

综述

神经细胞初级纤毛在中枢神经系统疾病中作用的研究进展

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摘要: 初级纤毛广泛存在于哺乳动物中枢神经系统中, 是神经细胞重要的胞外细胞器。初级纤毛中含有多种离子通道、G蛋白耦联受体、激酶等, 提示初级纤毛可感受胞外信号并将信号转导至细胞内, 从而引起细胞对外界刺激信号产生应答反应。近年来大量研究表明调控纤毛结构及功能的基因发生突变后, 会导致许多单基因的遗传性疾病。当神经细胞初级纤毛中激酶、G蛋白耦联受体以及离子通道的功能异常后, 往往会引起一系列的神经精神疾病、神经系统发育异常等神经系统疾病。本文就初级纤毛在神经系统疾病中作用的研究进展进行综述。

关键词: 初级纤毛; G蛋白耦联受体; 3型腺苷酸环化酶; 抑郁

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Advances on the role of primary cilia in neurological diseases

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Abstract: Primary cilium, widely distributed in mammalian central nervous system, is an important extracellular organelle of cells. The primary cilia contain a variety of ion channels, G-protein coupled receptors and different kinds of kinases, which indicates that primary cilia can detect extracellular signals and transduce them into cells to regulate various cellular and physiological processes. In humans, mutations of genes related to structure and function of primary cilia always cause various monogenetic diseases. Moreover, a series of neuropsychiatric diseases and neurodevelopmental dysplasia are caused by abnormal functions of G-protein coupled receptors, kinases and ion channels in primary cilia. This article reviews recent research progress on the role of primary cilia in related neurological diseases.

Key words: primary cilia; G-protein coupled receptors; type 3 adenylyl cyclase; depression

1 纤毛的基本结构和功能

纤毛是一种从原生物到哺乳动物非常保守的细胞器, 长度从几微米到2毫米不等^[1-3], 几乎所有类型的细胞表面都具有纤毛结构。纤毛结构大体上可以分为4部分: 纤毛基部、纤毛轴丝、纤毛基质和纤毛膜。纤毛基部位于纤毛的最低端, 并将纤毛基质和细胞质分割开来, 从而使位于细胞不同部位的受体、蛋白分子对细胞的调控更为精确。纤毛轴丝是由中心体的中心粒产生, 并向纤毛顶部逐渐

延伸的9对环形排列的微管组成, 同时被来自母中心粒的基体锚定在纤毛基部。纤毛基质填充于纤毛轴丝和纤毛膜之间。纤毛膜则包裹着轴丝, 与细胞膜紧密相连。根据纤毛轴丝的结构和功能不同, 纤毛可被分为“9+2”或“9+0”两种类型^[4](如图1)。一般情况下, “9+2”型纤毛为运动型纤毛(motile cilia), 由排列在外周的9根二联微管和中间的2根中心微管构成; “9+0”型纤毛为静止型纤毛(non-motile cilia), 也称初级纤毛(primary cilia)^[4], 中间缺少2根

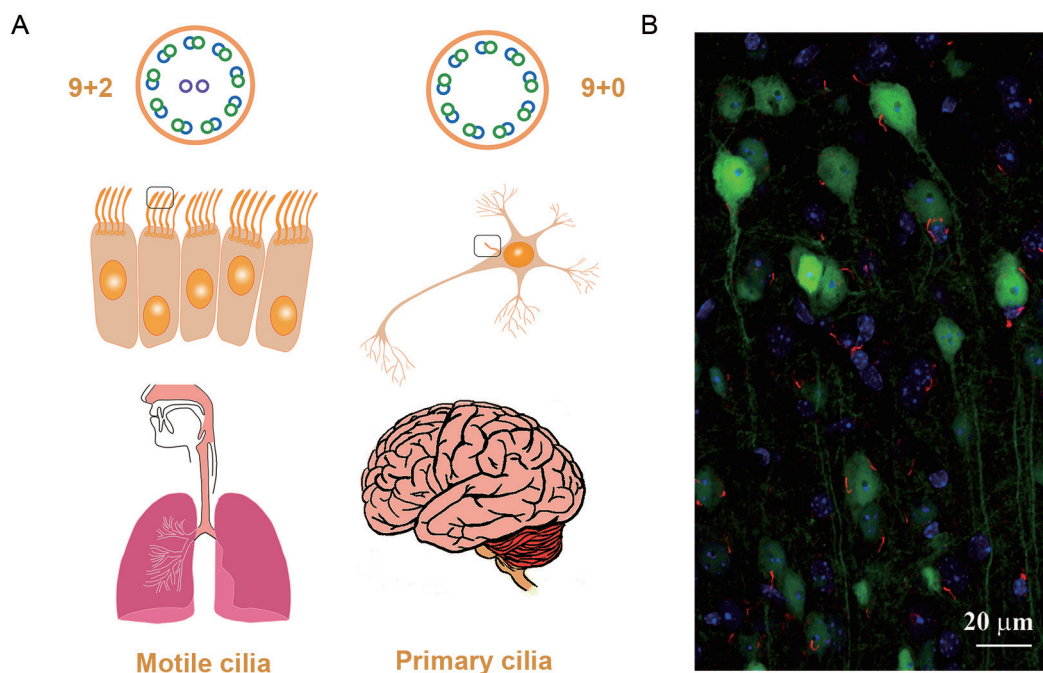


图 1. 纤毛的基本结构示意图和神经细胞初级纤毛的表达形态

Fig. 1. Ciliary structures and expression. *A*: Two types of ciliary structures in vertebrates: most cilia in cells of pseudostratified ciliated columnar epithelium are motile with ‘9+2’ arrangement; Most primary cilia in neurons are immotile with ‘9+0’ arrangement. *B*: Immunohistochemistry staining showing that primary cilia-expressing type 3 adenylyl cyclase (red) in mouse prefrontal cortex. Green: AAV-GFP-positive neurons. The nuclei were counterstained with DAPI. Scale bar, 20 μm .

中心微管，同时也缺少动力臂，因此不具有运动活性。运动型纤毛一般存在于呼吸道、输卵管、精子等需要通过纤毛的运动控制液体或物质流动的器官中。而初级纤毛主要位于密切感知并转导周围环境信息的各类上皮细胞和神经细胞中，如肾上皮细胞、嗅细胞和中枢神经系统中的神经元和胶质细胞等^[5]。本综述主要聚焦神经细胞初级纤毛，并对其形态和在神经系统疾病发生与发展中所发挥的重要作用进行综述。

初级纤毛在 19 世纪被首次报道^[6]，并广泛分布于哺乳动物体内的大多数细胞中。传统观念认为，初级纤毛是一种退化的细胞器，因而其长期被忽视。然而，近十年来大量研究表明初级纤毛作为细胞的一种感受装置，能感知细胞外机械和化学信号变化，并协助其转导至细胞内部从而引起细胞应答^[5]。初级纤毛在许多生理和病理过程中发挥了重要作用^[7,8]。既往研究表明初级纤毛膜中存在非离子通道型的 GABA 受体和谷氨酸受体^[9,10]，提示纤毛能够依赖于离子通道、受体以及下游的信号通路来行使功能。随后大量研究显示，初级纤毛中含有丰富的 G 蛋白耦联受体 (G-protein coupled receptors, GPCRs)，例

如 III 型生长激素抑制素受体 (type 3 somatostatin receptor, SSTR3)^[11,12]、VI 型血清素受体 (type 6 serotonin receptor, 5-HT6R)^[13] 和 I 型黑色皮质素受体 (melanin-concentrating hormone receptor 1, Mchr1)^[14] 等。这些表达在纤毛中的 GPCRs 大部分是 G_{α_s} 或 G_{α_i} 蛋白耦联受体，并且通过腺苷酸环化酶 (adenylyl cyclase, AC)/cAMP 信号通路将信号转导至神经细胞内^[9,10]。除 GPCRs 外，初级纤毛中表达的离子通道也参与了许多生理过程。研究表明初级纤毛中表达的多巴胺 D_5 受体与 $CaV_{1.2}$ -L 型钙离子通道存在功能性连接^[15]，激活 D_5 受体后，胞内钙离子浓度明显增高，同时伴随着肌动蛋白介导的纤毛增长现象^[16]。另有研究表明，初级纤毛中表达的 2 型加压素受体 (type 2 vasopressin receptor, V2R) 通过激活 AC，从而提高局部 cAMP 浓度，进而通过非选择性阳离子通道来调控纤毛内的 Ca^{2+} 信号^[17]。Bai 等人的研究也指出初级纤毛中的杂聚肽受体通过 TRPP2/TRCI 复合体，在感受机械信号以及调控胞内 Ca^{2+} 浓度等方面具有重要作用^[18]。纤毛内含有细胞纤毛内转运蛋白 (intraflagellar transport, IFT)，经“纤毛内运输”机制，将信号转导所需的蛋白质在细胞和纤毛之间进行双

向运输^[19], 而其基部含有扩散屏障, 可有效防止未经选择的分子扩散至细胞内^[20], 所以纤毛内的微环境与细胞内溶质是相对独立存在的。初级纤毛内基质的体积相较于神经细胞胞体是很小的, 而纤毛膜表面积相对较大, 可以镶嵌更多的受体蛋白; 同时初级纤毛内产生如 cAMP 的第二信使后, 较狭窄的纤毛区域可以富集更高浓度的第二信使; 从这种角度来说, 当营养物质、神经肽、形态发生素 (morphogens)、激素等胞外的信号分子存在时, 初级纤毛可以更加敏感和精确地感知信号^[21-23]。因此, 初级纤毛被认为是细胞的“天线”, 它可以快速检测和响应细胞外部的微环境变化, 并且将信号转导至细胞内部, 调节细胞活性、生长发育和其他生理过程, 是一种独特而且敏感的信号转导区域^[24]。

2 神经细胞初级纤毛的观察

神经细胞初级纤毛能够感知神经细胞外环境中的各种刺激, 如各种神经递质或调质的浓度、渗透压、pH 值甚至脑脊液流量等信息, 从而在中枢神经系统的发育、生理活动中发挥作用。然而, 由于缺乏初级纤毛的特异性标记物, 早期对初级纤毛的认识和功能研究也比较滞后。早期对初级纤毛的辨别主要依靠电镜的亚细胞结构的观察。1963 年, Dahl 观察到大鼠大脑皮层中神经细胞初级纤毛的存在^[25]。二十多年之后, Cohen 和 Meininger 报道在人的大脑皮层星形胶质细胞也观察到初级纤毛的亚细胞结构^[26]。自上世纪 90 年代以来, 随着科学技术的不断发展, 研究者开始使用免疫荧光的方法, 使用特异性的抗体对纤毛的结构以及表达在其中的不同蛋白质分子进行标记, 从而更加清晰地观察到纤毛的显微结构和不同蛋白分子的分布情况^[21-23, 27]。但这些方法的局限性在于, 不是所有的细胞都表达这些蛋白分子, 而基于某一蛋白的抗体可能不能标记所有的初级纤毛。最近, Sipos 等人运用不同的初级纤毛分子标记物抗体研究了小鼠大脑皮层中神经元、星形胶质细胞、小胶质细胞和少突胶质细胞等不同细胞类型中初级纤毛的表达情况, 研究结果表明, 针对 SSTR3 的抗体能够很好地标记神经元初级纤毛的形态, 而不能标记其他细胞类型的初级纤毛; 针对 ADP-核糖基化因子样蛋白 13B (ADP-ribosylation factor-like protein 13B, ARL13B) 的抗体则能很好地标记星形胶质细胞中的初级纤毛, 而无法标记其他细胞类型的初级纤毛; 而针对 3 型腺苷

酸环化酶 (type 3 adenylyl cyclase, AC3) 的抗体既能标记神经元中的初级纤毛, 也能标记星形胶质细胞的初级纤毛, 但后者信号要明显弱于在神经元中的标记, 这三种抗体均未能在中枢小胶质细胞和成熟的少突胶质细胞中观察到初级纤毛的形态^[28]。近些年来, 随着基因技术的兴起, 不断有研究者利用表达在纤毛中的一些特异性蛋白, 使用特定的转基因小鼠品系可以让我们直观地观察到纤毛的形态或检测钙信号变化。例如, O'Connor 等人使用绿色荧光蛋白 (GFP) 特异性标记神经细胞初级纤毛, 从而使研究者能够更为直观地观察到初级纤毛的改变^[29]。该转基因小鼠所携带的 GFP 与纤毛内特异性蛋白 SSTR3 相连, 研究者在深度麻醉的成年小鼠的大脑中观察到了包括神经细胞和上皮细胞脉络膜在内的许多细胞均带有初级纤毛^[29]。2016 年, Delling 等人应用钙成像技术研究钙信号对机械张力的反应。他们设计将钙指示剂 1.2 (calcium indicator 1.2) 基因编码进入初级纤毛中带有 mCherry (一种红色荧光蛋白) 标记的 ARL13B 基因中, 从而观察因为机械刺激引起的纤毛基质内的钙浓度改变。他们发现钙信号的变化与胚胎或成年小鼠肾脏纤毛是否感受到机械力无关^[30]。同样, 2015 年 Bangs 等人培育出一种用两种不同荧光标记初级纤毛的转基因小鼠 (ARL13B-mCherry:Centrin2-GFP), 即用 mCherry 标记初级纤毛中的 ARL13B, 用 GFP 标记纤毛中心体 (Centrin2), 他们使用这种转基因小鼠观察了初级纤毛和中心粒在胚胎发育全过程中的状态, 研究表明初级纤毛在胚胎第六天 (E6) 时出现在外胚层细胞, 但是内脏内胚层细胞以及滋养外胚层细胞含有中心体却缺少初级纤毛。ARL13B-mCherry:Centrin2-GFP 转基因小鼠可以清晰地标记外胚层, 甚至神经管内的神经上皮细胞也可进行清晰地观察^[31]。虽然我们己经可以使用带有荧光的转基因小鼠或特异性的抗体去标记初级纤毛, 但是仍然存在很多不足之处, 能够观察初级纤毛形态或检测纤毛中分子的实验方法也还远不能满足研究需求, 所以迫切需要寻找新的实验工具或新的转基因小鼠品系来检测和观察纤毛中的重要信号分子 (如 cAMP) 或者探究初级纤毛的形成过程。

3 神经细胞初级纤毛与神经系统疾病

人类中有许多和发育、退化等相关的单基因遗传疾病, 如 Bardet-Biedl 综合征、Joubert 综合征和 Meckel 综合征等, 均与初级纤毛的结构、长度的失

调相关,因而也统称这些疾病为“纤毛相关疾病”。近十年来的大量研究表明,神经细胞初级纤毛功能出现异常后,往往会产生精神疾病、智力障碍以及神经系统发育异常^[32],甚至与癌症的发生密切相关^[6,33,34]。纤毛中特异表达的受体、激酶等功能异常与这些神经系统疾病的发生高度相关。

3.1 初级纤毛中GPCRs与中枢神经疾病

在人类基因组中,GPCRs组成了庞大的蛋白质超家族,具有功能的GPCRs基因超过了800个^[35]。GPCRs可以被大致划分为6个类型:A类(视紫红质样受体家族)、B1类(分泌素受体家族)、B2类(黏附受体家族)、C类(谷氨酸受体家族)、F类(Frizzled/Smoothed受体家族)和味觉受体家族^[35,36]。绝大多数的GPCRs为视紫红质样受体家族,该类型的受体包含近700种受体,其中约有460种是嗅觉受体^[35]。在人体中,GPCRs具有重要的生理功能,可对多种信号做出应答反应,比如光子、多肽、蛋白质、激素、化学物质、脂质和糖类等。GPCRs也是一个广泛的治疗药物靶点,美国食品药品监督管理局(US Food and Drug Administration, FDA)认证的药物中约有1/3作用于GPCRs^[37]。许多GPCRs的下游效应分子定位于哺乳动物多种类型细胞的纤毛中^[38,39]。在啮齿动物神经细胞初级纤毛内,已被报道含有多种GPCR,如5-HT6R、SSTR3以及Mchr1等^[40-42](图2)。因此,初级纤毛可能在GPCRs介导的生理和病理过程中发挥重要功能。

近年来不断有研究显示,神经细胞初级纤毛中表达的多种蛋白分子的基因异常与许多纤毛疾病密切相关,初级纤毛的异常通常会造成神经细胞形态学的异常以及神经精神疾病^[4]。神经细胞初级纤毛中富含GPCRs以及其下游的效应蛋白^[43-45],这提示我们GPCRs可能作为信号传递的枢纽而存在。在关于小鼠Bardet-Biedl综合症的纤毛病模型研究中发现GPCRs在纤毛中起定位作用,当小鼠敲除编码BBS(Bardet-Biedl syndrome)蛋白复合体中的某些基因如*Bbs2*、*Bbs4*或*Bbs18*后,发现SSTR3、Mchr1、神经肽Y2受体(neuropeptide Y2 receptor, NPY2r)等GPCRs无法定位至神经细胞初级纤毛中,而*Bbs5*的缺失会导致多巴胺D1型受体在神经细胞初级纤毛中的运输和聚集^[40,46,47]。同时,TUB基因突变的纤毛病小鼠也出现了体型肥胖,部分GPCRs在神经纤毛中的定位异常^[47,48]。大量的研究表明神经细胞初级纤毛中的GPCRs在调控神经元生长和功能

方面起着重要作用。在胚胎时期抑制性中间神经元会迁移至特定脑区,并在神经环路中调控神经元的兴奋和抑制信号的传入。当中间神经元的微环路被破坏后,机体会出现一系列的神经发育失调现象,比如精神分裂症、自闭症(autism spectrum disorders, ASD)和智力障碍^[49]。5-HT6R是13种5羟色胺G蛋白耦联受体超家族的成员之一,且是唯一被报道表达在神经细胞初级纤毛中的受体亚型^[50,51],并与AC/cAMP信号通路具有紧密联系^[52]。很多临床数据均表明5-HT6R相关的激动剂和阻断剂具有抗抑郁的作用^[53]。2016年,Zhang等人发现前边缘皮质(prelimbic cortex, PrL)脑区中谷氨酸能神经元中的5-HT6R参与帕金森病引起的抑郁样行为,通过激活5-HT6R增加内侧前额叶皮质、杏仁核、缰核和腹侧海马中的多巴胺和去甲肾上腺素水平可以缓解帕金森病引起的抑郁样行为表现^[54,55]。同时相关报道指出,5-HT6R的内源性配体5-HT具有改善学习记忆以及认知功能的特性,并可能有助于治疗抑郁症相关的睡眠障碍和认知缺陷^[56]。Hu等人的研究揭示5-HT6R可以通过ARL13B来调控纤毛的长度,从而影响神经初级纤毛的功能,同时在阿尔茨海默病模型小鼠中也发现5-HT6R的阻断剂能够有效恢复小鼠的认知功能障碍^[57]。Wang等人通过改变5-HT6R的表达来调控初级纤毛的形态,发现5-HT6R异常后,初级纤毛中电压门控钠离子通道NaV_{1.2}异常,从而导致神经元兴奋性的改变^[58]。

除5-HT6R以外,神经细胞初级纤毛还表达其他GPCRs,这些GPCRs功能的异常也与神经系统疾病的发生密切相关。Omori等人的研究表明,下丘脑神经细胞初级纤毛中表达多种GPCRs,如神经肽FF受体1(neuropeptide FF receptor 1, NPF1R1)、催乳素释放激素受体(prolactin-releasing hormone receptor, PRLHR)和神经髓质U受体1(neuromedin U receptor 1, NMUR1),这些GPCR受体与摄食行为密切相关^[59]。此外,他们还发现一种短形式的2型多巴胺受体(a short form of dopamine receptor D2, DRD2S)能够被有效地转运到初级纤毛,并参与能量代谢的调节^[59]。Adamantidis等人的研究表明,Mchr1的缺失会影响海马NMDA受体介导的电流,从而导致认知功能受损^[60]。生长抑素受体家族有5个成员,均为GPCRs,其中只有SSTR3表达在神经细胞初级纤毛中。研究表明编码该受体的*sstr3*基因全身敲除小鼠出现了新物体识别受损,但是不影响新物体的位置记

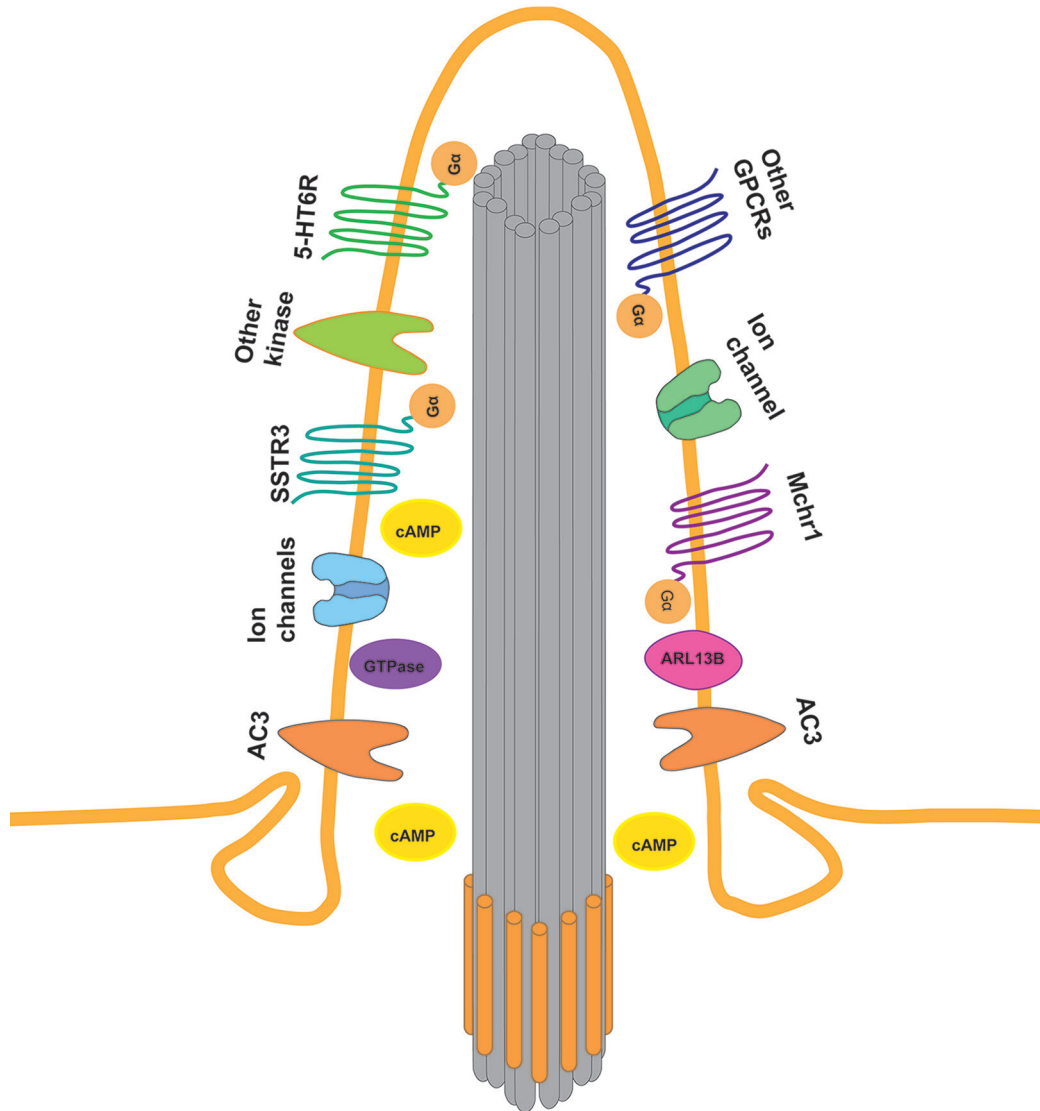


图 2. 神经细胞初级纤毛在神经系统疾病中发挥作用的可能机制

Fig. 2. Possible mechanism of the role of primary cilia in neurological diseases. The ciliary membrane is continuous with the plasma membrane. Specific signaling proteins are selectively targeted to and retained within neuronal cilia which contribute to several neurological diseases. These signaling proteins include enzymes, such as type 3 adenylyl cyclase (AC3) and ARL13B, the GPCRs, such as type 3 somatostatin receptor (SSTR3), type 6 serotonin receptor (5-HT6R), melanin-concentrating hormone receptor 1 (Mchr1) *etc.*, and ion channels. Dysfunction of these proteins in the primary cilia will cause aberrant intracellular downstream effectors, and ultimately influence the function of the neuron.

忆^[12]。随着研究的不断深入,有越来越多的 GPCRs 被发现表达在神经细胞的初级纤毛中,如 kisspeptin receptor 1 (Kiss1r)^[61]、NPY2r 和神经肽 Y5 受体 (neuropeptide Y5 receptor, NPY5r)^[46] 等,而对于这些 GPCRs 的功能研究也将会对某些神经系统疾病的发生机制提供新的实验证据。

3.2 初级纤毛中 GTPase (ARL13B) 与中枢神经系统疾病

在细胞接受外界信号后, GTPase 是介导细胞膜

和细胞骨架依赖性应答过程中的重要分子开关。研究表明纤毛中含有多种小 GTPase^[62],而在纤毛病 Joubert 综合征中 ARL13B 蛋白出现异常^[63], ARL13B 作为一种信号转导所必须的 GTPase,其作为纤毛中介导多种信号的关键蛋白分子之一而被广泛地报道^[64–70]。在某些器官中 ARL13B 是纤毛生成的关键分子^[71]。当条件性破坏 ARL13B 后,小鼠纹状体中神经环路的发育被扰乱^[72]。同样,当中间神经元缺失 ARL13B 时,会出现神经元树突和轴突数量的

减少、突触间联系中断以及突触活动性的减弱,同时会大量减少纤毛中 SSTR3 的含量并出现钙动力学紊乱;虽然 SSTR3 在纤毛中的表达可以修复因 ARL13B 缺乏所引起的形态学和突触连接缺陷,但是在非纤毛形式并具有正常活性 GTPase 的神经元中, SSTR3 的表达却不能修复 ARL13B 的缺乏造成的影响^[71],说明中间神经元中的 SSTR3 作为一种 GTPase 在抑制性神经元对下游神经细胞建立抑制作用和产生抑制性功能过程中十分重要。有关研究显示, AC3 作为腺苷酸环化酶的一种亚型与 SSTR3 共存于海马神经细胞初级纤毛^[73]以及嗅球细胞初级纤毛^[74]中,当纤毛中特定 GPCR 激活后,可通过与之耦联的 AC3 信号通路增加胞内 cAMP 的浓度,继而完成后续的一系列细胞应答反应^[45, 75]。ARL13B 也参与调控防止肌动蛋白聚合的 INPP5E 向纤毛的迁移^[65]。而 INPP5E 会在纤毛失去稳定之前耗尽,之后磷脂酰肌醇 4, 5-二磷酸转运至纤毛中,从而引发肌动蛋白的聚合和纤毛的断裂,并驱动细胞周期^[65]。Joubert 综合征是一种以“白齿症”为特征的常染色体隐性纤毛病^[76],其主要特征为内脏多器官、手、眼、大脑等的功能紊乱^[77, 78]。Joubert 综合征患者表现出大脑功能紊乱、智力障碍和发育迟缓等问题,在临床上也发现了一些纤毛病患者都表现出的临床症状,比如多囊肾、失明以及多指畸形。许多 Joubert 综合征患者 ARL13B 基因发生不同的突变,而这些突变均在某种程度上造成了神经系统发育障碍。ARL13B 发生纯合错义突变 c.[223 G > A] (p. (Gly75Arg) 后,产生 ARL13B 基因鸟苷酸交换活跃因子的明显丢失,但是却保留了其 GTPase 的活性^[79]。ARL13B 在调控 Shh 信号分子转运至纤毛内的过程中也发挥着重要的作用,同时也可诱导细胞进行增殖并存活^[80]。因为 Shh 信号分子存在于初级纤毛中,ARL13B 的破坏导致 Shh 信号传导障碍^[81, 82],所以 ARL13B 的消融而产生的的神经管细胞瘤,是由于 Shh 信号分子的部分缺失造成的^[83]。

3.3 初级纤毛中 AC3 与神经系统疾病

大多数的抗抑郁药品(比如选择性血清素再吸收抑制剂)均会通过 G 蛋白耦联的肾上腺素受体或血清素受体激发 AC 的活性发挥作用。而且,一些研究表明 AC 的活性与抑郁症直接相关^[84],同时,血小板 AC 的活性被认为是重度抑郁症(major depressive disorder, MDD)的生物学标记^[85, 86],而且血小板中主要的 AC 即为 AC3^[87]。在中枢神经系统中, AC3

广泛且特异性存在于神经细胞初级纤毛中^[9],同时也是神经细胞初级纤毛中调控 cAMP 信号通路的关键激酶^[9, 46]。最初, AC3 被报道表达在嗅上皮,并且是嗅觉信号传导中的关键蛋白^[88-90]。2007 年, Bishop 等人发现 AC3 在小鼠脑内皮层、海马、下丘脑、杏仁核、伏隔核等多个脑区广泛表达,且特异性表达在神经细胞的初级纤毛中^[45],从而推翻了在中枢神经系统中 AC3 是只存在于嗅觉纤毛的说法。Zhou 等人发现在小鼠前额叶皮质中 AC3 主要表达在神经元中,极少表达在小胶质细胞或星形胶质细胞中^[91]。Kumamoto 等人发现在成熟的神经元中初级纤毛参与调控树突的分支和突触的整合^[92],同时 Guadiana 等人在海马以及其他几个脑区中均发现初级纤毛中 AC3 产生的 cAMP 调控树突的生长以及突触间的活动性^[93]。

在中枢神经系统中, AC3 的生理功能还未完全明确,但是多个人类遗传学研究表明 AC3 与 MDD^[94]、ASD^[95, 96]等神经精神疾病高度相关。MDD 是一种复杂多样的精神疾病,其主要特征为持续的情感低落/兴趣减少、思维迟缓、无价值感或罪恶感、自杀观念等^[97]。通常,抑郁症患者还伴随认知障碍^[98],大脑体积减小^[99, 100],紊乱的睡眠模式^[101],空间感觉障碍^[102]等其他一系列异常。2012 年,精神病学权威杂志 *Mol Psychiatry* 发表了一篇关于人类基因组学的研究,通过对超过 2 000 名 MDD 患者的全基因组关联研究,发现 *ADCY3* 是与 MDD 最具相关性的基因之一^[94]。而且,抑郁症患者血液中 AC3 的含量明显下降^[103]。在全身敲除 AC3 (AC3KO) 的小鼠中也发现了一系列的类似 MDD 患者的特征和表现,如快速眼动睡眠时相延长、空间导向能力障碍、嗅觉减退、学习能力下降以及焦虑、抑郁样行为表现等^[104]。其中 MDD 的核心特征睡眠时程的改变以及快速眼动睡眠时相的增加所形成的睡眠紊乱现象^[105]均在 AC3KO 的小鼠中存在明显表现。同时,在 MDD 患者中往往会出现神经元的萎缩, AC3KO 小鼠的 CA3-CA1 脑区的突触活动以及突触联系相较于正常小鼠都存在明显的减少,而在空间导向障碍的同时出现长时程增强减弱^[104]。除此之外,在人类基因组学的研究中发现 AC3 也和 ASD^[95, 96]以及智力障碍^[106]具有高度相关性,但 AC3 参与的具体分子机制仍未被阐明。综上所述, AC3 在成年大脑中参与调控嗅觉信息的转导、情绪以及某些精神类疾病的发生和发展。

4 结论与展望

神经细胞初级纤毛作为一种细胞外细胞器, 曾经在很长的一段时间内被人们所忽视。但是近 20 年来, 不断有研究者发现其在调控神经精神疾病中的重要作用。同时, 众多研究者发现神经细胞初级纤毛中的多种 GPCRs (如 5-HT₆R、SSTR₃、Mchr1 等) 以及其下游的信号通路 (如 AC3/cAMP 信号通路), 在阿尔茨海默病、MMD、帕金森病、ASD 等多种神经精神疾病中发挥着重要的调控作用, 为我们攻克这些严重威胁人类生命和健康、难以治愈的神经精神疾病提供了新的思路和治疗靶点。而其中存在的多种受体、激酶以及离子通道也被发现在调控神经元活动、接受转导胞外信号的过程中存在重要的作用, 为研究非突触传递式的神经信号转导提供了一种新的思路。但是就目前来讲, 科学研究在该领域中还有很长的路要走, 对于神经细胞初级纤毛中多种信号通路的研究还不清楚, 其中的多种蛋白以及信号通路上下游分子之间的关系还不明确。而神经细胞初级纤毛的特殊结构以及重要功能, 必定会成为我们治愈许多神经精神疾病的新希望。

* * *

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参考文献

- Afzelius BA, Gargani G, Romano C. Abnormal length of cilia as a possible cause of defective mucociliary clearance. *Eur J Respir Dis* 1985; 66(3): 173–180.
- Niggemann B, Muller A, Nolte A, Schnoy N, Wahn U. Abnormal length of cilia - a cause of primary ciliary dyskinesia - a case-report. *Eur J Pediatr* 1992; 151(1): 73–75.
- Serafini SM, Michaelson ED. Length and distribution of cilia in human and canine airways. *Bull Eur Physiopathol Respir* 1977; 13(4): 551–559.
- Lee JE, Gleeson JG. Cilia in the nervous system: linking cilia function and neurodevelopmental disorders. *Curr Opin Neurol* 2011; 24(2): 98–105.
- Guemez-Gamboa A, Coufal NG, Gleeson JG. Primary cilia in the developing and mature brain. *Neuron* 2014; 82(3): 511–521.
- Bloodgood RA. From central to rudimentary to primary: The history of an underappreciated organelle whose time has come. The primary cilium. *Methods Cell Biol* 2009; 94: 3–52.
- Braun DA, Hildebrandt F. Ciliopathies. *Cold Spring Harb Perspect Biol* 2017; 9(3). pii: a028191. doi: 10.1101/cshperspect.a028191.
- Youn YH, Han YG. Primary cilia in brain development and diseases. *Am J Pathol* 2018; 188(1): 11–22.
- Qiu L, LeBel RP, Storm DR, Chen X. Type 3 adenylyl cyclase: a key enzyme mediating the cAMP signaling in neuronal cilia. *Int J Physiol Pathophysiol Pharmacol* 2016; 8(3): 95–108.
- Sterpka A, Chen X. Neuronal and astrocytic primary cilia in the mature brain. *Pharmacol Res* 2018; 137: 114–121.
- Wang ZS, Li V, Chan GCK, Phan T, Nudelman AS, Xia ZG, Storm DR. Adult type 3 adenylyl cyclase-deficient mice are obese. *PLoS One* 2009; 4(9): e6979.
- Einstein EB, Patterson CA, Hon BJ, Regan KA, Reddi J, Melnikoff DE, Mateer MJ, Schulz S, Johnson BN, Tallent MK. Somatostatin signaling in neuronal cilia is critical for object recognition memory. *J Neurosci* 2010; 30(12): 4306–4314.
- Brodsky M, Lesiak AJ, Croicu A, Cohenca N, Sullivan JM, Neumaier JF. 5-HT₆ receptor blockade regulates primary cilia morphology in striatal neurons. *Brain Res* 2017; 1660: 10–19.
- Green JA, Gu C, Mykytyn K. Heteromerization of ciliary G protein-coupled receptors in the mouse brain. *PLoS One* 2012; 7(9): e46304.
- Jin X, Mohieldin AM, Muntean BS, Green JA, Shah JV, Mykytyn K, Nauli SM. Cilioplasm is a cellular compartment for calcium signaling in response to mechanical and chemical stimuli. *Cell Mol Life Sci* 2014; 71(11): 2165–2178.
- Upadhyay VS, Muntean BS, Kathem SH, Hwang JJ, Aboualawi WA, Nauli SM. Roles of dopamine receptor on chemosensory and mechanosensory primary cilia in renal epithelial cells. *Front Physiol* 2014; 5: 72.
- Raychowdhury MK, Ramos AJ, Zhang P, McLaughlin M, Dai XQ, Chen XZ, Montalbetti N, Del Rocio Cantero M, Ausiello DA, Cantiello HF. Vasopressin receptor-mediated functional signaling pathway in primary cilia of renal epithelial cells. *Am J Physiol Renal Physiol* 2009; 296(1): F87–F97.
- Bai CX, Giamarchi A, Rodat-Despoix L, Padilla F, Downs T, Tsiokas L, Delmas P. Formation of a new receptor-operated channel by heteromeric assembly of TRPP2 and TRPC1 subunits. *EMBO Rep* 2008; 9(5): 472–479.
- Rosenbaum JL, Witman GB. Intraflagellar transport. *Nat Rev Mol Cell Biol* 2002; 3(11): 813–825.
- Endicott SJ, Brueckner M. NUP98 sets the size-exclusion diffusion limit through the ciliary base. *Curr Biol* 2018;

- 28(10): 1643–1650.
- 21 Wheatley DN, Feilen EM, Yin Z, Wheatley SP. Primary cilia in cultured-mammalian-cells - detection with an antibody against detyrosinated alpha-tubulin (Id5) and by electron-microscopy. *J Submicrosc Cytol Pathol* 1994; 26(1): 91–102.
- 22 Roperto F, Mingozzi C, Varricchio E, Di Guardo G, Bruno F. Primary cilium expression in cells from normal and pathological caprine skin. *J Submicrosc Cytol Pathol* 1999; 31(1): 169–171.
- 23 Oishi I, Kawakami Y, Raya A, Callol-Massot C, Izpisua Belmonte JC. Regulation of primary cilia formation and left-right patterning in zebrafish by a noncanonical Wnt signaling mediator, *duboraya*. *Nat Genet* 2006; 38(11): 1316–1322.
- 24 Singla V, Reiter JF. The primary cilium as the cell's antenna: Signaling at a sensory organelle. *Science* 2006; 313(5787): 629–633.
- 25 Dahl HA. Fine structure of cilia in rat cerebral cortex. *Z Zellforsch Mikrosk Anat* 1963; 60: 369–386.
- 26 Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A. Regeneration of a germinal layer in the adult mammalian brain. *Proc Natl Acad Sci U S A* 1999; 96: 11619–11624.
- 27 Wheatley DN, Wang AM, Strugnelli GE. Expression of primary cilia in mammalian cells. *Cell Biol Int* 1996; 20(1): 73–81.
- 28 Sipos É, Komoly S, Ács P. Quantitative comparison of primary cilia marker expression and length in the mouse brain. *J Mol Neurosci* 2018; 64(3): 397–409.
- 29 O'Connor AK, Malarkey EB, Berbari NF, Croyle MJ, Haycraft CJ, Bell PD, Hohenstein P, Kesterson RA, Yoder BK. An inducible CiliaGFP mouse model for in vivo visualization and analysis of cilia in live tissue. *Cilia* 2013; 2(1): 8.
- 30 Delling M, Indzhukulian AA, Liu X, Li Y, Xie T, Corey DP, Clapham DE. Primary cilia are not calcium-responsive mechanosensors. *Nature* 2016; 531(7596): 656–660.
- 31 Bangs FK, Schrode N, Hadjantonakis AK, Anderson KV. Lineage specificity of primary cilia in the mouse embryo. *Nat Cell Biol* 2015; 17(2): 113–122.
- 32 Fliegauf M, Benzing T, Omran H. Mechanisms of disease - When cilia go bad: cilia defects and ciliopathies. *Nat Rev Mol Cell Biol* 2007; 8(11): 880–893.
- 33 Green JA, Mykytyn K. Neuronal ciliary signaling in homeostasis and disease. *Cell Mol Life Sci* 2010; 67(19): 3287–3297.
- 34 Marley A, von Zastrow M. A simple cell-based assay reveals that diverse neuropsychiatric risk genes converge on primary cilia. *PLoS One* 2012; 7(10): e46647.
- 35 Fredriksson R, Lagerstrom MC, Lundin LG, Schiöth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol* 2003; 63(6): 1256–1272.
- 36 Pandey-Szekeres G, Munk C, Tsonkov TM, Mordalski S, Harpoe K, Hauser AS, Bojarski AJ, Gloriam DE. GPCRdb in 2018: adding GPCR structure models and ligands. *Nucleic Acids Res* 2018; 46(D1): D440–D446.
- 37 Hauser AS, Attwood MM, Rask-Andersen M, Schiöth HB, Gloriam DE. Trends in GPCR drug discovery: new agents, targets and indications. *Nat Rev Drug Discov* 2017; 16(12): 829–842.
- 38 Mykytyn K, Askwith C. G-protein-coupled receptor signaling in cilia. *Cold Spring Harb Perspect Biol* 2017; 9(9): pii: a028183. doi: 10.1101/cshperspect.a028183.
- 39 Schou KB, Pedersen LB, Christensen ST. Ins and outs of GPCR signaling in primary cilia. *EMBO Rep* 2015; 16(9): 1099–1113.
- 40 Berbari NF, Lewis JS, Bishop GA, Askwith CC, Mykytyn K. Bardet-Biedl syndrome proteins are required for the localization of G protein-coupled receptors to primary cilia. *Proc Natl Acad Sci U S A* 2008; 105(11): 4242–4246.
- 41 Brailov I, Bancila M, Brisorgueil MJ, Miquel MC, Hamon M, Verge D. Localization of 5-HT₆ receptors at the plasma membrane of neuronal cilia in the rat brain. *Brain Res* 2000; 872(1–2): 271–275.
- 42 Handel M, Schulz S, Stanarius A, Schreff M, Erdtmann-Vourliotis M, Schmidt H, Wolf G, Holtt V. Selective targeting of somatostatin receptor 3 to neuronal cilia. *Neuroscience* 1999; 89(3): 909–926.
- 43 Tabibian JH, Masyuk AI, Masyuk TV, O'Hara SP, LaRusso NF. Physiology of cholangiocytes. *Compr Physiol* 2013; 3(1): 541–565.
- 44 Green JA, Schmid CL, Bley E, Monsma PC, Brown A, Bohn LM, Mykytyn K. Recruitment of beta-arrestin into neuronal cilia modulates somatostatin receptor subtype 3 ciliary localization. *Mol Cell Biol* 2016; 36(1): 223–235.
- 45 Bishop GA, Berbari NF, Lewis J, Mykytyn K. Type III adenylyl cyclase localizes to primary cilia throughout the adult mouse brain. *J Comp Neurol* 2007; 505(5): 562–571.
- 46 Domire JS, Green JA, Lee KG, Johnson AD, Askwith CC, Mykytyn K. Dopamine receptor 1 localizes to neuronal cilia in a dynamic process that requires the Bardet-Biedl syndrome proteins. *Cell Mol Life Sci* 2011; 68(17): 2951–2960.
- 47 Loktev AV, Jackson PK. Neuropeptide Y family receptors traffic via the bardet-biedl syndrome pathway to signal in neuronal primary cilia. *Cell Rep* 2013; 5(5): 1316–1329.
- 48 Sun X, Haley J, Bulgakov OV, Cai X, McGinnis J, Li T.

- Tubby is required for trafficking G protein-coupled receptors to neuronal cilia. *Cilia* 2012; 1(1): 21.
- 49 Marin O. Interneuron dysfunction in psychiatric disorders. *Nat Rev Neurosci* 2012; 13(2): 107–120.
- 50 Gerard C, Martres MP, Lefevre K, Miquel MC, Verge D, Lanfumey L, Doucet E, Hamon M, el Mestikawy S. Immunolocalization of serotonin 5-HT₆ receptor-like material in the rat central nervous system. *Brain Res* 1997; 746(1–2): 207–219.
- 51 Mitchell ES, Neumaier JF. 5-HT₆ receptors: a novel target for cognitive enhancement. *Pharmacol Ther* 2005; 108(3): 320–333.
- 52 Fukuo Y, Kishi T, Yoshimura R, Kitajima T, Okochi T, Yamanouchi Y, Kinoshita Y, Kawashima K, Naitoh H, Umene-Nakano W, Inada T, Kunugi H, Kato T, Yoshikawa T, Ujike H, Nakamura J, Ozaki N, Iwata N. Serotonin 6 receptor gene and mood disorders: Case-control study and meta-analysis. *Neurosci Res* 2010; 67(3): 250–255.
- 53 Wesolowska A, Jastrzebska-Wiesek M. Behavioral pharmacology: potential antidepressant and anxiolytic properties. *Int Rev Neurobiol* 2011; 96: 49–71.
- 54 Zhang YM, Zhang L, Wang Y, Sun YN, Guo Y, Du CX, Zhang J, Yao L, Yu SQ, Liu J. Activation and blockade of prefrontal 5-HT₆ receptors produce different effects on depressive-like behaviors in unilateral 6-hydroxydopamine-induced Parkinson's rats. *Neuropharmacology* 2016; 110(Pt A): 25–36.
- 55 Liu KC, Li JY, Tan HH, Du CX, Xie W, Zhang YM, Ma WL, Zhang L. Serotonin(6) receptors in the dorsal hippocampus regulate depressive-like behaviors in unilateral 6-hydroxydopamine-lesioned Parkinson's rats. *Neuropharmacology* 2015; 95: 290–298.
- 56 Meneses A. Memory formation and memory alterations: 5-HT₆ and 5-HT₇ receptors, novel alternative. *Rev Neurosci* 2014; 25(3): 325–356.
- 57 Hu L, Wang B, Zhang Y. Serotonin 5-HT₆ receptors affect cognition in a mouse model of Alzheimer's disease by regulating cilia function. *Alzheimers Res Ther* 2017; 9(1): 76.
- 58 Wang B, Hu L, Sun Z, Zhang Y. Cilia function is associated with axon initial segment morphology. *Biochem Biophys Res Commun* 2019; 516(1): 15–21.
- 59 Omori Y, Chaya T, Yoshida S, Irie S, Tsujii T, Furukawa T. Identification of G protein-coupled receptors (GPCRs) in primary cilia and their possible involvement in body weight control. *PLoS One* 2015; 10(6): e0128422.
- 60 Adamantidis A, Thomas E, Foidart A, Tyhon A, Coumans B, Minet A, Tirelli E, Seutin V, Grisar T, Lakaye B. Disrupting the melanin-concentrating hormone receptor 1 in mice leads to cognitive deficits and alterations of NMDA receptor function. *Eur J Neurosci* 2005; 21(10): 2837–2844.
- 61 Koemeter-Cox AI, Sherwood TW, Green JA, Steiner RA, Barbari NF, Yoder BK, Kauffman AS, Monsma PC, Brown A, Askwith CC, Mykytyn K. Primary cilia enhance kisspeptin receptor signaling on gonadotropin-releasing hormone neurons. *Proc Natl Acad Sci U S A* 2014; 111(28): 10335–10340.
- 62 Li YJ, Hu JH. Small GTPases and cilia. *Protein Cell* 2011; 2(1): 13–25.
- 63 Li YJ, Ling K, Hu JH. The emerging role of Arf/Arl small GTPases in cilia and ciliopathies. *J Cell Biochem* 2012; 113(7): 2201–2207.
- 64 Mariani LE, Bijlsma MF, Ivanova AI, Suciuc SK, Kahn RA, Casparly T. Arl13b regulates Shh signaling from both inside and outside the cilium. *Mol Biol Cell* 2016; 27(23): 3780–3790.
- 65 Humbert MC, Weihbrecht K, Searby CC, Li YL, Pope RM, Sheffield VC, Seo S. ARL13B, PDE6D, and CEP164 form a functional network for INPP5E ciliary targeting. *Proc Natl Acad Sci U S A* 2012; 109(48): 19691–19696.
- 66 Li YJ, Zhang Q, Wei Q, Zhang YX, Ling K, Hu JH. SUMOylation of the small GTPase ARL-13 promotes ciliary targeting of sensory receptors. *J Cell Biol* 2012; 199(4): 589–598.
- 67 Li YJ, Wei Q, Zhang YX, Ling K, Hu JH. The small GTPases ARL-13 and ARL-3 coordinate intraflagellar transport and ciliogenesis. *J Cell Biol* 2010; 189(6): 1039–1051.
- 68 Zhang Q, Li Y, Zhang YX, Torres VE, Harris PC, Ling K, Hu JH. GTP-binding of ARL-3 is activated by ARL-13 as a GEF and stabilized by UNC-119. *Sci Rep* 2016; 6: 24534.
- 69 Cevik S, Hori Y, Kaplan OI, Kida K, Toivenon T, Foley-Fisher C, Cottell D, Katada T, Kontani K, Blacque OE. Joubert syndrome Arl13b functions at ciliary membranes and stabilizes protein transport in *Caenorhabditis elegans*. *J Cell Biol* 2010; 188(6): 953–969.
- 70 Casparly T, Larkins CE, Anderson KV. The graded response to sonic hedgehog depends on cilia architecture. *Dev Cell* 2007; 12(5): 767–778.
- 71 Seixas C, Choi SY, Polgar N, Umberger NL, East MP, Zuo XF, Moreiras H, Ghossoub R, Benmerah A, Kahn RA, Fogelgren B, Casparly T, Lipschutz JH, Barral DC. Arl13b and the exocyst interact synergistically in ciliogenesis. *Mol Biol Cell* 2016; 27(2): 308–320.
- 72 Guo J, Otis JM, Higginbotham H, Monckton C, Cheng J, Asokan A, Mykytyn K, Casparly T, Stuber GD, Anton ES. Primary cilia signaling shapes the development of interneuronal connectivity. *Dev Cell* 2017; 42(3): 286–300.e4.
- 73 Guadiana SM, Parker AK, Filho GF, Sequeira A, Semple-Rowland S, Shaw G, Mandel RJ, Foster TC,

- Kumar A, Sarkisian MR. Type 3 adenylyl cyclase and somatostatin receptor 3 expression persists in aged rat neocortical and hippocampal neuronal cilia. *Front Aging Neurosci* 2016; 8: 127.
- 74 Luo J, Chen XM, Pan YW, Lu S, Xia ZG, Storm DR. The type 3 adenylyl cyclase is required for the survival and maturation of newly generated granule cells in the olfactory bulb. *PLoS One* 2015; 10(3): e0122057.
- 75 Wang ZS, Phan T, Storm DR. The type 3 adenylyl cyclase is required for novel object learning and extinction of contextual memory: role of camp signaling in primary cilia. *J Neurosci* 2011; 31(15): 5557–5561.
- 76 Doherty D. Joubert syndrome: insights into brain development, cilium biology, and complex disease. *Semin Pediatr Neurol* 2009; 16(3): 143–154.
- 77 Thomas S, Cantagrel V, Mariani L, Serre V, Lee JE, Elkhartoufi N, de Lonlay P, Desguerre I, Munnich A, Boddaert N, Lyonnet S, Vekemans M, Lisgo SN, Caspary T, Gleeson J, Attie-Bitach T. Identification of a novel ARL13B variant in a Joubert syndrome-affected patient with retinal impairment and obesity. *Eur J Hum Genet* 2015; 23(5): 621–627.
- 78 Cantagrel V, Silhavy JL, Bielas SL, Swistun D, Marsh SE, Bertrand JY, Audollent S, Attie-Bitach T, Holden KR, Dobyms WB, Traver D, Al-Gazali L, Ali BR, Lindner TH, Caspary T, Otto EA, Hildebrandt F, Glass IA, Logan CV, Johnson CA, Bennett C, Brancati F, International Joubert Syndrome Related Disorders Study Group, Valente EM, Woods CG, Gleeson JG. Mutations in the cilia gene ARL13B lead to the classical form of Joubert syndrome. *Am J Hum Genet* 2008; 83(2): 170–179.
- 79 Rafiullah R, Long AB, Ivanova AA, Ali H4, Berkel S, Mustafa G, Paramasivam N, Schlesner M, Wiemann S, Wade RC, Bolthausen E, Blum M, Kahn RA, Caspary T, Rappold GA. A novel homozygous ARL13B variant in patients with Joubert syndrome impairs its guanine nucleotide-exchange factor activity. *Eur J Hum Genet* 2017; 25(12): 1324–1334.
- 80 Shao J, Xu L, Chen L, Lu Q, Xie X, Shi W, Xiong H, Shi C, Huang X, Mei J, Rao H, Lu H, Lu N, Luo S. Arl13b promotes gastric tumorigenesis by regulating smo trafficking and activation of the hedgehog signaling pathway. *Cancer Res* 2017; 77(15): 4000–4013.
- 81 Falcon-Urrutia P, Carrasco CM, Lois P, Palma V, Roth AD. Shh signaling through the primary cilium modulates rat oligodendrocyte differentiation. *PLoS One* 2015; 10(7): e0133567.
- 82 Larkins CE, Aviles GD, East MP, Kahn RA, Caspary T. Arl13b regulates ciliogenesis and the dynamic localization of Shh signaling proteins. *Mol Biol Cell* 2011; 22(23): 4694–4703.
- 83 Bay SN, Long AB, Caspary T. Disruption of the ciliary GT-Pase Arl13b suppresses Sonic hedgehog overactivation and inhibits medulloblastoma formation. *Proc Natl Acad Sci U S A* 2018; 115(7): 1570–1575.
- 84 Tabakoff B, Hoffman PL. Transducing emotionality: the role of adenylyl cyclases. *Biol Psychiatry* 2012; 71(7): 572–573.
- 85 Menninger JA, Tabakoff B. Forskolin-stimulated platelet adenylyl cyclase activity is lower in persons with major depression. *Biol Psychiatry* 1997; 42(1): 30–38.
- 86 Hines LM, Tabakoff B; WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence Investigators. Platelet adenylyl cyclase activity: A biological marker for major depression and recent drug use. *Biol Psychiatry* 2005; 58(12): 955–962.
- 87 Katsel PL, Tagliente TM, Schwarz TE, Craddock-Royal BD, Patel ND, Maayani S. Molecular and biochemical evidence for the presence of Type III adenylyl cyclase in human platelets. *Platelets* 2003; 14(1): 21–33.
- 88 Challis RC, Tian HK, Wang J, He JW, Jiang JB, Chen XM, Yin WB, Connelly T, Ma LM, Yu CR, Pluznick JL, Storm DR, Huang LQ, Zhao K, Ma MH. An olfactory cilia pattern in the mammalian nose ensures high sensitivity to odors. *Curr Biol* 2015; 25(19): 2503–2512.
- 89 Chen XM, Xia ZG, Storm DR. Stimulation of electro-olfactogram responses in the main olfactory epithelia by airflow depends on the type 3 adenylyl cyclase. *J Neurosci* 2012; 32(45): 15769–15778.
- 90 Wong ST, Trinh K, Hacker B, Chan GC, Lowe G, Gaggari A, Xia Z, Gold GH, Storm DR. Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. *Neuron* 2000; 27(3): 487–497.
- 91 Zhou Y, Qiu L, Sterpka A, Wang H, Chu F, Chen X. Comparative phosphoproteomic profiling of type III adenylyl cyclase knockout and control, male, and female mice. *Front Cell Neurosci* 2019; 13: 34.
- 92 Kumamoto N, Gu Y, Wang J, Janoschka S, Takamaru KI, Levine J, Ge SY. A role for primary cilia in glutamatergic synaptic integration of adult-born neurons. *Nat Neurosci* 2012; 15(3): 399–405, S1.
- 93 Guadiana SM, Semple-Rowland S, Daroszewski D, Madorsky I, Breunig JJ, Mykytyn K, Sarkisian MR. Arborization of dendrites by developing neocortical neurons is dependent on primary cilia and type 3 adenylyl cyclase. *J Neurosci* 2013; 33(6): 2626–2638.
- 94 Wray NR, Pergadia ML, Blackwood DH, Penninx BW, Gordon SD, Nyholt DR, Ripke S, MacIntyre DJ, McGhee KA, Maclean AW, Smit JH, Hottenga JJ, Willemsen G, Middeldorp CM, de Geus EJ, Lewis CM, McGuffin P,

- Hickie IB, van den Oord EJ, Liu JZ, Macgregor S, McEvoy BP, Byrne EM, Medland SE, Statham DJ, Henders AK, Heath AC, Montgomery GW, Martin NG, Boomsma DI, Madden PA, Sullivan PF. Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. *Mol Psychiatry* 2012; 17(1): 36–48.
- 95 Skafidas E, Testa R, Zantomio D, Chana G, Everall IP, Pantelis C. Predicting the diagnosis of autism spectrum disorder using gene pathway analysis. *Mol Psychiatry* 2014; 19(4): 504–510.
- 96 RK CY, Merico D, Bookman M, J LH, Thiruvahindrapuram B, Patel RV, Whitney J, Deflaux N, Bingham J, Wang Z, Pellecchia G, Buchanan JA, Walker S, Marshall CR, Uddin M, Zarrei M, Deneault E, D'Abate L, Chan AJ, Koyanagi S, Paton T, Pereira SL, Hoang N, Engchuan W, Higginbotham EJ, Ho K, Lamoureux S, Li W, MacDonald JR, Nalpathamkalam T, Sung WW, Tsoi FJ, Wei J, Xu L, Tasse AM, Kirby E, Van Etten W, Twigger S, Roberts W, Drmic I, Jilderda S, Modi BM, Kellam B, Szego M, Cytrynbaum C, Weksberg R, Zwaigenbaum L, Woodbury-Smith M, Brian J, Senman L, Iaboni A, Doyle-Thomas K, Thompson A, Chrysler C, Leef J, Savion-Lemieux T, Smith IM, Liu X, Nicolson R, Seifer V, Fedele A, Cook EH, Dager S, Estes A, Gallagher L, Malow BA, Parr JR, Spence SJ, Vorstman J, Frey BJ, Robinson JT, Strug LJ, Fernandez BA, Elsabbagh M, Carter MT, Hallmayer J, Knoppers BM, Anagnostou E, Szatmari P, Ring RH, Glazer D, Pletcher MT, Scherer SW. Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. *Nat Neurosci* 2017; 20(4): 602–611.
- 97 Nelson JC, Charney DS. The symptoms of major depressive-illness. *Am J Psychiatry* 1981; 138(1): 1–13.
- 98 Murrrough JW, Iacoviello B, Neumeister A, Charney DS, Iosifescu DV. Cognitive dysfunction in depression: Neurocircuitry and new therapeutic strategies. *Neurobiol Learn Mem* 2011; 96(4): 553–563.
- 99 Arnone D, McKie S, Elliott R, Juhasz G, Thomas EJ, Downey D, Williams S, Deakin JF, Anderson IM. State-dependent changes in hippocampal grey matter in depression. *Mol Psychiatry* 2013; 18(12): 1265–1272.
- 100 Koolschijn PC, van Haren NE, Lensvelt-Mulders GJ, Hulshoff Pol HE, Kahn RS. Brain volume abnormalities in major depressive disorder: a meta-analysis of magnetic resonance imaging studies. *Hum Brain Mapp* 2009; 30(11): 3719–3735.
- 101 Palagini L, Baglioni C, Ciapparelli A, Gemignani A, Riemann D. REM sleep dysregulation in depression: State of the art. *Sleep Med Rev* 2013; 17(5): 377–390.
- 102 Cornwell BR, Salvatore G, Colon-Rosario V, Latov DR, Holroyd T, Carver FW, Coppola R, Manji HK, Zarate CA, Grillon C. Abnormal hippocampal functioning and impaired spatial navigation in depressed individuals: evidence from whole-head magnetoencephalography. *Am J Psychiatry* 2010; 167(7): 836–844.
- 103 Redei EE, Andrus BM, Kwasny MJ, Seok J, Cai X, Ho J, Mohr DC. Blood transcriptomic biomarkers in adult primary care patients with major depressive disorder undergoing cognitive behavioral therapy. *Transl Psychiatry* 2014; 4: e442.
- 104 Chen XM, Luo J, Leng YH, Yang YM, Zweifel LS, Palmiter RD, Storm DR. Ablation of type III adenylyl cyclase in mice causes reduced neuronal activity, altered sleep pattern, and depression-like phenotypes. *Biol Psychiatry* 2016; 80(11): 836–848.
- 105 Pillai V, Kalmbach DA, Ciesla JA. A meta-analysis of electroencephalographic sleep in depression: evidence for genetic biomarkers. *Biol Psychiatry* 2011; 70(10): 912–919.
- 106 Saeed S, Bonnefond A, Tamanini F, Mirza MU, Manzoor J, Janjua QM, Din SM, Gaitan J, Milochau A, Durand E, Vaillant E, Haseeb A, De Graeve F, Rabearivelo I, Sand O, Queniat G, Boutry R, Schott DA, Ayesha H, Ali M, Khan WI, Butt TA, Rinne T, Stumpel C, Abderrahmani A, Lang JC, Arslan M, Froguel P. Loss-of-function mutations in *ADCY3* cause monogenic severe obesity. *Nat Genet* 2018; 50(2): 172–174.