A novel small thienoindazole-derivative compound induces chondrogenic differentiation without promoting hypertrophy through production of Runx1


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Aiming at regeneration of permanent cartilage like joint cartilage, we have screened natural and synthetic compound libraries using stable lines of ATDC5 cells expressing green fluorescent protein (GFP) under the control of type II collagen promoter fused with four repeats of a Sox9 enhancer (COL2-GFP) as a monitoring system for chondrogenic differentiation. We found that a novel compound, a thienoindazole-derivative compound T-198946 (TM), most strongly induced the GFP fluorescence as early as after 48 h of treatment. TM was confirmed to enhance chondrogenic differentiation but inhibits the further hypertrophic differentiation in the cultures of immature mesenchymal C3H10T1/2 cells, determined by real-time RT-PCR analysis for the chondrogenic markers. To clarify its transcriptional targets and signal transduction mechanism, we screened for the target molecules of TM by the microarray analysis and revealed that Runx1 was most strongly induced by TM among 581 up-regulated genes including Sox5 and Sox6. Luciferase-reporter analyses using deletion, mutagenesis, and tandem-repeat of the COL2 promoter identified the core responsive element of Runx1 in the COL2 promoter to be between the −293 and −288 bp region containing a putative Runx-binding motif. The specific binding of Runx1 to this region was confirmed by EMSA and ChIP assays. For functional analyses, we performed adenoviral overexpression of the gene or the small interfering RNA in C3H10T1/2 cells. Although chondrogenic differentiation was enhanced by the Runx1 overexpression alone, it was further enhanced by co-transduction with Sox5, 6, and 9 (the Sox trio), without promoting the hypertrophy, similar to the effect of TM treatment. Gene-silencing of Runx1, Sox5/6, or Sox9 suppressed the TM effect on chondrogenic differentiation. Immunohistochemistry revealed that Runx1 and the Sox trio were co-localized in the proliferative and pre-hypertrophic chondrocytes of the mouse growth plate, and their physical interaction was confirmed by immunoprecipitation and two-hybrid analysis. Finally, full-thickness defects of mouse knee cartilage were completely filled with cartilaginous tissue after transplantation of cell-sheets of TM-treated chondrocytes, while the control cell-sheets did not. A novel small compound TM induces chondrogenic differentiation without promoting hypertrophy, through production of Runx1 that cooperatively functions with the Sox trio. TM will herald a new era of regenerative medicine of permanent cartilage, thus providing an epochal treatment of osteoarthritis.

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Osteomas are critical for optimal intramembranous bone formation in a tibial defect model of bone healing

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Osteal tissues contain a resident population of macrophages (OsteoMacs) that regulate osteoblast mineralisation in vitro. OsteoMacs form a canopy structure covering osteoblast bone forming surfaces (OBS) suggesting OsteoMacs regulate bone anabolic responses. Using the Maffia (Macrophage-Fas-Induced Apoptosis) transgenic mouse, in which the cfsr promoter drives the expression of AP20187 (www.ariad.com/regulationkits) ligand-inducible Fas-based suicide gene in macrophage/myeloid cells, we demonstrated that OsteoMacs are required for maintenance of OBS at sites of bone modelling. To definitively examine OsteoMacs contribution to vivo bone formation a tibial defect model was employed in which the defect site is filled with woven bone via intramembranous ossification 7 days post surgery. Immunohistochemistry demonstrated that F4/80+ OsteoMacs accumulated within the defect site, forming the canopy structure covering collagen type I (CTI)+ osteoblasts on woven bone surfaces. Osteonectins were performed in Maffia mice and a single injection of ligand or vehicle administered intra-defect at surgery. Healing was examined 7 days post surgery. Flow cytometry confirmed a 46±7% decrease in bone marrow F4/80+ macrophages in the contralateral limb of ligand treated mice. In ligand treated animals there was a striking reduction in the number of CTI+ osteoblasts and F4/80+ OsteoMac canopy within the defect. Quantitative immunohistochemical analysis of intra-defect CTI+ matrix area demonstrated a significant reduction (p = 0.03) in bone formation in ligand (20±6%) compared to vehicle (44±5%) treated mice. Osteoclasts express cfsr and therefore may be susceptible to apoptosis in ligand treated Maffia mice. To rule out that the decreased bone formation was indirectly due to inhibition of osteoclasts, osteonectins were performed in C57/Bl6 mice that were treated with OPG (1 mg/kg, initial intra-defect administration and subsequently subcutaneous injection every second day) to specifically inhibit osteoclasts. Immunohistochemical analysis of OPG treated animals demonstrated minimal change in intra-defect CTI+ woven bone deposition or defect healing. Distribution of F4/80+ OsteoMacs within the defect was unaffected by OPG treatment. These observations support that OsteoMacs, and not osteoclasts, play a critical role in driving and maintaining osteoblast function at sites of intramembranous bone deposition during growth and bone healing. Therefore OsteoMacs are novel participants in bone formation in vivo.

(1) www.ariad.com/regulationkits.

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Regulation of adipogenesis by Zfp467, a novel zinc-finger protein

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Broadening our current understanding of the molecular mechanisms of PTH induced bone formation will lead to the development of more effective treatments for bone disorders. Investigating gene regulation in differentiated mouse marrow stromal cells (Kusa 4Blb) treated with PTH(1–34) and probed onto an Affymetrix whole mouse genome microarray, has identified zinc-finger protein 467 (Zfp467) as a potential regulator of adipogenesis. Zfp467 belongs to the Krüppel-like family of transcription factors that bind to CC-rich DNA