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◇综 述◇

硬脑膜/颅缝间充质干细胞对颅骨发育的影响

安 冉[1,2,3,4,5*],刘 赞[1*] 综述 邵 国[6],张春阳[2,3,4,5],孙志刚[2] 审校

[[1] 内蒙古科技大学包头医学院,包头 014000;[2] 内蒙古科技大学包头医学院第一附属医院神经外科,包头 014010;[3] 包头医学院神经外科疾病研究所(转化医学),包头 014010;[4] 包头市神经外科临床医学研究所,包头 014010;[5] 内蒙古自治区骨组织再生与损伤修复工程技术中心,包头 014010;[6] 深圳市龙岗区第三人民医院转化医学中心,深圳 518100]

摘要 颅缝复合体由具有间充质的纤维组织、成骨前沿、下面的硬脑膜和上覆的骨膜组成。硬脑膜是一层保护大脑和脊髓的纤维结缔组织,拥有血管和淋巴管,对早期发育过程中颅骨成骨的进展,后期颅骨的形态和颅缝状态十分重要。颅缝间充质干细胞主要存在于颅缝中,具有增殖、分化为成骨细胞、生成颅骨和增强颅骨损伤后修复的能力。了解颅缝间充质干细胞与硬脑膜的作用机制,对颅骨生长发育和颅脑疾病的治疗非常重要。该文对硬脑膜和颅缝间充质干细胞在颅骨发育中的机制进行综述。

关键词 硬脑膜;颅缝间充质干细胞;发育;颅缝;颅骨;成骨

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颅缝是颅骨发育的生长中心[1],颅缝异常会导致颅骨发育异常,严重时会危及生命。引起颅缝异常的原因众多,如基因突变、硬脑膜发育不良、颅缝间充质干细胞缺失等[2-4]。研究表明,颅缝作为颅骨和大脑之间的调节者,通过释放可溶性因子和调节相关细胞活动发挥作用[5],颅缝间充质是对颅缝发育、颅骨生长和损伤修复至关重要的颅缝间充质干细胞的生态位[6-7],颅缝间充质干细胞过早消融会导致颅缝早闭[3],其在颅缝的开放和闭合过程中发挥重要作用[8]。硬脑膜是脑膜的最外层,是大脑和颅骨之间信号交流的沟通者[9]。在不同的动物模型和体外实验中发现硬脑膜对颅骨正常发育至关重要[10-13],硬脑膜缺失会出现颅骨畸形[4],硬脑膜调节颅缝的开闭状态[14-15]、细胞分化[16-17]等过程。现对颅缝、硬脑膜及颅缝间充质干细胞三者分别发挥作用的机制进行总结(图1),有望为颅缝早闭、颅骨缺损修复等颅脑疾病提供临床依据或新的治疗手段。

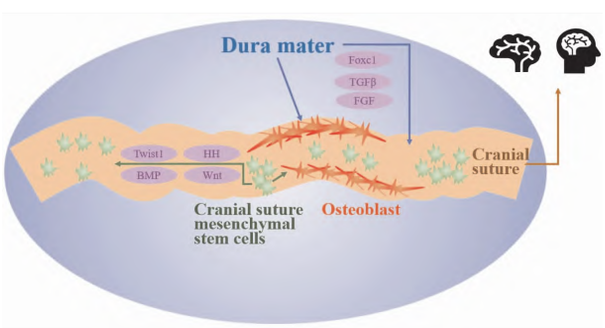

图1 硬脑膜/颅缝间充质干细胞对颅骨发育机制图

Fig.1 Mechanism of dural/cranial suture mesenchymal stem cells on cranial bone development map

1 颅缝及硬脑膜的起源及解剖学关系

颅骨由额骨、顶骨及枕骨等组成,起源于神经嵴和中胚层,其中只有额骨为双重起源,剩余骨均由中胚层衍生而来[18]。颅缝是相邻颅骨之间的纤维关节连接,它们不仅将不同胚胎来源的骨骼分开,而且它们本身是不同的来源。例如,源自神经嵴的颅缝

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作者简介:安 冉,女,硕士研究生;

张春阳,男,教授,主任医师,硕士生导师,通信作者,E-mail:zhangchunyang_1964@aliyun.com;

孙志刚,男,教授,主任医师,硕士生导师,通信作者,E-mail:sunzhg@21.cn

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包括额间缝和矢状缝,而冠状缝源自中胚层,人字缝的发育起源仍然未知。胚胎起源的差异可能导致不同颅缝间充质干细胞的不同功能[7,18]。

硬脑膜在胚胎期第 17.5 天由神经嵴衍生细胞形成[5,9],神经嵴细胞是一种干细胞,可以产生多种细胞类型和结构[19]。硬脑膜是位于颅骨下方一个致密的双层结缔组织,外层是位于颅骨内表面的骨膜,内层覆盖神经组织和蛛网膜下腔[20-22]。硬脑膜与颅缝间充质、成骨前沿、骨膜共同构成了颅缝复合体[23]。

2 颅缝间充质干细胞及其生理意义

研究[7,24-25]表明颅骨区域的主要间充质干细胞群位于颅骨的颅缝间充质内,称为颅缝间充质干细胞。目前,已有四种细胞被证实为颅缝间充质干细胞,包括 $Gli1^+$ 细胞、$Axin2^+$ 细胞、$Prrx1^+$ 细胞和 $Ctsk^+$ 细胞[7]。颅缝不仅是颅骨生长发育的关键部位,也是调节颅骨生长发育的干细胞的生态位。间充质干细胞几乎在所有被研究的组织中都有分布[26],它能够被诱导分化为多种细胞,如成骨细胞、软骨细胞和脂肪细胞,因此是骨组织工程中一种有潜力的细胞[27]。

$Axin2^+$ 细胞的干细胞特征表现为克隆能力,在研究人员所监测 1 年中,单细胞水平上检测到克隆扩增的 $Axin2^+$ 细胞。$Gli1^+$ 细胞也具有相同能力,此外,传代培养后,发现单个 $Gli1^+$ 细胞能够分化为骨、软骨和脂肪,表明它具有多向分化的能力[1,3]。以上结果表明它们是颅缝间充质中能够发挥作用的干细胞。

3 硬脑膜对颅骨成骨分化的影响

3.1 硬脑膜参与调控颅缝状态 硬脑膜是再骨化的充分必要条件,在维持颅缝开放与闭合中起关键作用[9]。Yu et al[28]发现在硬脑膜与颅骨之间放置了 10 nm 孔的 Parafilm 膜,6 周后发现颅缝几乎完全闭合,而未放置组颅缝则保持开放状态,表明硬脑膜有助于维持颅缝开放状态。制作大鼠颅缝旋转模型,在不破坏硬脑膜的情况下,将颅骨瓣分离后旋转 180°,原本开放的颅缝提前闭合[29]。

硬脑膜可以直接控制骨细胞的分化[30]。在用已闭合颅缝下方的硬脑膜和开放颅缝下方的硬脑膜分别与成骨细胞共培养,发现二者均可诱导成骨细胞的分化,只是已闭合颅缝下方硬脑膜组的成骨相关因子碱性磷酸酶、骨钙素的含量更高,成骨细胞增殖速度更快[31-32],而颅缝内的成骨分化由诱导与抑制信号的平衡控制[33],因此可能是硬脑膜向颅缝传递了成骨信号,促进成骨细胞成骨,进而影响颅缝状态。此外,硬脑膜等与颅缝相邻的结构为颅缝间充质干细胞发挥作用提供适宜的微环境[28],而颅缝间充质干细胞已被证实在颅缝再生中发挥重要作用[6]。

3.2 硬脑膜中相关因子对颅骨成骨的影响 硬脑膜中 Foxc1、成纤维细胞生长因子(fibroblast growth factor,FGF)、转化生长因子 β(transforming growth factor-β,TGF-β)等与颅骨相互作用,影响颅骨的生长发育[9,34-35]。Foxc1 在硬脑膜中表达,通过脑膜和颅骨之间的相互作用间接调节颅骨的早期生长[9],Foxc1 纯合突变体($Foxc1^{ch/ch}$)表现为脑积水,其硬脑膜和蛛网膜均未在颅骨顶端形成,颅骨顶端出现严重的骨缺损[10],这种现象证实了硬脑膜对颅骨早期发育的重要性。TGFβ 信号转导是脑膜正常发育所必需的,为硬脑膜在维持颅缝稳态中发挥指导作用[34-35],TGFβ 受体 2(transforming growth factor beta receptor 2,Tgfbr2)从早期开始就在脑膜间充质中表达,Tgfbr2 功能丧失可导致顶骨缺损[9]、颅骨小及下颌骨生长受限[36]。已知硬脑膜分泌的 FGF 对上覆颅骨发挥作用[37],已闭合的颅缝复合体中 FGF2 蛋白水平比开放状态中高[38],则表明 FGF2 影响颅缝状态,进而影响颅骨生长发育。硬脑膜细胞中成纤维细胞生长因子受体 2(fibroblast growth factor receptor 2,FGFR2)突变可促进成骨细胞的增殖和分化,并可能通过影响 Hippo/YAP-PI3K-AKT 增殖信号通路来影响颅缝早闭[15]。以上研究结果表明硬脑膜具有分泌各种因子调控颅缝状态、诱导成骨细胞增殖分化以及直接影响颅骨生长发育等功能。

4 颅缝间充质干细胞对颅骨成骨分化的影响

4.1 颅缝间充质干细胞对颅缝状态的调控 颅缝间充质干细胞在颅骨发育、缺损修复和再生中发挥重要作用。研究表明,颅缝间充质干细胞过早丢失会导致颅缝早闭[7,28,39]。他莫昔芬诱导 $Gli1^+$ 细胞消融后 1 个月,冠状缝和额前上颌缝闭合,诱导 2 个月后,所有颅缝均闭合[3]。Axin2 在颅缝的成骨前沿和骨膜中表达,在额中缝开始其正常的闭合过程之前,其表达逐渐减弱[40],通过 Wnt-Axin 调节网络[40]、与 Smad2/3 结合以刺激 TGFβ 信号传导[41]等,这可能是颅缝在出生后逐渐闭合的分子机制,并

且 Axin2 失活也会导致颅缝早闭[42]。应用颅缝间充质干细胞与生物可降解支架——改性甲基丙烯酸明胶相结合后置于原颅缝处可以逆转神经认知异常,并有助于颅缝再生[28]。因此,颅缝间充质干细胞可能是颅缝早闭的一种有前景的治疗方法。

颅缝间充质干细胞的机械敏感性影响颅缝状态。研究者利用弹簧扩张矢状缝,在 1、3、7、14、21、28 d 后的 μCT 图像和组织学染色结果显示从负荷后前 3 d,颅缝明显扩张,此时的 Gli1$^+$ 细胞快速增殖,第 3 天后骨边缘出现小突起并进一步延伸,在 1 个月内颅缝恢复到正常宽度,甚至有小部分区域闭合,这是 Gli1$^+$ 细胞分化为成骨细胞和骨细胞的结果[43]。

4.2 影响颅缝间充质干细胞成骨过程的分子机制

在颅缝间充质干细胞成骨的过程中存在多种信号通路,各种信号通路之间互相联系,建立起信号网络发挥作用。

Twist1 转录因子是公认的间充质细胞分化抑制剂[44],抑制成骨细胞分化,是骨形成和骨发育过程中影响间充质细胞增殖和分化的关键调节因子[45]。Twist1 在间充质干细胞中表达,包括颅缝间充质干细胞[46],其失活可促进颅缝间充质干细胞向成骨分化,导致颅缝早闭[47],例如,Saethre-Chotzen 综合征,它表现为复杂的颅缝闭合,最明显的是冠状缝、后额缝和人字缝[48]。Zhao et al[3] 检测 Twist1$^{+/-}$ 小鼠在颅缝闭合前后 Gli1$^+$ 细胞的表达,结果显示颅缝闭合前 Gli1$^+$ 细胞表达与对照组无明显差异,而颅缝闭合后 Gli1$^+$ 细胞表达显著减少,证明 Gli1$^+$ 细胞增殖分化为成骨细胞,促进新骨形成使得颅缝闭合,以上结果表明,Twist1 可以通过调控间充质干细胞来影响颅骨生长发育和损伤修复。此外,Twist1 是颅骨成骨细胞分化中转导 Wnt 信号传导所必需的[49],Twist1 在间充质干细胞成骨细胞分化中与 FGF 和骨形态发生蛋白(bone morphogenetic protein,BMP)信号传导相互作用[50]。

Wnt 信号促进颅缝间充质干细胞的成骨分化,导致颅缝早闭。应用 Wnt 激活剂后,颅缝间充质干细胞中 Runx2 和骨桥蛋白(osteopontin,OPN)表达水平显著增加,表明其向成骨细胞分化作用增强[28]。从机制上分析表明,Wnt 信号通路通过影响 FGF 和 BMP 信号通路的平衡影响颅骨发育过程中颅缝间充质干细胞的命运[7]。

Hedgehog(HH)信号分子通过平衡间充质干细胞与其他邻近细胞(如破骨细胞)的相互作用来调节骨稳态[51-52]。近年来,利用多种基因工程小鼠及体外实验显示 Indianhedgehog 治疗显著上调颅缝间充质内的主要间充质干细胞——Gli1$^+$ 细胞[51]的活性并增强成骨分化,而 HH 抑制剂治疗显著下调 Gli1$^+$ 细胞活性并抑制成骨分化,表明 Indianhedgehog 在诱导 Gli1$^+$ 细胞的成骨谱系中起着关键作用[3,7]。HH 诱导的成骨需要 BMP 信号介导来平衡间充质干细胞成骨与破骨细胞破骨,并且它们共同促进碱性磷酸酶的表达[52-53]。

BMP 信号通路已被证明是颅面发育模式的重要调节因子,影响颅缝间充质干细胞的成骨潜力、骨化和稳态[54],是工程骨再生中最成熟和最有效的生长因子[55]。BMP 信号通路通过 BMP 配体与 BMP 受体 I 型和 II 型的结合而转导,进一步激活细胞内 Smads 蛋白,Smads 蛋白磷酸化可以与 co-Smad4 结合成复合物,该复合物在细胞核中促进骨相关基因表达[54]。Gli1$^+$ 细胞产生的骨祖细胞在颅缝内显示出活跃的 BMP 信号传导活性。组织学分析证实,Gli1$^+$ 细胞中 BMP 受体 1A 型缺失导致矢状缝、冠状缝等多条颅缝变窄,Gli1$^+$ 细胞增殖和成骨分化活性增强[52]。有证据证明 BMP 受体 1A 型在颅面发育和颅缝早闭中保持颅缝间充质干细胞特性,BMP 受体 1A 型能够影响颅缝间充质干细胞产生异位骨组织,是小鼠和人颅缝间充质干细胞的表面标志物[7]。

5 展望

颅骨的生长发育及正常形态的形成对人的健康至关重要,硬脑膜和颅缝间充质干细胞在其中发挥重要作用。本文就硬脑膜、颅缝及颅缝间充质干细胞对颅骨发育的相关机制进行综述,硬脑膜和颅缝间充质干细胞影响颅缝的开放闭合状态,硬脑膜分泌因子直接或间接影响颅骨发育,Twist1、Wnt、HH 等信号形成信号网络调控颅缝间充质干细胞的成骨分化。硬脑膜和颅缝间充质干细胞在协调成骨细胞分化、颅骨生长过程中起关键作用,可能还存在其他机制影响该过程,仍需要进一步探究。

硬脑膜提供各种因子参与颅骨形成,调控颅缝状态[37],也有各种信号通路介导颅缝间充质干细胞向成骨细胞分化,从而影响颅缝的开放与闭合,但是这种机制是否是硬脑膜发出的信号,类似的研究很少,阐明硬脑膜和颅缝间充质干细胞之间的相互作用有利于更深刻地理解其中的作用机制,更好地进行临床转化,是下一步研究的主要方向。颅缝间充

质干细胞参与的颅缝再生需要与下面的硬脑膜进行物理接触[56],研究[57]表明,硬脑膜分泌的外泌体可以促进成骨细胞增殖、迁移和分化,并减少细胞凋亡,通过骨细胞中的 ALP、OCN 和 OPN 等成骨标志物表达变化表明其在颅骨成骨中发挥作用。

颅缝过早闭合会导致颅内压升高,进而引起视力损害、耳聋、癫痫发作和认知障碍等多种并发症,通常采用手术治疗,包括切除受影响的颅缝并进行颅骨重建。然而,这些手术对小儿患者具有挑战性,而且仅移除已闭合的颅缝往往会导致颅缝再次闭合,需要二次手术[24, 58],颅缝的融合不仅影响颅骨的形态,还会导致生长停滞[3],因此治疗颅缝早闭是临床急需攻克的难题。利用干细胞支架能使颅缝早闭小鼠颅缝再生,再生的颅缝恢复了正常的颅缝基因表达谱[28],这种治疗方式降低了颅内压,纠正了颅骨畸形,改善了神经认知功能。目前用于研究的间充质干细胞很多,包括骨髓间充质干细胞、脂肪间充质干细胞、脐带间充质干细胞及颅缝间充质干细胞等[26]。间充质干细胞具有自我更新、克隆扩增和多向分化的能力,是治疗各种疾病的细胞疗法中有潜力的细胞[59],其中骨髓间充质干细胞已被证明是细胞疗法的合格选择[44]。颅缝间充质干细胞在生长发育过程中能通过膜内成骨形成颅骨[60],其中 $Gli1^+$ 细胞及其衍生物可在硬脑膜中检测到[23]。因此,研究硬脑膜与颅缝间充质干细胞的关系将会是具有前景的研究热点,希望在不久的将来,颅缝间充质干细胞疗法可以成为治疗颅骨缺损和修复畸形的可靠方案,让更多颅脑疾病患者得到更好的治疗。

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Effects of dural/cranial suture mesenchymal stem cells on cranial bone development

An Ran[1,2,3,4,5], Liu Zan[1], Shao Guo[6], Zhang Chunyang[2,3,4,5], Sun Zhigang[2]

[[1]*Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou* 014000; [2]*Dept of Neurosurgery, The First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou* 014010; [3]*Institute of Neurosurgical Diseases (Translational Medicine), Baotou Medical College, Baotou* 014010; [4]*Clinical Medical Research Institute of Neurosurgery in Baotou City, Baotou* 014010; [5]*Engineering Technology Center for Bone Tissue Regeneration and Injury Repair of Inner Mongolia Autonomous Region, Baotou* 014010; [6]*Translational Medicine Center, The Third People's Hospital of Longgang District, Shenzhen City, Shenzhen* 518100]

Abstract The cranial suture complex is made up of fibrous tissue with mesenchyme, an osteogenic front, the dura mater beneath, and an overlying periosteum. The dura mater is a layer of fibrous connective tissue that protects the brain and spinal cord, containing blood and lymphatic vessels. It is important for the progression of cranial osteogenesis during early development, as well as the cranial morphology and the state of the cranial suture later in life. Cranial suture mesenchymal stem cells are primarily found in the cranial suture and can proliferate, differentiate into osteoblasts, generate cranial bone, and aid in the repair of cranial bone after injury. Understanding how cranial suture mesenchymal stem cells interact with the dura mater is critical for cranial growth and development, as well as the treatment of cranial diseases. This article reviews the roles of dura mater and cranial suture mesenchymal stem cells in cranial bone formation.

Key words dura mater; cranial suture mesenchymal stem cells; development; cranial suture; cranial; osteogenic

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Corresponding authors Zhang Chunyang, E-mail: zhangchunyang_1964@aliyun.com; Sun Zhigang, E-mail: sunzhg@21.cn