

· 基础研究 ·

TANGO2 全身敲除小鼠在生理条件下正常发育和繁殖

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[摘要] 目的: 利用运输和高尔基体组织蛋白2同系物(transport and Golgi organization protein 2 homolog, TANGO2)敲除小鼠阐明TANGO2在哺乳动物中的生理功能, 探究TANGO2是否是导致DiGeorge综合征发生的候选基因。方法: 制备TANGO2敲除小鼠模型, 通过实时荧光定量PCR检测该小鼠模型的基因敲除效率。观察并记录野生型、杂合和纯合敲除小鼠的表型和生长繁育情况。通过组织解剖和组织学分析, 观察野生型和敲除小鼠的心脏和大脑组织的形态结构。结果: TANGO2 mRNA在野生型小鼠的心脏和大脑中表达, 而在敲除小鼠相应组织中的表达水平显著降低。TANGO2敲除小鼠发育正常, 能够存活和繁殖, 没有明显的表型异常。与野生型同窝仔相比, 生存率没有显著差异。组织形态分析显示, 新生敲除小鼠和6月龄敲除小鼠的心脏和大脑与野生型之间无明显差异。结论: TANGO2敲除小鼠能够正常发育和繁殖, 并且缺乏人类中TANGO2相关疾病或DiGeorge综合征的相关表型。

[关键词] TANGO2; TANGO2相关疾病; DiGeorge综合征; 心脏发育; 神经发育

[中图分类号] R394.1

[文献标志码] A

[文章编号] 1007-4368(2023)06-780-07

doi: 10.7655/NYDXBNS20230605

Normal development and reproduction in TANGO2 knockout mice under physiological conditions

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[Abstract] **Objective:** The transport and Golgi organization protein 2 homolog (TANGO2) knockout mice were generated to elucidate its physiological function in mammals, and to explore whether TANGO2 is a candidate gene responsible for DiGeorge syndrome. **Methods:** The TANGO2 knockout mouse model was generated, and the knockout efficiency of the mouse model was detected by real-time quantitative PCR. The phenotype, growth and breeding of wild-type, TANGO2^{+/+} and TANGO2^{-/-} mice were observed and recorded. The morphological structures of heart and brain tissues in wild-type and knockout mice were observed through anatomic and histological analysis. **Results:** The TANGO2 mRNA was expressed in the heart and brain of wild-type mice, but its expression level was substantially reduced in the knockout mice. TANGO2 knockout mice could develop, survive and reproduce, and did not exhibit obvious phenotypic abnormalities. Histological analyses of the heart and brain showed no significant differences between knockout mice and wild-type mice at birth and 6-month-old. **Conclusion:** TANGO2 knockout mice grow and reproduce normally, and lack the phenotype associated with TANGO2-related disorders or DiGeorge syndrome in humans.

[Key words] TANGO2; TANGO2-related disease; DiGeorge syndrome; heart development; neurodevelopment

[J Nanjing Med Univ, 2023, 43(06): 780-785, 801]

运输和高尔基体组织蛋白2同系物(transport and Golgi organization protein 2 homolog, TANGO2)位

于人类22号染色体11.21位点上, 因此又称为C22orf25, 其在小鼠中的直系同源物被称为T10。2006年TANGO2在果蝇细胞系全基因组RNA介导的干扰筛选中被初次鉴定。2016年通过外显子组测序, 在3例婴儿期发作的疾病患者中检测到了TANGO2双等位基因突变, 3例患者都显现出脑萎缩

[基金项目] 国家重点研发计划专项(2019YFA0801601)

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Vazyme公司,美国),将1 μg 总RNA逆转录成cDNA。使用针对外显子V(TANGO2-Q-F:5'-GTCCTTGTCACTCAGCTCCT-3')和VI(TANGO2-Q-R:5'-CATGCTCCGTTTCAGTGAAGG-3')的引物,使用优化的PCR方案(95 $^{\circ}\text{C}$ 5 min预变性;然后95 $^{\circ}\text{C}$ 30 s,58 $^{\circ}\text{C}$ 退火30 s,72 $^{\circ}\text{C}$ 延伸30 s,45个循环)进行实时荧光定量PCR。使用 β -actin作为内参引物(β -actin-F:5'-CCTCTATGCCAACACAGTGC-3'; β -actin-R:5'-CCTGCTTGCTGATCCACATC-3')。PCR过程的SYBR荧光强度通过QuantStudio™5实时PCR系统(Applied Biosystems公司,美国)进行检测。使用 $\Delta\Delta\text{Ct}$ 分析方法计算mRNA相对含量。

1.2.3 苏木精-伊红(HE)染色

将新生和6月龄的WT和TANGO2^{-/-}小鼠的心脏和大脑组织固定在4%多聚甲醛的PBS溶液中,4 $^{\circ}\text{C}$ 过夜。PBS洗涤3次后,将固定好的样品进行脱水处理,并包埋在石蜡中。在石蜡切片机上将组织切成8 μm 厚的切片,并用苏木精和伊红染色。最后在体式镜(SZX10,Olympus公司,日本)上拍摄切片。

1.3 统计学方法

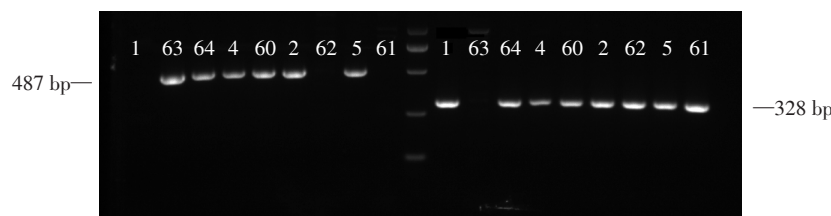
统计分析采用SPSS 23.0进行计算,统计图采用Origin 2018软件制作。数据以均值 $\pm$ 标准差($\bar{x} \pm s$)表示。使用成对 t 检验进行两组数据比较。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 mRNA水平上证实TANGO2敲除小鼠构建成功

通过PCR实验检测小鼠的基因型,WT小鼠只有1条487 bp的条带,TANGO2^{-/-}小鼠只有1条328 bp的条带,这两条条带在TANGO2^{-/-}小鼠中都能够显现(图2)。

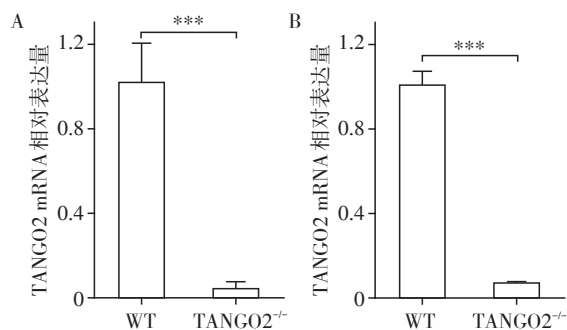
为了检验构建的TANGO2敲除小鼠模型的敲除效率,进行了WT和TANGO2^{-/-}小鼠心脏和大脑组织的实时荧光定量PCR。结果表明,TANGO2在WT小鼠的心脏和大脑中都有表达,而在同窝出生的TANGO2^{-/-}小鼠的相应组织中没有观察到TANGO2 mRNA的表达。这证明了TANGO2敲除小鼠模型构建成功(图3)。



1号、61号、62号小鼠的基因型是TANGO2^{+/+},2号、4号、5号、60号、64号小鼠的基因型是TANGO2^{+/+},63号小鼠的基因型是TANGO2^{-/-}。

图2 小鼠基因型鉴定

Figure 2 Mouse genotype identification



A:心脏中TANGO2 mRNA表达水平;B:大脑中TANGO2 mRNA表达水平。两组比较,*** $P < 0.001$ ($n=4$)。

图3 WT和TANGO2^{-/-}小鼠中TANGO2 mRNA的表达水平
Figure 3 The mRNA expression levels of TANGO2 in WT and TANGO2^{-/-} mice

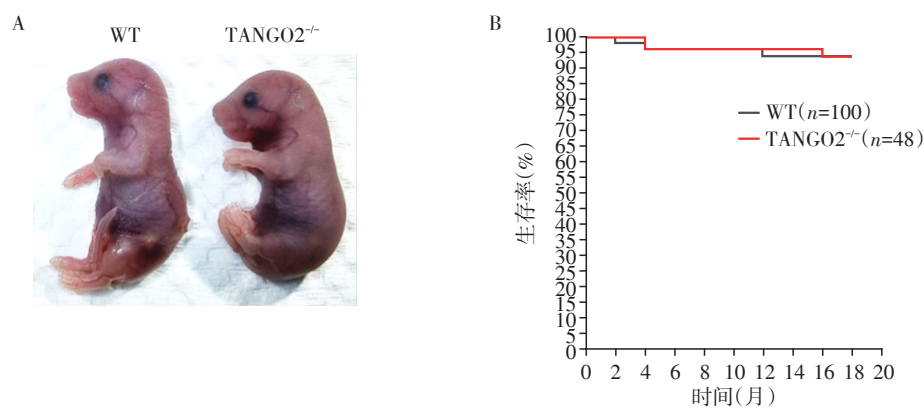
2.2 TANGO2敲除小鼠无明显表型异常

TANGO2^{-/-}小鼠能够正常生存和繁育后代,让适龄的TANGO2^{+/+}雌、雄小鼠交配,以产生TANGO2^{-/-}

小鼠进行表型观察。结果显示TANGO2^{-/-}小鼠能够以预期的孟德尔比例出生,WT、TANGO2^{+/+}和TANGO2^{-/-}出生小鼠比率分别为25%、51%和24%,且不存在明显的表型异常,说明敲除TANGO2对小鼠胚胎发育没有明显影响。出生后的TANGO2^{-/-}小鼠发育正常,未出现发育迟缓的现象,能够正常生活和繁殖,没有明显的表型异常或行为异常。TANGO2^{-/-}小鼠的生存率与WT小鼠相比没有表现出任何具有统计学意义的差异(图4)。

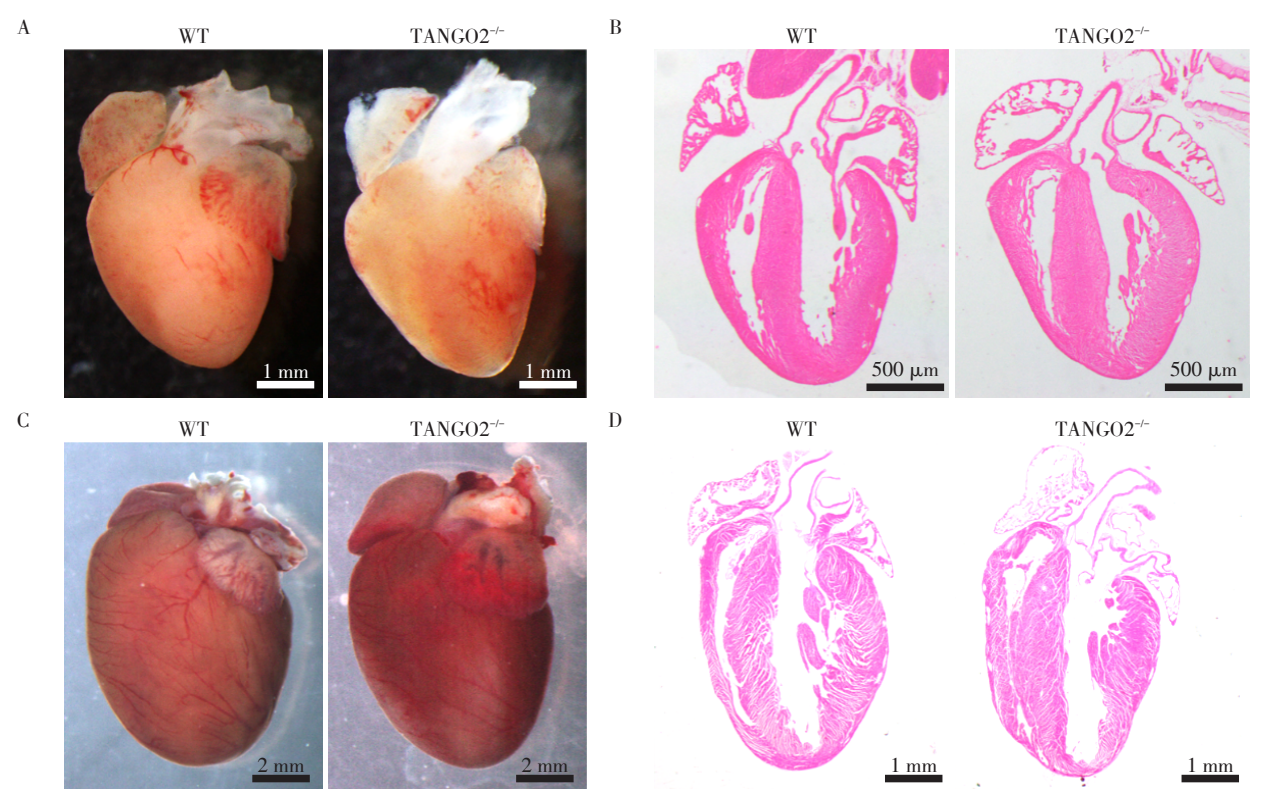
2.3 TANGO2敲除小鼠心脏和大脑表型正常

为了探究TANGO2^{-/-}小鼠的心脏是否存在结构异常,取新生和成年6月龄的WT和TANGO2^{-/-}小鼠的心脏进行表型观察。结果显示,新生和成年6月龄的TANGO2^{-/-}小鼠的心脏与WT相比不存在明显的差异,TANGO2^{-/-}小鼠的心脏结构正常,没有圆锥动脉干等异常结构(图5)。



A: WT和TANGO2^{-/-}新生小鼠的全身图;B: WT和TANGO2^{-/-}从出生到18个月的生存率。

图4 WT和TANGO2^{-/-}小鼠的总体表型
Figure 4 Total phenotype of WT and TANGO2^{-/-} mice



A: WT和TANGO2^{-/-}新生小鼠的心脏体式镜图(×12.5);B: WT和TANGO2^{-/-}新生小鼠的心脏HE图(×25);C: WT和TANGO2^{-/-}成年6月龄小鼠的心脏体式镜图(×6.3);D: WT和TANGO2^{-/-}成年6月龄小鼠的心脏HE图(×12.5)。

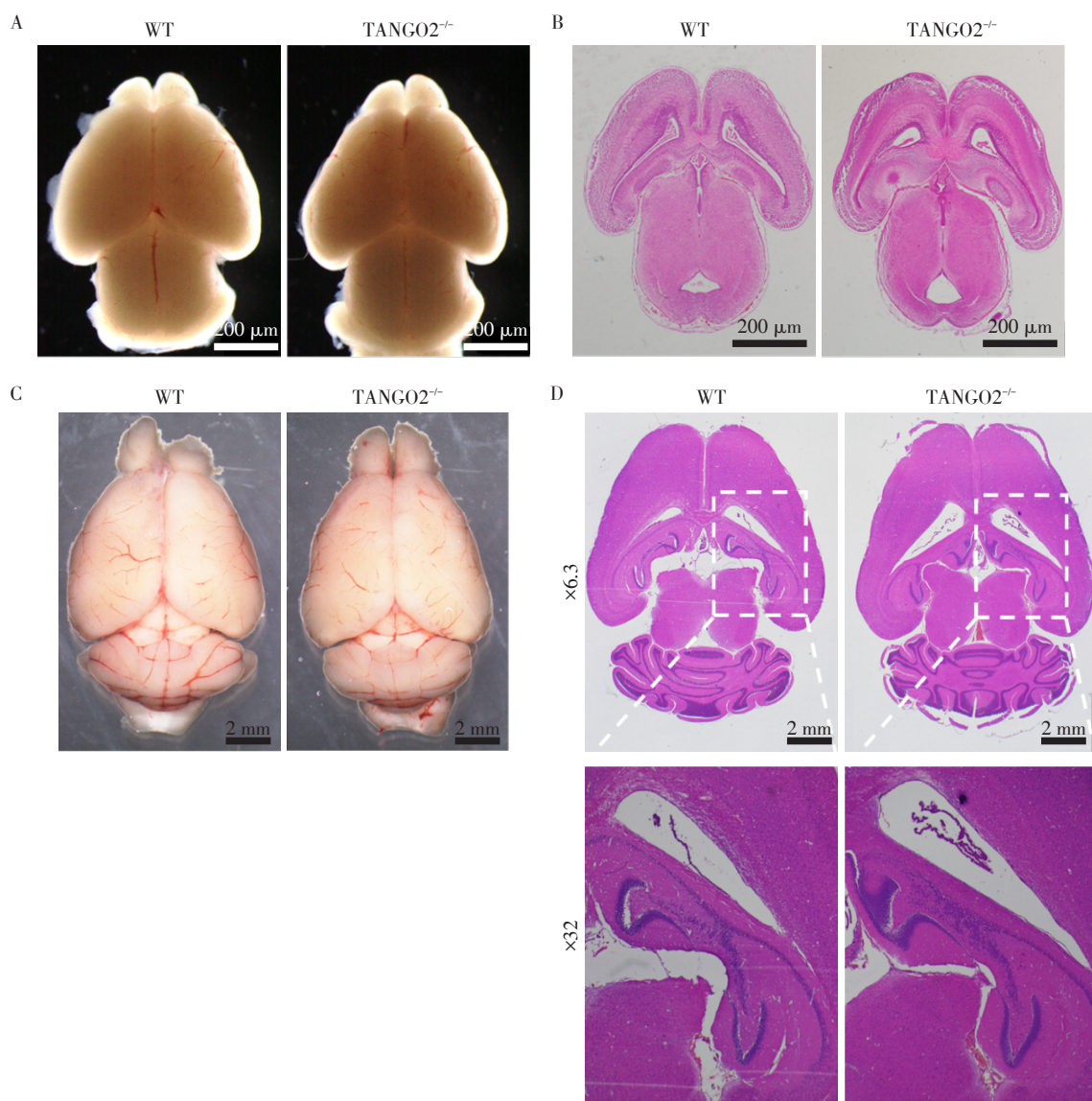
图5 WT和TANGO2^{-/-}小鼠的心脏表型
Figure 5 Cardiac phenotype of WT and TANGO2^{-/-} mice

接着观察了新生和成年6月龄WT和TANGO2^{-/-}小鼠的大脑。结果显示,与WT小鼠相比,TANGO2^{-/-}小鼠没有出现脑萎缩,大脑的嗅球、前脑、中脑、小脑等结构都没有萎缩或结构异常的表型,脑室也没有出现扩张的现象,海马结构正常(图6)。

综上所述,TANGO2敲除不会影响小鼠的心脏和大脑发育。

3 讨论

本研究未发现TANGO2在正常生理条件下的小鼠体内的作用。此外,无法证明新生和成年6月龄野生型和TANGO2敲除小鼠之间的心脏或神经发育相关的任何差异。基于TANGO2在心脏和大脑中的表达及其在人类中缺失会导致心脏缺陷和



A: WT和TANGO2^{-/-}新生小鼠的大脑体式镜图(×32);B: WT和TANGO2^{-/-}新生小鼠的大脑HE图(×32);C: WT和TANGO2^{-/-}成年6月龄小鼠的大脑体式镜图(×6.3);D: WT和TANGO2^{-/-}成年6月龄小鼠的大脑HE图。

图6 WT和TANGO2^{-/-}小鼠的大脑表型

Figure 6 Brain phenotype of WT and TANGO2^{-/-} mice

脑萎缩的表型,敲除小鼠明显缺乏与人类TANGO2相关疾病或DiGeorge综合征相关的表型,可能归因于在敲除动物模型中可能存在代偿机制抵消该基因敲除的影响。在TANGO2敲除小鼠中,可能存在线粒体功能或高尔基体转运涉及的蛋白质或机制替代了TANGO2的缺失。基因敲除后缺乏表型变化通常是由于旁系同源基因对其功能的补充。除TANGO2之外,小鼠心脏和大脑中还表达TANGO1、TANGO4、TANGO5、TANGO6、TANGO7和TANGO9等TANGO家族成员。TANGO1起到协助内质网货物运输的作用^[20];TANGO5也定位于内质网上,已知其具有调节脂质运输的作用^[21-22];TANGO4和TANGO7

发挥着调控细胞自噬的作用^[23-24];而TANGO6和TANGO9的具体作用仍不清楚。基于此,推测小鼠中TANGO2的功能可能被TANGO家族的其他成员替代。而且本研究仅在正常生理条件下评估了TANGO2在小鼠中的作用,不能排除TANGO2在小鼠体内发挥作用是条件依赖性的。人类病例报道,部分TANGO2相关疾病患者的初次发病是由麻醉或饥饿等触发因素诱导的^[20]。

前人研究中,DiGeorge综合征缺失区域中的一些其他基因在小鼠模型中的纯和敲除会导致小鼠的心脏和神经出现明显异常。例如,心脏特异性的硫氧还蛋白还原酶2(thioredoxin reductase 2, Txnrd2)缺失

小鼠患有扩张型心肌病^[25]。T-盒转录因子1(T-box transcription factor 1, Tbx1)纯和敲除小鼠具有心脏圆锥动脉干、面部结构异常等表型^[26]。RAN结合蛋白1(RAN binding protein 1, Ranbp1)纯和敲除小鼠表现为严重的小头畸形,甚至胚胎前脑缺失^[27]。关于TANGO2的研究大多数是在体外细胞实验上进行的,本研究首次在小鼠中研究了TANGO2在生物体内的功能。但由于TANGO2敲除小鼠没有明显的表型异常,本研究对TANGO2的研究集中在组织和器官水平上,没有进一步深入细胞和细胞器水平。

总之,本研究构建了TANGO2全身敲除小鼠,并且首次报告了敲除小鼠能正常存活和繁殖,与野生型同窝仔相比没有显著差异,并且缺乏人类中TANGO2相关疾病或DiGeorge综合征的表型。

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[收稿日期] 2023-02-10
(本文编辑:陈汐敏)

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[收稿日期] 2023-02-14
(本文编辑:陈汐敏)