

C-type Natriuretic Peptide and Achondroplasia

C-type natriuretic peptide (CNP) is a member of a family of 3 related peptides—atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and CNP. They act by inducing accumulation of intracellular cGMP through 2 subtypes of guanylyl cyclase: guanylyl cyclase A for ANP and BNP and guanylyl cyclase B for CNP. Although the natriuretic peptides are known mainly for regulating the cardiovascular system, there is growing evidence that CNP is an important positive regulator of endochondral bone growth. For example, genetically engineered mice have short bones when null for CNP and long bones when CNP is overexpressed. In fact, growth plates in these mice are shortened and widened in a manner similar to that detected in mice with loss- and gain-of-function mutations for FGFR3, respectively. These observations led the group headed by Nakao to propose a functional relationship between CNP and FGF signaling in the growth plate, which they have now demonstrated by mouse genetics.

The group first generated transgenic mice in which CNP was overexpressed in the growth plate; expression of the gene encoding CNP, designated *Nppc*, was driven by the type II collagen cartilage-specific promoter (*Col2*). The *Col2-Nppc* transgenic mice displayed excessive skeletal growth that was mainly postnatal. Compared to non-transgenic littermates, the *Col2-Nppc* transgenic mice had longer body length, longer limb bones, a longer cranial base (measured as naso-occipital distance), and wider growth plates by histology.

Next, the *Col2-Nppc* transgenic mice were mated to another transgenic mouse strain in which the achondroplasia-activating mutation of FGFR3 was expressed in cartilage also under the control of the type II collagen promoter (*Col2-FGFR3^{ach}*). The latter mouse strain exhibits a dwarf phenotypic with characteristics of human achondroplasia and is considered an animal model for this condition. Offspring of this mating that carried both the *Col2-Nppc* and *Col2-FGFR3^{ach}* transgenes had near normal body lengths when measured over 10 weeks. At 3 months, measurements of cranial base length, femurs, and humeri were statistically the same as non-transgenic mice, indicating that over-expression of CNP in the growth plate had rescued the dwarfism caused by the achondroplasia transgene. There was also restoration of the shortened growth plate of the *Col2-FGFR3^{ach}* mice toward normal in the mice harboring both transgenes. Of note, the over-expression of CNP did not appear to rescue the reduced proliferation of growth plate chondrocytes detected in the *Col2-FGFR3^{ach}* mice.

To confirm the direct effect of CNP on bone growth, the authors treated cultured tibias from *Col2-FGFR3^{ach}* mice with different doses of CNP. Bone length showed a dose response to the CNP. The dose that restored bone

length to normal also restored synthesis of 2 markers of cartilage matrix biosynthesis—glycosaminoglycan and collagen—which were reduced in the *Col2-FGFR3^{ach}* mice to near normal.

The authors next examined the effect of CNP on FGFR3 signaling pathways in the tibial explants. No differences were observed in FGF-induced STAT1 signaling, which has been implicated in the control of chondrocyte proliferation. However, CNP reduced signaling through the MAP kinase-ERK pathway.

The model that Yasoda et al¹ constructed suggests that FGFR3 signals through STAT1 to down regulate chondrocyte proliferation and differentiation and through the MAP kinase-ERK pathway to negatively control matrix synthesis in the growth plate. They propose that CNP blocks the MAP kinase inhibitory signals of FGFR3 to increase matrix synthesis and thereby counters the restraining consequences of FGFR3 on bone growth. They speculate that these observations could form a basis for a new therapeutic approach to treating achondroplasia.

Yasoda A, Komatsu Y, Chusho H, et al. Overexpression of CNP in chondrocytes rescues achondroplasia through MAPK-dependent pathway. *Nat Med.* 2004;10:80-86.

Editor's Comment: *This is a very interesting paper that brings to the fore a growth plate regulatory circuit that has not been widely appreciated in the bone growth field. It also suggests that contrary to the popular view that activating FGFR3 mutations acts primarily through inhibition of chondrocyte proliferation and differentiation, they may also act by inhibiting the synthesis of the extracellular matrix that also contributes to bone growth.*

The idea that CNP could be used to stimulate growth in achondroplasia is intriguing. Obviously, this work needs to be confirmed and much more investigation done, but in theory, blocking a downstream pathway that propagates growth inhibitory FGFR3 signals has promise.

Of caution is that high levels of CNP likely generated in cartilage of the transgenic mice, which presumably would be needed to counter the effects of mutant FGFR3 in patients, may be very difficult to achieve in a therapeutic setting, especially without having adverse effects on other tissues that respond to CNP such as kidney, adrenal gland, and cardiovascular system or on other regulatory circuits that utilize MAP kinase-ERK pathways. Nevertheless, the unfolding of this story deserves considerable attention.

William A. Horton, MD