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Knock out of tmem38b by CRISPR/Cas9 in zebrafish unveils the in vivo role of Trimeric intracellular cation (TRIC) channel B on cell homeostasis

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Background/Introduction: *TMEM38B* encodes the endoplasmic reticulum (ER) potassium channel TRIC-B, which modulates calcium flux from the ER to the cytosol. Loss-of-function mutations in *TMEM38B* are responsible for the recessive bone disease osteogenesis imperfecta (OI) type XIV, characterized by altered collagen type I structure.

Purpose: To elucidate the link between impaired TRIC-B activity and OI, CRISPR/Cas9 was exploited to generate *tmem38b* knock out zebrafish.

Methods: *tmem38b* specific gRNA and Cas9 mRNA were injected in 1-2 cells zebrafish embryos. Mosaic fish were crossed with WT to obtain heterozygous from which homozygous were generated. Growth curve was evaluated from 5 days post fertilization (dpf) to 6 months post fertilization (mpf). Histomorphometry was performed on alizarin red stained fish. MicroCT were performed on adults to analyse bone properties. Transmission electron microscopy of skin and bone cells was carried out. The expression of the collagen specific heat shock protein Hsp47a/b was evaluated by whole mount immunohistochemistry. The experiments were approved by the Italian Ministry of Health.

Results: Two *tmem38b* zebrafish mutants were generated: one carrying a frameshift mutation resulting in a premature stop codon (*tmem38b*$^{-/-}$) and one with an in-frame deletion which eliminates Tric-b pore channel domain (*tmem38b*$^{\Delta 120\text{-}7/\Delta 120\text{-}7}$). A significant growth delay was detectable only in *tmem38b*$^{-/-}$ at 21 dpf and 1 mpf associated to reduced vertebral height and length ($p<0.05$). MicroCT analysis did not show any difference in bone volume, vertebral body thickness and vertebral body length in adult mutant fish. An increased ER cisternae size was observed in both mutants. Hsp47a/b expression was increased in mutants compared to WT (Hsp47a: WT 0%, *tmem38b*$^{-/-}$ 65%, *tmem38b*$^{\Delta 120\text{-}7/\Delta 120\text{-}7}$ 6% $p<0.05$; Hsp47b: WT 4%, *tmem38b*$^{-/-}$ 71% $p<0.05$; *tmem38b*$^{\Delta 120\text{-}7/\Delta 120\text{-}7}$ 55% $p<0.05$).

Conclusion(s): The *tmem38b* zebrafish mutants show enlargement of ER cisternae and increased expression of Hsp47 supporting a role of TRIC-B in cell homeostasis.

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Osteogenesis imperfecta and sclerostin antibody – Histological evaluation of the bone-tendon unit in the oim mouse

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Background/Introduction: Osteogenesis imperfecta (OI), a genetic disorder of type I collagen, is characterized by bone fragility and numerous spontaneous fractures. Tendon ruptures are reported in patients but the impact of OI on bone-tendon unit (BTU) is not known.

Purpose: This preliminary study aims to describe BTU in the oim mouse, an experimental model of type III (severe) OI, and to evaluate the effect of a sclerostin neutralizing antibody (Scl-Ab). Sclerostin is a potent inhibitor of osteogenesis via inhibition of the osteoblastic Wnt pathway and we previously showed positive effects on oim bones.

Methods: Histological longitudinal sections were made through the insertions of triceps brachialis, patellar ligament and triceps suralis of oim and wild type (WT) mice treated with either Vehicle or Scl-Ab for 9 weeks [approved by ethics committee for animal care of the university]. For each BTU, five mice per group were studied. The relative bone volume (BV/TV), the length of fibrocartilage (FC) - calcified fibrocartilage (CFC) interface and the proportion of thick collagen fibers were measured with Image J software.

Results: In oim mice, epiphyseal bone showed low BV/TV ratio (0.42+/-0.1) as well as deformations. The FC/CFC interface was significantly shorter than in WT group and triceps suralis tendons contained fibrocartilage islets. Treatment with Scl-Ab enhanced BV/TV (0.63+/-0.02) and the FC/CFC interface was lenghtened by 16% ($p<0.05$) in oim mice. The proportion of thick collagen fibers in tendons and CFC of treated oim were significantly lower than those of untreated mice (-46% in tendons, $p<0.05$).

Conclusion(s): This histological study highlighted original features of oim BTU which could explain tendon ruptures in patients. Scl-Ab appeared to impact positively bone epiphysis as well as FC/CFC interface and tendon composition. Higher resolution imaging and mechanical tests would confirm these preliminary data.

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