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PROTEOSTASIS IN THE EARLY SECRETORY COMPARTMENT AS A PATHOGENETIC MECHANISM AND THERAPEUTIC TARGET: ALTERED COLLAGEN BIOSYNTHESIS AND BONE DEVELOPMENT IN THE ABSENCE OF ERP44, A ZINC-REGULATED CHAPERONE

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The pathogenesis of most genetic diseases generally entails defects in protein synthesis, transport and homeostasis. In many cases, mutations in the protein to be transported (cargo) impede its arrival to the final destination. In others, the genetic defect lies in one of the factors that orchestrate the intracellular transport of macromolecules. A prototypic example is Multiple coagulation deficiency, a condition caused by mutations in LMAN1, a gene encoding a lectin (ERGIC53) that promotes the secretion of clotting factors 5 and 8 and IgM. We investigate the pathophysiology of protein secretion and the mechanisms that maintain redox homeostasis in and between cells. In this project, we focused on ERp44, a folding assistant that interacts with ERGIC53 and colocalizes with it in the early secretory compartment, coupling protein quality control with redox and calcium signaling in a pH-regulated manner (Anelli and Sitia, 2008; Vavassori et al., 2013; Watanabe et al., 2017).
To further investigate its pathophysiologic roles, we generated additional cellular and animal models. Reconstituting KO or KD cells with a panel of mutants, we discovered that ERp44 binds and is regulated by zinc (Watanabe et al., 2019). ERp44 determines the localization and function of ERAP1 and other zinc-containing secretory enzymes. Zinc depletion (obtained by specific chelators or by silencing Golgi-resident ZnT transporters) inhibits ERp44 movements and functions. Cycling rapidly between the ER and Golgi with ERGIC53 and KDEL receptors (Tempio et al., submitted), ERp44 controls the homeostasis and distribution of zinc in the secretory compartment.
At the organismic level, the majority of ERp44 KO mice are embryonically lethal. The few animals that survive are much smaller than their control littermates. Images obtained by TAC, MRI and immunohistochemistry reveal skeleton malformations at the level of the spinal column and skull, retarded bone growth, striking differences in the development and growth of long bones (femur and tibia), and reduced dimension of collagen fibers in tendons. The serum levels of zinc and alkaline phosphatase activity are altered in KO mice, suggesting that ERp44 controls collagen biogenesis and bone development, possibly modulating zinc homeostasis.

Ancor più che le grandi città, le nostre cellule sono incredibilmente affollate. Esse sono divise in compartimenti specializzati in particolari funzioni, che chiamiamo organuli. Ad esempio, i mitocondri producono energia, mentre i lisosomi sono sorte di isole ecologiche, che degradano in materiale danneggiato o in eccesso e lo riciclano immediatamente. Per il corretto funzionamento della cellula i miliardi di molecole che le compongono devono raggiungere il loro luogo di lavoro. Ogni tanto, le cose non vanno bene e si formano ingorghi: l'assenza o il ritardato arrivo di una proteina causano gravissime malattie.
Il nostro laboratorio studia i meccanismi che governano il traffico delle proteine nelle cellule, con particolare attenzione alle proteine destinate all'esportazione. Queste sono i principali mezzi di comunicazione tra cellule. In precedenza, abbiamo contribuito a comprendere come l'assenza di un controllore del traffico chiamato LMAN1 causi un rallentato trasporto di certi anticorpi e di due fattori della coagulazione in una forma di emofilia. Recentemente, abbiamo studiato ERp44, un controllore specializzato nella produzione di proteine molto complesse. Una di queste è il collagene, elemento

essenziale in tendini e ossa. Quando ERp44 manca scheletro e tendini si sviluppano male. I topolini che ne sono privi ci stanno rivelando l'esistenza di alcuni meccanismi compensatori. Stiamo ora stiamo studiando questi meccanismi per poterli modulare nella speranza di identificare bersagli farmacologici.

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Sindrome di Ehlers-Danlos

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