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Ca^{2+} 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

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In our previous studies, we have showed that Ca^{2+} plays an enhancing role by stimulating cellular condensation and by activating the Ca^{2+}/calmodulin-dependent kinase II(CaM kinase II) at the early step in chondrogenic differentiation. To investigate further the functional role of Ca^{2+}, chondroblasts of HH-stage 23/24 chick limb mesenchyme were micromass cultured and the effect of Ca^{2+} on the production of fibronectin(FN) and it's cell surface receptors, integrin α5β1, were analyzed. It appeared that extracellular Ca^{2+} markedly enhanced the synthesis of FN and integrin α5β1 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 disappeard at 24 hr, however, Ca^{2+} 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 Ca^{2+} 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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Ca^{2+}-ATPase Regulates the Intracellular Ca^{2+} in *in vitro* Chondrogenesis of Limb Bud Mesenchymal Cells

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Ca^{2+} is reported to be an important enhancing factor in chondrogenesis of chick limb bud mesenchymal cells *in vitro*, however, it is not clear how Ca^{2+} regulates chondrogenic processes. The plasma membrane Ca^{2+}-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 Ca^{2+}. In this study a functinal role of Ca^{2+}-ATPase in *in vitro* chondrogenesis was carried out. It appeared that Ca^{2+}-ATPase activity was increased during the chondrogenic differentiation. CaM antagonists like trifluoperazine (TFP), W-7, chlorpromazine decreased the Ca^{2+}-ATPase activity, while these antagonists promoted the chondrogenic differentiation. Nevertheless addition of CaM enhanced the Ca^{2+}-ATPase activity. Thus it can be said that increased cytosolic Ca^{2+} by inhibiting the Ca^{2+}-ATPase due to the blockage of CaM by TFP induces the chondrogenic differentiatinon.