*PCDHA*基因簇及其影响神经发育的研究进展

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摘要 原钙黏连素(PCDHs)家族属于Ca^{2+}依赖的细胞黏着糖蛋白, 在脑神经元网络搭建中扮演至关重要的角色。PCDHs家族在染色体上呈现簇状和非簇状分布, 簇内众多可变外显子在神经元内随机表达, 其丰富的蛋白变体组合锚定在神经元表面, 作为特有信号“密码”, 识别并介导轴突或树突之间的连接。该文综述了近些年国内外的研究报道, 阐述家族内成员*PCDHA*基因簇的研究进展, 包括*PCDHA*基因簇的基因结构和表达特征、蛋白变体的胞外域构成及其作用力的产生, 以及介导的信号通路等。*PCDHA*基因簇是精神类遗传缺陷病的潜在遗传位点, 其研究具有重要价值。

关键词 原钙黏连素; 可变外显子; 基因簇

Advances in *PCDHA* Gene Clusters and Their Influence on Neural Development

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Abstract The *PCDHs* family as Ca^{2+}-dependent cellular adhesion glycoprotein, plays a crucial role in the establishment of neural networks in the brain. The PCDHs family presents cluster and non-cluster distribution on chromosomes. Numerous variable exons within the cluster are randomly expressed in neurons. The combinations of the protein isoforms are abundant, and anchor on the neuron surface as their unique “code” to recognize and mediate the connections between axons or dendrites. This paper summarized reports published at home and abroad in recent years, and described the progress of *PCDHA* gene clusters, including the gene structure and expression characteristics, the extracellular structure of protein isoforms and the generation of their forces as well as the mediated signaling pathways. *PCDHA* gene cluster is a potential genetic locus of mental genetic defects, and the related studies are of great value.

Keywords *PCDHs*; variable exons; gene cluster

人原钙黏连素(protocadherins, PCDHs)家族位于5号染色体5q31区域, SHIMOJIMA等[4]报道, 亚显微水平5q31.3区域出现变异的幼儿, 表现出明显的肌张力低下, 喂养困难, 发育严重迟缓且伴有髓鞘延迟的癫痫/非癫痫性脑病; 面部特征上表现为额头狭窄、耳廓低落和异常、双侧上睑下垂、面颊水肿等。在该区域内分布的PCDHs家族和*NRG2*(neuregulin 2)都与神经发育功能相关。

PCDHs家族属于Ca^{2+}依赖的细胞黏着糖蛋白。该基因家族成员众多, 在染色体上呈现簇状和分散

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排列, 其簇状排列的成员包括*PCDHA*、*PCDHB*和*PCDHG*基因簇。基因簇之间结构和功能相近, 在进化上具有较高保守性, 其蛋白变体(isoform)数量对应人和小鼠: pcdhα分别为15和14个, pcdhβ分别为15和22个, pcdhγ分别为22和22个。这些蛋白变体锚定在神经细胞表面, 介导神经元之间的连接[1-2]。

在免疫系统中, 免疫球蛋白和T细胞受体(T cell receptor, *TCR*)基因在进化压力下形成独特机制, 即细胞在进行有丝分裂过程中发生DNA片段重排[3]; 在神经系统发育中, PCDHs家族在进化压力下同样形成特有的表达调控机制。本文将以*PCDHA*基因簇作为论述对象, 对近些年研究成果进行归纳, 阐述*PCDHA*基因簇的特殊基因结构及特有的表达模式。

1 *PCDHA*是精神类遗传缺陷病的潜在遗传位点

LACHMAN等[5]通过群体统计分析发现, *PCDHA*基因簇可变外显子的16.7 Kb缺失片段, 可能是造成精神分裂症(schizophrenia)和躁郁症(bipolar disorder)的遗传位点的原因。PEDROSA等[6]在患有精神分裂和躁郁症病人中鉴定出1个单碱基突变(single nucleotide polymorphisms, SNP), 位于*PCDHA*基因簇增强子中。除精神分裂症和躁郁症的报道外, *PCDHA*基因簇内基因片段缺失会影响人的音乐天赋[7]。此外, ANITHA等[8]在*PCDHA*基因簇中发现5个与自闭症显著关联的SNPs(rs251379、rs1119032、rs17119271、rs155806、rs17119346)。ROBERSON-NAY等[9]利用表观组关联分析发现, 同卵双胞胎的早期抑郁症可能与*PCDHA*基因簇有关。该基因簇第9外显子错义突变与先天无神经节性巨结肠病关联[10]。综上所述报道显示, PCDHA是精神类遗传缺陷病的潜在遗传位点。

2 *PCDHA*的表达特征

在基因水平上, *PCDHA*基因簇包含可变外显子(variable exons)和固定外显子(constant exons); 在转录水平上, 一个被随机选中的可变外显子会与固定外显子拼接成一个完整的转录本; 在翻译水平上, 随机表达的可变外显子翻译出胞外域和跨膜域等, 固定外显子翻译出胞内域(图1)。该蛋白合成以后锚定在神经元的表面, 参与介导神经元的自我识别和回避, 以及与其他功能神经元之间连接的建立[1,11-12]。

*PCDHA*基因簇在小鼠胚胎期前脑中广泛表达[13], 在大脑所有皮质层, 特别是在大脑新皮层的中间地带和边缘地带高表达[14], 在嗅觉神经元和嗅小体内也被检测到高表达[15]。在产蛋高峰期的母鸡中, 基因簇在脑和垂体等脑相关器官中高表达, 在脾脏中较高表达, 在其他器官如心、肝、肺、肾、卵巢和输卵管等器官中低表达[16]。

3 可变外显子在不同神经元中的角色

3.1 可变外显子的表达具有神经元特异性

小鼠*PCDHA*基因簇包含14个可变外显子, 其

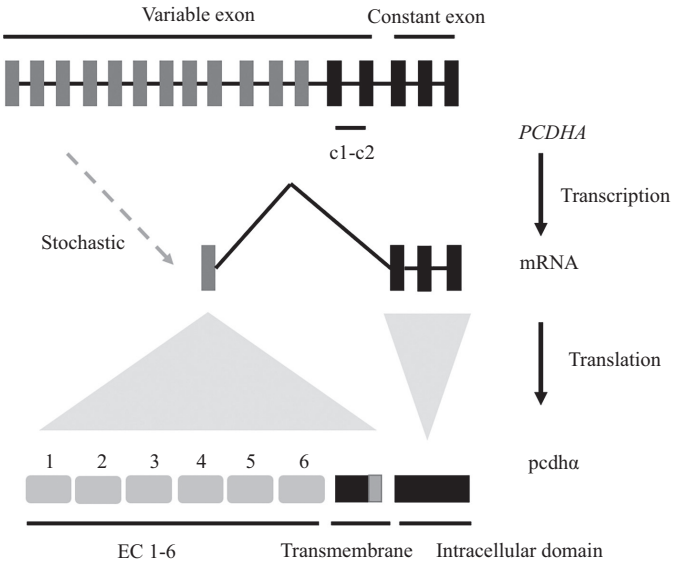


图1 *PCDHA*基因簇的表达模式图

Fig.1 Expression pattern of *PCDHA* gene cluster

中α1-α12之间具有高度序列相似性, 另外2个可变外显子αc1和αc2之间序列高度相似。在早期研究中, ESUMI等[17]巧妙利用不同品系小鼠杂交策略与单细胞RT-PCR技术, 发现不同浦肯野细胞中参与表达的可变外显子为α1-α12中不固定的2~3个, 每个细胞参与表达的可变外显子仅来自父本或母本一方。该结果暗示, *PCDHA*基因簇可变外显子的表达不仅具有随机选择性, 还具有等位基因上的表达特异性。不同的是, αc1和αc2都在浦肯野细胞中固定表达, 且无等位基因型上的表达特异性[18]。在嗅觉感受神经元(olfactory sensory neurons, OSNs)中, 可变外显子α1-α12的表达与浦肯野细胞中的表达类似, 但是可变外显子αc1和αc2呈现出不固定表达[15]。在羟色胺能神经元中, 仅有可变外显子αc1和αc2表达, α1-α12未参与表达[19]。以上结果显示, 可变外显子的表达存在特殊的调控机制, 且与神经元类型有关。

3.2 胞外域介导信号识别

基因簇的蛋白变体之间通常以二聚体的形式锚定在神经元表面, 通过顺式作用力(cis acting)组成1个信号识别单位(signal recognition unit)[20]。每个蛋白变体包括6个胞外域(EC 1-6)。信号识别单位的空间结构非常复杂, 其形成涉及生理学、生化学和结构学(图2)。有研究表明, 胞外域5与6产生的顺式作用力参与维持1个信号识别单位的空间结构, 这种结构有利于实现蛋白变体之间自由组合, 实现信号识别单位的多样性。在神经细胞建立连接过程中, 不同细胞的信号识别单位之间产生反式作用(trans acting)的亲和力, 这种亲和力的产生来于胞外域1到4。具体表现为胞外域1与胞外域4结合、胞外域2与胞外域3结合[21-22]。

由于胞外域所具有结构特异性, 其在信号识别过程中需双方完全匹配, 方能形成亲和力。有研究报道, 胞外域2和3对反式作用力的产生非常重要, 在信号识别中扮演关键角色[23-24], 而且在6个胞外域中, 这2个胞外域具有最为多样的氨基酸序列[25-26]。在动物研究中, HUANG等[16]通过对鸡可变外显子进行序列比对, 发现突变碱基主要集中在胞外域2上游、胞外域2内部和完整的胞外域3中(图3), 这提示这些区域在进化过程中相对不保守。

3.3 可变外显子的转录调控机制

*PCDHA*基因簇可变外显子上游包含一段保守的启动序列, 基因簇的下游远端有一个*HS5-1*增强子, CCCTC结合因子(CCCTC-binding factor, CTCF)促使启动子与增强子在染色质上形成环状结构, 启动可变外显子的随机转录[27], *RFX5*的敲除可增加CTCF结合因子水平, 促使*PCDHA*基因簇上调表达[28]。在小脑浦肯野氏细胞中, NOGUCHI等[29]通过敲入与敲除*PCDHA*基因簇可变外显子实验证实, 可变外显子α1-α12与αc1-αc2之间存在不同的转录调控机制。神经元内可变外显子的表达是随机的, 但不影响基因簇总体的表达水平; CANZIO等[30]报道, 可变外显子的随机表达与lncRNA的调控有关, 每个可变外显子都包含有2个CTCF结合序列, 其反义序列中包含lncRNA启动子, lncRNA随机被激活后在转录延伸过程中会削减模板链上CpG岛数量, 促使CTCF与靶序列结合, CTCF招募cohesin并与远端增强子形成DNA环状复合体, 实现可变外显子转录。

4 *PCDHA*基因簇敲除实验研究神经元发育

构建小鼠*PCDHA*基因簇敲除模型, 研究*PCDHA*基因簇在神经发育过程中的作用。敲除小鼠*PCDHA*基因簇后, 胚胎期皮质神经元的多极化迁移能力减

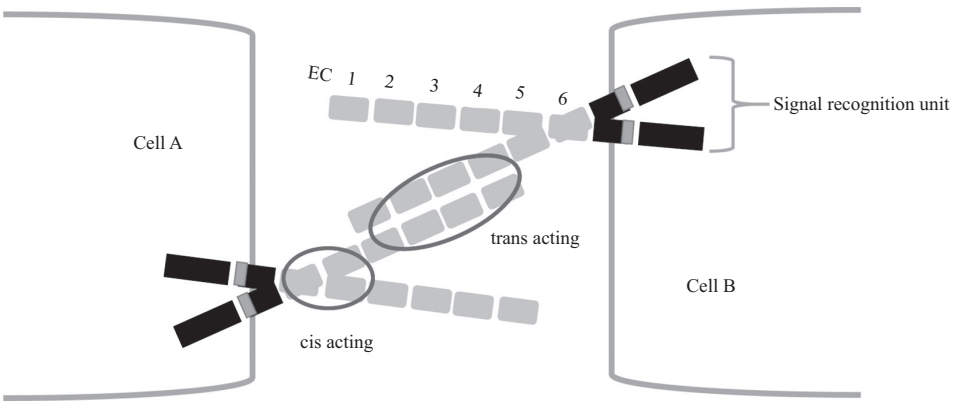
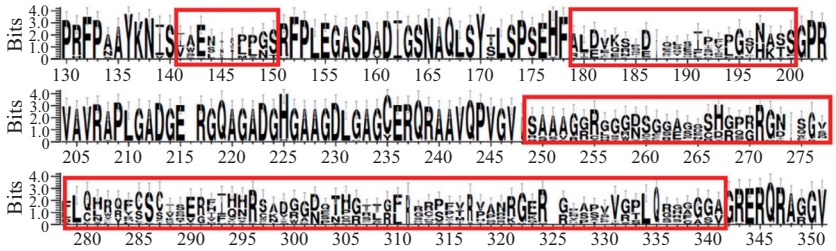


图2 pcdhα介导的细胞间连接

Fig.2 pcdhα-mediated intercellular connection



红色框表示高度变异区域。

The red box represents hypervariable region.

图3 鸡pcdhα蛋白变体多重比对的结果

Fig.3 Multiple comparisons of chicken pcdhα variants

弱[14], 嗅觉神经元不能聚合导致嗅小球数量增多、体积变小[15]; 在17.5天胚胎小鼠海马神经元中, 其有髓鞘神经纤维的比例明显下降[31]; 此外, 在海马体不同区域内, 5-羟色胺能神经元的投射也出现紊乱[32]; 小鼠出现条件反射性恐惧(contextual fear conditioning)和空间工作性记忆(spatial working memory)等行为特征[33]。以上报道表明, 敲除*PCDHA*基因簇会造成神经元发育异常。

5 *PCDHA*基因簇涉及的信号通路

*PCDHA*基因簇的蛋白变体锚定在神经细胞的膜表面, 作为信号分子参与许多信号通路。在神经元迁移过程中, *PCDHA*基因簇除成员*PCDHAC2*外, 均参与介导WAVE/Pyk2/Rac1通路[14]。蛋白变体的膜内域与Pyk2结合后抑制Pyk2磷酸化[34-35], 使Rac1解脱抑制。Rac1与蛋白变体结合激活WAVE复合体[36-37]。在中枢神经系统的交感神经元中, 信号识别单位与Ret结合形成功能复合体, 该复合体促使酪氨酸磷酸化, 维持自身稳定并激活下游通路[38]。在前列腺癌细胞中, miR-193a-5p靶向*PCDHA*基因簇的表达可抑制*PCDHA*基因簇表达[39]。

6 *PCDHA*基因簇在畜禽中的研究报道

在农业动物中, 鸡QTL数据库显示, *PCDHA*基因簇所在区域靠近开产日龄相关数量性状基因座(quantitative trait locus, QTL)[40]。之前有报道称台湾地方红毛鸡具有较高的产蛋性能, 其脑垂体中*PCDHA*基因簇的mRNA水平显著高于其他鸡品种[41], 另外相关性分析的结果显示, *PCDHA*基因簇表达水平跟产蛋率存在相关性[42-43]。HUANG等[16]发现, 在*PCDHA*基因簇内含子中拷贝数变异与欣华鸡开产日龄和280天产蛋数显著关联, 并推测*PCDHA*基因簇的结构变异通过可变外显子剂量效应, 介导神经系统发育从而影响产蛋性能。

7 结语

动物神经中枢系统的调节机制非常复杂和精细, 而*PCDHA*等基因簇在神经网络的搭建中扮演非常重要的角色。从自然进化的角度上讲, *PCDHA*基因簇复杂的基因结构与表达机制是动物神经系统不断进化的结果。在进化压力下, 基因簇可能通过非等位同源重组的方式, 进化出序列相似、数量众多的外显子, 这些外显子受到神经元特殊的转录机制调控, 随机转录并翻译后, 蛋白变体再次组合形成二聚体, 成为神经元天然的“身份密码”。随着人们对PCDHs家族的了解不断深入, 发现基因结构变异或重要区域内碱基突变, 会影响神经元间信号识别, 通过诱导并激活下游通路, 造成机体的神经功能障碍, 如自闭症、躁郁症等。另外, 神经系统的发育与动物的行为学反应、繁殖及生产性能等有一定联系, 因此, 在农业动物研究中, PCDHs家族一样具有重要的研究价值。

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