，其中6个上调基因，主要有Tac1、Serpinb6a、Ephx1、Cml3、Olr606；113个下调基因，主要有Acvr1、Btbd1、Leprotl1、Unc119、Canx。3000m缺氧组最显著上调差异表达基因为TAC1，7000m缺氧组最显著上调差异表达基因为MT1A。

2.4 TAC1和MT1A基因相对表达量

2.4.1 基因芯片差异表达：利用基因芯片在差异表达基因的筛选中，选择ratio值＞2为上调基因，ratio值＜0.5为下调基因。3000m缺氧组，TAC1上调5.12倍；7000m缺氧组，TAC1上调2.31倍；3000m缺氧组，MT1A轻度下调；7000m缺氧组，MT1A上调2.58倍（图3）。

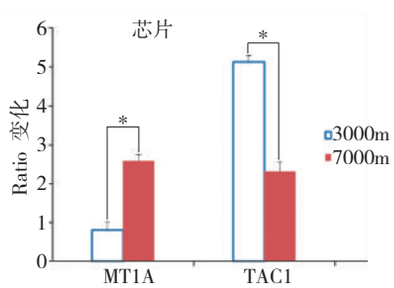
表2 3000m缺氧组部分差异表达基因

调节类型/基因名称	定义	ratio(G/Z)
上调		
TAC1	tachykinin 1 (Tac1), mRNA.	5.120492
Rgs9	regulator of G-protein signaling 9 (Rgs9), mRNA.	3.414064
Serpinb6a	serine (or cysteine) peptidase inhibitor, clade B, member 6a (Serpinb6a), mRNA.	3.063928
Adora2a	adenosine A2a receptor (Adora2a), mRNA.	2.859086
Penk1	proenkephalin 1 (Penk1), mRNA.	2.729999
Ephx1	epoxide hydrolase 1, microsomal (Ephx1), transcript variant 1, mRNA.	2.702339
Rxrg	retinoid X receptor gamma (Rxrg), mRNA.	2.634897
Gng7	guanine nucleotide binding protein (G protein), gamma 7 (Gng7), mRNA.	2.60403
Gpr88	G-protein coupled receptor 88 (Gpr88), mRNA.	2.565351
Accn4	amiloride-sensitive cation channel 4, pituitary (Accn4), mRNA.	2.528048
Rps9	ribosomal protein S9 (Rps9), mRNA.	2.436904
Scn4b	sodium channel, type IV, beta (Scn4b), mRNA.	2.424059
Capn6	calpain 6 (Capn6), mRNA.	2.40135
Cklf	chemokine-like factor (Cklf), mRNA.	2.378757
Olr1460	olfactory receptor 1460 (Olr1460), mRNA.	2.339164
Cml3	camello-like 3 (Cml3), mRNA.	2.303987
Olr784	olfactory receptor 784 (Olr784), mRNA.	2.298848
Olr606	olfactory receptor 606 (Olr606), mRNA.	2.291437
Acads	acyl-Coenzyme A dehydrogenase, C-2 to C-3 short chain (Acads), nuclear gene encoding mitochondrial protein, mRNA.	2.284816
Dusp7	dual specificity phosphatase 7 (Dusp7), mRNA.	2.228697
Olr555	olfactory receptor 555 (Olr555), mRNA.	2.224829
Abhd1	abhydrolase domain containing 1 (Abhd1), mRNA.	2.222107
Olr757	olfactory receptor 757 (Olr757), mRNA.	2.163918
Mme	membrane metallo endopeptidase (Mme), mRNA.	2.130548
Scnn1b	sodium channel, nonvoltage-gated 1 beta (Scnn1b), mRNA.	2.129986
Tbc1d10a	TBC1 domain family, member 10a (Tbc1d10a), mRNA.	2.091622
Olr898	olfactory receptor 898 (Olr898), mRNA.	2.078992
Ppp1r1b	protein phosphatase 1, regulatory (inhibitor) subunit 1B (Ppp1r1b), mRNA.	2.074502
Krt4	keratin 4 (Krt4), mRNA.	2.03998
下调		
Cttn	cortactin isoform B (Cttn), mRNA.	0.09585392
Hs3st1	Heparin sulfate (glucosamine) 3-O-sulfotransferase 1 (Hs3st1), mRNA.	0.09412675
Tmeff1	transmembrane protein with EGF-like and two follistatin-like domains 1 (Tmeff1), mRNA.	0.08339847
Fzd1	frizzled homolog 1 (Drosophila) (Fzd1), mRNA.	0.07779302
Zmym3	zinc finger, MYM-type 3 (Zmym3), mRNA.	0.0712728
Pex7	peroxisomal biogenesis factor 7 (Pex7), mRNA.	0.07096106
Grp	gastrin releasing peptide (Grp), mRNA.	0.07081508
Canxc	alnexin (Canx), mRNA.	0.06539713
Unc119	UNC-119 homolog (C. elegans) (Unc119), mRNA.	0.05697402
Leprotl1	leptin receptor overlapping transcript-like 1 (Leprotl1), mRNA.	0.03620305
Abi1	abl-interactor 1 (Abi1), mRNA.	0.02229726
Cir	CBF1 interacting corepressor (Cir), mRNA.	0.02085734
Btbd1	BTB (POZ) domain containing 1 (Btbd1), mRNA.	0.02064504
Acvr1	activin A receptor, type I (Acvr1), mRNA.	0.01752698
Siah1a	seven in absentia 1A (Siah1a), mRNA.	0.009230856

表3 7000m缺氧组部分差异表达基因

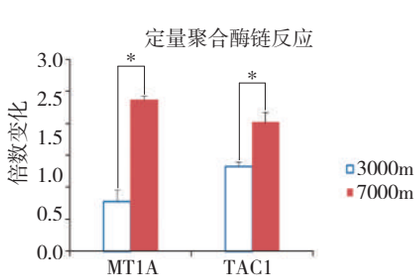
调节类型/基因名称	定义	ratio(G/Z)
上调		
MT1A	metallothionein 1a (Mt1a), mRNA.	2.584722
Cml3	camello-like 3 (Cml3), mRNA.	2.433586
Wfdc1	WAP four-disulfide core domain 1 (Wfdc1), mRNA.	2.372134
Tfpi	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor) (Tfpi), mRNA.	2.358224
Vwf	von Willebrand factor (Vwf), mRNA.	2.326911
TAC1	tachykinin 1 (TAC1), mRNA.	2.312457
Egr2	early growth response 2 (Egr2), mRNA.	2.296247
Podxl	podocalyxin-like (Podxl), mRNA.	2.283256
C1qc	complement component 1, q subcomponent, C chain (C1qc), mRNA.	2.26332
Dusp7	dual specificity phosphatase 7 (Dusp7), mRNA.	2.244265
Tnfrsf11b	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin) (Tnfrsf11b), mRNA.	2.237252
Olr606	olfactory receptor 606 (Olr606), mRNA.	2.202652
Ephx1	epoxide hydrolase 1, microsomal (Ephx1), transcript variant 1, mRNA.	2.189385
Btg2	B-cell translocation gene 2, anti-proliferative (Btg2), mRNA.	2.165379
Cbs	cystathionine beta synthase (Cbs), mRNA.	2.154165
Tac4	tachykinin 4 (TAC4), mRNA.	2.123191
Phyhd1	phytanoyl-CoA dioxygenase domain containing 1 (Phyhd1), mRNA.	2.075506
Stxbp2	syntaxin binding protein 2 (Stxbp2), mRNA.	2.067438
Sult1a1	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1 (Sult1a1), mRNA.	2.033228
Junb	jun B proto-oncogene (Junb), mRNA.	2.019629
Olr898	olfactory receptor 898 (Olr898), mRNA.	2.017068
下调		
Yy1	YY1 transcription factor (Yy1), mRNA.	0.09926891
Adcy8	adenylate cyclase 8 (brain) (Adcy8), mRNA.	0.09703359
Idi1	isopentenyl-diphosphate delta isomerase 1 (Idi1), mRNA.	0.09631847
Hs3st1	Heparin sulfate (glucosamine) 3-O-sulfotransferase 1 (Hs3st1), mRNA.	0.09327485
Tgm2	transglutaminase 2, C polypeptide (Tgm2), mRNA.	0.09201901
Ubfd1	ubiquitin family domain containing 1 (Ubfd1), mRNA.	0.09084779
Gsk3b	glycogen synthase kinase 3 beta (Gsk3b), mRNA.	0.08317615
Mcart1	mitochondrial carrier triple repeat 1 (Mcart1), nuclear gene encoding mitochondrial protein, mRNA.	0.07846506
Apeh	N-acylaminoacyl-peptide hydrolase (Apeh), mRNA.	0.07596575
Fmo1	flavin containing monooxygenase 1 (Fmo1), mRNA.	0.06852455
Tmeff1	transmembrane protein with EGF-like and two follistatin-like domains 1 (Tmeff1), mRNA.	0.06510857
Mgl1	macrophage galactose N-acetyl-galactosamine specific lectin 1 (Mgl1), mRNA.	0.05151329
Fxr1	fragile X mental retardation, autosomal homolog 1 (Fxr1), mRNA.	0.02322636
Tpm4	tropomyosin 4 (Tpm4), mRNA.	0.0216937
Leprotl1	leptin receptor overlapping transcript-like 1 (Leprotl1), mRNA.	0.006983034
Atad1	ATPase family, AAA domain containing 1 (Atad1), mRNA.	0.003625701
Zfp238	zinc finger protein 238 (Zfp238), mRNA.	0.002556105

图3 基因芯片检测3000m、7000m缺氧组TAC1与MT1A表达情况



ratio变化为实验组扫描信号值与对照组扫描信号值的比值,*7000m缺氧组ratio变化同3000m缺氧组比较,$P < 0.05$

图4 qRT-PCR测定3000m、7000m缺氧组TAC1与MT1A表达情况



ratio变化为实验组扫描信号值与对照组扫描信号值的比值,*7000m缺氧组ratio变化同3000m缺氧组比较,$P < 0.05$

2.4.2 定量逆转录-聚合酶链反应(qRT-PCR)差异表达:采用2-△△CT法对3组大鼠TAC1和MT1A基因表达进行相对定量分析,β-肌动蛋白为内参基因,将qRT-PCR结果带入2-△△CT公式。3000m缺氧组,TAC1上调1.32倍,7000m缺氧组,TAC1上调2.02倍;3000m缺氧组,MT1A经检验差异没有显著性,表达水平无变化。7000m缺氧组,MT1A上调2.37倍(图4),缺氧组TAC1和MT1A两个基因的表达量与对照组比较,都是上调的。

3 讨论

缺氧性脑损伤是一个复杂的病理生理过程,在该过程中,细胞因子网络系统从多方面、多阶段参与调控和维持内环境稳态,而细胞因子复杂的网络体系又受到基因的动态级联调控,越来越多的研究表明,脑损伤可引起多种基因在中枢神经系统表达改变,且在一些特定基因之间存在内在的调控关系,因此,本实验通过大鼠脑缺氧损伤模型探讨基因表达谱的改变。石蜡切片显示,3000m缺氧组神经元排列较为规则,部分轴突断裂或消失;7000m缺氧组细胞固缩变形,胞浆深染,轴突断裂或消失(图1)。证明此次实验所建立的Wistar大鼠缺氧模型是成功的,可用于大鼠缺氧损伤的研究。

研究表明3000m缺氧组共有已知差异表达基因215个,其中29个上调,186个下调。7000m缺氧组共有已知差异表达基因205个,其中21个上调,184个下调。7000m缺氧组在相同缺氧时间段内,其差异表达基因数目却是减少的,这可能提示了脑损伤后诱发的病理生理的瀑布效应伴随着复杂的分子效应,且有一个时间上的分子窗,正好为基因治疗提供了机会[5]。Li H等[6]建立了中度和重度的大鼠脑损伤模型,在伤后0.5h、4h和24h,用基因芯片检测伤侧海马,发现重度伤者在相同受伤时间段内,其差异表达基因数目却是减少的,与本实验结果相似。两组差异表达基因中共表达基因有120个,其中7个上调,113个下调。表明在缺氧损伤的各个阶段某些基因始终发挥着重要调节作用。

将3000m和7000m所有差异表达基因加入数据库通过David进行GO term富集分析,主要分为3个层面,即生理过程,细胞组分和分子功能。缺氧损伤广泛影响细胞的生理过程,3000m缺氧组主要涉及的生理过程包括调节炎症反应、神经系统过程、生长发育、细胞间信号转导、神经冲动的传导。7000m缺氧组主要涉及的生理过程包括神经冲动的传导、稳态过程、细胞内环境的调节、离子稳态、化学平衡。表明不同缺氧状态下基因表达谱存在差异,许多相关基因是成簇作用调控机体生命过程的。提示损伤诱导大鼠脑皮质积极地启动自身修复机制,调节内环境、合成蛋白质、调节轴突的生长和重塑等。Zhou D等[7]发现小鼠慢性间歇性和慢性持续性缺氧的差异表达基因主要涉及细胞周期、生长因子、受体、神经肽,其中部分基因24h可返回到正常表达水平。

在3000m缺氧组已知的差异表达基因中,表达上调最显著的基因是TAC1。7000m缺氧组已知的差异表达基因中表达上调最显著的是MT1A,两组差异表达基因共表达基因上调最显著的基因之一是TAC1。qRT-PCR已证实TAC1和MT1A表达均上调,与芯片结果相符。提示TAC1对缺氧较为敏感,轻、重度高原缺氧表达均上调。而MT1A对缺氧反应较为迟钝,在7000m重度缺氧时,显著上调,而在轻度缺氧条件下,机体反而轻度下调MT1A基因。基因芯片探测3000m缺氧组TAC1上调5.12倍,明显高于PCR验证的上调1.32倍,可能与芯片扫描误差有关,有待进一步证实。将GO富集分析结果中涉及TAC1和MT1A的生理过程整理成表(表4—5),可见TAC1主要涉及神经系统过程、有机物反应、细胞间信号传递、稳态调节。MT1A主要涉及细胞内环境、细胞内离子稳态、稳态过程。提示TAC1和MT1A在缺氧损伤过程中对组织修复,再生,内环境平衡,神经信号的转导有积极的促进作用。

TAC1速激肽类是一种活性肽,可以编码除了NκB以外速激肽家族的所有物质,从而兴奋神经。速激肽家族主要有P物质(SP)、神经肽A、神经肽B、神经肽K和神经肽γ[8]。其中,P物质是广泛分布于神经纤维内的一种神经肽,具有扩张血管、保护神经等功能。当缺氧损伤发生时,TAC1编码的P物质在中枢末梢释放增加,与NK1受体结合,通过直接或间接促进谷氨酸等的释放发挥痛觉传递作用[9]。Berner J[10]等通过将小鼠的tac1基因敲除,得出P物

质和神经肽A对于呼吸系统的形成和可塑性具有重要的意义[11]。速激肽、P物质和NKA不足会导致TAC1敲除的新生小鼠在吗啡诱导的呼吸抑制过程中出现更加严重的缺氧反应。提示TAC1与呼吸系统缺氧反应关系密切。已经证实,从啮齿动物研究表明P物质在主要的免疫细胞中储存和产生,包括巨噬细胞、白细胞、淋巴细胞和树突状细胞[12]。Helyes Z等[13]通过探讨内毒素诱导的气道炎症和由此产生的支气管高反应性TAC1(-/-)组,NK1(-/-)组,和双基因敲除[TAC1(-/-)/NK1(-/-)]组中小鼠IL-6和TNF-α的表达。发现在TAC1(-/-)组和双基因敲除[TAC1(-/-)/NK1(-/-)]组IL-6和TNF表达明显降低。表明Tac1在炎症损伤方面的调节,对机体起着重要的保护作用。

MT1A是一种金属硫蛋白,在哺乳动物组织中已经发现MT有四个亚型,MT-I和MT-II是大多数组织中两个比较主要的亚型。易结合锌、铜、铁等金属元素,发挥生理作用[14]。MT可以诱导多种细胞因子,如白细胞介素1(IL-1),白细胞介素6(IL-6),肿瘤坏死因子(TNF-α)等,参与免疫和炎症反应。MT可以诱导锌离子和白细胞介素6的产生和释放,同时IL-6也受到MT的反馈调节。从而改变机体的免疫力[15]。Liu X等[16]发现内皮素-1(ET-1)利用自分泌或旁分泌机制通过ET-A受体在化学感受Ⅰ型细胞和免疫细胞中发挥促炎症作用。已经证实,高糖诱导ET-1在血管内皮细胞中的表达增强可以调节其他应力反应蛋白如金属硫蛋白(MT)[17—18]。在ET-1基因启动子区有HIF-1α的结合位点,低氧可以通过诱导低氧诱导因子(HIF-1α)的产生而调节ET-1基因的表达[19]。Mazurek B等[20]将新生小鼠的耳蜗解剖经过正常氧,缺氧,复氧三个阶段后发现组织中MT1A基因的表达普遍降低。提示MT1A与缺氧损伤密切相关。

综上所述,本实验表明轻度缺氧时机体主要负责感知疼痛、调节炎症反应、生长发育正向调控及神经冲动传导方面的基因簇上调。而在重度缺氧时,机体负责离子平衡、稳态过程、细胞化学平衡方面的基因簇上调,形成复杂的基因网络调控系统。表明缺氧程度不同,基因的表达谱也是不同的。TAC1对缺氧较为敏感,轻、重度高原缺氧表达均上调。TAC1主要负责神经系统过程、有机物反应、细胞间信号传递、稳态等方面的调控。而MT1A对缺氧反应较为迟钝,在7000m重度缺氧时,显著上调,而在轻度缺氧条件,机体反而轻度下调MT1A基因。MT1A主要负责细胞内环境、细胞内离子稳态、稳态过程等方面的调控。这一发现为进一步研究高原脑病基因网络调控系统、发现药靶基因以及早期生物标志物提供了基础数据和研究方向。各基因簇之间的内在联系仍是一个复杂的,多因素参与的过程,需进一步探讨。

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表4 TAC1涉及的生理过程

Top Functions		count	genes
neurological system process	神经系统过程	32	ALS2, OLR784, ADORA2A, OLR757, CTNND2, OLR555, TAC1, ADORA1, OLR1460, APLP2, CTNNB1, GPX1, SPRY2, GRIN2B, SYN2, SV2B, SCNN1B, GABRG2, UNC119, OLR898, LIN7B, GRIN2A, EPHX2, PCDH8, OLR606, SOD2, PNOC, FYN, PDCL, RAB14, RGS9, APBB1
response to organic substance	有机物反应	20	ADORA2A, ACADS, GRIN2A, MGP, TAC1, EPHX1, DCN, CTNNB1, PRSS8, GPX1, GRIN2B, FYN, SULT1A1, MGEA5, NEUROD1, MAPK9, RGS9, BMP7, SDF4, GNG7
cell–cell signaling	细胞间信号传递	17	ALS2, GABRG2, ADORA2A, DLGAP4, GRIN2A, FZD1, LIN7B, TAC1, PCDH8, RIMS2, ADORA1, CTNNB1, GRIN2B, PNOC, SYN2, RAB14, SV2B
homeostatic process	稳态调节	16	ADORA2A, SLC9A2, GRIN2A, TAC1, ADORA1, LOC498453, APLP2, CTNNB1, SOD2, GSR, GPX1, GRIN2B, MGEA5, NEUROD1, NCOR1, DLG1
behavior	行为	15	ALS2, GABRG2, ADORA2A, GRIN2A, CTNND2, TAC1, PCDH8, ADORA1, APLP2, SOD2, GRIN2B, PPP1R1B, CKLF, APBB1, GNG7
synaptic transmission	突出转导	14	ALS2, GABRG2, ADORA2A, LIN7B, GRIN2A, TAC1, PCDH8, ADORA1, CTNNB1, GRIN2B, PNOC, SYN2, RAB14, SV2B
transmission of nerve impulse	神经冲动传导	14	ALS2, GABRG2, ADORA2A, LIN7B, GRIN2A, TAC1, PCDH8, ADORA1, CTNNB1, GRIN2B, PNOC, SYN2, RAB14
response to endogenous stimulus	内源性刺激反应	14	ACADS, ADORA2A, GRIN2A, MGP, TAC1, CTNNB1, PRSS8, GPX1, SULT1A1, MGEA5, MAPK9, RGS9, BMP7, GNG7
chemical homeostasis	化学稳态	12	GRIN2B, ADORA2A, SLC9A2, GRIN2A, MGEA5, TAC1, NEUROD1, NCOR1, ADORA1, APLP2, SOD2, DLG1
response to wounding	损伤反应	11	GPX1, JUB, NRP1, MTPN, GRIN2A, EPHX2, TAC1, DCN, SCNN1B, SOD2, ACVR1
cellular homeostasis	细胞内稳态	11	GPX1, GSR, GRIN2B, ADORA2A, GRIN2A, MGEA5, TAC1, ADORA1, APLP2, SOD2, DLG1
ion homeostasis	离子稳态	10	GRIN2B, ADORA2A, SLC9A2, GRIN2A, MGEA5, TAC1, ADORA1, APLP2, SOD2, DLG1
positive regulation of developmental process	正向调节生长发育	9	ASCL1, METRN, PDLIM7, TAC1, NEUROD1, MAPK9, BMP7, CTNNB1, ACVR
regulation of membrane potential	调节细胞膜潜能	8	GRIN2B, ADORA2A, GRIN2A, MGEA5, TAC1, ADORA1, SOD2, DLG1

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表5 Mt1a涉及的生理过程

Top Functions		count	genes
cellular homeostasis	细胞内环境	14	EGR2, GRIK2, TAC1, APLP2, SOD2, EDNRB, GSR, GPX1, GRIN2B, MT1A, TGM2, PLLP, UGT8, DLG1
cellular ion homeostasis	细胞内离子稳态	12	EDNRB, EGR2, MT1A, GRIN2B, GRIK2, TGM2, TAC1, PLLP, UGT8, APLP2, SOD2, DLG1
homeostatic process	稳态过程	15	EGR2, GRIK2, TAC1, LOC498453, APLP2, SOD2, EDNRB, GSR, GPX1, MT1A, GRIN2B, TGM2, PLLP, UGT8, DLG1
cellular chemical homeostasis	细胞内稳态	12	EDNRB, EGR2, MT1A, GRIN2B, GRIK2, TGM2, TAC1, PLLP, UGT8, APLP2, SOD2, DLG1
ion homeostasis	离子稳态	12	EDNRB, EGR2, MT1A, GRIN2B, GRIK2, TGM2, TAC1, PLLP, UGT8, APLP2, SOD2, DLG1
chemical homeostasis	化学稳态	12	EDNRB, EGR2, MT1A, GRIN2B, GRIK2, TGM2, TAC1, PLLP, UGT8, APLP2, SOD2, DLG1
response to inorganic substance	无机物反应	9	GPX1, TNFRSF11B, MT1A, CPOX, ALG2, MGP, ABAT, CBX6, SOD2
response to metalion	金属反应	7	GPX1, TNFRSF11B, MT1A, CPOX, ALG2, MGP, ABAT
di-, tri-valent inorganic cation homeostasis	2价3价阳离子稳态	7	EDNRB, MT1A, GRIK2, TGM2, TAC1, APLP2, SOD2
cellular homeostasis	细胞内环境	14	EGR2, GRIK2, TAC1, APLP2, SOD2, EDNRB, GSR, GPX1, GRIN2B, MT1A, TGM2, PLLP, UGT8, DLG1

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