ROLE OF PiT2 IN BONE AND ADIPOSE TISSUES INTER-COMMUNICATION ?

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The Bone Marrow Adipose Tissue (BMAT) has lately attracted a lot of attention within the scientific community, in particular regarding its intimate relationship with skeletal health. We know that BMAT volume is inversely correlated with bone volume during aging and in some clinical conditions such as postmenopausal osteoporosis, anorexia nervosa or diabetes. Recently, we have shown how the invalidation of the sodium-phosphate co-transporter PiT2 leads to an impaired endochondral and intramembranous ossification. In addition, we identified PiT2 as a novel and major determinant for bone quality and strength. Because of the profound relationship existing between bone and BMAT, we hypothesize that BMAT is altered in PiT2KO mice and impacts on skeletal health.

To characterize the BMAT phenotype, we first examined BMAT volume in the tibia from *PiT2WT* and *PiT2KO* mice by immunohistochemistry and by osmium tetroxide micro-computerized tomography (OsO_4 µ-CT) analyses. Perilipin immunostaining was performed on mouse tibial sections at post-natal day 16 (n=8 *PiT2WT*, n=7 *PiT2KO*). In the *PiT2KO* mice, we observed a 3-fold increase in the number of perilipin+ adipocytes in the proximal and the distal MAT ($p<0.0036$ and $p<0.0076$, respectively). Our preliminary OsO_4 µ-CT analyses also show an increase in BMAT volume in the 18-week-old *PiT2KO* mice compared to *PiT2WT* mice (Adipose volume/total volume: 3.94% (WT, n=1) and 7.79% (*PiT2KO*, n=2). Three-dimensional visualization of BMAT is currently being analyzed by using contrast-enhanced high-resolution microCT (CE-CT) on tibia from 3- and 16-week-old females (n=6 and 7 PiT2KO and n=6 and 4 WT, respectively).

Despite the BMAT increase, plasma levels of Adiponectin were unchanged, suggesting that the metabolic activity of the PiT2-deficient adipocytes from the bone marrow was different from the one observed in some clinical conditions where BMAT is also increased (such as anorexia nervosa).

The inverse correlation between BMAT and bone volumes could originate from the ability of the Bone marrow Mesenchymal Stromal Cells (BMSCs) to differentiate either in adipocytes or osteoblasts. Thus, we are currently investigating the reciprocal relationship between osteoblasts and adipocytes by analysing the effect of PiT2 deficiency on BMSCs cultured in a co-differentiation medium.

To elucidate the molecular mechanisms involved in the action of PiT2 in adipogenesis, we performed a yeast two-hybrid screen to identify putative PiT2 proteic partners. Interestingly, some of them are known to be involved in skeletal and/or adipose tissues physiology, further suggesting the possibility that PiT2 may have a role at the interface between bone and BMAT. We have validated the interaction of some of these putative partners through co-immunoprecipitation. Of note, preliminary western blot data suggest than some of them have a reduced expression in the PiT2-deficient tibia compared to controls (n=6 WT and n=4 PiT2KO). These first results unravel new molecular action of PiT2 that could be independent from its Pi-transport ability. Altogether, our results may identify PiT2 as a new player in the communication between bone and adipose tissues and reveal the *PiT2KO* mice as a new model to better understand the regulation of BMAT and its role in bone physiology.

Keywords: PiT2, bone, bone marrow adipose tissue.