C-Type Natriuretic Peptide Negatively Cross-Talks with Fibroblast Growth Factor Receptor 3 Signaling in ATDC5 Cells

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Objective and Method　We have reported that C-type natriuretic peptide (CNP) stimulates endochondral ossification and corrects the shortened body length of achondroplasia model mouse, which is caused by constitutive active fibroblast growth factor receptor 3 (FGFR3).

In order to examine the molecular basis of these findings, using ATDC5 cells, mouse chondrogenic cell line, we investigated, 1) the effect of basic FGF on CNP- stimulated intracellular guanosine 3', 5'-cyclic monophpsphate (cGMP) production by radioimmunoassay and 2) the effect of CNP on basic FGF-stimulated Erk1/2 phospholilation in MAP kinase cascade by western blot analysis.

Result　1) In ATDC5 cells, CNP stimulated the production of cGMP in a dose-dependent manner (10^{-9}～10^{-7}M CNP) with a 30-fold increase of the basal level at 10^{-7}M CNP. This increase was inhibited by about 60% with the addition of 10～100ng/mL of basic FGF. 2) Erk1/2 phospholilation was barely detectable at the basal level but was noticeably stimulated with the addition of basic FGF (1～10ng/mL). CNP (10^{-7}～10^{-6}M) inhibited the basic FGF-induced Erk1/2 phospholilation in a dose-dependent manner without decreasing the amount of Erk protein. 10^{-4}M 8-Bromo cGMP also inhibited Erk1/2 phospholilation.

Discussion　Basic FGF/FGFR3 is known as the negative regulator of endochondral ossification. We have shown that basic FGF inhibited the CNP-stimulated cGMP production by disrupting a signaling pathway through GC-B while CNP antagonized the basic FGF activation of the MAP kinase cascade. These results indicate the presence of a negative cross-talk between CNP/GC-B and MAP kinase pathways and suggest the efficacy of CNP as therapeutic reagent for achondroplasia.