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

정재철, 박대규, 강신성
경북대학교 자연과학대학 생명과학

In our previous study, we showed that the enhancing role of TGF-β2 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, α3β1 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 similar 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.

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

김수동*, 인혜정, 손중경†, 강신성
경북대학교 자연과학부 생명과학과, †사범대 생명교육과

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.