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· 基础研究 ·

## CKIP-1对小鼠颅颌面软硬组织生长发育的影响

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**【摘要】** 目的 探讨酪蛋白激酶2相互作用蛋白1(the casein kinase 2 interacting protein-1, CKIP-1)基因对颅颌面部软硬组织的影响,为颅颌面相关疾病的机制研究和治疗提供基础。方法 6月龄雄性CKIP-1基因敲除小鼠(KO)作为实验组,野生型小鼠(WT)作为对照组,用Micro-CT对小鼠颅颌面部硬组织(顶骨、鼻骨、切牙、磨牙)的骨量进行分析,另外用HE染色和甲苯胺蓝染色分析颌面部软组织(鼻软骨、唇黏膜、舌)的组织形态差异。结果 CKIP-1基因可负调控小鼠颅颌面骨松质骨的骨量及牙齿的牙本质矿化,相较WT小鼠,KO小鼠的顶骨板障层厚度增加93%,而皮质骨未见明显差异;鼻骨松质骨厚度增加160%,皮质骨无显著差异;牙釉质厚度未见异常,但牙髓腔变小,牙本质厚度增加48%;HE及甲苯胺蓝染色分析发现KO小鼠的鼻翼软骨板厚度增加57%,并发现局部出现异常骨化;唇黏膜角化层增厚170%;舌肌纤维直径增加45%。结论 CKIP-1基因对小鼠颌面部多种软硬组织的生长发育均有不同程度的影响。

**【关键词】** 酪蛋白激酶2相互作用蛋白1; 颅颌面组织; 生长发育; 基因敲除小鼠; 皮质骨; 松质骨; 软骨; 动物表型观察; 组织学分析

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**The effects of casein kinase 2 interacting protein-1 on the growth and development of craniomaxillofacial soft and hard tissues in mice** TANG Mingyue, HU Ao, CAI Bolei, GAO Ye, LIU Fuwei, LV Qianxin, JIN Dan, HOU Yan, WANG Le, ZHANG Zhouyang, KONG Liang. State Key Laboratory of Military Stomatology, National Clinical Research Center for Oral Diseases, Shanxi Clinical Research Center for Oral Diseases, Department Oral and Maxillofacial Surgery, Stomatological hospital, Air Force Medical University, Xi'an 710000, China

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**【Abstract】 Objective** To investigate the effect of casein kinase 2 interacting protein-1 (CKIP-1) on craniofacial soft tissues and hard tissues, to provide the basis for the study and treatment of craniomaxillofacial related diseases.

**Methods** 6-month-old male CKIP-1 knockout (KO) mice were selected as the experimental group, and wild-type (WT) mice were selected as the control group. The craniomaxillofacial hard tissues (parietal bone, nasal bone, incisors and molars) were analyzed through micro-CT, and the morphological changes of maxillofacial soft tissues (nasal cartilage, lip mucosa and tongue) were analyzed through HE staining and toluidine blue staining. **Results** CKIP-1 negatively regulated bone mass of cancellous bone of cranial and maxillofacial bones and dentin mineralization. Compared with the WT mice, the thickness of the parietal baffle layer increased by 93% in KO mice, while cortical bone showed no significant difference between the two groups. The nasal cancellous bone thickness increased by 160% in KO-mice, while cortical bone showed no significant difference between the two groups; the enamel thickness was normal, but the pulp cavity became smaller and the dentin thickness increased by 48%. Compared with the WT mice, the HE staining and to-

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luidine blue staining analyses of the soft tissues revealed that the thickness of the alar cartilage plate of KO mice increased by 57%, and local ossification was found within the cartilage plate. The thickness of the keratinized layer of the labial mucosa increased by 170% in KO mice and the muscle fiber diameter of the lingual muscle increased by 45%.

**Conclusion** CKIP-1 genes have different effects on the growth and development of various soft and hard tissues in the maxillofacial region of mice.

**【Key words】** casein kinase 2 interacting protein 1; craniofacial tissue; growth and development; gene knockout mice; cortical bone; cancellous bone; cartilage; animal phenotype observation; histological analysis

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**【Competing interests】** The authors declare no competing interests.

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人类颅骨和面部的生长发育涉及数百个基因,其控制着多种组织细胞的迁移、增殖和分化<sup>[1-2]</sup>。由于小鼠与人有90%的同源基因,因此基因敲除小鼠是研究目标基因生物学功能和相关疾病机理的重要工具。酪蛋白激酶2相互作用蛋白1(the casein kinase 2 interacting protein-1, CKIP-1)是在胎儿肝脏基因表达谱中首次发现的一种转录因子,在细胞形态、分化、凋亡等分子功能方面,以及在骨形成、肿瘤发生、免疫调节等生物学过程中均发挥作用<sup>[3-9]</sup>。通过对CKIP-1基因敲除小鼠的研究,发现CKIP-1基因负调控长骨骨量增长导致骨质疏松<sup>[10]</sup>;或能抑制血管中泡沫细胞形成及胆固醇代谢,发挥抗动脉粥样硬化作用<sup>[11]</sup>。然而颅骨属于扁骨,其胚胎发育过程与长骨不同,不经过软骨钙化阶段,因此有其特殊性。与长骨来源的骨髓间充质干细胞相比,颌面部的骨髓间充质干细胞在促进成骨方面对CKIP-1具有更高的敏感性,说明CKIP-1可能与颌骨畸形或颌骨缺损等疾病有关<sup>[12]</sup>;但CKIP-1基因对颅颌面组织生长发育的影响目前缺乏系统的研究。本课题组前期发现,CKIP-1基因敲除小鼠出现颌骨发育过度以及耳发育畸形等变化<sup>[13]</sup>,提示CKIP-1对颅颌面的发育可能有重要影响。本研究对6月龄同窝CKIP-1基因敲除小鼠及野生型小鼠颅颌面多种硬组织(顶骨、鼻骨、牙)及软组织(鼻软骨、唇黏膜、舌)进行系统的组织学及形态学分析,探讨CKIP-1在颅颌面生长发育中的作用。

## 1 材料和方法

### 1.1 动物、试剂和仪器

选取CKIP-1杂合子(Aa)C57小鼠(由军事医

学科学院构建),进行交配,选取同窝6月龄雄性CKIP-1基因敲除小鼠AA(KO)及野生型小鼠aa(WT),普通谷物鼠饲料喂养(各6只,体重18~25g,同窝雄性对照)。

Micro CT(Siemens Inveon Micro-CT,西门子,德国);计算机图像分析系统(Inveon Acquisition Workplace,西门子,德国);无水乙醇(国药集团,中国);二甲苯(国药集团,中国);HE染液(servicebio,中国);甲苯胺蓝染色液(雷根生物,中国);中性树胶(国药集团,中国);4%多聚甲醛(碧云天,中国);EDTA(富宇化学试剂,中国),苏木精(Merck KGaA,德国),伊红(Merck KGaA,德国)。

脱水机(JKT,俊杰电子,中国);包埋机(JB,俊杰电子,中国);病理切片器(RM2235,Leica,德国);冻台(RM2016,俊杰电子,中国);组织摊片机(KD-PH,科迪仪器,中国);烤箱(GFL-230,莱玻瑞,中国);倒置光学显微镜(DMI6000,徕卡,德国);载玻片(Servicebio,中国);镊子、眼科剪、组织剪、刀柄、刀片、50 mL离心管(上海手术器械厂,中国)。

### 1.2 方法

**1.2.1 动物处死** 第四军医大学口腔医院实验动物伦理委员会批准(批准号:2016伦审字012号)。经1%戊巴比妥钠(50 mg/kg)适度麻醉处死后,应用显微外科设备在超净工作台上解剖小鼠,取颌面部组织(顶骨、鼻骨、鼻软骨、牙齿、唇黏膜,舌),4%多聚甲醛固定备用。

**1.2.2 Micro-CT扫描** 取材用4%多聚甲醛固定小鼠颅骨及颌骨,并进行Micro-CT扫描(Inveon Acquisition Workplace;80 kV,500  $\mu$ A,400 ms),扫描层厚为10.56  $\mu$ m。选区小鼠顶骨、鼻骨、切牙、磨

牙进行三维重建。

1.2.3 HE染色 在常温下用4%多聚甲醛固定24 h;脱钙、石蜡包埋和脱蜡后,将样品切成3~4 μm厚的切片。石蜡切片在室温下用苏木精染色15 min,并用蒸馏水洗涤3次,每次1 min。用1%盐酸酒精分化并用蒸馏水洗涤3次后,苏木精染色切片在室温下用0.5%伊红染色3 min, PBS溶液洗涤。DMI6000倒置光学显微镜观察染色切片。

1.2.4 甲苯胺蓝染色 样品常温下4%多聚甲醛固定24 h;脱钙、石蜡包埋和脱蜡后,将样品切成3~4 μm厚的切片。蒸馏水浸洗2~3次;甲苯胺蓝染色液染20~30 min。随后水洗,洗去多余染色试剂;95%酒精分化,100%酒精脱水, DMI6000倒置光学显微镜观察染色切片。

### 1.3 统计学分析

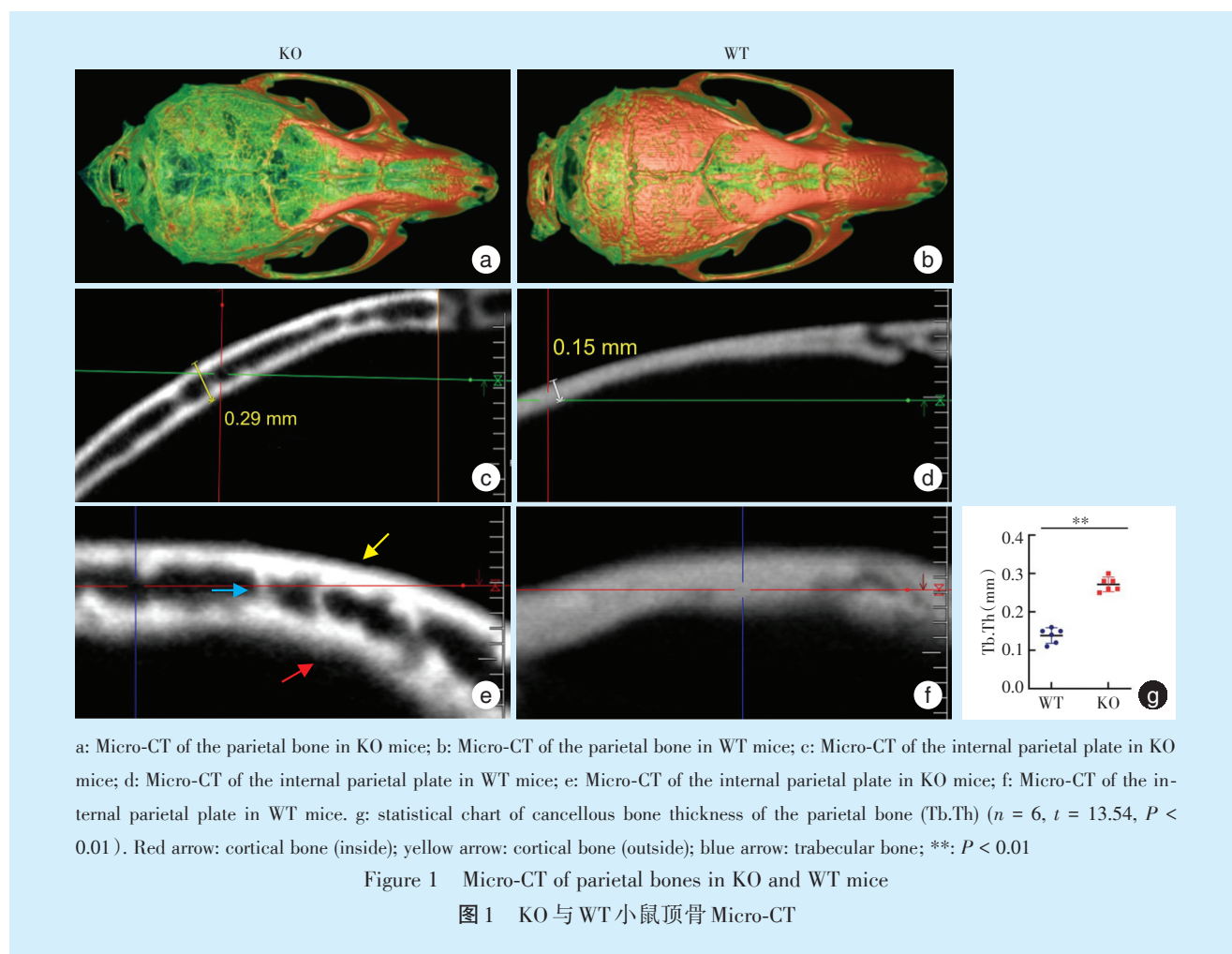
用 Image Pro-Plus 6.0 分析软件定量分析软组

织(鼻软骨、唇、舌)HE染色表达及甲苯胺蓝染色情况,采用SPSS 17.0统计软件进行独立 *t* 检验及 Mann-whitney *U* 检验分析,  $P < 0.05$  为差异有统计学意义。

## 2 结果

### 2.1 顶骨影像学分析

通过 Micro-CT 对小鼠顶骨区域进行三维重建, KO 小鼠与 WT 小鼠顶骨大体观察无显著差异(图 1a、1b)。KO 小鼠顶骨内板厚 0.29 mm(图 1c), WT 小鼠顶骨内板厚 0.15 mm,几乎看不到板障层(图 1d)。通过放大观察发现 KO 小鼠内板与外板之间的板障(即松质骨,图 1e 蓝色箭头)厚度较 WT 小鼠(图 1f)增加, KO 小鼠顶骨内板较 WT 小鼠增厚 93%( $t = 13.54, P < 0.01$ )(图 1g)。而皮质骨未见明显差异。



### 2.2 鼻骨影像学分析

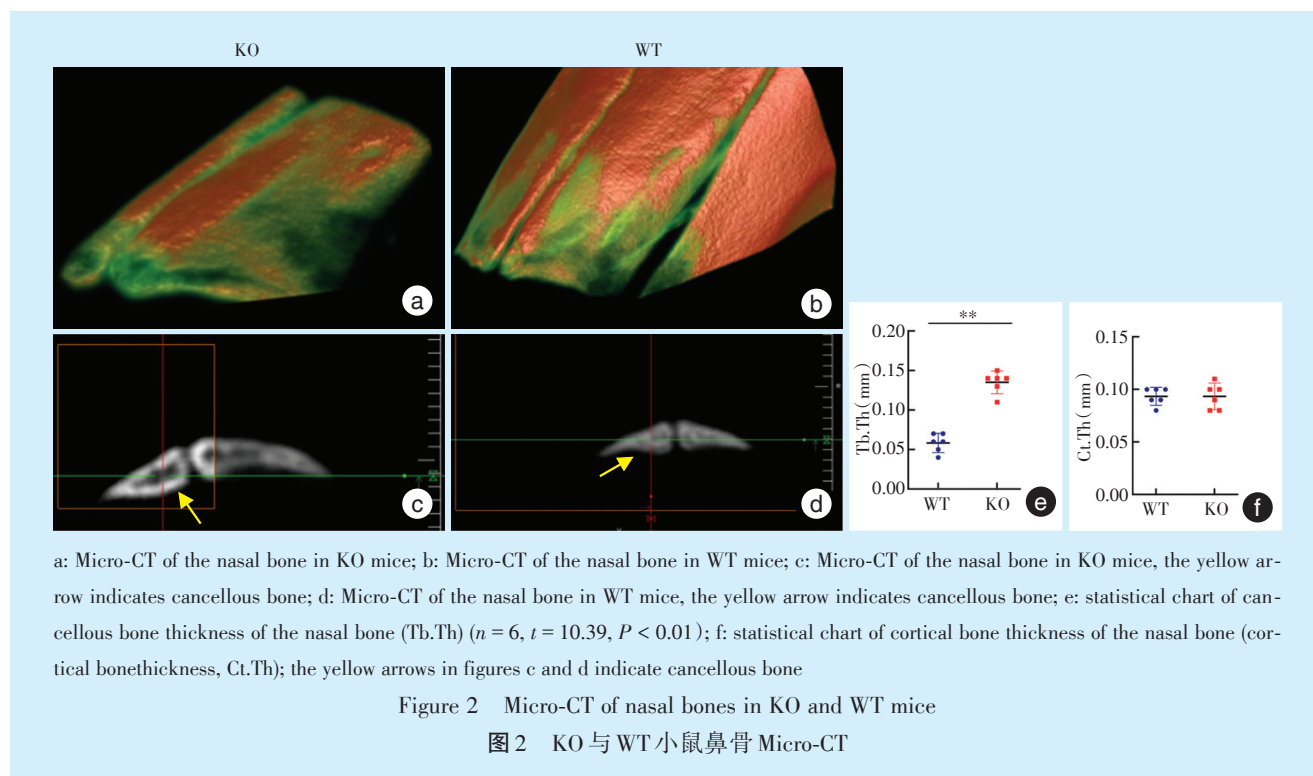
通过 Micro-CT 对小鼠鼻骨区域进行三维重建,

KO 小鼠与 WT 小鼠鼻骨大体观察无显著差异(图 2a、2b),高倍放大观察发现 KO 小鼠松质骨(图 2c



黄色箭头)厚度较WT小鼠(图2d黄色箭头)增加160%(图2e)( $n=6, t=10.39, P<0.01$ ),而皮质骨

未见明显差异(图2f)。



### 2.3 牙齿的影像学分析

通过Micro-CT对WT及KO小鼠的切牙及磨牙分别进行三维重建,切牙纵断面观察(图3a、3b)发现KO小鼠切牙牙髓腔明显减小;横断面观察(图3c、3d)与纵断面趋势相同。对磨牙进行观察发现KO小鼠较WT鼠牙本质厚度增加48%( $n=6, t=11.15, P<0.01$ )(图3e~3g),但牙釉质厚度( $n=6, t=0.26, P=0.79$ )、整牙平均矿化密度(BMD)( $P=0.67$ )以及冠长、根长和冠根比差异均无统计学意义(图3h、3i、表1)。

### 2.4 鼻弹性软骨的组织学分析

HE染色及甲苯胺蓝染色观察定量分析结果显示,KO小鼠鼻翼软骨板(黄色虚线区域)增厚57%( $n=6, t=15.36, P<0.01$ )(图4)。

### 2.5 唇黏膜的组织学分析

通过HE染色及定量分析,低倍镜下观察KO小鼠与WT小鼠唇黏膜角化层形态差异显著,KO小鼠角化层更厚且固有层乳头狭长(图5a、5b),KO小鼠相比于WT小鼠唇黏膜角化增厚170%(图5c)( $n=6, P<0.01$ , Mann-whitney  $U$ 检验),高倍镜下放大观察发现KO小鼠部分区域出现病毒空泡(图5d),而WT小鼠则未发现(图5e)。

### 2.6 舌肌的组织学分析

通过HE染色及定量分析,低倍镜下观察KO及WT小鼠舌肌纤维(图6a、6b),发现KO小鼠纤维束较粗;高倍镜下放大观察(图6c、6d)趋势相同,定量检测KO组较WT组舌肌纤维直径增粗45%( $n=6, t=12.2, P<0.01$ )(图6e)。

## 3 讨论

课题组前期研究发现CKIP-1<sup>-/-</sup>(KO)小鼠的股骨及下颌骨骨量较WT小鼠发生增加,本实验通过Micro-CT进一步对颅颌面相关硬组织进行了对比分析,验证了颅颌面部骨骼骨量变化趋势与全身相同并进行了定量分析。文献报道,CKIP-1作为泛素化调节因子1(Smad ubiquitination regulatory factor 1, Smurf 1)的调控因子,可激活Smurf1,泛素化降解骨形成相关蛋白Smad 1/5、BMP受体、Runx2以及磷酸化形式的MEKK2等<sup>[14]</sup>,从而调控骨量变化。课题组推测CKIP-1是通过该通路抑制颅颌面顶骨、鼻骨的松质骨骨量增加。本研究中,通过对牙齿的三维重建及定量分析,发现KO小鼠较WT小鼠牙本质厚度显著增加且差异具有统计学意义,在临床中,牙本质的薄弱或导致垂直根折,若

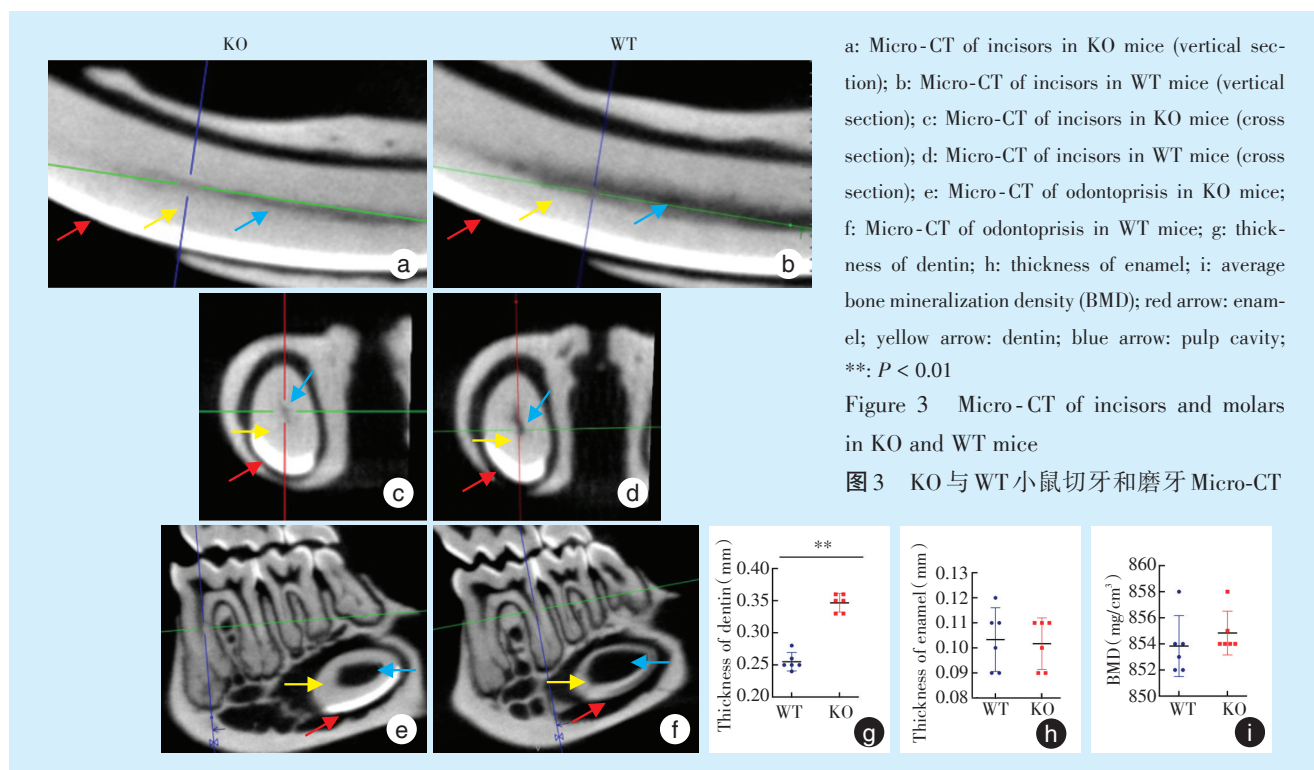
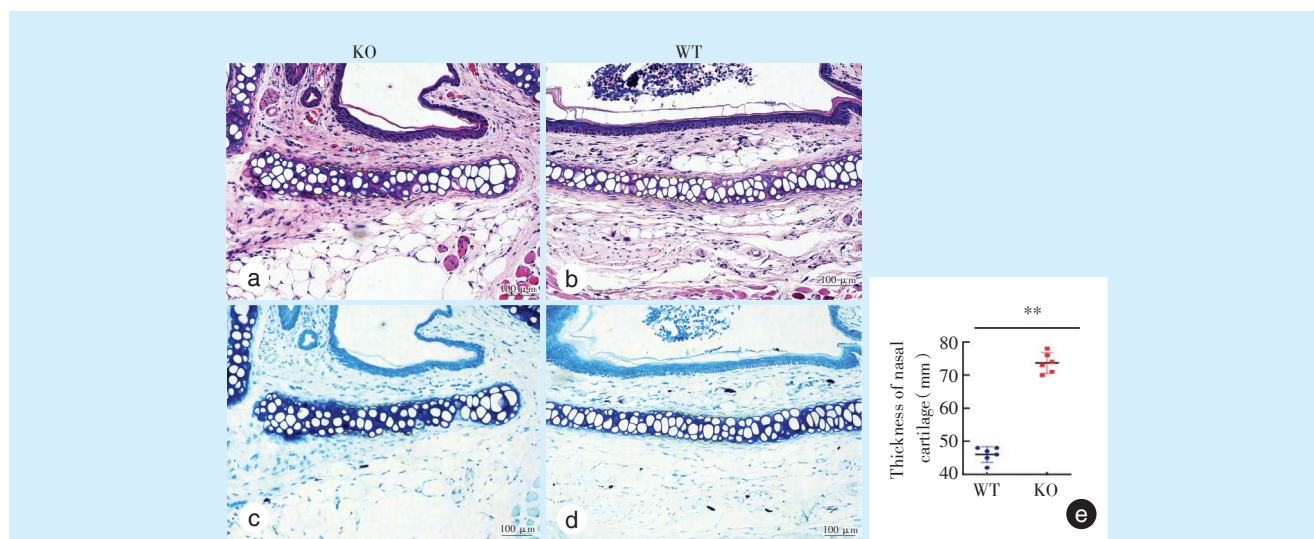


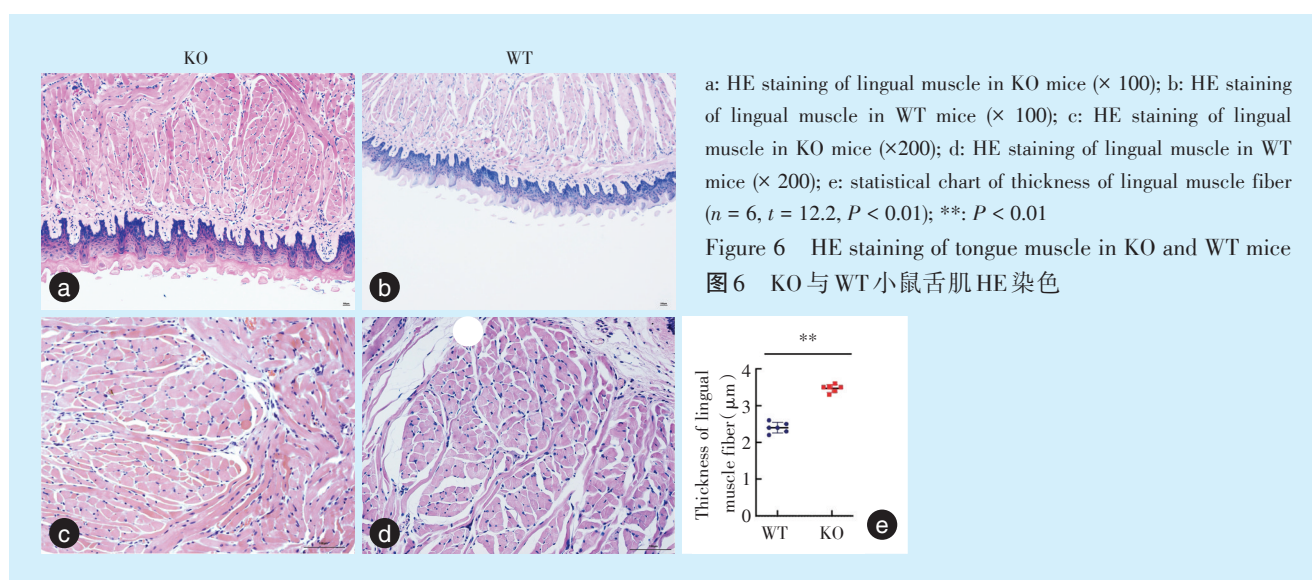
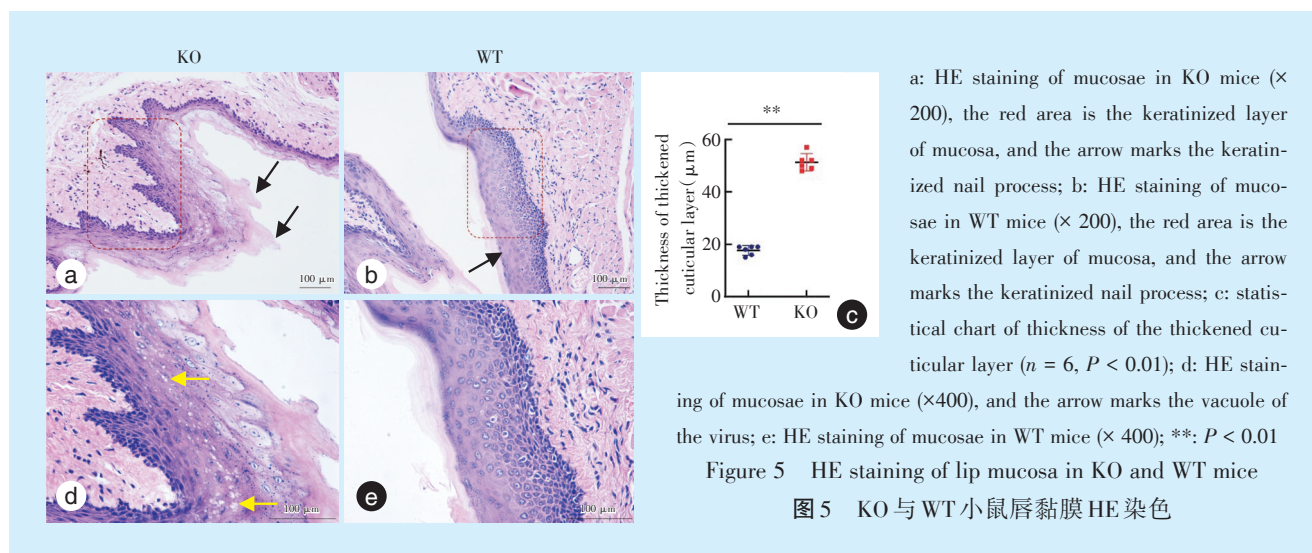
表1 KO与WT小鼠切牙和磨牙形态对比

Table 1 Comparison of incisor and molar morphology between KO and WT mice

Groups	Incisors			Molars		
	Crown length (mm)	Root length (mm)	Crown root ratio (%)	Crown length (mm)	Root length (mm)	Crown root ratio (%)
KO	3.26 ± 0.14	7.15 ± 0.28	45.67 ± 3.6	0.78 ± 0.05	1.27 ± 0.19	61.83 ± 8.75
WT	3.20 ± 0.08	7.19 ± 0.28	44.59 ± 2.6	0.75 ± 0.11	1.27 ± 0.12	59.80 ± 13.81
<i>t</i>	-1.684	0.439	-1.325	-1.859	0	-0.693
<i>P</i>	0.104	0.663	0.196	0.073	1	0.495







不及时拔除会导致牙周组织损伤,更严重者会引起牙槽骨吸收。引起垂直根折的发病因素众多<sup>[15]</sup>,这一发现有助于进一步了解垂直根折的发病因素,进而可以更好地预防该疾病。

鼻软骨在面中部的生长过程中起着重要的作用,是防止鼻骨塌陷的垂直支柱,鼻软骨缺损的修复依然是鼻重建手术中的主要挑战,组织工程技术的发展为软骨再生提供了一个新的视角。本研究中,KO小鼠鼻软骨厚度显著增加;由于鼻软骨为弹性软骨,推测CKIP-1可能影响软骨细胞的增殖和分化,这为鼻软骨组织工程提供了新的理论依据。CKIP-1影响软骨机制目前还未见报道,但文献中指出,骨关节中TGF- $\beta$ 可通过调控Smad 1/5与Smad 2/3之间的平衡调控软骨骨化程度,在骨组织中CKIP-1可通过负调控Smad 1/5促进骨形成,

推测在CKIP-1影响软骨基质分泌能力可能与TGF- $\beta$ 通路存在一定的关系<sup>[16]</sup>。

本研究对唇黏膜的HE染色及定量分析结果显示,KO小鼠唇黏膜角化增厚且不规则。结合课题组前期对皮肤的研究,这一现象与全身皮肤趋势相同;Nrf2通路与皮肤角化及恶变的关系密切,但具体机制尚不清楚<sup>[17]</sup>。研究显示,发现CKIP-1通过下调Keap1,抑制高葡萄糖(high glucose, HG)诱导的肾小球内皮细胞(glomerular endothelial cells, GMCs)和糖尿病小鼠肾脏中纤维连接蛋白(fibronectin, FN)和细胞间粘附分子1(intercellular cell adhesion molecule-1, ICAM-1)的上调,影响Nrf 2和Keap 1的多泛素化,促进Nrf 2-ARE通路<sup>[18-19]</sup>。由此可推测CKIP-1可能参与Nrf 2相关通路造成皮肤角化。

本研究对舌肌HE染色及定量分析的结果显示, KO小鼠舌肌中纤维组织增厚, 排列更加紧密。Safi等<sup>[20]</sup>研究发现CKIP-1在C2C12细胞成肌分化中上调, CKIP-1通过其PH域与磷脂酰肌醇3-磷酸结合, 并以PI3K依赖的方式定位于质膜, 但舌肌组织中是否也是通过这一机制促进肌细胞分化, 有待于进一步验证。

本研究通过Micro-CT和组织学分析全面探索了CKIP-1对颅颌面组织生长发育的影响, 描述了CKIP-1对颅颌面多种软硬组织的生长发育产生的影响, 为深入研究颅颌面生长发育的相关机制及相关疾病的治疗提供研究基础。

**【Author contributions】** Tang MY performed the experiments and wrote the article. Hu A, Cai BL, Gao Y analyzed the data. Liu FW, Lv QX, Jin D, Hou Y, Wang L, Zhang ZY assisted in the performing of experiments. Kong L designed the study and revised the article. All authors read and approved the final manuscript as submitted.

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