哺乳动物腺苷酸环化酶的表达调控与功能

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摘要 腺苷酸环化酶(adenylate cyclase, AC)能够直接催化ATP(adenosine triphosphate)生成第二信使cAMP(cyclic AMP), 参与调节细胞一系列信号应答反应。在哺乳动物中, AC由10个不同基因分别编码的异构体组成的, 其中包括9种整合膜蛋白(AC1~9)和1种可溶性蛋白(AC10), 它们之间在不同器官组织或细胞中的表达与分布特征及其调控机制存在差异, 能够发挥多种多样的生理功能, 并对健康与疾病发生具有显著影响。该文就腺苷酸环化酶的研究进展作一综述。

关键词 腺苷酸环化酶; 基因表达; 调控; 功能

Expression, Regulation and Function of Adenylate Cyclases in Mammals

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Abstract Adenylate cyclase (AC) catalyzes the conversion of ATP (adenosine triphosphate) to cAMP (cyclic AMP) which is a universal second messenger and involved in a wide range of cellular signaling responses. In mammals, a total of ten AC isoforms encoded by different genes are identified, and defined by two distinct types, nine transmembrane enzymes (AC1-9) and one soluble protein (AC10). These AC isoforms feature differences in spatial and temporal expression and distribution among different organs/tissues or cells, as well as in the mechanisms of regulation, and exert various physiological functions and obvious influences in health and diseases, which are summarized in this article.

Keywords adenylate cyclase; gene expression; regulation; function

腺苷酸环化酶(adenylate cyclase, AC)几乎存在于生物界所有细胞中, 它能够在外界信号刺激和细胞内代谢变化条件下催化ATP(adenosine triphosphate)生成cAMP(cyclic AMP)和焦磷酸(pyrophosphate, PPi), 从而调节各种生理反应[1]。在哺乳动物中, AC是G蛋白偶联受体(G protein-coupled receptors, GPCRs)超级家族作用途径下游的关键信号分子, 在功能上涉及发育学、神经生理学和药理学等诸多领域的信号作用通路[2-3]。cAMP则是第二信使, 能够控制细胞代谢、分泌与基因转录等一系列细胞活动[4]。近些年来, 人们对哺乳动物ACs的研究取得了诸多进展, 尤其是利用基因敲除或修饰动

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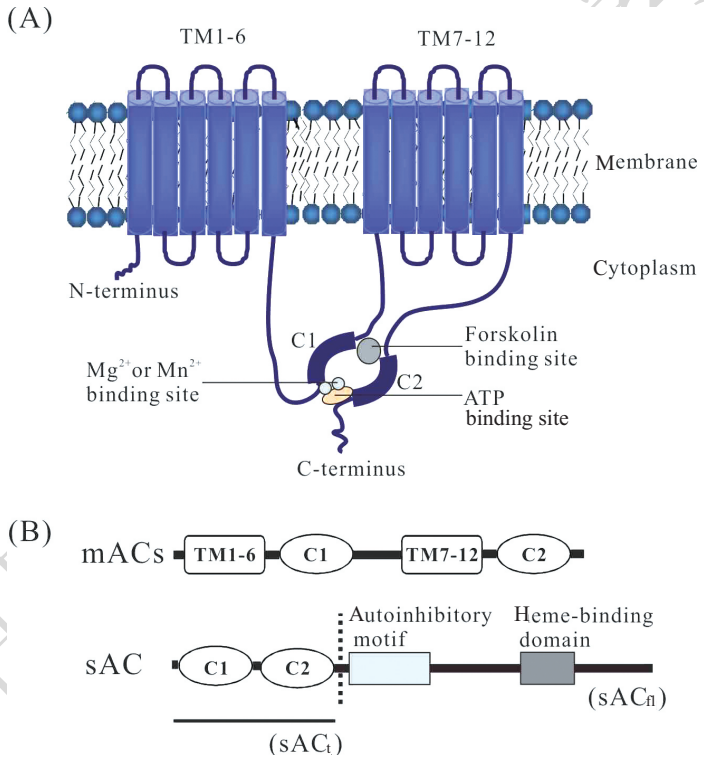
物模型对这些基因的功能与调控及其在健康与疾病中的作用进行了有意义的探讨。

1 ACs的种类与结构特点

在哺乳动物中, AC包括10种异构体(isoforms)或同工酶(isoenzymes), 分别由位于不同染色体或染色体臂上的基因编码, 它们均属于核苷酸环化酶III型(class III nucleotidyl cyclases)家族成员[1,4]。其中, 9种异构体(AC1~9)属于整合膜蛋白(membrane-bound AC, mAC), 另外1种(AC10)属于可溶性蛋白(soluble AC, sAC)[1]。

在蛋白质结构特征上(图1), 每种mAC均含有C1和C2两个高度同源(氨基酸序列相似性达50%~90%)的环化酶同源结构域(cyclase homology domain, CHD)以及2个分别由6个跨膜肽段组成的跨膜结构域TM1~6和TM7~12, 这些结构域交互排列形成了一个相似的TM-CHD结构单元。C1和C2具有高度相似的三级结构, 它们一起可以形成一个假对称的界面(pseudosymmerical interface), 并在界面上由两者的互补残基共同参与构成了AC催化活性位点。由于C1在进化过程中丢失了部分必需的催化残基, 结果导致仅具有一个催化活性中心, 需要两个金属离子(Mg^{2+}或Mn^{2+})作为辅助因子参与起作用。mACs在细胞膜上将其肽链的N-端(N-terminus)和C-端(C-terminus)均伸入细胞质中, 而不同异构体之间N-端的序列和长度差别比较明显。TM1~6和



A: mAC包含TM1~6和TM7~12两个跨膜结构域以及C1和C2两个环化酶同源结构域, 它的肽链N-端和C-端均伸入细胞质中。C1和C2可以形成一个假对称的界面, 由它们的氨基酸残基共同组成酶催化活性位点, 需要两个金属离子(Mg^{2+}或Mn^{2+})作为辅助因子, ATP结合在C1-C2界面的一侧, 而毛猴素等调节物质的结合可以引起上述界面的构像(conformation)发生变化, 从而对AC酶活性产生影响。B: 与mACs相比, sAC缺少跨膜结构域, C1和C2直接串联在一起, 位于肽链的氨基末端区域。sAC包含由全长mRNA及其选择性剪接产生的变异体分别编码的酶蛋白sAC_{fl}和sAC_t; 其中, sAC_t仅由C1和C2组成, 而sAC_{fl}在C1和C2的下游C-端包含一个自抑模体和一个血红素结合域。 sAC_t具有比sAC_{fl}显著提高的酶活性。

A: mACs each has two domains of six transmembrane spans, TM1-6 and TM7-12, and two of cyclase homology domain , C1 and C2, as well as the N- and C-terminus located in the cytoplasm. C1 and C2 may form a catalytic active site at the pseudosymmetrical interface by residues from both domains, with the requirement of two metal cofactors (Mg^{2+} or Mn^{2+}) for catalytic activity. ATP binds at one of two pseudosymmetric binding sites at the C1-C2 interface, while the binding of forskolin and other modulators may regulate AC activity by inducing a conformational change. B: in comparison with mACs, sAC is lack of any transmembrane domain, and the C1 and C2 are directly connected in tandem and located in the region of N-terminus. The sAC gene produces alternatively spliced mRNAs coding for the full-length form of sAC_{fl} and a truncated one of sAC_t, respectively; of which, sAC_t consists almost exclusively of the C1 and C2, whereas sAC_{fl} has the C1 and C2 followed by the C-terminal residues containing an autoinhibitory motif and a heme-binding domain. The enzymatic activity of sAC_t is much higher than sAC_{fl}.

图1 哺乳动物腺苷酸环化酶的结构示意图(根据参考文献[1,7-8]修改)

Fig.1 Schematic representation of domain architecture of mammalian adenylate cyclases (modified from references [1,7-8])

TM7~12决定mAC的细胞膜靶向定位和CHDs定向以及寡聚化[1,4]。

sAC能够通过mRNA的选择性剪接(alternative splicing)机制产生不同的变异体, 其中全长的sAC_{fl}在其肽链的氨基末端区域含有两个明显的CHDs串联在一起。紧随其后的下游C-端是一段长度大约为1 100氨基酸残基的序列, 含有一个自抑模体(autoinhibitory motif)和一个血红素结合域(heme-binding domain), 但无任何明显的跨膜结构域。而一个截短表达的sAC_t仅由两个CHDs组成, 它具有比sAC_{fl}显著提高(10倍以上)的酶活性。与mACs相比, sAC的氨基酸序列显著分化, 并且在结构组成上也更像细菌来源的环化酶[1,5]。

目前认为, AC酶活性是由两个同源或异源的CHDs形成的二聚体决定的。其催化中心位于该二聚体的接缝界面, 使其催化速率明显地受该界面的结构运动影响, 这种在结构上的重新定向过程是AC活性调节的一种主要机制[6]。

2 ACs在不同器官组织或细胞中的表达与分布

迄今, 不同学者已利用RT-PCR、免疫组织化学和原位杂交等技术对哺乳动物不同AC异构体的表达与分布特征进行了不少研究[7,9-18]。他们发现, 这些异构体均能够在绝大多数被检测的不同器官组织或细胞中普遍表达, 但在表达水平上存在差异, 说明它们能够在一定的器官组织或细胞中优势表达(表1)。此外, 一些异构体的表达水平在生长发育过程中或在一定刺激条件下也会发生变化[3,12,19], AC3的表达也受表观遗传学机制所调节[20]。

据报道, 在心脏中, AC5和AC6是能够高水平表达的异构体, AC1不表达, 其他mACs异构体均存在一定水平的表达[7,9-10,12-18]。在肾脏中, 除AC8不表达外, 其他mACs异构体均能够表达, 其中AC4和AC6似乎具有一定的表达优势。在肝脏中, AC2和AC8不表达, AC6与其他mACs异构体相比表现出较高的表达水平。在肺器官中, 除AC1不表达外, AC2、AC3、AC7和AC8较其他mACs异构体的表达水平较高。在肌肉组织中, AC7和AC9的表达水平较高, AC1、AC2、AC6和AC8次之, AC3和AC5不表达。在肾上腺中, 能够检测到AC1、AC5、AC6、AC8和AC9的表达, 其他mACs异构体似乎不表达。在睾丸中, sAC是最主要的表达形式, 所有9种mACs异构体也分别存在一定水平的表达。此外还发现, AC3、

表1 哺乳动物AC异构体在不同器官组织或细胞中的表达(根据参考文献[7,9-10,12-18]修改)

Table 1 Expression of AC isoforms among different organs/tissues or cells in mammals (modified from references [7,9-10,12-18])

AC异构体 AC isoform	表达的器官/组织或细胞 Expressed organs/tissues or cells
AC1	Brain, adrenal gland (medulla), kidney, muscle, ovary, liver, placenta, spleen, testis, peripheral blood leukocytes
AC2	Brain, lung, muscle, heart, kidney, ovary, uterus, testis, placenta, spleen, prostate, small intestine, thymus, bone marrow stromal cells
AC3	Brain, olfactory epithelium, pancreas, lung, heart, testis, brown adipose tissue, kidney, ovary, liver, uterus, male germ cells, placenta, spleen, prostate, small intestine, peripheral blood leukocytes, thymus, bone marrow stromal cells
AC4	Widespread (brain blood vessels, kidney, ovary, liver, cholangiocytes, heart, lung, brown adipose tissue, uterus, testis, placenta, spleen, prostate, small intestine, peripheral blood leukocytes, thymus, bone marrow stromal cells)
AC5	Brain (striatum), heart, kidney, liver, lung, testis, adrenal gland, brown adipose tissue, uterus, cholangiocytes, placenta, spleen, prostate, ovary, small intestine, thymus
AC6	Widerspread (brain, heart, kidney, liver, lung, testis, muscle, adrenal gland, brown adipose tissue, uterus, cholangiocytes, placenta, spleen, prostate, ovary, small intestine, thymus, bone marrow stromal cells)
AC7	Widespread (brain, platelets, heart, kidney, liver, lung, testis, muscle, uterus, cholangiocytes, placenta, spleen, ovary, peripheral blood leukocytes, thymus, bone marrow stromal cells)
AC8	Brain, pancreas, lung, testis, muscle, adrenal gland, uterus, brown adipose tissue, heart, ovary, cholangiocytes
AC9	Widespread (brain, testis, heart, lung, muscle, kidney, liver, adrenal gland, uterus, brown adipose tissue, cholangiocytes, placenta, spleen, prostate, ovary, small intestine, peripheral blood leukocytes, thymus, bone marrow stromal cells)
AC10(sAC)	Widespread/all detected tissues (brain, testis, heart, kidney, liver, muscle, cholangiocytes)

对应的异构体在下划线的组织器官或细胞中高水平表达。

Underlined organ/tissue or cells expressed this form at high level.

AC4、AC5、AC6和AC9能够在棕色脂肪组织(brown adipose tissue)中表达, AC2、AC3、AC4、AC6、AC7和AC9能够在骨髓间充质干细胞(bone marrow stromal cells, BMSCs)中表达以及一些异构体分别能够在卵巢、胎盘、脾、外周血白细胞、子宫、前列腺、小肠、胸腺、胰腺、血小板、雄性生殖细胞、胆管上皮细胞等其他各种器官组织或细胞中表达(表1)。

中枢神经系统(central nervous system, CNS)及其相关组织或细胞是ACs发挥功能的重要场所。研究表明, 除了发现AC4主要在血管中表达并存在于主要嗅觉上皮(main olfactory epithelium, MOE), 还发现一些异构体在脊髓(AC3、AC7)、纹状体(AC7, AC8)、海马(AC7)等组织中明显缺乏表达外, 所有mACs在皮层、基底神经节、丘脑、小脑、脑干、嗅觉系统等各种脑组织或中枢神经系统中表达, 但它们的表达水平存在差别[3,9,12-13,19,21-22]。此外, 从不同的发育时期或条件变化来看, AC1在脑组织发育或神经生长成熟过程中的表达水平变化较大, 而AC8在成年脑组织中的表达会发生较大的变化, 其他异构体的表达则在总体上表现比较稳定。从不同种类哺乳动物的脑组织进行的比较分析来看, mACs的时空表达特征基本相似, 但也存在细微差别。特别有趣的是, 调节特性不同的mACs似乎趋向于在同一组织区域中共同表达, 而调节特性相同的mACs则倾向于局限在不同组织区域中表达[3,19]。因此, 哺乳动物的不同mACs在脑功能活动中似乎能够发挥精细而巧妙的协同调节功能。

最后, 应该指出的是, sAC似乎在迄今已报道的所有被检测的器官组织或细胞中表达, 包括脑、睾丸、心脏、肾脏、肝脏、肌肉和胆管上皮细胞(表1)以及星形胶质细胞(astrocytes)、海马、视觉皮层、小脑中神经元的树突小荆棘(dendritic spines)和轴突末端(axon terminals)、背根神经节(dorsal root ganglion, DRG)、脊髓中正在发育的神经元等[11,23-24]。

3 ACs的亚细胞定位与调控

3.1 mACs

mACs含有的跨膜结构使其靶向于细胞膜上发挥功能。mACs是GPCRs-G蛋白信号作用通路下游的主要效应物(effector), G蛋白被激活后产生游离的α亚基(Gα)和βγ亚基二聚体($G_{\beta\gamma}$), 它们分别能够直接调节mACs的活性。其中, 刺激型α亚基($G\alpha_s$)对所有九种mACs(AC1~9)均产生激活作用。抑制型α亚基($G\alpha_i$)除对AC2、AC4和AC7无影响外, 对其余的异构体均产生抑制作用。$G_{\beta\gamma}$抑制AC1、AC3和AC8, 也能够在一定条件下对AC2、AC4~7产生激活作用。此外, 钙离子(Ca^{2+})/钙调蛋白(calmodulin, CaM)能够有选择性地对这些异构体产生直接的调节作用[2,25-26]。通常根据mACs的调节特性将它们划分成4组(表2): 第I组包括AC1、AC3和AC8, 它们均能够由钙活化的CaM诱导激活; 第II组包括AC2、AC4和AC7, 能够被$G_{\beta\gamma}$激活; 第III组包括AC5和AC6, 能够被亚微摩尔水平或生理浓度的游离Ca^{2+}以及$G\alpha_i$所抑制; 第IV组仅包括AC9, 它与AC1~8的序列相似性较低, 并且在植物中发现的一种小分子化合物——毛喉素(forskolin)可以显著地直接激活AC1~8, 却对AC9的激活作用甚微。

mACs能够通过磷酸化(phosphorylation)、糖基化(glycosylation)和S-亚硝基化(S-nitrosylation)等蛋白质修饰作用, 产生抑制或激活效应[2]。一些异构体(AC1、AC5、AC6、AC8)的二聚体化与G蛋白的调节作用有关[15]。此外, 迄今已发现不少蛋白质因子能够与mACs产生互作而影响其功能, 包括SOCE(store-operated Ca^{2+}-entry)装置和电压门控钙离子通道(voltage-gated Ca^{2+} channels, VGCCs)[25]、A型激酶锚定蛋白(A-kinase anchoring proteins, AKAPs)[27]、膜联蛋白A4(annexin A4)[28]、Snapin和Ric8a等[29]。这些蛋白质互作因子可能会使AC-cAMP信号通路与各种细胞机器发生关联, 或者协调其他信号分子对AC-cAMP信号的调控[30]。研究表明, 一氧化氮(nitric oxide, NO)信号通路影响mACs的活性[2,31]; 对Ca^{2+}敏感的mACs(AC1、AC3、AC5、AC6、AC8)能够富集地存在于细胞膜的脂筏(lipid rafts)之中, 并与SOCE位点区室化(compartmentalization), 这将非常有利于这些mACs对Ca^{2+}浓度水平的变化作出快速反应[32-33]。通过在脂筏和非脂筏域中的差异定位, 不同的AC异构体能够参与特异的信号通路和细胞应答[34]。对mACs调控机制研究进展的详细了解, 可进一步参考有关学者在过去已发表的一系列综述文章[7,15,17,35-36]。

3.2 sAC

sAC缺少跨膜结构, 其存在于细胞质及其包含的亚细胞器(细胞核和线粒体等)中, 并且能够发生

表2 哺乳动物AC异构体的调节特性(根据参考文献[25-26]修改)

Table 2 Regulatory properties of mammalian AC isoforms (modified from references [25-26])

AC异构体 AC isoform	分组 group	$G\alpha_s$	$G\alpha_i$	$G_{\beta\gamma}$	钙 Calcium	蛋白激酶 Protein kinase	毛喉素 Forskolin
mAC	Group I:				stimulated		
	AC1	↑	↓	↓	↑ CaM	↑ PKC_α ↑ RTK	↑
	AC3	↑	↓	↓	↑ CaM	↓ CaMK IV ↑ PKC_α	↑
	AC8	↑	↓	↓	↑ CaM	↓ CaMK II ↓ PKA	↑
	Group II:				insensitive		
	AC2	↑	—	↑ $^{\$}$	—	↑ PKC_α	↑
	AC4	↑	—	↑ $^{\$}$	—	↑ PKC_α	↑
	AC7	↑	—	↑ $^{\$}$	—	↑ PKC_α	
	Group III:				inhibited	↑ $PKC_{\alpha,\zeta}$	
	AC5	↑	↓	↑ $^{\$}$	↓ free Ca^{2+}	↑ RTK ↓ PKA ↓ $PKC_{\delta,\varepsilon}$	↑ ↑
	AC6	↑	↓	↑ $^{\$}$	↓ free Ca^{2+}	↑ RTK ↓ PKA	
	Group IV:						
	AC9	↑	↓ (weak)	—	↓ via CaN	↓ PKC_α ↑ CaMK II	↑ (weak)
sAC	AC10*	—	—	—	stimulated	—	—

AC活性上调(↑), 下调(↓), 或未变化(–)。$^{\$}$ $G_{\beta\gamma}$的刺激作用依赖于$G\alpha_s$或毛喉素的共激活。*AC10/sAC可被重碳酸盐激活。CaM: 钙调蛋白; CaN: 钙调磷酸酶; CaMK IV: 钙调蛋白激酶IV; CaMK II: 钙调蛋白激酶II; RTK: 受体酪氨酸激酶; PKC: 蛋白激酶C; PKA: 蛋白激酶A; $G\alpha_s$: 刺激型G蛋白α亚基; $G\alpha_i$: 抑制型G蛋白α亚基; $G_{\beta\gamma}$: G蛋白βγ异二聚体亚基。

AC activity is stimulated (↑), inhibited (↓), or not modified (–). $^{\$}$ $G_{\beta\gamma}$ stimulation is conditional upon $G\alpha_s$ or forskolin co-activation. *AC10/sAC is activated by bicarbonate. CaM: calmodulin; CaN: calcineurin; CaMK IV: CaM kinase IV; CaMK II: CaM kinase IV; RTK: receptor tyrosine kinases; PKC: protein kinase C; PKA: protein kinase A; $G\alpha_s$: stimulatory G protein α subunits; $G\alpha_i$: inhibitory G protein α subunits; $G_{\beta\gamma}$: G protein βγ subunits of heterodimer.

动态转移以及定位在“微结构域(microdomain)”之中, 从而对cAMP的生成和信号作用进行精细的时空调控[37-39]。与mACs的调节机制有很大不同, sAC并不能够对$G\alpha_s$和毛喉素等传统上所认知的AC激活剂产生反应, 却被HCO_3^-和Ca^{2+}直接激活, 并对细胞内ATP的浓度变化比较敏感[40]。Ca^{2+}能够提高sAC对其反应底物ATP-Mg^{2+}的亲和力, 而HCO_3^-可以提高酶反应速率和减弱高浓度ATP-Mg^{2+}底物产生的抑制效应, 这两者对sAC具有协同的激活作用[41]。 sAC也受蛋白质之间互作的调节, 它能够与精子特异Na^+/H^+逆向转运蛋白(sperm-specific Na^+/H^+ exchanger, sNHE)互作, 调节HCO_3^-介导的激活作用[42]。

4 ACs在生理活动与健康和疾病发生中的作用

近年来, 利用基因敲除(knockout, KO)、RNA干扰(RNA interference, RNAi)、转基因过表达(over-expression)、突变体、小分子抑制剂(small molecule inhibitors)、关联分析和嵌合子小鼠等一系列方法和技术, 正在不断地揭示出不同AC异构体所发挥的各种各样的生理作用及其与健康和疾病的关系。

4.1 mACs

4.1.1 AC1、AC3和AC8 这些异构体能够使细胞内Ca^{2+}与cAMP信号系统发生偶联, 从而具有非常重要的生理功能。AC1和AC8是在中枢神经系统中优势表达的两个主要异构体, 它们在学习与记忆等适应性行为以及疼痛和脑发育中产生显著影响[43-44]。研究表明, *AC1*缺失或扰乱会影响躯体感觉皮层(somatosensory cortex)中神经分布的“模式化(patterning)”或“barrelfield”的形成[45]、丘脑突触(thalamocortical synapses)的成熟和躯体感觉运动行为(somatosensorimotor behaviors)[46]、视网膜映射的细化(retinotopic refinement)[47]、远程场景记忆(remote contextual memory)[48]以及多种形式的突触

可塑性(synaptic plasticity)[49-50]。AC8与AC1经常表现相同或冗余的生理作用, 但在某些方面也存在明显的差异。研究发现, *AC8*敲除会显著抑制突触前沉默(presynaptic silencing)诱导发生之后的正常突触功能的恢复, 而*AC1*敲除在这方面的影响甚微[51]。*AC1*敲除会影响参考记忆(reference memory), 而*AC8*敲除却影响记忆保持(memory retention)和快速获得新的空间信息以及工作/情景样记忆(working/episodic-like memory)[52]。*AC1*或*AC8*敲除均能够减轻慢性肌肉疼痛[44], 利用AC1的选择性抑制剂NB001能够对肠易激综合征(irritable bowel syndrome, IBS)诱导的自发性疼痛(一种主要形式的慢性内脏疼痛)以及神经病理性与发炎性等慢性疼痛产生明显的镇痛效果[53-54]。AC1和AC8参与调节可卡因(cocaine)或鸦片(opiate)等诱导的行为可塑性或上瘾, 对它们的敲除可使小鼠降低或失去对鸦片、可卡因、冰毒(methamphetamine, 即甲基苯丙胺)等诱导产生的依赖性以及降低多巴胺反应[55-57]。AC1和AC8也在脑神经对酒精的敏感性中发挥关键的调节作用, 它们的缺失会显著增加纹状体中细胞的凋亡(apoptosis), 加剧小鼠对酒精诱导的神经退行性变(neurodegeneration)[58-59]。此外, *AC1/AC8*双敲除小鼠表现活动减退(hypoactive)、减少的蔗糖偏好(sucrose preference)和神经营养信号(neurotrophic signaling)的改变以及冲动性(impulsivity)降低和社交性(sociability)增加等行为特征, 而*AC1*超表达的转基因小鼠则产生相反的表现[60-61]。

AC8在糖诱导胰腺β细胞分泌胰岛素(insulin)的活动中发挥调节作用, 参与能量稳态和营养稳态的控制[62-63]。*AC8*(而不是*AC1*)敲除或抑制会降低糖诱导的胞质钙和胰岛素分泌的增加, 导致小鼠对葡萄糖的耐受性变差[64]。*AC8*敲除小鼠对红藻氨酸(kainic acid)和毛果芸香碱(pilocarpine)诱发癫痫作用的敏感性降低, 而且使它们在海马中诱导的退行性神经元和苔藓纤维发芽(mossy fiber sprouting)显著减少, 说明AC8与癫痫发生(epileptogenesis)有关[65]。

AC3是嗅觉信号转导通路的必要成分, 该基因的缺失会显著降低主要嗅球(main olfactory bulb)的大小和成体神经发生(adult neurogenesis)的水平、增加颗粒细胞(granule cells)的凋亡和扰乱新形成颗粒细胞的成熟[66]、改变嗅球地形图(topographical map)[67-68]以及导致主要嗅觉表皮中一些基因的表达水平发生显著变化[69]。*AC3*缺失小鼠表现为周围性和行为性嗅觉丧失(anosmia)[22]以及母性行为(闻仔鼠尿、建筑巢穴、攻击性)的异常[70]。AC3在能量稳态中也发挥明显作用, 该基因缺失的成年小鼠表现肥胖症状[71]。而AC3功能获得型突变小鼠可防止饮食诱导的肥胖, 表现出体重和脂肪量的明显降低以及比较低的基础胰岛素和糖水平[72]。一种GLP-1(glucagon-like peptide-1)类似物 —— 利拉鲁肽(liraglutide), 可使肝脏中*AC3*的表达升高, 引起体重降低和胰岛素抵抗(insulin resistance)的改善[73]。AC3参与调节肾脏和肠道的跨细胞Mg^{2+}运输, 在肾中对它的特异性删除会导致小鼠尿液和肾中泌尿的Mg^{2+}浪费增加、血清的Mg^{2+}浓度在低Mg^{2+}饮食条件下显著下降[74]。*AC3*在小鼠中整体敲除会趋于增加尿量和钠排泄, 而肾小球滤过率(glomerular filtration rate, GFR)却降低50%[75]。AC3似乎与致瘤性有关, 它在HEK293细胞中的过表达会增加细胞迁移、侵袭、增殖和克隆形成, 而利用shRNA降低人胃癌细胞中AC3的表达则会抑制肿瘤生长[20]。

4.1.2 AC2、AC4和AC7 目前, 尚无*AC2*基因敲除或修饰动物模型的研究报道。研究发现, *AC2*表达的上调与胰腺和小肠神经内分泌肿瘤(neuroendocrine tumors, NETs)等癌症有关[76]。AC2在动脉导管(ductus arteriosus)的脉管张力和重塑过程中具有一定的调节作用[77], 它似乎也与长期吗啡处理引起的耐受性有关[78]。与AC2一样, 人们迄今对AC4的生理作用仍缺乏了解。通过对*AC4*在小鼠的集尿管(collecting duct)中进行特异性敲除的研究表明, 该基因的缺失并未使小鼠发生任何显著的生理表型变化[79]。

研究表明, AC7在淋巴细胞(lymphocytes)和巨噬细胞(macrophages)中高度表达, 参与影响先天性和适应性免疫应答。*AC7*缺失小鼠表现严重的胚胎致死(超过90%)、对脂多糖(lipopolysaccharide, LPS)诱导的内毒素性休克异常敏感, 并对T细胞依赖性和非依赖性抗原产生妥协的抗体反应和记忆T细胞的减少、使巨噬细胞能够对LPS反应而产生更多的促炎性细胞因子TNF-α(tumor necrosis factor-alpha)[80]。酵母聚糖(zymosan)通过AC7激活PKA(protein kinase A)而在发炎中调节先天免疫的应答, *AC7*缺失会使源于骨髓的原代巨噬细胞丧失酵母聚糖介导的cAMP和PKA活性升高, 致使TNF-α的产生增加[81]。AC7有

利于急性髓细胞白血病(acute myeloid leukemia)的发生, 而该基因的缺失会导致这些白血病细胞的生长减少、程序化死亡升高和c-Myc表达降低[82]。

4.1.3 AC5和AC6 目前人们普遍认为, AC5和AC6是调节寿命和胁迫抗性的基本要素。*AC5*敲除可使小鼠平均寿命延长30%、明显改善老龄化诱导的骨密度降低和心肌病等症状[83]。对AC5的选择性抑制也有利于减轻各种胁迫或刺激因素诱导的心肌细胞凋亡, 使寿命延长[84-85]。而*AC5*过表达却损害心脏承受压力的能力[86], 引起心肌细胞中肌质网(sarcoplasmic reticulus)的Ca^{2+}超载和氧化胁迫而诱导心律失常(arrhythmias)[87]。AC5能够在中枢神经系统的背侧纹状体(dorsal striatum)和伏隔核(nucleus accumbens)高度集中表达, 与多巴胺(dopamine)受体D1和D2以及μ阿片受体(mu opioid receptors, MOR)等共存, 可能会参与影响运动功能(motor function)、奖励(reward)和情绪(emotion)等[88]。*AC5*缺失或抑制会使吗啡(morphine)的主要行为效应减轻和阿片受体选择性激动剂的效应丧失[89], 增加小鼠的酒精消费和降低酒精敏感性[90], 产生抗焦虑样反应(anxiolytic-like response)[91], 获得对含有嗅觉线索食物的行为偏好[92], 抑制多巴胺前体L-DOPA(L-3,4-dihydroxyphenylalanine, 用于治疗帕金森病)诱导的运动障碍(dyskinesia)[93], 显著减轻各种急性(包括热、机械、神经损伤)和慢性(包括炎症和非炎症性)疼痛反应[94]以及防止饮食诱导的肥胖和胰岛素抵抗[95]。此外, *AC5*突变与舞蹈症(chorea)和肌张力障碍(dystonia)等运动障碍有关[96]。

与AC5一样, AC6也是在哺乳动物心脏中介导β-肾上腺素能受体(β-adrenergic receptors, β-AR)刺激信号的主要异构体, 但它们之间介导的功能却很不相同, 尤其表现在对逆境胁迫的反应。AC6在心脏中表达的激活或提高, 可以改善缺血性心肌病和严重充血性心力衰竭的心脏功能[97-98]、提高心肌梗塞和心肌病的成活率[99-100]以及降低压力过载心脏的扩张和功能障碍[101]。*AC6*敲除还会使β-AR持续刺激(与心肌病有关)期间的死亡率增加[102], 以及破坏骨机械力转导而影响装载诱导骨骼的适应[103]。在多囊性肾病(polycystic kidney disease, PKD)中, AC6对囊肿形成和肾损伤发挥明显的调节作用, 该基因的缺失可降低肾的大小和囊肿形成、使肾功能改善、增加成活率[104]。AC6在集尿管中参与调节钠和水的运输, 它的缺失会使尿渗透压降低、尿排出量和液体摄取增加[105]。最后, AC6能够与Snapin和Snap25结合, 调节神经突延伸(neurite extension)[106]。

4.1.4 AC9 据报道, *AC9*敲除会引起免疫系统发生改变, 提高IgG_1对卵清蛋白挑战的反应[7]。利用siRNA抑制单核细胞中*AC9*的表达, 会显著增加TNF-α的释放[107]; 而*AC9*表达水平的下调或抑制也能够促进T细胞和宫颈癌细胞的增殖, 并减少细胞凋亡[108-109]。在急性前髓细胞白血病(acute promyelocytic leukemia, APL)细胞系NB4中, 对*AC9*表达水平的下调可抑制ATRA(all-trans retinoic acid)诱导的APL细胞分化[110]。此外, *AC9*表达水平在NF1恶性外周神经鞘肿瘤(peripheral nerve sheath tumor)细胞中也表现升高[111]。

4.2 sAC

由于碳酸酐酶在生理系统或活细胞中无处不在, 使HCO_3^-、CO_2和pH三者之间几乎处于瞬态均衡的状态, 参与许多生物过程的调节。sAC能够被HCO_3^-直接激活, 从而在生理上发挥CO_2/HCO_3^-/pH敏感器的功能[112]。*sAC*基因的破坏不会影响精子发生, 却严重损伤精子活力、导致雄性不育, 而雌性突变小鼠表现育性正常[113]。sAC参与调节线粒体呼吸和激活细胞凋亡的线粒体途径, 它在线粒体中能够响应代谢产生的CO_2, 调节ATP和活性氧(reactive oxygen species, ROS)生成, 从而对养分的有效性做出反应[114]。成年大鼠心肌细胞在模拟离体缺血(simulated *in vitro* ischaemia, SI)条件下, 会导致sAC易位到线粒体和线粒体的去极化(depolarization)以及随后在模拟再灌注(simulated reperfusion, SR)期间的细胞凋亡, 而在SI(非SR)期间利用抑制剂或shRNA对*sAC*进行的抑制均可显著降低SI/SR诱导的线粒体损伤和细胞凋亡[115]。此外, *sAC*的抑制也可以消除模拟离体缺血或酸中毒(acidosis)诱导冠状动脉内皮细胞(coronary endothelial cells)依赖于线粒体途径的凋亡[116]以及抑制氧化胆固醇(oxysterol)或氧化胁迫诱导血管平滑肌细胞(vascular smooth muscle cells, VSMC)凋亡的线粒体途径和ROS生成[117-118]。同时, 研究还发现, sAC在细胞质(而不是线粒体)中超表达, 会显著促进氧化胆固醇处理诱导的细胞凋亡和ROS生成[118]。

sAC在星形胶质细胞中高度表达, 它能够被电碳酸氢钠协同转运蛋白(electrogenic $NaHCO_3$

cotransporter, NBC)介导的HCO_3^-内流所激活, 导致cAMP升高, 从而诱导糖原分解和糖酵解的增强, 使乳酸释放到细胞外间隙而被神经元吸收, 作为维持突触功能的能源底物, 因而sAC使脑的能量代谢与神经元偶联[23]。sAC(而不是mAC)能够促进PC12细胞响应神经生长因子(nerve growth factor, NGF)和佛波酯(phorbol ester, PMA)引起的快速迁移, 它是NGF诱导小分子G蛋白Rap1激活所必需的[119]。sAC在轴突发育中能够响应神经生长导向因子Netrin-1而产生cAMP, 它的超表达会促进生长锥(growth cones)的发生和轴突的生长(axonal outgrowth), 而对它的抑制则起相反的作用[24]。sAC生成的cAMP是脑源性神经营养因子(brain-derived neurotrophic factor, BDNF)克服髓鞘结合糖蛋白(myelin-associated glycoprotein, MAG)介导神经突生长(neurite outgrowth)的抑制所必需的。而在大鼠和小鼠神经中过表达sAC, 可以诱导髓鞘中神经突生长和促进轴突再生(axonal regeneration)[120]。在视网膜神经节细胞(retinal ganglion cells, RGC)中, sAC抑制会显著降低RGC的存活和轴突生长; 而对mACs的特异性抑制或*AC1/AC8*双敲除, 并未使RGC发生变化[121]。sAC在无色素睫状上皮细胞(nonpigmented ciliary epithelial cells)内高度表达, 而重碳酸盐的产生在房水(aqueous humor, AH)生产过程中起关键作用, 对sAC的抑制可导致眼内压(intraocular pressure, IOP)显著增加[122]。

sAC在一些癌细胞的增殖中发挥作用, 影响肿瘤的恶性程度。研究表明, sAC在前列腺癌(prostate cancer)细胞中表达水平升高, 对它的抑制可导致细胞周期停滞、凋亡以及放射敏感性提高[123-124]。sAC超表达则会促进细胞增殖, 使放射效果显著降低[123]。在正常皮肤中, sAC的染色会均匀地分布于整个角质形成细胞(keratinocytes), 而在牛皮癣/银屑病(psoriasis)、寻常疣(verruca vulgaris)和皮肤晒伤引起的原位鳞状细胞癌(squamous cell carcinoma *in situ*, SCCIS)等一些过度增生性皮肤疾病中, sAC的染色主要集中在角质形成细胞的核中。进一步的实验证明, sAC可在细胞分化中迁移至细胞核, 似乎能够调节基因的表达而发挥作用[39]。有人已经发现, sAC在细胞核的表达与黑素细胞(melanocytes)从良性到癌症的转变有关[125]。sAC介导的信号通路也与乳腺癌细胞的增殖有关[126]。在胰腺β细胞中, 糖代谢会导致CO_2、ATP和Ca^{2+}等三种细胞内信号的产生, sAC是正常糖代谢过程中刺激胰岛素分泌所必需的[40]。在INS-1E胰岛素瘤细胞中, sAC能够依赖钙离子流入(calcium influx)而被糖激活介导cAMP的升高, GLP-1则诱导mACs介导cAMP的升高[127]。研究也发现, sAC发生的碱基置换突变与吸收性高尿钙症(absorptive hypercalciuria)有关, 导致肾结石的形成和脊柱骨密度降低[128]。sAC还参与调节白细胞跨内皮迁移(transendothelial migration, TEM)[129]、影响肾远端质子分泌(renal distal proton secretion)[130]。

5 结语与展望

在哺乳动物中, ACs是cAMP信号作用通路的关键控制酶, 近年来对ACs的基因表达与调控、蛋白质结构和生理功能等方面的研究已取得了显著进展, 使其成为令人感兴趣的药物研发目标。例如, AC1、AC3和AC8可作为处理智力迟钝(mental retardation)、疼痛、上瘾(addication)、焦虑(anxiety)、抑郁(depression)以及神经退行性变疾病[如阿尔茨海默病(Alzheimer's disease)等]的治疗靶点[43]。AC5的抑制剂则对心脏胁迫、老龄化、糖尿病和肥胖症等具有潜在的治疗应用性[86,95]。此外, 针对不同的mACs研发特异性的抑制剂, 将有助于解决$G\alpha_{i/o}$偶联受体介导AC"超敏化"引起的药物滥用和成瘾这一当前全球面临的普遍问题[2]。近年来, 人们已建议将sAC作为雄性避孕(male contraceptive)、II型糖尿病(type 2 diabetes)、青光眼(glaucoma)、前列腺癌、黑色素瘤(melanoma)的治疗靶点或诊断标记[5,7,40,122,124,127]。sAC也正在被应用于对银屑病与鳞状细胞癌的诊断标记和药物研发靶点[1,125]。

但是, 也应该指出, 由于不同种类的AC异构体通常能够在同一器官组织或细胞中共同表达, 不利于区分它们之间在功能与调节等方面存在的差异。一些研究也可能会因为不同异构体之间存在补偿效应或者未知反应和误靶, 导致在实验结果上的缺陷。同时, 迄今对ACs介导的信号作用通路与分子调控机制的了解, 大多数报道仍然局限于在离体条件下对细胞模型的研究, 今后应充分利用各种动物模型和基因修饰等技术与方法开展这方面的工作, 以便充分揭示某一特定的AC异构体或不同异构体之间的互作在不同器官组织或细胞类型中发挥作用的实

际意义, 从而为特异性药物研发以及人类健康与疾病治疗服务。

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