D-53 Tyrosine Phosphorylation of FAK in Integrin-mediated Signaling during Chondrogenic Differentiation

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Chondrogenic differentiation is initiated by a cellular condensation and proliferation followed by accumlation of extracellular matrix. The interaction of cells with FN mediated by integrins, *i.e.*α5β1 is believed to correlate with the onset of chondrogenesis but the signaling mechanism of integrins is still obscure because their short cytoplasmic domains do not possess endogenous catalytic activity. Recently it has been reported that binding of integrins to extracellular ligands activates FAK, which then generates a tyrosine phosphorylation cascade within the cell. To elucidate a possible functional relationship between FAK and the integrin-mediated signaling during chondrogenesis, we investigated the tyrosine phosphorylation state of FAK in *in vitro* micromass culture of wing bud mesenchymal cells. Tyrosine phosphorylation of FAK was sequencially increased to day 3 of the culture after then decreased, which is consistent with the expression pattern of FN as an extracellular ligand for integrin, α5β1, while the expression level of FAK was not changed during chondrogenesis. Moreover, tyrosine phosphorylated FAK was associated with cytoskeletal talin and vinculin. These data suggest that the cell and FN interaction plays important role for chondrogenesis and their intracellular signaling occurs via FAK activation, which is mediated by talin.

D-54 Induction of Nitric Oxide Synthase in Monocyte and Macrophage Cell Lines by Cell Adhesion

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Nitric oxide(NO) appears to be an important and pleiotropic bioregulator of immune responses, especially as a potent antitumoral mediator. Vitamin E-succinate(VES), α-tocoperol, is known for anticarcinogenic effect as an antioxidant, while the precise mechanism of its effect is not fully understood. It was proposed that VES induction of monocyte cell adhesion is the result from increased amounts of fibronectin(FN) binding to VES induced cell surface FN-receptor. Using human monocytic U937 and mouse macrophage Raw264.7 cell lines, we analyzed the effect of VES and FN-coating on the generation of NO. High level of nitrites were detected for induction of inducible nitric oxide synthase(iNOS) in cell supernatants as soon as 24-48 hours following cell cultured with VES or in FN-coated well, but not in untreated cells. iNOS activity were completely blocked in cultured cells by N-G-monomethyl-L-arginine. Moreover, combination with VES and FN- coating drastically enhanced the NO production compared to that induced with VES, FN-coating, and LPS/IFN-γ(a common NO inducer), respectively. The protein level of NOS analyzed by Western blotting showed the same result. In cytokine-stimulated cells, activation of NF-kB is a common regulatory component in iNOS gene expression. In this study, the DNA-binding activities of NF-kB proteins as determined by EMSA were increased with treatment of VES or/and FN-coating like as with treatment of LPS/IFN-γ. These data suggest that VES and FN function synergistically in induction of iNOS by cell adhesions through FN and its receptor. However, the precise role of VES in iNOS induction is remained to be elucidated.