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Transforming Growth Factor-β_2 Promotes the Integrin $\alpha_5\beta_1$ Expression at Condensation Period of Chondrogenic Differentiation *in vitro.*

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In our previous study, we showed that the enhancing role of TGF-β_2 in the chondrogenesis of limb bud mesenchymal cells is occured 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, $\alpha_3\beta_1$ and $\alpha_5\beta_1$, of TGF-β_2-treated chondroblast cultures were analyzed. In control cultures the integrin subunit α_3 , α_5 and β_1 expression was continuously increased from 6 hr to 96 hr of cultures. On the other hand the expression of integrin α_5 was remarkably increased up to 24 hr and then rapidly decreased in treated cultures. Expressions of intergrin α_3 were continuously increased during differentiation of chondroblasts in treated culture, however, the expressed amount of α_3 was weaker than that of control culture. Nevertheless, β_1 pattern of treated culture was similat to that of control. These results with our previous data indicate that TGF-β_2 enhances chondrogenic differentiation by promoting interaction of FN and integrin $\alpha_5\beta_1$ at condensation period, followed by down-regulation of this recptor.

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A Role of Ca^{2+}/CaM Kinase II in the Chondrogenic Differentiation of Chick Mesenchymal Cells *in vitro*

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Ca^{2+} is reported to be an important enhancing factor in chondrogenesis of chick limb bud mesenchyme. However, it is not clear how Ca^{2+} regulates chondrogenic process. To investigate further the functional role of Ca^{2+}, chondroblasts of HH-stage 23/24 chick limb mesenchyme were micromass cultured in the presence of KN-62, an inhibitor of Ca^{2+}/CaM kinase II, and the effect this treatment on the chondrogenesis were analysed. It was found that Ca^{2+}/CaM kinase II activity increased along with the chondrogenic differentiation and addition of Ca^{2+} promoted the enzyme activity in control culture. KN-62 inhibited chondrogenesis in dose-dependent manner and it diminished promoting effect of Ca^{2+} on chondrogenesis. Moreover, the inhibitory effct of KN-62 on chondrogenesis by KN-62 was most effective when treated for the first 24 hrs. These data indicate that Ca^{2+} might play an enhancing role through modulation of Ca^{2+}/CaM kinase II at the early stage of chondrogenic differentiation. Currently we are purifing the substrate for this enzyme from cultured chondroblast.