CELL | TMCO1：内质网中的钙过载激活钙离子通道

利用基因敲除小鼠模型证实，TMCO1是一个内质网Ca2+过载激活的Ca2+通道。这一重要的研究发现发布在2016年5月19日的《Cell》杂志上，题为“TMCO1 Is an ER Ca(2+) Load-Activated Ca(2+) Channel” 。

钙离子(Ca2+)是一种多用途的细胞内信号，控制了许多不同的细胞功能，例如收缩、分泌、记忆形成、基因转录、细胞生长和细胞死亡等。细胞钙离子稳态的维持主要是通过内质网进行的。而内质网中钙离子库的内稳态对于维持细胞内钙离子信号和细胞生理功能的至关重要。钙释放激活钙离子通道（CRAC）负责钙离子库排空后的钙离子内流和补充，不过细胞要如何处理内质网中过剩的钙离子呢？

中国科学院动物研究所唐铁山课题组与美国克利夫兰州立大学周爱民课题组、中国科学院动物研究所陈佺课题组、中国科学院上海药物研究所李扬课题组等合作，综合运用多种实验手段，在阐释细胞应对内质网钙离子过载机制方面获得突破性进展。

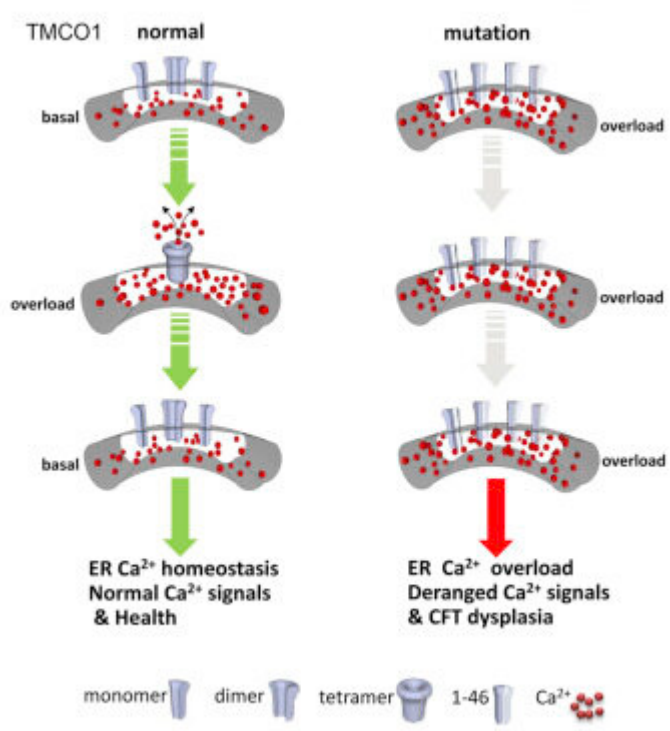

Fig1. CLAC通道维持内质网钙库钙稳态的工作模型

TMCO1在需要响应超负荷的钙离子时发生同源四聚化，而在钙离子耗尽的情况下分解，在巨大的脂质体上形成了一个钙离子选择性离子通道。因此细胞可以通过TMCO1可逆的聚合/解聚，实时监测并释放内质网中过多的钙离子以维持钙库钙稳态。他们将这种内质网钙过载激活的钙通道命名为CLAC (Ca2+ Load Activated Ca2+)通道。

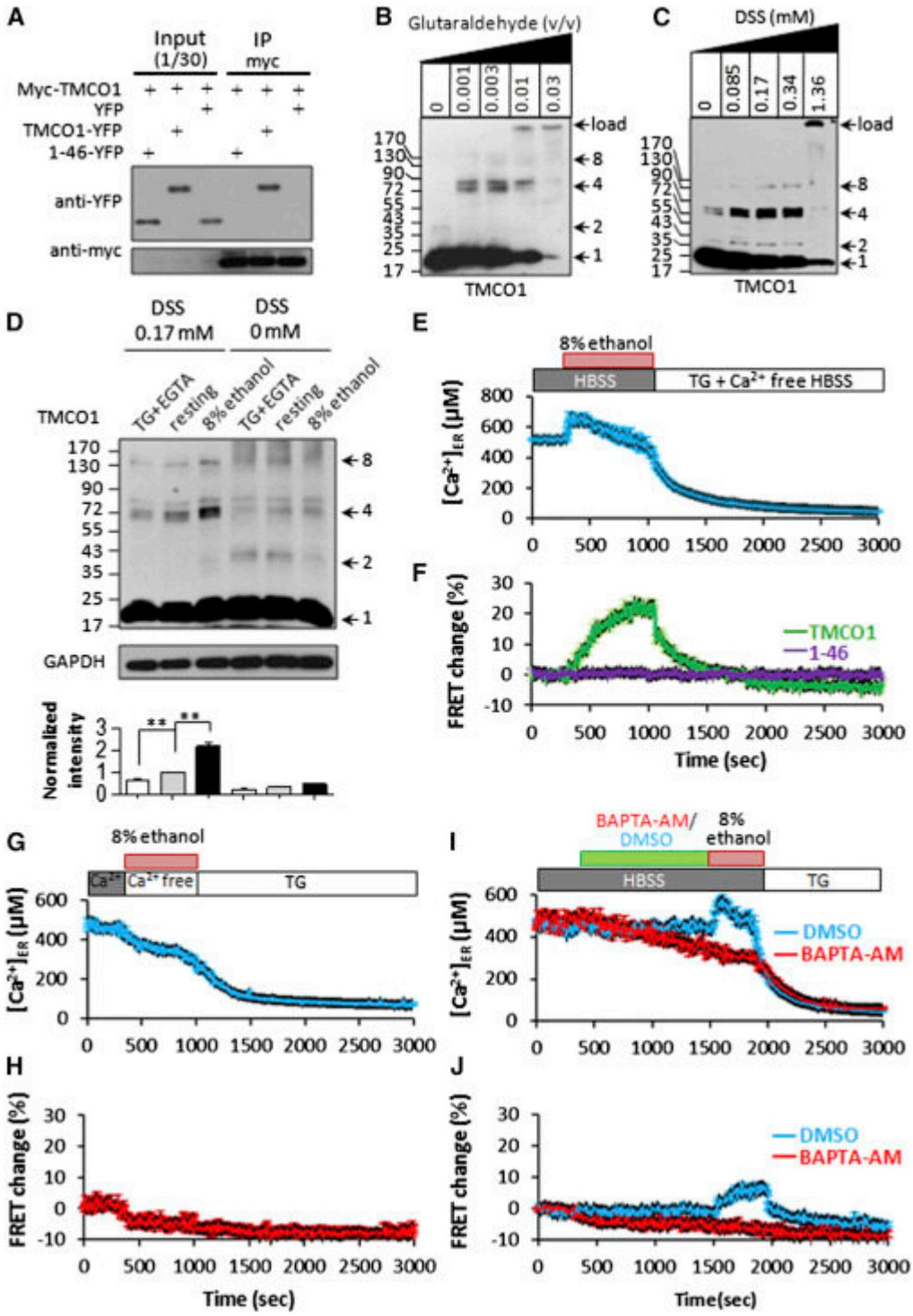

Fig2. TMCO1 Undergoes Oligomerization and Forms Tetramers in Response to ER Ca2+ Overload. (A) Co-immunoprecipitation experiment (coIP) shows TMCO1 forms a complex with itself. The input lanes contained 1/30 of lysates used in coIP reactions. Representative of at least three independent experiments. (B and C) Determination of the TMCO1 stoichiometry via chemical cross-linking. Increasing concentrations of glutaraldehyde (GA) (B) and disuccinimidyl suberate (DSS) (C) were used as indicated. Numbers represents the state of oligomerization: 1, monomer; 2, dimer; 4, tetramer; 8, octamer. Representative of at least three independent experiments. (D) Similar experiments as (C) were performed on HeLa cell lysates prepared from resting cells, cells pre-treated with TG in Ca2+ -free medium (TG+EGTA), and cells pre-treated with 8% ethanol in Ca2+-containing medium (8% ethanol). Representative of at least three independent

experiments. (E) D1ER indicated ER Ca2+ concentration manipulated by 8% ethanol in Ca2+ containing medium and by TG in Ca2+ free medium (mean±SEM, n = 9). Each trace line is shown in mean±SEM. (F) Time course of mean FRET ratio changes measured from individual cells expressing CFPand YFP-tagged TMCO1 (green trace line, n = 16) or 1-46 (purple trace line, n = 6) after application of ethanol and TG. The FRET/CFP ratio before treatment was considered as basal, and FRET changes were represented in percentiles: 1003 (R-Rbasal)/R basal. FRET increase means the assembly of TMCO1 and vice versa. Each trace lineis shown in mean ±SEM. (G and H) ER Ca2+changes manipulated by 8% ethanol and TG in Ca2+ free medium (n = 21). FRET signal change of TMCO1 were shown in (H) (n =25). Each trace line is shown in mean±SEM. (I) ER Ca2+ changes manipulated by 8% ethanol and TG in 40mM of BAPTA-AM loaded cells (red trace line, n = 12). DMSO were used as control (blue trace line, n = 10). Each trace line is shown in mean±SEM. (J) FRET changes of TMCO1 were monitored according to the same procedure as above. Curve for control (DMSO, blue trace line, n = 9) and pre-treatment with BAPTA-AM (red trace line, n = 12) were shown. Each trace line is shown in mean±SEM.

TMCO1基因位于人类1号染色体，编码239个氨基酸的蛋白。研究表明TMCO1缺陷综合征（TMCO1基因功能缺失性突变）会导致颅面畸形、骨骼异常、精神发育迟缓、共济失调和许多其他的临床症状，与CFT 发育不良（cerebro-facio-thoracic dysplasia）相关。利用TALEN技术，构建Tmco1基因敲除小鼠（该模型由上海南方模式生物构建），该基因敲除小鼠模拟了人类CFT发育不良的主要临床表型，如：骨骼发育迟缓、智力迟钝、共济失调等，同时内质网中钙离子严重的错误运转。

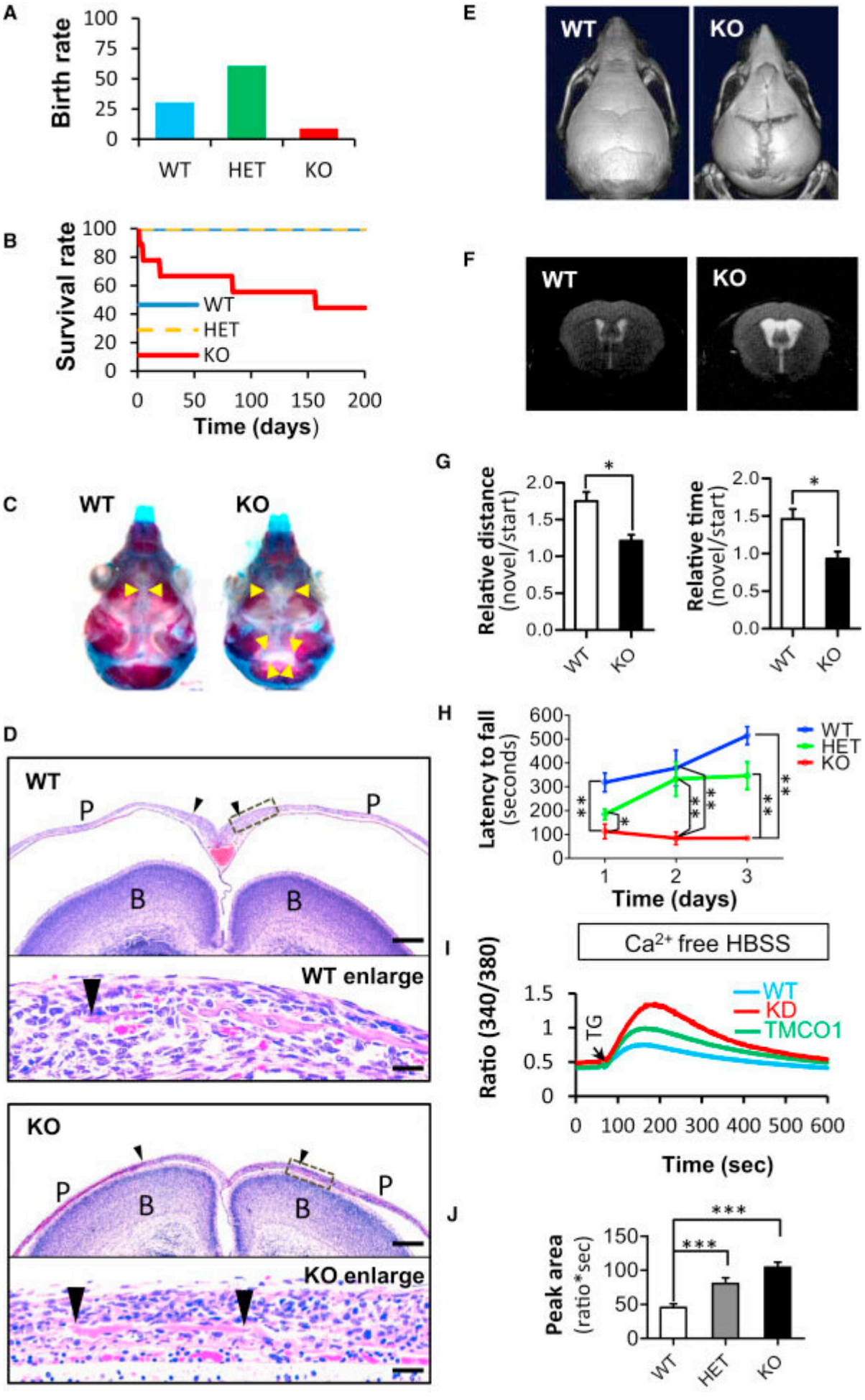
A
Birth rate
100
75
50
25
0
WT
HET
KO
B
Survival rate
100
80
60
40
20
0
WT
HET
KO
0
50
100
150
200
Time (days)
C
WT
KO
D
WT
P
P
B
B
WT enlarge
KO
P
P
B
B
KO enlarge
E
WT
KO
F
WT
KO
G
Relative distance (novel/start)
2.0
1.5
1.0
0.5
0.0
*
WT
KO
Relative time (novel/start)
2.0
1.5
1.0
0.5
0.0
*
WT
KO
H
Latency to fall (seconds)
600
500
400
300
200
100
0
1
2
3
Time (days)
WT
HET
KO
I
Ca2+ free HBSS
Ratio (340/380)
1.5
1
0.5
0
TG
WT
KD
TMCO1
0
100
200
300
400
500
600
Time (sec)
J
Peak area (ratio*sec)
150
100
50
0
***
***
WT
HET
KO

Fig3. TMCO1Knockout Mice Show a Delayed Osteogenesis, Mental Retardation, Ataxia, and Severe ER Ca2+ Mishandling. (A) Percentage of each genotype at birth. WT, wild-type; HET, heterozygote; KO, TMCO1-knockout. Data are calculated from an overall 112 pups from heterozygous matings. (B) Survival rate of each genotype. Data are calculated from an overall 112 pups from heterozygous matings. (C) Alizarin Red S and Alcian Blue staining of the skulls of P4 mice. KO mouse skulls exhibit a significantly wider open frontier suture (between the two arrowheads) than its WT littermates. (D) Histological analysis of P1 WT and KO mouse skulls. HE stainings show a significant wider gap between the left and right osteogenic fronts at the sagittal suture of KO pups (lower panels, pointed out with arrows) when compared with their WT littermates (upper panels, pointed out with arrows), and discontinuous osteoid deposition is seen at KO osteogenic fronts (lower enlarged panel, pointed out with arrows), revealing a delayed osteogenesis in P1 KO mice. Enlarged section was indicated with gray box in first and third panels. B, brain; P, parietal bone. Scale bars, 250mm (first and third panels); 25mm (second and fourth panels). (E) Micro-CT of the skulls of 5-month-old WT and KO mice. KO skull shows obvious gaps along the midline interfrontal suture, transverse coronal and lambdoid sutures. Representative data from at least three KO mice are shown. (F) Magnetic resonance imaging (MRI) of brains of 5-month-old mice show enlarged ventricles in KO mouse. (G) Y-maze task assay of adult WT and KO mice. Relative distance walked (left panel) and relative time spent (right panel) of KO mice (black column, n = 6) in novel arm over start arm decrease significantly (*p < 0.05) than WT mice (white column, n = 10). (H) Rotarod performance of adult WT and KO mice. An average latency to fall from the accelerating rotarod is shown for WT mice (blue, n = 10), HET mice (green, n = 8) and the KO mice (red, n = 6) in 3 consecutive days. For each group of mice, the results are shown as mean±SEM. *p < 0.05; **p < 0.01. (I and J) Overload of ER Ca2+ in primary osteoblasts cultured fromTMCO1-KO mice. Curves represent TG-induced Ca2+ transients in WT (blue trace line, n = 34), KD (red trace line, n = 28), and HET (green line, n = 23) primary osteoblasts. (J) Histogram shows the average peak area of TG-triggered Ca2+ transients (mean±SEM) in each group. Each curve in (I) is an average of Ca2+ responses from all cells in each group. ***p < 0.001.

这些研究结果都表明，TMCO1作为一种内质网膜蛋白，防止了内质网钙离子库的钙离子过度负载，保护了内质网钙离子内稳态的稳定。

CLAC通道的发现不仅在钙信号调控领域取得了原创性的突破，也为脑-颅面-胸发育异常综合症的治疗提供了重要线索。鉴于钙库钙稳态失衡在阿尔兹海默病和帕金森病病理发生中的重要作用，CLAC通道的发现也为这两种人类疾病的治疗提供了潜在的靶点。

全文下载