Transforming Growth Factor-β₂ Promotes the Integrin α₅β₁ Expression at Condensation Period of Chondrogenic Differentiation in vitro.

In our previous study, we showed that the enhancing role of TGF-β₂ in the chondrogenesis of limb bud mesenchymal cells is occued by stimulating the expression of fibronectin (FN) necessary for the initiation of limb bud chondrogenesis at the early step in in vitro chondrogenesis. To investigate further these events, the expression pattern of fibronectin receptors, α₅β₁ and α₅β₁, of TGF-β₂-treated chondroblast cultures were analyzed. In control cultures the integrin subunit α₃, α₅ and β₁ expression was continuously increased from 6 hr to 96 hr of cultures. On the other hand the expression of integrin α₅ was remarkably increased up to 24 hr and then rapidly decreased in treated cultures. Expressions of integrin α₃ were continuously increased during differentiation of chondroblasts in treated culture, however, the expressed amount of α₃ was weaker than that of control culture. Nevertheless, β₁ pattern of treated culture was similiar to that of control. These results with our previous data indicate that TGF-β₂ enhances chondrogenic differentiation by promoting interaction of FN and integrin α₅β₁ at condensation period, followed by down-regulation of this receptor.

A Role of Ca²⁺/CaM Kinase II in the Chondrogenic Differentiation of Chick Mesenchymal Cells in vitro

Ca²⁺ is reported to be an important enhancing factor in chondrogenesis of chick limb bud mesenchyme. However, it is not clear how Ca²⁺ regulates chondrogenic process. To investigate further the functional role of Ca²⁺, chondroblasts of HH-stage 23/24 chick limb mesenchyme were micromass cultured in the presence of KN-62, an inhibitor of Ca²⁺/CaM kinase II, and the effect this treatment on the chondrogenesis were analysed. It was found that Ca²⁺/CaM kinase II activity increased along with the chondrogenic differentiation and addition of Ca²⁺ promoted the enzyme activity in control culture. KN-62 inhibited chondrogenesis in dose-dependent manner and it diminished promoting effect of Ca²⁺ on chondrogenesis. Moreover, the inhibitory effect of KN-62 on chondrogenesis by KN-62 was most effective when treated for the first 24 hrs. These data indicate that Ca²⁺ might play an enhancing role through modulation of Ca²⁺/CaM kinase II at the early stage of chondrogenic differentiation. Currently we are purifying the substrate for this enzyme from cultured chondroblast.