IJAE
Vol. 123, n. 1 (Supplement): 129, 2018
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Muscle-to-bone crosstalk: the Wnt/β-catenin pathway is a candidate mechanism mediating the signalling between C2C12 muscle cells and 2T3 osteoblasts

Maria Sara Magarò[1], Jessika Bertacchini[1], Francesco Potì[2], Marta Checchi[3], Marta Benincasa[1], Paola Sena[1] and Carla Palumbo[1]

[1] University of Modena and Reggio Emilia, Department of Biomedical, Metabolic Science and Neuroscience – Section of Human Morphology, Modena, Italia
[2] Department of Medicine and Surgery, Unit of Neurosciences, University of Parma, Parma, Italia
[3] University of Modena and Reggio Emilia, Department of Biomedical, Metabolic Science and Neuroscience – Section of Human Morphology, 41124, Modena, Italia

Bone and muscle have been recognized as endocrine organs since they produce and secrete "hormone like factors", osteokines and myokines, that can mutually influence each other and other tissues, giving rise to "bone-muscle crosstalk" [1]. Recently, evidences emerged that myokines have profound effects on osteocytes in culture and vice versa [2][3]. Besides, skeletal muscle secreted factors have been found to promote osteocytes and osteoblasts viability through activation of the Wnt/β-catenin pathway [2]. To determine whether myokines can potentially regulate osteogenesis, we conditioned the differentiation of 2T3 osteoblasts with 25% medium from early (day 1), mid (day 7) or late (day 14) myotubes. Osteoblast conditioned differentiation was investigated from different viewpoints: i) analysis of mRNA and protein levels of marker genes of differentiation; ii) characterization of the functional maturation by studying the deposition of mineralized matrix and the activity of alkaline phosphatase enzyme. To date, we have demonstrated that the early conditioned medium (day 1, CM 1) decreased significantly the mineralization degree of osteoblasts compared to control. Besides, CM 1 induced statistically significant increase in the expression of Sclerostin, both SOST mRNA transcription and protein synthesis during osteoblast differentiation. Sclerostin is a secreted glycoprotein mainly expressed in bone and cartilage matrix, considered a negative regulator of bone growth due to its role as an antagonist of the Wnt/β-catenin pathway. It has also been found to inhibit the pre-osteocyte differentiation of osteoblasts in vitro [4]. These data prompted us to analyze the protein content of CM1 and surprisingly we found that myotubes at day 1 and 4 of differentiation secrete Sclerostin. Therefore, we hypothesize that muscle cell-secreted Sclerostin may act as an endocrine negative regulator of 2T3 differentiation through inhibition of Wnt/β-catenin signaling and such effect may be enhanced by the induced osteoblast isoform acting in an autocrine loop.

Further experiments are needed to test the involvement of Sclerostin as mediator of the effects of the early CM. Thus, we are planning the following experiments: i) 2T3 differentiation with recombinant Sclerostin combined with its antibody-mediated neutralization, ii) overexpression of Sclerostin in 2T3 cells, iii) treatment of C2C12 cells with SOST siRNA and 2T3 conditioned differentiation with C2C12 siRNA-treated CM.

References

[1] Hamrick (2011) A role for myokines in muscle-bone interactions, Exerc Sport Sci Rev 39(1) 43-7.
[2] Jähn et al. (2012) Skeletal muscle secreted factors prevent glucocorticoid-induced osteocyte apoptosis through activation of β-catenin, Eur Cell Mater 24 197-209
[3] Huang et al. (2017) Crosstalk between MLO-Y4 osteocytes and C2C12 muscle cells is mediated by the Wnt/β-catenin pathway, JBMR Plus 1(2) 86-100.
[4] Atkins et al. (2011) Sclerostin is a locally acting regulator of late-osteoblast/preosteocyte differentiation and regulates mineralization through a MEPE-ASARM-dependent mechanism, J Bone Miner Res 26(7) 1425-36.

Key words

Bone-muscle interactions, Sclerostin, Wnt/β-catenin, osteoblast differentiation.

http://www.fupress.com/ijae