E105 The pattern of protein phosphorylation by protein kinase C during chondrogenesis *in vitro*

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Protein kinase C (PKC) is known to be related to differentiation of chick limb bud mesenchyme *in vitro*. In this study, we assayed the phosphorylation pattern by PKC during chondrogenesis. Cells prepared from HH-stage 23/24 chick wing buds were cultured for 3 days and *in vitro* phosphorylation was performed in PKC activating condition. Sixty two- and 65-kDa proteins were phosphorylated specifically, which proteins were observed mailny in the membrane fraction. As chondrogenesis proceeds, phosphorylation of 65-kDa protein was increased, while phosphorylaiton of 62-kDa protein showed little change. Treatment of lysophosphatidylcholine (LPC), which activates some PKC isoforms and sustains activation of PKC, to cells cultured for the first 24 hr promoted chondrogenesis and increased the phosphorylation of 62-kDa protein *in vitro*. However, under the same condition PMA suppressed the phosphorylation of 62-kDa protein. When isotypes of PKC were assayed by western blot analysis, PKC-α and γ (cPKC) were translocated to membrane in 1 day-cultured cells by LPC treatment, which is consistent with the phosphorylation pattern of 62-kDa protein. These results suggest that phosphorylation of 62-kDa protein by PKC may play a role at the early stage of chondrogenesis of chick limb mesenchyme.

E106 Potentiation of the activity of nitric oxide by phorbol ester in HL-60 human myeloid cell

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Exposure of cells to sodium nitroprusside (SNP; 0.5 to 2 mM), a NO generating agent, induced DNA fragmentation of HL-60 cells, an early event in apoptosis. Moreover, SNP in combination with phorbol ester markedly increased the extent of DNA fragmentation (by 5-6 fold) while not affecting phorbol ester-induced cellular differentiation to monocytic lineage as determined by plastic adherence and CD 14 expression. However, superoxide dismutase and catalase did not suppress the phorbol ester plus SNP-induced DNA fragmentation, indicating that peroxinitrite, a strong oxidant generated from the interaction of NO and superoxide, does not involve in this process, and the stage-2 tumor promoter mezrein also mimicked the effect of phorbol ester. Under the same conditions, SNP in combination with phorbol ester caused apoptosis in other human myeloid cell line, U-937 cells, but not induced apoptosis in normal peripheral blood mononuclear cells. Taken together, these findings suggest that exposure of HL-60 cells to phorbol ester renders them more susceptible to NO-induced DNA damage and that this phenomenon contributes to the synergistic cytotoxic effects of NO and PKC in myeloid leukemia cells.