

Notch信号通路在体节分割时钟中的研究进展

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摘要 体节分割时钟(somite segmentation clock)是脊椎动物胚胎发育过程中调控体节周期性形成的分子振荡系统, 驱动体节沿胚胎前后轴按固定时间间隔依次生成。Notch信号通路在此过程中发挥关键作用, 通过调控*Hes7*和*Dll1*等基因的振荡表达, 精确协调体节分割时钟的周期与体节边界的形成。这一过程依赖于各个分子精密的交互作用, 其中任何环节受到干扰都可能导致体轴发育异常。近年来, 研究者们借助于单细胞转录组测序、光遗传学和活细胞成像等新型研究技术, 在探究Notch信号通路参与体节分割时钟的过程及其分子机制方面取得了显著进展。该文旨在梳理Notch信号通路在体节分割时钟中的作用, 探讨其失调可能引发的疾病, 并基于当前的技术手段, 展望Notch信号通路在体节分割时钟中仍需研究的问题, 以期对体节发育及其相关疾病的研究提供新的思路和视角。

关键词 Notch信号通路; 体节分割时钟; 基因调控; 胚胎发育

Research Progress on the Notch Signaling Pathway in the Somite Segmentation Clock

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Abstract Somite segmentation clock is a molecular oscillation system that regulates the periodic formation of somites during the development of vertebrate embryos. It drives the somites to generate along the anterior-posterior axis of the embryo at a fixed time interval. The Notch signaling pathway plays a crucial role in this process by regulating the oscillatory expression of genes such as *Hes7* and *Dll1*, accurately coordinating the period of somite segmentation clock with the formation of body segment boundaries. This process relies on the precise interaction of various molecules, and any disturbance of these regulatory steps can lead to abnormal body axis development. In recent years, significant progress has been made in exploring the involvement of the Notch signaling pathway in the process and molecular mechanisms of somite segmentation clock through new research techniques such as single-cell transcriptome sequencing, optogenetics, and live cell imaging. This review aims to clarify the role of the Notch signaling pathway in somite segmentation clock, explore the diseases that may be caused by its dysregulation, and based on current technological means, look forward to the issues that still need to be studied in the Notch signaling

收稿日期: 2025-02-23

接受日期: 2025-05-20

国家自然科学基金(批准号: 32060139)、广西自然科学基金(批准号: 2020GXNSFBA297026)和桂林医科大学研究生创新项目(批准号: GYYK2025024)资助的课题

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Received: February 23, 2025

Accepted: May 20, 2025

This work was supported by the National Natural Science Foundation of China (Grant No.32060139), the Guangxi Natural Science Foundation (Grant No.2020GXNSFBA297026), and the Graduate Research Program of Guilin Medical University (Grant No.GYYK2025024)

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pathway in somite segmentation clock, in order to provide new ideas and perspectives for the research of somite development and related diseases.

Keywords Notch signaling pathway; somite segmentation clock; gene regulation; embryonic development

Notch信号通路是一种进化上保守的细胞间通讯机制,它通过Notch受体与配体的相互作用^[1],影响细胞增殖、分化和凋亡等多种生物学过程,对体节形成具有重要意义^[2]。体节分割时钟(somite segmentation clock, 亦称分节时钟)作为一种时间调控方式,通过调控基因的有序表达和周期性振荡,确保体节的正确形成,对于肌肉骨骼系统的发育、轴向延伸以及整体形态和比例的维持都具有不可或缺的作用^[3]。在胚胎发育过程中,Notch信号通路与体节分割时钟紧密相连,Notch信号通路的激活和抑制是体节分割时钟调控基因表达振荡的关键因素,它影响着体节边界的确立和细胞分化的精确调控^[4]。因此,Notch信号通路和体节分割时钟共同确保了体节的正确形成和有序排列,一旦这一过程发生异常,则可能导致包括脊柱侧凸、骨骼和神经系统发育不全等在内的先天性疾病^[5]。近年来,随着光遗传学、活细胞成像和类器官等多种新技术的涌现,在体外模拟体节分割时钟已成为可能,为深入解析这一复杂过程提供了强有力的工具。本文总结了Notch信号通路在体节分割时钟中的作用,探讨其失调可能引发的疾病,并基于当前的技术手段,展望未来仍需研究的方向。

1 Notch信号通路与体节分割时钟

1.1 切割的Notch信号通路与振荡的体节分割时钟

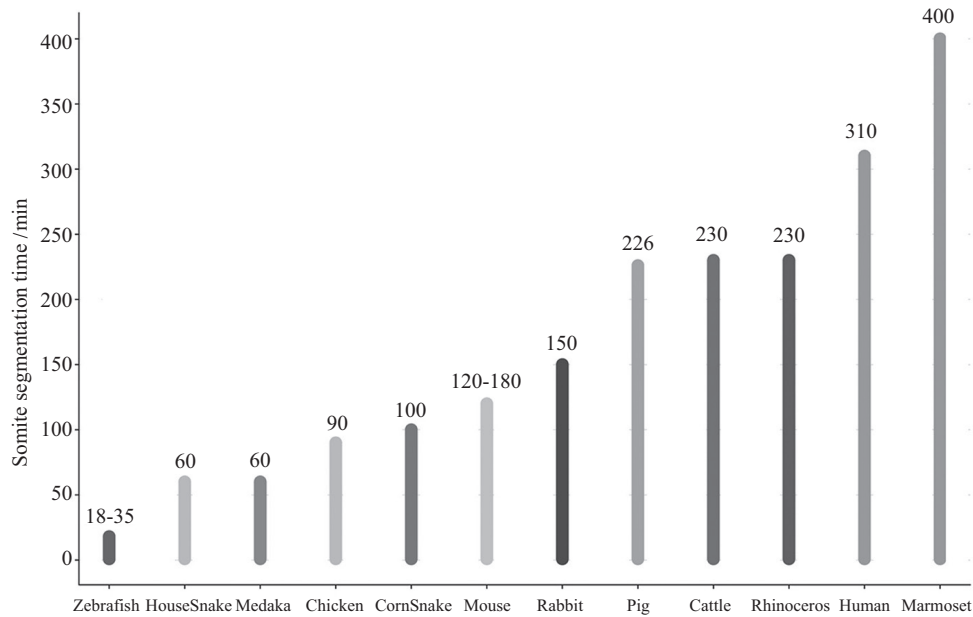
Notch信号通路是一种在多细胞生物中广泛存在的细胞通讯信号系统,其涉及Notch受体与配体之间的相互作用,以及由此触发的细胞内信号转导过程。Notch信号通路的主要组分包括Notch受体、DSL(delta/serrate/Lag-2)配体以及下游效应分子^[6]。Notch受体家族包括4个同源异构体,它们各自是单次跨膜的异二聚体蛋白,由一个大的细胞外亚基和一个小的跨膜及细胞内亚基组成。配体Delta-like(Dll1、Dll3、Dll4)和Serrate/Jagged(Jagged 1、Jagged 2)则属于DSL家族,它们与Notch受体结合以启动信号传递过程^[7]。当配体与邻近细胞的Notch受体结合后,Notch受体启动了一系列分子剪切事件。在细胞外区域,配体结合首先诱导了由ADAM金属蛋白酶介

导的切割,释放了Notch受体的细胞外部分。随后,剩余的跨膜片段在细胞内被 γ -分泌酶进一步切割,这一过程释放出了Notch的细胞内域(Notch intracellular domain, NICD)。NICD作为一个转录共激活因子,通过核定位信号进入细胞核内^[8],与转录因子CSL(CBF1/suppressor of hairless/Lag-1)家族的成员形成复合物。这一复合物结合到靶基因的启动子区域,从而激活或促进下游靶基因的表达^[9]。

体节分割时钟是脊椎动物胚胎发育过程中的一种分子振荡机制,它确保体节能够按照一定的时间间隔和正确的顺序逐一形成^[10]。在每一对体节的形成过程中,体节分割时钟都会以固定时间从尾部开始扫过整个准体节中胚层(presomitic mesoderm, PSM),然后在头部位置调控体节的生成^[11]。大量研究表明,体节分割时钟主要由Notch、Wnt和成纤维细胞生长因子(fibroblast growth factor, FGF)通路的基因构成^[4,6,10,12]。这些基因呈现一种周期性的振荡表达模式,其振荡周期与体节发生的周期相一致,此种特殊的表达模式驱动了PSM细胞的同步振荡介导形成了体节^[13]。体节分割时钟在脊椎动物中具有高度的保守性。从鱼类到哺乳动物,体节分割时钟基因的表达模式和调控机制都表现出相似性^[14]。图1例举了迄今为止研究的比较清楚的12个物种体节分割时钟时长,从图中可知人类体节分割时钟的周期是小鼠的2倍左右,这与小鼠和人类胚胎在发育时间上的差异是一致的^[15]。MARSHALL等^[16]认为体节分割时钟时长的差别可能可以解释小鼠和大象之间巨大的体型差距,物种身体结构越复杂其胚胎发育所需时间越长,体节分割时钟的存在确保了体节的正常生成,从而保障了体轴的发育。

1.2 细胞内的转录反馈环路

体节分割时钟的核心组分是一类在胚胎轴线上周期性波动表达的基因,其动态调控依赖于Notch信号通路中的关键下游基因,包括*Hes1*、*Hes7*和*Hey*基因家族等^[17]。这些基因通过复杂的转录反馈环路形成精确的振荡网络。以*Hes1*和*Hes7*为例,*Hes1*通过直接抑制自身启动子区域的转录,形成一个典型的负反馈环路,其表达水平随时间呈现周期



此处时间指不同物种体节分割时钟的振荡周期时长。

Here, the time refers to the oscillation duration of the segmentation clock in different species.

图1 12个物种的体节分割时钟时长(根据参考文献[14-16]总结并绘制)

Fig.1 Duration of the somite segmentation clock in 12 species (summarize and draw based on the references [14-16])

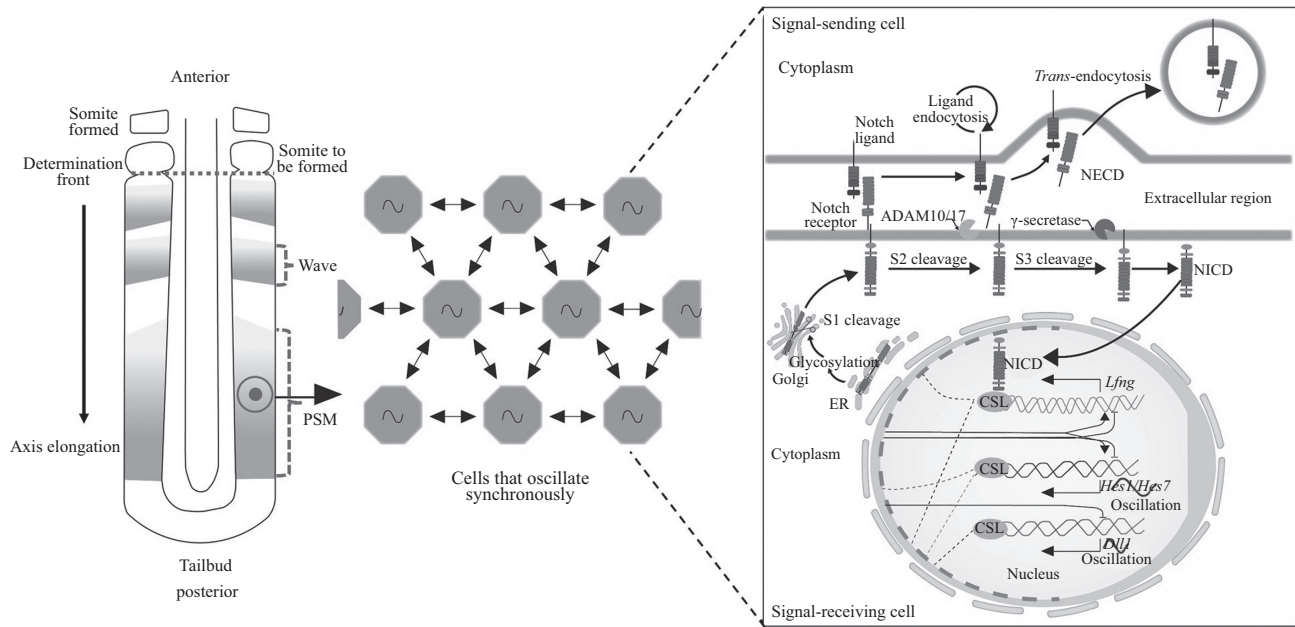
性上升与下降;而Hes7则通过双重机制参与调控:一方面直接抑制细胞周期蛋白基因 *Cyc1*和 *Cyc2*的转录,另一方面间接影响自身的表达稳定性。这种交叉调控网络使得时钟基因的振荡频率与体节分节的时空模式同步。同时,当Hes/Her在细胞内积累至阈值浓度时,它们不仅抑制自身基因转录,还会通过交叉抑制其他时钟基因^[18](图2)。

此外,Notch信号通路的配体与受体的相互作用还通过配体-受体介导的旁分泌信号在细胞间形成动态梯度,从而在空间维度上精确指导体节的形成。Dll1在PSM中呈现振荡性表达,其蛋白产物通过旁分泌作用激活邻近细胞的Notch受体,进而诱导下游Hes/Her基因家族的表达^[19]。RNA修饰也会影响时钟基因的动态平衡。高水平Hes1蛋白会直接抑制pri-miR-9的转录;反之,当Hes1蛋白水平低时,pri-miR-9转录活跃。Hes1蛋白与pri-miR-9 mRNA的振荡相位相反,这种反相关对抑制细胞分化具有重要作用^[20]。Hes7的3'UTR也受到miRNA的调控,从而维持其振荡表达与体节分节的时空协调;Hes7是通过快速降解实现负反馈环路的精准时序控制的,其蛋白稳定性是体节分割钟振荡周期和体节分节模式形成的关键。破坏Hes7的3'UTR会导致负反馈环路失效,引发体节畸形^[21]。这些多层次

的调控机制的协同作用,使体节分割时钟在时空维度上实现高精度同步,确保体节边界形成的准确性与可重复性(图2)。

1.3 细胞间的协调振荡

除基因在一个细胞内的表达调控之外,体节边界的形成是由一群细胞共同决定的结果,这种协调一致的行动需要细胞间的局部通讯。早期研究通过观察原肠胚后部PSM细胞的动态行为发现,PSM区的细胞先启动Hes/Her基因的表达,这些细胞通过Notch信号把振荡信号传递给前面的细胞,当信号传到最前端的细胞时,所有细胞的Hes/Her基因几乎同时停止振荡,体节边界就精准形成了^[22]。这一现象启发了LEWIS等^[23]提出经典的“同步振荡”模型:相邻细胞通过Delta/Notch信号通路的相互作用,同步彼此的Hes/Her基因振荡,从而实现群体细胞的分割协调性。机制研究表明,Hes7蛋白通过抑制Dll1的转录,驱动Dll1在细胞内的周期性表达;而Dll1则通过旁分泌作用激活邻近细胞的Notch受体,进而上调相邻细胞中Hes/Her基因家族的表达水平。这种机制将相邻细胞的振荡器连接成一个同步网络,确保整个PSM区的细胞以相同频率振荡,最终在体节边界处实现同步化停滞^[24](图2)。后续研究进一步揭示了Notch信号通路的时空特异性调控是维持振荡同



在胚胎发育的早期,成对的体节逐渐从PSM的前端(anterior)“发芽”。尾芽(tailbud)是位于胚胎后端(posterior)的原肠胚形成部位,它不断用祖细胞“补充”PSM。体节分割的周期性受分子振荡器的调节,该分子振荡器驱动循环基因从PSM的后部到前部表达。随着每个周期的振荡,时钟基因表达域向前移动形成“波”(wave),到达PSM的前端,形成体节的边界(determination front)。这种循环基因表达的周期与体节形成的周期相匹配,从而使得体节不断形成、体轴延长(axis elongation)。细胞的同步振荡由Notch信号通路通过信号发送细胞(signal sending cell)和信号接收细胞(signal receiving cell)调控。Notch受体存在于信号接收细胞的表面,是一种跨膜蛋白,由Notch胞外结构域(Notch extracellular domain, NECD)、跨膜结构域(Notch transmembrane domain, TMD)和Notch胞内结构域(Notch intracellular domain, NICD)组成。Notch信号的传递经过“三重切割”,Notch最初作为无活性的单链前体转运到内质网,在内质网中Notch受体的EGF样结构域(epidermal growth factor-like repeats)发生糖基化。然后将糖基化的Notch单链转运到高尔基体。在高尔基体中,转化酶裂解Notch跨膜区细胞外段中的S1位点,形成NECD和TMD两个不同的片段。这些片段随后形成异二聚体形状的成熟Notch受体,随后被转运到细胞表面。到达细胞表面后,Notch异二聚体跨膜受体与相邻细胞上的Notch跨膜配体(Dll1)结合。然后,Notch受体的S2切割位点被ADAM金属蛋白酶家族切割。放出部分细胞外片段,被 γ -分泌酶在S3切割位点裂解,导致Notch可溶性NICD的释放。随后, NICD易位到细胞核与转录因子CSL(CBF1/suppressor of hairless/Lag-1)相互作用,促进*Lfng*、*Hes7/Hes1*和*Dll1*的转录。*Lfng*编码的糖基转移酶,能识别并糖基化修饰Notch受体上的EGF重复序列同时调节*Dll1*的转录,来调控邻近细胞中Notch信号的激活。*Hes1/Hes7*则形成一个负反馈振荡网络,同时调控受体Dll1的表达水平,调节着整个细胞的振荡节奏。

During early embryonic development, paired somites progressively “bud” from the anterior end of the presomitic mesoderm. The tailbud—located at the posterior end as a site of gastrulation—continuously replenishes the PSM with progenitor cells. The periodicity of somite segmentation is regulated by a molecular oscillator that drives cyclic gene expression from the posterior to anterior PSM. With each oscillatory cycle, the expression domain of clock genes advances anteriorly, forming an expression wave that reaches the anterior PSM to establish the somite boundary (determination front). This cyclic gene expression period matches somite formation rhythm, enabling sequential somite generation and axis elongation. The synchronous oscillation of cells is regulated by the Notch signaling pathway through signal sending cells and signal receiving cells. The Notch receptor is present on the surface of signal receiving cells as a transmembrane protein, consisting of the Notch extracellular domain, the Notch transmembrane domain, and the Notch intracellular domain. The transmission of Notch signals involves “triple cleavage.” Initially, Notch is transported to the endoplasmic reticulum as an inactive single-chain precursor, where the epidermal growth factor-like repeats of the Notch receptor undergo glycosylation. The glycosylated Notch single chain is then transported to the Golgi apparatus. In the Golgi apparatus, furin cleaves the S1 site in the extracellular segment of the Notch transmembrane region, forming two distinct fragments: NECD and TMD. These fragments subsequently form a mature Notch receptor in the shape of a heterodimer and are then transported to the cell surface. Upon reaching the cell surface, the Notch heterodimeric transmembrane receptor binds to the Notch transmembrane ligand (Dll1) on adjacent cells. Subsequently, the S2 cleavage site of the Notch receptor is cleaved by an ADAM metalloprotease family member, releasing a portion of the extracellular fragment. The γ -secretase then cleaves the S3 site, leading to the release of the soluble NICD. The NICD then translocates to the nucleus, where it interacts with the transcription factor CSL, promoting the transcription of *Lfng*, *Hes7/Hes1*, and *Dll1*. The glycosyltransferase encoded by *Lfng* recognizes and glycosylates the EGF repeats on the Notch receptor while regulating the transcription of *Dll1*, thereby modulating the activation of Notch signaling in neighboring cells. *Hes1/Hes7* form a negative feedback oscillation network, simultaneously regulating the expression level of the receptor Dll1, which controls the oscillation rhythm of the entire cell.

图2 Notch信号通路在体节分割时钟中的核心作用(根据参考文献[8,18,22]总结并绘制)

Fig.2 The core role of the Notch signaling pathway in the somite segmentation clock (summarize and draw based on the references [8,18,22])

步性的关键,在PSM前部,Delta表达水平较低,从而允许体节边界形成;而在后部,高Delta表达维持细胞间同步振荡。数十年来,该模型在多种脊椎动物模型(斑马鱼、鸡胚、小鼠)中得到验证,成为体节分割的经典理论。

近年来,DIAS等^[25]和KLEPSTAD等^[26]的研究相继挑战了经典同步振荡模型,提出体节边界形成的“自组织”假说。他们通过活细胞成像与计算机模拟发现,在特定培养条件下,PSM区仍能自发形成类似体节的周期性结构,且其空间模式与Hes/Her/Delta基因的振荡无关。这一现象提示,体节形成可能本质上是由细胞机械张力和代谢状态等内在属性以及间充质向上皮转化过程中产生的物理堆积等外源性约束力共同作用的结果^[27]。细胞在迁移过程中因密度变化产生机械压缩力,可能通过YAP/TAZ通路等转录依赖途径触发局部细胞命运转变,从而形成体节边界^[28-29]。该模型强调的“自下而上”调控机制为理解体节发育提供了新视角:体节可能并非严格依赖基因振荡的“时钟”,而是通过细胞群体在物理化学约束下的自组织行为实现边界划分。这一假说后续还需通过单细胞测序解析自组织过程中基因表达的时空特征,或利用物理模型模拟无振荡条件下的体节形成效率进行进一步验证。

1.4 Notch信号通路参与体节分割过程

最新的实验研究提供了Notch信号通路参与体节分割的直接证据^[30]。在动物模型中,Notch1基因敲除的小鼠表现出明显的体节缺陷,而Delta-like1过表达的斑马鱼则出现了体节过多的情况^[31]。在体外实验中,利用胚胎干细胞或胚胎体细胞培养系统,研究人员能够重现体节分割时钟的振荡现象,并通过操纵Notch信号通路的相关分子,观察到了基因表达振荡的改变。通过在胚胎体细胞中过表达NICD,可以观察到时钟基因表达模式的改变^[32]。结合这些实验研究,可以看到Notch信号通路与体节分割时钟的相互作用具有以下具体证据。首先,Notch信号通路的关键分子在体节形成区域的表达具有时空特异性,这与体节分割时钟的节律性表达模式相吻合^[33]。其次,Notch信号通路的遗传操作会导致体节分割时钟的紊乱,影响胚胎的正常发育^[34]。最后,在去除复杂体内环境的条件下,体外的实验模型中仍能观察到Notch信号通路对体节分割时钟的真实影响。

在体节发育中,Notch信号通路主要在以下几

个方面中发挥作用。①维持体节分割时钟基因的周期性表达。在胚胎的轴线区域,体节分割时钟基因如Hes1、Hes7等通过转录反馈环路实现周期性表达。Notch信号通路通过调节这些基因的表达,参与维持体节分割时钟的节律性。Hes1和Hes7的蛋白产物可以抑制自身及其他体节分割时钟基因的表达,而NICD通过激活Hes1和Hes7的转录,使这些基因的表达呈现出周期性变化^[35]。②调节细胞命运和边界形成。在体节形成过程中,Notch信号通路通过调节细胞命运,确保了体节边界的正确形成。Delta配体在边界细胞中高表达,而Notch受体在邻近细胞中活跃^[36]。这种不对称表达模式使得边界细胞获得更高的Notch信号活性,从而抑制其成为肌肉细胞,而邻近细胞则分化为肌肉细胞。此外,Notch信号通路还参与调控体节前缘细胞的增殖和分化,进一步影响体节的形成。③协调胚胎轴向延伸。在胚胎轴向延伸过程中,Notch信号通路与Wnt、FGF等其他信号通路相互作用,共同调控胚胎的生长和发育。Notch信号通路通过影响细胞增殖、分化和凋亡等过程,参与胚胎轴向器官的形成。例如,在神经管发育过程中,Notch信号通路与Wnt信号通路协同作用,调控神经前体细胞的增殖和分化^[37]。④调控细胞黏附和迁移。在体节形成过程中,细胞黏附和迁移对于胚胎的正确发育至关重要。Notch信号通路通过调节细胞黏附分子的表达,影响细胞间的黏附作用^[38]。此外,Notch信号通路还参与调控细胞迁移,确保细胞在胚胎发育过程中到达正确的位置^[39]。

2 新技术推进Notch通路与体节时钟研究

2.1 单细胞转录组测序和基因编辑技术

单细胞RNA测序(single-cell RNA sequencing, scRNA-seq)技术的应用为研究Notch信号通路在体节分割时钟中的作用提供了新的思路。VAN DEN BRINK等^[40]通过整合单细胞和空间转录组测序技术,在胚状体中识别了尿囊细胞(allantoic cells)等多种新的胚胎细胞类型,并揭示了Notch信号通路关键调节因子在胚胎和胚状体中的表达相似性,证实了胚状体可作为研究体节分割时钟的系统模型。MOK等^[41]利用scRNA-seq技术对鸡胚前体节中胚层区域进行分析,发现Notch信号通路相关基因Foxd1在PSM区后部富集,Olfml3则在体节中线域分泌,这种异质性支持了Notch信号在体节分节中的分区调

控模型,即Notch在不同区域通过协同YAP、Wnt等其他通路实现干细胞维持与体节分化的平衡。整合scRNA-seq和RNA断层扫描技术(RNA tomography)的方法在体轴延伸中首次捕捉到此类空间异质性,提供了从单细胞到组织尺度的新视角。

CRISPR基因编辑技术的应用进一步推动了Notch信号通路在体节分割时钟中的功能研究。MATSUDA等^[42]通过基因组编辑技术,针对脊柱分割缺陷患者中发现的Notch信号通路关键基因(如*HES7*、*LFNG*、*DLL3*和*MESP2*)进行了深入研究。研究发现,这些基因的突变会显著影响分节钟的振荡、同步和分化特性,同时引发*FGF4*、*FGF18*和*DUSP*等下游基因的上调,揭示Notch信号通路在体节分割中的核心调控作用。此外,最近的研究表明,使用CRISPR/Cas系统制备的*NCSTN*基因条件性敲除小鼠,经采用三种不同剂量的他莫昔芬和不同的给药方式(腹腔注射、皮下注射和灌胃注射)诱导敲除*NCSTN*基因后,*Notch1*和*Hes1*的表达量显著下降,该模型可作为研究Notch信号通路相关皮肤和神经系统疾病的动物模型^[43],进一步证实Notch信号通路在发育和疾病中的重要性。

2.2 光遗传技术

光遗传学技术通过光控手段精确调控特定细胞活性,并结合荧光蛋白标记实时监测Notch信号通路组分的动态分布与相互作用。SHIMOJO等^[44]利用光遗传学技术诱导Notch配体*Dll1*的振荡性表达,发现这种振荡性表达对维持神经祖细胞的稳定性至关重要,提示Notch信号通路的动态调控在细胞命运决定中发挥关键作用。YOSHIOKA-KOBAYASHI等^[45]进一步构建了光遗传学发送器-接收器系统,通过光控诱导发送器细胞中*Dll1*的表达,并利用*Hes1*报告系统监测接收器细胞的响应。研究发现,Notch信号通路的关键调控因子*Lfng*能够延迟细胞间Notch信号的传递过程,揭示Notch信号在细胞间通讯中的精细调控机制。此外,光遗传学技术还证实,*Dll1*的振荡性表达能够在相邻细胞间传递信息,这种传递可能因信号转导延迟而导致反相或同相的振荡模式^[46],为理解体节分割时钟的同步化机制提供重要线索。

在体节发育过程中,Notch信号通路的精确调控尤为重要。研究表明,在前体节中胚层中,*Dll1*的持续表达会导致体节及其衍生物(如脊椎和肋骨)的严重融合,而*Dll1*的振荡性表达则是维持正常体节分

割的关键。类似地,在大脑发育中,*Dll1*的持续表达会抑制神经祖细胞的增殖,进一步体现了Notch信号通路动态调控的重要性。这些发现不仅揭示了*Dll1*振荡性表达在组织形态发生中的作用,也为理解Notch信号通路在体节分割时钟中的功能提供了新的理论依据。

2.3 活细胞成像和3D/4D成像技术

体节分割时钟的动态过程涉及复杂的细胞行为和组织重塑,而活细胞成像和3D/4D成像技术的发展为研究Notch信号通路在这一过程中的作用提供了前所未有的时空分辨率。MARTINS等^[47]率先利用3D和4D成像技术,揭示了体节形成过程中单个细胞的运动模式,包括中间区域的上皮化以及间质细胞的重排,这些发现为理解Notch信号通路在细胞间通讯和组织重塑中的作用奠定了基础。MCCOLL等^[48]通过定量分析和4D细胞成像追踪,捕捉到真皮肌原细胞向头侧中间域的定向运动,并发现这一过程是骨骼肌形成的起始点,提示Notch信号通路可能在细胞定向迁移和组织模式化中发挥关键作用。

YOSHIOKA-KOBAYASHI等^[32]开发了一种小鼠前体节中胚层(PSM)组织的单细胞活细胞成像系统,能够量化每个PSM细胞的瞬时振荡相位及其同步程度。研究发现,在缺乏Notch信号通路关键调控因子*Lfng*的情况下,*Hes7*的振荡在单细胞水平上出现去同步化和衰减,表明*Lfng*通过Notch信号通路调控细胞间的耦合过程,从而维持体节分割时钟的同步性。这一发现直接揭示了Notch信号通路在体节分割时钟中的主导地位。此外,MOK等^[49]在鸡胚中使用4D活细胞成像技术,直观地观测到体节发育组织中的上皮-间质转化过程,为Notch信号通路在体节形态发生中的功能提供了细胞动力学证据。这些研究表明,Notch信号通路不仅通过调控基因表达的振荡模式影响体节分割时钟,还通过介导细胞间通讯和组织重塑参与体节的形成过程。

2.4 微流控和类器官培养技术

微流控技术通过精确调控流体流动,能够实现内源性信号的同步化振荡,并通过构建通路调节剂的空间梯度,从而深入分析信号梯度对体节发生过程的影响^[50]。LIU等^[51]利用微流控技术将多能干细胞来源的PSM组织置于微加工的槽中,通过施加外源性形态素梯度,成功诱导了从头到尾的自发性体节形成。这一研究不仅揭示了细胞和组织生物力学

在体节形成中的调节作用,还为研究Notch信号通路在体节分割时钟中的时空动态调控中提供了新的方法。

类器官培养技术能够从干细胞或成体组织中培育出具有特定器官结构和功能特性的三维组织,为模拟体节形成过程提供了高度仿真的模型。YAMAN等^[52]通过诱导人类多能干细胞生成轴向延伸的类器官,发现体节分化基因 *MESP2* 的周期性激活在空间和时间上与类器官 PSM 的分割时钟波相吻合,提示Notch信号通路在体节分割时钟中的关键作用。此外, SANTOS等^[53]在人类类器官中发现,体节分割时钟的周期约为5小时,并观察到 *WNT3A* 和 *FGF8* 基因表达下调后体轴延伸终止的现象。这些研究揭示了Notch信号通路与其他信号通路(如Wnt和FGF)在体节分割时钟中的协同作用。

这些技术为研究Notch信号通路在体节分割时钟中的功能提供了强有力的工具,不仅能够模拟体节形成的动态过程,还能精确调控信号通路的时空表达,从而为揭示Notch信号通路在体节发育及相关疾病中的作用机制开辟了新的研究方向,为阐明Notch信号通路在体节分割时钟中的核心调控作用提供了直接的实验证据,同时也为相关发育异常疾病的机制研究和治疗策略开发奠定了重要基础。

3 Notch信号通路在体节分割时钟异常导致的疾病

3.1 Notch通路异常关联多种先天脊柱畸形

Notch信号通路的异常会导致体节分割时钟的紊乱,从而引起一些与脊柱发育相关的先天性疾病。先天性脊柱侧凸(congenital scoliosis, CS)是一种常见的结构性畸形,越来越多的研究表明,Notch信号通路的异常激活或抑制与CS的发生密切相关。Notch信号通路通过调节椎体软骨和骨骼的形成,影响脊柱的正常发育^[54]。例如, *DLL3*、*MESP2*、*LFNG*、*HES7*等Notch信号通路相关基因的突变,已被证实与CS的发生有关。*DLL3*突变可导致Notch信号通路的过度激活,进而影响软骨细胞的分化和增殖,导致脊柱侧凸。*MESP2*是Notch信号通路的直接靶基因,其突变会影响椎体的正常形成。*LFNG*和*HES7*的突变则可导致体节分割时钟的紊乱,进而影响脊柱的对称性和正常排列^[55]。除脊柱侧凸外,Notch信号通路异常还与体节发育不全症(spondylo-

costal dysostosis, SCD)相关,这是一种以脊椎和肋骨发育异常为特征的疾病^[56]。患者通常表现为肋骨数量减少、脊椎骨融合或分裂不全,导致脊柱结构异常,这些症状与体节分割时钟的紊乱有关。遗传分析发现,大多数SCD病例是由*DLL3*的基因突变和截断引起的,该蛋白是分割时钟的关键调节因子,其他SCD病例可能是由*HES7*、*LFNG*和*MESP2*等基因突变引起的^[57]。

3.2 Notch通路异常致多器官发育疾病

Notch信号通路在体节分割时钟异常还可能致其他多种发育性疾病。例如,Alagille综合征(Alagille syndrome)是一种常染色体显性遗传病,其特征是胆管发育不全和心脏、面部、骨骼等多器官系统的异常。研究表明,*JAG1*和*NOTCH2*基因的突变是导致Alagille综合征的主要原因,这些基因编码的蛋白是Notch信号通路的重要组成部分^[58]。在心脏发育方面,Notch信号通路异常与先天性心脏缺陷(congenital heart defects, CHDs)有关,如心房和心室间隔缺损、主动脉缩窄等^[59],其在心脏瓣膜和血管发育中的作用失调可能导致瓣膜畸形和血管异常^[60]。此外,Notch信号通路异常还与皮肤疾病如先天性角化不良(dyskeratosis congenita)相关,这是一种以皮肤、骨髓和指甲发育异常为特征的遗传性疾病,其病因之一是*DKC1*基因突变,该基因编码的蛋白与Notch信号通路相互作用^[61]。在神经系统发育方面,Notch信号通路还与大脑皮质发育不全(cortical dysplasia)^[62]和神经管缺陷(neural tube defects, NTDs)相关,其失调可能导致神经元和胶质细胞的数量和排列异常^[63](表1)。

4 小结与展望

对Notch信号通路在体节分割时钟的研究深入揭示了细胞命运决定、细胞间通讯以及胚胎发育中周期性结构形成的分子机制,展现了细胞内及细胞间各分子间高度精细的协作模式。随着新技术的不断涌现,我们得以进一步阐明Notch信号通路在体节发育及相关疾病中的关键作用机制。同时,Notch信号通路在体节分割时钟的异常与多种人类疾病的发生密切相关。这些研究成果不仅加深了我们对疾病发病机理的理解,还为开发新型诊断工具与治疗策略开辟了道路。其中靶向Notch信号通路的调控分子已成为潜在的药物靶点, γ -分泌酶抑制剂是最早发现和最大的一类Notch通路的靶向药物,能阻

表1 Notch信号通路与体节分割时钟基因异常导致的常见疾病

Table 1 Common diseases caused by abnormalities in the Notch signaling pathway and somite segmentation clock

疾病名称 Disease name	主要症状 Main symptoms	Notch信号通路相关基因 Genes related to Notch signaling pathway
Congenital scoliosis	Structural deformities of the spine	<i>DLL3, MESP2, LFNG, HES7</i> ^[55]
Spondylocostal dysplasia	Abnormalities in the number or structure of vertebrae and ribs	<i>DLL3, HES7, LFNG, MESP2, MEOX1</i> ^[57]
Alagille syndrome	Incomplete development of the bile ducts and abnormalities in multiple organ systems	<i>JAG1, NOTCH2</i> ^[58]
Congenital heart defects	Septal defects of the atria and ventricles, aortic coarctation, etc	<i>NOTCH1, MFNG</i> ^[59]
Spondylocostal dysostosis	Abnormal development of the spine and ribs, reduced number of ribs, fusion of vertebrae	<i>DLL3, HES7, LFNG, MESP2</i> ^[55]
Dyskeratosis congenita	Abnormal development of the skin, bone marrow, and nails	<i>DKC1</i> ^[60]
Cortical dysplasia	Abnormalities in the number and arrangement of neurons and glial cells	<i>HEY1, NOTCH1, HES1, PAX5</i> ^[62]
Neural tube defects	Abnormal neural tube closure	<i>NOTCH1, NOTCH3, HES1, HES3</i> ^[63]
Klippel-Feil syndrome	Abnormal development of the cervical spine, restricted neck movement	<i>MEOX1, RIPPLY2</i> ^[64]
Adams-oliver syndrome	Congenital skin dysplasia, poor development of the skull	<i>NOTCH1, RBPJ, DLL4</i> ^[65]
Lehman syndrome	Lateral meningocele, midfacial hypoplasia, micrognathia, decreased muscle tone	<i>NOTCH3</i> ^[66]
Hajdu cheney syndrome	Abnormalities of the nervous system, craniofacial, and skeletal structures	<i>NOTCH2</i> ^[67]

止Notch受体的切割,使其无法产生可溶性的NICD,从而阻止NICD向细胞核的转移^[68]。 γ -分泌酶抑制剂主要包括DAPT、YO-01027、BMS 299897和LY-411575等。最近的研究表明通过系统性给予DAPT持续抑制Notch信号通路,对于延缓异位骨化形成具有良好的临床应用前景^[69];在小鼠中,通过使用 γ -分泌酶抑制剂Notch1也可促进股骨愈合^[70-71]。

当前尽管通过实验动物模型和诱导干细胞体外模拟的方法已经揭示了Notch信号通路参与体节分割时钟的过程,仍还有一些未明确的问题,这些问题横跨分子机制至胚胎发育的多个维度。①关于Notch信号通路与体节分割时钟如何在多样化的细胞类型及环境条件下维持精确且稳定的相互作用机制,目前尚缺乏深入理解^[72]。②Notch信号通路如何在三维空间内协调体节的形成,以及它与除经典的Wnt和FGF信号通路以外,其他分子和信号通路如何在这一空间内协作以共同调控胚胎发育模式的问题,仍需进一步探索^[73]。③体节分割时钟的启动与终止机制,以及这些机制如何与细胞周期进程及细胞命运决定过程相协调,仍不明确。④有观点认为蛋白质的降解速度决定了体节分割时钟的周期^[74],目前只对Notch信号通路中少数分子的降解过程比较明确,对于其他蛋白质分子的修饰与降解过程和

这些过程如何影响信号通路的活性与稳定性,也有待更为深入的研究。

致谢

感谢中南大学湘雅基础医学院范立青研究员在论文撰写过程中的指导和帮助。

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