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Ca²⁺ Enhanced the Cell-Cell Interactions via FN and Its Receptors at the Early Condensation Period of Chondrogenic Differentiation by Activating CREB through CaM II Kinase

김수동*, 인혜정, 박대규, 강신성
경북대학교 자연과학대학 생물학과

In our previous studies, we have showed that Ca²⁺ plays an enhancing role by stimulating cellular condensation and by activating the Ca²⁺/calmodulin-dependent kinase II(CaM kinase II) at the early step in chondrogenic differentiation. To investigate further the functional role of Ca²⁺, chondroblasts of HH-stage 23/24 chick limb mesenchyme were micromass cultured and the effect of Ca²⁺ on the production of fibronectin(FN) and its cell surface receptors, integrin $\alpha 5\beta 1$, were analyzed. It appeared that extracellular Ca²⁺ markedly enhanced the synthesis of FN and integrin $\alpha 5\beta 1$ from 6 hr to 72 hr of cultures, while KN-62, an specific inhibitor of CaM II kinase, drastically decreased the synthesis of both proteins. In control culture, CREB was detected at 6 hr and disappeared at 24 hr, however, Ca²⁺ stimulated the expression of CREB for longer period upto 48 hr and KN-62 blocked CREB production. These results suggest that the increment of intracellular Ca²⁺ enhanced the cell-cell interactions via FN and its receptor at the early condensation period of chondrogenic differentiation by stimulating FN and its receptor genes through activating CREB, which can be stimulated by CaM II kinase.

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Ca²⁺-ATPase Regulates the Intracellular Ca²⁺ in *in vitro* Chondrogenesis of Limb Bud Mesenchymal Cells

김수동*, 손종경, 강신성
경북대학교 자연과학대학 생물학과

Ca²⁺ is reported to be an important enhancing factor in chondrogenesis of chick limb bud mesenchymal cells *in vitro*, however, it is not clear how Ca²⁺ regulates chondrogenic processes. The plasma membrane Ca²⁺-ATPase, whose activity is regulated by calmodulin (CaM) through the binding at CaM-binding site in carboxyl-terminal, is an important enzyme regulating the intracellular Ca²⁺. In this study a functional role of Ca²⁺-ATPase in *in vitro* chondrogenesis was carried out. It appeared that Ca²⁺-ATPase activity was increased during the chondrogenic differentiation. CaM antagonists like trifluoperazine (TFP), W-7, chlorpromazine decreased the Ca²⁺-ATPase activity, while these antagonists promoted the chondrogenic differentiation. Nevertheless addition of CaM enhanced the Ca²⁺-ATPase activity. Thus it can be said that increased cytosolic Ca²⁺ by inhibiting the Ca²⁺-ATPase due to the blockage of CaM by TFP induces the chondrogenic differentiation.