PPARγ2-MEDIATED PROTEOLYTIC DEGRADATION OF B-CATENIN DETERMINES AN ANTI-OSTEOBLASTIC EFFECT OF ANTI-DIABETIC TZDS

Sima Rahman, P. Czernik, and B. Lecka-Czernik
University of Toledo

The transcription factor PPARγ2 is a key regulator of marrow mesenchymal stem cells (MSCs) differentiation. It positively regulates adipocyte and suppresses osteoblast differentiation. Anti-diabetic drugs thiazolidinediones (TZDs), which specifically activate PPARγ protein, upregulate fat production in the bone marrow and cause bone loss in animals and humans. In order to understand the mechanisms of TZD-induced bone loss, we performed microarray analysis of gene expression changes in a cellular model of PPARγ2-controled MSC differentiation, U-33/γ2 cells. Among the early responders to R were the proteins involved in Wnt signaling pathway, which is essential for MSC differentiation toward osteoblast and bone formation. The expression of WISP-1 and Tle3 was significantly altered within 2 h post treatment followed by the suppressive effect on the expression of multiple members of this pathway including Fzd receptors, Dkk1, Sfrp1, and Wif1 modulators, and Tcf3 and Tcf4 transcriptional effectors. Cellular silencing of WISP-1 and Tle3 using siRNA suggested that they are not the major mediators of R-induced suppression of osteoblast phenotype. Therefore, we tested whether R affects activity of b-catenin, a key mediator of Wnt signaling. We found that R-activated PPARγ2 induced proteolytic degradation of more than 90% of the unbound or active form of b-catenin as early as within 1 hr post treatment, not affecting a pool of protein bound (inactive) b-catenin. Consistent with this, R suppressed transcriptional activity of b-catenin, measured by the activity of TOP-FLASH construct in a luciferase gene reporter assay. Moreover, R suppressed alkaline phosphatase activity even in the presence of LiCl, which stabilizes an active form of b-catenin. To test whether b-catenin degradation is responsible for R anti-osteoblastic effects we modified PPARγ2 protein domains responsible for b-catenin degradation. Using transiently transfected marrow MSC with mutated PPARγ2 constructs, we are currently testing the hypothesis that a lack of PPARγ2 proteolytic activity for b-catenin protects Wnt pathway gene expression and osteoblast phenotype against the negative effects of anti-diabetic TZDs.