

Role of calcineurin in the *in vitro* regulation of cartilage differentiation and in the signal transduction pathways of human mononuclear cells

Protein phosphatases play equally important role as protein kinases in the regulation of protein phosphorylation processes of different signal transduction pathways controlling cell and tissue differentiation. We have shown the presence and the active function of calcineurin in micromass cultures and we observed that the mRNA level and the activity of the enzyme gradually decreased during the cartilage differentiation. Our results suggest a positive role of calcineurin in the regulation of *in vitro* chondrogenesis of chicken micromass cultures. We suppose that the reduced activity of calcineurin and the elevated activity of Erk1/2 during oxidative stress are likely mediators of the chondrogenesis inhibiting effect of oxidative stress. We also propose a negative regulatory role of calcineurin either in Erk1/2 pathway and/or in Sox9 phosphorylation. Our data support a positive role of Erk1/2 pathway in Sox9 phosphorylation, but under oxidative stress other Erk-activated factor(s) can oppose this positive effect of Sox9 on chondrogenesis.

We have shown that both PMA- and Ca^{2+} -treatments contribute to the decrease of calcineurin activity of T-cell enriched peripheral blood mononuclear cells without modulating the mRNA and protein levels of calcineurin. The calcineurin activity was more sensitive to Ca-ionophore than to the PMA-treatment as the inhibition of the activity was higher for Ca-ionophore. Since Gö6976 was able to reverse the inhibitory effect of PMA and Ca-ionophore applied alone or in combination on the calcineurin activity we suppose that the cPKC (PKC α , β , γ) isoenzymes can be involved in the inhibition of the enzyme.

The increase of intracellular Ca^{2+} concentration induced by Ca-ionophore significantly decreased the calcineurin activity. It seems that the reduced activity of calcineurin is more likely related to the activity of cPKC by calcium signal than the direct effect of calcium on calcineurin. Since the phosphorylation of calcineurin by PKC does not influence the phosphatase activity we assume the transducing role of Cabin 1 in the linking of PKC and calcineurin pathways. We provided evidence that the Cabin 1 hyperphosphorylated and activated by PKC takes part in the linking of cPKC and calcineurin pathways in peripheral mononuclear cells from healthy donors.