

## D111

### Transforming Growth Factor- $\beta_2$ 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- $\beta_2$  in the chondrogenesis of limb bud mesenchymal cells is occurred 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- $\beta_2$ -treated chondroblast cultures were analyzed. In control cultures the integrin subunit  $\alpha_3$ ,  $\alpha_5$  and  $\beta_1$  expression was continuously increased from 6 hr to 96 hr of cultures. On the other hand the expression of integrin  $\alpha_5$  was remarkably increased up to 24 hr and then rapidly decreased in treated cultures. Expressions of integrin  $\alpha_3$  were continuously increased during differentiation of chondroblasts in treated culture, however, the expressed amount of  $\alpha_3$  was weaker than that of control culture. Nevertheless,  $\beta_1$  pattern of treated culture was similar to that of control. These results with our previous data indicate that TGF- $\beta_2$  enhances chondrogenic differentiation by promoting interaction of FN and integrin  $\alpha_5\beta_1$  at condensation period, followed by down-regulation of this receptor.

## D112

### 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 effect of KN-62 on chondrogenesis by  $Ca^{2+}$  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 purifying the substrate for this enzyme from cultured chondroblast.