IDENTIFICATION OF A MECHANO-RESPONSIVE REGION IN THE PROMOTER OF TYPE X COLLAGEN GENE

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Introduction. The gene encoding collagen type X, α1(X), is important for skeletal development and aging. Its protein product is expressed specifically by hypertrophic chondrocytes from a growth plate of a long bone. This specificity is primarily regulated at the level of transcription. The defect of the type X collagen gene causes Schmid Metaphyseal Chondrodysplasia (SMCD) with short stature and brittle bones. Interestingly, although the type X collagen gene is mainly expressed during enchondral ossification, it is re-expressed by articular chondrocytes during osteoarthritic pathogenesis. However, it is not clear which factor modulates type X collagen gene expression by chondrocytes. Mechanical load is an important factor in the chondrocyte microenvironment to influence cell metabolism and gene expression. Mechanical load leads to deformation of cartilage matrix and generates strain signal, which is sensed by chondrocytes embedded within extracellular matrix. Mechanical signals then activate mechanotranduction pathways to alter gene expression. Previously, we identified type X collagen gene as one of the mechano-responsive genes in cartilage. The level of type X collagen mRNA was dramatically up-regulated by cyclic stretch-induced matrix deformation in a three-dimensional (3D) chondrocyte culture we established recently. In this study, we analyzed the promoter region of chicken type X collagen gene using molecular techniques. We identified a mechano-responsive region within the type X collagen gene promoter.

Methods. Two populations of hypertrophic chondrocytes were isolated for the study. Early hypertrophic chondrocytes were isolated from cephalic parts of chick sterna from 17-day embryos, while late hypertrophic chondrocytes were isolated from hypertrophic zone of tibiotarsal growth plates of 15-day chick embryos. Four µg of plasmids pCAT I/II containing the chicken collagen X gene promoter fused to a reporter gene (chloramphenical acetyl transferase, CAT), and 1 µg of β-galactosidase internal control plasmid pSV-β-gal, were cotransfected into cells with 25 µg LipofectAMINE reagent in serum-free growth medium (OPTI-MEM, GIBCO, BRL) without antibiotic reagent. Transfected cells were suspended in Ham-12 containing 10% FBS and seeded in 3D collagen sponges (Gelform, Upjohn) before they were stretched with 5% elongation by a computer controlled Bio-Stretcher at 60 cycles/min, 15min/hr for 2 days. Cells were harvested from sponges by digestion of the collagen sponge with 0.03% collagenase for 20 minutes. Total RNA was extracted from cells cultured in sponges with RNase mini kit (QIAGEN). Real-time quantitative RT-PCR (Biotechnology Institute) was performed to quantify the mRNA level of the type X collagen. CAT (pg) and β-galactosidase (OD₆₅₀) were measured by using CAT ELISA kit (Roche, Germany) and β-Gal ELISA (Mannheim, Boehringer). Normalized levels of CAT expression were calculated by CAT (pg) / β-Gal (OD₆₅₀).

Results. Cyclic deformation of collagen matrix greatly stimulated the mRNA level of type X collagen in early hypertrophic chondrocytes, which just started synthesizing type X collagen. Under cyclic stretch conditions, the type X collagen mRNA level was 2 to 4 fold higher than that of non-stretched cells, as measured by real-time quantitative RT-PCR (Fig. 1). In contrast, cyclic stretch did not increase the type X collagen mRNA level in late hypertrophic chondrocytes, which were already synthesizing a high level of type X collagen. Thus, mechanical signals stimulated type X collagen gene expression, but did not raise the maximal level of type X collagen mRNA in hypertrophic chondrocytes. To understand the mechanism underlying mechanical stimulation of type X collagen gene expression at the transcriptional level, two type X promoter-reporter gene constructs, pCAT I and pCAT II were transfected into early hypertrophic chondrocytes. pCAT I contained nucleotides from –562 bp to +86 bp of the collagen X promoter region ligated to a CAT reporter gene, while pCAT II contained an additional 2.6 kb upstream promoter sequence (~3,200 to –563) nucleotide elements that exerted responsiveness to mechanical signals. Sequence analysis of this mechano-responsive region revealed that it contained three repeats of stretch/shear stress responsive elements (SSRE) GAGACC, with two repeats surrounding one repeat at the opposite orientation. Currently we are testing whether these SSRE repeats are involved in mechanical responsiveness of the type X collagen gene by deletion analysis and site-directed mutagenesis.

Discussion. In this study, we have shown that the cyclic matrix deformation stimulated type X collagen mRNA level in early hypertrophic chondrocytes, but not in fully differentiated hypertrophic chondrocytes. This suggests that regulation of type X collagen gene expression at the transcriptional level consists of at least two components: a factor(s) that determines the level of expression in a tissue-specific manner and a modulator(s) that further adjusts the level of expression according to mechanical environment. Consistent with this hypothesis, our promoter analysis indicates that the promoter of type X collagen gene contains at least two functional regions, one of which (~562 bp to +86 bp) is required for basal level of expression, and the other one (~3,200 bp to –562 bp) is a mechano-responsive region. The former region does not contain any SSRE and is not responsive to mechanical regulation, while the latter region contains three SSRE repeats and is responsive to mechanical stimulation. Interestingly, SSRE repeats may also mediate mechanical response in the promoters of PDGF, tenascin, and type XII collagen. SMCD results from haploinsufficiency of type X collagen, since we have shown that mechanical signals may up-regulate type X collagen mRNA by at least two fold, understanding the mechanism of mechanical stimulation of type X collagen may help treat and prevent SMCD by raising the type X collagen level in early hypertrophic chondrocytes.