Foxc对颅颌面骨组织发育的调控作用

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摘要 *Foxc*(forkhead box C)基因在人、鼠等多个物种间高度保守, 其亚家族成员*Foxc1*基因和*Foxc2*基因参与了细胞内多条重要信号转导通路, 与人体神经、骨骼、循环系统的发育和功能密切相关。近年来的研究表明, *Foxc1*基因和*Foxc2*基因在颅颌面组织发育过程中发挥着重要的调控作用, 可能共同参与了极为复杂的牙和颅颌面骨组织发育调控网络。该文就近年来*Foxc*基因对颅颌面骨组织发育过程的调控作用研究进展进行综述, 以期为相关领域研究的开展提供参考。

关键词 Foxc1; Foxc2; 颅颌骨; 颅颌面畸形; 骨骼发育

Regulation of Forkhead Box C Genes on Craniomaxillofacial Bone Tissue Development

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Abstract The forkhead box C (*Foxc*) genes are highly conserved in various species such as humans and rodents. The subfamily members of *Foxc* genes, namely *Foxc1* and *Foxc2*, which have been proven to be participated in several important signal transduction pathways, and are closely related to the development and function of the human neural, skeletal and circulatory system. Recent studies have suggested that *Foxc1* and *Foxc2* may play an important and cooperative role in regulation of the development of the craniomaxillofacial bone tissue and teeth. In this paper, the research progress of the regulation of *Foxc* genes on craniomaxillofacial bone tissue development is reviewed.

Keywords forkhead box C1; forkhead box C2; craniomaxillary bone; craniomaxillofacial malformation; skeletal development

颅颌面部正常的外形与功能对人类饮食、呼吸、言语及心理生理健康均具有重要影响。近年来, 颅颌面发育畸形的发生率逐渐增高, 已达到全身先天性畸形的30%以上。目前, 临床治疗手段仅能部分恢复患者外形和功能, 极大地降低了患者的生活质量, 增加了患者及其家庭的经济负担[1]。哺乳动物颅颌面部骨骼主要是由胚胎发育期出现的颅神经嵴细胞(cranial neural crest cells, CNCCs)衍化而来[1-3]。目前的研究表明, 颅颌面骨组织发育过程涉及CNCCs的迁移、位置确定、增殖分化及信号调控等一系列复杂的生物学事件。许多经典信号通路, 如转化生长因子-β(transforming growth factor-β, TGF-β)、骨形

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态发生蛋白(bone morphogenetic protein, BMP)、成纤维细胞生长因子(fibroblast growth factor, FGF)等信号转导通路均参与了CNCCs的迁移、增殖和分化等过程, 相应信号分子及关键基因的异常可引起组织表型变异和器官发生畸形[1,4-5]。

1 Foxc与颅颌面骨组织发育

含有翼状螺旋结构的叉头盒(forkhead box, Fox)蛋白首次在果蝇中被发现, 是一个在进化上高度保守的重要转录因子家族, 其典型结构特征是含有一段由100个氨基酸组成并能与特定DNA序列结合的被称为“Winged helix”或“Forkhead”的DNA结合结构域。迄今为止, 已经从真菌、细菌及多种真核生物中鉴定出了超过100种*Fox*基因, 并将其归属于19个亚家族中, 其不同亚家族却有着不同的生物学功能。目前的研究显示, Fox超家族广泛地参与了生物的生长发育过程, 其功能主要包括调控胚胎发育、调控细胞增殖、分化和体内稳态调节等[6]。

Foxc1(forkhead box C1)基因和*Foxc2*基因作为*Foxc*基因两个亚家族的唯一成员, 均只包含有一个单独编码的外显子而没有内含子, 且在人、小鼠等多个物种间高度保守。近年来的研究表明, Foxc1和Foxc2涉及TGF-β、BMP、FGF、Wnt/β-catenin等多条重要信号转导通路, 与人体神经、骨骼、循环系统的发育和功能密切相关[7-11]。Inman等[10]利用免疫组织学研究Foxc蛋白在小鼠胚胎颅颌面部的表达发现, *Foxc1*基因和*Foxc2*基因在小鼠胚胎E8.5天开始大量表达于胚胎头区的外间充质细胞中, 在E11.5天开始局限表达于眶部、颞部、上颌凸、原始Meckel软骨和产生腭侧突的外间充质细胞中, 随后, 在成骨及成软骨系统表达并一直持续到E18.5天。大量临床研究也证实, *Foxc1*基因和*Foxc2*基因突变分别可以导致Axenfeld-Rieger综合征(Axenfeld-Rieger syndrome, ARS)和淋巴水肿–重睫综合征(lymphedema-distichiasis syndrome, LDS)[12-14]。ARS患者可存在虹膜异常改变、角膜后胚胎环、房水排出结构异常、颅颌面骨组织及牙齿发育异常、心血管系统发育异常等全身表现[12]。LDS患者可存在淋巴及心血管系统发育异常、腭裂、脊柱畸形及脊髓硬膜外蛛网膜囊肿等全身表现[13-14]。尽管两种遗传性疾病的主要临床症状并不相同, 但均伴有不同程度的颅颌骨发育畸形, 将小鼠体内的*Foxc1*基因和*Foxc2*基因分别敲除后, 小鼠的颅颌面部骨骼也都出现严重的发育缺陷, 表现出颅骨缺失、上下颌短小, 腭裂等体征[15-17]。Murakami等[18]最近的研究指出, Foxc1能够通过刺激胶质瘤相关癌基因同源蛋白2(Gli family zinc finger protein 2, Gli2)的表达在软骨内成骨过程中发挥重要的作用, 阻断Foxc1-Gli2信号轴在小鼠发育过程中的表达, 可产生类似Axenfeld-Rieger综合征的颅颌面骨骼畸形表现。上述研究结果提示, Foxc1和Foxc2在颅颌面骨组织发育过程中可能发挥着重要的调控作用。

2 Foxc1和Foxc2对颅颌面骨组织发育调控作用

早期的研究显示, 成骨诱导因子BMP2刺激后成骨细胞的*Foxc1*和*Foxc2*基因的表达水平较对照组先后升高, 且细胞内Foxc1和Foxc2蛋白表达的上调与BMP2呈明显剂量依赖性关系。过表达*Foxc1*和*Foxc2*基因均可在体外促进多种细胞的骨向分化; 沉默*Foxc1*和*Foxc2*基因表达后, 细胞的骨向分化可受到明显抑制[19-22]。除BMP2外, BMP4与*Foxc1*和*Foxc2*基因的表达亦密切相关, BMP4可上调Foxc1表达进而促进C2C12细胞的成骨向分化, 但随着细胞的成骨分化成熟, Foxc1的表达量呈下降趋势[23]。在C2C12细胞内过表达Foxc2可引起BMP4表达增加, 成肌细胞系表达成骨分化标志物[24]。进一步的研究发现, Foxc1和Foxc2蛋白可以分别通过直接活化肌节同源框基因2(muscle segment homeobox 2, *Msx2*)和整合蛋白β1转录促进细胞的骨向分化过程[20,25-26]。Foxc2还可激活Wnt/β-catenin通路诱导骨髓间充质干细胞成骨分化, 抑制成脂相关蛋白过氧化物酶体增殖物激活受体γ-2(peroxisome proliferators-activated receptors γ-2, PPARγ-2)表达[27]。然而, 最近有研究发现, 上述Foxc2的引导分化作用可能还有Ihh(indian hedgehog)信号通路的参与[28]。值得注意的是, *Foxc2*基因还参与了人体脂肪代谢和能量转换, 其基因多态性与人体骨组织密度、胰岛素敏感性及女性血浆甘油三酯水平存在明显相关性[6,29]。最近, Kimberly等[10]和Sun等[15]分别发表了Foxc1调控小鼠颌骨和额骨发育的研究报道, 发现*Foxc1*基因突变或敲除后, 小鼠胚胎头部塑性阶段额部、第一鳃弓成骨节及周围细胞Msx2的表达显著上调, 小鼠上下颌骨近心端和额骨形成区的前体细胞骨向

分化提前, 最终导致小鼠上下颌骨近心端黏连骨化、额骨发育不全。Sun等[15]进一步利用小鼠胚胎成纤维细胞系C3H10T1/2和颅神经嵴细胞系O9-1研究BMP2刺激下细胞Msx2与*Foxc1*基因间的相互作用关系发现, Foxc1蛋白可以直接结合于*Msx2*启动子区域的一段52 bp的BMP响应元件, 抑制磷酸化Smad复合体1/5/8与Msx2的结合, 进而抑制Msx2上调。他们的研究结果证明, Foxc1可以通过负调节Msx2在BMP信号通路中的响应来抑制前体细胞的过早成骨分化, 在不同细胞背景下Foxc1可以产生相反的转录调控活性。然而, 由于*Foxc1*和*Foxc2*基因在胚胎发育过程中的调控作用广泛而极为复杂, 上述研究使用传统的非特异性*Foxc1*或*Foxc2*基因敲除方法获得的基因敲除小鼠表现为复杂的全身畸形, 且多会引起小鼠胚胎致死或出生致死。这不仅影响了对其进行后期的发育研究, 还对研究Foxc1或Foxc2在颅颌面骨组织发育和再生过程的调控作用产生严重干扰[10,15]。

此外, 由于Fox超家族中不少转录因子可以通过同源或异源二聚体的形式协同发挥转录调控作用, 而Foxc1和Foxc2蛋白氨基酸序列具有很高的同源性, 其DNA结合结构域仅有两个氨基酸残基的差异, 二者理论上可以识别相近的DNA序列从而在转录调控水平存在一定相关性。事实上, 以往的研究表明, *Foxc1*和*Foxc2*基因在包括上下颌骨在内的小鼠胚胎轴旁中胚层组织和神经嵴衍生组织发育过程中存在表达重合并产生相似的作用[30]。*Foxc1*和*Foxc2*基因敲除小鼠模型在表型上具有一定的相似之处, 表现为胚胎期或新生期致死, 少量存活小鼠存在心血管、骨骼、淋巴系统等多种畸形表现[30]。选择性敲除小鼠神经嵴细胞*Foxc2*基因, 小鼠角膜、角膜缘和结膜异常, 当同时敲除*Foxc1*基因后, 小鼠眼表组织畸形表现更为严重[31]。*Foxc1*和*Foxc2*基因在小鼠肾小球上皮细胞内表达, 并在维持肾脏功能中发挥着重要的作用, *Foxc1*基因突变小鼠主要表现为重肾双输尿管畸形, 颅骨、胸骨缺如; 而*Foxc2*基因突变小鼠表现为肾小球异常, 中轴骨畸形[32-33]。Foxc1和Foxc2还参与了淋巴系统的发生。*Foxc1*和*Foxc2*基因敲除小鼠体内细胞外调节蛋白激酶(extracellular regulated protein kinases, ERK)信号通路过度激活, 淋巴系统发育异常, 表现为类似LDS的症状[34]。此外, Foxc1和Foxc2蛋白可以通过与相同的Fox特异性DNA结合元件结合直接激活成血管相关基因整合蛋白β3, 前列腺凋亡反应蛋白4可以与Foxc1和Foxc2蛋白结合共同促进眼发育相关基因垂体同型框转录因子2(paired-liked homodomain transcription-factor 2, PITX2)的转录。上述研究更进一步提示, *Foxc1*基因和*Foxc2*基因可能共同参与了极为复杂的颅颌面骨组织发育调控网络[35-38]。

3 小结

上述研究结果显示, *Foxc1*基因和*Foxc2*基因在颅颌面骨组织发育过程中发挥着复杂的调控作用, 随着近年来基因条件性敲除、过表达技术的发展, *Foxc*基因在颅颌面组织(特别是牙和颅颌骨)发育过程中的调控作用机制将逐渐被揭开。系统而深入地研究*Foxc1*基因和*Foxc2*基因对颅颌面骨组织发育过程的调控机制将有助于揭示颅颌面发育畸形和骨代谢紊乱的发病机制, 并可能为针对这些疾病的预防和治疗提供病因学和治疗学理论依据, 具有显著的基础与临床研究意义。

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