Influence of $P2X_7$ receptors on the osteogenic differentiation of mesenchymal stromal/stem cells derived from human subcutaneous adipose tissue

P. Giuliani, Dept. of Medical, Oral and Biotechnological Sciences University of Chieti-Pescara, Chieti Italy
M. Zuccarini, Dept. of Medical, Oral and Biotechnological Sciences University of Chieti-Pescara, Chieti Italy
R. Ciccarelli, Dept. of Medical, Oral and Biotechnological Sciences University of Chieti-Pescara, Chieti Italy
M. Carluccio, Dept. of Medical, Oral and Biotechnological Sciences University of Chieti-Pescara, Chieti Italy
C. Morabito, Neuroscience, Imaging and Clinical Sciences; Aging Research Center and Translational Medicine University of Chieti-Pescara, Chieti, Italy. StemTeCh Group, Chieti, Italy.
M.A. Mariggiò, 2Neuroscience, Imaging and Clinical Sciences; Aging Research Center and Translational Medicine. University of Chieti-Pescara, Chieti, Italy. StemTeCh Group, Chieti, Italy.
E. Orioli, Dept. of Morphology, Surgery end Experimental Medicine. University of Ferrara, Ferrara Italy.
E. Adinolfi, Dept. of Morphology, Surgery end Experimental Medicine. University of Ferrara, Ferrara Italy.
P. Di Iorio, Dept. of Medical, Oral and Biotechnological Sciences University of Chieti-Pescara, Chieti Italy
F. Caciagli, Dept. of Medical, Oral and Biotechnological Sciences University of Chieti-Pescara, Chieti Italy

In different cellular/animal models, extracellular ATP seems to be able to influence bone formation/repair by interacting with a number of P2 receptors. Interestingly, several studies have recently pointed out the involvement of the ionotropic P2X7 receptors (P2X7R) in the osteogenic differentiation of mesenchymal stromal/stem cells (MSCs) derived from various sources, even though the results so far obtained are conflicting. Here, we investigated the expression of P2X7R and the effects exerted by its stimulation/inhibition in MSCs from human subcutaneous adipose tissue (S-ASCs), that are of particular interest for their potential therapeutic application in bone regenerative medicine. Undifferentiated S-ASCs displayed a faster doubling time, a greater proliferation rate and colony forming ability in comparison to MSCs from other sources as well as S-ASCs committed towards osteogenesis exhibited a greater differentiation that allowed a better colonization of a titanium scaffold compared to that obtained with MSCs derived from other tissues. Among the different P2X7R splice variants, we found that S-ASCs expressed the truncated P2X7RB, one of the two main splice variants, to a greater extent than the full length P2X7RA. Cell stimulation with 50-100 μM BzATP, a P2X7R agonist, caused an increase in both intracellular calcium concentration and cell migration (evaluated by scratch assay) without modifying cell proliferation (determined using trypan blue exclusion method). Furthermore, in S-ASCs submitted to osteogenic differentiation, BzATP (25-125 μM), added at each change of the culture medium, caused an early enhancement (7 days) and a later decrease (from 14 days onwards) of the calcium accumulation in the extracellular matrix (detected by Alizarin Red Staining) and of the expression of transcription factors (such as Runx2, alkaline phosphatase and osteopontin) associated with osteogenic differentiation. BzATP-induced effects in undifferentiated and differentiating S-ASCs were counteracted by A438079 (10 μM), a P2X7R antagonist, that, interestingly, increased cell differentiation when administered alone. Thus, considering the good accessibility of the adipose tissue, our findings indicate that S-ASCs are a useful model to study bone regenerative processes and that P2X7R are surely involved in the osteogenic differentiation of human S-ASCs, being its activity dependent on the stage this process.