MATRILIN: ESSENTIAL PERICELLULAR COMPONENT OF MECHANOTRANSDUCTION COMPLEX TO INDUCE INDIAN HEDGEHOG SIGNALING IN CARTILAGE

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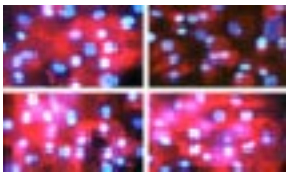
Introduction: It is known that cartilage is a mechano-sensitive tissue. Chondrocytes alter their metabolism and gene expression in response to mechanical signals. For example, it has been shown that dynamic loading may induce Indian hedgehog (Ihh) signaling in growth cartilage. Ihh, in turn, regulates chondrocyte proliferation and differentiation. However, it is not known how mechanical signals are transduced from the extracellular matrix (ECM) where the tissue deformation occurs, to the chondrocyte, and ultimately to the nucleus to regulate gene expression. In this study, we identify matrilin, a pericellular matrix molecule, as an essential component of mechanotransduction complex to transduce mechanical signals to the chondrocyte. Matrilins are novel ECM protein family which consists at least of four members. Cartilage matrix protein (CMP/matrilin-1), the first and the prototype of the matrilin family, is expressed specifically in cartilage together with matrilin-3. Furthermore, matrilin 1 and 3 co-oligomerize via c-terminal coiled coil domain. All the members of matrilin family contain matrix adhesive vWF A domains separated by EGF-like repeats. Matrilin-1 and 3 form pericellular filaments to connect ECM to chondrocytes via integrins. Previously we constructed a dominant negative mini-mat-3 cDNA, which contained only the coiled coil domain and EGF repeats, but lacked the functional A domains. Overexpression of this cDNA in chondrocytes sequested endogenous matrilin-1 and matrilin-3, thereby disrupting matrilin assembly and function in cartilage. Using this construct, in this study, we test the role of matrilins in mechanotransduction in cartilage.

Materials and Methods: Primary cultures of chick embryonic chondrocytes (CECs) were established from caudal part of 17-d embryonic sterna cartilage. Chondrocytes were cultured in Ham's F-12 medium containing 10% FBS. For primary cultures of chick embryonic fibroblasts (CEFs), skin fibroblasts from 9-d embryonic chicks were cultured in DMEM containing 10% FBS. A mini-matrilin-3 cDNA that lacks the A1 domain was cloned by RT-PCR from total RNA isolated from chick sterna cartilage(2). The mini-mat-3 was then cloned into a retroviral vector RCAS with V5 tag. In addition, a wild-type matrilin-1 and matrilin-3 was also cloned into RCAS without V5 tag. Transfection of CEF was performed by calcium phosphate method. After viral particles were collected from the conditioned medium of CEF, they were used to infect CEC. Immunofluorescent cytochemistry was performed using pAb p-27 against viral particle RCAS, a pAb against V-5-tag, mAb 1H1 against matrilin-1, mAb I-BA1 against type I collagen, and mAb II-II6B3 against type II collagen. Western blot analysis of the conditioned medium from CEC or CEF was performed using a pAb against V-5-tag, and a pAb D-2 against matrilin-1, under both reducing and non-reducing conditions. In addition, empty viral vector RCAS was used as a control for viral infection, and mock (no DNA) for control of transfection. For mechanical stress experiments, chondrocytes transfected by RCAS, RCAS/matrilin-1, and RCAS/mini-matrilin-3, were cultured in a 3D collagen culture, which was then loaded mechanically with a computer-controlled Bio-Stretch device, to induce 5% matrix deformation at 60 cycles/min, 15 min/hour. After two days of mechanical loading, mRNA was isolated from cultured chondrocytes. The mRNA levels of matrilin-1, matrilin-3, Type X collagen, and Ihh were determined by real time RT-PCR.

Results: Our hypothesis was that pericellular matrilin filaments were essential for transmission of stretch-induced matrix defoemation signals from extracellular matrix to chondrocytes. To test this hypothesis, the abundance of pericellular matrilin filaments were either decreased by transfection of a dominant negative mini-matrilin-3, or increased by transfection of a wild-type matrilin-1 or matrilin-3 into chondrocytes. Immunostaining with an antibody against endogenous matrilin-1 indicated that overexpression of mini-mat-3 abolished pericellular matrilin filaments, but did not affect intracellular matrilin production (Fig. 1). Overexpression of wild-type matrilin increased the abundance of pericellular matrilin filaments (Fig. 1). These transfected cells were then cultured in a 3D collagen culture system, to which dynamic mechanical loading was applied. The effects of alteration of matrilin filaments on mechano-responsiveness of chondrocytes were determined by quantifying the mRNA levels of mechano-responsive genes such as Ihh, type X collagen, and matrilin-1, which we identified previously. In mock-transfected, or vector RCAS transfected cells, Ihh mRNA level was increased 50 fold in response to mechanical stress (Fig. 2). This mechanical stimulation of Ihh mRNA was completely abolished by expression of a dominant negative mini-matrilin3 (mMT3) in chondrocytes, which lacked pericellular matrilin filaments (Fig. 2). In cells overexpressing wild-type matrilin-1 (MT1) or matrilin-3 (MT3), which produced excessive pericellular matrilin filaments, the extent of mechanical stimulation of Ihh mRNA level was reduced, but not abolished (Fig. 2). The mRNA data from other mechano-reponsive genes such as type X collagen and matrilin-1 presented the same pattern as that of Ihh mRNA, thus confirming our conclusion.

Discussion: In invertebrates such as c. elegans, it has been shown that a mechanotransduction complex consists several components, such as extracellular matrix molecules such as collagens, pericellular matrix molecules that contain EGF repeats, ion channels, and cytoskeletal proteins. However, identification of components of mechanotransduction complex in vertebrates has been very difficult, and thus very little information is available. In this study, we have identified matrilins as essential components to transduce mechanical signals in cartilage, including mechanical induction of indian hedgehog signals. Our data suggest that matrilin filaments are required for transducing matrix deformation signals to chondrocytes. Lack of functional matrilins completely abolishes the mechanoresponsiveness of chondrocytes. In chondrocytes that express excessive wild-type matrilins, mechanical responsiveness is decreased but not abolished. Therefore, the right amount of pericellular matrilins is essential for optimal transduction of mechanical stress signals in cartilage. Our data reveal the first functional role of matrilins as a component of mechanotransduction complex to transduce matrix deformation stress signals to chondrocytes.



Mock	mini-matrilin-3
RCAS	matrilin-1

Fig. 1

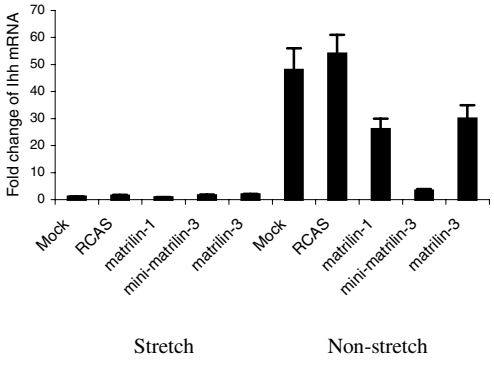


Fig. 2