P35 ROCK inhibitor down-regulates MMP-3 expression and increases aggregan production in human articular chondrocytes.

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<Objective> Chondrocytes lose their chondrocytic phenotypes in monolayer cultivation. We have reported that inhibition of the Rho family small GTPase ROCK promoted SOX9 expression and prevented the dedifferentiation of human articular chondrocytes. In the present study, we investigated the effect of ROCK inhibitor (ROCKi) on expression of catabolic MMP-3 and anabolic aggregan (AGN) in human articular chondrocytes. <Methods> Normal articular chondrocytes were cultured in the presence or absence of ROCKi (Y-27632, 1 uM). Expression of MMP-3 and AGN was assessed by quantitative real-time PCR analyses. Chondrogenic redifferentiation potential of monolayer-cultured chondrocytes was examined by pellet culture procedure using chondrogenic induction medium. <Results> ROCKi treatment suppressed gene expression of MMP-3 in monolayer-cultured chondrocytes. Continuous treatment of ROCKi (1 uM) prevented MMP-3 gene expression during the short- to long-term culturing periods. Relative expression level of AGN gene was increased by ROCKi treatment in monolayer-cultured chondrocytes. ROCKi pretreatment (1 uM) during monolayer culture influenced gene expression of MMP-3 and AGN in pellet-cultured chondrocytes. Chondrogenic pellets derived from ROCKi-untreated chondrocytes showed a lower expression level of MMP-3 and AGN compared with those derived from ROCKi-pretreated chondrocytes. The deposition of AGN was highly detected in a pellet derived from ROCKi-pretreated chondrocytes. The deposition of AGN was higher in ROCKi-pretreated pellets compared with untreated pellets. On the other hand, MMP3 deposition and its signal density were lower in ROCKi-pretreated chondrogenic pellets than in untreated pellets. <Conclusion> ROCKi suppressed expression of MMP-3 and stimulated AGN production in cultured articular chondrocytes. Our results suggest that ROCKi may have an important role in modulating the balance between degradation and production of cartilaginous extracellular matrix.