Estrogen Receptors Signaling in the Ferutinin-mediated Osteogenic Differentiation of Amniotic Fluid Stem Cells

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Ferutinin is a daucane sesquiterpene with a high estrogenic activity in vitro and in vivo (Appendino et al., 2002; Zavatti et al., 2009). Recently, we demonstrated that ferutinin is able to enhance proliferation and osteogenic differentiation of human amniotic fluid stem cells (AFSCs) (Zavatti et al., 2013). Morover, the oral administration in rats of this phytoestrogen improved the bone regeneration of critical-size bone defects filled with collagen-AFSCs constructs (Zavatti et al., 2015). The aim of this study was to investigated estrogen receptors and ERK signaling pathway in ferutinin-mediated osteogenic differentiation. AFSCs, isolated from supernumerary amniocentesis and immunoselected for c-Kit, were cultured in an osteogenic conditioned medium for 14 days and ferutinin was added at the concentration of 10^{-8}M. In both undifferentiated and differentiated AFSCs we identified the presence of estrogen receptor α (ERα) and G-protein coupled receptor 30 (GPR30) with a nuclear or cytoplasmatic localization, respectively. Alizarin Red S staining confirmed that ferutinin was able to stimulate osteogenic differentiation increasing extracellular matrix mineralization. To study estrogen receptors involved in this effect we used a selective ERα antagonist MPP (methyl-piperidino-pyrazole) 10^{-6}M and a selective GPR30 antagonist G15 10^{-6}M. Immunofluorescence experiments revealed in MPP-treated cells a reduced expression of osteopontin and osteocalcin compared to control and ferutinin-treated cells, whereas G15-treated cells showed a greater expression of these osteogenic markers demonstrating that ERα is positively involved in osteogenic differentiation. Western Blot experiments showed that ferutinin treatment increased the levels of p-ERK in AFSCs and this activation was repressed by the MEK inhibitor PD0325901. Also MPP repressed the activation of pERK induced by ferutinin. Moreover, addition of PD0325901 blocked the ferutinin-induced osteogenic differentiation. These results suggest that ferutinin through ERα promotes osteogenesis involving ERK signal pathway.

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